

RESILIENCE OF GRAPEVINE TO CLIMATE CHANGE: FROM PLANT PHYSIOLOGY TO ADAPTATION STRATEGIES

EDITED BY: Chiara Pastore, Chris Winefield, Maria Paz Diago and
Tommaso Frioni

PUBLISHED IN: Frontiers in Plant Science





frontiers

Frontiers eBook Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence.

The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714

ISBN 978-2-83250-009-5

DOI 10.3389/978-2-83250-009-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.

Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

RESILIENCE OF GRAPEVINE TO CLIMATE CHANGE: FROM PLANT PHYSIOLOGY TO ADAPTATION STRATEGIES

Topic Editors:

Chiara Pastore, University of Bologna, Italy

Chris Winefield, Lincoln University, New Zealand

Maria Paz Diago, University of La Rioja, Spain

Tommaso Frioni, Catholic University of the Sacred Heart, Piacenza, Italy

Citation: Pastore, C., Winefield, C., Diago, M. P., Frioni, T., eds. (2022). Resilience of Grapevine to Climate Change: From Plant Physiology to Adaptation Strategies. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-009-5

Table of Contents

- 05 Editorial: Resilience of grapevine to climate change: From plant physiology to adaptation strategies**
Chiara Pastore, Tommaso Frioni and Maria P. Diago
- 10 Biodiversity of Local *Vitis vinifera* L. Germplasm: A Powerful Tool Toward Adaptation to Global Warming and Desired Grape Composition**
Tommaso Frioni, Giovanni Bertoloni, Cecilia Squeri, Alessandra Garavani, Lily Ronney, Stefano Poni and Matteo Gatti
- 30 Optimal Ranges and Thresholds of Grape Berry Solar Radiation for Flavonoid Biosynthesis in Warm Climates**
Nazareth Torres, Johann Martínez-Lüscher, Etienne Porte and S. Kaan Kurtural
- 45 Day Temperature Has a Stronger Effect Than Night Temperature on Anthocyanin and Flavonol Accumulation in ‘Merlot’ (*Vitis vinifera* L.) Grapes During Ripening**
Yifan Yan, Changzheng Song, Luigi Falginella and Simone D. Castellarin
- 61 Improving Net Photosynthetic Rate and Rooting Depth of Grapevines Through a Novel Irrigation Strategy in a Semi-Arid Climate**
Xiaochi Ma, Pete W. Jacoby and Karen A. Sanguinet
- 73 *Vitis vinifera* L. Diversity for Cations and Acidity Is Suitable for Breeding Fruits Coping With Climate Warming**
Antoine Bigard, Charles Romieu, Yannick Sire and Laurent Torregrosa
- 86 Mitigating Heat Wave and Exposure Damage to “Cabernet Sauvignon” Wine Grape With Partial Shading Under Two Irrigation Amounts**
Johann Martínez-Lüscher, Christopher Cody Lee Chen, Luca Brillante and Sahap Kaan Kurtural
- 101 High Temperature and Elevated Carbon Dioxide Modify Berry Composition of Different Clones of Grapevine (*Vitis vinifera* L.) cv. Tempranillo**
Marta Arrizabalaga-Arriazu, Eric Gomès, Fermín Morales, Juan José Irigoyen, Inmaculada Pascual and Ghislaine Hilbert
- 119 Temperature Shift Between Vineyards Modulates Berry Phenology and Primary Metabolism in a Varietal Collection of Wine Grapevine**
Kelem Gashu, Noga Sikron Persi, Elyashiv Drori, Eran Harcavi, Nurit Agam, Amnon Bustan and Aaron Fait
- 142 Sunburn in Grapes: A Review**
Joanna M. Gambetta, Bruno P. Holzapfel, Manfred Stoll and Matthias Friedel
- 163 Evaluating Strategies for Adaptation to Climate Change in Grapevine Production—A Systematic Review**
Audrey Naulleau, Christian Gary, Laurent Prévot and Laure Hossard
- 183 Impacts of Pre-bloom Leaf Removal on Wine Grape Production and Quality Parameters: A Systematic Review and Meta-Analysis**
Joshua VanderWeide, Chris Gottschalk, Steven R. Schultze, Esmaeil Nasrollahiazar, Stefano Poni and Paolo Sabbatini

- 196 *Georgian Grapevine Cultivars: Ancient Biodiversity for Future Viticulture***
Maryam Sargolzaei, Laura Rustioni, Gabriele Cola, Valentina Ricciardi, Piero A. Bianco, David Maghradze, Osvaldo Failla, Fabio Quaglino, Silvia L. Toffolatti and Gabriella De Lorenzis
- 214 *Molecular Tools for Adapting Viticulture to Climate Change***
Éric Gomès, Pascale Maillot and Éric Duchêne
- 234 *Projected Wine Grape Cultivar Shifts Due to Climate Change in New Zealand***
Anne-Gaelle E. Ausseil, Richard M. Law, Amber K. Parker, Edmar I. Teixeira and Abha Sood
- 248 *Cold Hardiness Dynamics and Spring Phenology: Climate-Driven Changes and New Molecular Insights Into Grapevine Adaptive Potential***
Valeria De Rosa, Giannina Vizzotto and Rachele Falchi
- 261 *Accuracy of Interpolated Versus In-Vineyard Sensor Climate Data for Heat Accumulation Modelling of Phenology***
Paula Pipan, Andrew Hall, Suzy Y. Rogiers and Bruno P. Holzapfel
- 274 *Under-Vine Vegetation Mitigates the Impacts of Excessive Precipitation in Vineyards***
Justine Vanden Heuvel and Michela Centinari
- 288 *Melatonin Relieves Ozone Stress in Grape Leaves by Inhibiting Ethylene Biosynthesis***
Chuang Liu, Hui Kang, Yafang Wang, Yuxin Yao, Zhen Gao and Yuanpeng Du
- 300 *1-Aminocyclopropane-1-Carboxylate Deaminase-Producing Plant Growth-Promoting Rhizobacteria Improve Drought Stress Tolerance in Grapevine (Vitis vinifera L.)***
Bingbing Duan, Lin Li, Guoqiao Chen, Chenxing Su-Zhou, Yashan Li, Hasmik Merkeryan, Wei Liu and Xu Liu
- 315 *Missing Links in Predicting Berry Sunburn in Future Vineyards***
Christopher Bahr, Dominik Schmidt and Katrin Kahlen
- 323 *Potential Phenotyping Methodologies to Assess Inter- and Intravarietal Variability and to Select Grapevine Genotypes Tolerant to Abiotic Stress***
Luísa C. Carvalho, Elsa F. Gonçalves, Jorge Marques da Silva and J. Miguel Costa
- 339 *Physiological and Transcriptional Responses to Saline Irrigation of Young 'Tempranillo' Vines Grafted Onto Different Rootstocks***
Ignacio Buesa, Juan G. Pérez-Pérez, Fernando Visconti, Rebeka Strah, Diego S. Intrigliolo, Luis Bonet, Kristina Gruden, Maruša Pompe-Novak and Jose M. de Paz



OPEN ACCESS

EDITED AND REVIEWED BY
Leo Marcelis,
Wageningen University and
Research, Netherlands

*CORRESPONDENCE

Tommaso Frioni
tommaso.frioni@unicatt.it
Chiara Pastore
chiara.pastore@unibo.it
Maria P. Diago
maria-paz.diago@unirioja.es

SPECIALTY SECTION

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

RECEIVED 14 July 2022

ACCEPTED 26 July 2022

PUBLISHED 09 August 2022

CITATION

Pastore C, Frioni T and Diago MP
(2022) Editorial: Resilience of
grapevine to climate change: From
plant physiology to adaptation
strategies. *Front. Plant Sci.* 13:994267.
doi: 10.3389/fpls.2022.994267

COPYRIGHT

© 2022 Pastore, Frioni and Diago. This
is an open-access article distributed
under the terms of the [Creative
Commons Attribution License \(CC BY\)](#).
The use, distribution or reproduction
in other forums is permitted, provided
the original author(s) and the copyright
owner(s) are credited and that the
original publication in this journal is
cited, in accordance with accepted
academic practice. No use, distribution
or reproduction is permitted which
does not comply with these terms.

Editorial: Resilience of grapevine to climate change: From plant physiology to adaptation strategies

Chiara Pastore^{1*}, Tommaso Frioni^{2*} and Maria P. Diago^{3,4*}

¹Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, ²Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza, Italy, ³Department of Agricultural and Food Sciences, University of La Rioja, Logroño, Spain, ⁴Department of Viticulture, Institute of Grapevine and Wine Sciences, Logroño, Spain

KEYWORDS

abiotic stress, *Vitis vinifera* L., drought, viticulture, sunburn, berry ripening, phenology, breeding

Editorial on the Research Topic

Resilience of grapevine to climate change: From plant physiology to adaptation strategies

Introduction

High adaptability of grapevine (*Vitis vinifera* L.) allowed for the expansion of viticulture toward all the main continents over the last centuries, establishing communities whose identity, culture and value system rely on their intimate links with the wine production. However, today the sector is probably facing the most complicated challenges since the post-phyllloxera era. Climate change is already posing serious to the industry sustainability, and climate projections seem to predict that worst times have yet to come. In such a scenario, viticulture needs to adapt rapidly to ensure satisfying growers remunerability, keeping intact the links with local traditions and quality of products (Pallioti et al., 2014; Van Leeuwen et al., 2019). In particular, the scientific community is tasked to provide new solutions that can defend this system and solve the main issues of growers under the unpredictability of climatic conditions.

The present Research Topic collect 22 papers, produced by 42 groups spread over 13 different countries. The article collection includes 13 original research articles, eight reviews, and one perspective paper, targeting physiological, molecular and cellular basis of: i. *Vitis vinifera* susceptibility to the most frequently occurring limiting conditions; ii. potential aspects on which agronomic adaptation strategies could rely. Papers included in the collection addressed all the main issues linked to the effects of altered environmental conditions on vine and grape physiology, contributed to fill the specific knowledge gaps, and proposed new solutions and alternatives for the industry.

Warming trends and vine phenology

One of the main effects of climate change is the compression of grapevine phenology and the advance in harvest dates (Palliotti et al., 2014). Some of the studies included in this collection specifically targeted the description of the effects of rising temperatures on grapevine vegetative and reproductive development rates. First, Pipan et al. proposed different interpretation of weather data in order to calculate heat summation and to model grapevine phenological development. They found that interpolated climate data can be suitable to drive phenological models, but vineyard topography and orography could affect their confidence. In the same framework, Gashu et al. reported that different cultivars exhibit varying sensibility to temperatures, and that the phenological compression observed in specific environmental conditions, can be offset when weather patterns change.

The work by Ausseil et al. highlighted that advancement of ripening due to warming trends is due to an advance in flowering and veraison time, whereas the time-window between veraison and harvest is less affected. In this framework, climatic models can help viticulture to re-arrange cultivars at local or national scale.

However, effects of climate change on phenology do not regard only late season phenology. Increase of spring frost occurrence is for sure one of the unexpected consequences of warming trends. It is linked to the advance in budbreak time recorded in many wine regions due to the increase in temperatures at the end of winter (false springs) and it is one of the most destructive phenomena related to climate change (Poni et al., 2022). In their review, De Rosa et al. tried to make the point about links between cold hardiness, air and soil temperatures, and genetic signaling behind the different varietal behaviors in budbreak time, in order to assist breeders toward the selection of genotypes exhibiting a postponed unlock of bud dormancy, and an increased frost tolerance.

Understanding effects of climate change on vine and berry responses to identify best counter-actions

Climate change poses new challenges and threats for viticulture, since the composition of berries and quality of wine depend on the main climatic factors, such as water status, radiation, temperature and greenhouse gases (CO₂) concentration. The effects of climate change are visible on vines and on berries in both primary and secondary metabolism, even altering the relationship between vine phenology and grapevine varietal performance. Temperature increases and more frequent and longer drought periods, are expected shortly in viticultural areas. Even upon the occurrence of small differences in the seasonal mean daily temperature (+ 1.5°C), strong changes

on wine grapevine performance and berry primary metabolism may be induced, causing, in warmer environment, earlier onset of phenological events, accelerated vegetative development and sometimes slower (Gashu et al.) or more frequently faster (Allegro et al., 2021) ripening of the berries, which are more intense in red cultivars than in white ones. Even with the increase in atmospheric CO₂, grape maturity may advance, hastening sugar accumulation and malic acid breakdown, also with differential responses for different clones of the same cultivar (Arrizabalaga-Arriazu et al.). Under higher solar exposure, flavonoids exhibit different sensitivities to degradation, with flavonols being the only compounds that could be positively affected by solar radiation, while anthocyanin depletion is often observed (Torres et al.). In the context of climate change, the night temperatures seem to exert less effects than day temperature on anthocyanin and flavonol accumulation. No effects or very limited ones occur, in fact, on anthocyanins and flavonols in condition of differential night temperatures (Yan et al.). Increasingly frequent drought stress events, have led to evaluate the possibility of alternative water uses, that are often high in salts. In these conditions, the use of rootstocks that are able to mitigate the effect on the scion of high salinity water is necessary as leaf gas exchanges can be reduced and an excess of Cl⁻ and Na⁺ accumulation in the leaves can occur. Anyway, no clear effects on grape berry soluble solids and phenolic compounds accumulation following irrigation with saline water and effects on vine physiology and berry composition should be still elucidated with long-term experiments (Buesa et al.). As temperature and drought increase with climate change, the frequency of extreme thermal events, as heat waves is set to increase. Sunburn is the result in grapevine berries of the complex interplay of environment and grapevine architecture affecting both the local heat impact on the berry surface and the susceptibility of berry. Sunburn damages appear in the berries as a consequence of photooxidative damage that is exacerbated by thermal stress. In these cases, berry response is accomplished through an increased production of antioxidants, HSPs, carotenoids, and polyphenols (Gambetta et al.). With a large variability depending on geographical locations, also the risk of damage due to spring frosts is globally increasing, being a potential risk for grapevine cultivation. Spring frost events, in cold winter regions can cause significant crop losses. To the contrary, warmer regions can be affected by low rates of budburst and lower productivity due to insufficient chilling during winter (De Rosa et al.).

Identifying resilient plant material to face current and forthcoming vineyard limiting conditions

Genetic diversity of *Vitis* spp. is for sure one of the main points of strength of viticulture and a key-factor for

the historical expansion of grapevine cultivation all over the world. The selection of the most adequate plant material for the establishment of a new vineyard is considered the fundament of viticulture long-term adaptation strategies to climate change (Palliotti et al., 2014). Notably, when choosing a specific rootstock, cultivar, or clone, a grower is taking a decision which is going to produce a repeated effect over years from the vineyard plantation to its end-life. Decisions taken today should then consider both current environmental circumstances, as well as those that could take place in the forthcoming 20 or 30 years. Under changing climates and environmental unpredictability, this obviously represents an additional challenge (Palliotti et al., 2014).

Water availability and quality is one of the main issues under climate change conditions. Rootstocks represent the first interface of vine with available water and nutrients. Buesa et al. tested the performance of young Tempranillo vines grafted to the new M1 and M4 rootstock, as compared to the commercial 1,103 Paulsen (1103P), according to a saline irrigation treatment. They found that while showing reduced gas exchanges parameters, M4 was able to preserve better fruit composition than M1 and 1103P.

Grape acidity is one of the fruit composition traits most susceptible to warming trends. Malic acid is indeed quickly oxidized *via* respiration, which is directly dependent to night and day temperatures. Advance of veraison and high temperatures foster the decrease of acidity, which is a key-component of grapes quality for the production of white and sparkling wines (Poni et al., 2018). In their work, Frioni et al. evaluated fruit ripening course of 16 local minor cultivars vs. the locally most common variety. Results highlighted that local germoplasm could hide relevant potentialities in terms of adaptation of viticulture to climate change.

For sure, varietal traits that were considered undesired or of limited interests in the past can be now the focus of renewed interests. In these terms, the work of Sargolzaei et al. assumes high relevance. They went back to *Vitis vinifera* L. domestication sites, in the Caucasus, looking for genotypes exhibiting late harvest or genetic tolerance to pathogens, in order to introduce these accessions in forthcoming breeding programs.

Interestingly, Gashu et al., comparing a set of cultivars in two different sites, reported that varying environmental conditions could stress out or compress differences in the time of onset of veraison, or in malic acid degradation rates.

In terms of plant material, last level for driving vineyard tolerance to environmental pressure is intra-varietal variability. Arrizabalaga-Arriazu et al. tested the effects of elevated air CO₂ concentration and temperatures on fruit composition of different clones of Tempranillo. They found that, in such conditions, different clones, while exhibiting similar organic acid degradation rates, had significantly different sugars accumulation patterns and anthocyanins accumulation rates.

Overall, a significant number of the works included in this collection focused on genetic resources, varietal selection and rearrangement, and new insights for breeding. Altogether, the authors provided a wide overlook of the relevance of grapevine genetic diversity for the adaptation of viticulture to climate change.

Modern soil and canopy management in the climate change scenario

In the last few decades, climate change has already been affecting the regional suitability of grapevines. Modeling approaches can be useful to describe the future situation of grapevine cultivation worldwide (Ausseil et al.) being, at the same time, a promising tool to prevent the risks caused by extreme thermal events (Bahr et al.). The availability of accurate climate data is actually necessary in order to evaluate adaptation strategies and to establish how to manage vineyard soil and vine canopy, select vineyard site, choose the most suited cultivar in a particular environment and predict phenological development (Pipan et al.). Quite often, a combination of adaptation strategies provides better solutions, even if only a small number of studies have developed approaches to quantify feasibility and effectiveness of adaptation strategies and have assessed their economic impacts, especially at vineyard scale (Naulleau et al.). Among the modern soil strategies management, in arid regions with unstable climatic patterns, the Direct Root-Zone irrigation (DRZ) could allow economizing water and ensuring grape production through the induction in the vine of the production of deeper roots and the improvement of the photosynthetic rate and the enhancement of grapevine adaptation (Ma et al.). Plant growth-promoting rhizobacteria (PGPRs) are bacterial groups obtained from rhizosphere soil, that can promote plant growth by means of biological control against soil-borne pathogens, biological nitrogen fixation, and root growth promotion (Pii et al., 2015), but also mitigate environmental stresses through different mechanisms. One of the mechanisms involves the production of ACC deaminase, that is able to lower ethylene levels and enhance growth, which is in general reduced by excessive ethylene under environmental stressful conditions, in particular drought. For these reasons, the application of PGPRs could be a promising strategy for mitigate the effects of drought stress. Recent researches demonstrated that the inoculation in the soil of ACC-deaminase producing PGPRs, as a single strain or in mixed combination, can affect vine phytohormones biosynthesis and induce the ROS defense system contributing to the response to the drought stress (Duan et al.). In some grape growing regions, mainly across most of the United States, excessive precipitation events have greatly increased due to climate change, with detrimental impacts on plants and soil

in vineyard due to an increase of the erosivity of soils. In these situations, either natural or seeded under-vine vegetation (UVV) can help mitigate many of the problems associated with excessive precipitation, providing vegetative coverage to reduce the force of raindrops, increasing soil organic matter and enhancing soil microbial diversity (Vanden Heuvel and Centinari). Concerning the modern strategies of vine canopy management, in vertically shoot positioned trellis the impact of heat waves and exposure of berries can be mitigated through a partial shading (~60% of solar radiation) of the cluster zone obtained with the application of shading nets, that are able to lower the temperature of 3.9°C in the shaded clusters in comparison to the exposed ones. In combination with water supply, this practice could also avoid berry dehydration during the last part of ripening with beneficial effects on anthocyanins and flavonols, in comparison to fully exposed clusters (Martínez-Lüscher et al.). Interestingly, as reviewed by VanderWeide et al. performing pre-bloom leaf removal to achieve high fruit quality in challenging growing climates seems to be a good strategy, since berry composition significantly improves following pre-bloom defoliation due to the decrease in yield and in bunch rot disease. Ozone (O₃) in the troposphere is a highly oxidizing atmospheric pollutant. In addition to climate change, elevated O₃ concentration severely affects the growth and development of plants, including grapevine (Blanco-Ward et al., 2021). O₃ stress induces the release of large amounts of ethylene by the leaves and canopy treatments with melatonin could significantly inhibit the ethylene response mediated by O₃ stress inducing a positive response in photosynthesis and ROS scavenging systems (Liu et al.).

Emerging methodologies and molecular tools to explore new frontiers in viticulture

One of the key strategies to adapt to climate change (Naulleau et al.) is the breeding and growing of alternative varieties, better adapted to abiotic stresses or with improved aptitudes in acidity, therefore more suitable for winemaking. Toward this end, insight on the diversity of grape solutes known to be influenced by temperature, such as K⁺, Mg²⁺, Ca²⁺, NH₄⁺ has been revealed (Bigard et al.) in 12 different *Vitis vinifera* L. cultivars, that were characterized in their berries, as well as their effect in berry acidity. It was shown that a significant genotypic diversity is prevalent in *Vitis vinifera* L. for fruit composition at physiological ripe stage and that parameters determining berry growth and acids accumulation are susceptible of been manipulated by crossbreeding. In this direction, the state of the art of the molecular tools and their usefulness to understand grapevine response to environmental stress, genetics and genomics of grapevine stress tolerance, and

how to control and modulate the genome and its expression are reviewed (Gomès et al.). Regarding this very last strategy, the molecular drivers of cold hardiness loss (particularly critical to cope with late frost damage) and the mechanisms that control deacclimation and budbreak to modulate bud phenology are presented (De Rosa et al.), together with their variability in distinct genotypes. On the other side of the equation, heat waves are more recurrent, particularly in warm regions. These are certainly driving heat and water stress and are often associated with berry sunburn, a disorder causing severe yield loss and decline in berry quality. In this volume, a new modeling approach, which integrates functional-structural plant information and management practices over time has been proposed to identify sunburn-reducing strategies in a given vineyard (Bahr et al.). Moreover, the potential and current methods to improve field phenotyping of grapevine to support the characterization of inter- and intra-varietal diversity, as the closing loop stage of breeding, particularly with regards to tolerance to heat and water stress are also discussed (Carvalho et al.).

Challenges ahead and perspectives

In the short and mid-term, challenges derived from climate change effects (e.g., phenology shifts, decoupling of grape ripening, berry sunburn, heat and water stress, increase of adverse events such as late frost and hail phenomena, occurrence of new pests and diseases, etc.) will remain or even will get aggravated. Nevertheless, the knowledge about adaptation strategies to cope with them is also substantially increasing and will continue to grow, as demonstrated by the collection of works included in this volume, which cover all sort of strategies, from breeding and canopy management to the state of the art of molecular approaches and phenotyping tools. Although not extensively referred to in this volume, the fast development of electronics, artificial intelligence, internet of things, and sensors in general, considered disruptive technologies, may contribute to advanced, unprecedented monitoring of the crop and the final triggering of precision viticulture, aimed at improving the sustainability and profitability of grapegrowing. This may play a significant role in the optimization of resources (e.g., water) as well as in the reduction of chemical inputs for fertilization and spraying.

In summary, while all this research is commendable, the derived knowledge will not become fruitful if effective training and extension activities, to transfer it to the viticulturists and winemakers worldwide are not put in place. This is certainly one of the cornerstones of the effective and successful adaptation of the grape and wine industry to the challenges driven by climate change.

Author contributions

TF, MD, and CP have made a substantial, direct, and intellectual contribution to the paper and approved it for publication in *Frontiers in Plant Science*. All authors contributed to the article and approved the submitted version.

Acknowledgments

Topic Editors wish to express their gratitude to all the authors who contributed to the Research Topic. Special thanks to the *Frontiers in Plant Science* Editorial Board who gave us the opportunity to build up the article collection, and to the Editorial Office for the prompt assistance.

References

- Allegro, G., Pastore, C., Valentini, G., and Filippetti, I. (2021). The evolution of phenolic compounds in *Vitis vinifera* L. Red berries during ripening: analysis and role on wine sensory—a review. *Agronomy*. 11, 999. doi: 10.3390/agronomy11050999
- Blanco-Ward, D., Ribeiro, A., Paoletti, E., and Miranda, A. I. (2021). Assessment of tropospheric ozone phytotoxic effects on the grapevine (*Vitis vinifera* L.): a review. *Atmospher. Environ.* 244, 117924. doi: 10.1016/j.atmosenv.2020.117924
- Pallioti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., and Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. *Scientia Horticulturae*. 178, 43–54. doi: 10.1016/j.scienta.2014.07.039
- Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., and Crecchio, C. (2015). Microbial interactions in the rhizosphere: beneficial influences of plant

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol. Fertil. Soils*. 51, 403–415. doi: 10.1007/s00374-015-0996-1

Poni, S., Gatti, M., Pallioti, A., Dai, Z., Duchêne, E., Truong, T. T., and Tombesi, S. (2018). Grapevine quality: a multiple choice issue. *Scientia Horticulturae*. 234, 445–462. doi: 10.1016/j.scienta.2017.12.035

Poni, S., Sabbatini, P., and Pallioti, A. (2022). Facing spring frost damage in grapevine: recent developments and the role of delayed winter pruning—a review. *Am. J. Enol. Vitic. ajev.2022.22011*. doi: 10.5344/ajev.2022.22011

Van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy*. 9, 514. doi: 10.3390/agronomy9090514



Biodiversity of Local *Vitis vinifera* L. Germplasm: A Powerful Tool Toward Adaptation to Global Warming and Desired Grape Composition

Tommaso Frioni*, Giovanni Bertoloni, Cecilia Squeri, Alessandra Garavani, Lily Ronney, Stefano Poni and Matteo Gatti

Dipartimento di Scienze delle Produzioni Vegetali Sostenibili, Università Cattolica del Sacro Cuore, Piacenza, Italy

OPEN ACCESS

Edited by:

Claudio Bonghi,
University of Padua, Italy

Reviewed by:

Yuejin Wang,
Northwest A&F University, China
Osvaldo Failla,
University of Milan, Italy
Sara Amâncio,
University of Lisbon, Portugal
Birsen Çakir,
Ege University, Turkey

*Correspondence:

Tommaso Frioni
tommaso.frioni@unicatt.it

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 06 February 2020

Accepted: 21 April 2020

Published: 14 May 2020

Citation:

Frioni T, Bertoloni G, Squeri C,
Garavani A, Ronney L, Poni S and
Gatti M (2020) Biodiversity of Local
Vitis vinifera L. Germplasm:
A Powerful Tool Toward Adaptation
to Global Warming and Desired Grape
Composition.
Front. Plant Sci. 11:608.
doi: 10.3389/fpls.2020.00608

Global warming is endangering maintenance of optimal grape composition in white varieties aimed at sparkling wine making due to difficulties to maintain adequate acidity and fresh aromas. These troubles are being faced by the main white variety of the Colli Piacentini district, named Ortrugo. Its vegetative and reproductive behavior was compared over 3 years with that of other minor autochthonous white varieties. Criteria set for adequate grape composition under sparkling vinification (total soluble solids at 20–21°Brix) and titratable acidity (TA) \geq 6.5 g/L combined with Principal Component Analysis (PCA) on the measured variables allowed a thinning down of the initial group of 17 to 7 varieties including Ortrugo, Bucalò, Barbesino, Lecco, Melara, Santa Maria and Molinelli. PCA isolated Ortrugo's behavior for inadequacy to maintain sufficient TA at harvest mostly due to extremely low malic acid concentration. However, time trend analyses of accumulation and degradation patterns of tartaric and malic acids disclosed that, in Ortrugo, the most limiting factors were more intense post-veraison tartaric acid dilution and a lower malic acid pool at veraison as compared to any other variety. Conversely, Molinelli and Barbesino proved to be ideal material for sparkling wine purposes, as they associated to desirable agronomic features a strong ability to retain high TA with a well-balanced tartrate-to-malate ratio. Our study emphasizes that often neglected or superficially evaluated germplasm genetic resources might hide strong potential for adapting to challenges imposed by climate change in that representing an excellent tool for adaptation strategies.

Keywords: autochthonous cultivars, minor varieties, viticulture, climate change, titratable acidity, malic acid

INTRODUCTION

Effects of climate change on viticulture have been the object of a massive flux of scientific literature since the mid-80s' (Mozell and Thach, 2014; Palliotti et al., 2014; Schultz, 2016; Van Leeuwen et al., 2019; De Ollas et al., 2019). Global warming is also, albeit slowly, re-designing the geographical distribution of cultivars (i.e. former cool areas can nowadays accommodate medium or late ripening varieties) whereas warm areas are often facing excessive light and heating availability as compared to the need of traditionally grown varieties (Jones and Webb, 2010; Schultz, 2016; Van Leeuwen et al., 2019). Moreover, the issue of "meteorological drought" is rising all across Europe (Spinoni et al., 2016) and territories that have been dry farmed for ages are now facing

the uncomfortable issue of having to provide supplemental water with irrigation (Van Leeuwen et al., 2019). Other dramatic impacts of climate change in warm viticulture areas include: (i) increased advancement and compression of all phenological stages; according to Sadras and Petrie (2012) number of days (d) elapsing between average harvest dates for Chardonnay and Cabernet Sauvignon cultivars has decreased from 21 days at the beginning of the 90 s to only 9 days; (ii) increasing frequency of extreme events (e.g. mild winters, severe summer drought, hot spells, unexpected unseasonal flooding etc.) that might have serious effects on viticulture profitability. A suitable example is recurrent frost damage fostered by very early bud push and, afterward, a longer time window during which a damaging frost event might still occur; (iii) within a global change scenario, ideal grape composition for some specific wines is definitely more difficult to achieve (Poni et al., 2018).

In the case of white varieties, and especially to those grown to produce sparkling or spumante wines, the challenge thrown by climate change is of utmost complexity. If the wine target is a sparkling, a desirable grape composition at harvest is often as follows: a total soluble concentration (TSS) between 20 and 21°Brix, a titratable acidity (TA) \geq 6.5–7.0 g/L, a pH \leq 3.2 and, hopefully, healthy and turgid berries. Among such desired characteristics, increased heat summations – including also a marked increase of night temperatures – primarily endanger the maintenance of adequate acidity. It is well established (Ruffner, 1982; Ford, 2012) that, while tartaric acid concentration is essentially unaffected by temperature, the rapid loss in malic acid starting at the onset of berry softening is mainly temperature driven (Ford, 2012).

If the goal is to maintain adequate total acidity at harvest with a good balance between the two main organic acids of the grape berry, then genetic variation in organic acid metabolism should be investigated and eventually exploited. It has been known for some time that species within the genus *Vitis* and individual varieties of the cultivated grapevine *V. vinifera* show ample variation in the natural acidity of berries (Duchêne, 2016; Poni et al., 2018; Famiani et al., 2016, 2018). Analysis of the acid composition in developing and ripe berries of 26 species of *Vitis* and 50 wine grape varieties of *V. vinifera* showed that, for a so called “early” sample, malic concentration ranged from a maximum of 0.85 g/100 mL in Pinot St George to the lowest 0.26 g/100 mL recorded in White Riesling. At late sampling, the majority of the varieties showed malate levels below 0.15 g/100 mL (Kliewer et al., 1967). Most importantly, though, some varieties showed greater than 50% loss of malate between early and late sampling, whereas others (e.g. White and Gray Riesling) retained malate at comparable levels across the sampling period.

Even if most of Italian and southern Europe viticulture is based on local varieties identifying the final products, the re-arrangement of cultivars and genotypes adopted within a wine region is something currently occurring especially as a consequence of climate change pressures (Palliotti et al., 2014; Mosedale et al., 2016). However, even if in literature some works report the evaluation of local biodiversity and the re-introduction of autochthonous minor cultivars as new tools to

improve the competitiveness of wine districts (Mannini, 2003; Storchi et al., 2007; Cruz-Castillo et al., 2009; Iorizzo et al., 2014; Biasi and Brunori, 2015; Urrestarazu et al., 2015; Gutiérrez-Gamboa et al., 2020), none of them has taken into account white varieties and the importance of maintaining acidity in grapes in relation to high seasonal temperatures favoring abrupt organic acids depletion.

The local (autochthonous) white cultivar Ortrugo is currently grown over about 650 hectares in the Colli Piacentini wine district, in Northern Italy. Ortrugo has its own appellation and it is recognized as a high quality sparkling and spumante wine that is currently highly requested both nationwide and in export markets. Unfortunately, Ortrugo is extremely sensitive to post-veraison malate degradation and even in the presence of cultural practices aiming to screen cluster from direct radiation in summer (Gatti et al., 2015, 2019) in a typically warm season it is quite normal that TA in Ortrugo drops to unacceptable levels (e.g. \leq 6.0 g/L with malic acid often below 0.5 g/L). Within the same region where Ortrugo is grown, several autochthonous, yet minor, white varieties have been isolated and today they are maintained in a private vineyard collection (Fregoni et al., 2002) officially recognized by the Emilia Romagna Region (including Begano Bianco, Bervedino, Marsanne, Melara, Ortrugo and Santa Maria, officially registered in the National Catalog of Grapevine Varieties RNVV, Barbesino, Bucalò, Calòra, Bianchetta di Diolo, Bianchetta di Bacedasco, Colombina, Molinelli, Lecco, Lisöra, Sticiucaera Bianca currently not listed in the RNVV). “Minor” in the present case defines a varietal that, for a number of reasons (e.g. specialization in viticulture focusing on the most productive biotypes with progressive erosion of the local biodiversity, adverse climatic regimes leading to poor ripening of late varieties and higher disease pressure, as well as conservative policy making in terms of modifications of current appellation regulations), have been progressively neglected by growers, resulting in a drastic reduction of planted surfaces that is also conducive to the risk of total loss of propagation material.

Hypothesis pursued in this study is that biodiversity hidden within a population of local varieties can aid at solving the scarce performances of traditionally selected grapevine cultivars under the current climate change scenario. In this specific case, cv. Ortrugo, almost flattening acidity at harvest under warming trends pressures, was tested versus 16 other minor white varieties for vegetative parameters, yield and fruit composition at harvest; “developmental sampling” of berries across multiple time points in the growing seasons was undertaken to provide comparison of acid levels vs other parameters of technological maturity and to explore the physiological and metabolic reasons hidden behind differential ripening patterns.

MATERIALS AND METHODS

Experimental Site and Treatment Layout

The study was carried out for 3 years (2017–2019) in a vineyard germplasm collection planted in 2003 at the Mossi Estate (Albareto, Ziano Piacentino, Italy, 44° 97′ 93″ N 09 40′ 99″ E, 270 m asl) where, along with Ortrugo, 16 more white

minor varieties (**Supplementary Figure S1**) are reunited. All varieties, grafted on K5BB rootstock, are planted at 2.2×2 m spacing (between row and within row distance, respectively), with coupled vines in the row for a resulting density of 4545 plants/hectare. Rows are directed along maximum soil slope (about 12%) assuming a SE-NW orientation and vines are trained to a unilateral Guyot with about 10 nodes on the primary horizontal cane and two more on a spur left for annual cane renewal. According to the relative abundance of the propagation material, each variety is present in at least one or two adjacent rows. The experiment was conducted on 30 vines per genotype (510 vines total) randomly chosen within the available plots. Each year, thinning was applied between BBCH 14–15 to maintain one primary shoot per node and four test vines per variety were randomly chosen along the row(s) in 2017 and then maintained also for the two following seasons. These selected vines were used for detailed assessment of vegetative growth, yield components and grape composition at harvest, whereas the others were used for veraison-to-harvest berry samplings. The vineyard is typically non-irrigated, whereas fertilization and disease and pest management were uniform across the whole vineyard surface and conducted according to local sustainable practices. The minimum, mean, and maximum daily air temperature ($^{\circ}\text{C}$) and daily rainfall (mm) from 1 January (DOY 1) to 31 December (DOY 365) were recorded in each season by a nearby weather station.

Vegetative Growth and Yield Components

Upon completion of leaf fall (end of November) all test vines were pruned and the removed 1-year old pruning weight immediately recorded in the field with a portable digital scale.

Each season, in late spring (end of May – beginning of June), number of inflorescences borne on each shoot was recorded according to position of the shoot on the horizontal cane. Total vine fruitfulness was then calculated as a ratio of total inflorescences on total shoots.

At harvest, test vines were individually picked and total cluster number per vine counted. Concurrently, three representative clusters per vine, usually inserted on basal, median and apical cane portions, were taken to the laboratory for further subsampling. Fruits were individually weighted and the main rachis length measured in order to calculate the compactness index expressed as cluster mass-to-rachis length ratio (Tello and Ibanez, 2014). From each of the three clusters, one 50 berry subsample was taken by careful cutting each berry at the pedicel with small sharp scissors and then crushed. The obtained must was then used for technological maturity determinations as described in the next paragraph. In each year, the yield to total pruning weight ratio (kg/kg), otherwise known as Ravaz index (Ravaz, 1911) was calculated on a single vine basis.

Grape Composition

Each year, from veraison (TSS ~ 4.5 to 5 Brix) until harvest, three 50-berry samples were taken weekly from extra vines of each variety. Test vines were excluded to not alter natural dynamic

of grape ripening due to progressive reduction of the pending yield. During sampling, it was assured that the removed berries were taken from clusters located on both sides of the row and, within each cluster, the top, median, and bottom portions were also represented. Sampled berries were brought to the laboratory, weighed, and crushed to obtain a juice. Musts were analyzed immediately for TSS using a temperature-compensated desk refractometer, whereas pH and TA were measured by titration with 0.1 N NaOH to a pH 8.2 end point and expressed as g/L of tartaric acid equivalents.

In each season, all varieties were picked at similar ripening aiming at a final TSS of about 20–21 $^{\circ}$ Brix and a TA ≥ 6.5 g/L. Resulting harvest dates were 22 August 2017, 30 August 2018 and 5 September 2019. TSS, TA, and pH were determined on the remaining berries of each of the three sampled clusters according to the standard methods described above.

HPLC Analysis

To assess tartaric and malic acid concentrations in all samples taken seasonally and at harvest, an aliquot of the must was diluted four times, then filtered through a $0.22 \mu\text{m}$ polypropylene syringe for high-performance liquid chromatography (HPLC) analysis and transferred to auto-sampler vials. All solvents were of HPLC grade. Water Milli-Q quality, acetonitrile, and methanol were obtained from VWR. L-(+)-tartaric acid and L-(-)-malic acid standards were purchased from Sigma-Aldrich. The chromatographic method was developed using an Agilent 1260 Infinity Quaternary LC (Agilent Technology) consisting of a G1311B/C quaternary pump with an inline degassing unit, G1329B autosampler, G1330B thermostat, G1316B thermostated column compartment, and a G4212B diode array detector (DAD) fitted with a 10 mm path, $1 \mu\text{L}$ volume Max-Light cartridge flow cell. The instrument was controlled using the Agilent Chemstation software version A.01.05. The organic acids' analysis used an Allure Organic Acid Column, 300×4.6 mm, $5 \mu\text{m}$ (Restek). Separation was performed in isocratic conditions using water, pH-adjusted to 2.5 using ortho-phosphoric acid, at a flow rate of 0.8 mL/min. The column temperature was maintained at $30 \pm 0.1^{\circ}\text{C}$, and $15 \mu\text{L}$ of sample was injected. The elution was monitored at 200 to 700 nm and detected by UV-vis absorption with DAD at 210 nm. Organic acids were identified using authentic standards, and quantification was based on peak areas and performed by external calibration with standards.

Statistical Analysis

Vine performance data were subjected to a two-way analysis of variance (ANOVA) using the SigmaStat software package (Systat Software, Inc.). Homogeneity of error variances for data taken on the same individuals over different years was assessed with Bartlett's test. The year was considered as a random variable, and the error term for the treatment factor was the year \times treatment interaction mean square. Since variances were in all cases homogeneous, the year \times treatment effects were tested using the pooled error mean square as an error term (Gomez and Gomez, 1984). Treatment comparison was performed using the Student-Neuman-Keuls test at $p \leq 0.05$. Year \times treatment interaction was partitioned only when the F test was significant.

TABLE 1 | Vegetative growth, yield components and Ravaz Index recorded over 3 years (2017–2019) in 17 *Vitis vinifera* L. varieties including the reference cultivar Ortugo. Data were taken on four vines per variety.

Varietal	Shoots/ vine	Total pruning weight/vine (g)	Cane fruitfulness (clusters/shoot)	Basal cane fruitfulness (clusters/shoot)	Yield/vine (kg)	Clusters/ vine	Cluster Weight (g)	Cluster compactness (g/cm)	Berry weight (g)	Ravaz Index (kg/kg)
Barbesino	11.8 ab	449 ab	1.3 bcd	1.0 abcd	2.05 bcde	13.2 bc	158 cdefg	13.13 ab	1.98 bcdef	4.77 cde
Bervedino	12.3 ab	233 bcd	1.6 a	1.6 a	3.47 a	19.5 a	181 cdef	22.87 a	1.98 bcdef	18.68 b
Besgano Bianco	9.9 b	581 a	0.9 defg	0.7 bcd	2.12 bcde	9.2 bcd	231 abc	15.64 ab	3.43 a	4.54 cde
Bianchetta di Bacedasco	11.5 ab	381 abc	0.8 efgh	0.6 cde	2.22 bcde	10.5 bcd	216 abcd	13.69 ab	2.21 bcd	7.22 cde
Bianchetta di Diolo	11.6 ab	373 abc	1.0 def	1.1 abcd	3.00 ab	11.3 bcd	282 a	22.57 a	3.36 a	10.48 c
Bucalò	12.7 ab	253 bcd	0.6 hi	0.7 bcd	1.04 ef	7.7 de	139 efg	12.45 b	1.80 defg	5.19 cde
Calora	11.1 b	457 ab	1.0 defg	0.8 bcd	2.57 abc	13.1 bc	208 bcde	12.11 b	2.37 b	6.25 cde
Colombina	10.4 b	452 ab	1.1 def	0.8 bcd	2.14 bcde	12.3 bcd	177 cdef	12.05 b	1.52 g	5.85 cde
Lecco	10.8 b	297 bcd	0.5 i	0.4 de	0.42 f	4.4 e	100 g	8.65 b	1.96 bcdef	1.72 e
Lisöra	11.1 b	188 cd	1.4 abc	1.4 ab	1.46 cde	13.4 b	117 fg	13.25 ab	2.00 bcdef	9.14 cd
Marsanne	12.3 ab	273 bcd	1.2 cde	1.3 abc	1.88 bcde	12.6 bcd	141 defg	9.67 b	1.72 efg	7.16 cde
Melara	12.7 ab	413 ab	0.7 fghi	0.2 e	1.24 def	8 cde	165 cdefg	11.93 b	2.14 bcde	3.66 de
Molinelli	13.8 a	398 abc	1.5 ab	1.6 a	1.83 bcde	17.3 a	113 fg	7.97 b	1.62 fg	6.43 cde
Ortugo	11.8 ab	308 bcd	0.7 ghi	0.5 de	2.37 bcd	8.1 cde	270 ab	18.23 ab	1.86 cdefg	8.60 cd
Santa Maria	11.1 b	441 ab	1.0 defg	0.7 bcd	1.53 cde	9.5 bcd	165 cdefg	13.01 ab	2.23 bc	3.64 de
Stolucera Bianca	12.1 ab	149 d	0.9 defg	0.7 bcd	2.09 bcde	11.7 bcd	174 cdefg	11.57 b	2.17 bcd	23.54 a
Verdea	10.4 b	532 a	1.0 def	0.7 bcd	2.36 abc	10.1 bcd	231 abc	13.41 ab	1.94 cdef	4.85 cde
Year										
2017	11.5 a	299 b	1.1 a	1.0 a	2.33 a	13 a	177 b	14.86 a	2.14 b	12.14 a
2018	12.1 a	415 a	0.9 c	0.7 b	1.88 b	9 c	216 a	13.24 a	2.26 a	5.70 a
2019	11.4 a	390 a	1.0 b	1.0 a	1.85 c	12 b	156 c	13.17 a	2.05 b	5.48 b
Varietal	***	***	***	***	***	***	***	***	***	***
Year	*	***	***	**	**	***	***	ns	**	***
V × Y	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Within each column and factor (varietals and year) mean separation was performed using the Student-Newman-Keuls test. Probability levels are: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. ns implies non-significance.

Due to the high number of measured variables, a Principal Component Analysis (PCA) was also carried out twice using the XLSTAT statistical package (Addinsoft, New York, NY, United States). In the first run, observations were single vine data for the 7 selected varieties, whereas 11 variables were analyzed to include parameters representative of vine vigor, yield and grape composition. The second run was performed on the yearly data of the same varieties, additionally associating another set of seven variables representative of sugar and acid seasonal dynamics and climatic indices. In both cases, the chosen PCA was a Pearson correlation matrix, number of filter factors was set at 5 and the final data visualization was in the form of a distance bi-plot.

Repeated measures of the same parameters (berry mass, TSS, TSS/berry mass, TA, tartrate, malate) taken at different dates, throughout the season were analyzed with the Repeated Measure analysis of variance (ANOVA) routine embedded in the XLSTAT software package. The least squared (LS) mean method at $p \leq 0.05$ was used for multiple comparisons within dates.

RESULTS

Weather Trends and Indices

In 2017, total rainfall recorded from April to October summed up to 289 mm (Supplementary Figure S2 and Supplementary Table S1). Very limited rainfall occurred in summer months and,

in July and August, 13 days registered T_{\max} higher than 35°C. The Winkler Index (WI) calculated over a 10°C baseline was of 2143 Degree Days (DD) which lowered to 1984 DD if the calculating period was shortened at the end of September. The 2018 season registered a higher rainfall from April to October (433 mm) than 2017; yet summer temperatures were fairly high and WI resulted in 2200 DD (2014 from April to September). Year 2019 will be remembered for an unseasonal wet spring (about 300 mm fell in April and May out of 595 mm from April to October); at the same time June and July were very hot (peak T_{\max} of 39.5°C recorded on DOY 178). WI summed up to 2020 DD, whereas WI calculation restricted from April to September yielded 1963 DD.

Vine Growth and Yield Components

Shoot number per vine was very constant across years, whereas it ranged from 9.9 (Besgano Bianco) to 13.8 (Molinelli) among varieties with Ortugo setting at an intermediate position (11.8 shoots/vine) (Table 1). For data pooled over varieties, shoot fruitfulness had an overall mild decrease when calculated for the basal cane nodes (first three count nodes) vs. total nodes. However, among cultivars, Bervedino scored the highest fertility (1.6 inflorescences/shoot) and Lecco the lowest (0.5 inflorescences/shoot). In Melara only, restricting calculation of shoot fruitfulness to the first three nodes resulted in a much lower value (0.2 inflorescences/shoot) as compared to total cane value (0.7 inflorescences/shoot). Total pruning weight per vine

TABLE 2 | Parameters of technological maturity recorded at harvest over 3 years (2017–2019) in 17 *Vitis vinifera* L. varieties including the reference cultivar Ortugo.

Varietals	TSS (°Brix)	pH	TA (g/L)	Tartrate (g/L)	Malate (g/L)	Tartrate-to- malate ratio
Barbesino	21.2 ¹ bc	3.03 d	9.09 b	7.69 abc	2.51 cde	4.1 bcd
Bervedino	16.9 fg	3.03 d	6.96 cd	5.18 e	1.84 cde	3.4 bcd
Besgano Bianco	15.3 g	2.80 f	13.43 a	7.32 bcd	7.02 a	1.1 d
Bianchetta di Bacedasco	18.2 ef	3.25 ab	5.49 de	5.98 cde	1.55 de	4.2 bcd
Bianchetta di Diolo	15.4 g	3.04 d	9.51 b	5.81 de	4.01 b	1.6 cd
Bucalò	25.3 a	3.12 cd	6.93 cd	7.67 abc	1.66 de	5.0 b
Calora	18.5 e	3.13 cd	7.25 c	7.06 bcd	2.06 cde	3.9 bcd
Colombina	20.7 cd	2.99 de	6.86 cd	5.40 e	2.21 cde	2.6 bcd
Lecco	22.9 b	3.23 bc	6.54 cd	7.64 abc	1.97 cde	4.3 bcd
Lisöra	19.1 e	3.05 d	7.99 c	8.08 ab	2.25 cde	3.6 bcd
Marsanne	22.6 bc	3.34 a	4.64 e	5.92 de	1.51 e	4.7 bc
Melara	22.8 b	3.13 cd	6.38 cd	7.43 bcd	1.83 cde	5.3 b
Molinelli	22.2 bc	3.04 d	9.45 b	8.94 a	2.73 cd	3.5 bcd
Ortugo	20.8 cd	3.10 d	5.15 e	6.18 cde	0.46 f	18.3 a
Santa Maria	21.4 bc	3.28 ab	6.83 cd	5.95 de	2.96 c	2.4 bcd
Sticiucaera Bianca	19.4 de	3.07 d	7.56 c	6.61 bcde	2.42 cde	3.1 bcd
Verdea	15.6 g	2.91 e	10.35 b	6.13 cde	4.33 b	1.5 cd
Year						
2017	21.4 a	3.12 a	6.73 c	6.52 b	1.93 b	5.2 a
2018	18.8 b	3.03 b	7.97 b	7.69 a	2.80 a	5.0 a
2019	19.3 b	3.11 a	8.47 a	6.16 b	2.99 a	2.9 b
Varietal	***	***	***	***	***	***
Year	***	***	***	***	***	***
V × Y	ns	ns	***	***	**	**

Data were taken on four vines per variety. TTS = total soluble solids. TA = titratable acidity. Within each column and factor (varieties and year) mean separation was performed using the Student-Newman-Keuls test. Probability levels are: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. ns implies non-significance.

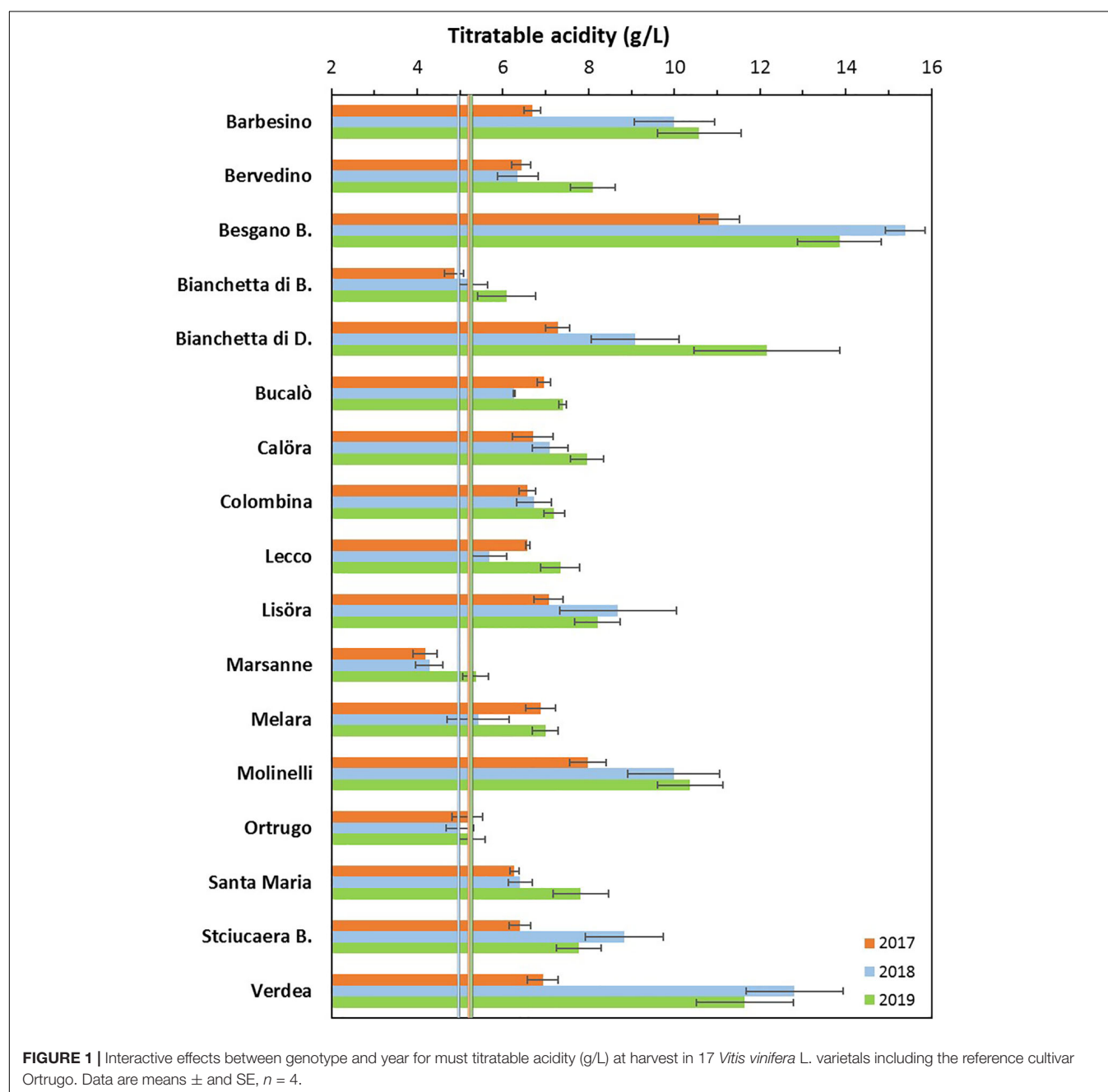
for data pooled over varieties showed that 2017 was the weakest season with only 299 g; large variation in pruning weight per vine occurred among genotypes with an almost four-fold difference between minimum (149 g) and maximum (581 g) values recorded in Stciucaera Bianca and Besgano Bianco, respectively, while Ortrugo set at 308 g.

Lack of significant $V \times Y$ interactions for any of the yield components reported in **Table 1** indicates that performance of different varieties was rather uniform across the three seasons. Yield per vine fell between 0.42 kg in Lecco and 3.47 kg in Bervedino and high variability among cultivars was recorded also for cluster weight (100–282 g span) and berry weight (1.52–3.43 g

span). Absolute values for cluster compactness also showed high variability among cultivars, yet within varieties mean separation highlighted very few significant differences. Likewise, the Ravaz index showed quite high variability as it went from 1.7 kg/kg (Lecco) to 18.8 kg/kg (Bervedino).

Grape Composition

Total soluble concentration recorded at harvest have shown that five varieties, and namely Bucalò, Lecco, Marsanne, Melara and Molinelli reached higher berry sugar concentration than the reference Ortrugo (20.8°Brix) (**Table 2**). Must pH in almost all cases was lower than the 3.3 threshold. Within-varieties



variability for acid components was very broad: TA ranged from the lowest 5.15 g/L scored by Ortrugo to the 13.4 g/L of Besgano Bianco; tartrate went from a minimum of 5.18 g/L in Bervedino to a maximum of 8.94 g/L in Molinelli and malate was the lowest in Ortrugo (0.46 g/L) and the highest in Besgano Bianco (7.0 g/L). **Table 2** data analysis also shows that a significant $V \times Y$ interaction occurred for TA, tartrate, malate and tartrate/malate ratio (**Figures 1–3**). Partitioning of such interactions disclosed, for TA, that the majority of varieties

was sensitive to seasonal effects leading to a lower TA retaining in the hot and dry 2017 as compared to the following two seasons (see for reference, main effects for year factor in **Table 2**). Some varieties, though, were less responsive to such climate-driven effect and their TA was relatively stable across years; among them Bucalò, Colombina, Lecco, Melara and Ortrugo (**Figure 1**). Tartaric acid concentration at harvest showed a somewhat different response to yearly effects among varieties; in fact, the pattern described above for TA was maintained

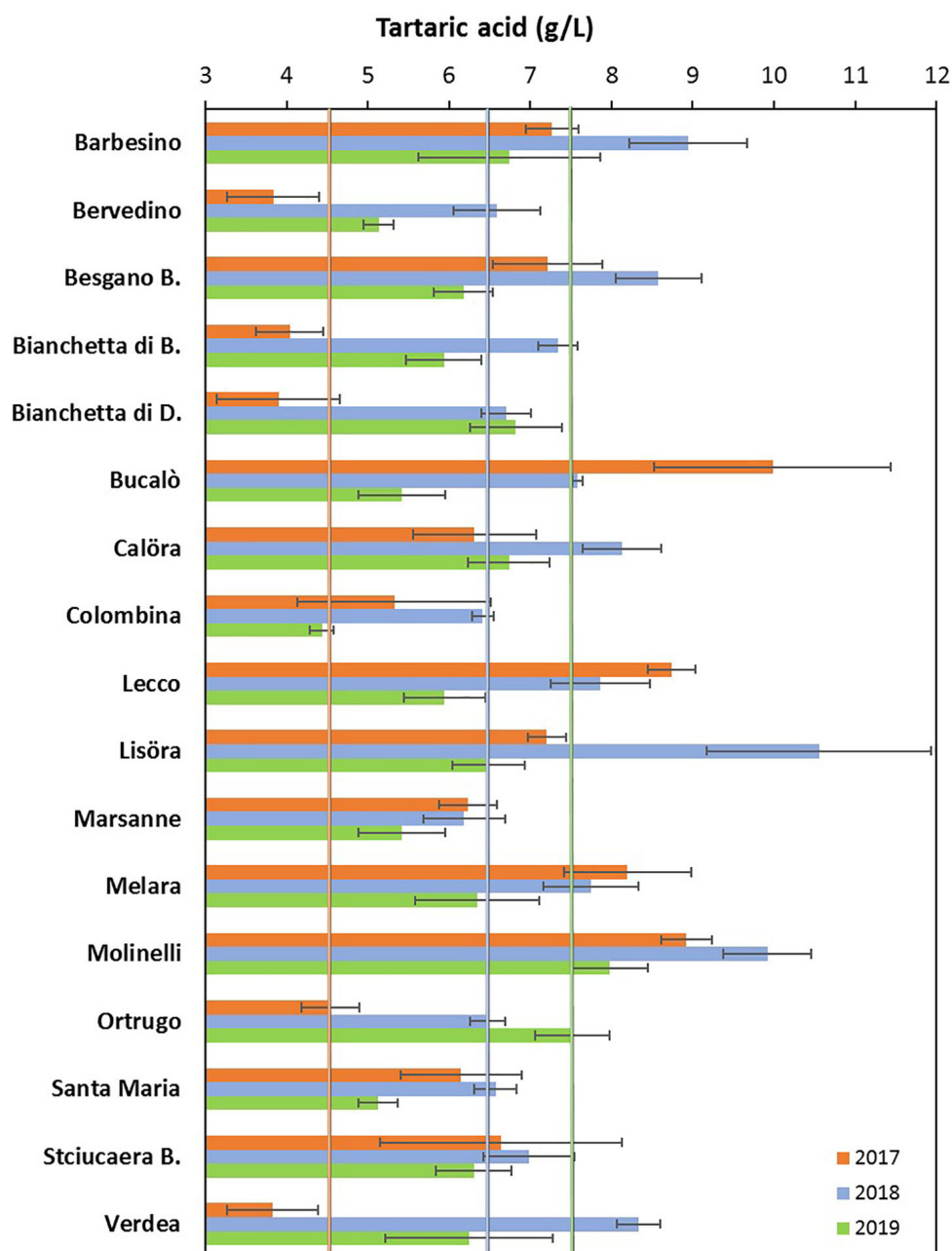


FIGURE 2 | Interactive effects between genotype and year for must tartaric acid concentration (g/L) at harvest in 17 *Vitis vinifera* L. varieties including the reference cultivar Ortrugo. Data are means \pm and SE, $n = 4$.

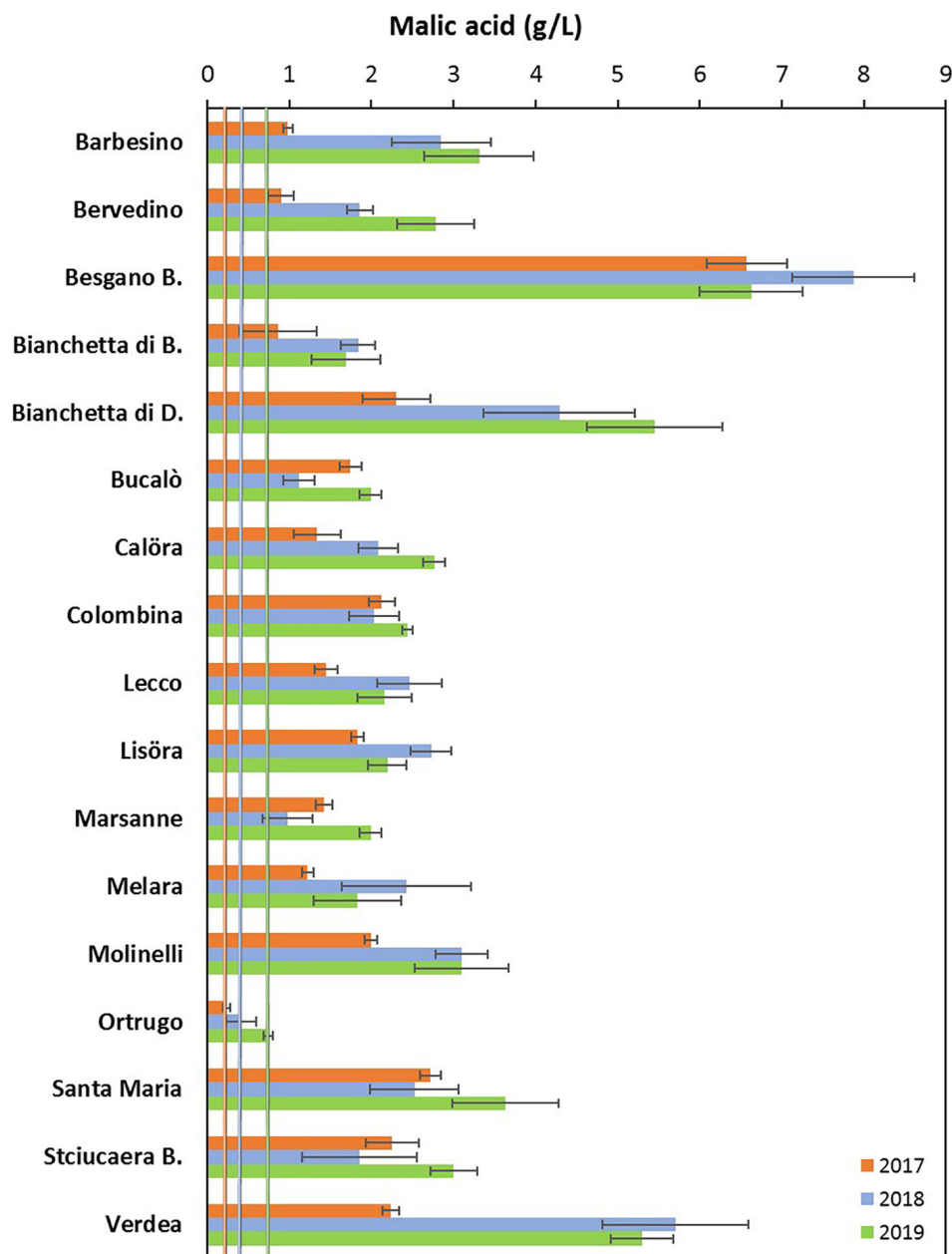


FIGURE 3 | Interactive effects between genotype and year for must malic acid concentration (g/L) at harvest in 17 *Vitis vinifera* L. varieties including the reference cultivar Ortrugo. Data are means \pm SE, $n = 4$.

only for Bervedino, Bianchetta di Bacedasco (Bianchetta di B.), Bianchetta di Diolo (Bianchetta di D.), Ortrugo and Verdea. In all remaining varieties, tartrate in 2017 was either similar or even higher than the concentration determined at harvest in 2018 and 2019 (Figure 2). Malate concentration at harvest confirmed high variability in terms of single varietal sensitivity to the climatic patterns; the hot and dry 2017 did not necessarily result in lowest malic acid concentration at harvest (cases of Besgano Bianco, Bucalò, Colombina, Marsanne, Santa Maria, Stciucaera Bianca) (Figure 3). Ortrugo had a very distinctive behavior, since in no

years the malic acid concentration reached the threshold of 1 g/L. Variation of the calculated tartrate/malate ratio was an obvious consequence of relative changes of the main acids and Ortrugo, with a ratio of 18.3, outclassed all remaining varieties whose ratios ranged between 1.1 and 5.3 (not shown).

When combinations of TSS and TA data were plotted together for all varieties and for data pooled over the 3 years (Figure 4), the reference Ortrugo along with Bianchetta di B. fell inside the bottom-left quadrant showing fairly adequate TSS and too low TA; six other genotypes (Barbesino, Molinelli, Santa Maria,

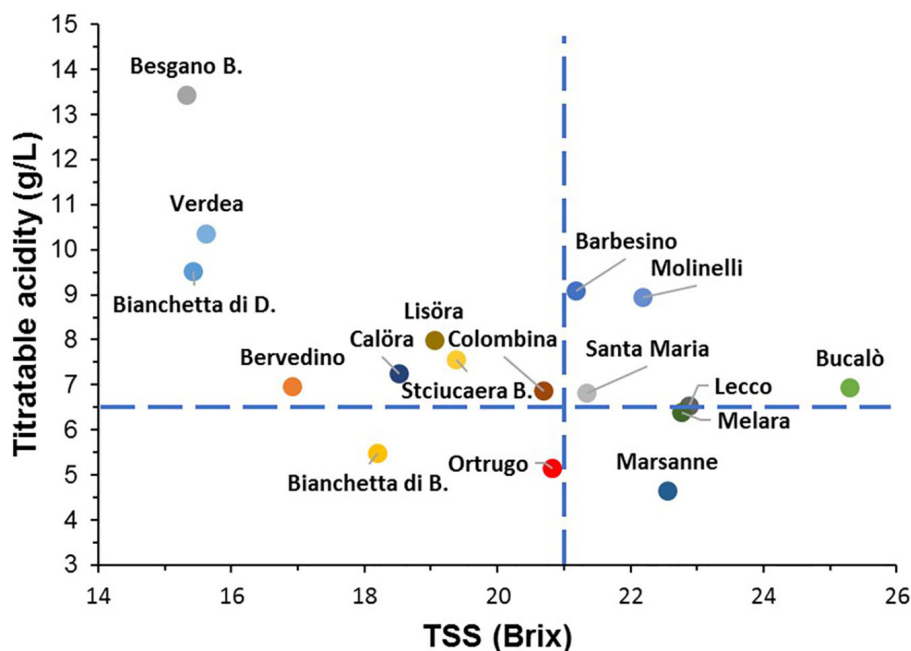


FIGURE 4 | Positioning of the 17 *Vitis vinifera* L. varieties including the reference cultivar Ortrugo as TSS/TA pair data recorded at harvest (data pooled over years). The blue dashed lines indicate requirement thresholds set at a TSS concentration of 21°Brix and a titratable acidity of 6.5 g/L.

Lecco, Bucalò, and Melara) were grouped within the top-right quadrant identifying requirements for optimal technological maturity in sparkling vinification (TSS ~ 20–21°Brix and TA \geq 6.5 g/L). For this reason, these six varieties and the reference Ortrugo were chosen to track seasonal variation of berry mass, TSS, TA, tartrate and malate (pH curves not shown) and were also subjected to Principal Component Analyses.

Post-veraison seasonal change in berry size showed, in any season, a significant varietal \times time interaction and, within each date, significant differences among genotypes (Figures 5A, 6A, 7A). Variation in fraction of berry size formed at the initial measurement over final berry size was mild for data pooled over years (46.7% in 2019 to 48.9% in 2017), whereas the same parameter showed larger variability across varieties ranging from a minimum of 40.7% in Molinelli to a maximum of 55.5% in Lecco. A common feature to every year was that at the very first sampling date large differences in berry size among varieties already occurred; in 2017 such differences tended to be smoothed over time and, at harvest, berry size of the pair Melara and Santa Maria was bigger than the other five varieties all grouped together. Conversely, in 2018 and 2019, initial variation in berry size among genotypes was overall maintained, in relative terms, until harvest.

Total soluble concentration tracked from veraison until harvest shared with berry mass significant varietal \times time interaction and, within each date, significant differences among varieties (Figures 5B, 6B, 7B). Notably, in any season, TSS already differed among cultivars at the very first sampling date with Santa Maria invariably having the highest sugar concentration. Such a pattern was quite drastically modified

during the ripening phase as, regardless of season, Bucalò always reached maximum TSS at harvest (26.5, 25.1, and 22.6°Brix in 2017, 2018, and 2019, respectively), whereas the lowest final sugar concentration were scored by Ortrugo in 2017 (20.7°Brix), Melara in 2018 (20.4°Brix) and Santa Maria in 2019 (18.4°Brix). Rate of TSS/day increments calculated from first date of sampling until harvest, showed that Bucalò always reached peak values, whereas the lowest rates were measured for Santa Maria and Ortrugo. When data were analyzed as TSS/berry, initial and end-of season values of the different varieties essentially mirrored berry weight trends (Figures 5C, 6C, 7C). In any season, Melara was the most efficient as total sugar accumulated per berry, whereas Ortrugo and Molinelli were the least.

Titratable acidity (TA) measured at first sampling around veraison showed already, every year, a large gap among varieties (Figures 8A, 9A, 10A). Across years, quite consistently, Bucalò had the highest TA values at the beginning of ripening, whereas Santa Maria and Ortrugo had the lowest acid pool. Barbesino and Molinelli were, by far, the two varieties that, regardless of the initial TA level, were able to maintain highest acidity at harvest. When evaluation was made in terms of maintenance, at harvest, of the minimum required TA concentration (6.5 g/L), Ortrugo was never able to meet the requirement. Rate of TA degradation, calculated as TA/day decrease from first sampling date until harvest for each season, was quite accelerated in 2017 (about 1 g/L*day) versus the ~0.6 g/L*day in the cooler 2019. Notably, Bucalò exhibited the fastest TA degradation rates and Barbesino the lowest, with Ortrugo setting at intermediate positions. When seasonal TSS and TA were plotted together with data pooled over years (Figure 11), an exponential model

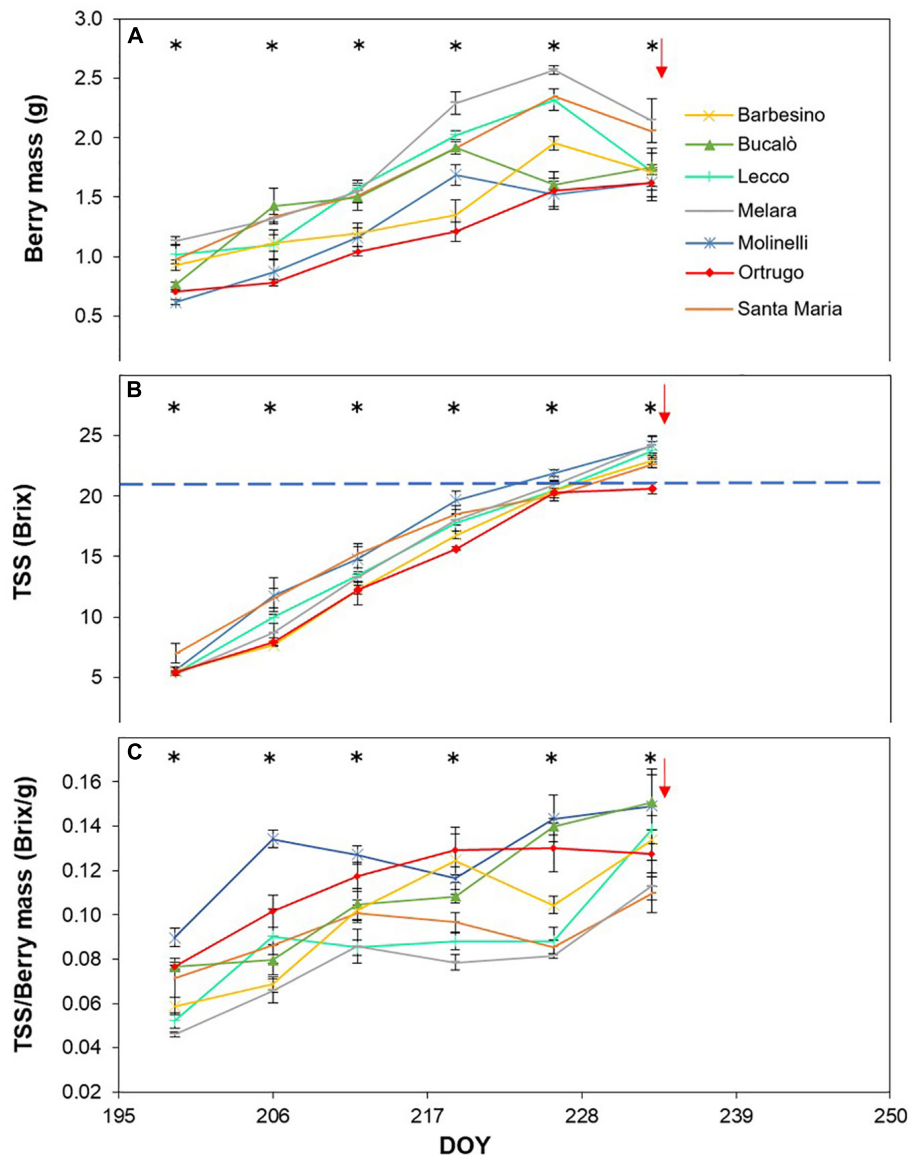


FIGURE 5 | Seasonal evolution of berry mass (A), total soluble solids (TSS) (B) and TSS/berry mass ratio (C) in 2017 for 7 selected *Vitis vinifera* L. varieties including the reference cultivar Ortrugo. Each point represents the average of three replicates \pm SE. Asterisks indicate within-date significant difference at $p \leq 0.05$. The blue dashed line indicates a TSS concentration of 21° Brix. Red arrows represent the date of harvest. DOY = Day of Year.

was fit showing that, over the entire range of represented TSS (about 5 to 25°Brix), Barbesino and Molinelli could account for about 3–5 g/L higher TA than values measured in the reference cultivar Ortrugo.

Seasonal tartaric acid variation differed quite substantially from the TA patterns (Figures 8B, 9B, 10B). The genotype Molinelli was the one showing, upon first sampling, maximum tartrate concentration (19–24 g/L) regardless of season. The same Molinelli along with Barbesino were the two varieties preserving maximum tartrate at harvest (Figures 8B, 9B, 10B and Table 2). Ortrugo showed overall high amounts of tartrate at the beginning and end of the seasonal sampling, scoring maximum pre-harvest concentration in 2019. Rate of tartrate degradation, in the fairly

warm 2017 and 2018 seasons, was the fastest in Molinelli and Ortrugo, whereas in 2019 Molinelli and Bucalò marked the most accelerated depletion.

Malate concentration followed from veraison to pre-harvest showed that, at veraison, Bucalò started with the largest pool in 2017 and 2018 (26.4 and 30.0 g/L, respectively), whereas maximum malate concentration was measured in Santa Maria in 2019 (Figures 8C, 9C, 10C). Conversely, every season, Molinelli and Ortrugo started with the lowest malate pools; yet, the fate of those initial amounts was quite different; while Ortrugo retained every year very low amounts (0.46 g/L on a 3 year basis, Table 2), Molinelli showed, even in the hot 2017, a very pronounced ability to preserve malic acid at harvest (2.73 g/L). Though, when the

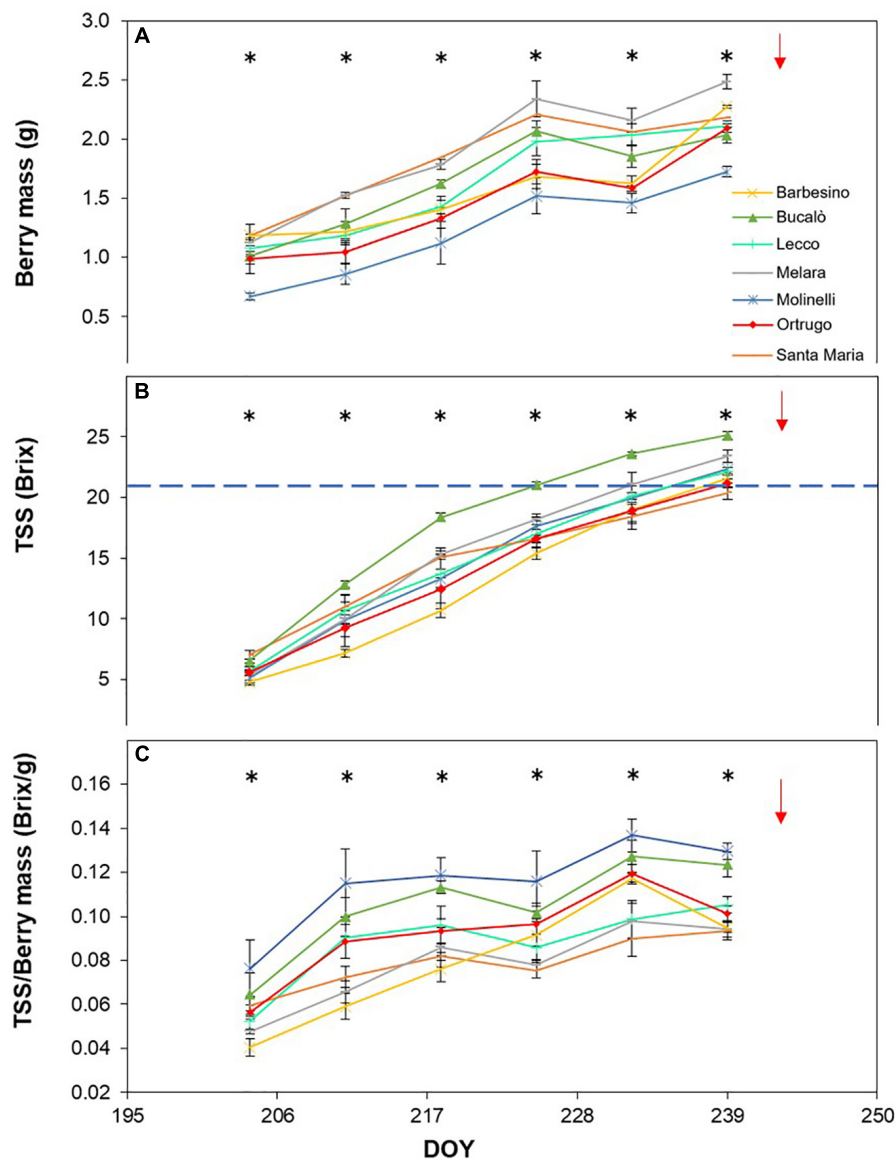


FIGURE 6 | Seasonal evolution of berry mass (A), total soluble solids (TSS) (B) and TSS/berry mass ratio (C) in 2018 for 7 selected *Vitis vinifera* L. varieties including the reference cultivar Ortrugo. Each point represents the average of three replicates \pm SE. Asterisks indicate within-date significant difference at $p \leq 0.05$. The blue dashed line indicates a TSS concentration of 21° Brix. Red arrows represent the date of harvest. DOY = Day of Year.

rate of malic degradation was evaluated as malate decrease/day from first date of sampling and harvest, Ortrugo showed quite higher resilience scoring the slowest rate in 2018 (0.496 g/L*day in 2018) and the second slowest rate in 2017 and 2019 after Santa Maria and Molinelli, respectively.

Principal Component Analysis

Principal component analysis was run first on data pooled over years for the seven selected varieties and for 11 variables representative of vegetative growth, yield and grape composition. PCA F1 and F2 dimensions were those covering the highest variability (69.06%) against 63.12% covered by F1 and F3 and only 52.40% explained by F2 and F3. Therefore, F1

and F2 dimensions were chosen to plot the correlation circle (Figure 12A) and the observation bi-plot (Figure 12B).

Looking at different reciprocal angles formed by the direction of the different PCA vectors, it was apparent that TSS (Brix) held a negative relationship with yield and pruning weight per vine ($r = -0.675$ and -0.605 , respectively, and the same negative linear model was found for tartrate vs. berry weight ($r = -0.668$). Interestingly, the same significant relationship was not shown when berry weight was regressed against malate ($r = 0.192$). A positive correlation was found for TA vs cluster number ($r = 0.833$) and for malic acid and pruning weight ($r = 0.663$).

The observation bi-plot allowed a quite clear separation of different varieties. The reference Ortrugo is isolated from

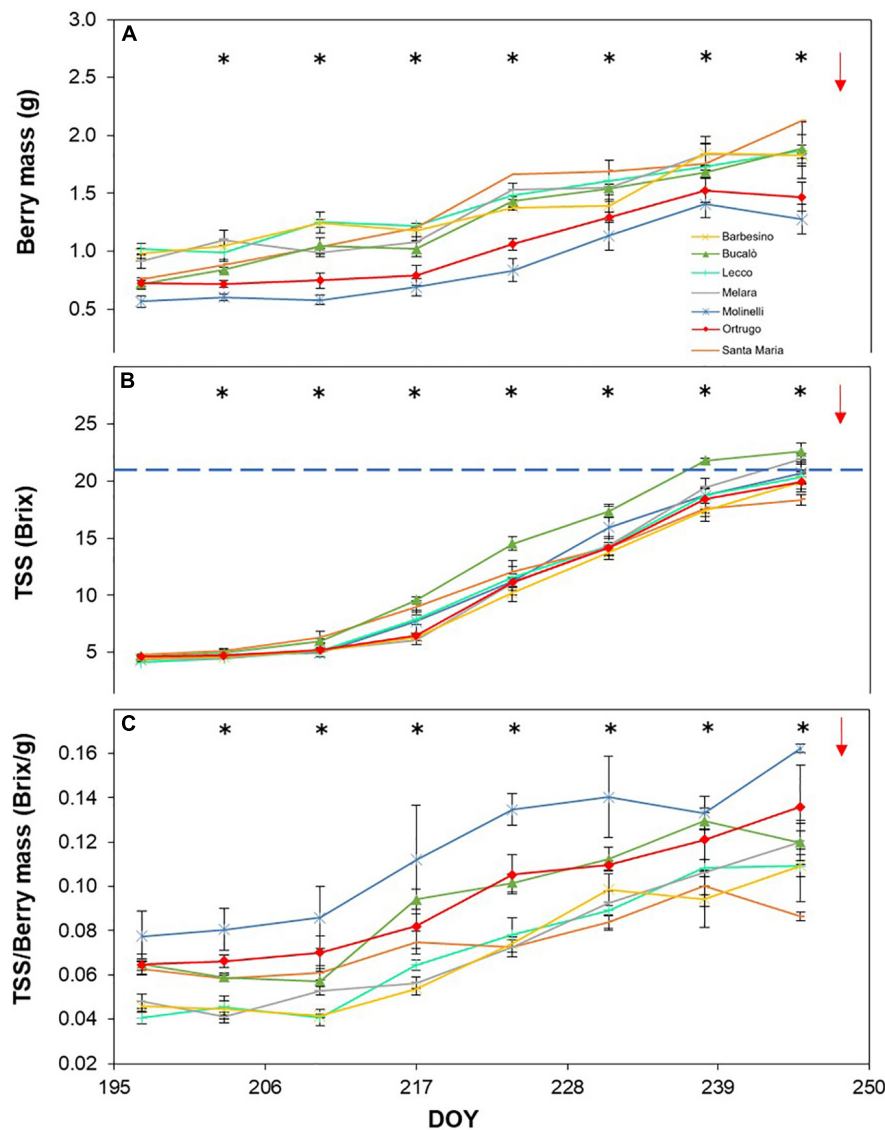


FIGURE 7 | Seasonal evolution of berry mass (A), total soluble solids (TSS) (B) and TSS/berry mass ratio (C) in 2019 for 7 selected *Vitis vinifera* L. varieties including the reference cultivar Ortrugo. Each point represents the average of three replicates \pm SE. Asterisks indicate within-date significant difference at $p \leq 0.05$. The blue dashed line indicates a TSS concentration of 21° Brix. Red arrows represent the date of harvest. DOY = Day of Year.

the remaining varieties due to an inverse correlation between tartrate/malate ratio (very high) and malate (very low) and a good attitude to crop under a fairly low vigor status. The bottom-right quadrant of the observation bi-plot also isolates cvs. Molinelli and Barbesino, these sharing a distinct attitude of maintaining high TA primarily through high tartrate retention. Yield and sugar accumulation are sufficient whereas vine vigor is higher than Ortrugo. Then a third group, albeit more dispersed, encompasses Bucalò, Melara, Santa Maria and Lecco which are reunited by the capacity to achieve high sugar concentration and content, high pH and berry size. Their crop load is generally moderate or low.

A second PCA run was performed on the same seven varieties using yearly data of TSS and acid components rates as well as of DD/day and $(T_{\max} - T_{\min})/\text{day}$ within the time period

comprised between first grape sampling date and harvest. This latter corresponded to 35, 38, and 51 days in 2017, 2018, and 2019, respectively.

The correlation circle (Figure 12C) showed some expected positive correlations (e.g. those between DD/day and $(T_{\max} - T_{\min})/\text{day}$ vs. daily rates of TSS increment and TA degradation). A good correlation ($r = 0.704$) was also found between DD/day and rate of malate degradation, whereas tartrate degradation was less responsive to active temperatures. Interestingly, no correlation was found between daily degradation rates of malic and tartaric acids.

The observation bi-plot which maintained single season behavior of different varieties was quite effective at separating 2019 behavior versus the 2017 and 2018 patterns with varieties

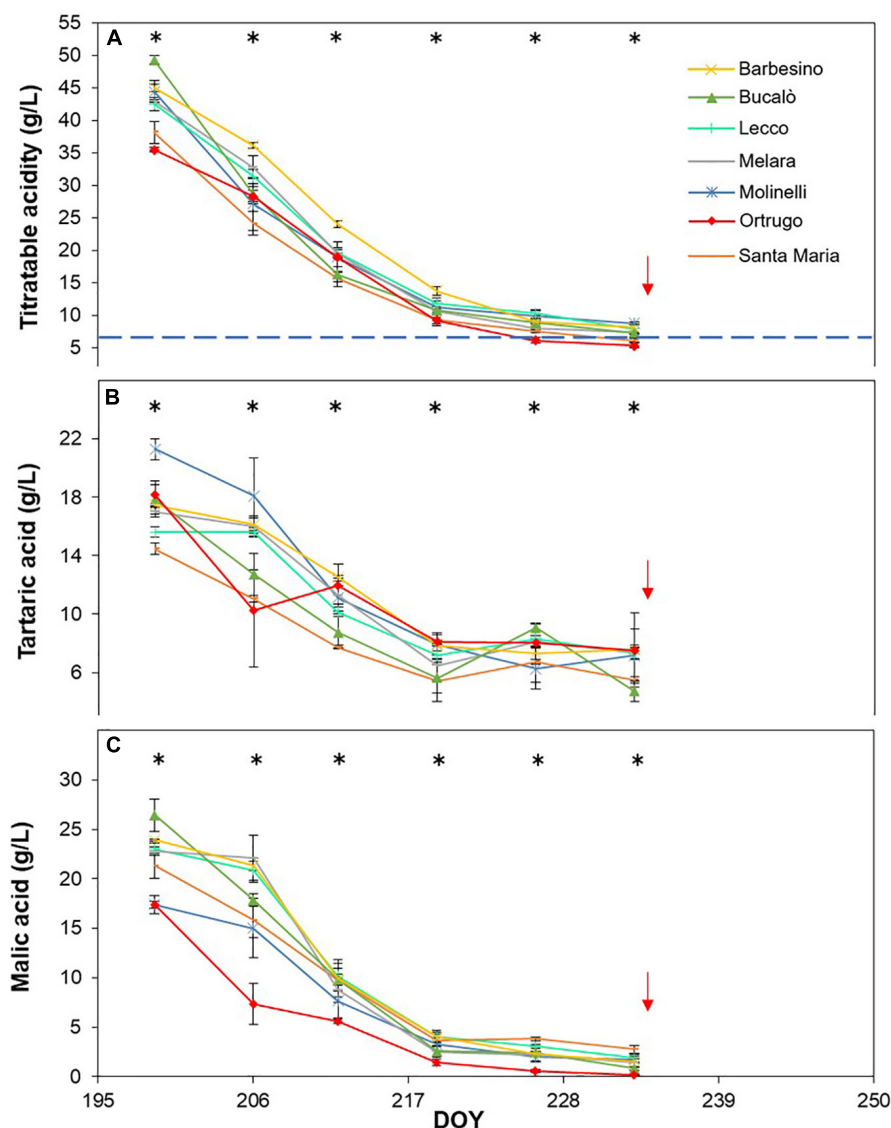


FIGURE 8 | Seasonal evolution of titratable acidity (A), tartaric acid (B) and malic acid (C) in 2017 for 7 selected *Vitis vinifera* L. varieties including the reference cultivar Ortrugo. Each point represents the average of three replicates \pm SE. Asterisks indicate within-date significant difference at $p \leq 0.05$. The blue dashed line indicates a titratable acidity of 6.5 g/L. Red arrows represent the date of harvest. DOY = Day of Year.

that tended to mix. Though, in both 2017 and 2018, Ortrugo and Molinelli consistently separated due to their characteristic to show faster rates of diminishing tartaric acid concentration; by contrast Melara, Lecco and Bucalò grouped together for their high rates of malate depletion. Then, Barbesino and Santa Maria positioned themselves according to year; Barbesino in 2018 showed strong retention capacity for tartaric acid, whereas Santa Maria in 2017 had quite high rates of tartaric acid decrease.

DISCUSSION

Criteria chosen in our work to establish minimum desirable requirements for sparkling wine making (TSS around 20–21°Brix

and TA ≥ 6.5 g/L) were quite effective to narrow down to 6 varieties, from an initial batch of 16, the group size within which reliable alternatives to the reference Ortrugo could have been found. Data shown in **Tables 1, 2** as well as PCA run on 11 variables representative of growth, yield and grape composition (**Figure 12B**) have clearly isolated the behavior of Ortrugo vs. the remaining varieties, confirming its weaknesses at maintaining, regardless of the seasonal weather, enough acidity to allow proper sparkling wine making. Such inadequacy manifests through a very high tartrate-to-malate ratio, which, in turn, originates from extremely low malic acid retention at harvest. However, final TA recorded at harvest is the result of complex interactions among factors affecting synthesis, dilution and degradation of different acids throughout the season.

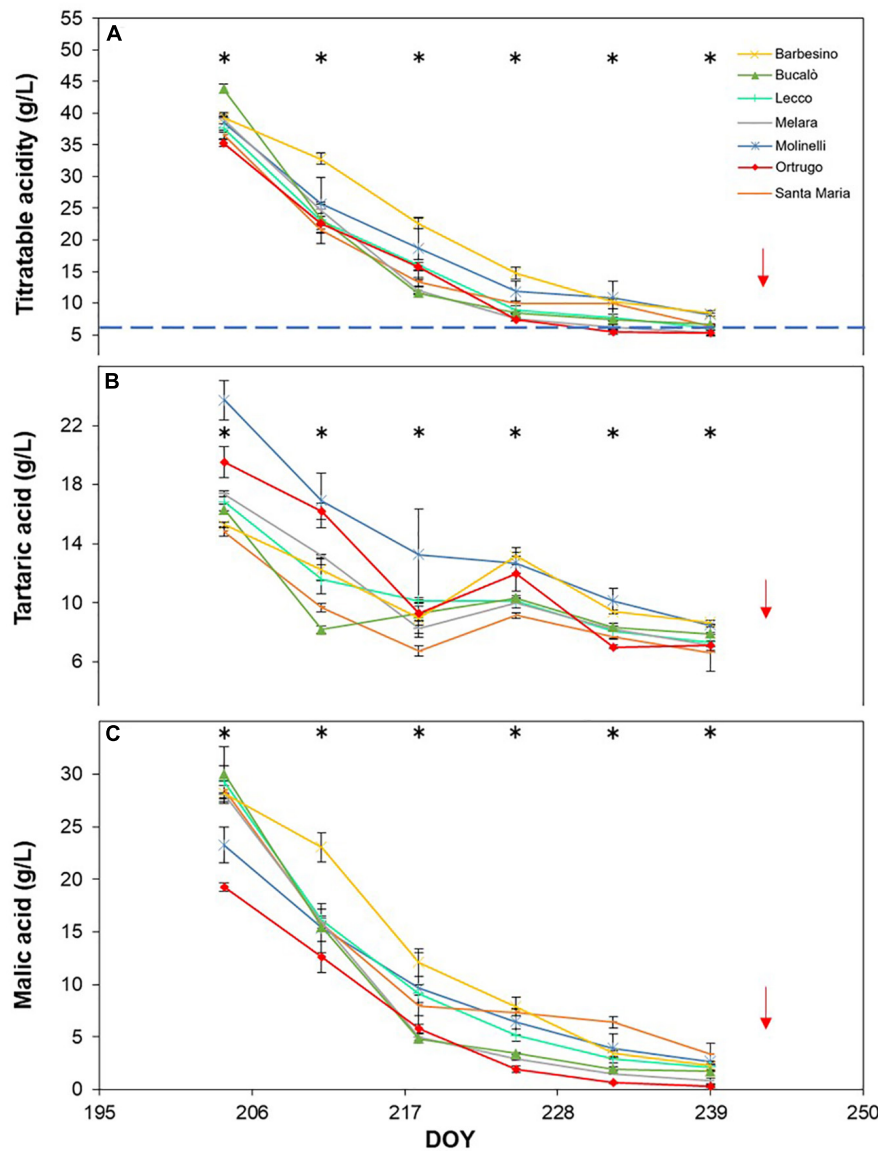


FIGURE 9 | Seasonal evolution of titratable acidity (A), tartaric acid (B) and malic acid (C) in 2018 for 7 selected *Vitis vinifera* L. varieties including the reference cultivar Ortrugo. Each point represents the average of three replicates \pm SE. Asterisks indicate within-date significant difference at $p \leq 0.05$. The blue dashed line indicates a titratable acidity of 6.5 g/L. Red arrows represent the date of harvest. DOY = Day of Year.

Pre-veraison tartaric acid pool available in Ortrugo berries seems adequate as the measured 18.2 g/L (average over 3 years) ranks second after the top scorer Molinelli (21.4 g/L). It has been well established in literature (Walker and Famiani, 2018) that ascorbic acid must be considered the true intermediate precursor in grape tartrate biogenesis. This was resolved in an experiment where young leaves were fed with $^{14}\text{CO}_2$ or [U- ^{14}C]-sucrose and the accumulation of radiolabel could be followed over time into glucose/ascorbate-2-keto-L-idonate/L-idonate/5-keto-D-gluconate and finally tartaric acid (Hawker, 1969). Moreover, while it has been ascertained that the immature berry itself is the main, if not unique, site of tartaric acid biosynthesis (Hale, 1962) it has been shown in a molecular study that levels of

transcripts encoding L-IdnDH were down-regulated in berries grown in the dark using clusters inserted in light-excluding boxes (De Bolt et al., 2008). Such scientific evidence leads to conclude that abundance of glucose substrate as well as an open canopy allowing good cluster exposure to light might be beneficial for accumulation of tartaric acid in the green berry. As a matter of fact, Ortrugo seems to satisfy both requirements; on a 3 year basis, it is the varietal showing the highest amount of total sugar per vine (493 g/vine) and both Ravaz index (8.6) and total pruning weight per vine (308 g/m of row length) are quite typical of low vigor conditions, ruling out the possibility of excessive cluster shading by a too dense canopy. According to Ruffner (1982), tartaric acid increases in the green berry until about 4 weeks after

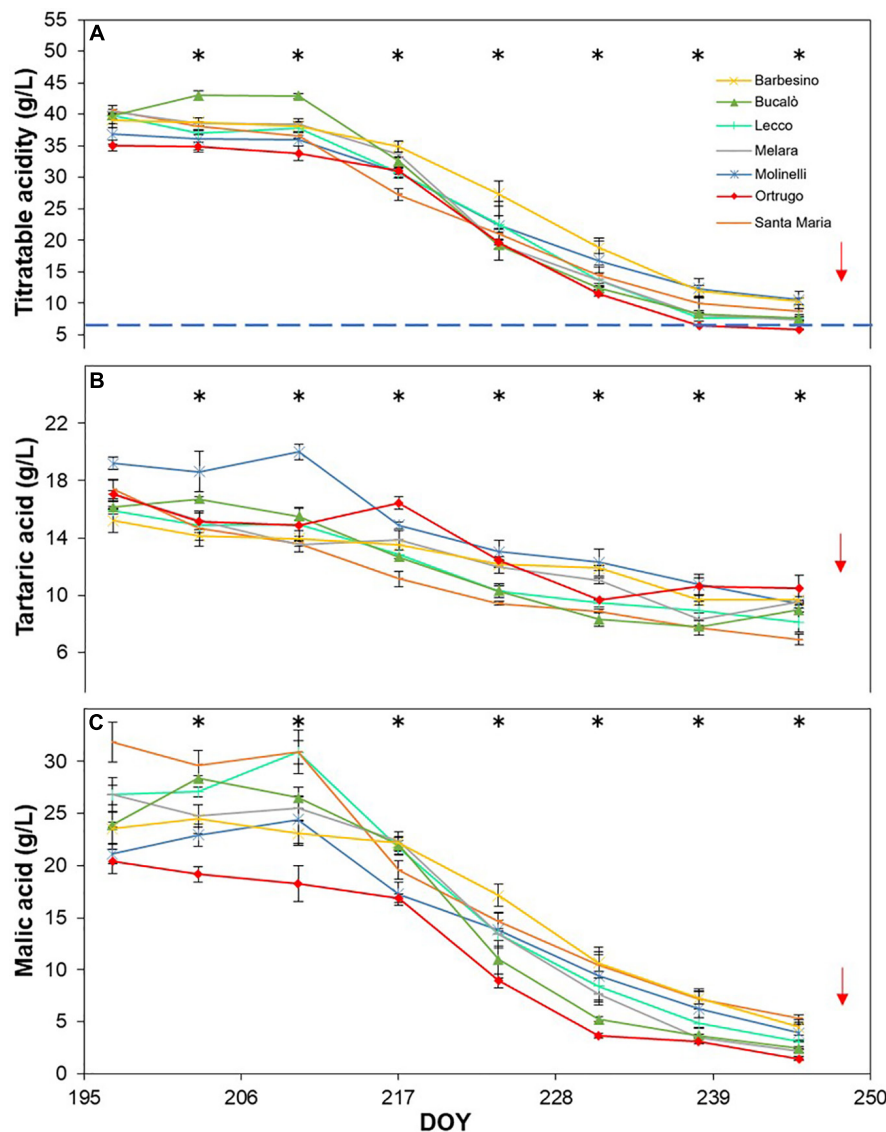


FIGURE 10 | Seasonal evolution of titratable acidity (A), tartaric acid (B) and malic acid (C) in 2019 for 7 selected *Vitis vinifera* L. varieties, including the reference cultivar Ortrugo. Each point represents the average of three replicates \pm SE. Asterisks indicate within-date significant difference at $p \leq 0.05$. The blue dashed line indicates a titratable acidity of 6.5 g/L. Red arrows represent the date of harvest. DOY = Day of Year.

flowering. Thereafter, its synthesis is halted and further changes in concentration are almost exclusively due to increasing berry volume during the post lag growth phase, leading to dilution of tartaric acid (Ford, 2012). Thermal stability is also common and shared knowledge of physiological biochemistry of tartaric acid whose concentration in the berry is essentially unaffected by temperature; some pioneer work alluding to a “respiration” of tartaric acid at temperatures above 30°C (Peynaud and Maurié, 1958) never found subsequent validation. Further, if dilution is the main player in the dynamic of tartaric acid decrease after veraison, observation-bi plot shown in **Figure 12D** clearly indicates that daily increment in berry fresh mass (BM/day) and daily decrease in tartaric acid concentration (HT/day) were correlated and such correlation was mostly reflected by 2017

and 2018 Ortrugo data. Overall, in term of tartaric acid seasonal balance, main weakness for Ortrugo was quite high dilution rates rather than a limitation in the initial pre-veraison build up.

As for malic acid, Ortrugo started each season with the lowest amounts as compared to any other varieties; initial malic concentration in green berries measured in Ortrugo was 19.0 g/L (data pooled over years) with a maximum gap vs. Bucalò (starting at 26.8 g/L). Malate biosynthesis starts in the immature berries about 7 days after flowering whereas the onset of softening marks the beginning of a rapid loss (Coombe, 1995; Ollat et al., 2002). Hale (1962) demonstrated that malate is synthesized in the berry and not imported from any other part of the vine. There is also a shared consensus that the primary pathway for malic acid formation in the green berry is

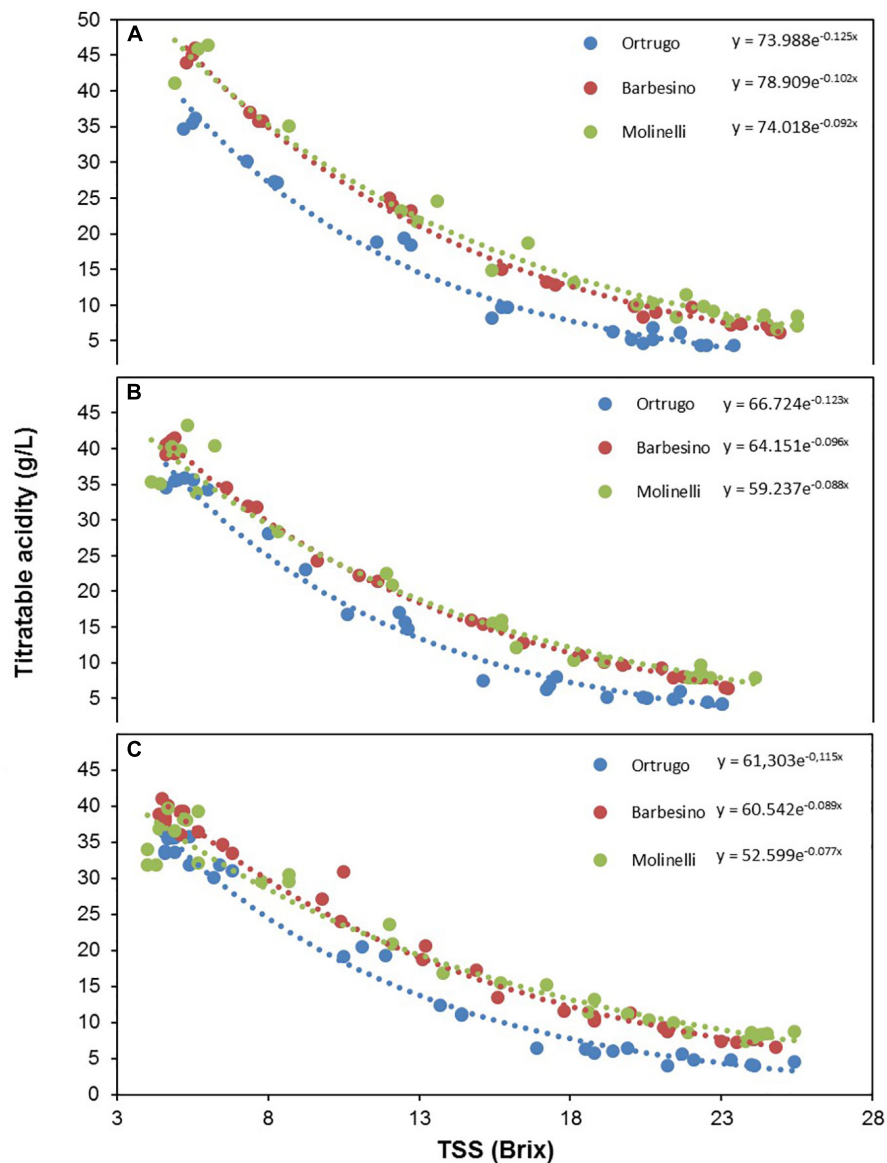


FIGURE 11 | Seasonal variation of titratable acidity expressed as a function of total soluble solids (TSS) in 2017 (A), 2018 (B), and 2019 (C), in Ortrugo (blue), Barbesino (red) and Molinelli (green) grapes. Data were fit to the following equations: Ortrugo 2017 $y = 73.99e^{-0.12x}$ $R^2 = 0.97$; Ortrugo 2018 $y = 66.72e^{-0.12x}$ $R^2 = 0.98$; Ortrugo 2019 $y = 61.30e^{-0.11x}$ $R^2 = 0.98$; Barbesino 2017 $y = 78.91e^{-0.10x}$ $R^2 = 0.99$; Barbesino 2018 $y = 64.15e^{-0.10x}$ $R^2 = 0.99$; Barbesino 2019 $y = 60.54e^{-0.09x}$ $R^2 = 0.99$; Molinelli 2017 $y = 74.02e^{-0.09x}$ $R^2 = 0.97$; Molinelli 2018 $y = 59.24e^{-0.09x}$ $R^2 = 0.98$; Molinelli 2019 $y = 52.60e^{-0.08x}$ $R^2 = 0.98$.

through the cytoplasmic enzyme phospho-enol-piruvate (PEP) carboxylase which catalyzes the B-carboxylation of PEP arising from glycolysis, forming oxalacetic acid; such reaction is often referred as “dark fixation of CO_2 ” (O’Leary, 1982). Indeed, several other enzymes have been associated with malate breakdown, including those associated with gluconeogenesis, respiration and fermentation (Ruffner, 1982; Famiani et al., 2016). As related to the effect of environmental factors on malate accumulation, although no evidence has been reported in terms of sensitivity to radiation, Buttrose et al. (1971) have confirmed that high temperature during the pre-veraison period led to lower levels of malate accumulation. According to the above scenario, reasons

explaining why Ortrugo shows a scarcer malate pool at pre-veraison, if compared to other varieties, still remain in the realm of speculation. Indeed, taking the time window between 1 June and 15 July within which the first stage of berry growth and lag phase can be reasonably placed, it seems true that the cooler 2018 (average daily $T_{\text{max}} = 27.9^\circ\text{C}$ and $\text{DD} = 567$) did result in higher malate accumulation in all varieties except for Ortrugo, therefore showing its quite low sensitivity to such thermal trigger. Then, if it is true that rates of dark (night) respiration are also linked to net photosynthesis rates during the preceding day (Poni et al., 2006), canopies having high carbon balance might be favored at accumulating malate. However, in this study we neither recorded

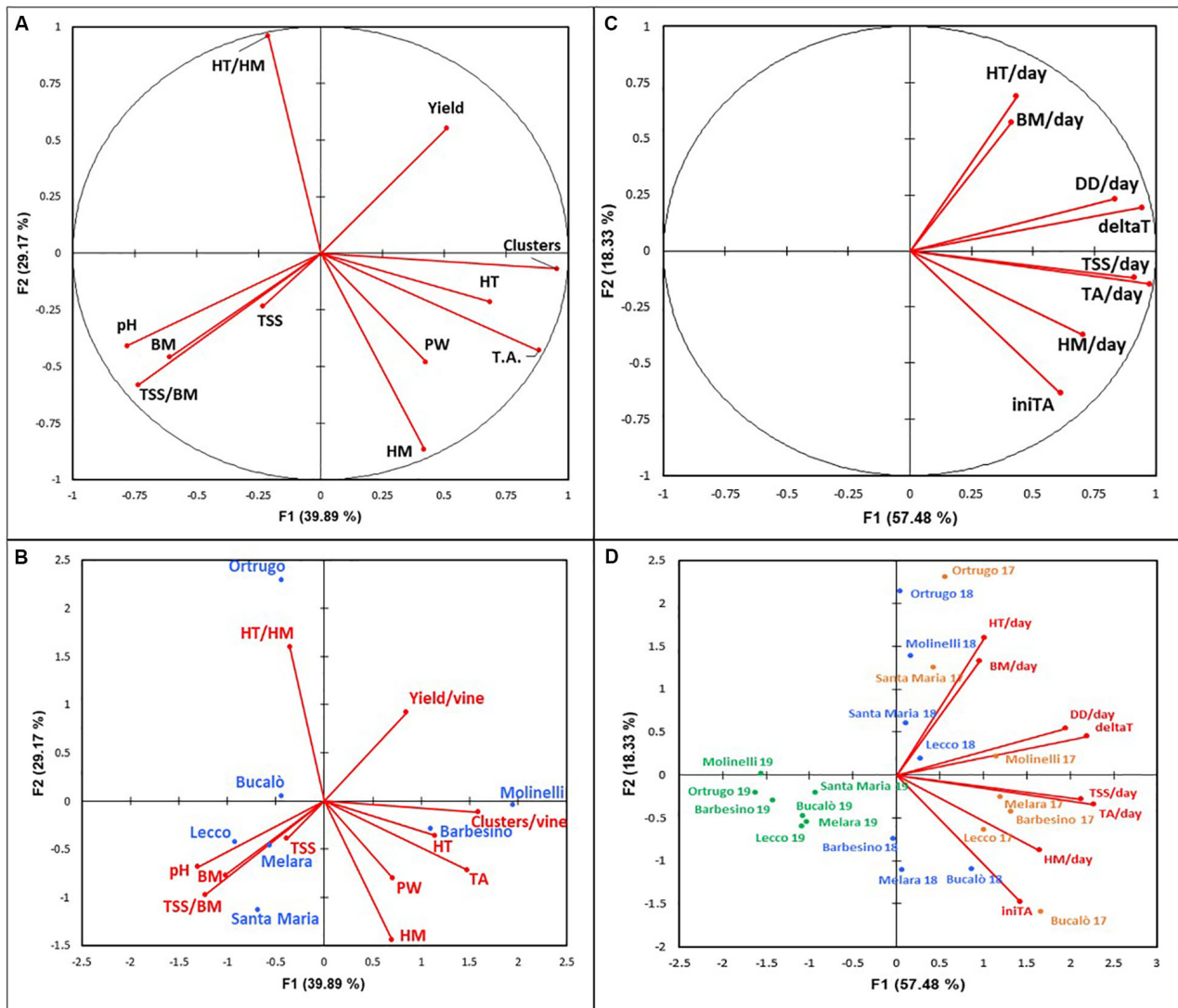


FIGURE 12 | (A,B) Principal component analysis (PCA) of 11 variables (axes F1 and F2: 69.06%) for 7 selected *Vitis vinifera* L. varieties, including the reference cultivar Ortrugo (data pooled over 3 years). Panel A represents correlation circle. Panel B represents the biplot with variables and varieties. HT/HM, tartaric acid/malic acid ratio; HT, tartaric acid; TA, titratable acidity; PW, pruning weight; HM, malic acid; TSS, total soluble solids; TSS/BM, total soluble solids/berry mass ratio; BM, berry mass. **(C,D)** Principal component analysis (PCA) of 8 variables (axes F1 and F2: 75.81%) for 7 selected *Vitis vinifera* L. varieties, including the reference cultivar Ortrugo (data pooled over 3 years). Panel C represents the correlation circle. Panel D represents the biplot with variables and varieties. Varieties are reported according to their behaviour in the 3 years of trial (2017 in orange, 2018 in blue, 2019 in green). HT/day, tartaric acid per day; BM/day, berry mass per day; DD/day, degree day per day; deltaT, (Tmax – Tmin)/day; TSS/day, total soluble solids per day; TA/day, titratable acidity per day; HM/day, malic acid per day; iniTA, initial titratable acidity.

leaf gas exchange parameters nor did we estimate canopy light interception which, to a certain extent, is a good estimator of total canopy photosynthesis (Poni et al., 2003). The closer approximation in our trial is given by the Ravaz index, values of which ranging between 5 and 6 are supposed to correspond to adequate vine balance (Howell, 2001); among the seven selected varieties, though, Ortrugo is the one having the highest ratio (8.60) suggesting a vine balance leading to a quite limited canopy supply function as compared to sink demand.

Actually, besides the low malate pool at veraison, Ortrugo has also shown a very scarce ability in preserving a sufficient malic

acid share from degradation. Over 3 years, the concentration at the end of the season was just 2.5% of that found at first samplings, whereas other genotypes such as Barbesino and Molinelli maintained higher fractions (7.6 and 8.8%, respectively, of the concentration at veraison). Post-veraison malate degradation is enhanced by high temperatures (Buttrose et al., 1971), whereas an active role of the light regime has never been demonstrated in contrast to the effects seen on tartaric acid and ascorbate (Dokoozlian and Kliever, 1996). As a confirmation, PCA reported in **Figures 12C,D** shows no correlation between daily loss of malic and tartaric acid

throughout the post-veraison period until harvest. However, Ortrugo does not have any faster consumption of the initial malic acid pool since rates of malic degradation were, on a 3 year basis, the lowest (0.45 g/L*day) among the seven varieties. Although Ortrugo's weakness at retaining adequate TA at harvest has already been reported in some specific papers (Gatti et al., 2015, 2019), this study clarifies that such limitation primarily rises from fairly low malic acid accumulation pre-veraison and intense dilution of tartaric acid after veraison. **Figure 11**, showing the decreasing trend of TA in Ortrugo, Molinelli and Barbesino vs increasing TSS, is quite self-explanatory: the three patterns do not differ in terms of slopes rather for a given intercept values that stays quite constant throughout the whole ripening season.

Looking for alternative genotypes ensuring the economic sustainability of the productive process, a satisfying yield is still a basic requirement (Howell, 2001). In our work, most of the minor local genotypes from the Colli Piacentini area stood out for an average productivity comparable to Ortrugo, the main varietal elected for the local appellation (**Table 1**). Considering that Ortrugo, in the most favorable vintages, sets very close to the maximum yield allowed of 12 tons/ha, all those minor cultivars exhibiting yields similar to Ortrugo can be considered performant genotypes in terms of average productivity. In particular, the good basal nodes fruitfulness demonstrated by Bervedino, Molinelli and Lisöra (**Table 1**), make these cultivars very prone to a modern vineyard management based on spur-pruning and mechanization of pre-pruning operations (Poni et al., 2016).

PCA analyses shown in **Figure 12B** isolate within the bottom-right quadrant the behavior of Molinelli and Barbesino. Molinelli stands out as an ideal cultivar for sparkling wine purposes as it exhibits desirable agronomic features (such as balanced cropload, small and loose clusters, fairly high fruitfulness of the basal nodes), fast sugar accumulation and a sort of extraordinary ability to retain TA with a well-balanced tartrate-to-malate ratio. Notably, Molinelli retained enough malic acid (about 2 g/L) even in the hot 2017 not because its daily degradation rate was slower than Ortrugo, rather because the initial pool was higher. Similar performances were also shown by Barbesino, which, however, showed more sensitivity than Molinelli to malic acid degradation. Current appellation regulation for Ortrugo allows a maximum 10% of "other varieties" to be blended and the mix with Molinelli or Barbesino, once registered, seems highly recommended.

Finally, PCA analyses allowed the grouping of the four remaining varietals (Bucalò, Lecco, Santa Maria and Melara) showing a generally high rate of sugar accumulation and maintenance of adequate TA. However, possible use of such genotypes seems to be spoiled by low productivity that, especially in Lecco, Bucalò and Melara, diminishes the interest as alternate choices for Ortrugo.

CONCLUSION

A detailed 3 year study on agronomic performance of 16 minor white varietals of the Colli Piacentini grape district was

performed to assess if any of them could provide a reliable alternative to the local main white cultivar Ortrugo, showing major limitations at maintaining sustained acidity at harvest in the context of global warming. In regard to the mechanisms leading Ortrugo to present, at harvest, low TA with just traces of malic acid, it has been ascertained that the two most limiting factors are post veraison intense dilution of tartaric acid and too low malic acid accumulated pre-veraison.

Data processing assisted with both repeated measures and principal components analyses allowed a thinning down of the initial group of 16 genotypes to 7 varietals (Molinelli, Barbesino, Bucalò, Melara, Lecco, Santa Maria and the reference Ortrugo) showing ability to combine at harvest adequate TSS (20–21°Brix) and minimum required TA (≥ 6.5 g/L). Then, further analyses have shown that especially Molinelli and Barbesino, regardless of season, performed very efficiently as optimal technological maturity for sparkling wine making, productivity, vine balance and cluster morphology. Such two minor varietals stand out as ideal alternatives to Ortrugo and could contribute to the enhancement of the industry competitiveness by posing new products with an unaffected link to the district identity.

Our work demonstrates that grapevine intra-specific biodiversity hides prominent potentialities for viticulture adaptation strategies to climate change and renew the emphasis on the value of genetic resources conservation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

TF, MG, and SP designed and supervised the research. TF, GB, AG, CS, LR, and MG performed the research and analyzed the data. TF, GB, MG, and SP drafted the manuscript. CS, AG, and LR critically revised the manuscript. LR verified quality of written English. All authors read and approved the final manuscript.

FUNDING

Research carried out within the ValorInVitis project funded by the Emilia Romagna Region under the RDP program (PSR Emilia Romagna 2014–2020 Mis. 16.1.01 FA 2A). Grant no. 5004320.

ACKNOWLEDGMENTS

The authors want to thank Mossi 1558 estate for the technical support in the vineyard, Maria Giulia Parisi, Valentina Bronzoni,

Luca Bricchi, and Noémie Chéron for the technical help and Maurizio Zamboni, Alberto Vercesi, Roberto Miravalle, Marco Profumo, Mauro Mazzocchi, Claudio Gazzola, Andrea Pradelli, and Andrea Illari for topic discussion. Special thanks to Luigi Mossi who reunited the varieties and built the vineyard.

REFERENCES

- Biasi, R., and Brunori, E. (2015). The on-farm conservation of grapevine (*Vitis vinifera* L.) landraces assures the habitat diversity in the viticultural agro-ecosystem. *Vitis* 54, 265–269.
- Buttrose, M., Hale, C., and Kiewer, W. M. (1971). Effect of temperature on the composition of Cabernet Sauvignon berries. *Am. J. Enol. Vitic.* 22, 71–75.
- Coombe, B. G. (1995). Adoption of a system for identifying grapevine growth stages. *Austral. J. Grape Res.* 1, 104–110. doi: 10.1111/j.1755-0238.1995.tb00086.x
- Cruz-Castillo, J. G., Franco-Mora, O., and Famiani, F. (2009). Presence and uses of wild grapevine (*Vitis* spp.) in the central region of Veracruz in Mexico. *J. Int. Sci. Vigne Vin* 43, 77–81.
- De Bolt, S., Ristic, R., Iland, P. G., and Fod, C. M. (2008). Altered light interception reduces grape berry weight and modulates organic acid biosynthesis during development. *HortScience* 43, 957–961. doi: 10.21273/hortsci.43.3.957
- De Ollas, C., Morillón, R., Fotopoulos, V., Puértolas, J., Ollitrault, P., Gómez-Cadenas, A., et al. (2019). Facing climate change: biotechnology of iconic mediterranean woody crops. *Front. Plant Sci.* 10:427. doi: 10.3389/fpls.2019.00427
- Dokoozlian, N., and Kiewer, W. M. (1996). Influence of light on grape berry growth and composition varies during fruit development. *J. Am. Soc. Hortic. Sci.* 121, 869–874. doi: 10.21273/jashs.121.5.869
- Duchêne, E. (2016). How can grapevine genetics contribute to the adaptation to climate change? *OENO One* 50:98. doi: 10.20870/oeno-one.2016.50.3.98
- Famiani, F., Farinelli, D., Frioni, T., Palliotti, A., Battistelli, A., Moscatello, S., et al. (2016). Malate as substrate for catabolism and gluconeogenesis during ripening in the pericarp of different grape cultivars. *Biol. Plant.* 60:155. doi: 10.1007/s10535-015-0574-2
- Famiani, F., Paoletti, A., Proietti, P., Battistelli, A., Moscatello, S., Cruz-Castillo, J. G., et al. (2018). The occurrence of phosphoenolpyruvate carboxykinase (PEPCK) in the pericarp of different grapevine genotypes and in grape leaves and developing seeds. *J. Hortic. Sci. Biotechnol.* 93, 456–465. doi: 10.1080/14620316.2017.1417748
- Ford, C. M. (2012). “The biochemistry of organic acids in the grape,” in *The Biochemistry of the Grape Berry*, eds H. Geros, M. M. Chaves, and S. Delrot (Sharjah: Bentham Science Publishers), 67–88. doi: 10.2174/978160805360511201010067
- Fregoni, M., Zamboni, M., and Colla, R. (2002). *Caratterizzazione Ampelografica Dei Vitigni Autoctoni Piacentini*. Piacenza: Grafiche Lama.
- Gatti, M., Garavani, A., Cantatore, A., Parisi, M. G., Bobeica, N., Merli, M. C., et al. (2015). Interactions of summer pruning techniques and vine performance in the white *Vitis vinifera* cv. Ortrugo. *Austral. J. Grape Wine Res.* 21, 80–89. doi: 10.1111/ajgw.12107
- Gatti, M., Garavani, A., Krajczek, K., Ughini, V., Parisi, M. G., Frioni, T., et al. (2019). Mechanical mid-shoot leaf removal on Ortrugo (*Vitis vinifera* L.) at pre- or mid-veraison alters fruit growth and maturation. *Am. J. Enol. Vitic.* 70, 88–97. doi: 10.5344/ajev.2018.18055
- Gomez, K. A., and Gomez, A. A. (1984). *Statistical Procedures for Agricultural Research*. Hoboken, NJ: John Wiley & Sons.
- Gutiérrez-Gamboa, G., Liu, S. Y., and Pszczółkowski, P. (2020). Resurgence of minority and autochthonous grapevine varieties in South America: a review of their oenological potential. *J. Sci. Food Agric.* 100, 465–482. doi: 10.1002/jsfa.10003
- Hale, C. R. (1962). Synthesis of organic acids in the fruit of the grape. *Nature* 195, 917–918. doi: 10.1038/195917a0
- Hawker, J. (1969). Changes in the activity of malic enzyme, malate dehydrogenase, phosphoenolpyruvate carboxylase and pyruvate decarboxylase during the development of a non-climatic fruit (the grape). *Phytochemistry* 8, 9–23.
- Howell, G. S. (2001). Sustainable grape productivity and the growth-yield relationship: a review. *Am. J. Enol. Vitic.* 52, 165–174.
- Iorizzo, M., Macciola, V., Testa, B., Lombardi, S. J., and De Leonardi, A. (2014). Physicochemical and sensory characteristics of red wines from the rediscovered autochthonous *Tintilia* grapevine grown in the Molise region (Italy). *Eur. Food Res. Technol.* 238, 1037–1048. doi: 10.1007/s00217-014-2186-z
- Jones, G., and Webb, L. (2010). Climate change, viticulture, and wine: challenges and opportunities. *J. Wine Res.* 21, 103–106. doi: 10.1080/09571264.2010.530091
- Kiewer, W. M., Howarth, L., and Omori, M. (1967). Concentrations of tartaric acid and malic acid and their salts in *Vitis vinifera* grapes. *Am. J. Enol. Vitic.* 18, 42–54.
- Mannini, F. (2003). “Italian indigenous grapevine cultivars: guarantee of genetic biodiversity and economic resources,” in *Proceedings of the I International Symposium on Grapevine Growing, Commerce and Research*, Leuven, Vol. 652, 87–95. doi: 10.17660/actahortic.2004.652.9
- Mosedale, J. R., Abernethy, K. E., Smart, R. E., Wilson, R. J., and Maclean, I. M. (2016). Climate change impacts and adaptive strategies: lessons from the grapevine. *Glob. Change Biol.* 22, 3814–3828. doi: 10.1111/gcb.13406
- Mozell, M. R., and Thach, L. (2014). The impact of climate change on the global wine industry: challenges & solutions. *Wine Econ. Policy* 2, 81–89. doi: 10.1016/j.wep.2014.08.001
- O’Leary, M. (1982). Phosphoenolpyruvate carboxylase: an enzymologist’s view. *Annu. Rev. Plant Physiol.* 33, 297–315. doi: 10.1146/annurev.pp.33.060182.001501
- Ollat, N., Carde, J. P., Gaudillere, J. P., Barrieu, F., Diakou-Verdin, P., and Moing, A. (2002). Grape berry development: a review. *OENO One* 36:970. doi: 10.20870/oeno-one.2002.36.3.970
- Palliotti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., and Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. *Sci. Hortic.* 178, 43–54. doi: 10.1016/j.scienta.2014.07.039
- Peynaud, E., and Maurié, A. (1958). Synthesis of tartaric and malic acids by grape vines. *Am. J. Enol. Vitic.* 9, 32–36.
- Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T. T., et al. (2018). Grapevine quality: a multiple choice issue. *Sci. Hortic.* 234, 445–462. doi: 10.1016/j.scienta.2017.12.035
- Poni, S., Magnanini, E., and Bernizzoni, F. (2003). Degree of correlation between total light interception and whole-canopy net CO₂ exchange rate in two grapevine growth systems. *Austral. J. Grape Wine Res.* 9, 2–11. doi: 10.1111/j.1755-0238.2003.tb00226.x
- Poni, S., Palliotti, A., and Bernizzoni, F. (2006). Calibration and evaluation of a STELLA software-based daily CO₂ balance model in *Vitis vinifera* L. *J. Am. Soc. Hortic. Sci.* 131, 273–283. doi: 10.21273/jashs.131.2.273
- Poni, S., Tombesi, S., Palliotti, A., Ughini, V., and Gatti, M. (2016). Mechanical winter pruning of grapevine: physiological bases and applications. *Sci. Hortic.* 204, 88–98. doi: 10.1016/j.scienta.2016.03.046
- Ravaz, M. L. (1911). L’effeuillage de la vigne. *Ann. d. L’Ecole Natl. D’Agric. Montpellier* 11, 216–244.
- Ruffner, H. P. (1982). Metabolism of tartaric and malic acids in *Vitis*: a review – part a. *Vitis* 21, 247–259.
- Sadras, V. O., and Petrie, P. R. (2012). Predicting the time course of grape ripening. *Austral. J. Grape Wine Res.* 18, 48–56. doi: 10.1111/j.1755-0238.2011.00169.x
- Schultz, H. R. (2016). Global climate change, sustainability, and some challenges for grape and wine production. *J. Wine Econ.* 11, 181–200. doi: 10.1017/jwe.2015.31
- Spinoni, J., Naumann, G., Vogt, J., and Barbosa, P. (2016). *Meteorological Droughts in Europe: Events and Impacts: Past Trends and Future Projections*. Ispra: JRC Science Hub publisher.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.00608/full#supplementary-material>

- Storchi, P., Pieri, M., Valentini, P., Bucelli, P., Faviere, V., and Giannetti, F. (2007). Evaluation of indigenous germplasm through “Environment × Genotype” interaction. *Acta Hortic.* 754, 91–96. doi: 10.17660/actahortic.2007.754.11
- Tello, J., and Ibanez, J. (2014). Evaluation of indexes for the quantitative and objective estimation of grapevine bunch compactness. *Vitis* 53, 9–16.
- Urrestarazu, J., Miranda, C., Santesteban, L. G., and Royo, J. B. (2015). Recovery and identification of grapevine varieties cultivated in old vineyards from Navarre (Northeastern Spain). *Sci. Hortic.* 191, 65–73. doi: 10.1016/j.scienta.2015.04.029
- Van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change inviticulture and potential adaptations. *Agronomy* 9:514. doi: 10.3390/agronomy9090514
- Walker, R. P., and Famiani, F. (2018). Organic acids in fruits: metabolism, functions and contents. *Hortic. Rev.* 45, 371–430. doi: 10.1002/9781119431077.ch8

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Frioni, Bertoloni, Squeri, Garavani, Ronney, Poni and Gatti. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Optimal Ranges and Thresholds of Grape Berry Solar Radiation for Flavonoid Biosynthesis in Warm Climates

Nazareth Torres^{†‡}, Johann Martínez-Lüscher^{†‡}, Etienne Porte and S. Kaan Kurtural^{*†‡}

Department of Viticulture and Enology, University of California Davis, Davis, CA, United States

OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Jun Wang,
China Agricultural University,
China
Marianna Fasoli,
E. & J. Gallo Winery,
United States

*Correspondence:

S. Kaan Kurtural
skkurtural@ucdavis.edu

[†]These authors have contributed
equally to this work

*ORCID:

Nazareth Torres
orcid.org/0000-0002-0597-4635
Johann Martínez-Lüscher
orcid.org/0000-0002-3077-1346
S. Kaan Kurtural
orcid.org/0000-0001-9578-831X

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 19 April 2020

Accepted: 08 June 2020

Published: 23 June 2020

Citation:

Torres N, Martínez-Lüscher J, Porte E
and Kurtural SK (2020) Optimal
Ranges and Thresholds of Grape
Berry Solar Radiation for Flavonoid
Biosynthesis in Warm Climates.
Front. Plant Sci. 11:931.
doi: 10.3389/fpls.2020.00931

In commercial wine grape production, canopy management practices are applied to control the source-sink balance and improve the cluster microclimate to enhance berry composition. The aim of this study was to identify the optimal ranges of berry solar radiation exposure (exposure) for upregulation of flavonoid biosynthesis and thresholds for their degradation, to evaluate how canopy management practices such as leaf removal, shoot thinning, and a combination of both affect the grapevine (*Vitis vinifera* L. cv. Cabernet Sauvignon) yield components, berry composition, and flavonoid profile. Three experiments were conducted in Oakville, CA, USA. First experiment assessed changes in the grape flavonoid content driven by four degrees of exposure. In the second experiment, individual grape berries subjected to different exposures were collected from two cultivars (Cabernet Sauvignon and Petit Verdot). The third experiment consisted of an experiment with three canopy management treatments (i) LR (removal of 5 to 6 basal leaves), (ii) ST (thinned to 24 shoots per vine), and (iii) LRST (a combination of LR and ST) and an untreated control (UNT). Berry composition, flavonoid content and profiles, and 3-isobutyl 2-methoxypyrazine were monitored during berry ripening. Although increasing canopy porosity through canopy management practices can be helpful for other purposes, this may not be the case of flavonoid compounds when a certain proportion of kaempferol was achieved. Our results revealed different sensitivities to degradation within the flavonoid groups, flavonols being the only monitored group that was upregulated by solar radiation. Within different canopy management practices, the main effects were due to the ST. Under environmental conditions given in this trial, ST and LRST hastened fruit maturity; however, a clear improvement of the flavonoid compounds (i.e., greater anthocyanin) was not observed at harvest. Methoxypyrazine berry content decreased with canopy management practices studied. Although some berry traits were improved (i.e. 2.5° Brix increase in berry total soluble solids) due to canopy management practices (ST), this resulted in a four-fold increase in labor operations cost, two-fold decrease in yield with a 10-fold increase in anthocyanin production cost per hectare that should be assessed together.

Keywords: anthocyanin, canopy management, kaempferol, leaf removal, methoxypyrazines, shoot thinning

INTRODUCTION

In vineyard production systems, canopy management practices are usually employed to control the source-sink balance and improve the cluster microclimate leading to an improved grape composition and resultant wines (Sivilotti et al., 2016). Canopy density is usually controlled during the dormant season through the winter pruning. Additional canopy management practices may be applied during berry development. Fruit-zone leaf removal and especially, shoot thinning have been widely used in order to increase the cluster exposure to solar radiation, reduce crop load as well as decreasing the pest pressures (Terry and Kurtural, 2011; Provost and Pedneault, 2016; Sivilotti et al., 2016), increasing flavonoid content (Martínez-Lüscher et al., 2019) and diminishing herbaceous aromas (Koch et al., 2012). Nevertheless, when high air temperature and excessive radiation combine, detrimental effects on berry acidity and flavonoid content have been reported in warm climate regions (Martínez-Lüscher et al., 2017).

Leaf removal consists of removing basal leaves around the clusters in the east or north side during grape development increasing the cluster exposure to solar radiation. It is well known that an early leaf removal (before flowering) increased total soluble solids, anthocyanins, and flavonols (Tarara et al., 2008; Diago et al., 2012; Gatti et al., 2012; Pastore et al., 2013; Bogicevic et al., 2015; Cook et al., 2015; Bubola et al., 2017). However, some authors reported increases in titratable acidity in Sangiovese (Gatti et al., 2012) and Teran (Bubola et al., 2017) cultivars while other authors found decreases in acidity with basal leaf removal on Tempranillo (Diago et al., 2012). Conversely, Sivilotti et al. (2016) reported a positive effect of leaf removal applied after flowering on Merlot grapevine by improving cluster integrity by reducing incidence of *Botrytis*, and lower herbaceous aromas without affecting yield and cluster mass. Contrariwise, Pastore et al. (2013) reported that defoliation at veraison reduced the anthocyanin content and increased the impact of sunburn. In fact, these authors found that leaf removal induced a general delay in the transcriptional ripening program, which was particularly apparent for structural and regulatory genes involved in the anthocyanin biosynthesis.

Clearly, vineyard location, cultivar (Tardaguila et al., 2010), timing of leaf removal (Pastore et al., 2013; Sivilotti et al., 2016), method (Diago et al., 2012), and degree of leaf removal (Feng et al., 2015), the growing season (Sivilotti et al., 2016), among others, are all factors influencing how leaf removal affects grapevine berry composition and integrity.

On the other hand, shoot thinning has been related to increased cluster and berry mass and the number of berries per cluster, with a reduction on yield (Sun et al., 2012; Jogaiah et al., 2013). Conversely, Wang et al. (2019) observed that shoot thinning had relatively minor impacts on yield components because of a compensatory effect due to the lower cluster number with concomitant increase in cluster mass. Contrarily, shoot thinning practices on grapevine did not show a great impact on berry primary metabolism (Sun et al., 2012; Wang et al., 2019), however, secondary metabolites were affected by them (Terry and Kurtural, 2011). In fact, we recently reported an increase of two-fold in the flavonol content of Merlot berries

when leaf or shoot removal was applied mainly by increasing the proportion of quercetin and kaempferol derivatives in detriment of the myricetin derivatives (Martínez-Lüscher et al., 2019).

Berry composition is dependent on a complex balance between compounds derived from primary and secondary metabolism. Between secondary metabolites, flavonoids (i.e., anthocyanins and flavonols) play an important role in the quality and the antioxidant properties of grapes (Torres et al., 2016; Samoticha et al., 2018) and are very responsive to environmental factors such as solar exposure (Blancquaert et al., 2019). Anthocyanin compounds are responsive of the berry color, and flavonols act as a UV shields, contribute to the wine antioxidant capacity, color stability, and hue through co-pigmentation with anthocyanins (Gómez-Míguez et al., 2006). On the other hand, the methoxypyrazines are wine key odorants contributing to their herbaceous characteristics and have been related to unripe berries and poor-quality wines when these are not part of the wine typicity (Roujou de Boubée et al., 2000). Since they can be present in grape berry and wines at high levels, they may have an important sensorial impact on wine quality (Ryona et al., 2008). Among methoxypyrazines, the 3-isobutyl-2-methoxypyrazine (IBMP) is considered the most relevant to wine flavor due to its correlation with the intensity of the bell pepper character of wines (Roujou de Boubée et al., 2000) and its content at harvest seems to be dependent of the solar exposure (Scheiner et al., 2010; Koch et al., 2012; Sivilotti et al., 2016).

The differences found in the literature about the effect of manipulating the canopy architecture on the flavonoid and aromatic content due to different solar exposure of berries in warm climates opens an important field of research. Therefore, we aimed to find the optimal ranges of berry solar exposure estimated as percent of kaempferol (Martínez-Lüscher et al., 2019) for flavonoid synthesis up regulation and the thresholds for their degradation, and to evaluate how canopy management practices such as leaf removal, shoot thinning and a combination of both affect the grapevine yield components, berry composition, flavonoid profile, and herbaceous aromas.

MATERIAL AND METHODS

Plant Material and Experimental Design

Experiment 1: Berry Microclimate Affect Berry Quality and Determines Berry Skin Flavonoid Composition at Harvest

An experiment was performed in 2017 on 7-year Cabernet Sauvignon vines (clone FPS08) grafted onto 110 Richter rootstock (*V. rupestris* × *V. berlandieri*) with NW-SE row orientation and a vine spacing of 2 m × 2.4 m (vine × row) in a commercial vineyard in Oakville, CA (38.427° N 122.410° W). Individual berries were sampled at harvest according to their position in the canopy and overexposure based on visual appearance. Each independent replicate was a sample of 75 berries collected from up to 50 plants each (200 in total), these plants being potentially the same for all exposures. From each sample, 55 berries were used for must analyses and berry mass, and the remaining 20 berries were stored

at -20°C for analyses of flavonoids. Thus, four observational treatments with four replicates consisted in two rows of 25 vines each were established: (i) non-exposed berries collected from interior clusters (Exp-); (ii) exposed but free of signs of overexposure, collected from northeast exposed clusters (Exp+ Deg-); (iii) exposed and with mild signs of sunburn, collected from southwest exposed clusters (Exp+ Deg+); and (iv) exposed and with severe signs of sunburn with signs of damage collected from southwest exposed clusters (Exp+ Deg++). These treatments are provided visually in **Figure 1**.

Experiment 2: Relationship Between Canopy Porosity and Berry Anthocyanin and Flavonol Content in a Commercial Vineyard

In the second experiment, individual grapes from different cluster positions (interior, exposed from the west side of the canopy, exposed from the east side, and overexposed from the

east side) were collected from two cultivars (Cabernet Sauvignon and Petite Verdot) grown in a commercial vineyard in Oakville, CA (38.427°N 122.410°W) in 2017. Cabernet Sauvignon grapevines (FPS clone 04 grafted onto St. George and spaced $1.2\text{ m} \times 1.2\text{ m}$ (vine \times row)) and Petit Verdot grapevines (clone 400-ENTAV-INRA grafted onto 101 to 14 Mgt and spaced $1.8\text{ m} \times 1.2\text{ m}$) were 21 and 9-years old, respectively. The exposure of each individual grape was estimated with fish-eye lens photography from the grape perspective pointing the zenith. The images were processed in R (version 3.2.5-6). After applying a thresholding condition to the blue channel of all images, they were converted into binary pixels (black/white). Thus, the percent of binary pixels capturing the sky was used to calculate the percentage of canopy porosity as reported previously (Martínez-Lüscher et al., 2019). Then, those berries were collected at harvest, and their flavonoid content was analyzed with reversed-phase high performance liquid chromatography.

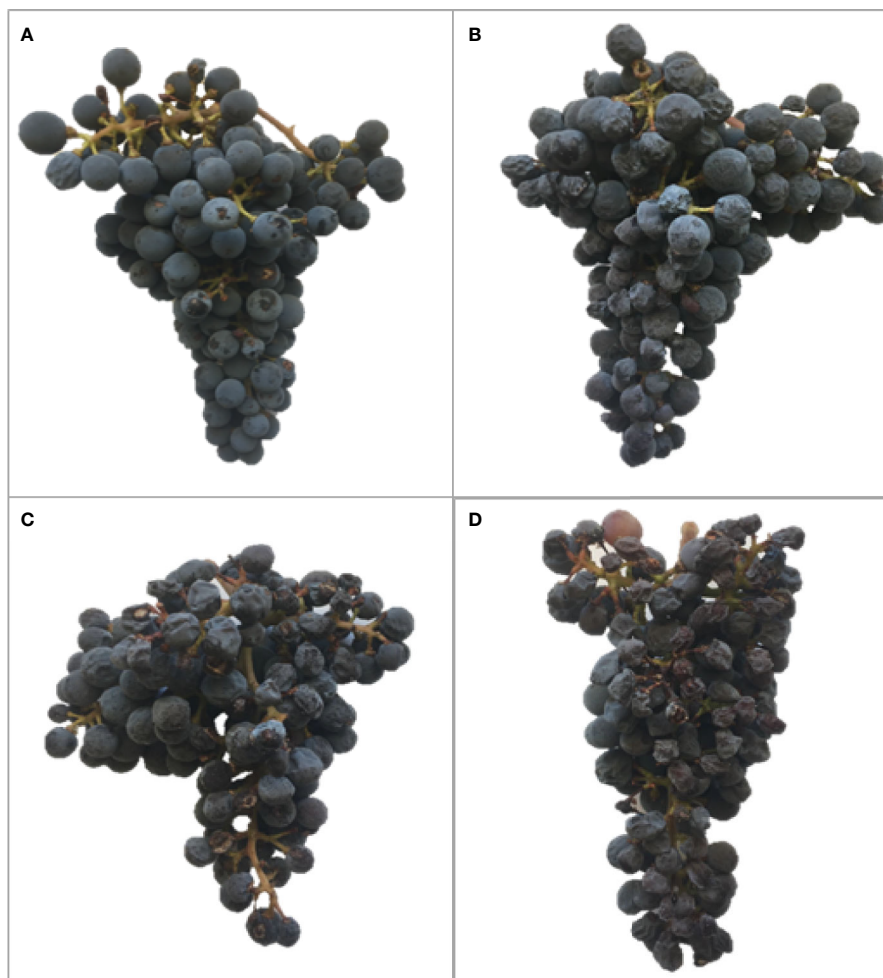


FIGURE 1 | Examples of harvested clusters from Cabernet Sauvignon with different degree of exposure: **(A)** Exp- (Non exposed berries collected from clusters in canopy interior), **(B)** Exp+ Deg- (exposed but not degraded, collected from Northeast exposed clusters), **(C)** Exp+ Deg+ (exposed and degraded, collected from Southwest exposed clusters) and **(D)** Exp+ Deg++ (exposed and very degraded grapes with signs of damage collected from Southwest exposed clusters), collected in Oakville, CA in 2017.

Figure S1 shows that the % of kaempferol was a good estimator of the canopy porosity as we previously reported in Martínez-Lüscher et al. (2019).

Experiment 3: Response of Berry Chemistry, Flavonoid Metabolism, and Methoxypyrazine Degradation During Berry Ripening to Canopy Management Practices

The experiment was conducted in 2019 in Oakville, CA (38.428 N, 122.409 W) with row orientation NW-SE. The vineyard was spaced 2 m × 2.4 m with Cabernet Sauvignon grapevines (clone FPS08) on 110R rootstock. The grapevines were trained to a vertically shoot-positioned system with a cordon height 96 cm above vineyard floor, trained to a bilateral cordon, and pruned to 1-bud spurs. Plants were irrigated weekly with 2-drip emitters per vine, with the capacity to deliver 3.8 L of water per hour. The experiment was designed as a randomized complete block with three canopy management practices: (i) removal of 5 to 6 basal leaves on the NE side (LR); (ii) thinned to 24 shoots per vine (ST); and (iii) a combination of LR and ST (LRST) and an untreated control (no shoot thinning or leaf removal, UNT), with four replicates each consisting in 5 grapevines, 3 of which were sampled and the 2 on distal ends were treated as border plants. The ST and LR treatments were applied on 11 June 2019. Harvest commenced when the berry TSS reached to *ca.* 24°Brix on 23 September (112 DAF). The sampling time points were as follows: 2 weeks before veraison (11 July), veraison (1 August), 2 weeks after veraison (15 August), 3 weeks after veraison (20 August), 5 weeks after

veraison (3 September), and harvest (23 September), were chosen to cover the response of the berry metabolism to cultural practices and the concomitant increase in exposure.

Weather Conditions

Weather data (Table 1) were obtained from the California Irrigation Management Information System, CIMIS, station (#77, Oakville, CA) located on site during the growing seasons covered by the experiments and the reference period 1999 to 2019 (California Department of Water Resources, 2020). Number of days with temperatures above 30°C were counted for the 2017 and 2019 growing seasons.

Canopy Architecture, Yield Components, and Labor Operations Costs of Experiment 3

Leaf area index (LAI) was measured on 21 June to characterize grapevine canopy growth and converted into leaf area on by a smartphone based program, VitiCanopy, coupled with an iOS system (Apple Inc., Cupertino, CA, USA) (De Bei et al., 2016). The gap fraction threshold was set to 0.75, extinction coefficient was set to 0.7, and sub-divisions were 25. A “selfie-stick” was used for an easy access to place the device about 75 cm underneath the canopy. The device was positioned with the maximum length of the screen being perpendicular to the cordon, and the cordon being in the middle of the screen according to previous work (De Bei et al., 2016; Yu and Kurtural, 2020). In each experimental unit, three images were taken to capture half canopy of each vine, and analyzed by the software. The relationship between leaf dry mass and area was

TABLE 1 | Weather conditions during the growing seasons of 2017, 2019 and the average for the same period in the last 20 years (1999–2019).

Month	April	May	June	July	August	September	October	
Year	Mean daily temperature (°C)							Mean
2017	20.6	22.9	18.6	17.8	22.2	29.6	18.8	21.5
2019	12.9	15.3	17.3	17.2	15.4	22.6	12.5	16.2
1999–2019	13.5	16.1	18.9	19.6	19.2	18.5	15.7	17.3
	Solar radiation (W m ⁻²)							Total
2017	195	263	289	299	247	201	159	1653
2019	246	270	342	330	288	251	203	1930
1999–2019	225	271	306	309	273	227	161	1772
	Precipitation (mm)							Total
2017	85.3	0.0	7.4	0.0	0.0	1.0	4.6	98.3
2019	12.5	88.9	0.0	0.2	0.0	1.5	0.2	103.3
1999–2019	44.3	26.2	5.3	0.2	0.0	2.1	37.9	116.1
	Minimum daily temperature (°C)							Mean
2017	6.8	8.2	10.7	10.8	12.2	11.3	5.7	9.4
2019	8.8	8.4	11.2	11.1	12.3	9.7	4.9	9.5
1999–2019	6.5	8.2	9.9	11.0	10.9	9.4	7.5	9.1
	Maximum daily temperature (°C)							Mean
2017	21.5	26.3	29.3	31.3	30.5	30.4	27.9	28.2
2019	23.3	22.4	29.2	29.9	31.2	29.4	26.6	27.4
1999–2019	21.3	24.5	28.2	29.6	29.6	29.3	25.6	26.9
	Days with temperature over 30 °C (no)							Total
2017	0	1	10	13	16	11	13	64
2019	0	3	0	11	12	18	13	57

Weather data were obtained from the CIMIS weather station #77 (Oakville, CA) located at the research site.

determined on a subsample of leaves of different sizes using a leaf area meter (Li-Cor 3300, Lincoln, NE USA). Total leaf area was calculated by defoliating one grapevine per treatment replicate after harvest and using the regressive relationship between leaf dry mass and leaf area. At harvest, clusters were manually removed, counted, and weighed on a top-loading balance. Leaf area to fruit ratio was calculated by dividing leaf area with crop weight. Dormant pruning weight was collected during the dormant season (16 December); and crop load was calculated as the ratio between yield per vine (kg) and the pruning mass (kg) of each vine. Labor operations costs and gross income per hectare were calculated based on yield and net returns per hectare (California Department of Food and Agriculture, 2020; Kurtural et al., 2020) and methods presented elsewhere (Kurtural et al., 2019). Anthocyanin productivity (unit cost to produce anthocyanin) was calculated as reported by Cook et al. (2015).

Berry Mass and Chemistry

At each sampling point and experiment, 55 berries were randomly collected from the middle of each treatment-replicate and kept on ice until they were measured. Berries were weighed, and mean berry mass was determined as the average mass of the counted berries. These berries were used to determine the total soluble solids (TSS), the pH, and the titratable acidity (TA). TSS was measured as °Brix, with a digital refractometer (Atago PR-32 Palette digital refractometer, ATAGO USA, Bellevue, WA, United States). The juice pH and TA was determined with an autotitrator (862 Compact Titrosampler, Herisau, Switzerland) using sodium hydroxide to titrate to an end point of pH 8.3, and it was expressed as $\text{g}\cdot\text{L}^{-1}$ of tartaric acid.

Berry Flavonoid Content and Composition

For each sampling point in each experiment, 20 berries were collected, gently peeled, and berry skins were freeze-dried (Cold Trap 7385020, Labconco, Kansas City, MO, United States). Dried tissues were ground with a tissue lyser (MM400, Retsch, Germany). Fifty mg of the resultant powder was extracted in methanol: water: 7 M hydrochloric acid (70:29:1, V/V/V) to simultaneously determine flavonol and anthocyanin concentration and profile as previously described Martínez-Lüscher et al. (2019). Briefly, extracts were filtered (0.45 μm , Thermo Fisher Scientific, San Jose, CA, United States) and analyzed using an Agilent 1260 series reversed-phase high performance liquid chromatography (HPLC) system (Agilent 1260, Santa Clara, CA, United States) coupled to a diode array detector. Separation was performed on a reversed-phase C18 column LiChrospher® 100, 250 mm \times 4 mm with a 5- μm particle size and a 4-mm guard column of the same material at 25°C with elution at 0.5 ml per minute. The mobile phase was designed to avoid co-elution of anthocyanins and flavonols (Martínez-Lüscher et al., 2019) consisted in a constant 5% of acetic acid and the following gradient (v/v) of acetonitrile in water: 0 min 8%, at 25 min 12.2%, at 35 min 16.9, at 70 min 35.7%, 65% between 70 and 75 min, and 8% between 80 and 90 min. The identification of flavonoid compounds was conducted by determining the peak area of the absorbance at 280, 365, and 520 nm for flavan-3-ols,

flavonols and anthocyanins, respectively. Identification of individual flavan-3-ols, anthocyanins, and flavonols were made by comparison of the commercial standard retention times found in the literature. Commercial standards of epicatechin, malvidin-3-O-glucoside, and quercetin-3-O-glucoside (Sigma-Aldrich, St. Louis, MO) were used for the quantification of flavan-3-ols, anthocyanins, and flavonols, respectively. The determination of proanthocyanidins was performed using an Agilent HPLC-DAD (1100 series, Agilent, Santa Clara, CA) after an acid catalysis in the presence of excess phloroglucinol (Kennedy and Jones, 2001), with minor modifications described in Martínez-Lüscher et al. (2017).

Quantification of 3-Isobutyl-2-Methoxypyrazine With GC-MS

The 3-isobutyl-2-methoxypyrazine (IBMP) was quantified by a stable isotope dilution assay (SIDA) using headspace solid phase microextraction coupled to a gas chromatograph and a mass spectrometer (HS-SPME-GC-MS) as described Chapman et al. (2004) and Koch et al. (2010) with some modifications. Briefly, 20 berries per treatment-replicate from Experiment 3 were randomly collected from the clusters of three vines in the middle of each treatment-replicate on both side of the canopy, by cutting the pedicel with a pair of scissors and frozen at -80°C until analysis. Pedicels were removed by hand and berries were placed in 50 ml conical tubes. 10 ml of pure water and 100 μl of deuterated ($[\text{D}_3]$) IBMP isotope ($5 \text{ pL}\cdot\text{L}^{-1}$) were added into the tube. Then, samples were ground with a tissue homogenizer Power Gen 1800D (Fisher Scientific, PA-USA) and centrifuged at 3000 rpm for 10 min. 10 ml of the supernatant was pipetted into 20 ml SPME vials containing 3 g of sodium chloride.

Samples were analyzed with an Agilent 6890N gas chromatograph equipped with a split/splitless injector coupled to a 5973 mass selective detector (MSD) (Agilent Technologies, Santa Clara, CA, USA). A Gerstel MPS2 autosampler (Gerstel Inc., Columbia, MD) and a HP 5MS capillary column (30 mm \times 0.25 mm and 0.25 film thickness) were used for head space (HS) sampling. Then, HS samples were exposed to a 23-gauge, 2 cm divinylbenzene/carboxen/polymethylsiloxane (DVB/CARB/PDMS) SPME fiber for 30 min at 40°C with continuous agitation for extraction. SPME fiber was desorbed at 260°C in splitless for 5 min into the GC-MS inlet with a 0.7 mm straight glass liner. Inlet flow was set to $50 \text{ mL}\cdot\text{min}^{-1}$ for another 5 min. Oven temperature was held at 40°C for 5 min, then ramped $2.5^{\circ}\text{C}\cdot\text{min}^{-1}$ up to 80°C , $5^{\circ}\text{C}\cdot\text{min}^{-1}$ up to 110°C , $25^{\circ}\text{C}\cdot\text{min}^{-1}$ up to 230°C and finally kept steady at 230°C for 5 min. The MSD interface was kept at 280°C and the carrier gas was Helium at a constant pressure of 4.77 psi with an initial flow of $0.8 \text{ mL}\cdot\text{min}^{-1}$. Selected ion monitoring was used at mass of $m/z=124$ for IBMP and $m/z=127$ for $[\text{D}_3]\text{IBMP}$.

Statistical Analysis

Statistical analyses were carried out using the R-Studio version 3.6.1 (RStudio: Integrated Development for R, Boston, MA, USA) for Windows. All data were subjected to Shapiro-Wilk's

normality test (Royston, 1995). Correlations between variables were calculated with the Pearson's test by using the same software. Segmented regression analysis was used to determine the point of inflection in the relationship between increasing exposure (percent kaempferol as described in Martínez-Lüscher et al. (2019)) and the berry skin anthocyanin and flavonol content with “segmented” 0.5-0.3 R package (Muggeo, 2008). Data were normally distributed and, subsequently, were submitted to an analysis of variance (ANOVA) to assess the statistical differences between the treatments applied in each experiment performed. Means \pm standard errors (SE) were calculated, and when the F value was significant ($P \leq 0.05$), a Duncan's new multiple range *post hoc* test was executed using “agricolae” 1.2-8 R package (de Mendiburu, 2016). When data were not normally distributed, a Kruskal-Wallis test was conducted. Percentage data were transformed according to the suggestion of the most likelihood test, into arcsine root square before ANOVA or Kruskal-Wallis tests.

RESULTS

Effect of Different Solar Exposure on Berry Mass, Must Composition and Berry Skin Flavonoids

The growing season of 2017 was warmer and drier compared to the reference data for the same period within the last 20 years (Table 1). Thereby, average daily temperature was 4°C higher and rainfall was 18 mm less. Grape berry mass differed significantly depending on the degree of exposure (Table 2). Overexposed berries (Exp+ Deg+ and Exp+ Deg++) were the smallest due to overexposure resulting in dehydration thereby reducing berry mass. Neither total soluble solids nor titratable acidity changed regardless of the degree of exposure to which berries were subjected. However, the juice pH of the Exp+ Deg+

and Exp+ Deg++ berry must was greater ($p=0.022$) compared to Exp- and Exp+ Deg- berries.

Berry skin flavonoid content and composition were also affected by the degree of exposure (Table 2). The berry anthocyanin content of Exp- was similar to Exp+ Deg-. However, overexposed berries resulted in berry anthocyanin content that was 70% and 90% lower when compared to the Exp- berries. Grape berry exposure to solar radiation not only affected the anthocyanin content but also modified the ratio between the tri- and di-substituted anthocyanins leading to a less stable profile in all treatments with exposed berries. Likewise, berry skin flavonol content and composition were strongly affected by the degree of exposure to solar radiation. Therefore, in Exp+ Deg- flavonol content was two-fold greater than Exp-, albeit they abruptly decreased in overexposed grapes (Exp+ Deg+ and Exp+ Deg++) where flavonol content was 25% and 50% lower when compared to Exp- berries. Furthermore, in overexposed berries the proportion of kaempferol and quercetin significantly increased while the proportion of myricetin decreased.

Regarding proanthocyanidins in berries, mild exposure did not affect their content in Exp+ Deg- compared to Exp- berries. However, greater solar exposure (Exp+ Deg+ and Exp+ Deg++) decreased proanthocyanidin content in berries but to a lesser extent compared to Exp- (45% and 60%, respectively). Finally, the content of flavan-3-ols was severely reduced in Exp+ Deg++ berries (47% lower than the flavan-3-ol content in Exp- berries).

Assessing the Canopy Porosity Threshold for Optimum Berry Flavonoid Content

The analyses performed on single berries from two varieties confirmed the obtained response in anthocyanins and flavonols in Cabernet Sauvignon (Figure 2, Table S1). Thus, exposure affected the accumulation/degradation of these flavonoids. Exposed berries from the East side of the canopy decreased 8%

TABLE 2 | Effect of different degree of exposure on the berry mass and composition, and berry skin flavonoid profile of Cabernet sauvignon grapevine berry at harvest: i) Exp- (non-exposed berries collected from clusters in canopy interior), ii) Exp+ Deg- (exposed but not degraded, collected from Northeast exposed clusters), iii) Exp+ Deg+ (exposed and degraded, collected from Southwest exposed clusters), and iv) Exp+ Deg++ (exposed and very degraded grapes with signs of damage collected from Southwest exposed clusters) and collected in Oakville, CA in 2017.

	Degree of exposure				ANOVA
	Exp-	Exp+ Deg-	Exp+ Deg+	Exp+ Deg++	P value
Berry mass (g)	1.14 \pm 0.04 a	1.18 \pm 0.03 a	1.00 \pm 0.03 b	0.86 \pm 0.03 c	<0.0001
pH	3.40 \pm 0.01 b	3.42 \pm 0.01 b	3.55 \pm 0.03 a	3.51 \pm 0.06 ab	0.022
Titratable acidity (g•L ⁻¹)	7.98 \pm 0.11	7.93 \pm 0.14	7.40 \pm 0.33	7.70 \pm 0.51	ns
TSS (°Brix)	24.35 \pm 0.4	23.63 \pm 0.21	24.98 \pm 0.76	25.03 \pm 0.71	ns
Total anthocyanins (mg•berry ⁻¹)	2.23 \pm 0.04 a	2.11 \pm 0.11 a	0.63 \pm 0.09 b	0.27 \pm 0.02 c	<0.0001
Ratio 3'4'5'/3'4'	10.91 \pm 0.38 a	9.30 \pm 0.38 b	9.51 \pm 0.29 b	7.74 \pm 0.18 c	<0.0001
Total flavonols (mg•berry ⁻¹)	0.106 \pm 0.006 b	0.196 \pm 0.008 a	0.081 \pm 0.008 c	0.054 \pm 0.003 d	<0.0001
% Kaempferol	5.80 \pm 0.47 c	9.08 \pm 0.59 b	11.53 \pm 0.55 a	13.23 \pm 0.56 a	<0.0001
% Myricetin	35.71 \pm 0.55 a	28.98 \pm 0.92 b	20.97 \pm 1.74 c	12.95 \pm 0.86 d	<0.0001
% Quercetin	42.33 \pm 0.76 d	49.24 \pm 0.73 c	53.37 \pm 1.59 b	61.56 \pm 0.91 a	<0.0001
Total proanthocyanidins (mg•berry ⁻¹)	4.58 \pm 0.1 a	4.98 \pm 0.27 a	2.48 \pm 0.18 b	1.94 \pm 0.23 b	<0.0001
Total flavan-3-ols (mg•berry ⁻¹)	0.017 \pm 0.001 a	0.016 \pm 0.001 A	0.011 \pm 0.000 b	0.009 \pm 0.001 b	<0.0001

Values represent means separated by Duncan's new multiple range test (at $P = 0.05$). Within columns, means followed by different letters are significantly different as affected by the degree of exposure. ns, not significant ($P > 0.05$).

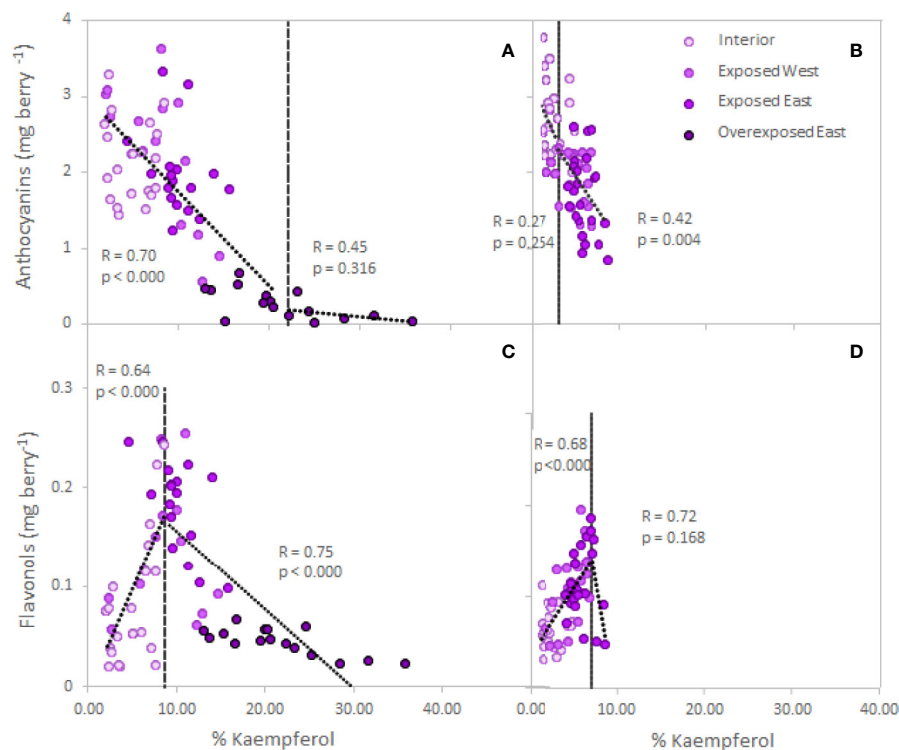


FIGURE 2 | Relationship between grape skin anthocyanin (A, B) and flavonol (C, D) content (mg per berry) and increasing exposure (% of kaempferol, Martínez-Lüscher et al., 2019) in Cabernet Sauvignon (A, C) and Petit Verdot (B, D) single berries collected from the cluster interior (Exp-), exposed West (Exp+ Deg-), exposed East (Exp+ Deg+) and overexposed East (Exp+ Deg++). Grey dashed lines are the breaking points determined through segmented regression.

and 36% of the anthocyanin content in Cabernet Sauvignon and Petit Verdot, respectively. Thus, Petit Verdot seemed to be more sensitive to higher level of solar exposure and degraded anthocyanins. Overexposed berries of Cabernet Sauvignon resulted in an 87% decrease of the berry skin anthocyanins when compared to the interior berries (Table S1). Berry skin anthocyanins and increasing exposure showed a significant trend below the 22% of kaempferol (Figure 2A). Conversely, analysis of the segmented regression on Petit Verdot berries did not show a clear trend below the 3.2% of Kaempferol and after the point of inflection, anthocyanins started to degrade (Figure 2B). Regarding flavonol content, no differences were observed between cultivars (cultivar, $p = 0.978$, Table S1). Conversely, when exposure increased to ca. 60% the content of flavonols in exposed berries of both canopy sides and in both cultivars; the overexposed berries had the lowest flavonol content (Table S1). Thus, our data revealed a strong positive relationship between the berry skin flavonols and the percentage of kaempferol until 8.6% of kaempferol proportion for Cabernet Sauvignon ($R = 0.64$, $p < 0.0001$) and 7.2% Petit Verdot ($R = 0.68$, $p < 0.0001$) (Figures 2C, D). However, beyond these thresholds, flavonols started to degrade, and there was an indirect relationship between the flavonol content and the percentage of kaempferol for both cultivars, this relationship being significant only for Cabernet Sauvignon (Figures 2C, D).

Different Solar Exposure Driven by Canopy Management Affects Grapevine Performance and Berry Quality

The weather conditions during the execution of this experiment were highlighted by greater maximum daily temperatures when compared to the reference period (1999–2019). This was more prominent during the driest months (Table 1). Moreover, global solar radiation received at the experimental site was to ca. 200 W m⁻² greater than the total solar radiation recorded within the reference period (Table 1).

The LR and ST decreased leaf area index (LAI) and increased canopy porosity. The combinatory effect of LR and LT treatments caused a 58% reduction of LAI and a 45% increase of canopy porosity (Table 3). However, neither leaf area nor pruning mass showed significant differences between treatments. On the other hand, yield components were mostly affected by the shoot thinning treatments (Table 3). Thus, shoot thinned vines showed lower number of clusters, yield, and Ravaz Index (RI), and increased leaf area to fruit ratio per vine as expected. The extent of yield reductions was 55% and 47% for ST and LRST vines, respectively (Tables 3 and 4).

Berry mass was not significantly affected by canopy management practices during the berry ripening although vines subjected to LRST tended to result in smaller berries (Figure 3A). The most influential effects observed on berry

TABLE 3 | Effect of the canopy management practices untreated control (UNT), leaf removal (LR), shoot thinning (ST), and a combination of both (LRST) on the canopy architecture, and yield components of Cabernet sauvignon grown in Oakville, CA in 2019.

	Cultural practice				ANOVA
	UNT	LR	ST	LRST	p value
<i>Canopy architecture</i>					
LAI	1.68 ± 0.078 a	1.32 ± 0.152 b	1.18 ± 0.076 b	0.98 ± 0.113 c	<0.0001
Percent canopy porosity	21.0 ± 1.4 c	24.6 ± 1.7 b	26.8 ± 1.7 b	30.4 ± 1.4 a	<0.0001
Leaf area (m ² ·vine ⁻¹)	5.70 ± 0.93	4.82 ± 0.49	4.76 ± 0.68	4.13 ± 0.81	ns
Pruning mass (kg·vine ⁻¹)	1.38 ± 0.12	1.26 ± 0.14	1.08 ± 0.10	1.00 ± 0.12	ns
<i>Yield components</i>					
Clusters per vine	107 ± 10 a	95 ± 19 a	48 ± 2 b	44 ± 1 b	<0.0001
Cluster mass (g)	87.37 ± 3.91	86.83 ± 9.21	107.33 ± 16.08	99.56 ± 13.47	ns
Yield (kg·vine ⁻¹)	9.36 ± 0.61 a	8.63 ± 1.55 a	5.12 ± 0.88 b	4.43 ± 0.58 b	<0.0001
Leaf area to fruit ratio (m ² ·kg ⁻¹)	0.60 ± 0.03 b	0.57 ± 0.04 b	0.93 ± 0.02 a	0.93 ± 0.07 a	<0.0001
Ravaz Index (RI) (kg·kg ⁻¹)	6.87 ± 0.77 a	6.90 ± 0.68 a	4.76 ± 0.18 b	4.52 ± 0.67 b	<0.0001

Values represent means separated by Duncan's new multiple range test (at $P = 0.05$). Within columns, means followed by different letters are significantly different as affected by the canopy management practices of leaf removal and shoot thinning and their interactions. ns, not significant ($P > 0.05$).

TABLE 4 | Cost estimates on labor operations costs of canopy management practices and cost to produce one unit of anthocyanin and IBMP of Cabernet Sauvignon grapevine subjected to untreated control (UNT), leaf removal (LR), shoot thinning (ST), and a combination of both (LRST) in Oakville, CA in 2019 (Kultural et al., 2020).

	Cultural practice				ANOVA
	UNT	LR	ST	LRST	P value
<i>Cultural practices labor operation cost (\$/Ha)</i>					
Dormant pruning	1,336.27	1,336.27	1,336.27	1,336.27	–
Shoot thinning	0	0	738.53	738.53	–
Leaf removal	0	2,067.39	0	2,067.39	–
Total	1,336.27	3,403.66	2,074.8	4,142.19	–
Yield (Mg/Ha)	19.5 ± 0.64 a	17.97 ± 1.62 a	10.66 ± 0.92 b	9.23 ± 0.61 b	<0.0001
Gross income (\$/Ha)	\$170,702	\$157,308	\$93,317	\$80,798	–
Anthocyanin productivity (\$/kg)	29.69 ± 1.78 c	92.09 ± 7.57 b	92.10 ± 7.57 b	223.42 ± 0.42 a	<0.0001
IBMP productivity (\$/μg)	15.80 ± 0.45 b	42.37 ± 5.76 b	53.41 ± 12.55 b	180.74 ± 26.69 a	<0.0001

Values represent means separated by Duncan's new multiple range test (at $p = 0.05$). Within columns, means followed by different letters are significantly different as affected by the canopy management practices of leaf removal and shoot thinning and their interactions.

chemistry were due to shoot thinning treatments (Figure 3). Therefore, shoot thinned vines had greater total soluble solids and lower titratable acidity from mid-ripening to harvest. However, no significant effect was observed on the must pH (Figures 3B–D).

Shoot thinned grapevines had higher anthocyanin content at veraison (Figure 4A). However, we did not measure any changes to anthocyanin content at harvest as affected by the canopy management practices applied. Although anthocyanin content was not affected, anthocyanin composition was modified by treatments from mid-ripening to harvest (Figure 4B–F). Berry skins of ST and LRST grapevines showed a lower 3'4'5'/3'4' ratio leading to increased proportion of cyanidins and peonidins (Figures 4C, E) in detriment of malvidins which was the most abundant anthocyanin found in berry skins (Figure 4B). During the monitored period, different canopy management practices modified berry flavonol content (Figure 5A). The berries from LRST grapevines showed the greatest berry skin flavonol content, while, at harvest, the flavonol content of LR, ST, and LRST was similar and greater when compared to the UNT content. Not only canopy management practices modified flavonol content but they also affected their composition. The LRST treatment

had a higher proportion of kaempferol and quercetin from mid-ripening to harvest (Figures 5B, C) and lower of myricetin after veraison (Figure 5D).

As expected, berry IBMP content decreased throughout ripening with all the canopy management practices tested in this study (Figure 6). However, we found the significant differences among treatments after veraison and at harvest. The LRST treatment resulted in the lowest IBMP content from mid-ripening to harvest.

Correlation analysis between the monitored variables at harvest revealed a strong relationship between canopy architecture variables (LAI and canopy porosity) and berry flavonol content (Figure 7). Moreover, canopy porosity was strongly correlated to the kaempferol proportion in berry skins ($r = 0.75$, $p = 0.001$). On the other hand, a lower yield due to canopy management practices was related to decreased IBMP and increased flavonol content ($r = 0.56$, $p = 0.025$ and $r = -0.61$, $p = 0.012$, respectively). Finally, a strong relationship was found between TSS and TA with the leaf to fruit ratio ($r = 0.81$, $p < 0.001$ and $r = -0.62$, $p = 0.011$). Finally, a higher solar exposure estimated as the kaempferol proportion was strongly correlated with decreased anthocyanin berry contents ($r = -0.69$, $p = 0.003$) and yield ($r = -0.69$, $p = 0.002$).

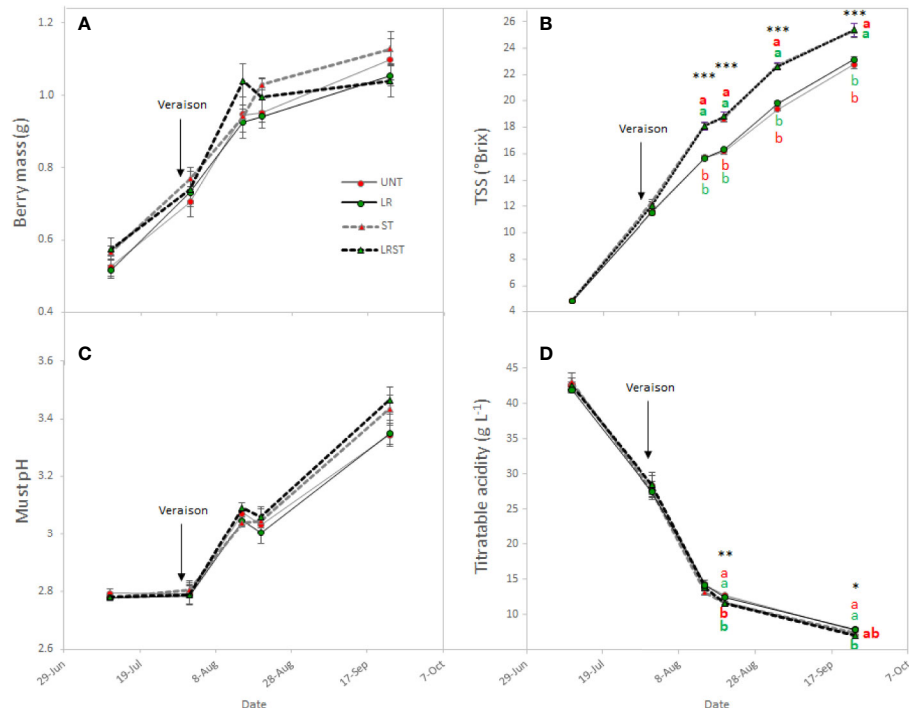


FIGURE 3 | Effect of canopy management practices (UNT, untreated; LR, leaf removal; ST, shoot thinning and LRST, LR and ST combined) on berry mass (A) Brix (B), must pH (C), and titratable acidity (D) the growing season. Values represent means \pm SE ($n = 4$). At each time point, different letters indicate significant differences ($p < 0.05$) between canopy management practices according to the one-way ANOVA followed by Duncan's new multiple range test. *, **, and *** indicate significance at 5%, 1%, and 0.1% probability levels, respectively.

Analysis of labor operations cost of canopy management practices indicated that the most expensive canopy management practices was the LRST (Table 4) where growers received a 53% lower income per hectare. Thereby, productivity data provided evidence that the cost of producing a kg of anthocyanin and removing a μg of IBMP was 10-fold greater in LRST compared to UNT per ha (Table 4).

DISCUSSION

Effects of Canopy Management Practices on Canopy Architecture and Yield Components

Yield components were mainly affected by shoot thinning practices, decreasing the number of clusters and yield per vine leading to unbalanced vines ($\text{RI} < 5$) according to the previous studies (Sun et al., 2012; Jogaiah et al., 2013). Yield per meter of row is increased quasilinearly with the increase in shoot density per meter of row as indicated by previous studies (Terry and Kurtural, 2011; Geller and Kurtural, 2013). The lack of effect of LR on yield was corroborated by several studies (Pastore et al., 2013; Mijowska et al., 2016; Yu et al., 2016) when a late leaf removal was applied. Moreover, Yu et al. (2016) and Cook et al. (2015) reported that grapevines may produce more leaves than

required, especially in warm climates, therefore, the increase in canopy gaps and the diminution of external leaf layers did not elicit decreases in yield as they were not severe enough reductions to the functional leaf area. The RI between 5 and 10 is considered optimum for vine balance (Bravdo et al., 1985; Terry and Kurtural, 2011). Therefore, RI and leaf area to fruit ratio data reported with the grapevines subjected to shoot thinning (ST and LRST) were under cropped that led to lower yields.

In our study, Cabernet Sauvignon vines were not able to modulate their vegetative biomass in response to canopy management practices applied. Previous studies showed that pruning mass values up to 1 kg/m of row were considered optimal under warm climate (Terry and Kurtural, 2011). In our experiment the pruning mass per meter of all treatments ranged from 0.5 (in LRST) to 0.7 (in UNT) kg/m without differences between treatments. Moreover, although the shoot counts were obviously different between treatments, we did not find differences in the pruning mass, that suggested lower lateral expansion and/or reduced shoot diameter with an increasing number of shoots as previously reported Brillante et al. (2018). Consequently, we found that the mass of each shoot ranged from 28 and 25 g in UNT and LR, respectively, to 45 and 42 g in ST and LRST, respectively, corroborating work by Brillante et al. (2018).

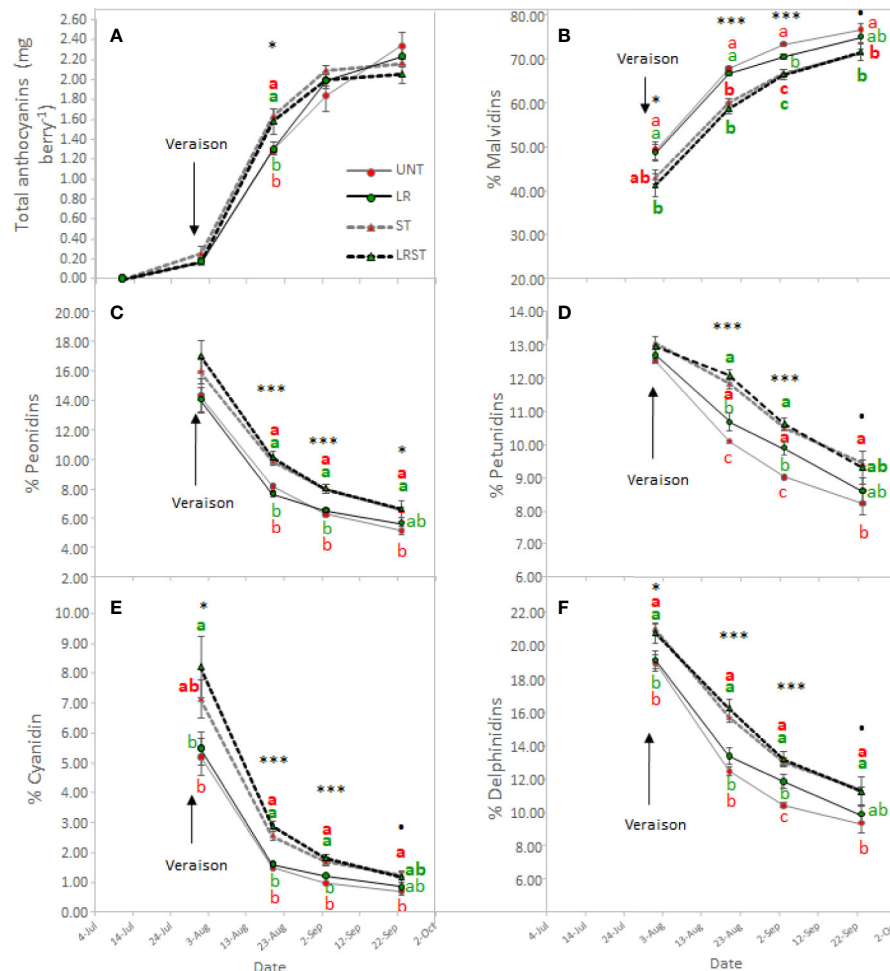


FIGURE 4 | Effect of canopy management practices (UNT, Untreated; LR, Leaf removal; ST, Shoot thinning; LRST, LR and ST combined) on berry skin anthocyanin content (A), percent Malvidin (B), percent Peonidin (C), percent Petunidin (D), percent Cyanidin (E) and percent Delphinidin (F) during the growing season. Values represent means \pm SE ($n = 4$). At each time point, different letters indicate significant differences ($p < 0.05$) between canopy management practices according to the one-way ANOVA followed by Duncan's new multiple range test. *, **, and *** indicate significance at 10%, 5%, and 0.1% probability levels, respectively.

Effects of the Cluster Microclimate on the Physico-Chemical Attributes of Berries

Martínez-Lüscher et al. (2017) reported negligible variation of berry mass of Cabernet Sauvignon due to higher solar exposure under irrigated viticulture. Similarly, berry masses remained unaffected by a higher solar exposure of the cluster due to canopy management practices unless they were directly exposed to sunlight where berries may suffer dehydration as previously reported by Mijowska et al. (2016). This has been attributed to the effect of the higher temperatures with subsequent increases in berry transpiration that affected cell division and elongation (reviewed by Uhlig, 1998).

Under our experimental conditions, shoot thinning treatments hastened berry ripening by enhancing the TSS to *ca.* 2.5°Brix and decreasing must titratable acidity by 0.6 g·L⁻¹ at harvest. Thus, overexposure has been related with higher pH due to the elevated temperature that berries overcome and the

subsequent organic acid degradation (Sweetman et al., 2014). Nevertheless, Wang et al. (2019) recently suggested that changes on the source-to-sink ratio induced by shoot thinning might have more influence on berry maturity than the change in the microclimate (higher light interception and canopy porosity) they reported.

Effects of the Cluster Microclimate on the Berry Flavonoid Content and Profile and IBMP Content

Cultural practices have been related to increased anthocyanin content (Diago et al., 2012; Gatti et al., 2012; Bubola et al., 2017). However, in agreement with other studies (Sivilotti et al., 2016; Pastore et al., 2017), under our experimental conditions, berry anthocyanin content did not increase due to LR, ST or LRST. Similarly, anthocyanin content was not affected by mild-exposure in berries collected from the commercial vineyard

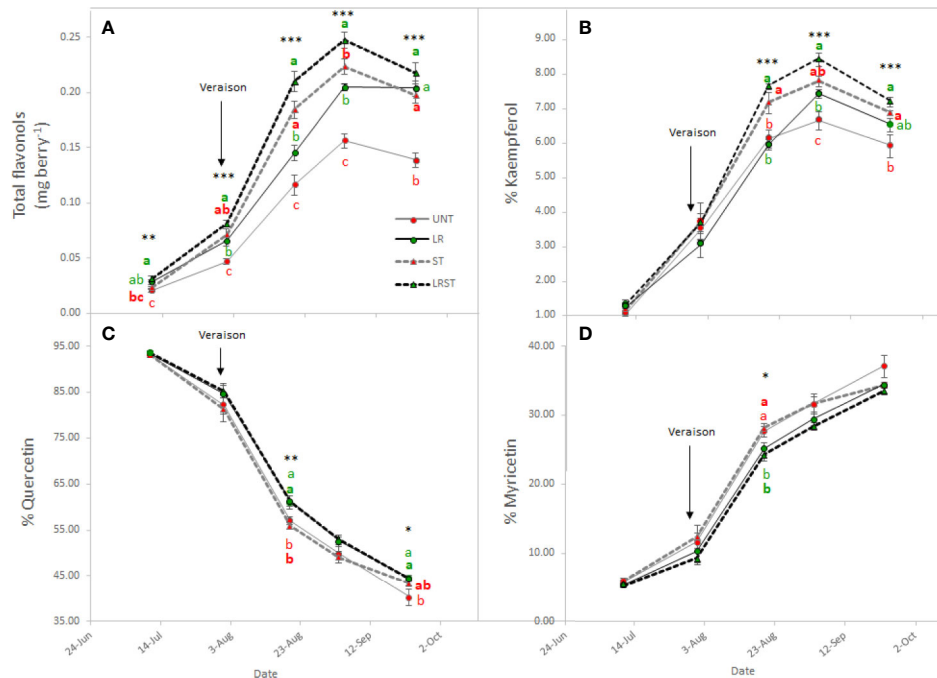


FIGURE 5 | Effect of canopy management practices (UNT: Untreated, LR: Leaf removal, ST: Shoot thinning and LRST: LR and ST combined) on berry skin flavonol content (A), percent Kaempferol (B), percent Quercetin (C), and percent Myricetin (D) during the growing season. Values represent means \pm SE ($n = 4$). At each time point, different letters indicate significant differences ($p < 0.05$) between canopy management practices according to the one-way ANOVA followed by Duncan's new multiple range test. *, **, and *** indicate significance at 5%, 1%, and 0.1% probability levels, respectively.

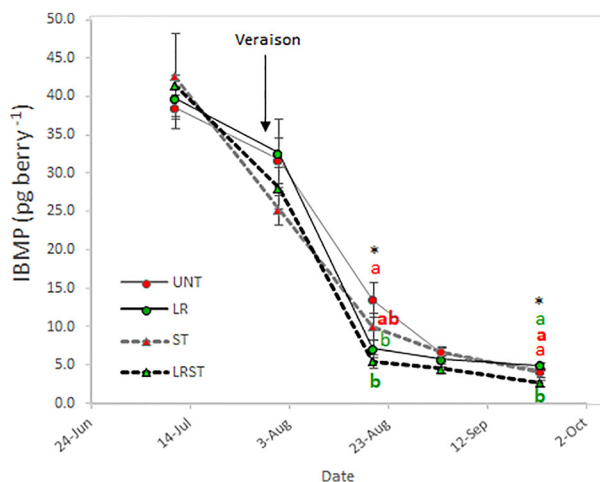


FIGURE 6 | Effect of canopy management practices (UNT: Untreated, LR: Leaf removal, ST: Shoot thinning and LRST: LR and ST combined) on berry IBMP content during the growing season. Values represent means \pm SE ($n = 4$). At each time point, different letters indicate significant differences ($p < 0.05$) between canopy management practices according to the one-way ANOVA followed by Duncan's new multiple range test. * indicate significance at 5% probability level.

either. Increasing exposure was detrimental for anthocyanin content as the overexposed berries were subjected to higher temperatures that may have impaired their accumulation (Martínez-Lüscher et al., 2017). The anthocyanin berry content at harvest is the result between synthesis and degradation rates. It was reported anthocyanin synthesis may be up-regulated by greater exposure (Matus et al., 2009). Therefore, ST and LRST increased the anthocyanin content at mid-ripening because of the increasing solar exposure (higher kaempferol proportion). Additionally, it was recently highlighted that some members of the dihydroflavonol reductase and UFGT genes required for anthocyanin biosynthesis were moderately up-regulated in LR treated berries leading to increases of anthocyanin content at mid-ripening (Sun et al., 2017). However, at harvest, no significant effect of canopy management practices on anthocyanin content was found, and this result is corroborated by Pastore et al. (2017) who reported no beneficial effect due to higher cluster exposure in warm climates. Although cultural practices may induce different cluster temperatures by increasing exposure, we did not find a clear relationship between exposure (% of kaempferol) and cluster temperature when kaempferol proportion are low (Figure S2) suggesting that results of this work were mainly explained by different exposures. Nevertheless, under elevated temperatures, a down-regulation of anthocyanin biosynthesis and enhanced rates of degradation have been

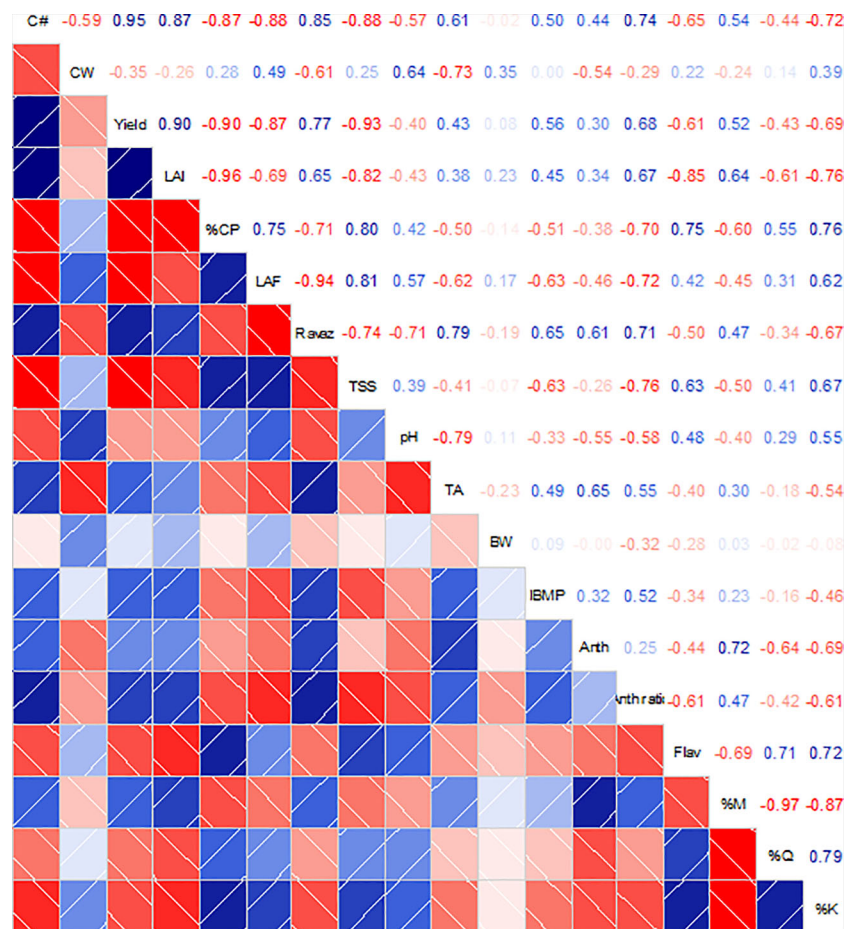


FIGURE 7 | Correlation matrices among grapevine canopy architecture, yield components, berry mass and must compositions and flavonoid content and profile from Cabernet Sauvignon grapevines subjected to different canopy management practices (UNT, untreated; LR, Leaf removal; ST, Shoot thinning; LRST, LR and ST combined) at harvest. Upper panel shows the R values for the Pearson's correlation analysis. Intensity of blue or red colors in the upper and lower panels represents the significance of the relationship between variables. White lines in lower panel represent the regression curves for each pair of variables. C#, cluster number; CW, cluster mass; LAI, Leaf Area Index; %CP, % canopy porosity; LAF, leaf area to fruit ratio; RI, Ravaz Index; TSS, total soluble solids; TA, titratable acidity; BW, berry mass; Anth, total anthocyanins; Flav, total flavonols; %M, % myricetin; %Q, % quercetin; %K, % kaempferol; %Dp, % delphinidin; %Cy, % Cyanidin; %Pt, % Petunidin; %Pn, % Peonidin; %Mv, % Malvidin.

reported (Movahed et al., 2016). Those authors suggested that high temperature induced anthocyanin degradation by enhancing the expression of VviPrx31 and consequently the peroxidase activity. Likewise, overexposed berries (Exp+ Deg+ and Exp+ Deg++) with kaempferol proportions greater than 10% were subjected to higher temperatures that dramatically decreased anthocyanin content.

Matus et al. (2009) reported that flavonol content increased by two-fold in exposed berries compared to non-exposed. Our results corroborated this finding partially, depending on the level and duration of exposure, canopy position of the berries, and orientation of the vineyard. Therefore, when flavonol proportion was below 10% of kaempferol, flavonol content increased; but would decrease after this inflection point due to degradation. Matus et al. (2009) further indicated that this increase in flavonol may be driven by the up-regulation of MYB12 and flavonols

synthase 4 (FLS4) due to the greater exposure suggesting that FLS4 could be a target of MYB12 in grapevine. Accordingly, Sun et al. (2017) found that increased accumulation of flavonols in light exposure berries, were accompanied by the up-regulation of several genes of the FLS gene family suggesting that they may be functionally redundant in response to light signal.

During the experiment conducted in the 2019 growing season, the kaempferol proportion increased in LR and ST treatments, but largest increase was measured when ST and LR were applied concurrently. Likewise, the higher the degree of exposure degree a greater kaempferol accumulation was observed during the 2017 growing season. The increase in kaempferol in total proportion of flavonols was accompanied with a concomitant decrease of quercetin and myricetin proportions. These results are corroborated with our previous work performed on Merlot and Cabernet Sauvignon. (Martínez-

Lüscher et al., 2019), and by others on Cabernet Sauvignon, Nero d'Avola, Raboso Piave, and Sangiovese in Italy (Pastore et al., 2017). We previously reported the proportion of kaempferol was a feasible tool for accounting the solar radiation received by berry due to the greater canopy porosity (Martínez-Lüscher et al., 2019) and this corresponded to the 1930 W·m⁻² of global radiation accumulated at the research site in Experiment 3. On the other hand, the higher proportion of quercetin derivatives in detriment of myricetin derivatives found in LR vines has been related to downregulation of F3'5'H family genes (Sun et al., 2017).

Previous work on red grapevine berries, indicated that IBMP content decreased with greater solar exposure due to the canopy management practices during berry ripening (Ryona et al., 2008; Dunlevy et al., 2013). In our work, the lowest IBMP content was measured in LRST berries. Our results indicated a negative and linear relationship between leaf to fruit ratio and IBMP content. Conversely, the relationship between kaempferol proportion and IBMP was not significant. Therefore, our data suggested that the decrease of IBMP content was better explained by changes in the source-sink balance rather than differences in solar exposure. Likewise, Koch et al. (2012) provided evidence that solar exposure affected IBMP content to a greater extent when canopy porosity was enhanced before fruit set and not during berry ripening corroborating our results. The lower berry IBMP content was explained by a diminution of the accumulation rates rather than increased rates of degradation (Ryona et al., 2008) due to canopy management practices and restriction of applied water between fruit set and veraison (Brillante et al., 2018) in a warm climate.

Labor Operations Costs of Canopy Management Practices

The total operating costs per hectare of a Cabernet Sauvignon vineyard in Napa County, CA U.S.A. is approximately US\$ 40,382 (Kurtural et al., 2020). The labor operations costs of canopy management practices per hectare are 25% of the total costs. Our data indicated that although some berry traits were improved by the removal of shoots (2.5% increase in TSS) and leaves or the more common practice of doing them concurrently, their profitability is not ensured in warm climates. The unit cost to produce one unit of anthocyanin increased by about 10-fold with the additional canopy management practices. Therefore, the unfavorable leaf area to fruit ratios increased the cost of producing anthocyanins as previously reported by Cook et al. (2015) in Merlot grapevine grown in a warm climate. Likewise, the diminution of accumulation rates of IBMP were not as economically effective as once thought due to loss in yield and reduction in gross income per hectare for the grower. Finally, the breaking points determined through segmented regression analysis indicated that although increases in solar exposure (kaempferol proportion greater than 6.4% and 7% for anthocyanins and IBMP, respectively) led to significant IBMP content decreases ($r = -0.95$, $p = 0.011$), however, we were unable to elucidate this effect on anthocyanin content ($r = -0.24$, $p = 0.434$, **Figure S3**).

CONCLUSION

Since the effect of canopy management practices lead to higher solar exposure in hot climates that might be deleterious on grape quality, we aimed to elucidate the thresholds for maintaining anthocyanin content, while waiting for the target TSS required for fermentations and green aroma removal without compromising the yield. Although increasing canopy porosity through canopy management practices can be helpful for other purposes such as pest protection, this may not be the case of flavonoid compounds when a certain proportion of kaempferol is attained. Our data from these trials revealed different sensitivities to degradation within the flavonoid groups, flavonols being the only monitored compounds that were upregulated by solar radiation. Anthocyanin depletion was observed in all the trials with increasing solar radiation exposure (*i.e.* greater proportion of kaempferol). Under our experimental conditions, ST and LRST hastened fruit maturity; however, a clear improvement of the flavonoid compounds (*i.e.* greater anthocyanin content) was not observed at harvest. On the other hand, all the canopy management practices studied (LR, ST, and LRST) decreased IBMP from mid-ripening to harvest. Therefore, although some berry traits (*i.e.*, increase of 2.5°Brix and lower IBMP content) were improved due to canopy management practices (ST and LRST), this came with costs of labor and yield and gross income reduction that decreased flavonoid productivity per hectare; and these all should be assessed together when taking the decision to apply these treatments in hot climates.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SK acquired the funding. NT, JM-L, EP, and SK conducted the experiments and analyzed the data. NT wrote the first version of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The authors acknowledge the USDA-NIFA Specialty Crop Research Initiative award no. 2015-51181-24393, University of California Agriculture and Natural Resources Cooperative Extension Specialist Funds, Department of Viticulture and Enology Harold Olmo Research Trust, Rossi Endowment. The authors declare that this study received funding from Syngenta Crop Protection USA LLC and Allied Grape Growers. The funders were not involved in the study design, collection, analysis, and interpretation of data, the writing of

this article or the decision to submit it for publication. The authors also acknowledge Constellation Brands United States for in-kind support and access to vineyards during the execution of the trial.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.00931/full#supplementary-material>

FIGURE S1 | Relationship between the % of Kaempferol and the % of Canopy porosity from berries collected from Cabernet Sauvignon (A) and Petit Verdot (B) varieties at harvest in Oakville in September 2017.

FIGURE S2 | Relationship between the % of Kaempferol and cluster temperature at mid ripening in Cabernet Sauvignon subjected to different canopy management

practices (UNT: Untreated, LR: Leaf removal, ST: Shoot thinning and LRST: LR and ST combined). Cluster temperature means separated by Duncan's new multiple range test (at $P = 0.05$). Within columns, means followed by different letters are significantly different as affected by the canopy management practices of leaf removal and shoot thinning and their interactions.

FIGURE S3 | Relationship between grape skin anthocyanin (A) and IBMP (B) content (mg and pg per berry, respectively) and increasing exposure (% of kaempferol, Martínez-Lüscher et al., 2019) in Cabernet Sauvignon subjected to different canopy management practices (UNT: Untreated, LR: Leaf removal, ST: Shoot thinning and LRST: LR and ST combined). Black lines are the breaking points determined through segmented regression.

TABLE S1 | Effect of the different degree of exposure on the skin flavonoid content of Cabernet Sauvignon and Petit Verdot berries collected from different orientations (Interior, Exposed from the West side of the canopy, Exposed from the East side and Overexposed from the East side of the canopy) in Oakville, CA in 2017. Values represent means separated by Kruskal-Wallis test (at $p = 0.05$). Within columns, means followed by different letters are significantly different as affected by the combination of degree of exposure and cultivar

REFERENCES

- Blancaert, E. H., Oberholster, A., Ricardo-da-Silva, J. M., and Deloire, A. J. (2019). Grape Flavonoid Evolution and Composition Under Altered Light and Temperature Conditions in Cabernet Sauvignon (*Vitis vinifera* L.). *Front. Plant Sci.* 10, 1062. doi: 10.3389/fpls.2019.01062
- Bogicevic, M., Maras, V., Mugoša, M., Kodžulović, V., Raičević, J., Šućur, S., et al. (2015). The effects of early leaf removal and cluster thinning treatments on berry growth and grape composition in cultivars Vranac and Cabernet Sauvignon. *Chem. Biol. Tech. Agric.* 2, 13. doi: 10.1186/s40538-015-0037-1
- Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. (1985). Effect of crop level and crop load on growth, yield, must and wine composition, and quality of Cabernet Sauvignon. *Am. J. Enol. Vitic.* 36, 125–131.
- Brillante, L., Martínez-Lüscher, J., and Kurtural, S. K. (2018). Applied water and mechanical canopy management affect berry and wine phenolic and aroma composition of grapevine (*Vitis vinifera* L. cv. Syrah) in Central California. *Sci. Hortic.* 227, 261–271. doi: 10.1016/j.scienta.2017.09.048
- Bubola, M., Sivilotti, P., Janjanin, D., and Poni, S. (2017). Early Leaf Removal has a Larger Effect than Cluster Thinning on Grape Phenolic Composition in cv. Teran. *Am. J. Enol. Vitic.* 68:2. doi: 10.5344/ajev.2016.16071
- California Department of Food and Agriculture. Cdfa (2020). *Grape Crush Final Report 2019*.
- California Department of Water Resources 2020. *CIMIS Weather Observation Data: University of California at Davis*. www.cimis.water.ca.gov (accessed Jan 18, 2020).
- Chapman, D. M., Thorngate, J. H., Matthews, M. A., Guinard, J. X., and Ebeler, S. E. (2004). Yield effects on 2-methoxy-3-isobutylpyrazine concentration in cabernet sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. *J. Agric. Food Chem.* 52, 5431–5435. doi: 10.1021/jf0400617
- Cook, M. G., Zhang, Y., Nelson, C. J., Gambetta, G., Kennedy, J. A., and Kurtural, S. K. (2015). Anthocyanin composition of Merlot is ameliorated by light microclimate and irrigation in Central California. *Am. J. Enol. Vitic.* 66, 266–278. doi: 10.5344/ajev.2015.15006
- De Bei, R., Fuentes, S., Gilliam, M., Tyerman, S., Edwards, E., Bianchini, N., et al. (2016). VitiCanopy: a free computer App to estimate canopy vigor and porosity for grapevine. *Sensors*, 16: E585. doi: 10.3390/s16040585
- de Mendiburu, M. F. (2016). *Package 'Agricolae.' Statistical Procedures for Agricultural Research. Version 1.3-0*.
- Diago, M. P., Ayestarán, B., Guadalupe, Z., Poni, S., and Tardáguila, J. (2012). Impact of Prebloom and Fruit Set Basal Leaf Removal on the Flavonol and Anthocyanin Composition of Tempranillo Grapes. *Am. J. Enol. Vitic.* 63, 3. doi: 10.5344/ajev.2012.11116
- Dunlevy, J. D., Soole, K. L., Perkins, M. V., Nicholson, E. L., Maffei, S. M., and Boss, P. K. (2013). Determining the Methoxypyrazine Biosynthesis Variables Affected by Light Exposure and Crop Level in Cabernet Sauvignon. *Am. J. Enol. Vitic.* 64, 450–458. doi: 10.5344/ajev.2013.13070
- Feng, H., Yuan, F., Skinkis, P. A., and Qian, M. C. (2015). Influence of cluster zone leaf removal on Pinot noir grape chemical and volatile composition. *Food Chem.* 173, 414–423. doi: 10.1016/j.foodchem.2014.09.149
- Gómez-Míguez, M., González-Manzano, S., Escribano-Bailón, M. T., Heredia, F. J., and Santos-Buelga, C. (2006). Influence of different phenolic copigments on the color of malvidin 3-glucoside. *J. Agric. Food Chem.* 54, 5422–5429. doi: 10.1021/jf0604586
- Gatti, M., Bernizzoni, F., Civardi, S., and Poni, S. (2012). Effects of Cluster Thinning and Preflowering Leaf Removal on Growth and Grape Composition in cv. Sangiovese. *Am. J. Enol. Vitic.* 63, 3. doi: 10.5344/ajev.2012.11118
- Geller, J. P., and Kurtural, S. K. (2013). Mechanical Canopy and Crop-Load Management of Pinot gris in a Warm Climate. *Am. J. Enol. Vitic.* 64, 65–73. doi: 10.5344/ajev.2012.12045
- Jogaiah, S., Striegler, K. R., Bergmeier, E., and Harris, J. (2013). Influence of Canopy Management Practices on Canopy Characteristics, Yield, and Fruit Composition of 'Norton' Grapes (*Vitis aestivalis* Michx). *Int. J. Fruit Sci.* 13, 441–458. doi: 10.1080/15538362.2013.789267
- Kennedy, J. A., and Jones, G. P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* 49, 1740–1746. doi: 10.1021/jf001030o
- Koch, A., Doyle, C. G., Matthews, M. A., Williams, L. E., and Ebeler, S. E. (2010). Analysis of 2-methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. *Phytochemistry* 71, 2190–2198. doi: 10.1016/j.phytochem.2010.09.006
- Koch, A., Ebeler, S. E., Williams, L. E., and Matthews, M. A. (2012). Fruit ripening in *Vitis vinifera*: light intensity before and not during ripening determines the concentration of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon berries. *Physiol. Plant* 145, 275–285. doi: 10.1111/j.1399-3054.2012.01572.x
- Kurtural, S. K., Beebe, A. E., Martínez-Lüscher, J., Zhuang, S., Lund, K. T., McGourty, G., et al. (2019). Conversion to mechanical pruning in vineyards maintains fruit composition while reducing labor costs in 'Merlot' grape production. *HortTechnology* 29, 128–139. doi: 10.21273/HORTTECH04204-18
- Kurtural, S. K., Steward, D., and Sumner, D. (2020). Sample costs to establish a vineyard and produce wine grapes Napa County Crush District 4. *Univ. Calif. Coop. Ext. Serv. Bul. GR-VN16*, 1–27.
- Martínez-Lüscher, J., Chen, C. C. L., Brillante, L., and Kurtural, S. K. (2017). Partial solar radiation exclusion with color shade nets reduce the degradation of organic acids and flavonoids of grape berry (*Vitis vinifera* L.). *J. Agric. Food Chem.* 65, 10693–10702. doi: 10.1021/acs.jafc.7b04163
- Martínez-Lüscher, J., Brillante, L., and Kurtural, S. K. (2019). Flavonol Profile Is a Reliable Indicator to Assess Canopy Architecture and the Exposure of Red Wine Grapes to Solar Radiation. *Front. Plant Sci.* 10, 10. doi: 10.3389/fpls.2019.00010

- Matus, J. T., Loyola, R., Vega, A., Peña-Neira, A., Bordeu, E., Arce-Johnson, P., et al. (2009). Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin and flavonol synthesis in berry skins of *Vitis vinifera*. *J. Exp. Bot.* 60, 853–867. doi: 10.1093/jxb/ern336
- Mijowska, K., Ochmian, I., and Oszmianski, J. (2016). Impact of Cluster Zone Leaf Removal on Grapes cv. Regent Polyphenol Content by the UPLC-PDA/MS Method. *Molecules* 21, 1688. doi: 10.3390/molecules21121688
- Movahed, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., et al. (2016). The grapevine VvPrx31 peroxidase as a candidate gene involved in anthocyanin degradation in ripening berries under high temperature. *J. Plant Res.* 129, 513–526. doi: 10.1007/s10265-016-0786-3
- Muggeo, V. M. R. (2008). Segmented: an R package to fit regression models with broken-line relationships. *R. News* 8, 20–25.
- Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, M., Tornielli, G. B., and Filippetti, I. (2013). Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. *BMC Plant Biol.* 13, 30. doi: 10.1186/1471-2229-13-30
- Pastore, C., Allegro, G., Valentini, G., Muzzi, E., and Filippetti, I. (2017). Anthocyanin and flavonol composition response to veraison leaf removal on Cabernet sauvignon. Nero d'avola, Raboso Piave and Sangiovese *Vitis vinifera* L. cultivars. *Sci. Hortic.* 218, 147–155. doi: 10.1016/j.scienta.2017.01.048
- Provost, C., and Pedneault, K. (2016). The organic vineyard as a balanced ecosystem: Improved organic grape management and impacts on wine quality. *Sci. Hortic.* 208, 43–56. doi: 10.1016/j.scienta.2016.04.024
- Roujou de Boubée, D., Van Leeuwen, C., and Dubourdieu, D. (2000). Organoleptic Impact of 2-Methoxy-3-isobutylpyrazine on Red Bordeaux and Loire Wines. Effect of Environmental Conditions on Concentrations in Grapes during Ripening. *J. Agric. Food Chem.* 48, 4830–4834. doi: 10.1021/jf000181o
- Royston, P. (1995). Remark AS R94: A remark on Algorithm AS 181: The W test for normality. *Appl. Stat.* 44, 547–551. doi: 10.2307/2986146
- Ryona, I., Pan, B. S., Intrigliolo, D. S., Lakso, A. N., and Sacks, G. L. (2008). Effects of Cluster Light Exposure on 3-Isobutyl-2-methoxypyrazine Accumulation and Degradation Patterns in Red Wine Grapes (*Vitis vinifera* L. Cv. Cabernet Franc). *J. Agric. Food Chem.* 56, 10838–10846. doi: 10.1021/jf801877y
- Samoticha, J., Jara-Palacios, M. J., Hernández-Hierro, J. M., Heredia, F. J., and Wojdylo, A. (2018). Phenolic compounds and antioxidant activity of twelve grape cultivars measured by chemical and electrochemical methods. *Eur. Food Res. Technol.* 244, 1933–1943. doi: 10.1007/s00217-018-3105-5
- Scheiner, J. J., Sacks, G. L., Pan, B., Ennahli, S., Tarlton, L., Wise, A., et al. (2010). Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red winegrapes. *Am. J. Enol. Vitic.* 61, 358–364.
- Sivilotti, P., Herrera, J. C., Lisjak, K., Česnik, H. B., Sabbatini, P., Peterlunger, E., et al. (2016). Impact of Leaf Removal, Applied Before and After Flowering, on Anthocyanin, Tannin, and Methoxypyrazine Concentrations in 'Merlot' (*Vitis vinifera* L.) Grapes and Wines. *J. Agric. Food Chem.* 64, 4487–4496. doi: 10.1021/acs.jafc.6b01013
- Sun, Q., Sacks, G. L., Lerch, S. D., and Vanden Heuvel, J. E. (2012). Impact of Shoot and Cluster Thinning on Yield, Fruit Composition, and Wine Quality of Corot noir. *Am. J. Enol. Vitic.* 63, 50–56. doi: 10.5344/ajev.2011.11029
- Sun, R.-Z., Cheng, G., Li, Q., He, Y.-N., Wang, Y., Lan, Y.-B., et al. (2017). Light-induced Variation in Phenolic Compounds in Cabernet Sauvignon Grapes (*Vitis vinifera* L.) Involves Extensive Transcriptome Reprogramming of Biosynthetic Enzymes, Transcription Factors, and Phytohormonal Regulators. *Front. Plant Sci.* 8, 547. doi: 10.3389/fpls.2017.00547
- Sweetman, C., Sadras, V. O., Hancock, R. D., Soole, K. L., and Ford, C. M. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65, 5975–5988. doi: 10.1093/jxb/eru343
- Tarara, J. M., Lee, J., Spayd, S. E., and Scagel, C. F. (2008). Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes. *Am. J. Enol. Vitic.* 59, 235–247.
- Tardaguila, J., Martínez de Toda, F., Poni, S., and Diago, M. P. (2010). Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. *Am. J. Enol. Vitic.* 61, 372–381.
- Terry, D. B., and Kurtural, S. K. (2011). Achieving vine balance of Syrah with mechanical canopy management and regulated deficit irrigation. *Am. J. Enol. Vitic.* 62, 426–437. doi: 10.5344/ajev.2011.11022
- Torres, N., Goicoechea, N., Morales, F., and Antolin, M. C. (2016). Berry quality and antioxidant properties in *Vitis vinifera* L. cv. Tempranillo as affected by clonal variability, mycorrhizal inoculation and temperature. *Crop Pasture Sci.* 67, 961–977. doi: 10.1071/CP16038
- Uhlig, B. A. (1998). Effects of solar radiation on grape (*Vitis vinifera* L.) composition and dried fruit colour. *J. Hortic. Sci. Biotech.* 73, 111–123. doi: 10.1080/14620316.1998.11510953
- Wang, X., De Bei, R., Fuentes, S., and Collins, C. (2019). Influence of Canopy Management Practices on Canopy Architecture and Reproductive Performance of Semillon and Shiraz Grapevines in a Hot Climate. *Am. J. Enol. Vitic.* 70, 360–372. doi: 10.5344/ajev.2019.19007
- Yu, R., and Kurtural, S. K. (2020). Proximal sensing of soil electrical conductivity provides a link to soil-plant water relationships and supports the identification of plant water status zones in vineyards. *Front. Plant Sci.* 11, 244. doi: 10.3389/fpls.2020.00244
- Yu, R., Cook, M. G., Yacco, R. S., Watrelot, A. A., Gambetta, G., Kennedy, J. A., et al. (2016). Effects of Leaf Removal and Applied Water on Flavonoid Accumulation in Grapevine (*Vitis vinifera* L. cv. Merlot) Berry in a Hot Climate. *J. Agric. Food Chem.* 64, 8118–8127. doi: 10.1021/acs.jafc.6b03748

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Torres, Martínez-Lüscher, Porte and Kurtural. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Day Temperature Has a Stronger Effect Than Night Temperature on Anthocyanin and Flavonol Accumulation in ‘Merlot’ (*Vitis vinifera* L.) Grapes During Ripening

OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Alessandra Ferrandino,
University of Turin, Italy
Inmaculada Pascual,
University of Navarra, Spain

***Correspondence:**

Simone D. Castellarin
simone.castellarin@ubc.ca

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 28 April 2020

Accepted: 03 July 2020

Published: 24 July 2020

Citation:

Yan Y, Song C, Falginella L and
Castellarin SD (2020) Day
Temperature Has a Stronger Effect
Than Night Temperature on
Anthocyanin and Flavonol
Accumulation in ‘Merlot’ (*Vitis vinifera*
L.) Grapes During Ripening.
Front. Plant Sci. 11:1095.
doi: 10.3389/fpls.2020.01095

Yifan Yan¹, Changzheng Song¹, Luigi Falginella² and Simone D. Castellarin^{1*}

¹ Wine Research Centre, The University of British Columbia, Vancouver, BC, Canada, ² Research Center, Vivai Cooperativi Rauscedo, Rauscedo, Italy

Flavonoids impart color and mouthfeel to grapes and wine and are very sensitive to environmental conditions. Growth chamber experiments were performed to investigate the effect of temperature regimes and the differences between day/night temperatures on anthocyanins and flavonols in Merlot grapes. Among the regimes tested, the ones with diurnal 20°C determined the highest levels of anthocyanins and flavonols. Higher diurnal temperatures decreased those levels but increased the proportion of methoxylated and acylated species. When regimes with the same day temperature but different night temperatures were compared, differences between day/night temperatures did not affect anthocyanins, unless a difference of 25°C between day and night temperatures was imposed. When regimes with the same night temperature but different day temperatures were compared, the regime with higher day temperature had a lower anthocyanin level. No relationships were observed between the effects of temperature regimes on anthocyanin level and the expression of key anthocyanin genes. However, the effects on anthocyanin acylation level were consistent with the effects on the acyltransferase expression, and the effects on flavonol level were consistent with the effects on the expression of key flavonol genes. This study indicates that, in Merlot grapes, anthocyanins and flavonols are mostly sensitive to day temperatures.

Keywords: abiotic stress, anthocyanins, flavonols, flavonoids, viticulture

INTRODUCTION

Flavonoids constitute the major portion of phenolic compounds in grapes and include anthocyanins and flavonols, as well as flavan-3-ols and proanthocyanidins (also known as tannins) (Teixeira et al., 2013). In red grape varieties, anthocyanins are produced in the berry skin during ripening, act as attractants for seed dispersal, and determine the color of the wines (Shirley, 1996). Flavonols are largely accumulated during blooming and ripening, and function as UV protectors (Carbonell-Bejerano et al., 2014). In red wines, they can co-pigment with anthocyanins to affect color stability, while in white wines, they directly affect the yellowish color (Castillo-Muñoz et al., 2007). Moreover, anthocyanins and flavonols act as antioxidants and have health benefits when consumed, such as cardiovascular disease prevention, obesity control, and diabetes alleviation (He and Giusti, 2010).

Anthocyanins are classified into different subgroups based on their chemical structures: as 3′4′- and 3′4′5′-substituted, according to the number of hydroxyl groups on the B-ring, and as methoxylated or non-methoxylated, according to the presence or absence of methoxyl groups. Likewise, flavonols are classified into 3′-substituted, 3′4′-substituted, and 3′4′5′-substituted, or methoxylated and non-methoxylated according to the same criteria as described above. In grapes, anthocyanins and flavonols are normally accumulated as stable glycosylated forms (Castillo-Muñoz et al., 2007). Furthermore, the 6′-hydrogen on the sugar moiety can be substituted by aliphatic or aromatic acids to generate acylated anthocyanins and flavonols (Mazza and Miniati, 1993). An increased number of hydroxyl groups on the B-ring shifts the anthocyanin color from red to blue, while methoxylation and acylation shift the color to red and blue, respectively (Lachman and Hamouz, 2005; Liu et al., 2018). Generally, glycosylation, methoxylation, and acylation increase the thermal stability of flavonoids (Jackman and Smith, 1996).

The accumulation of anthocyanins and flavonols during berry development is greatly affected by environmental factors (Mori et al., 2007; Matus et al., 2009; Azuma et al., 2012), which suggests that their levels and profiles change among seasons and wine regions, and may also be affected by the predicted climatic changes (Jones et al., 2012).

Among the environmental factors, temperature has arguably the most profound effect on anthocyanin accumulation in grapes (Jones et al., 2012). Early studies showed that high day and night temperature regime (37/32°C) completely inhibited coloration in Emperor grapes (Kliewer, 1977) and that high day temperature (35°C) strongly reduces anthocyanin concentration, while the effect of night temperature changes in relation to the day temperature considered (Kliewer and Torres, 1972). Temperature effects on anthocyanin accumulation also depend on the developmental stages when the berries experience a particular temperature. Yamane et al. (2006) suggested that the most sensitive stage for grape berry to temperature treatment was 1 to 3 weeks after the onset of ripening, defined by viticulturists as veraison (shift of berry color from green to yellow/red). A recent study by Gaiotti et al. (2018) indicated that grapes exposed to cool night temperatures (10–11°C, compared with 15–20°C in

control) from 12 days before veraison to the end of veraison enhanced anthocyanin accumulation but only in one out of the two seasons. Anthocyanin composition was also altered by temperature regimes, with the methoxylated and acylated proportion being increased by high temperature treatments (Mori et al., 2005; Tarara et al., 2008; De Rosas et al., 2017).

In contrast to anthocyanins, flavonol accumulation was originally reported to be unaffected by temperatures (Spayd et al., 2002; Azuma et al., 2012). However, recent studies showed that high temperatures (30–40°C) during berry ripening exerted negative effects on flavonol accumulation (Degu et al., 2016; Pastore et al., 2017b). Exposure to high temperatures affected the various flavonols differently: for instance, an increase of 6 or 9°C from ambient temperature caused the complete absence of kaempferol 3-O-glucoside, an 85% reduction of quercetin 3-O-glucoside, and a 65% reduction of myricetin 3-O-glucoside (Pillet, 2011).

It remains unclear whether temperature affects the synthesis of flavonoids directly or whether high temperatures promote degradative events (Mori et al., 2007). Though several studies reported that the expression of phenylpropanoid and flavonoid genes [including *phenylalanine ammonia lyase* (*VviPAL*), *chalcone synthase* (*VviCHS*), *chalcone isomerase* (*VviCHI*), *UDP-glucose: flavonoid 3-O-glucosyltransferase* (*VviUFGT*), and the transcription factors (TFs) *VviMybA1* and *VviMybA2*], were down-regulated by high temperatures (30–35°C, compared with 15–20°C) (Yamane et al., 2006; Azuma et al., 2012; Movahed et al., 2016), other studies reported that the expression of flavonoid genes (*VviUFGT* and *VviMybA*) was not affected (Mori et al., 2007) or up-regulated by high temperatures (Rienth et al., 2014a), yet decreased levels of anthocyanins were observed. Mori et al. (2007) reported a reduced anthocyanin concentration in berries exposed to a high temperature condition (35°C, compared with 25°C) despite similar *VviUFGT* expression and higher UFGT enzyme activity. This indicates that the lower anthocyanin level under high temperature conditions might result from a higher degradation rate rather than a lower biosynthesis rate.

Cohen et al. (2008) and Gaiotti et al. (2018), as well as anecdotal knowledge from the industry, suggest that the difference between day/night temperatures affects the flavonoid concentration in grapes, with greater differences promoting flavonoid concentration. However, those experiments did not provide definitive evidence, and a large knowledge gap remains on the role of night temperature and the difference between day/night temperatures in anthocyanin and flavonol accumulation. This topic is even more important nowadays since the increase of night temperatures due to climate change in the past half century was faster than that of day temperatures, which suggests a further decrease in the difference between day/night temperatures in future years (Stocker et al., 2014).

In this study, growth chamber experiments that compared a wide range of temperature regimes were performed to assess the effect of temperatures on anthocyanins and flavonols in Merlot grapes, with a focus on the impact of the difference between day/night temperatures. Five temperature regimes imposed from veraison to harvest, which ranged from 20 to 35°C during the

day, from 5 to 30°C at night, and from 5 to 25°C as a day/night difference, were compared in each experiment. The goal of our research was to identify the optimum temperature for anthocyanin and flavonol accumulation in Merlot grapes, as well as to determine if the temperature difference between day/night affected this accumulation. We hypothesized that a larger difference between day/night temperatures and a lower night temperature might be beneficial for anthocyanin and flavonol accumulation in grapes. Gene expression analysis was also carried out in order to assess the extent of temperature effects on transcriptional regulation of anthocyanin and flavonol synthesis during day and night.

MATERIALS AND METHODS

Experimental Design and Plant Material

Three experiments were conducted in three consecutive years from 2017 to 2019. All three experiments considered own-rooted grapevines of the same variety and clone (*Vitis vinifera* L. cv. Merlot, cl. 347), and utilized five growth chambers.

Experiment 1

In 2017, Merlot fruiting cuttings were obtained according to Mullins and Rajasekaran (1981) with some modifications. Three-node mature canes were collected during the dormant season from a commercial vineyard located in the Okanagan Valley, British Columbia, Canada (49°25' N, 119°56' W), in February 2017. Cuttings were placed within a heat-controlled container (25°C at the base), at 4°C in darkness; at the bottom of the container, rooting was induced using the ROOTS® Liquid Stimulator (Wilson, Canada) with Greenhouse Potting Mix (Grower's Nursery Supply Inc, Salem, USA), perlite, and sand (v/v, 3:1:0.5). Afterwards, pre-rooted cuttings were transferred into 7.5 L pots containing Greenhouse Potting Mix and moved to the greenhouse. Vines were grown at $22.5 \pm 0.9/19.8 \pm 0.5$ (day/night temperature, °C) and $54.5 \pm 1.6\%$ relative humidity (RH). Environmental solar radiation was supplemented with Greenpower LED (Phillips, Canada) top lighting in order to guarantee $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ of Photosynthetically Active Radiation (PAR) at the table surface for 16 h per day. Plants were irrigated on alternate days and supplemented with a nutrition solution (Relab Den Haan, Hoorn, Netherlands) twice per week in order to avoid any water deficit or nutrient shortage. One shoot per plant was retained and topped after inflorescence development; one cluster per plant was selected at fruit set, while the other clusters were removed. One lateral shoot was left to support the cluster (Mullins and Rajasekaran, 1981).

At 60 days after anthesis (DAA), vines with approximately 50% of the berries showing partial or full red pigmentation (veraison) were selected. Shoot tips were topped to 20 primary leaves and grapevines were transferred into Conviron BDR 16 (Conviron, Winnipeg, Canada) growth chambers. Each chamber contained 12 plants that were divided into four biological replicates. The temperature regimes applied were: i) 20/10 (day/night temperature, °C); ii) 20/15; iii) 25/15; iv) 35/25; v)

35/30. For the sake of simplicity, they were categorized as low (20/10 and 20/15), intermediate (25/15), and high (35/25 and 35/30) temperature regimes (Table S1), according to the diurnal temperature. These regimes were chosen to generate two day/night temperature differences: $\Delta T = 5^\circ\text{C}$ (20/15 and 35/30) and $\Delta T = 10^\circ\text{C}$ (20/10, 25/15, and 35/25). Two intermediate 1-h stages were set during the day/night and the night/day transitions in order to mimic gradual temperature decrease/increase. The day/night cycle was 14 h/8 h (plus the two 1-h transition stages). Mean PAR at the pot level was set at 400, 100, and $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ in all chambers during the day, transition, and night stages, respectively. Plants were irrigated the same way as in greenhouse and new secondary shoots were removed through the duration of the experiment. Light intensity around the clusters was measured with a PAR sensor (Kipp and Zonen, Delft, Holland); RH was measured with a humidity probe (Campbell Scientific, Edmonton, Canada); berry surface temperature was measured with an infrared thermal gun (NAPA, Canada).

Berry sampling was carried out at five time-points throughout ripening. Eight berries per biological replicate were randomly collected at 62, 76, 89, 104, and 118 DAA (harvest date) at 4 PM for metabolites and gene expression analyses. Additionally, at 76 and 89 DAA (mid-ripening stages), berry samples were also collected at 4 AM, in order to investigate whether diurnal and nocturnal conditions affected flavonoid gene expression. Berries were removed from clusters by carefully cutting the pedicel, immediately flash-frozen in liquid nitrogen, and stored at -80°C until analysis. By the end of the experiment < 18% of the berries had been removed from the clusters.

Experiment 2

In 2018, one-year Merlot rooted cuttings obtained from the previous season were pruned to five to six dormant buds on a single cane and grown in 11.25 L pots filled with Greenhouse Potting Mix. Two shoots with two to three clusters each were maintained per vine after fruit-set; the other shoots were removed. The vines were grown in the greenhouse under the same conditions as in Experiment 1 until veraison (69 DAA), when the primary shoots were topped to 20 leaves, and 20 vines at a similar developmental stage were moved into growth chambers (four biological replicates per chamber, one plant per replicate). The five treatments considered were: i) 20/10 (day/night temperature, °C); ii) 20/15; iii) 25/15; iv) 25/20; v) 30/20. They were categorized according to the diurnal temperature as low (20/10 and 20/15), intermediate (25/15 and 25/20), and high (30/20) temperature regimes (Table S1). These regimes were chosen to generate two day/night temperature differences: $\Delta T = 5^\circ\text{C}$ (20/15 and 25/20) and $\Delta T = 10^\circ\text{C}$ (20/10, 25/15, and 30/20). Day/night cycles, light intensities, and irrigation strategies were the same as described for Experiment 1. Secondary shoots were kept during the experiment. Light, temperature, and RH were measured as described in Experiment 1; berry surface temperature was not recorded in Experiment 2.

Samples of 20 berries were randomly collected from each biological replicate at 79, 93, 113, and 135 DAA at 4 PM, and at 93 and 113 DAA at 4 AM. Berry samples were collected and

stored as described in Experiment 1. An additional sample was collected from four randomly selected grapevines out of the 20 used for the experiment at 69 DAA, before the grapevines were moved into growth chambers. By the end of the experiment <20% of the berries had been removed from the clusters.

Experiment 3

In 2019, one-year Merlot rooted cuttings obtained from the previous season (2018) were grown in the greenhouse as described in Experiment 2 and 20 vines at a similar developmental stage were moved into growth chambers (four biological replicates per chamber, one plant per replicate) at veraison (67 DAA). The five temperature regimes tested were: i) 20/5 (day/night temperature, °C); ii) 20/15; iii) 30/5; iv) 30/15; v) 30/25. They were categorized according to the diurnal temperature as low (20/5 and 20/15) and high (30/5, 30/15, and 30/25) temperature regimes (**Table S1**). These regimes were chosen to generate three day/night temperature differences: $\Delta T = 5^{\circ}\text{C}$ (20/15 and 30/25), $\Delta T = 15^{\circ}\text{C}$ (20/5 and 30/15), and $\Delta T = 25^{\circ}\text{C}$ (30/5). Growth chamber conditions and irrigation strategies were the same as in Experiment 1 and 2. Secondary shoots were kept during the experiment. Light, temperature, and RH were measured as described in Experiment 1. Samples of 20 berries were collected—as described in Experiment 2—at harvest (113 DAA at 4 PM).

Berry Sample Preparation

The frozen berry samples were weighed, peeled, and deseeded using a scalpel and tweezers. Berry tissues were kept frozen using liquid nitrogen. Skin, flesh, and seed weights were recorded. The skin and the flesh were ground into fine powder under liquid nitrogen using a mortar and pestle and an electronic mill (A11 S001, IKA Inc., Wilmington, USA), respectively. The skin powder was used for anthocyanin and flavonol quantification (in Experiments 1, 2, and 3) and gene expression analysis (in Experiment 1). The flesh powder was used for the determination of total soluble solids (TSS) and titratable acidity (TA).

Berry Total Soluble Solids and Titratable Acidity

An aliquot of 2 g flesh powder was thawed at room temperature for 20 min, and the juice was collected by centrifugation at 10,000g for 10 min. Berry TSS were measured with a digital refractometer (Reichert A2R200, Reichert GmbH, Seefeld, Germany) and reported as °Brix; TA was measured by titration with 0.1 N NaOH using a 50-ml alkali burette until pH 8.2 and expressed as tartaric acid equivalents (g/L).

Anthocyanin and Flavonol Analysis

Anthocyanins and flavonols were extracted with aqueous acidified methanol (v/v, methanol:water:formic acid, 49.5:49.5:1) and analyzed by high-performance liquid chromatography and mass spectrometry (HPLC-MS), according to Downey and Rochfort (2008) with slight modifications. Approximately 0.15 g of skin powder was extracted in 1.5 ml of the extraction solution for 20 min using a sonicator (FS20H, Fisher Scientific, Ottawa, Canada) followed by 10-min centrifugation at 13,000 g at room

temperature. The supernatant was filtered (0.22 $\mu\text{m} \times 13 \text{ mm}$, PVDF Millex Filter, Sigma-Aldrich, Oakville, Canada) and transferred into an amber vial (Agilent Technologies, Mississauga, Canada) using a syringe (Luer-Lok Tip Syringe, Sigma-Aldrich, Oakville, Canada). The pellet was then extracted for a second time with the same procedures described above. The two fractions of the supernatant were combined. Five μL of the extract was injected into an Agilent 1100 Series LC coupled to an MSD Trap XCT Plus System (Agilent Technologies, Mississauga, Canada) and a Diode Array Detector (DAD, Agilent Technologies, Mississauga, Canada). Chromatographic separation was carried out by an Agilent ZORBAX SB-C18 Column (1.8 μm , 4.6 \times 50 mm) (Agilent Technologies, Mississauga, Canada) with the temperature maintained at 67.0°C. The mobile phases were aqueous formic acid (v/v, 98:2; Solvent A) and acetonitrile/formic acid (v/v, 98:2; Solvent B). The LC separation used a binary solvent gradient, with a flow rate of 1.20 ml/min. The gradient conditions were 0.20 min, 5.0% solvent B; 6.00 min, 20.0% solvent B; 9.00 min, 80.0% solvent B; 10.00 min; 90.0% solvent B; 10.10 min, 90.0% solvent B; 11.00 min, 5.0% solvent B; and stopped at 11.50 min. Mass spectra were generated *via* electrospray ionization (ESI) in both positive and negative modes. Compound identification was conducted by i) comparing their retention times with those of standards (3-O-glucosides of cyanidin, peonidin, delphinidin, petunidin, and malvidin, 3-O-glucosides of kaempferol, quercetin, myricetin, isorhamnetin, and syringetin, all from Extrasynthese, Genay, France), ii) matching the mass spectra of identified peaks with anthocyanin and flavonol compounds retrieved from published papers, and iii) comparing their elution order (Mazza et al., 1999; Castillo-Muñoz et al., 2007; Downey and Rochfort, 2008). Uncertain peaks were then verified by MS/MS analysis. Anthocyanin and flavonol quantifications were based on UV-vis spectra at wavelengths of 520 and 353 nm and expressed as malvidin 3-O-glucoside and quercetin 3-O-glucoside equivalents, respectively, as commonly reported (among others, Castillo-Muñoz et al., 2007; Downey and Rochfort, 2008). Calibration curves were constructed using five gradient concentrations of malvidin 3-O-glucoside (1–250 $\mu\text{g/ml}$) (Extrasynthese, Genay, France) and quercetin 3-O-glucoside (0.5–10 $\mu\text{g/ml}$) (Extrasynthese, Genay, France) solutions.

RNA Extraction and Gene Expression Analysis

Samples collected in Experiment 1 at 76 and 89 DAA during the day and at night were used for transcript analysis. Total RNA was extracted from 0.2 g of skin powder using SpectrumTM Plant Total RNA Kit (Sigma-Aldrich, Oakville, Canada) according to the manufacturers' instructions. The quality and integrity of the extracted RNA was assessed by gel electrophoresis, and the amount was assessed by a spectrophotometer (NanoDrop-1000, Thermo Fisher Scientific, Waltham, USA). One μg of extracted RNA was retrotranscribed using the InvitrogenTM SuperScriptTM IV ViloTM Master Mix (Thermo Fisher Scientific, Waltham, USA) following manufacturers' instructions.

Transcript abundance was assessed by qRT-PCR on a 7500 Real-Time PCR Systems (Applied BiosystemsTM, ThermoFisher

Scientific, Waltham, USA) according to Wang et al. (2019). Specific primers for the tested genes were reported in **Table S2**. *VviUbiquitin* was chosen as the reference gene as reported in Bogs et al. (2006).

Statistical Analysis

Statistical analyses were performed using SPSS v23.0 (IBM, New York, USA). A one-way ANOVA test was used to evaluate the effect of temperature regimes on all the parameters assessed within each specific sampling point. Separation of means was performed using an LSD test. A two-way ANOVA test was used to evaluate the effect of the temperature regimes and the sampling time, as well as their interactions on the gene expression level. Linear regression analysis was conducted between TSS and total anthocyanin concentration (expressed as $\mu\text{g/g}$ skin FW) to test the relationship between sugar and anthocyanin accumulation during berry ripening, and an ANCOVA test was conducted to assess the significant differences between the coefficients of the linear regression curves from the above test.

RESULTS

Temperature Effect on Berry Development, Sugar, and Acid Accumulation

Berry weight at harvest was generally not affected by the temperature regimes, except for the 25/15 regime in Experiment 1, which had a larger berry than the 35/25 and 35/30 regimes (**Table S3**). Skin weight was generally higher in the low than the high temperature regimes. In Experiment 1, it was higher in the 20/10 and 20/15 berries than in berries from any other regimes; in Experiment 2, it was higher in the 20/10 than the 30/20 berries; while in Experiment 3, it was higher in the 20/15 than the 30/5 berries. No difference in the skin-to-berry weight ratio was found in Experiment 1, while a higher ratio was found in the 20/10 and 20/15 berries than in the 25/15, 25/20, and 30/20 berries in Experiment 2, and in the 20/5 and 20/15 berries than in the 30/15 berries in Experiment 3. Seed weight at harvest was not affected by the temperature regimes.

The effect of the difference between day/night temperatures ($\Delta T = 5$ or 10°C in Experiments 1 and 2, $\Delta T = 5, 15$, or 25°C in Experiment 3) was only observed in the skin-to-berry weight ratio in Experiments 2 and 3. Berries exposed to regimes with the same night temperature had a higher skin-to-berry weight ratio when exposed to a smaller difference between day/night temperatures (e.g., 20/15, $\Delta T = 5^\circ\text{C}$, vs. 25/15, $\Delta T = 10^\circ\text{C}$ in Experiment 2; 20/15, $\Delta T = 5^\circ\text{C}$, vs. 30/15, $\Delta T = 15^\circ\text{C}$ in Experiment 3).

At harvest, berries exposed to the high temperature regimes generally had lower TSS than the ones exposed to the intermediate and low temperature regimes. TSS difference among the temperature regimes were not significant in Experiment 1, while higher TSS were observed in the 20/15 than the 25/20 berries in Experiment 2 and in the 20/5 and 20/15 berries than in the 30/15 berries in Experiment 3 (**Table 1**).

TABLE 1 | Temperature effects on berry total soluble solids (TSS) and titratable acidity (TA) at harvest in Experiments 1, 2, and 3.

Experiment	Temperature regimes (day/night temperature, $^\circ\text{C}$)	TSS ($^\circ\text{Brix}$) [‡]	TA (g/L)
Experiment 1 (118 DAA)*	20/10	19.3 \pm 0.6	6.34 \pm 0.16 a
	20/15	20.6 \pm 1.0	6.14 \pm 0.15 a
	25/15	19.9 \pm 1.2	5.47 \pm 0.12 b
	35/25	19.4 \pm 0.8	4.80 \pm 0.02 c
	35/30	17.3 \pm 0.8	4.75 \pm 0.27 c
Experiment 2 (135 DAA)	20/10	20.7 \pm 0.9 ab	7.02 \pm 0.11 a
	20/15	20.8 \pm 0.8 a	5.98 \pm 0.16 b
	25/15	20.5 \pm 0.2 ab	4.70 \pm 0.24 c
	25/20	18.2 \pm 1.1 b	5.05 \pm 0.50 c
	30/20	19.1 \pm 0.5 ab	4.63 \pm 0.35 c
Experiment 3 (113 DAA)	20/5	21.5 \pm 0.8 a	9.14 \pm 0.40 a
	20/15	22.4 \pm 1.0 a	7.84 \pm 0.57 b
	30/5	20.1 \pm 0.3 ab	4.97 \pm 0.19 c
	30/15	18.9 \pm 0.3 b	4.30 \pm 0.26 c
	30/25	20.9 \pm 0.9 ab	4.24 \pm 0.22 c

*DAA refers to days after anthesis; [‡]Values reported are the mean \pm standard error (SE, $n = 4$). Different letters indicate significantly different means according to an LSD test ($p \leq 0.05$).

Similar results were observed when the evolution of TSS was considered. In Experiment 1, the 20/15 berries had higher TSS than the 35/30 berries at 104 DAA (**Figure S1A**), while in Experiment 2, the 20/10 and 20/15 berries had higher TSS than the 25/20 berries at 113 DAA (**Figure S1B**).

At harvest, TA was significantly and consistently affected by the temperature regimes in all experiments. TA level progressively decreased with the increase in temperatures (**Table 1**). This was also clear when the evolution of TA was assessed (**Figures S1C, D**). Soon after treatment applications (16 days in Experiment 1, i.e., 76 DAA; 24 days in Experiment 2, i.e., 93 DAA), the low temperature regimes (i.e., 20/10 and 20/15) displayed higher TA levels than other regimes; this higher level in the low temperature regimes was maintained until harvest.

The difference between day/night temperatures had no effect on TSS. When regimes with the same day but different night temperatures were compared, the difference between day/night temperatures affected TA levels only in the low temperature regimes, with a larger difference between day/night temperatures increased the TA level (e.g., 20/10, $\Delta T = 10^\circ\text{C}$ vs. 20/15, $\Delta T = 5^\circ\text{C}$ in Experiment 2; 20/5, $\Delta T = 15^\circ\text{C}$ vs. 20/15, $\Delta T = 5^\circ\text{C}$ in Experiment 3) (**Table 1; Figures S1C, D**). Opposite results were observed when regimes with the same night, but different day temperatures were compared. TA levels were higher in berries exposed to regimes with a smaller difference between day/night temperatures (e.g., 20/15, $\Delta T = 5^\circ\text{C}$, vs. 25/15, $\Delta T = 10^\circ\text{C}$ in Experiments 1 and 2; 20/15, $\Delta T = 5^\circ\text{C}$, vs. 30/15, $\Delta T = 15^\circ\text{C}$ in Experiment 3). Similar results were observed during berry ripening (**Figures S1C, D**).

Temperature Effects on Anthocyanin Levels

Anthocyanin levels at harvest were significantly affected by the temperature regimes; the three experiments consistently indicated that the low-temperature regimes and the high-

^aDp, O, Pl, Pe, Mw refer to ovariectomized, peonidin, petunidin, and malvidin, respectively; gic refers to 3-O-glucoside, ac-glc refers to 3-O-16''-acetylglucosides, and cou-glc refers to 3-O-16''-coumaroylglucosides; 34''-sub, 34''-15''-sub, Meth, Non-meth, Non-ac, and Cou-glc refer to the relative concentration of total 34''-substituted anthocyanins, 34''-5''-substituted anthocyanins, non-methoxylated anthocyanins, non-methoxylated anthocyanins, 34''-5''-substituted anthocyanins, methoxylated anthocyanins, and 3-O-16''-p-coumaroylglucosides to total anthocyanins; Anthocyanin peaks were integrated by UV-vis spectra at 520 nm; anthocyanin concentration were expressed as µg/g skin FW based on malvidin 3-O-glucoside equivalents. Values reported are the mean ± standard error (SE, n = 4). Different letters indicate significantly different means within each experiment according to an LSD test ($p \leq 0.05$). ^bDAA refers to days after anthesis.

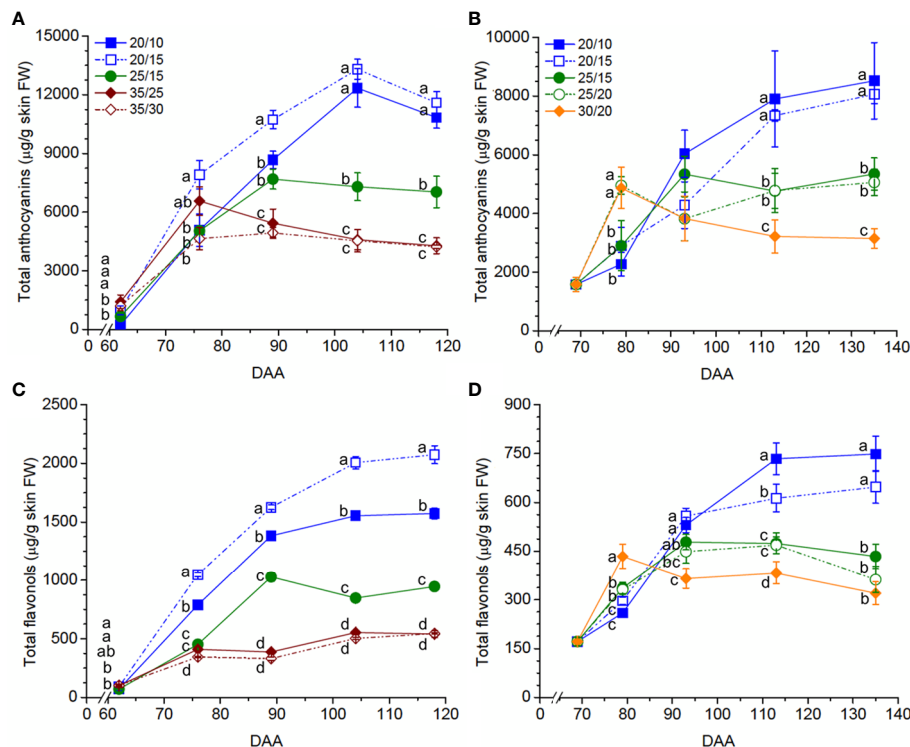


FIGURE 1 | Temperature effects on anthocyanin (A, B) and flavonol (C, D) concentration ($\mu\text{g/g}$ skin FW) in Merlot grapes in Experiments 1 (A, C) and 2 (B, D). Values reported are the mean \pm standard error (SE, $n = 4$). Different letters indicate significant different means according to an LSD test ($p \leq 0.05$). Legends in (A) indicate the temperature regimes in (A, C); legends in (B) indicate the temperature regimes in (B, D). DAA refers to days after anthesis.

in Experiment 3. When regimes with the same night but different day temperatures were compared, the relative concentrations of methoxylated, acylated, and 3'4'5'-substituted anthocyanins were higher in temperature regimes with a larger difference between day/night temperatures (e.g., 25/15, $\Delta T = 10^\circ\text{C}$, vs. 20/15, $\Delta T = 5^\circ\text{C}$, in Experiments 1 and 2; 30/15, $\Delta T = 15^\circ\text{C}$, vs. 20/15, $\Delta T = 5^\circ\text{C}$ in Experiment 3) with two exceptions. No significant differences were observed in the proportion of methoxylated anthocyanins between the 20/15 and 25/15 berries in Experiment 1; the proportion of 3'4'5'-substituted anthocyanins were higher in the 20/5 and 20/15 berries than in the 30/5 and 30/15 berries, respectively, in Experiment 3.

Temperature Effects on Flavonol Levels

The low temperature regimes consistently promoted flavonol level at harvest in the three experiments; the intermediate temperature regimes caused an intermediate flavonol level in Experiment 1 but comparable levels with those of the high temperature regime in Experiment 2 (Table 3). Similar results were observed during berry ripening in Experiments 1 and 2 (Figures 1C, D; Figures S2C, D; Figures S3C, D).

When regimes with the same day but different night temperatures were compared, a higher flavonol level was observed in the low temperature regime with a smaller difference between day/night temperatures (i.e., 20/15, $\Delta T =$

5°C , vs. 20/10, $\Delta T = 10^\circ\text{C}$) in Experiment 1 but not in Experiments 2 and 3 (Table 3). This was observed throughout the season in Experiment 1 (Figure 1C, Figure S2C, Figure S3C). On the other hand, when regimes with the same night but different day temperatures were compared, a smaller difference between day/night temperatures (e.g., 20/15, $\Delta T = 5^\circ\text{C}$, vs. 25/15, $\Delta T = 10^\circ\text{C}$, in Experiments 1 and 2; 20/15, $\Delta T = 5^\circ\text{C}$, vs. 30/15, $\Delta T = 15^\circ\text{C}$ in Experiment 3) was found to increase the flavonol level, except that no differences were found between the 25/20 and 30/20 berries in Experiment 2 in general.

Temperature Effects on Flavonol Profiles

Eleven flavonols were profiled in Merlot berry skin (Tables 3 and S4) in the three experiments. At harvest, the quercetin was the most abundant flavonol (constituting of 55.29%, 50.31%, 52.53% of total flavonols in Experiments 1, 2, and 3, respectively), and was present as glucoside, glucuronide, galactoside, and (rhamnosyl)glucoside (Table 3).

The temperature regimes affected the relative concentration of flavonol subfamilies among experiments, with the proportion of methoxylated (isorhamnetin and syringetin) and 3'4'5'-substituted (myricetin and syringetin) flavonols promoted by the high temperature regimes at harvest (Table 3). At the same stage, a lower proportion of 3'4'-substituted flavonols was observed in the high temperature regimes, but no differences

M, Q, K, I, S refer to myricetin, quercetin, kaempferol, isorhamnetin, and syringetin, respectively; glc refers to 3-O-glucoside, gal refers to 3-O-galactoside, ac-glc refers to 3-O-(6"-acetyl)glucoside, and ram-glc refers to 3-O-(6"-ramnosyl)glucoside; 4-sub, 3,4'-sub, 3,4''-sub, Meth, and Non-meth refer to the relative concentration of 4'-substituted flavonols, 3,4'-substituted flavonols, methoxylated flavonols, and non-methoxylated flavonols; *Flavonoid peaks were identified by UV-vis spectra at 353 nm; flavonoid concentrations were expressed as $\mu\text{g/g}$ skin FW based on quercetin 3-O-glucoside equivalents. Values reported are the mean \pm standard error (SE, $n = 4$). Different letters indicate significantly different means within each experiment according to an LSD test ($p \leq 0.05$). *DAA refers to days after anthrax.

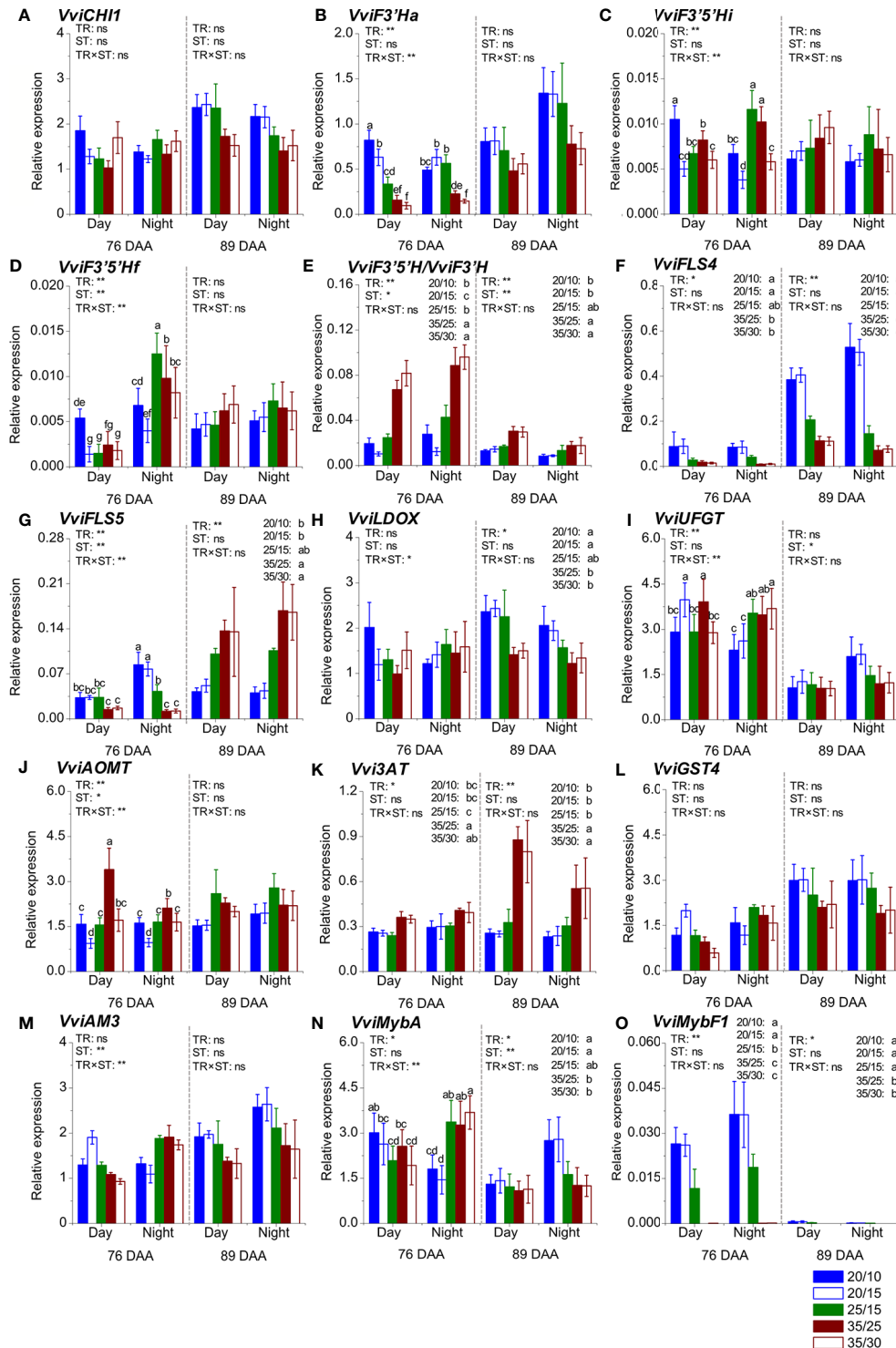


FIGURE 2 | Expression analyses of flavonoid genes in Experiment 1. Expression level of general flavonoid pathway genes (A–D); ratio of *VviF3'5'H* to *VviF3'H* expression (E); expression level of flavonoid biosynthesis genes (F, G); expression level of flavonoid pathway transcription factors (N, O) during the day and night at 76 and 89 DAA. Values were reported as relative expression levels to the expression of the reference gene *VviUbiquitin*. TR and ST indicate temperature regimes and sampling time (day or night), respectively. Values reported are the mean \pm standard error (SE, $n = 4$). Two-way ANOVA was used to assess the effect of temperature regimes, sampling time, and the interaction effect between temperature regimes and sampling time. *, **, and ns stand for $p \leq 0.05$, $p \leq 0.01$, and not significant, respectively. Different letters indicate significantly different means at each time point according to an LSD test ($p \leq 0.05$). DAA refers to days after anthesis.

berries during the day, and in the 25/15, 35/25, 35/30 berries at night. Despite the trend toward higher expression levels in the 20/15 and 20/10 berries than in the 35/25 and 35/30 berries at 89 DAA at night, the differences were not significant.

The highest and the lowest expression level of *anthocyanin O-methyl transferase* (*VviAOMT*) (**Figure 2J**) was found in the 35/25 and 20/15 berries, respectively, during the day at 76 DAA. Neither the temperature regimes nor the sampling time affected the expression at 89 DAA. The expression of *anthocyanidin 3-O-glucoside 6''-O-acyltransferase* (*Vvi3AT*) (**Figure 2K**) was consistently promoted by the high temperature regimes (*i.e.*, 35/25 and 35/30), particularly at 89 DAA.

Despite an interaction effect between the temperature regimes and the sampling time on the expression level being observed at 76 DAA, the expression of *VviMybA*—a key anthocyanin TF—was generally found to be higher in the low-temperature regimes (*i.e.*, 20/10 and 20/15) during the day, and in the intermediate- and high-temperature regimes (*i.e.*, 25/15, 35/25, and 35/30) at night (**Figure 2N**). At 89 DAA, the expression of *VviMybA* was ~2-fold higher in berries exposed to the low temperature regimes (*i.e.*, 20/10 and 20/15) than in berries exposed to other regimes. The TF that regulates flavonol biosynthesis, *VviMybF1*, was modulated similarly as its target *VviFLS4* (**Figure 2O**).

The difference between day/night temperatures did not affect the expression of most genes when regimes with the same day temperature were compared, except for *VviF3'Ha*, *VviF3'5'Hi*, *VviF3'5'Hf*, *VviUFGT*, and *VviAOMT*, which were affected at 76 DAA. Generally (except for *VviUFGT*), a larger difference between day/night temperatures (*e.g.*, 20/10, $\Delta T = 10^{\circ}\text{C}$, *vs.* 20/15, $\Delta T = 5^{\circ}\text{C}$) increased the expression level. When regimes with the same night temperature were compared, it was found that a smaller difference between day/night temperatures (*e.g.*, 20/15, $\Delta T = 5^{\circ}\text{C}$, *vs.* 25/15, $\Delta T = 10^{\circ}\text{C}$) increased the expression of *VviFLS4*, *VviLDOX*, and *VviMybF1* at one of the stages at least, while a larger difference between day/night temperatures (*e.g.*, 25/15, $\Delta T = 10^{\circ}\text{C}$, *vs.* 20/15, $\Delta T = 5^{\circ}\text{C}$) increased *Vvi3AT* expression at both stages. The expression of *VviFLS5* was increased in the 20/15 ($\Delta T = 5^{\circ}\text{C}$) berries at 76 DAA, but decreased in the same regime at 89 DAA, compared to the 25/15 berries ($\Delta T = 10^{\circ}\text{C}$).

DISCUSSION

The negative effect of elevated temperatures on anthocyanin accumulation has been well documented in grapes (Kliewer and Torres, 1972; Mori et al., 2007; Tarara et al., 2008; De Rosas et al., 2017). Our study employs a large range of temperature regimes, and the results indicate that the optimum temperature for anthocyanin accumulation in Merlot berries is around 20°C during the day. Mori et al. (2007) reported that high day temperature (35°C) reduced the total anthocyanin content in Cabernet Sauvignon berries to less than half of that in control (25°C). It is also observed in our study that a 5°C increase in day temperature from 20 to 25°C severely reduces the anthocyanin level (37%, in Experiment 1 and 2). Recently, De Rosas et al.

(2017) reported that a small increase in temperature (+2.5–3°C from an average of 24°C in controls) resulted in a ~40% and 28% to 41% loss of anthocyanins in Bonarda and Malbec berries, respectively.

Temperature was often reported to have no effect on flavonol accumulation. However, in our study, it has been observed that flavonol accumulation is strongly inhibited by the high temperature regimes. In addition, there is a reduction in flavonol accumulation in the intermediate temperature regimes as well, compared to the low temperature regimes (*e.g.*, 25/15 in Experiment 1, 25/15 and 25/20 in Experiment 2). Inconsistencies among studies might be attributed to the milder temperature treatments applied (Spayd et al., 2002), different grape varieties studied (Mori et al., 2005), different scales of the study conducted, *e.g.*, field studies (Cohen et al., 2008)—where various environmental factors can interact to temperature—or *in vitro* systems (Azuma et al., 2012)—where cultured tissues might affect cell metabolic responses. In this study, despite the consistent effects of temperature regimes on the total flavonol levels observed across the three experiments, these levels were on average lower in Experiments 2 (–56%) and 3 (–43%) than in Experiment 1. Flavonol biosynthesis in grapes is sensitive to light (Teixeira et al., 2013). Despite light intensity been set at the same level in the growth chambers, incident light measured at the cluster level was lower in Experiments 2 and 3 than in Experiment 1 (**Table S6**), likely determining the lower flavonol levels.

The reduction in berry weight observed with high temperature regimes in Experiment 1 was potentially caused by the higher vapour pressure deficit in the growth chambers with high temperatures (that were also characterized by lower humidity) as in Sweetman et al. (2014). Previous studies showed that higher vapour pressure deficits increase berry transpiration reducing berry water content and size (Zhang and Keller, 2015). In this study, temperature regimes also induced changes in the skin-to-berry weight ratio. Both concentrations and content per berry of anthocyanins and flavonols are similarly affected by the temperature regimes. Thus, differences in berry size and skin-to-berry weight ratio observed among the temperature regimes—which have been reported to affect anthocyanin concentration (Wong et al., 2016)—have a minor role in determining the differences in anthocyanin and flavonol concentration in the current study.

Our study reveals strong effects of high temperatures on anthocyanin and flavonol profiles. Higher relative abundance of methoxylated anthocyanins and flavonols, as well as acylated anthocyanins, are part of the Merlot response to high temperatures. Similarly, relative concentrations of methoxylated and acylated anthocyanins were increased in Malbec and Bonarda grapes grown under high temperatures (2.5–3°C higher than control temperatures) (De Rosas et al., 2017) and in Pinot noir grapes grown under high night temperature (30°C, *vs.* 15°C in control) (Mori et al., 2005).

Methoxylation and acylation have been known to enhance the chemical and thermal stability of anthocyanins (Liu et al., 2018). Therefore, the modification of anthocyanin and flavonol profiles under high temperatures might be promoted by the biosynthesis of more stable forms (*e.g.*, methoxylated anthocyanins and

flavonols) with a higher resistance to thermal degradation (Sadilova et al., 2006). However, the altered profiles under the high temperature regimes might also be due to an unpaired degradation that affected more severely the non-methoxylated and non-acylated species.

In this study, caffeoyl glucosides were not detected in Merlot grapes, a consistent finding with several other studies (Tarara et al., 2008; Dimitrovska et al., 2011; Cook et al., 2015; Hilbert et al., 2015; Sivilotti et al., 2016). However, caffeoyl glucosides have been found in Merlot grapes (Mazza et al., 1999; Liang et al., 2014; Shi et al., 2016; Shi et al., 2018). This suggests that growing conditions including environmental factors and viticultural strategies, or the clone chosen may determine the presence and/or the levels of caffeoyl glucosides in Merlot grapes. However, it is worth noting that caffeoyl glucosides are generally a small fraction (0.03–0.77% across 64 red grape varieties) of total anthocyanins (Mattivi et al., 2006), and when present it is unlikely that they would affect the general response of anthocyanins to temperature regimes.

The cultivation systems (e.g., potted vines grown in the growth chamber vs. vines grown in open fields) may affect the physiological response of grapes to temperature regimes; therefore, caution is required when interpreting the results of this study. However, in this study, anthocyanin levels and profiles as well as temperature-related changes were similar to the ones reported in open field studies (Tarara et al., 2008).

The expression of *VviUFGT*, which determines anthocyanin accumulation during ripening, is not affected by the temperature regimes in our study. Consistent with this finding, Mori et al. (2007) reported that the transcript abundance of *VviUFGT* was not affected by high temperature (35°C), while the activity of UFGT enzyme was increased, although the accumulation of anthocyanins decreased. These results suggest that the lower anthocyanin level in berries under the high temperature regimes might result from a different mechanism (post-transcriptional regulation or metabolite degradation). Moreover, Lecourieux et al. (2019) revealed that the transcriptome and proteome correlate poorly in heat-stressed berries and highlighted the importance of analysing the proteome for understanding the metabolite responses in heat-stress berries. Unlike with anthocyanins, the down-regulation of key flavonol genes such as *VviFLS4* (also named as *VviFLS1* in Lecourieux et al., 2017 and Sun et al., 2017) and *VviMybF1* in berries under the high temperature regimes matched the decreased level of flavonols, which suggests a transcriptional control of flavonols in response to the temperature regimes. Lecourieux et al. (2017) reported a strong inhibition of *VviFLS4* expression under heat stress during berry ripening, which led to reduced flavonol accumulation. *VviMybF1* is known to be the specific TF of *VviFLS4* (Czemmel et al., 2009; Czemmel et al., 2017), and a synchronous down-regulation of *VviFLS4* and *VviMybF1* has been observed in the high temperature regimes in the present study.

Anthocyanin accumulation in plants depends on the turnover between biosynthesis and degradation (Niu et al., 2017; Liu et al., 2018), and the latter was shown to be enhanced under high temperature conditions (Mori et al., 2007; Movahed et al., 2016;

Niu et al., 2017). Mori et al. (2007) reported the degradation of anthocyanins in grape berries incubated at 35°C by isotope tracing. Niu et al. (2017) investigated the biosynthesis and degradation of anthocyanins in plums, showing that even though the activity of the anthocyanin biosynthetic enzymes was increased by high temperature, 79% of anthocyanins were degraded by a class III peroxidase after 9 days of exposure at 35°C. In grapes, a recent study suggested a direct role of peroxidases on anthocyanin catabolism (Movahed et al., 2016). Protocatechuic acid, phloroglucinol acid, and 4-hydroxybenzoic acid have been reported as major degradation products of anthocyanins in black carrot, strawberry, and elderberry extracts subjected to high temperature (Sadilova et al., 2006; Sadilova et al., 2007). In our study, protocatechuic acid and 4-hydroxybenzoic acid, as well as other anthocyanin degradation products such as gallic acid and syringic acid (Yang et al., 2018), were detected in Merlot grapes by LC/MS-MS analysis (Figure S8, Table S5). However, since the abovementioned phenolic acids are commonly detected in unripe grapes (Eyduran et al., 2015), whether these compounds are accumulated in the berry from anthocyanin catabolism or *via* other pathways requires further investigation.

Despite the weak relationship between the changes induced by the temperature regimes on anthocyanin accumulation and gene expression (data not shown), the up-regulation of *Vvi3AT* matches the increased proportion of acylated anthocyanins observed in berries exposed to the high temperature regimes, suggesting a regulatory mechanism at the transcriptional level. However, the expression pattern of *VviAOMT* does not explain much about the increased methoxylated anthocyanin proportion in the high temperature regimes. This could be explained by a higher stability of the methoxylated forms at high temperatures (Jackman and Smith, 1996; Liu et al., 2018). However, as different methoxylation levels among temperature regimes were established soon after treatment application (Figure S5C), the time points chosen for gene expression analysis might have missed the stage when *VviAOMT* was modulated by temperatures, as well as we cannot exclude that other enzymes could have contributed to the final proportion of methoxylated anthocyanins under the different temperature regimes (Fournier-Level et al., 2011; Giordano et al., 2016). The relative concentration of 3'4'5'-substituted anthocyanins and flavonols is higher in the intermediate- and high-temperature regimes with comparison to the low temperature regimes in Experiments 1 and 2, as previously reported (Tarara et al., 2008; Pillet, 2011; Azuma et al., 2012; Pastore et al., 2017a), which matches the increased ratio of the *VviF3'5'5'Ha* (cumulative expression levels of *VviF3'5'5'Hi* and *VviF3'5'5'Hf*) to *VviF3'5'5'Ha*. However, a lower proportion of 3'4'5'-substituted anthocyanins is observed in berries exposed to the high temperature regimes in Experiment 3 at harvest (113 DAA). In previous studies, either an increase or decrease of the 3'4'5'-substituted proportion have been reported in response to high temperatures (Mori et al., 2007; Tarara et al., 2008; Pastore et al., 2017b). These discrepancies may be related to the developmental stages when treatments were applied. When the temperature treatments were applied one week before veraison, high temperature (30°C compared to 20°C)

induced a lower proportion of 3'4'5'-substituted anthocyanins in Pinot noir grapes *via* the up-regulation of *VviF3'H* expression (Mori et al., 2007). Similar results were reported in Sangiovese grapes exposed to high temperatures from one week before veraison (Pastore et al., 2017b). On the other hand, when temperature treatments were applied at veraison, high temperatures induced a higher proportion of 3'4'5'-substituted anthocyanins (Tarara et al., 2008). In the current study, temperature treatments were applied at the same time (veraison) in all experiments; however, small differences in the timing of treatment applications may have occurred and resulted in the inconsistent results observed for the 3'4'5'-substituted proportion in the response to the treatments among experiments. It is noteworthy that, in Experiment 2, despite that the proportion of 3'4'5'-substituted anthocyanin is higher in the high temperature regimes at harvest (135 DAA), it was not at 113 DAA (Figure S5B). This indicates that the effects of the temperature regimes on the anthocyanin profile can change depending on the developmental stage of the grapes.

Previous studies suggested that a larger difference between day/night temperatures, due to lower night temperatures, favoured anthocyanin accumulation (Mori et al., 2005; Gaiotti et al., 2018). In our study, it has been observed that comparing the regimes with the same day but different night temperatures, the difference between day/night temperatures has no effect on either anthocyanin and flavonol accumulation or their profiles, except for flavonol levels that are higher in the 20/15 than in the 20/10 berries in Experiment 1. Remarkably, the difference between day/night temperatures affects anthocyanin levels, only when a day/night temperature difference of 25°C (30/5, in Experiment 3) is applied, compared with day/night temperature differences of 5°C (30/25) or 15°C (20/15). When the grapevines are subjected to regimes with the same night but different day temperatures, an effect on anthocyanin and flavonol accumulation is observed. The lack of effects of day/night temperature differences when the same day temperature is considered (except for 20/10 *vs.* 20/15 for flavonols in Experiment 1 and 30/5 *vs.* 30/15 and 30/25 for anthocyanins in Experiment 3), as well as the fact that within the regimes that

have the same night temperature, anthocyanins, and flavonols change according to day temperatures, suggest that day temperature has a stronger effect than night temperature on anthocyanin and flavonol biosynthesis or/and degradation. A stronger effect of day than night temperature on anthocyanin accumulation was recently postulated by Gouot et al. (2018).

Previous studies have shown a circadian control on the expression of the flavonoid pathway genes and related TFs in *Arabidopsis* seeds (Harmer et al., 2000). In our study, out of 14 genes tested, six (*VviF3'5'Hf*, *VviFLS5*, *VviUFGT*, *VviAM3*, *VviMybA*, and *VviAOMT*) are affected by the sampling time (day *vs.* night) regardless of the temperature regimes and most of the genes that change in their expression between day/night have higher expression levels at night. Rienth et al. (2014b) showed that the expression pattern of the flavonoid pathway genes was distinct between day/night at veraison, but only a few showed consistent patterns at other developmental stages. Our study focuses on the expression analysis of two developmental stages (76 and 89 DAA), and clear trends are observed only for the six genes reported above; further studies that consider more developmental stages during fruit ripening are required to better understand a potential circadian regulation of the expression of flavonoid genes and its impact on flavonoid biosynthesis.

TSS and TA level are useful indicators of grape ripeness and quality. Overall, TSS levels at harvest were lower under high temperature regimes as in Kliever (1977) and Mori et al. (2005), although the differences were not statistically significant in Experiment 1. Sugars regulate the anthocyanin accumulation in grape berry, and increased sugar level at veraison was reported to trigger the anthocyanin biosynthesis (Dai et al., 2014). Linear regression analysis reveals that there is a decoupling between sugar and anthocyanin levels under the high temperature regimes (Figure 3). There was a much more distinct decoupling of TSS and anthocyanin accumulation in regimes with the same night but different day temperatures, whereas this decoupling was absent or minor between regimes with the same day but different night temperatures (Figure 3, Tables S7 and S8). Sadras and Moran (2012; 2013) reported that the decoupling

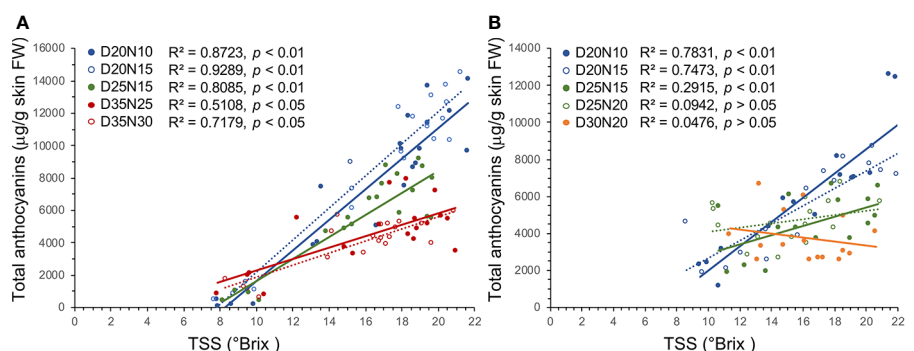


FIGURE 3 | Linear regression analysis between sugar and anthocyanin accumulation during berry ripening in Merlot grapes exposed to different temperature regimes in Experiments 1 (A) and 2 (B).

of anthocyanin and sugar accumulation under elevated temperatures (1–3°C increase) during early stages of berry development was due to a delayed onset of anthocyanin accumulation during berry ripening. However, the decoupling of TSS and anthocyanin accumulation observed in the current study might be either the result of a lower rate of anthocyanin accumulation and/or a higher rate of anthocyanin degradation in the high temperature regimes (**Figure 3**). Arrizabalaga et al. (2018) suggested that the lower anthocyanin levels in grapes grown under high day/night temperature conditions (28/18°C compared with 24/14°C) were due to a relatively lower rate rather than a delayed rate of anthocyanin accumulation.

In this study, the TA decrease during berry ripening was stronger under high temperature regimes. The TA decrease during ripening is normally attributed to the degradation of malic acid which is increased by elevated temperatures (Ruffner et al., 1976; Bergqvist et al., 2001; Rienth et al., 2016). Sweetman et al. (2014) showed an increased sensitivity of malate metabolism to heating treatments during the day than at night. Consistently, in this study, regimes with the same high day temperature and different night temperatures resulted in similar TA levels (**Table 1**; **Figure S1**).

In conclusion, the application of the temperature regimes from veraison to harvest strongly affected anthocyanin and flavonol accumulation and shifted their profiles. Lower temperatures promoted anthocyanin and flavonol accumulation. Despite our initial hypothesis, no or limited effects of the difference between day/night temperatures were observed on anthocyanins and flavonols when regimes with the same day but different night temperatures were compared. On the contrary, when regimes with the same night but different day temperatures were compared, it was observed that a larger difference between day/night temperatures decreased the anthocyanin levels but increased the relative concentration of methoxylated and acylated anthocyanins as well as methoxylated and 3'4'5'-substituted flavonols. Consequently, we conclude that day temperature exerts stronger effects than night temperature on anthocyanin and flavonol accumulation in Merlot grapes. Despite the gene expression analysis was conducted only in one of the three experiments, this study reveals inconsistent responses to the temperature regimes among anthocyanin genes, which indicates that the major effect observed on anthocyanin levels and profiles might result from anthocyanin degradation at high temperatures and from a different rate of degradation among anthocyanin species. Conversely, the effect of the temperature regimes on flavonol levels was consistent with that observed on the levels of transcripts of key flavonol genes, thus suggesting a major regulatory mechanism at the transcriptional level. Gene expression was assessed only at two developmental stages that corresponded to the maximum rates of anthocyanin accumulation (**Figure 1**) and highest gene expression of anthocyanin related genes (Castellarin et al., 2007; Castellarin and Di Gaspero, 2007); however, the expression levels at other ripening stages might also have affected the anthocyanin levels and profiles at harvest. Molecules previously reported as final products of anthocyanin degradation were detected but they could not be quantified. For these reasons, further studies will be aimed on integrating a comprehensive characterization of gene expression

profiles, enzyme activities, and the evolution of degradation products during grape ripening.

DATA AVAILABILITY STATEMENT

All data sets presented in this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

YY performed all the experiments and drafted the manuscript. CS collaborated with YY in performing the greenhouse and lab experiments. LF contributed to the data analysis and critically reviewed the manuscript. SC designed the study, analysed the data with YY and LF, and wrote the manuscript with YY. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by Natural Science and Engineering Research Council of Canada (NSERC RGPIN-2015-04760), the Canada Research Chair (950-230913) program, and China Scholarship Council (CSC).

ACKNOWLEDGMENT

We would like to thank Lufiani Lina Madilao for supervising the LC/MS-MS analysis and Nolwenn Paugam for helping processing the samples.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.01095/full#supplementary-material>

FIGURE S1 | Temperature effects on total soluble solids (TSS, **A** and **B**) and titratable acidity (TA, **C** and **D**). Values reported are the mean \pm standard error (SE, $n = 4$). Different letters indicate significantly different means according to an LSD test ($p \leq 0.05$). Legend in (**A**) indicates the temperature regimes in (**A**, **C**); legend in (**B**) indicates the temperature regimes in (**B**, **D**). DAA refers to days after anthesis.

FIGURE S2 | Temperature effects on anthocyanin (**A**, **B**) and flavonol (**C**, **D**) concentration ($\mu\text{g/g}$ berry FW) in Merlot grapes in Experiments 1 (**A**, **C**) and 2 (**B**, **D**). Values reported are the mean \pm standard error (SE, $n = 4$). Different letters indicate significant different means according to an LSD test ($p \leq 0.05$). Legend in (**A**) indicates the temperature regimes in (**A**, **C**); legend in (**B**) indicates the temperature regimes in B and D. DAA refers to days after anthesis.

FIGURE S3 | Temperature effects on anthocyanin (**A**, **B**) and flavonol (**C**, **D**) content ($\mu\text{g/berry}$) in Merlot grapes in Experiments 1 (**A**, **C**) and 2 (**B**, **D**). Values reported are the mean \pm standard error (SE, $n = 4$). Different letters indicate

significant different means according to an LSD test ($p \leq 0.05$). Legend in (A) indicates the temperature regimes in (A, C); legend in (B) indicates the temperature regimes in (B, D). DAA refers to days after anthesis.

FIGURE S4 | Temperature effects on the evolution of the relative concentration of acylated anthocyanins during berry ripening in Experiments 1 (A, C, E) and 2 (B, D, F). Values represent the mean \pm standard error (SE, $n = 4$). Different letters indicate significantly different means according to an LSD test ($p \leq 0.05$). Legend in (A) indicates the temperature regimes in (A, C, E); legend in (B) indicates the temperature regimes in (B, D, F). DAA refers to days after anthesis.

FIGURE S5 | Temperature effects on the evolution of the relative concentration of 3',4',5'-substituted (A, B) and methoxylated (C, D) anthocyanins during berry ripening in Experiments 1 (A, C) and 2 (B, D). Values represent the mean \pm standard error (SE, $n = 4$). Different letters represent significantly different means according to an LSD test ($p \leq 0.05$). Legend in (A) indicates the temperature regimes in (A, C) legend in (B) indicates the temperature regimes in (B, D). DAA refers to days after anthesis.

REFERENCES

- Arrizabalaga, M., Morales, F., Oyarzun, M., Delrot, S., Gomès, E., Irigoyen, J. J., et al. (2018). Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. *Plant Sci.* 267, 74–83. doi: 10.1016/j.plantsci.2017.11.009
- Azuma, A., Yakushiji, H., Koshita, Y., and Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta* 236 (4), 1067–1080. doi: 10.1007/s00425-012-1650-x
- Bergqvist, J., Dokoozlian, N., and Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. *Am. J. Enol. Viticult.* 52 (1), 1–7.
- Bogs, J., Ebadi, A., McDavid, D., and Robinson, S. P. (2006). Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. *Plant Physiol.* 140 (1), 279–291. doi: 10.1104/pp.105.073262
- Carbonell-Bejerano, P., Diago, M. P., Martínez-Abaigar, J., Martínez-Zapater, J. M., Tardaguila, J., and Núñez-Olivera, E. (2014). Solar ultraviolet radiation is necessary to enhance grapevine fruit ripening transcriptional and phenolic responses. *BMC Plant Biol.* 14 (1):183. doi: 10.1186/1471-2229-14-183
- Castellarin, S. D., and Di Gaspero, G. (2007). Transcriptional control of anthocyanin biosynthetic genes in extreme phenotypes for berry pigmentation of naturally occurring grapevines. *BMC Plant Biol.* 7 (1):46. doi: 10.1186/1471-2229-7-46
- Castellarin, S. D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., and Di Gaspero, G. (2007). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell Environ.* 30 (11), 1381–1399. doi: 10.1111/j.1365-3040.2007.01716.x
- Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., and Hermosín-Gutiérrez, I. (2007). Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *J. Agric. Food Chem.* 55 (3), 992–1002. doi: 10.1021/jf062800k
- Cohen, S. D., Tarara, J. M., and Kennedy, J. A. (2008). Assessing the impact of temperature on grape phenolic metabolism. *Anal. Chim. Acta* 621 (1), 57–67. doi: 10.1016/j.aca.2007.11.029
- Cook, M. G., Zhang, Y., Nelson, C. J., Gambetta, G., Kennedy, J. A., and Kurtural, S. K. (2015). Anthocyanin composition of Merlot is ameliorated by light microclimate and irrigation in central California. *Am. J. Enol. Viticult.* 66 (3), 266–278. doi: 10.5344/ajev.2015.15006
- Czemmel, S., Stracke, R., Weisshaar, B., Cordon, N., Harris, N. N., and Bogs, J. (2009). The grapevine R2R3-MYB transcription factor *VvMYB1* regulates flavonol synthesis in developing grape berries. *Plant Physiol.* 151 (3), 1513–1530. doi: 10.1104/pp.109.142059
- Czemmel, S., Höll, J., Loyola, R., Arce-Johnson, P., Alcalde, J. A., Matus, J. T., et al. (2017). Transcriptome-wide identification of novel UV-B and light modulated flavonol pathway genes controlled by *VvMYB1*. *Front. Plant Sci.* 8:1084:1084. doi: 10.3389/fpls.2017.01084
- Dai, Z. W., Meddar, M., Renaud, C., Merlin, I., Hilbert, G., and Gomès, E. (2014). Long-term *in vitro* culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. *J. Exp. Bot.* 65 (16), 4665–4677. doi: 10.1093/jxb/ert489
- De Rosas, I., Ponce, M. T., Malovini, E., Deis, L., Cavnagnaro, B., and Cavnagnaro, P. (2017). Loss of anthocyanins and modification of the anthocyanin profiles in grape berries of Malbec and Bonarda grown under high temperature conditions. *Plant Sci.* 258, 137–145. doi: 10.1016/j.plantsci.2017.01.015
- Degu, A., Ayenew, B., Cramer, G. R., and Fait, A. (2016). Polyphenolic responses of grapevine berries to light, temperature, oxidative stress, abscisic acid and jasmonic acid show specific developmental-dependent degrees of metabolic resilience to perturbation. *Food Chem.* 212, 828–836. doi: 10.1016/j.foodchem.2016.05.164
- Dimitrovska, M., Bocevska, M., Dimitrovski, D., and Murkovic, M. (2011). Anthocyanin composition of Vranec, Cabernet Sauvignon, Merlot and Pinot Noir grapes as indicator of their varietal differentiation. *Eur. Food Res. Technol.* 232 (4), 591–600. doi: 10.1007/s00217-011-1425-9
- Downey, M. O., and Rochfort, S. (2008). Simultaneous separation by reversed-phase high-performance liquid chromatography and mass spectral identification of anthocyanins and flavonols in Shiraz grape skin. *J. Chromatogr. A* 1201 (1), 43–47. doi: 10.1111/j.1755-0238.2004.tb00008.x
- Eyduran, S. P., Akin, M., Ercisli, S., Eyduran, E., and Maghradze, D. (2015). Sugars, organic acids, and phenolic compounds of ancient grape cultivars (*Vitis vinifera* L.) from Igdir province of Eastern Turkey. *Biol. Res.* 48 (1), 2. doi: 10.1186/0717-6287-48-2
- Fournier-Level, A., Huguency, P., Verriès, C., This, P., and Ageorges, A. (2011). Genetic mechanisms underlying the methylation level of anthocyanins in grape (*Vitis vinifera* L.). *BMC Plant Biol.* 11 (1), 179. doi: 10.1186/1471-2229-11-179
- Gaiotti, F., Pastore, C., Filippetti, I., Lovat, L., Belfiore, N., and Tomasi, D. (2018). Low night temperature at veraison enhances the accumulation of anthocyanins in Corvina grapes (*Vitis vinifera* L.). *Sci. Rep.* 8 (1), 1–13. doi: 10.1038/s41598-018-26921-4
- Giordano, D., Provenzano, S., Ferrandino, A., Vitali, M., Pagliarini, C., Roman, F., et al. (2016). Characterization of a multifunctional caffeoyl-CoA O-methyltransferase activated in grape berries upon drought stress. *Plant Physiol. Biochem.* 101, 23–32. doi: 10.1016/j.plaphy.2016.01.015
- Gouot, J. C., Smith, J. P., Holzapfel, B. P., Walker, A. R., and Barril, C. (2018). Grape berry flavonoids: A review of their biochemical responses to high and extreme high temperatures. *J. Exp. Bot.* 70 (2), 397–423. doi: 10.1093/jxb/ery392
- Harmer, S. L., Kay, S. A., Hogenesch, J. B., Straume, M., and Bay, S. A. (2000). Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. *Science* 290 (5499), 2110–2113. doi: 10.1126/science.290.5499.2110
- He, J., and Giusti, M. M. (2010). Anthocyanins: Natural colorants with health-promoting properties. *Annu. Rev. Food Sci. Technol.* 1, 163–187. doi: 10.1146/annurev.food.080708.100754

- Hilbert, G., Soyer, J. P., Molot, C., Giraudon, J., Milin, M., and Gaudillere, J. P. (2015). Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis* 42 (2), 69.
- Jackman, R. L., and Smith, J. L. (1996). "Anthocyanins and betalains," in *Natural Food Colorants* (Boston, MA: Springer), 244–309. doi: 10.1007/978-1-4615-2155-6_8
- Jones, G. V., Reid, R., and Vilks, A. (2012). "Climate, grapes, and wine: Structure and suitability in a variable and changing climate," in *The Geography of Wine* (Dordrecht: Springer), 109–133. doi: 10.1007/978-94-007-0464-0_7
- Kliewer, W. M., and Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. *Am. J. Enol. Viticult.* 23 (2), 71 LP–71 77.
- Kliewer, W. M. (1977). Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. *Am. J. Enol. Viticult.* 28 (2), 96–103.
- Lachman, J., and Hamouz, K. (2005). Red and purple coloured potatoes as a significant antioxidant source in human nutrition - A review. *Plant Soil Environ.* 51 (11), 477–482.
- Lecourieux, F., Kappel, C., Pieri, P., Charon, J., Pillet, J., and Lecourieux, D. (2017). Dissecting the biochemical and transcriptomic effects of a locally applied heat treatment on developing Cabernet sauvignon grape berries. *Front. Plant Sci.* 8, 53. doi: 10.3389/fpls.2017.00053
- Lecourieux, D., Kappel, C., Claverol, S., Pieri, P., Feil, R., and Lecourieux, F. (2019). Proteomic and metabolomic profiling underlines the stage- and time-dependent effects of high temperature on grape berry metabolism. *J. Integr. Plant Biol.* 1–27. doi: 10.1111/jipb.12894
- Liang, N. N., Zhu, B. Q., Han, S., Wang, J. H., Pan, Q. H., Reeves, M. J., et al. (2014). Regional characteristics of anthocyanin and flavonol compounds from grapes of four *Vitis vinifera* varieties in five wine regions of China. *Food Res. Int.* 64, 264–274. doi: 10.1016/j.foodres.2014.06.048
- Liu, Y., Tikunov, Y., Schouten, R. E., Marcelis, L. F., Visser, R. G., and Bovy, A. (2018). Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: A review. *Front. Chem.* 6:52:52. doi: 10.3389/fchem.2018.00052
- Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., and Velasco, R. (2006). Metabolite profiling of grape: Flavonols and anthocyanins. *J. Agric. Food Chem.* 54 (20), 7692–7702. doi: 10.1021/jf061538c
- Matus, J. T., Loyola, R., Vega, A., Peña-Neira, A., Bordeu, E., and Alcalde, J. A. (2009). Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin and flavonol synthesis in berry skins of *Vitis vinifera*. *J. Exp. Bot.* 60 (3), 853–867. doi: 10.1093/jxb/ern336
- Mazza, G., and Miniati, E. (1993). "Anthocyanins in *Vitis vinifera*," in *Anthocyanins in Fruits, Vegetables, and Grains* (Boca Raton, FL, USA: CRC Press Inc.), 150–153.
- Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., and Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. *J. Agric. Food Chem.* 47 (10), 4009–4017. doi: 10.1021/jf990449f
- Mori, K., Saito, H., Goto-Yamamoto, N., Kitayama, M., Kobayashi, S., and Hashizume, K. (2005). Effects of abscisic acid treatment and night temperatures on anthocyanin composition in Pinot noir grapes. *Vitis-Geil Weilerhof* 44 (4), 161–165.
- Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58 (8), 1935–1945. doi: 10.1093/jxb/erm055
- Movahed, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., et al. (2016). The grapevine VviPrx31 peroxidase as a candidate gene involved in anthocyanin degradation in ripening berries under high temperature. *J. Plant Res.* 129 (3), 513–526. doi: 10.1007/s10265-016-0786-3
- Mullins, M. G., and Rajasekaran, K. (1981). Fruiting cuttings: Revised method for producing test plants of grapevine cultivars. *Am. J. Enol. Viticult.* 32 (1), 35 LP–35 40.
- Niu, J., Zhang, G., Zhang, W., Goltsev, V., Sun, S., Wang, J., et al. (2017). Anthocyanin concentration depends on the counterbalance between its synthesis and degradation in plum fruit at high temperature. *Sci. Rep.* 7 (1), 1–16. doi: 10.1038/s41598-017-07896-0
- Pastore, C., Allegro, G., Valentini, G., Muzzi, E., and Filippetti, I. (2017a). Anthocyanin and flavonol composition response to veraison leaf removal on Cabernet Sauvignon, Nero d'Avola, Raboso Piave and Sangiovese *Vitis vinifera* L. cultivars. *Sci. Hortic.* 218, 147–155. doi: 10.1016/j.scienta.2017.01.048
- Pastore, C., Dal Santo, S., Zenoni, S., Movahed, N., Allegro, G., and Tornielli, G. B. (2017b). Whole plant temperature manipulation affects flavonoid metabolism and the transcriptome of grapevine berries. *Front. Plant Sci.* 8, 929. doi: 10.1186/1471-2229-13-30
- Pillet, J. (2011). *Impact du microclimat sur le métabolisme de la baie de raisin* (Bordeaux 2: Doctoral dissertation).
- Rienth, M., Torregrosa, L., Kelly, M. T., Luchaire, N., Pellegrino, A., and Romieu, C. (2014a). Is transcriptomic regulation of berry development more important at night than during the day? *PloS One* 9 (2), e88844. doi: 10.1371/journal.pone.0088844
- Rienth, M., Torregrosa, L., Luchaire, N., Chatbanyong, R., Lecourieux, D., and Romieu, C. (2014b). Day and night heat stress trigger different transcriptomic responses in green and ripening grapevine (*Vitis vinifera*) fruit. *BMC Plant Biol.* 14 (1), 108. doi: 10.1186/1471-2229-14-108
- Rienth, M., Torregrosa, L., Sarah, G., Ardisson, M., Brillouet, J. M., and Romieu, C. (2016). Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome. *BMC Plant Biol.* 16 (1), 164. doi: 10.1186/s12870-016-0850-0
- Ruffner, H. P., Hawker, J. S., and Hale, C. R. (1976). Temperature and enzymic control of malate metabolism in berries of *Vitis vinifera*. *Phytochemistry* 15 (12), 1877–1880. doi: 10.1016/S0031-9422(00)88835-4
- Sadilova, E., Stintzing, F. C., and Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *J. Food Sci.* 71 (8), C504–C512. doi: 10.1111/j.1750-3841.2006.00148.x
- Sadilova, E., Carle, R., and Stintzing, F. C. (2007). Thermal degradation of anthocyanins and its impact on color and in vitro antioxidant capacity. *Mol. Nutr. Food Res.* 51 (12), 1461–1471. doi: 10.1002/mnfr.200700179
- Sadras, V. O., and Moran, M. A. (2012). Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. *Aust. J. Grape Wine R.* 18 (2), 115–122. doi: 10.1111/j.1755-0238.2012.00180.x
- Sadras, V. O., and Moran, M. A. (2013). Nonlinear effects of elevated temperature on grapevine phenology. *Agric. For. Meteorol.* 173, 107–115. doi: 10.1016/j.agrformet.2012.10.003
- Shi, P. B., Yue, T. X., Ai, L. L., Cheng, Y. F., Meng, J. F., Li, M. H., et al. (2016). Phenolic compound profiles in grape skins of Cabernet Sauvignon, Merlot, Syrah and Marselan cultivated in the Shacheng area (China). *South Afr. J. Enol. Vitic* 37 (2), 132–138. doi: 10.21548/37-2-898
- Shi, P., Song, C., Chen, H., Duan, B., Zhang, Z., and Meng, J. (2018). Foliar applications of iron promote flavonoids accumulation in grape berry of *Vitis vinifera* cv. Merlot grown in the iron deficiency soil. *Food Chem.* 253, 164–170. doi: 10.1016/j.foodchem.2018.01.109
- Shirley, B. W. (1996). Flavonoid biosynthesis: 'New' functions for an 'old' pathway. *Trends Plant Sci.* 1 (11), 377–382. doi: 10.1111/j.1755-0238.2012.00180.x
- Sivilotti, P., Herrera, J. C., Lisjak, K., Baša Česnik, H., Sabbatini, P., Peterlunger, E., et al. (2016). Impact of leaf removal, applied before and after flowering, on anthocyanin, tannin, and methoxypyrazine concentrations in 'Merlot' (*Vitis vinifera* L.) grapes and wines. *J. Agric. Food Chem.* 64 (22), 4487–4496. doi: 10.1021/acs.jafc.6b01013
- Spayd, S., Tarara, J., Mee, D., and Ferguson, J. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Viticult.* 53 (3), 171–182.
- Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M. M., Allen, S. K., Boschung, J., et al. (2014). "Climate change 2013: The physical science basis", in *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge: Cambridge University Press), 1535.
- Sun, R. Z., Cheng, G., Li, Q., He, Y. N., Wang, Y., Lan, Y. B., et al. (2017). Light-induced variation in phenolic compounds in Cabernet Sauvignon grapes (*Vitis vinifera* L.) involves extensive transcriptome reprogramming of biosynthetic enzymes, transcription factors, and phytohormonal regulators. *Front. Plant Sci.* 8, 547. doi: 10.3389/fpls.2017.00547
- Sweetman, C., Sadras, V. O., Hancock, R. D., Soole, K. L., and Ford, C. M. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65 (20), 5975–5988. doi: 10.1093/jxb/eru343
- Tarara, J. M., Lee, J., Spayd, S. E., and Scagel, C. F. (2008). Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in 'Merlot' grapes. *Am. J. Enol. Viticult.* 59 (3), 235–247. doi: 10.3390/ijms10125350

- Teixeira, A., Eiras-Dias, J., Castellarin, S., and Gerós, H. (2013). Berry phenolics of grapevine under challenging environments. *Int. J. Mol. Sci.* 14 (9), 18711–18739. doi: 10.3390/ijms140918711
- Wang, J., Abbey, T., Kozak, B., Madilao, L. L., Tindjau, R., and Castellarin, S. D. (2019). Evolution over the growing season of volatile organic compounds in viognier (*Vitis vinifera* L.) grapes under three irrigation regimes. *Food Res. Int.* 108512. doi: 10.1016/j.foodres.2019.108512
- Wong, D. C. J., Gutierrez, R. L., Dimopoulos, N., Gambetta, G. A., and Castellarin, S. D. (2016). Combined physiological, transcriptome, and cis-regulatory element analyses indicate that key aspects of ripening, metabolism, and transcriptional program in grapes (*Vitis vinifera* L.) are differentially modulated accordingly to fruit size. *BMC Genomics* 17 (1), 416. doi: 10.1186/s12864-016-2660-z
- Yamane, T., Seok, T. J., Goto-Yamamoto, N., Koshita, Y., and Kobayashi, S. (2006). Effects of temperature on anthocyanin biosynthesis in grape berry skins. *Am. J. Enol. Viticult.* 57 (1), 54–59. doi: 10.1007/s10623-017-0337-5
- Yang, P., Yuan, C., Wang, H., Han, F., Liu, Y., Wang, L., et al. (2018). Stability of anthocyanins and their degradation products from cabernet sauvignon red wine under gastrointestinal pH and temperature conditions. *Molecules* 23 (2), 354. doi: 10.3390/molecules23020354
- Zhang, Y., and Keller, M. (2015). Grape berry transpiration is determined by vapor pressure deficit, cuticular conductance, and berry size. *Am. J. Enol. Vitic* 66 (4), 454–462. doi: 10.5344/ajev.2015.15038
- Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Copyright © 2020 Yan, Song, Falginella and Castellarin. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Improving Net Photosynthetic Rate and Rooting Depth of Grapevines Through a Novel Irrigation Strategy in a Semi-Arid Climate

Xiaochi Ma^{1,2*}, Pete W. Jacoby¹ and Karen A. Sanguinet¹

¹ Department of Crop and Soil Sciences, Washington State University, Pullman, WA, United States, ² Department of Plant Sciences, University of California – Davis, Davis, CA, United States

OPEN ACCESS

Edited by:

Chris Winefield,
Lincoln University, New Zealand

Reviewed by:

Honghai Luo,
Shihezi University, China
Hipólito Medrano,
University of the Balearic Islands,
Spain
José Manuel Mirás-Avalos,
University of Santiago de Compostela,
Spain

*Correspondence:

Xiaochi Ma
xchma@ucdavis.edu

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 23 June 2020

Accepted: 13 August 2020

Published: 27 August 2020

Citation:

Ma X, Jacoby PW and Sanguinet KA
(2020) Improving Net Photosynthetic
Rate and Rooting Depth of Grapevines
Through a Novel Irrigation
Strategy in a Semi-Arid Climate.
Front. Plant Sci. 11:575303.
doi: 10.3389/fpls.2020.575303

Direct root-zone irrigation (DRZ) is a novel subsurface irrigation strategy initially tested in vineyards for economizing water and securing grape production in arid regions with unstable climatic patterns. However, studies are lacking on the responses of grapevine leaf carbon assimilation and deep rooting patterns to the novel irrigation strategy, which are essential for optimizing grapevine growth and alleviating extreme water stress during periods of heat and drought. Thus, a two-year field study was conducted in a commercial vineyard of Cabernet Sauvignon (*Vitis vinifera* L.) under a semi-arid climate in Washington, USA to compare the differences in leaf gas exchange and root distribution along the 0–160 cm soil profile, combined with measurements of specific leaf area and total carbon and nitrogen content in leaves and shoots to compare DRZ and traditional surface drip irrigation (SD) under three watering regimes. Compared to SD, significantly higher rates of net CO₂ assimilation, stomatal conductance and transpiration in leaves, which positively correlated to midday stem water potential, were found in grapevines irrigated through DRZ in both years. Meanwhile, DRZ reduced total root number by 50–60% and root length density (RLD) by 30–40% in the upper 60 cm soil at high (0.75–0.80 crop evapotranspiration) and moderate (0.60–0.65 crop evapotranspiration) irrigation rates, but no significant differences were found at low (0.45–0.50 crop evapotranspiration) irrigation rate between DRZ and SD. Higher root number and RLD were detected under DRZ within 60–160 cm soil depths, accompanied by a decreased ratio of total carbon to nitrogen content in leaves with slightly increased specific leaf area. Decreased rainfall and increased temperature in 2018 possibly amplified the positive effects of DRZ. Our study indicates that grapevines under DRZ could develop deeper roots for water uptake, which helps ameliorate water stress and improve the photosynthetic rate as well as enhance grapevine adaptation to semi-arid climates.

Keywords: direct root-zone irrigation, drought, photosynthesis, leaf gas exchange, root development, *Vitis vinifera*, minirhizotron, water management

INTRODUCTION

Grapevines (*Vitis* spp.) are one of the most important horticultural crops worldwide in terms of economic and social values. In many wine-growing regions, efficient water management in vineyards helps regulate vegetative growth of grapevines and optimize the balance between yield and berry quality (Bernardo et al., 2018). For arid regions where the limited precipitation hardly supports grapevine growth, irrigation plays a significant role in offsetting the water deficit (Alexandratos and Bruinsma, 2012; Fraga et al., 2018; Malek et al., 2018). In contrast, excessive and highly localized irrigation leads to soil hypoxia and salinity, excessive leaching, and increased energy use for pumping, which might also cause adverse effects on grapevine growth and production, groundwater contamination and a rapid decline in groundwater levels (Drew, 1997; Scanlon et al., 2012; Kisekka et al., 2019; Zhang et al., 2020). Moreover, unstable climate patterns and increased demand of agricultural water for food production intensify the pressure on global water resources (Mbow and Rosenzweig, 2019). Thus, development of efficient irrigation strategies is necessary to sustain viticulture and improve water productivity, achieving “more crop per drop” (Davies and Bennett, 2015; Costa et al., 2016).

Compared to surface irrigation systems, the application of deficit irrigation through regulated subsurface micro-irrigation systems could be a more efficient means to regulate grapevine growth, while enhancing crop water use efficiency (WUE_c , yield per unit area per unit of applied water) and sustaining the vineyard management (e.g. reduce evapotranspiration, restrict water availability for weed growth), especially in arid areas with limited water supply (Ayars et al., 2015; Ma et al., 2020). With upgraded irrigation equipment and scheduling tools in recent decades, the benefits have been demonstrated through studies both on annual row crops (Bhattarai et al., 2008; Zaccaria et al., 2017; Murley et al., 2018) and woody perennial crops (Zhang et al., 2017; Martínez-Gimeno et al., 2018; Paris et al., 2018; Pisciotto et al., 2018). However, additional improvements in subsurface irrigation systems, such as easier access for belowground maintenance and convenient adjustments to water delivery depth, are required for maximizing the benefits of the investment, since the initial costs for subsurface irrigation are usually higher than those for traditional surface irrigation systems (Payero et al., 2005; Lamm et al., 2012; Ma et al., 2020).

Direct root-zone irrigation (DRZ) is a novel subsurface micro-irrigation system which was initially tested in vineyards and showed promise for improving WUE_c and sustaining grape yield and quality in a semi-arid climate (Jacoby and Ma, 2018). Compared to traditional irrigation systems, DRZ flexibly adjusts the installation position and water delivery depth, concurrently providing easier access for belowground system maintenance (Ma et al., 2019). In a scenario of climatic change, grapevines have a high demand for supplemental irrigation especially in areas with seasonal drought (Bonada et al., 2015; Bernardo et al., 2018; Douthe et al., 2018). However, more details regarding the physiological performance of grapevines under DRZ are needed to finely tune its application in vineyards and provide empirical

evidence to avoid detrimental effects on grapevine growth from improper water deficit that is regulated by irrigation.

The root system is indispensable for plant growth and survival owing to its function in water and nutrient uptake (Volder et al., 2004; Osmont et al., 2007; Volder et al., 2009), while woody portions of the root system provide structure support for aboveground growth (Comas et al., 2010). Irrigation significantly affects root growth especially in arid regions as it influences soil water availability (Sánchez-Blanco et al., 2019), which also influences plant water status and leaf gas exchange (Koundouras et al., 2008; Ko and Piccinini, 2009). Previous studies showed that DRZ restricts shallow root growth to potentially minimize the negative influence of fluctuations in precipitation and soil moisture within the top soil profile (Ma et al., 2020). Additionally, the deep root system of grapevines is vital for sustainable growth due to its potential to support grapevine throughout periods of drought and heat during the summer months (Savi et al., 2018). However, the effects of DRZ and other subsurface irrigation strategies on root growth in the deep soil profile (below drip pipes) and how it correlates to leaf gas exchange and water status in perennial crops (e.g. grapevine) are not clear.

We previously found that DRZ irrigation rate and not delivery depth was crucial to maintain grapevine water status and mitigate stress (Ma et al., 2019). In addition, we found that DRZ increased grape yields by 9–12% compared to traditional surface drip irrigation (SD) and that grapevine rooting was decreased in the top 60 cm soil profile suggesting that DRZ promoted deeper root growth (Ma et al., 2020). To provide further insight into root development of grapevines particularly in the deep soil profile and to measure the correlations between root growth, leaf gas exchange and whole-plant water status under DRZ compared to SD, a two-year field study (2017–2018) was conducted in a commercial vineyard in southcentral Washington, USA. Root distribution along the 0–160 cm soil profile was measured. Leaf gas exchange was monitored, and it was correlated to whole-plant water status which was measured through midday stem water potential ($\Psi_{\text{stem-md}}$) and was recently reported (Ma et al., 2020). In addition, leaf area, as well as total carbon and nitrogen contents in leaves and shoots were measured to determine the differences in nutrient assimilation between SD and DRZ. We hypothesized that grapevines irrigated through DRZ have proportionally increased rooting at depth, concurrently with higher photosynthetic carbon assimilation rates which positively correlate to the diurnal plant water status.

MATERIALS AND METHODS

Field Site Description

This study was conducted for two consecutive growing seasons (2017–2018) in a commercial vineyard of ten-year old, own-rooted *Vitis vinifera* L. cv. Cabernet Sauvignon in the Red Mountain American Viticultural Area (AVA) near Benton City, Washington (46°16'59" N, 119°26'33" W, 228 m a.s.l.). Mature and own-rooted Cabernet Sauvignon was selected as our

experimental material because it is well adapted to irrigation and has been the top produced grape variety in Washington since 2015 with a high economic value (Washington State Wine Commission, USA, 2020). The vineyard rows were oriented north-south, with a spacing of 1.8 m and 2.5 m, respectively between vines and rows. A three-wire trellis was applied, and the vertical distances were 100 cm, 140 cm and 180 cm between the soil surface and each wire. Soil on our experimental vineyard was of the Aridisol order and classified as a Hezel loamy fine sand, consisting of 80% sand, 17% silt, and 3% clay along 0–40 cm profile (United States Department of Agriculture-Natural Resources Conservation Service, USA. Web Soil Survey, 2019), with 0.56% total carbon and 0.056% total nitrogen content based on soil chemical analysis. Daily precipitation and air temperature were recorded through an automated weather station operated by the Washington AgWeatherNet statewide system (AgWeatherNet, <https://weather.wsu.edu/>) located near the vineyard in Benton City, WA (approximately 1 km from the study site). Phenological stages of grapevines for all treatments were recorded based on the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale for bud break (stage 09), flowering (stage 65), fruit set (stage 71), veraison (stage 81) and harvest (stage 89) (Lorenz et al., 1995). To map the development of vines over the course of the study, all dates of phenological stage were reported as the average of all treatments (Pisciotta et al., 2018; Ma et al., 2019).

Irrigation Systems and Experimental Design

Before all treatments started, all grapevines were drip-irrigated through a commercial SD system, which was installed at the same time of vineyard establishment. Another surface drip line was installed in each experimental row as part of tested DRZ and SD systems before the 2015 growing season. Grapevines had acclimated to both tested irrigation systems for two years (2015–2016) before conducting this two-year study. The vertical distance was 40 cm between the suspended drip line and bottom wire of the trellis and was 60 cm between the suspended drip line and the soil surface. Two pressure compensating emitters (CETA, Antelco, Longwood, FL, USA) were used by both irrigation systems for each tree and were located approximately 40 cm on either side of vine trunk, with a flow rate of $4 \text{ L h}^{-1} \text{ vine}^{-1}$. For the DRZ system, a hole with a diameter of 25.4 mm was drilled vertically to the 60 cm soil layer. The PVC tube (Schedule 40, 20 mm inner diameter) was cut into a length of 100 cm which was vertically inserted into the hole for water delivery, with a 40 cm length above ground. A PVC cap (Schedule 40, 21mm inner diameter) for each PVC tube was previously drilled to allow passage of feeder line, which connects the surface drip line with a drip emitter inside the tube. Details on designs and installation of SD and DRZ systems were described by Ma et al. (2020).

The experiment was implemented as a split-plot design in a randomized complete block design with three blocks. Irrigation rate was the whole-plot factor forming a complete block based on evapotranspiration for Cabernet Sauvignon ($\text{ETc} = \text{ETo} \times \text{Kc}$) from bud break to harvest. The data for reference crop (grass)

evapotranspiration (ETo) were collected through the same automated weather station mentioned above and were calculated using Penman-Monteith equation (Allen et al., 1998). The average crop coefficient ($\text{Kc} = 0.5$) was developed based on previous studies on wine grapes in southcentral Washington (Evans et al., 1993; Keller et al., 2016). Three levels of irrigation were applied from fruit set to harvest: high rate (0.75–0.80 ETc), moderate rate (0.60–0.65 ETc), and low rate (0.45–0.50 ETc). Irrigation amounts during different periods of phenological stages are shown in **Supplementary Table 1**. Irrigation method (either SD or DRZ) was the subplot factor and only one irrigation method was assigned to each subplot. Each subplot involved three contiguous rows with five vines in each row (3 rows \times 5 vines = 15 vines per subplot), and measurements were only taken on the three central vines in the central row, with twelve buffer vines alongside to avoid unwarranted interference from neighboring treatments. All treatments (irrigation rate \times irrigation method) were replicated three times.

Irrigation Scheduling

All grapevines were irrigated from bud break to postharvest, with treatments implemented from fruit set to harvest in each year. Fertigation was implemented through surface drip lines ($4 \text{ L h}^{-1} \text{ vine}^{-1}$) and was controlled by the vineyard manager. Liquid fertilizer (25% N, 0% P, 0% K, 3% S) was applied once for about 24 h between bud break and fruit set to avoid any interaction effects between irrigation method and fertilization, which was beyond the scope of this study. The irrigation interval (3–7 days) was determined by the vineyard manager based on weather, soil water content and long-standing guidelines to reach commercial production goals. Generally, vines were irrigated when soil water content in commercial plots (within 20 m to the boundary of treatment plots) decreased to $4 \pm 1 \text{ mm}$, $11 \pm 1 \text{ mm}$, and $12 \pm 1 \text{ mm}$, respectively at a 20, 40, and 60 cm soil depth which were continuously monitored by EnviroSCAN probes (Sentek technologies, Australia). In the experimental site, two EnviroSCAN probes, respectively in SD- and DRZ-treated plots under the high irrigation rate, were used to monitor the changes in water content at 60 cm soil layer between late June and early July in 2017, which are presented in **Supplementary Figure 1**. Irrigation events for different treatment plots on the same day were started simultaneously. Battery powered controllers (11,000 L series, Galcon, Kfar Blum, Israel) were used for reducing the water amounts to designated rates, and actual amounts of applied water were recorded through small mechanical water meters (D.L. Jerman Co., Hackensack, NJ, USA) installed in each experimental row. After harvest, two more rounds of full irrigation, each for 24 h, were applied to refill soil moisture for helping grapevines prevent frost damage in winter. After that, no more irrigation was applied until the bud break of the following growing season.

Leaf Gas Exchange

Measurements of leaf gas exchange were taken from fruit set to harvest in each growing season. One leaf (nodes 6–8 from the shoot tip) from each of the three central grapevines in each subplot was selected for leaf gas exchange measurement using the

LCi-SD portable photosynthesis system (ADC BioScientific Ltd., Hoddesdon, UK). The broad leaf chamber was used with a window area of 6.25 cm². Air flow rate was 200 ml min⁻¹ and reference CO₂ concentration was set at 400 μmol mol⁻¹. Prior to taking measurements on a leaf, the chamber was closed and status of all the sensors inside the chamber were checked through readings on the display. Generally, the reading for ambient CO₂ concentration should stabilize to give similar level of reference CO₂ concentration. Readings for ambient H₂O, photosynthetically active radiation (PAR) and chamber temperature should be also stable. Measurements were made after all of these checks were satisfactory. A portion of the leaf was enclosed in the chamber which took up to 2 min to stabilize its new microclimate inside the chamber and make readings. Leaves were mature, fully expanded, and exposed to sunlight. Net rate of CO₂ assimilation (A), stomatal conductance (g_s), transpiration rate (E) and intrinsic water use efficiency (WUE_i, defined as the ratio between A and g_s) were measured on sunny and clear days with incident PAR on leaf surface > 1,700 μmol m⁻² s⁻¹, typically right before the irrigation, between 10:00 am and 12:00 noon.

In Situ Root Observation

One of the three central vines in the middle row of each subplot was selected for *in situ* root imaging, comprising three treatment replicates for analyses of root number (count tube⁻¹) and root length density (RLD, mm cm⁻²). RLD was defined as the total root length per unit of root image area. Minirhizotron tubes (length × diameter = 180 cm × 6.35 cm) were installed at a distance of 30 cm from the vine trunk with an angle of 15° to the vine trunk, allowing observation of roots within a 0–160 cm soil profile. The exposed top of the root tubes (approximately 8–10 cm) was covered with aluminum tape and sealed with vinyl caps to avoid disturbance from light on root growth and to prevent light scattering and interference for imaging. Eight root images were taken at a dpi of 300 along the length of each tube using the CI-600 In Situ Root Imager (CID Bio-Science, Camas, WA, USA) operated by a tablet computer with CI-600 software installed (<https://cid-inc.com/support/CI-600/software/>). Root images were taken at phenological stages of fruit set, veraison, and harvest in each year. The size of each root image was 21.5 cm long and 19.6 cm wide, with approximately 0.8 cm overlap of adjacent images to guarantee the scan of the entire root area of interest. Root images were analyzed individually by using RootSnap! Image Analysis Software version 1.3.2.25 (CID Bio-Science, Camas, WA, USA). Details in operation of the root imager and root image analysis were described by Ma et al. (2019).

Measurements of Leaf Area, Carbon and Nitrogen Contents in Leaves and Shoots

To help understand the leaf and shoot development of grapevines under DRZ, preliminary experiments were conducted at harvest in 2018 for investigating the influences of the DRZ on leaf size and carbon and nitrogen contents in leaves and shoots. Two mature leaves (one from the east side and another from the west side) on each grapevine were randomly

selected for specific leaf area (SLA) measurement. Leaves were sampled by severing the petiole with a razor blade, then leaves from the same grapevine were put into a sampling bag and stored on ice. All samples were brought back to lab immediately for measuring leaf size by using the LI-3100C Area Meter (LI-COR Biosciences, NE, USA). After leaf size measurement, all leaf samples were put into an air-dryer at 60°C for at least 48 h. Dry samples were weighed for leaf biomass. SLA for each grapevine was calculated as:

$$SLA \text{ (cm}^2\text{g}^{-1}\text{)} = \frac{\text{leaf size (cm}^2\text{)}}{\text{leaf biomass (g)}}$$

Meanwhile, other sets of leaves and shoots were sampled for total carbon (C) and nitrogen (N) content analyses. Twelve leaves and twelve shoots from three central vines in the same subplot, with four leaves and four shoots collected per vine, were mixed and put into an air-dryer at 60°C for at least 48 h. All dried samples were milled into powder, and around 0.2 g powder per dry sample was sent for total carbon and nitrogen content analyses by using the TruSpec Micro analyzer (LECO Corp., MI, USA).

Statistical Analyses

Data were analyzed separately by year. A two-way analysis of variance (ANOVA) adjusted for split-plot design was used to detect treatment effects on leaf gas exchange, root growth, leaf area, and total C and N contents, followed by Tukey's HSD test as a post-hoc analysis for comparisons between different treatment groups. A one-way ANOVA was used to detect differences in total root number and RLD within each range of soil depth (20 cm intervals along 0–160 cm soil profile; 0–60 cm and 60–160 cm soil profiles) between two irrigation methods (SD and DRZ) at each irrigation rate. Statistical analyses were run by using R 3.4.3 statistical software package (www.r-project.org) at p-value = 0.05. Correlation analyses were performed to evaluate the strength of relationships between leaf gas exchange and Ψ_{stem-md} in grapevines. Linear equations and correlation coefficients were calculated with SigmaPlot 12.5 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Weather Trends

Weather patterns were different between the two years of the study (**Figure 1**). Total precipitation was 33% lower in 2018 (150.1 mm) than in 2017 (222.5 mm). Cumulative precipitation before bud break (Stage 09) and between bud break and fruit set (Stage 71) were 42% and 64% lower, respectively in 2018 than in 2017. Precipitation was extremely limited from fruit set to harvest (Stage 89) in both years and was similar after harvest. Annual temperature was 1.2°C higher in 2018 as compared to 2017. Although average temperature before bud break was 2°C higher in 2018 than in 2017, average temperatures near bud break (14 days prior to bud break) were similar between years. Between the stages of bud break and flowering, the average

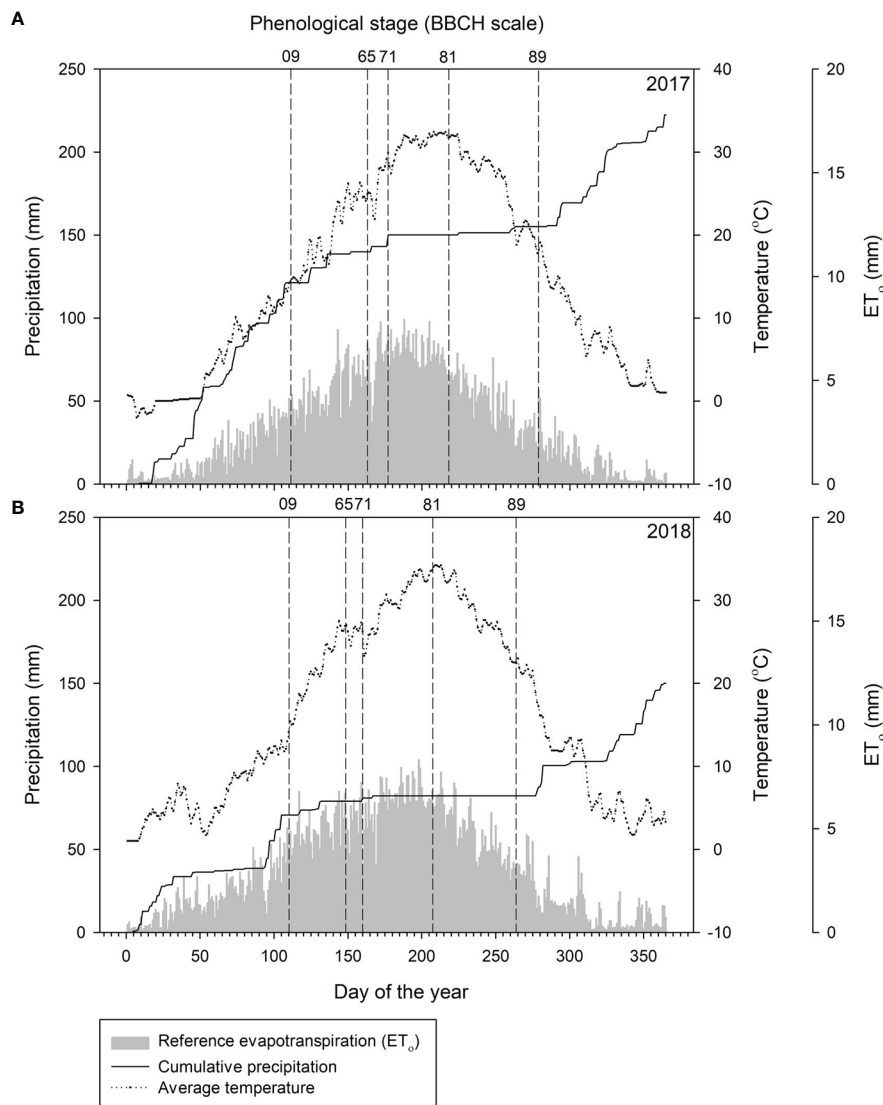


FIGURE 1 | Cumulative daily precipitation (mm, solid lines), daily temperature (°C, dotted lines), daily reference evapotranspiration (ET_o, mm, gray bars), and day of the year of phenological stage (dashed lines) in Red Mountain, WA, USA in **(A)** 2017 and **(B)** 2018. Weather data were collected from AgWeatherNet at Washington State University (<http://weather.wsu.edu/>). Phenological stages were based on BBCH scale for bud break (stage 09), flowering (stage 65), fruit set (stage 71), veraison (stage 81) and harvest (stage 89).

temperature was 1.4°C higher in 2018 than in 2017. The annual reference evapotranspiration (ET_o) was higher in 2018 (1155.5 mm) than in 2017 (1064.8 mm); however, accumulated ET_o from bud break to harvest was similar between years.

Leaf Gas Exchange

Both irrigation rate and method significantly influenced leaf gas exchange in both years (**Figure 2**). In general, grapevines irrigated through DRZ had higher rate of net CO₂ assimilation (A), accompanied by higher rates of stomatal conductance (g_s) and transpiration (E) (**Figures 2A–F**). Meanwhile, decreases in irrigation rate reduced A, g_s, and E (**Figures 2A–F**). Intrinsic

water use efficiency (WUE_i) had opposite relationships with irrigation rate and method compared to the other three parameters, as decreases in irrigation rate as well as DRZ strategy improved WUE_i (**Figures 2G, H**). The most significant differences between treatment effects were found during the hottest time of each growing season, usually from mid-July to early September. On average, DRZ significantly improved A, g_s and E by 16–24, 16–32, and 12–30%, respectively during those periods. Significant linear correlations ($P < 0.001$) were found between leaf gas exchange and midday stem water potential ($\Psi_{\text{stem-md}}$) during late growing season in both years, as higher $\Psi_{\text{stem-md}}$ was accompanied by increased A, g_s, E and decreased

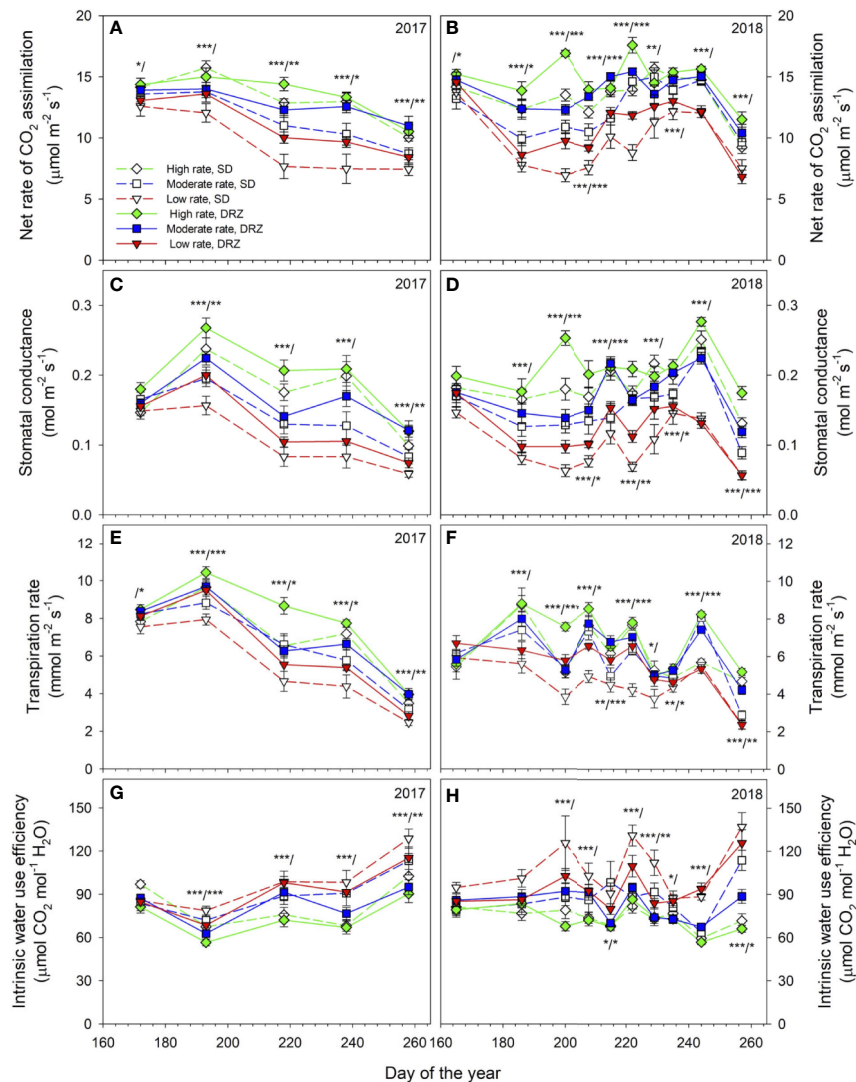


FIGURE 2 | Leaf gas exchange under surface drip (SD, dashed lines) and direct root-zone (DRZ, solid lines) irrigation in 2017–2018. **(A, B)**: net CO_2 assimilation rate **(A)**, $\mu\text{mol m}^{-2} \text{s}^{-1}$; **(C, D)**: stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$); **(E, F)**: transpiration rate **(E)**, $\text{mmol m}^{-2} \text{s}^{-1}$; and **(G, H)**: intrinsic water use efficiency (WUE_i , $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$). Three irrigation rates were set based on crop evapotranspiration (ET_c) for Cabernet Sauvignon: Diamonds with green lines, squares with blue lines, and triangles with red lines represent irrigation rates at high ($0.75\text{--}0.80 \text{ ET}_c$), moderate ($0.60\text{--}0.65 \text{ ET}_c$) and low ($0.45\text{--}0.50 \text{ ET}_c$), respectively. Asterisks on the left side of the slash indicate statistically significant differences between effects of irrigation rate, and ones on the right side of the slash indicate statistically significant differences between effects of irrigation method. *, ** and *** represent statistical differences at $P \leq 0.05$, 0.01 and 0.001 , respectively. Error bars represent standard error ($n = 9$).

WUE_i . Correlations were the strongest at harvest compared to other phenological stages within each year and were stronger in 2018 than in 2017 (Figure 3).

Root Distribution

Treatment effects on root number and RLD were found in both years. Compared to SD, irrigation through the DRZ system significantly reduced total root number in the 0–160 cm soil profile by 20% at fruit set (right after treatments were applied) and by 23% at veraison in 2017. Similar patterns were also found at fruit set (11% decrease) and veraison (16% decrease) in 2018, although those decreases were not statistically significant.

Decreases in irrigation rate from high to low significantly reduced total root number in the 0–160 cm soil profile for grapevines irrigated through the SD system; however, no significant reduction was found in grapevines irrigated through the DRZ system. When the whole soil profile (0–160 cm) was considered, differences in irrigation rate and method showed no significant effects on RLD over the course of this study; however, higher total root number and RLD were found in 60–160 cm soils under DRZ than under SD at the moderate rate, with significant differences found in 2017 (Supplementary Figures 2 and 3). Comparisons of root number and RLD between SD and DRZ within 20 cm intervals along the 0–160 cm soil profile at

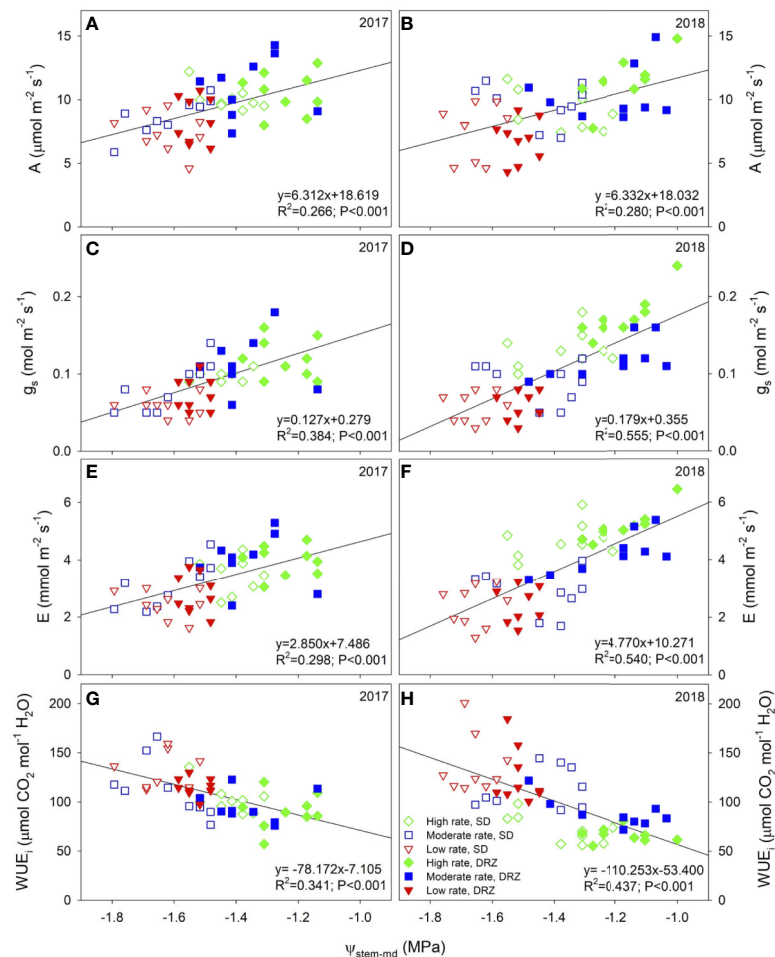


FIGURE 3 | Linear correlations between leaf gas exchange and midday stem water potential ($\Psi_{\text{stem-md}}$, MPa) in 2017 and 2018. Positive correlations were found between (A, B) $\Psi_{\text{stem-md}}$ and net CO₂ assimilation rate (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), (C, D) $\Psi_{\text{stem-md}}$ and stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), and (E, F) $\Psi_{\text{stem-md}}$ and transpiration rate (E, $\text{mmol m}^{-2} \text{s}^{-1}$). Negative correlation was found between (G, H) $\Psi_{\text{stem-md}}$ and intrinsic water use efficiency (WUE_i, $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$). Open symbols represent the surface drip irrigation (SD), and closed symbols represent the direct root-zone irrigation (DRZ). Three irrigation rates were set based on crop evapotranspiration (ET_c) for Cabernet Sauvignon: Green diamonds, blue squares, and red triangles represent irrigation rates at high (0.75–0.80 ET_c), moderate (0.60–0.65 ET_c) and low (0.45–0.50 ET_c), respectively. R^2 : coefficient of determination. Data were from measurements on Day of the year 258 and 257, respectively in 2017 and 2018 and were pooled within each year ($n = 54$).

fruit set and veraison are shown in **Supplementary Figures 4–7**. No significant increases in total root number and RLD under DRZ were found within each of 20 cm intervals along 60–160 cm soil profile across all irrigation rates except the low rate at harvest in 2018, where grapevines irrigated through DRZ had significantly higher root number at 60–80 cm soil depth (**Figure 4**).

However, the most significant treatment effects both on root number and RLD were found within 0–60 cm soil profile. DRZ significantly reduced total root number in top 60 cm soil at high and moderate irrigation rates in both years, with 50–60% less total root number for grapevines under DRZ compared to SD, but no significant decrease was found at low irrigation rate (**Supplementary Figure 2**). For grapevines irrigated through

SD, decreased irrigation rate from high to low significantly reduced total root number in the 0–60 cm soil profile, as 60 and 46% fewer roots were found at harvest in 2017 and 2018, respectively (**Figure 4**). However, grapevines under DRZ showed no significant differences in total root number in the 0–60 cm soil profile between high and low irrigation rates in both years (**Figure 4**). Compared to SD, DRZ also reduced root length density (RLD) by 30–40% in the upper 60 cm soil at high and moderate irrigation rates, but no decrease was detected at low irrigation rate in both years (**Supplementary Figure 3**). More specifically, significant differences in RLD between irrigation methods were found in the top 0–20 cm soil profile, as DRZ reduced RLD by 50–60% compared to SD at high and moderate rates in 2018 (**Figure 5**).

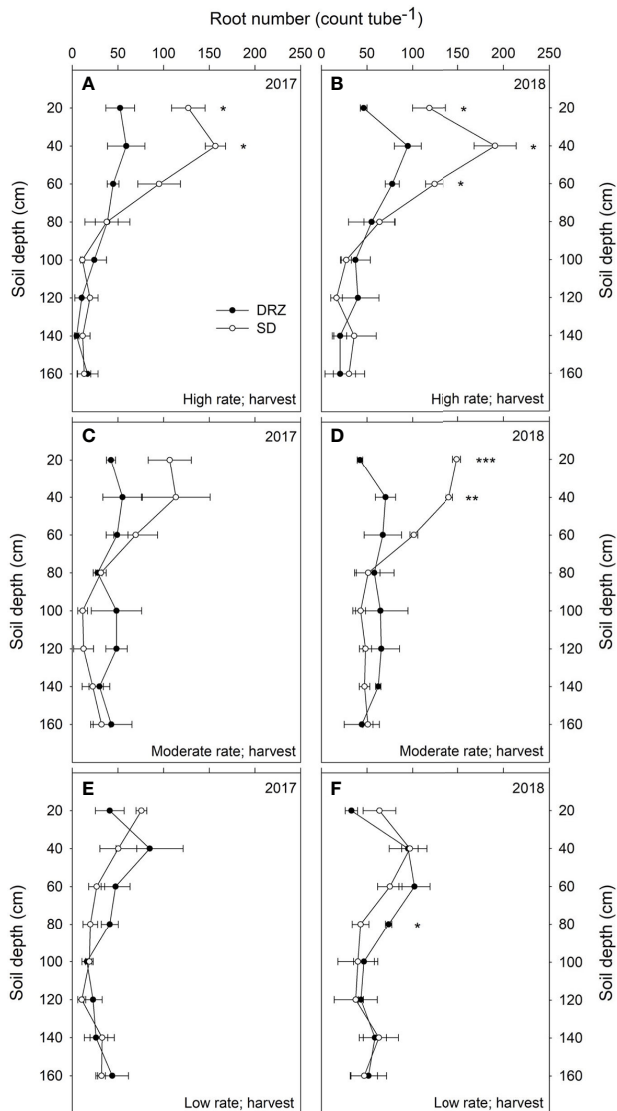


FIGURE 4 | Total root number (count tube⁻¹) along the 0–160 cm soil profile under surface drip (SD, open circles) and direct root-zone (DRZ, closed circles) irrigation at harvest in 2017 and 2018. Three irrigation rates were set based on crop evapotranspiration (ET_c) for Cabernet Sauvignon: **(A, B)** high rate: 0.75–0.80 ET_c ; **(C, D)** moderate rate: 0.60–0.65 ET_c ; and **(E, F)** low rate: 0.45–0.50 ET_c . *, ** and *** represent statistical differences at $P \leq 0.05$, 0.01 and 0.001, respectively. Error bars represent standard error ($n=3$).

Specific Leaf Area, Total Carbon and Nitrogen Contents in Leaves and Shoots

Specific leaf area (SLA) was slightly higher for grapevines irrigated through DRZ than SD within each irrigation rate, especially at the low rate, where 5.3% higher SLA was found (**Supplementary Figure 8**). Decreases in irrigation rates from high to moderate and from high to low reduced SLA by 4.4 and 8.7%, respectively, with a 3.3% more reduction from high to low rate for grapevines irrigated through SD compared to DRZ (**Supplementary Figure 8**). Significant differences in total N

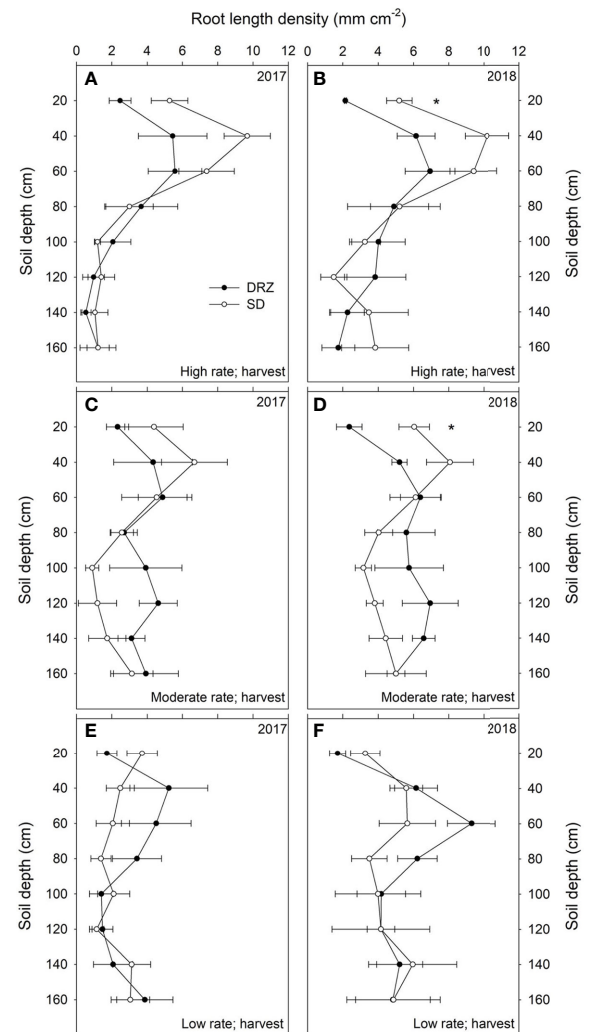


FIGURE 5 | Root length density (RLD, mm cm⁻²) along the 0–160 cm soil profile under surface drip (SD, open circles) and direct root-zone (DRZ, closed circles) irrigation at harvest in 2017 and 2018. Three irrigation rates were set based on crop evapotranspiration (ET_c) for Cabernet Sauvignon: **(A, B)** high rate: 0.75–0.80 ET_c ; **(C, D)** moderate rate: 0.60–0.65 ET_c ; and **(E, F)** low rate: 0.45–0.50 ET_c . * represents statistical differences at $P < 0.05$, 0.01 and 0.001, respectively. Error bars represent standard error ($n = 3$).

content and C:N ratio in leaves were found between the two irrigation methods, as 8.4% higher total N content and 6.5% lower C:N ratio in leaves were found with adoption of DRZ (**Table 1**). No significant differences in total C content in leaves and total C and N contents in shoots were found.

DISCUSSION

Given current climate change scenarios, it has become evident that more efficient use of sparse water resources is of paramount importance for viticulture sustainability (Medrano et al., 2015; Fraga et al., 2018). Many perennial crops such as grapevines,

TABLE 1 | Mean total carbon (C) percentage, total nitrogen (N) percentage and carbon to nitrogen ratio (C:N ratio) in leaves and shoots as influenced by irrigation rate and method using two-way analysis of variance (ANOVA).

Main effect	Leaf			Shoot		
	C (%)	N (%)	C:N ratio	C (%)	N (%)	C:N ratio
Irrigation rate						
High	46.07	1.47	31.43	48.88	0.45	109.75
Moderate	46.89	1.52	30.93	49.07	0.47	105.82
Low	46.84	1.47	32.01	48.90	0.47	103.24
Irrigation method						
SD	46.27	1.43 ^b	32.51 ^a	48.95	0.46	107.87
DRZ	46.92	1.55 ^a	30.41 ^b	48.96	0.47	104.66
ANOVA						
Rate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Method	n.s.	*	*	n.s.	n.s.	n.s.
Rate × Method	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s., not significant at $P > 0.1$.

*, **, *** significant difference at $P \leq 0.05$, 0.01 and 0.001 , respectively.

Within each year, means of main factors followed by different letters are significantly different at $P \leq 0.05$ according to the Tukey's HSD test.

almonds, apples, and pomegranates, in particular rely on supplemental irrigation to maintain growth and yield during periods of drought stress (Romero et al., 2004; Zhang et al., 2017; Pisciotto et al., 2018; Zhong et al., 2019). While there has been a positive shift from less sustainable methods of irrigation such as spray or furrow irrigation to more sustainable drip irrigation methods, improvements are still needed to enhance water productivity (Ayars et al., 2015). Direct root-zone irrigation (DRZ) was introduced recently as an efficient subsurface drip irrigation strategy to improve water use efficiency and sustain grape production in semi-arid regions (Ma et al., 2019). This study further advanced our knowledge of the novel irrigation strategy by investigating the eco-physiological responses of Cabernet Sauvignon grapevines to the DRZ and found improved leaf gas exchange with deep root development compared to the traditional surface drip irrigation (SD). These findings will help guide efficient grape cultivation in semi-arid climates with DRZ which could be also adopted in other agroecosystems.

This study found significant improvements in leaf gas exchange of grapevines under DRZ compared to SD in both years with different weather patterns. Those improvements were possibly attributed to higher water availability within the root zone under DRZ from fruit set to harvest, as the higher soil water content was detected in the 60 cm soil layer where the water was delivered (**Supplementary Figure 1**). Al-Omran et al. (2005) also found increased water content in soil layers treated by subsurface drip irrigation which benefitted crop performance in sandy soil. Delivering water through subsurface irrigation systems could provide more water for crop growth by reducing soil water evaporation (Ayars et al., 1999), thus grapevines irrigated through DRZ had more stomata that remained open for gas exchange and experienced less diurnal water stress, which was indicated by higher midday stem water potential ($\Psi_{\text{stem-md}}$) compared to SD with a progress of water deficit (Ma et al., 2020). Influences of irrigation method on leaf gas exchange were amplified in the summer with limited precipitation, higher temperature and reference evapotranspiration (ET_o), as they induce the stomatal closure and reduction of plant water

potential (Limousin et al., 2013), thus the soil water availability became a major limiting factor that significantly influenced the photosynthetic capacity of grapevines. Compared to the wet growing season of 2017, decreased cumulative precipitation accompanied by increased average temperature and ET_o in 2018 probably reduced soil water availability for grapevine growth, which intensified water stress in grapevines as revealed by decreased $\Psi_{\text{stem-md}}$ across all treatments (Ma et al., 2020). Therefore, improvements in leaf gas exchange for grapevines under DRZ were more significant in 2018 than in 2017. Due to a major role of water availability on grapevine growth in arid climates, influences of irrigation rate on leaf gas exchange were consistently significant from fruit set to harvest in both years, which are in accordance with previous studies (Chaves, 2004; Costa et al., 2007; Keller et al., 2016). These findings indicate that a precise regulation of soil water content through DRZ is vital for optimizing the leaf CO_2 assimilation of grapevines to cope with heat- and drought-induced adversities in semi-arid climates.

Observation of root growth showed increased total number and root length density (RLD) of deep roots (60–160 cm soil layers) compared to shallow roots (0–60 cm soil layers) at moderate and low irrigation rates than at high rate, revealing that deeper root systems in grapevines could be developed through regulated deficit irrigation as proposed in previous studies (Dry et al., 2000; Costa et al., 2007; Romero et al., 2012). Recent studies observed the water uptake patterns of grapevine and other plant species in semi-arid regions, and found that with progressed water stress a large proportion of water was derived from deep soils (Wang et al., 2017; Savi et al., 2019). Deeper root distributions have been detected for grapevines with increased drought tolerance (Smart et al., 2006; Fort et al., 2017), indicating that deep root systems may access groundwater in deeper soil to maintain higher leaf photosynthetic rate and to relieve plant water stress, which is consistent with our findings (Ma et al., 2019; Ma et al., 2020).

Significant differences in root distribution between DRZ and SD were also found as the irrigation method influences plant rooting patterns (Basso et al., 2003; Romero et al., 2004). In this study, DRZ

affected root growth by adjusting the water availability in different soil layers. DRZ significantly reduced root development in the topsoil, partly due to limited irrigation water that was available in surface soil, which is consistent with previous studies on root distribution of different crops under subsurface drip irrigation (Phene et al., 1991; Machado et al., 2003; Romero et al., 2004; Al-Omran et al., 2005; Kong et al., 2012; Pisciotta et al., 2018). Moreover, higher root number and RLD were found under DRZ compared to SD within 60–160 cm soil profile, indicating that DRZ possibly increases root growth by improving water availability below 60 cm soil depth. Differences in root distribution might also exist below 160 cm soil depth, although the majority of grapevine roots (e.g. >80%) are reported to be in the upper 100 cm of soil in managed agricultural systems (Smart et al., 2006). However, this study could not make any further conclusions. A portion of roots might remain near the subsurface emitters, as significantly higher root number was found under DRZ within 60–120 cm soil depth, where they can easily access irrigation water (Romero et al., 2004). Although the eco-physiological responses of grapevine roots to DRZ emphasized in the current study indicate the changes in soil water availability, one caveat is that direct observations of the soil water distribution along the entire soil profile are limited. Future studies will better elucidate the relationship between deep root development and soil moisture distribution in response to DRZ.

Soil type and texture could be another important factor influencing the impacts of DRZ on grapevines. Thus far, the DRZ system has been tested only in sandy soil, which is a highly permeable soil type. Although significant effects were found, this soil type possibly compromises the positive influences of DRZ on improvement in soil water availability due to its lower water holding capacity compared to clay soils. Thus, more significant treatment effects may be detected in a less permeable soil type, similar to the findings of Al-Omran et al. (2005) where soil moisture content was increased by adding clay deposits. Future research should focus on a comprehensive comparison of soil water content between different soil types.

Reduced water availability to some extent restricts root ability for water and nutrient uptake, which might lower the nutrient concentration, such as carbon (C) and nitrogen (N), in leaves and shoots for regulating plant development (Saud et al., 2017). In our study, no earlier basal leaf abscission occurred between veraison and harvest in both years, and no significant differences in total C and N contents between different irrigation rates were found in leaves and shoots, indicating that no extreme water stress was reached that could severely hamper the grapevine growth. However, grapevines irrigated through DRZ experienced less water stress, which was also indicated by higher leaf N content, lower C:N ratio in mature leaves and slightly higher specific leaf area in 2018. These patterns are consistent with findings reported previously in cowpea and sorghum (Anyia and Herzog, 2004; Chen et al., 2015). In this scenario, grapevine possibly invested a greater portion of resources to accelerate aboveground growth rather than root development. Instead of producing new roots for improvement in aboveground growth, DRZ might also elongate the root lifespan for water and nutrient uptake through increased soil water availability (McCormack

and Guo, 2014). Those aspects are also worth investigating in future studies.

CONCLUSION

This study found higher photosynthetic carbon assimilation rates in grapevines irrigated through the direct root-zone irrigation (DRZ) compared to surface drip irrigation (SD) and provided insights into rooting patterns under subsurface irrigation with seasonal drought. Grapevine alters rooting patterns under DRZ by significantly restricting shallow root growth and encouraging root development in the deeper soil profile. Deep rooting patterns could help grapevines take water from deeper layers for optimizing grapevine growth and alleviating water stress during periods of heat and drought. Future studies need to investigate the relationship between grapevine rooting patterns and dynamics of soil water distribution in different soil types and in different grapevine varieties to help them better adapt to arid climates.

DATA AVAILABILITY STATEMENT

The datasets supporting the conclusion of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

XM, PJ, and KS designed and supervised the research. XM performed the research and analyzed the data. XM drafted the manuscript. PJ and KS critically revised the manuscript and verified quality of written English. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by USDA Western Sustainable Agriculture Research and Education Program Graduate Student Grant (GW17-058); Washington State Department of Agriculture Specialty Crop Block Program Project (K1768); Washington State Grape and Wine Research Program (Nos. 3019-3818; 3019-6818); Northwest Center for Small Fruit Research (No. 2072-21000-047-16); USDA National Institute of Food and Agriculture, Hatch project (No. 1014527).

ACKNOWLEDGMENTS

We wish to thank Scott Williams, the general manager of Kiona Vineyards and Winery in Washington state, USA, for providing the field site for this study and for irrigation, vineyard maintenance, and labor during grape harvest. We also want to thank Jessica

Braden and Soil Plant Waste Analytical Lab in Department of Crop and Soil Sciences at Washington State University for performing C/N analyses of grapevine tissues, and thank Dr. Markus Keller for providing us valuable suggestions for this research and assistance in grape storage and leaf area measurement.

REFERENCES

- Alexandratos, N., and Bruinsma, J. (2012). *World agriculture towards 2030/2050: The 2012 revision* (Rome: FAO). ESA working paper No. 12-03. doi: 10.22004/ag.econ.288998
- Allen, R. G., Pereira, L. S., Raesk, D., and Smith, M. (1998). "Crop evapotranspiration — Guidelines for computing crop water requirements," in *Irrigation and Drainage* (Rome, Italy: FAO), 56.
- Al-Omran, A. M., Sheta, A. S., Falatah, A. M., and Al-Harbi, A. R. (2005). Effect of drip irrigation on squash (*Cucurbita pepo*) yield and water-use efficiency in sandy calcareous soils amended with clay deposits. *Agric. Water Manage.* 73, 43–55. doi: 10.1016/j.agwat.2004.09.019
- Anyia, A., and Herzog, H. (2004). Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *Eur. J. Agron.* 20, 327–339. doi: 10.1016/S1161-0301(03)00038-8
- Ayars, J. E., Phene, C. J., Hutmacher, R. B., Davis, K. R., Schoneman, R. A., Vail, S. S., et al. (1999). Subsurface drip irrigation of row crops: a review of 15 years of research at the Water Management Research Laboratory. *Agric. Water Manage.* 42, 1–27. doi: 10.1016/S0378-3774(99)00025-6
- Ayars, J. E., Fulton, A., and Taylor, B. (2015). Subsurface drip irrigation in California—Here to stay? *Agric. Water Manage.* 157, 39–47. doi: 10.1016/j.agwat.2015.01.001
- Basso, L. H., Hopmans, J. W., Jorge, L. A., de, C., de Alencar, C. M., and Moura e Silva, J. A. (2003). Grapevine root distribution in drip and microsprinkler irrigation. *Sci. Agric.* 60, 377–387. doi: 10.1590/S0103-90162003000200024
- Bernardo, S., Dinis, L.-T., Machado, N., and Moutinho-Pereira, J. (2018). Grapevine abiotic stress assessment and search for sustainable adaptation strategies in Mediterranean-like climates. A review. *Agron. Sustain. Dev.* 38, 66. doi: 10.1007/s13593-018-0544-0
- Bhattarai, S. P., Midmore, D. J., and Pendergast, L. (2008). Yield, water-use efficiencies and root distribution of soybean, chickpea and pumpkin under different subsurface drip irrigation depths and oxygenation treatments in vertisols. *Irrig. Sci.* 26, 439–450. doi: 10.1007/s00271-008-0112-5
- Bonada, M., Jeffery, D. W., Petrie, P. R., Moran, M. A., and Sadras, V. O. (2015). Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines: Temperature and water effects on grapes and wines. *Aust. J. Grape Wine Res.* 21, 240–253. doi: 10.1111/ajgw.12142
- Chaves, M. M. (2004). Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J. Exp. Bot.* 55, 2365–2384. doi: 10.1093/jxb/erh269
- Chen, D., Wang, S., Xiong, B., Cao, B., and Deng, X. (2015). Carbon/nitrogen imbalance associated with drought-induced leaf senescence in sorghum bicolor. *PLoS One* 10, e0137026. doi: 10.1371/journal.pone.0137026
- Comas, L. H., Bauerle, T. L., and Eissenstat, D. M. (2010). Biological and environmental factors controlling root dynamics and function: effects of root ageing and soil moisture. *Aust. J. Grape Wine Res.* 16, 131–137. doi: 10.1111/j.1755-0238.2009.00078.x
- Costa, J. M., Ortuño, M. F., and Chaves, M. M. (2007). Deficit irrigation as a strategy to save water: Physiology and potential application to horticulture. *J. Integr. Plant Biol.* 49, 1421–1434. doi: 10.1111/j.1672-9072.2007.00556.x
- Costa, J. M., Vaz, M., Escalona, J., Egipto, R., Lopes, C., Medrano, H., et al. (2016). Modern viticulture in southern Europe: Vulnerabilities and strategies for adaptation to water scarcity. *Agric. Water Manage.* 164, 5–18. doi: 10.1016/j.agwat.2015.08.021
- Davies, W. J., and Bennett, M. J. (2015). Achieving more crop per drop. *Nat. Plants* 1, 15118. doi: 10.1038/nplants.2015.118
- Douthe, C., Medrano, H., Tortosa, I., Mariano Escalona, J., Hernandez-Montes, E., and Pou, A. (2018). Whole-plant water use in field grown grapevine: Seasonal and environmental effects on water and carbon balance. *Front. Plant Sci.* 9, 1540. doi: 10.3389/fpls.2018.01540
- Drew, M. C. (1997). Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 223–250. doi: 10.1146/annurev.arplant.48.1.223
- Dry, P. R., Loveys, B. R., and Düring, H. (2000). Partial drying of the rootzone of grape. II. Changes in the pattern of root development. *Vitis* 39, 9–12. doi: 10.5073/vitis.2000.39.9-12
- Evans, R. G., Spayd, S. E., Wample, R. L., Kroeger, M. W., and Mahan, M. O. (1993). Water use of *Vitis vinifera* grapes in Washington. *Agric. Water Manage.* 23, 109–124. doi: 10.1016/0378-3774(93)90035-9
- Food and Agriculture Organization (2018). Available at: <https://www.fao.org/faostat/en/#data/> (Accessed December 31, 2018).
- Fort, K., Fraga, J., Grossi, D., and Walker, M. A. (2017). Early measures of drought tolerance in four grape rootstocks. *J. Am. Soc. Hort. Sci.* 142, 36–46. doi: 10.21273/JASHS03919-16
- Fraga, H., García de Cortázar Atauri, I., and Santos, J. A. (2018). Viticultural irrigation demands under climate change scenarios in Portugal. *Agric. Water Manage.* 196, 66–74. doi: 10.1016/j.agwat.2017.10.023
- Jacoby, P., and Ma, X. (2018). Introducing direct root-zone deficit irrigation to conserve water and enhance grape quality in the Pacific Northwest. *Crops Soils* 51, 34–58. doi: 10.2134/cs2018.51.0510
- Keller, M., Romero, P., Gohil, H., Smithyman, R. P., Riley, W. R., Casassa, L. F., et al. (2016). Deficit irrigation alters grapevine growth, physiology, and fruit microclimate. *Am. J. Enol. Vitic.* 67, 426–435. doi: 10.5344/ajev.2016.16032
- Kisekka, I., Kandelous, M. M., Sanden, B., and Hopmans, J. W. (2019). Uncertainties in leaching assessment in micro-irrigated fields using water balance approach. *Agric. Water Manage.* 213, 107–115. doi: 10.1016/j.agwat.2018.10.012
- Ko, J., and Piccinini, G. (2009). Characterizing leaf gas exchange responses of cotton to full and limited irrigation conditions. *Field Crops Res.* 112, 77–89. doi: 10.1016/j.fcr.2009.02.007
- Kong, Q., Li, G., Wang, Y., and Huo, H. (2012). Bell pepper response to surface and subsurface drip irrigation under different fertigation levels. *Irrig. Sci.* 30, 233–245. doi: 10.1007/s00271-011-0278-0
- Koundouras, S., Tsiatas, I. T., Zioziou, E., and Nikolaou, N. (2008). Rootstock effects on the adaptive strategies of grapevine (*Vitis vinifera* L. cv. Cabernet-Sauvignon) under contrasting water status: Leaf physiological and structural responses. *Agric. Ecosyst. Environ.* 128, 86–96. doi: 10.1016/j.agee.2008.05.006
- Lamm, F. R., Bordovsky, J. P., Schwankl, L. J., Grabow, G. L., Enciso-Medina, J., Peters, R. T., et al. (2012). Subsurface drip irrigation: Status of the technology in 2010. *Trans. ASABE* 55, 483–491. doi: 10.13031/2013.41387
- Limousin, J.-M., Bickford, C. P., Dickman, L. T., Pangle, R. E., Hudson, P. J., Boutz, A. L., et al. (2013). Regulation and acclimation of leaf gas exchange in a piñon-juniper woodland exposed to three different precipitation regimes: Rainfall manipulation in piñon-juniper woodland. *Plant Cell Environ.* 36, 1812–1825. doi: 10.1111/pce.12089
- Lorenz, D. H., Eichhorn, K. W., Bleiholder, H., Klose, R., Meier, U., and Weber, E. (1995). Growth stages of the grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*)—Codes and descriptions according to the extended BBCH scale†. *Aust. J. Grape Wine Res.* 1, 100–103. doi: 10.1111/j.1755-0238.1995.tb00085.x
- Ma, X., Sanguinet, K. A., and Jacoby, P. W. (2019). Performance of direct root-zone deficit irrigation on *Vitis vinifera* L. cv. Cabernet Sauvignon production and water use efficiency in semi-arid southcentral Washington. *Agric. Water Manage.* 221, 47–57. doi: 10.1016/j.agwat.2019.04.023
- Ma, X., Sanguinet, K. A., and Jacoby, P. W. (2020). Direct root-zone irrigation outperforms surface drip irrigation for grape yield and crop water use efficiency while restricting root growth. *Agric. Water Manage.* 231, 105993. doi: 10.1016/j.agwat.2019.105993
- Machado, R. M. A., do Rosário, M., Oliveira, G., and Portas, C. A. M. (2003). Tomato root distribution, yield and fruit quality under subsurface drip irrigation. *Plant Soil* 255, 333–341. doi: 10.1007/978-94-017-2923-9_32

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.575303/full#supplementary-material>

- Malek, K., Adam, J., Stockle, C., Brady, M., and Rajagopalan, K. (2018). When Should Irrigators Invest in More Water-Efficient Technologies as an Adaptation to Climate Change? *Water Resour. Res.* 54, 8999–9032. doi: 10.1029/2018WR022767
- Martínez-Gimeno, M. A., Bonet, L., Provenzano, G., Badal, E., Intrigliolo, D. S., and Ballester, C. (2018). Assessment of yield and water productivity of clementine trees under surface and subsurface drip irrigation. *Agric. Water Manage.* 206, 209–216. doi: 10.1016/j.agwat.2018.05.011
- Mbow, C., and Rosenzweig, C. (2019). “Chapter 5: food security,” in *Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems*. Available at: <https://www.ipcc.ch/srccl/> (Accessed June 8, 2020).
- McCormack, M. L., and Guo, D. (2014). Impacts of environmental factors on fine root lifespan. *Front. Plant Sci.* 5, 205. doi: 10.3389/fpls.2014.00205
- Medrano, H., Tomás, M., Martorell, S., Escalona, J., Pou, A., Fuentes, S., et al. (2015). Improving water use efficiency of vineyards in semi-arid regions. A review. *Agron. Sustain. Dev.* 35, 499–517. doi: 10.1007/s13593-014-0280-z
- Murley, C. B., Sharma, S., Warren, J. G., Arnall, D. B., and Raun, W. R. (2018). Yield response of corn and grain sorghum to row offsets on subsurface drip laterals. *Agric. Water Manage.* 208, 357–362. doi: 10.1016/j.agwat.2018.06.038
- Osmond, K. S., Sibout, R., and Hardtke, C. S. (2007). Hidden branches: Developments in root system architecture. *Annu. Rev. Plant Biol.* 58, 93–113. doi: 10.1146/annurev.arplant.58.032806.104006
- Paris, P., Di Matteo, G., Tarchi, M., Tosi, L., Spaccino, L., and Lauteri, M. (2018). Precision subsurface drip irrigation increases yield while sustaining water-use efficiency in Mediterranean poplar bioenergy plantations. *For. Ecol. Manage.* 409, 749–756. doi: 10.1016/j.foreco.2017.12.013
- Payero, J. O., Dean Yonts, C., Irmak, S., and Tarkalson, D. (2005). *Advantages and disadvantages of subsurface drip irrigation* (University of Nebraska Lincoln, Extension), EC776. Available at: <https://digitalcommons.unl.edu/extensionhist/4787> (Accessed May 24, 2020).
- Phene, C. J., Davis, K. R., Huttmacher, R. B., Bar-Yosef, B., Meek, D. W., and Misaki, J. (1991). Effect of high frequency surface and subsurface drip irrigation on root distribution of sweet corn. *Irrig. Sci.* 12, 135–140. doi: 10.1007/BF00192284
- Pisciotta, A., Di Lorenzo, R., Santalucia, G., and Barbagallo, M. G. (2018). Response of grapevine (Cabernet Sauvignon cv) to above ground and subsurface drip irrigation under arid conditions. *Agric. Water Manage.* 197, 122–131. doi: 10.1016/j.agwat.2017.11.013
- Romero, P., Botia, P., and Garcia, F. (2004). Effects of regulated deficit irrigation under subsurface drip irrigation conditions on vegetative development and yield of mature almond trees. *Plant Soil* 260, 169–181. doi: 10.1023/B:PLSO.0000030193.23588.99
- Romero, P., Dodd, I. C., and Martinez-Cutillas, A. (2012). Contrasting physiological effects of partial root zone drying in field-grown grapevine (*Vitis vinifera* L. cv. Monastrell) according to total soil water availability. *J. Exp. Bot.* 63, 4071–4083. doi: 10.1093/jxb/ers088
- Sánchez-Blanco, M. J., Ortuño, M. F., Bañon, S., and Álvarez, S. (2019). Deficit irrigation as a strategy to control growth in ornamental plants and enhance their ability to adapt to drought conditions. *J. Hortic. Sci. Biotechnol.* 94, 137–150. doi: 10.1080/14620316.2019.1570353
- Saud, S., Fahad, S., Yajun, C., Ihsan, M. Z., Hammad, H. M., Nasim, W., et al. (2017). Effects of Nitrogen Supply on Water Stress and Recovery Mechanisms in Kentucky Bluegrass Plants. *Front. Plant Sci.* 8, 983. doi: 10.3389/fpls.2017.00983
- Savi, T., Petruzzellis, F., Martellos, S., Stenni, B., Dal Borgo, A., Zini, L., et al. (2018). Vineyard water relations in a karstic area: deep roots and irrigation management. *Agric. Ecosyst. Environ.* 263, 53–59. doi: 10.1016/j.agee.2018.05.009
- Savi, T., Petruzzellis, F., Moretti, E., Stenni, B., Zini, L., Martellos, S., et al. (2019). Grapevine water relations and rooting depth in karstic soils. *Sci. Total Environ.* 692, 669–675. doi: 10.1016/j.scitotenv.2019.07.096
- Scanlon, B. R., Faunt, C. C., Longuevergne, L., Reedy, R. C., Alley, W. M., McGuire, V. L., et al. (2012). Groundwater depletion and sustainability of irrigation in the US High Plains and Central Valley. *Proc. Natl. Acad. Sci.* 109, 9320–9325. doi: 10.1073/pnas.1200311109
- Smart, D. R., Schwass, E., Lakso, A., and Morano, L. (2006). Grapevine rooting patterns: A comprehensive analysis and a review. *Am. J. Enol. Vitic.* 57, 89–104.
- United States Department of Agriculture-Natural Resources Conservation Service, USA. Web Soil Survey (2019). Available at: <https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm/> (Accessed May 24, 2019).
- Volder, A., Smart, D. R., Bloom, A. J., and Eissenstat, D. M. (2004). Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness. *New Phytol.* 165, 493–502. doi: 10.1111/j.1469-8137.2004.01222.x
- Volder, A., Anderson, L. J., Smart, D. R., Bloom, A. J., Lakso, A. N., and Eissenstat, D. M. (2009). Estimating nitrogen uptake of individual roots in container- and field-grown plants using a ¹⁵N-depletion approach. *Funct. Plant Biol.* 36, 621. doi: 10.1071/FP08330
- Wang, J., Fu, B., Lu, N., and Zhang, L. (2017). Seasonal variation in water uptake patterns of three plant species based on stable isotopes in the semi-arid Loess Plateau. *Sci. Total Environ.* 609, 27–37. doi: 10.1016/j.scitotenv.2017.07.133
- Washington State Wine Commission, USA (2020). *Grape production report*. Available at: <https://www.washingtonwine.org/trade/documents> (Accessed Jul 26, 2020).
- Zaccaria, D., Carrillo-Cobo, M. T., Montazar, A., Putnam, D., and Bali, K. (2017). Assessing the viability of sub-surface drip irrigation for resource-efficient alfalfa production in central and southern California. *Water* 9:837. doi: 10.3390/w9110837
- Zhang, H., Wang, D., Ayars, J. E., and Phene, C. J. (2017). Biophysical response of young pomegranate trees to surface and sub-surface drip irrigation and deficit irrigation. *Irrig. Sci.* 35, 425–435. doi: 10.1007/s00271-017-0551-y
- Zhang, Y. F., Li, Y. P., Sun, J., and Huang, G. H. (2020). Optimizing water resources allocation and soil salinity control for supporting agricultural and environmental sustainable development in Central Asia. *Sci. Total Environ.* 704, 135281. doi: 10.1016/j.scitotenv.2019.135281
- Zhong, Y., Fei, L., Li, Y., Zeng, J., and Dai, Z. (2019). Response of fruit yield, fruit quality, and water use efficiency to water deficits for apple trees under surge-root irrigation in the Loess Plateau of China. *Agric. Water Manage.* 222, 221–230. doi: 10.1016/j.agwat.2019.05.035

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Ma, Jacoby and Sanguinet. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Vitis vinifera L. Diversity for Cations and Acidity Is Suitable for Breeding Fruits Coping With Climate Warming

Antoine Bigard^{1,2}, Charles Romieu^{1,3}, Yannick Sire² and Laurent Torregrosa^{1,2,3*}

¹ AGAP, University of Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France, ² UE INRAE de Pech Rouge, University of Montpellier, INRAE, Gruissan, France, ³ GENOVIGNE, University of Montpellier, IFV, INRAE, Institut Agro, Montpellier, France

The selection of grapevine varieties is considered to be the smartest strategy for adapting the viticulture to climate warming. Present knowledge of the diversity of grape solutes known to be influenced by temperature is too limited to perform genetic improvement strategies. This study aimed to characterize the diversity for major cations (K^+ , Mg^{2+} , Ca^{2+} , NH_4^+) of the *Vitis vinifera* fruit and their effect on acidity. Two developmental stages were targeted: the end of green growth, when organic acids reach a maximum, and the physiological ripe stage defined by the stopping of solutes and water import at the maximum volume of the berry. Twelve varieties and 21 microvines from the same segregating population were selected from preliminary phenotyping. The concentration of cations depended on the stage of fruit development, the genotype and the environment with GxE effects. In the ripe grape, K^+ concentration varied from 28 to 57 mmol.L⁻¹ with other cations being less concentrated. Combined with the variation in organic acids, cation concentration diversity resulted in titratable acidity of the ripe fruit ranging from 38 to 215 meq.L⁻¹. These results open new perspectives for the selection of varieties to mitigate the adverse effects of climate warming on grape quality.

Keywords: fleshy fruit, grape, cations, acidity, fruit quality, climate changes

OPEN ACCESS

Edited by:

Tommaso Frioni,
Catholic University of the Sacred
Heart, Piacenza, Italy

Reviewed by:

Zhanwu Dai,
Chinese Academy of Sciences, China
Elisabetta Oddo,
University of Palermo, Italy

*Correspondence:

Laurent Torregrosa
laurent.torregrosa@supagro.fr

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 18 April 2020

Accepted: 20 July 2020

Published: 18 September 2020

Citation:

Bigard A, Romieu C, Sire Y and
Torregrosa L (2020) *Vitis vinifera* L.
Diversity for Cations and Acidity Is
Suitable for Breeding Fruits Coping
With Climate Warming.
Front. Plant Sci. 11:01175.
doi: 10.3389/fpls.2020.01175

INTRODUCTION

With a world production ranging from 75 to 85 million tons a year, grapes are one of the most commonly eaten fleshy fruits. At a global level, viticulture is mainly dedicated to table grape production (www.oiv.int, www.fao.org), but the production of juice, dried grapes or wines can also be important to local economies. For instance, wine production in France represents an annual economic balance of more than 11 billion € (www.franceagrimer.fr).

The grape is mainly composed of water (75–85% of the fresh weight), sugars (10–15%), organic acids (0.2–1%), minerals (0.1–0.5%), polyphenolic compounds (0.1–0.2%) and aroma compounds (<0.1%). The development of the berry involves two growth periods (Mullins et al., 1992). The first phase results from cell division and a first run of vacuolar expansion due to the accumulation of organic acids (Kliewer, 1965; Ojeda et al., 1999). During this phase, inorganic compounds are accumulated, e.g. Ca^{2+} with a central role in cell wall structure, but also K^+ , NH_4^+ , and Mg^{2+} as counter-ions for vacuolar anions (Doneche and Chardonnay, 1992; Mpelasoka et al., 2003; Bashir and Kaur, 2018).

After a lag phase, berries soften, phloem unloading shifts from the symplasmic to the apoplasmic pathway (Zhang et al., 2006), triggering the sudden acceleration of sugar import and a second phase of water import, known as ripening (Matthews et al., 1987). The organization of the sugar import pathway remains uncertain and it was proposed that it could be energized by the discharge of a phloem potassium battery (Nieves-Cordones et al., 2020).

Cations participate in many aspects of fruit development through the regulation of various metabolic pathways (Ramesh-Kumar et al., 2006; Maathuis, 2009; Song et al., 2018). For instance, K^+ regulates almost 60 enzymes, including protein synthesis, oxidative metabolism and photosynthesis (Vicente et al., 2009). Magnesium is a major constituent of the chlorophyll and serves important biochemical functions in protein synthesis. It is also involved in the regulation of energetic metabolism as a constituent of the Mg-ATP or Mg-ADP complex and in the regulation of the Calvin cycle (Vicente et al., 2009). In plants, Ca^{2+} is primarily associated with the cell wall pectin materials with a role in turgor regulation associated with organ rheological properties. It is also a mediator of plant responses, such as abiotic stress signaling. Potassium is the predominant cation in plants (Doneche and Chardonnay, 1992; Bonomelli and Ruiz, 2010; Bashir and Kaur, 2018). Given their sequential accumulation, organic acids (up to 250 mmol.L^{-1}) and sugars (up to 1 mol.L^{-1}) are the main contributors of the osmotic potential during, respectively, green stage and ripening, far above inorganic compounds (Storey, 1987). Indeed, K^+ remains below 100 mmol.L^{-1} , and both magnesium and calcium remain under 5 mmol.L^{-1} all throughout grapevine fruit development.

Grape juice acidity is dependent on cations which neutralize and precipitate a fraction of organic acids (Champagnol, 1984). The balance between acidity and sugars is known to be a major determinant of wine organoleptic quality (La Rosa, 1955; Jaime-Baro, 1973; Kourakou, 1974; Du Plessis, 1984). Climate changes have already impacted vine development and grape composition (Ojeda et al., 2017; Drappier et al., 2017). The impact of environmental factors on grapevine reproductive development and on the accumulation of primary metabolites has been extensively described (Butrose, 1969a; Butrose, 1969b; Webb et al., 2007; Dai et al., 2011; Greer, 2012; Xu et al., 2014; Rienth et al., 2016; Luchaire et al., 2017; Torregrosa et al., 2017). However, the impact of environmental factors on cation accumulation has received little attention. A panel of viticultural practices can be implemented to modify the balance between primary metabolites and cations, but these practices have been shown to induce deleterious effects on plant development or secondary metabolite accumulation (Champagnol, 1984; Greer et al., 2011; Bobeica et al., 2015). Similarly, post-harvest corrections of the acido-basic balance can improve (most often in increasing) acidity, from organic acid addition to cation removal through electrodialysis (Escudier et al., 2012; Sweetman et al., 2014).

The use of genetic diversity and breeding appear as smart options for selecting genotypes better adapted to global warming (Ollat et al., 2015; Gascuel et al., 2017; Torregrosa et al., 2017).

However, modern viticulture only uses a limited fraction of the potential diversity (Wolkovich et al., 2018). For instance, the 30 first varieties propagated in France accounted for 85% of all plants produced in 2017 (www.franceagrimer.fr). There are several studies on the diversity for fruit size, primary and secondary metabolites in *V. vinifera* (Boursiquot et al., 1995; Shiraishi et al., 2010; Houel et al., 2013; Preiner et al., 2013; Teixeira et al., 2013; Yinshan et al., 2017) or in segregating progenies (Doligez et al., 2006; Liu et al., 2006; Liu et al., 2007; Mejia et al., 2007; Duchêne et al., 2012; Doligez et al., 2013; Duchêne et al., 2013; Chen et al., 2015; Costantini et al., 2015; Houel et al., 2015). However, while there are some reports about the capacity of the rootstock to modulate K^+ and Mg^{2+} scion nutritional status (Kodur et al., 2009), little is known about the genetic diversity of cation composition of the grapevine fruit.

Recently, Bigard et al. (2019) pointed out the impossibility to unambiguously define developmental stages in unsynchronized berry populations. Attempts were made to compare genotypes more precisely by sorting berries at precise physiological stages, i.e. the onset, and the arrest, of phloem unloading through the apoplasmic pathway (Bigard et al., 2018). Using the same approaches, we have characterized the diversity for the major cation concentration (i.e. Potassium, Calcium, Magnesium and Ammonium) in grapevine fruit and the resulting acidity.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Berries from two *Vitis vinifera* subsets (Table S1) were analysed. The first subset included: i) in 2016, 12 ungrafted accessions established on non-irrigated sandy soils at the Grapevine Biological Resources Centre of Vassal (www6.montpellier.inrae.fr/vassal) among which ii) 6 genotypes were re-phenotyped in 2017 at the experimental vineyard of Institut Agro of Montpellier (en.montpellier-supagro.fr/research/experimental-research-platforms/pierre-galet-experimental-vineyard), all grafted on SO4 rootstock, established on gravelly soils. Five to twenty replicated plants were available each year, managed by spur pruning, vertical shoot positioning (VSP) and fertirrigation. The number of clusters was reduced to 4-8 per vine by cluster thinning to avoid source/sink unbalance effects.

The second subset included a progeny of 21 microvines derived from a cross between the Picovine 00C001V0008 (*Vvgai1/Vvgai1*) bearing the *Dwarf and Rapid Cycling and Flowering* (DRCF) trait (Chaib et al., 2010) and the Ugni Blanc fleshless berry mutant (Fernandez et al., 2006). These microvine plants were grown in pot (3-6 years old) in semi-controlled conditions at the INRA experimental centre of Pech-Rouge (France) in 2016. In 2017, 6 of the 21 microvines were re-phenotyped at the Montpellier SupAgro campus. Lateral branches were systematically removed to standardize vegetative and reproductive development, maintaining a single proleptic shoot per plant as described in Luchaire et al. (2017) and Torregrosa et al. (2019).

Both subsets of genotypes were exposed to different growing conditions to assess G×E effects: microvines were maintained at 15/25 +/- 3°C night/day temperatures and watered at full PET (potential evapotranspiration) in greenhouse while macrovines were grown outdoors for 2 years in 2 environments differing for soils and climate conditions (Table S2). In the manuscript, the terms experiments, environment, or year are indifferently used to represent the variations due to environment *sensu lato* (E).

Fruit Sampling Methods

Samples at key stages of berry development were obtained as described in Bigard et al. (2018). For varieties, in 2016, individual berry softening was monitored by hand on 9 pre-selected bunches. When the first soft berries were detected, it was assumed that all the remaining ones had reached the green lag-phase, and 4–30 hard berries were sampled. Then, 3, 4 and 5 weeks later, 2–54 berries were sampled on the same bunches. Unfortunately, all berries were already soft at the beginning of the experiment on Trousseau, so the green stage is missing for this genotype. From the 3 dates of sampling, only the samples displaying the maximum average berry volume were analyzed for cations. In 2017, the same sampling methodology was repeated at the green stage. Berry growth was then non-destructively monitored upon immersing in water two reference clusters per genotype 3 times a week (Torregrosa et al., 2008). Triplicates (3 x 30 berries) were sampled at 3-day intervals when berry growth started to slow down. In 2017, samples for green stages of Trousseau and Muscat d'Alexandrie were lost.

For microvines, plants were grown until displaying all reproductive stages from flowering to berry shriveling (Torregrosa et al., 2019). In 2016 and 2017, 2–11 green lag-phase berries were sampled per cluster as described above for macrovines. Regarding ripe stage, in 2016, 2–13 berries were collected from 3 successive bunches above the one exhibiting the first signs of shrivelling. In 2017, 5–8 berries were sampled on clusters 3 to 5 levels below the bunch showing berry softening.

Sample Preparation

For both genotype subsets, samples displaying the maximum average berry volume were selected for cation analyses. In 2016, berries were ground with a mortar and pestle at room temperature and frozen at -30°C. Before analyses, samples were first heated at 60°C for 30 min. Crude samples were then vigorously vortexed for 30 seconds and centrifuged 5 min at 18,500 g at 20°C. Clear supernatant was 10X-diluted with 0.2 N HCl, and then filtered on 0.2 µm cellulose acetate filters and finally analyzed. In 2017, after weighing, berries were immersed in 4 volumes of 0.25 N HCl, de-seeded and incubated for 2 days at room temperature. After a gentle shaking, supernatants were 10X-diluted with water and stored at -30°C. Then, for analysis, samples were defrosted at room temperature and vigorously shaken.

Cations Analysis and Titratable Acidity Calculation

Samples were centrifuged for 3 min at 12000 rpm (20°C) and then 10 µl clear supernatant was directly injected via a Waters® 717 (Waters, www.waters.com) device for HPLC through a

Waters® IC-Pak Cation M/D 3.9x150 mm column (20°C) eluted at 1ml/min flow rate with 0.004 N HNO₃ as mobile phase. Then, K⁺, Ca²⁺, Mg²⁺ and NH₄⁺ concentrations were measured using a Shimadzu® CDD-10A conductometer (Shimadzu, www.shimadzu.fr) and the Waters® EMPOWER-3 peak integration software. Recalculated titratable acidity (RTA) was expressed in meq.L⁻¹, as the sum of malic and tartaric acids (Bigard et al., 2018) minus K⁺, these elements being respectively the main anions and the major cation (Boulton, 1980).

Data Analysis and Graphic Representations

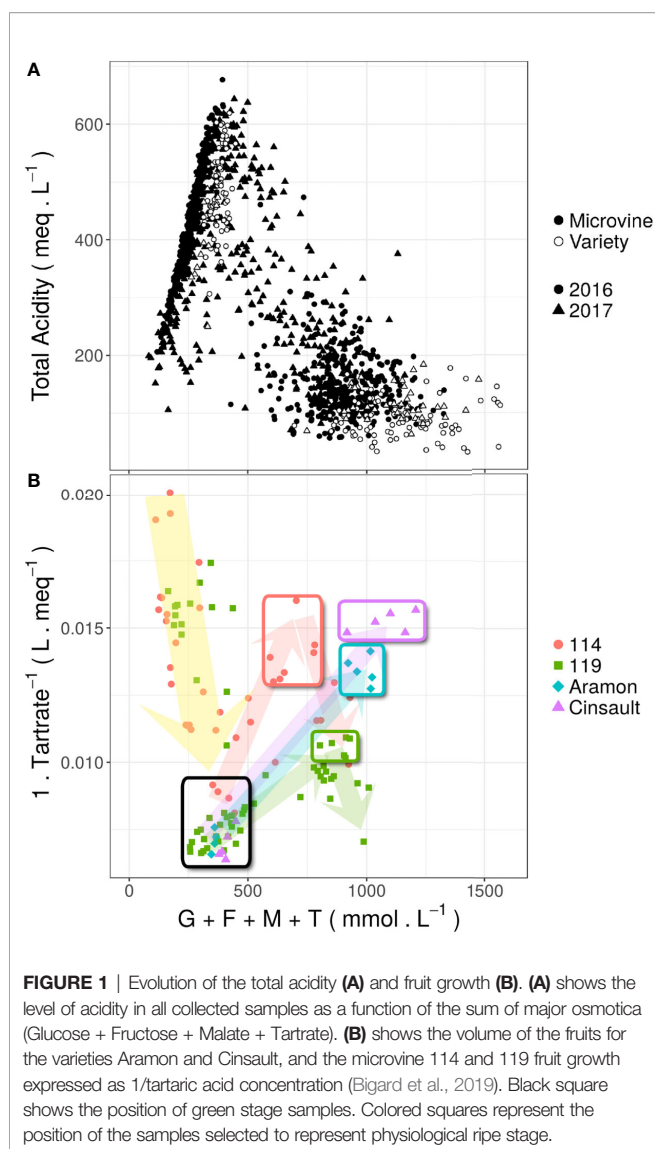
Statistical analysis was conducted using R-software version 3.4.3 (R Core Team, 2017). Raw data and R codes will be provided upon request. Statistical analysis of G, E and G×E interactions were based on genotypes repeated in 2016 and 2017 and determined using an ANOVA II test for parametric data subsets and a Two-way ordinal regression test for non-parametric data subsets. Correlations were calculated using a Pearson's correlation coefficient test.

RESULTS AND DISCUSSION

The Complexity of the Sampling Strategy

The changes in total acidity (Figure 1A), berry growth (Figure 1B) and malic acid (Figure 3 in Bigard et al., 2018) with respect to the accumulation of major osmotica are proxies to assess the advancement of fruit ripening. For both genotype subsets (Figure 1A), berry total acidity falls in the expected order of magnitude. The developmental stage of the different samples can be outlined using berry growth (Figure 1B), which is correlated to the dilution of the tartaric acid (Bigard et al., 2019). For microvines, samples are distributed according to the classical development program of the grapevine fruit from green to over-ripe stages. The selection performed on the base of berry volume allowed for the refining of targeted stages (Figure 1B), the berry volume virtually doubling between green and ripe stage, as widely accepted for most *V. vinifera* varieties (Houel et al., 2013; Bigard et al., 2018).

For all species forming clusters of small fruits such as redcurrant, blueberry, blackcurrant, dates, coffee or grapevine, the phenotyping is complicated by the heterogeneity and asynchrony of single fruit development (Lobos and Hancock, 2015; Lobos et al., 2017). A cluster is composed of berries at different developmental stages (Coombe, 1992; Shahood, 2017; Bigard et al., 2019; Shahood et al., 2019). In genetic and most physiological studies, a grape phenological stage corresponds to a mix of berries representing the heterogeneity of the fruits at plot level (Preiner et al., 2013; Houel et al., 2015; Yinshan et al., 2017; Duchêne et al., 2020). Obviously, the concept of developmental stage remains equivocal in such heterogeneous samples. Indeed, a phenological stage must be defined by intrinsic physiological parameters, such as the onset and the arrest of sugar loading, which may occur at different harvest dates or brix, according to the genotype (Bigard et al., 2019; Shahood et al., 2019).



The sampling procedure implemented here aimed to obtain samples representative of the onset and at the arrest of the second growth period which must be clearly distinguished from the following shriveling phase. Our approach includes two intrinsic limitations. Firstly, the sampling of the last hard berries to represent the green stage and the collection of spatial (microvines, Luchaire et al., 2017) or temporal (varieties) series of berries to select the ripe stage, both presume that all fruits are synchronized. Secondly, a sample averaging unsynchronized berries can only provide an approximation of the maximum concentration reached by a berry (Bigard et al., 2019). Despite these limitations, the interpretation of Figures 1A, B, and the Figure 3 of Bigard et al. (2018), suggests that proposed methods can reduce some usual averaging artifacts in genotypic diversity studies.

Potassium

Varieties and microvines displayed very limited changes in K^+ concentration during ripening (Figure S5). Since the volume of

the *V. vinifera* berry doubles during ripening (Houel et al., 2013; Bigard et al., 2018), this implies a very faint K^+ accumulation rate, with respect to major organic osmotica. For varieties which were grown outdoors, K^+ concentrations ranged from 21 (Muscat d'Alexandrie) to 43 (Béclan) mmol.L^{-1} at green stage and from 35 (Couston) to 54 (Petit Manseng) mmol.L^{-1} at ripe stage (Figure 2) with very little evolution during ripening for most varieties (Figure S6). Microvines grown in greenhouse displayed similar K^+ concentrations, ranging from 25 (microvine n°73) to 47 (microvine n°199) mmol.L^{-1} at green stage and from 28 (microvine n° 117) to 57 (microvine n°349) mmol.L^{-1} at ripe stage with slight evolution during ripening for most lines (Figure S7). At the green stage, statistical analyses (Tables S3, S4) showed an effect of G, E and GxE on K^+ accumulation for both varieties and microvines. At the ripe stage, G and GxE effects were statistically significant in varieties with no E effect. The strong G effect compared to the small GxE effect suggests a genetic control of this trait at the ripe stage in varieties. In the microvine subset, both G and E effects were statistically significant without interaction showing the strong genetic control of this trait inside a progeny.

Potassium concentrations observed here are either higher (Mpelasoka et al., 2003) or lower (Storey, 1987; Rogiers et al., 2017) than previously reported in other GxE backgrounds. It is known that agronomic factors (type of soil, fertilization, irrigation...) modulate K^+ soil availability and influence its accumulation in grapes (Champagnol, 1984). Temperature during ripening can also modulate K^+ concentration in ripe grapes (Mira de Orduña, 2010). Moreover, methodological factors can also make the comparison difficult. Indeed, around 50% of berry K^+ is located in the skin, the extractability of which critically relies on the protocol, and may dramatically increase during ripening, due to marked modification in cell wall structure and tightness (Possner and Kliever, 1985). Furthermore, K^+ is prone to precipitation as potassium bitartrate in the juice, as the pH increases during ripening (Rienth et al., 2016). Hence the quantification of this element is dependent on the care taken to avoid its precipitation prior to analysis. In this study, sample preparation performed at high temperatures or in acid conditions and high dilution levels limited the precipitation of K^+ . However, as the first steps of sample preparation were different in 2016 and 2017, it cannot be excluded that extraction efficiency varied between the 2 years. Nevertheless, even this potentially limits the discussion on causality of year-to-year variations, Dumas et al. (2020) showed that the methods of extraction have little impact on genotypic comparisons.

Considering the diversity of genotypes and environments of this study, the variability for K^+ content in the ripe fruit is quite moderate, suggesting a strong homeostasis for this element. There have been significant advances in the understanding of the mechanisms of K^+ transport and accumulation in the last 10 years (Rogiers et al., 2017; Vilete et al., 2020). Compared to other reports, our data reveals 2 interesting biological features: i) the low increase of K^+ concentration during ripening and ii) the lack of environmental effect, when compared to sugars (Bigard et al., 2018). Differences with the previous reports could result from the

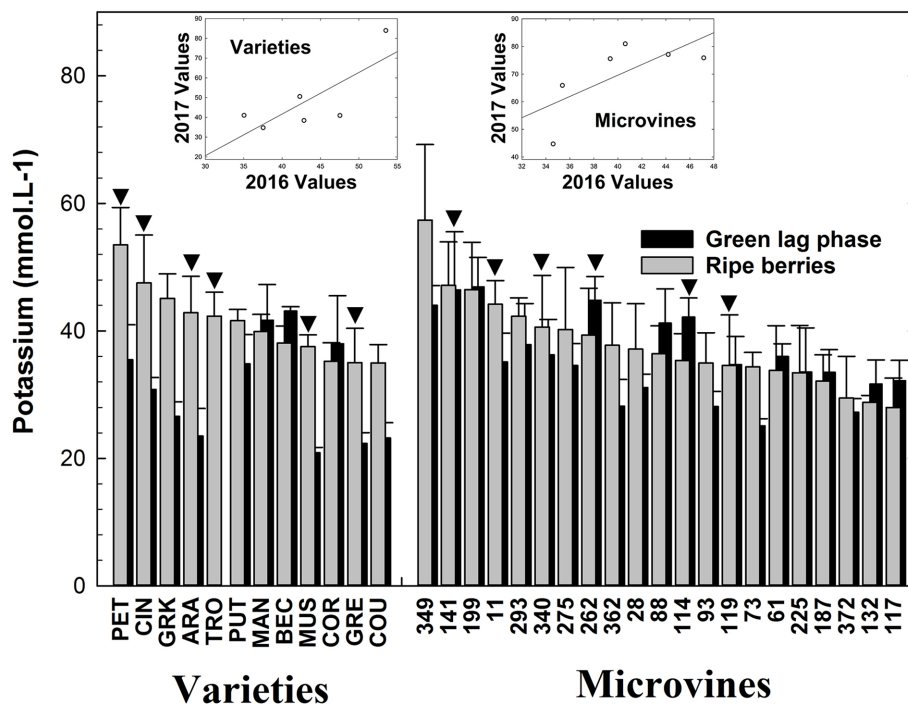


FIGURE 2 | K^+ concentrations in the grapevine fruit at the end of green growth and at physiological ripe stage. Bar chart represents 2016 mean values with the corresponding SE. Genotypes experimented in 2016 and 2017 are indicated by a black down-pointing triangle. Inserted plots show the relationships between the mean values of both years (see **Tables S3, S4** for detailed numeric values and statistics).

sampling strategies. Generally, the determination of the ripe stage is based on unsorted berries harvested at technological maturity, after solutes and water importation stops. Here, we have taken care to assess solute concentrations before shriveling, that concentrates all the solutes of the berry, while their transport is definitively stopped.

Magnesium

Magnesium was less accumulated than potassium in all genotypes. At both fruit developmental stages, varieties accumulated less Mg^{2+} than microvines (**Figure S8**). At the green stage, Mg^{2+} ranged from 0.9 (Cinsaut) to 2.9 (Béclan) $mmol.L^{-1}$ for varieties and from 2.1 (microvine n° 114) to 6.6 (microvine n° 61) $mmol.L^{-1}$ for microvines (**Figure 3**). At the ripe stage, values ranged from 0.9 (Cornifesto) to 2.4 (Petit Manseng) $mmol.L^{-1}$ for varieties and from 2.2 (microvine n° 119) to 4.9 (microvine n° 362) $mmol.L^{-1}$ for microvines. At the green stage, statistical analyses (**Tables S3, S4**) showed an effect of G, E and GxE for both varieties and microvines, with a lower significance for E effects in microvines. At the ripe stage, statistical analyses showed a significant effect of E, G without GxE interaction for varieties, and G, E and GxE effects for microvines. As for K^+ , Mg^{2+} concentration evolved little during the ripening growth period (**Figures S9, S10**). In this study, Mg^{2+} concentrations were found higher in the microvine progeny than in varieties, which exhibited similar values than previously reported (Mpelasoka et al., 2003).

This could be due to the specific condition of cultivation of microvines which were grown in a greenhouse and on their own roots.

Calcium

This element is accumulated at a much lower rate than K^+ and Mg^{2+} in grapes (**Figure 4**). Varieties and microvines tended to display a similar range of Ca^{2+} concentrations values either in green or ripe fruits (**Figure S11**). At the green stage, concentrations in Ca^{2+} ranged from 1.1 (Cinsaut) to 10.5 (Béclan) $mmol.L^{-1}$ for varieties and from 0.8 (microvine n° 114) to 3.2 (microvine n° 362) $mmol.L^{-1}$ for microvines. At the ripe stage, values ranged from 0.1 (Mandilaria) to 3.2 (Béclan) $mmol.L^{-1}$ for varieties and from 0.4 (microvine n° 119) to 2 (microvine n° 372) $mmol.L^{-1}$ for microvines. The concentrations in Ca^{2+} tended to decrease in macrovine and microvines (**Figures S12, S13**). At the green stage, statistical analyses (**Tables S3, S4**) showed an effect of G, E and GxE for both varieties and microvines. At the ripe stage, statistical analyses showed a significant effect of E, G and GxE interaction for varieties, and G, E without GxE interaction for microvines also suggesting a possible control of this trait during breeding.

In this study, Ca^{2+} content in green and ripe berries was found higher than previously reported (Mpelasoka et al., 2003; Bonomelli and Ruiz, 2010; Bashir and Kaur, 2018). Interestingly, conversely to K^+ and Mg^{2+} , on both subsets, the concentration in Ca^{2+} decreased significantly during ripening while the volume of

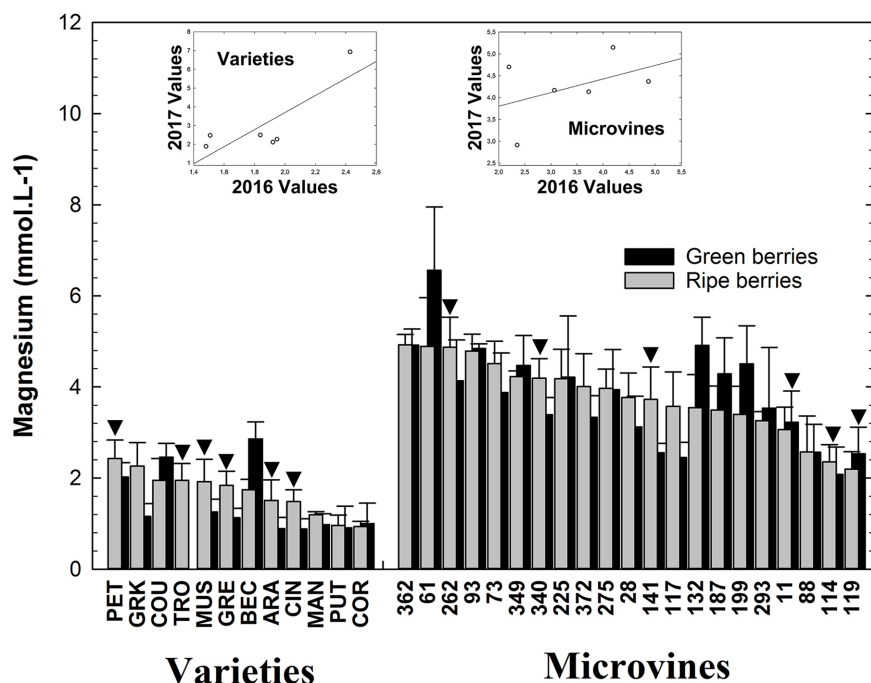


FIGURE 3 | Mg^{2+} concentrations of the grapevine fruit at the end of green growth and at physiological ripe stage. Bar chart represents 2016 mean values with the corresponding SE. Genotypes experimented in 2016 and 2017 are indicated by a black down-pointing triangle. Inserted plots show the relationships between the mean values of both years (see **Tables S3, S4** for detailed numeric values and statistics).

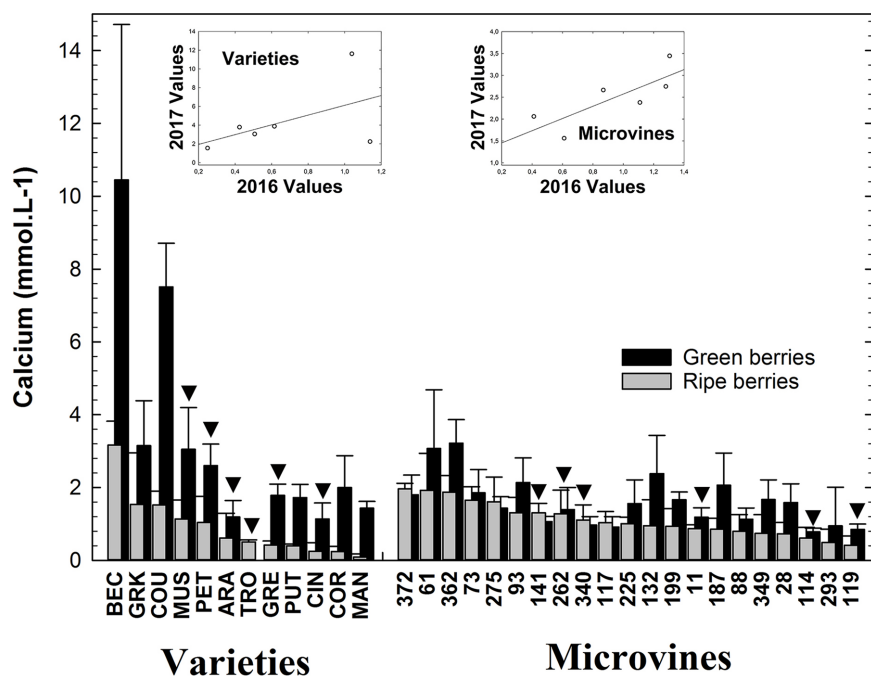


FIGURE 4 | Ca^{2+} concentrations in the grapevine fruit at the end of green growth and at physiological ripe stage. Bar chart represents 2016 mean values with the corresponding SE. Genotypes experimented in 2016 and 2017 are indicated by a black down-pointing triangle. Inserted plots show the relationships between the mean values of both years (see **Tables S3, S4** for detailed numeric values and statistics).

the berry usually doubled (Bigard et al., 2018). Due to a poor mobility of Ca^{2+} in the phloem (Hocking et al., 2016), this element is mainly accumulated during green berry growth to support cell division and structure. During grape ripening, which is associated with a second phase of growth by vacuolar expansion (Dai et al., 2011), this element is being diluted.

Ammonium

Varieties and microvines displayed a similar range of NH_4^+ concentrations values either in green or ripe fruits (**Figure S14**) with a clear tendency to decrease for both subset during ripening (**Figures S15, S16**). For varieties, NH_4^+ concentrations ranged from 0.9 (Béclan) to 19.6 (Grenache) mmol.L^{-1} at green stage and from 0 (Mandilaria) to 5.5 (Cornifesto) mmol.L^{-1} at ripe stage (**Figure 5**). In microvines, NH_4^+ ranged from 9.5 (microvine n°114) to 33.7 (microvine n°372) mmol.L^{-1} at green stage and from 3 (microvine n°349) to 15 (microvines n°141) mmol.L^{-1} at ripe stage. Statistical analyses (**Table S4**) showed an effect of G and GxE on NH_4^+ at green stage for the microvine line. Only microvine 114 kept high NH_4^+ concentrations for both years. For varieties (**Table S3**), they showed an effect of G, E and GxE. At the ripe stage, statistical analyses showed a significant effect of E, G with GxE interaction for both varieties and microvines. Grape displays the same range of NH_4^+ concentrations as other fleshy fruits such as red fleshy fruits, e.g. Strawberry (Taghavi et al., 2004), Blackberry or Raspberry (Strik and Bryla, 2015).

Ammonium is a source of yeast assimilable nitrogen (YAN) conditioning grape juice fermentation. Its accumulation is dependent on agronomical (cover grass, yield, fertilization) and environmental factors making the comparison between data obtained in different experimental contexts difficult. In this study, despite some effects of the year and the interaction GxE, we have observed that a fraction of the phenotypic diversity for this trait is genotype-dependent. In some genotypes, the amounts accumulated during berry development (up to 15 mmol.L^{-1}) potentially cover yeast needs while in some varieties the level of NH_4^+ could be limiting for wine processing (Salmon, 1996; Taillandier et al., 2007).

Recalculated Titratable Acidity (RTA)

As largely documented in grapevine, the acidity significantly decreased during ripening for both subsets (**Figures S17-S19**). Recalculated titratable acidity at the green stage ranged from 360 (Mandilaria) to 580 (Petit Manseng) meq.L^{-1} for varieties and from 318 (microvine n°293) to 578 (microvine n°73) meq.L^{-1} for microvines (**Figure 6**). At the physiological ripe stage, RTA varied from 38 (Trousseau) to 134 (Petit Manseng) meq.L^{-1} for varieties and from 64 (microvine n°349) to 215 (microvine n°73) meq.L^{-1} for microvines. At the green stage, statistical analyses (**Tables S3, S4**) showed an effect of G, E and GxE for varieties and a statistically significant effect on G with no E effect and no interactions for microvines. At the ripe stage, statistical analyses showed a significant effect of E, G with GxE

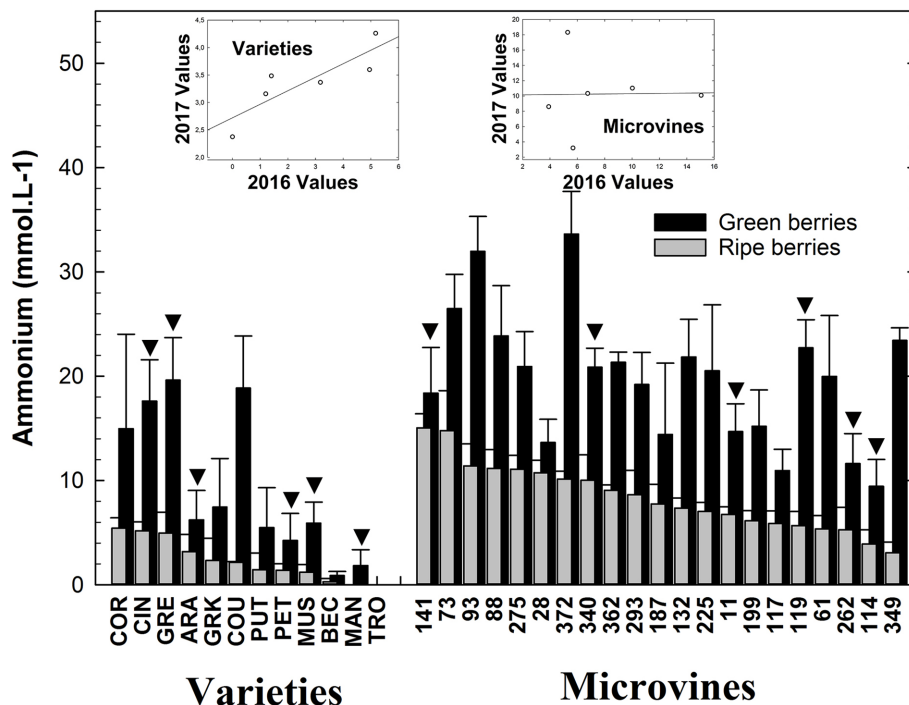


FIGURE 5 | NH_4^+ concentrations in the grapevine fruit at the end of green growth and at physiological ripe stage. Bar chart represents 2016 mean values with the corresponding SE. Genotypes experimented in 2016 and 2017 are indicated by a black down-pointing triangle. Inserted plots show the relationships between the mean values of both years (see **Tables S3, S4** for detailed numeric values and statistics).

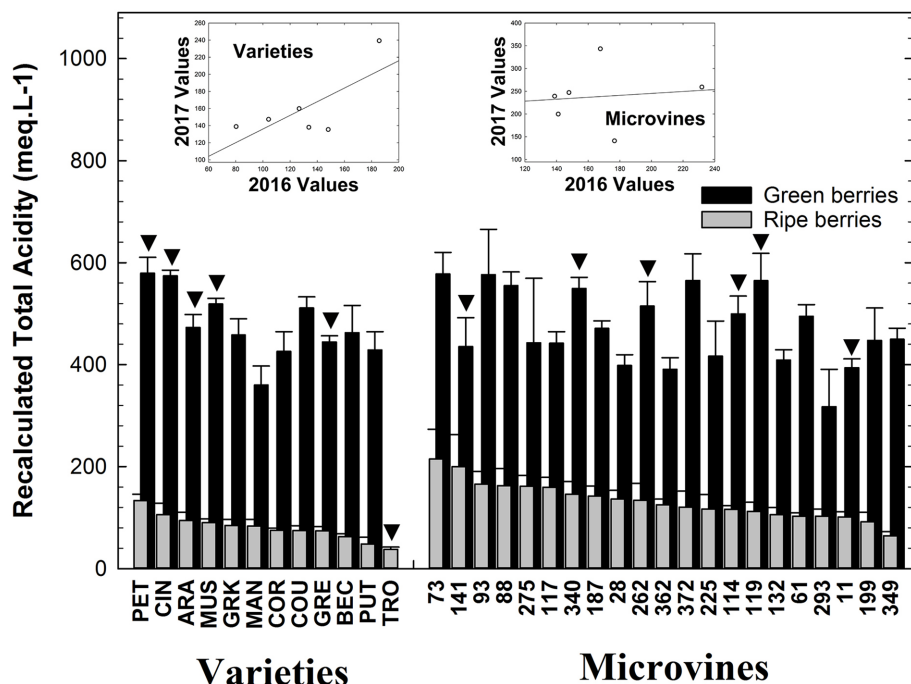


FIGURE 6 | Recalculated Total Acidity (meq.L⁻¹) the grapevine fruit at the end of green growth and at physiological ripe stage. Bar chart represents 2016 mean values with the corresponding SE. Genotypes experimented in 2016 and 2017 are indicated by a black down-pointing triangle. Inserted plots show the relationships between the mean values of both years (see **Tables S3, S4** for detailed numeric values and statistics).

interaction for both varieties and microvines. Those results are concomitant with the E, G and GxE effects obtained in Bigard et al. (2018) at the ripe stage for malate + tartrate concentration, showing the difficulty of finding a genetic control of this trait.

Grape acidity is a major challenge in viticulture (Champagnol, 1984; Sweetman et al., 2014; Ollat et al., 2018). The effect of temperature on grape acidity is well documented (Kliwer and Lider, 1970; Butrose et al., 1971; Seguin et al., 2004; Rienth et al., 2016). Following the report of Bigard et al. (2018) that presented the genetic diversity for anions (i.e. organic acids), here we analyzed the cation variations providing an overview of the diversity of the main determinants of the acidity of the grapes.

Correlations Between Traits

For microvines at the green stage, Mg²⁺ was correlated with Ca²⁺ in 2016 (0.73, *p*-Value < 0.05) and NH₄⁺ with the malic/tartaric acid ratio in 2017 (0.66, *p*-Value < 0.05). Several significant correlations appeared at ripe stage during both years: between Ca²⁺ and K⁺ (0.77, *p*-Value < 0.05), between RTA and NH₄⁺ (0.73, *p*-Value < 0.05), and between RTA and K⁺ (0.62, *p*-Value < 0.05). Glucose was also correlated with Mg²⁺ both years (0.66 and 0.75, *p*-Value < 0.05), malic acid with NH₄⁺ in 2016 (0.71, *p*-Value < 0.05). For varieties, at green stage in 2016, Ca²⁺ was correlated to Mg²⁺ (0.79, *p*-Value < 0.05) and tartaric acid to Mg²⁺ (0.65, *p*-Value < 0.05). At the ripe stage, very strong correlations were found in both years: between Ca²⁺ and Mg²⁺ (0.96, *p*-Value < 0.05),

between K⁺ and Mg²⁺ (0.86, *p*-Value < 0.05), between K⁺ and Ca²⁺ (0.81, *p*-Value < 0.05). In 2016, glucose was correlated to Mg²⁺ (0.70, *p*-Value < 0.05). In 2017, K⁺ was correlated to berry weight (-0.61, *p*-Value < 0.05) and tartaric acid was correlated to K⁺ (0.71, *p*-Value < 0.05), to Ca²⁺ (0.79, *p*-Value < 0.05) and to Mg²⁺ (0.72, *p*-Value < 0.05). All cations analysed except NH₄⁺, had an absolute correlation higher than 0.60 with RTA (*p*-Value < 0.05).

In this study, the strong impact of the environment was clearly visible as values were higher in 2016 than 2017 for cations accumulation. The highest correlations were found between Mg²⁺ and Ca²⁺ in varieties, both cations being known to have similar patterns of accumulation as expected from their common transport by xylem, and their absence from phloem sap (Glad et al., 1992a; Glad et al., 1992b).

In the range of the genotypes studied here, while the level of sugar concentrations increased by a factor 10 during ripening (Bigard et al., 2018), only a weak increase of K⁺ concentration was observed. In this respect, the Petit Manseng, a variety which accumulates a huge amount of sugars in the fruit (Bigard et al., 2018) only exhibited little variations of K⁺ concentration during ripening. This observation doesn't support the hypothesis of an interdependent import of sugars and K⁺ during ripening (Coetzee et al., 2019; Duchêne et al., 2020; Nieves-Cordones et al., 2020). Because the berries shrivel after the arrest of phloem unloading (Du Plessis, 1984; Bigard et al., 2019), an important part of the co-variations of sugars and K⁺ concentration during ripening could be linked to water loss. Hence the importance of

clearly defining the stages of sampling so as not to confuse the import and the concentration of fruit solutes.

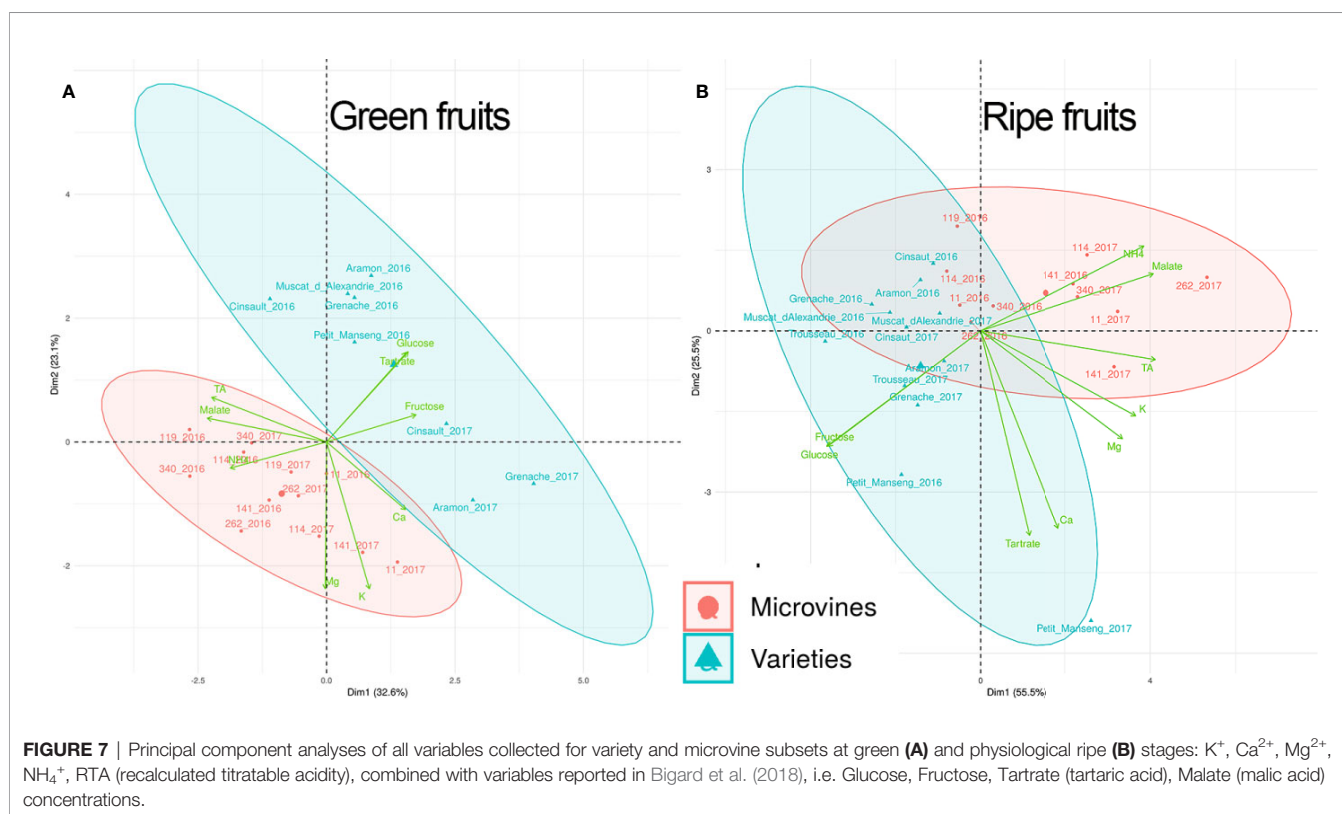
Cation and Acidity Diversity in a Breeding Perspective

The variables collected in this study at green and ripe stages for 12 accessions repeated in 2016 and 2017 (K^+ , Mg^{2+} , Ca^{2+} , NH_4^+ , Titratable acidity) with the data of primary metabolites (Glucose, Fructose, Tartaric and Malic acids) from Bigard et al. (2018) were submitted to a PCA (Figure 7). At the green stage, PCA (Figure 7A) represents 55.7% of the variability observable. This analysis shows that varieties accumulated more sugars than microvines, with less NH_4^+ . The total acidity (but not Tartaric acid) and cations (except NH_4^+) appear to be genotype-dependent in both genotype subsets. At the ripe stage, PCA (Figure 7B) represents 81% of the variability observable, which is higher than the green stage PCA. Both sugars were well correlated, as reported in Bigard et al. (2018). Another interesting observation is the link between malic acid and NH_4^+ , which was already mentioned in the correlation section, in particular at the ripe stage. It is empirically known in viticulture (Champagnol, 1984) that soil and fertilization management or the use of vigorous rootstocks, can increase the content in nitrogen of the grapes and also increase malic acid accumulation, as the results of complex interactions between plant vigor and microclimate.

To establish a selection strategy, it is critical to assess the genetic potential of the targeted species (Lobos et al., 2017; Torregrosa et al., 2017). This includes the estimation of the

available genetic diversity and the possibility to segregate targeted traits. For grapevine, as for other fruit crops, both the nutritional and the organoleptic components need to be considered. Indeed, in countries where nutrition is not secured either in quantity or quality, it may be critical for breeding fruit crops to focus on nutrient concentrations (Luby, 2009). This is particularly true for the regions where fresh grapes or derived products (juice, raisins) are a significant part of the human diet, such as Asia or South America (www.OIV.int). For fruits and non-fermented derivative products, vitamins and antioxidant compounds but also sugars and minerals are important nutritional components (Simon, 2014).

The amount of sugar at the ripe stage in grapes at physiological ripe stage can vary from 0.8 to 1.4 mol.L⁻¹ depending on the genotype (Bigard et al., 2018), which represents a significant potential of calories. The dietary reference index for K^+ is 120 mmol.day⁻¹ (IOM, 2004). Potassium is the predominant cation of most fleshy fruits where it can be accumulated up to 75 mmol.Kg⁻¹ in Banana for instance (Wall, 2006) and up to 60 mmol.Kg⁻¹ in grapes (this study). Magnesium is the second abundant cation in fleshy fruits (Wall, 2006), with a dietary reference index of 13 mmol.day⁻¹ (IOM, 2000). In some genotypes, we have shown here that Mg^{2+} can be accumulated up to 5 mmol.Kg⁻¹ at physiological ripe stage. So table grape and derivative products (juice or raisins), can be a source of energy, K^+ and Mg^{2+} for human nutrition. For Ca^{2+} , in regards to the level of the dietary reference index (250 mmol.day⁻¹, IOM, 2004) and considering the concentrations observed here (less than 3 mmol.Kg⁻¹ at physiological ripe



stage), grape consumption in fresh or as juice can only be a minor contributor to the human diet.

Regarding organoleptic properties, either for fresh grapes or derivative products (juice or wine), one important parameter to be discussed in light of our data is the sugar/acidity balance (Boulton, 1980; Du Plessis, 1984). Breeding programs are ongoing in Brazil and in France to select grape juice or wine varieties with improved sugar/acidity balance (Ritschel et al., 2014; Escudier et al., 2016). Due to several mechanisms, climate warming tends to increase the concentration in sugars in the grape at ripe stage (Ollat et al., 2018; Arrizabagala et al., 2018; Torregrosa et al., 2017) while decreasing the acidity (Lakso and Kliever, 1978; Romieu et al., 2018; Van Leeuwen et al., 2019). In this study, PCA analysis showed that sugars and K^+ are orthogonal at both developmental stages (Figure 7). The lack of univocal relationship between sugar and K^+ accumulation during the phloem unloading period was recently reported by Bigard et al. (2019). This result is now confirmed from a genetic point of view. Consequently, it seems rather unlikely that the selection of low K^+ accumulator genotypes would help in reducing the excessive sugar concentrations triggered by global warming, as proposed by Duchêne et al. (2020).

In Bigard et al. (2018), we have shown that either sugar or organic acid concentration can be independently selected in existing variety germplasms or in segregating populations. Here, we showed that cation concentrations display a significant genetic diversity and that this parameter can provide additional phenotypic diversity for the acidity of the grape. This diversity would be an effective alternative to physical or chemical methods currently allowed to improve grape must or juice acidity (Escudier et al., 2012; Sweetman et al., 2014). Indeed, while European regulations (EEC-606/2009) limit the addition of organic acids from 20–33 meq.L⁻¹ depending on the product and the region, the OIV codex determines a maximum of 54 meq.L⁻¹ (OENO 3/99 and OENO 13/01) for these corrections. The correction of cation concentrations by ion exchange resins or by bipolar membrane electrodialysis is also limited to 54 meq.L⁻¹ (CEE 53/2011, OENO 360/2010) for conventional wines. Electro-membrane based and chemical processes cannot be combined and no correction is allowed for the production of organic wines.

The data reported here in complement to the report of Bigard et al. (2018), shows that a significant genotypic diversity is prevalent in *V. vinifera* for fruit composition at physiological ripe stage. This study also shows that parameters determining berry growth, organic and inorganic solute accumulation, i.e. sugar loading, organic acid synthesis and dilution, and major cation importation can be manipulated by crossbreeding. This opens interesting perspectives for the selection of grape varieties displaying specific fruit composition traits, with some of them being potentially useful to mitigate some adverse effects of climate warming on grape quality.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

LT, CR, and AB designed the experiments. AB, YS, and CR performed the experiments. AB, LT, and CR drafted and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the CIVB (Comité Interprofessionnel des Vins de Bordeaux), the Jean Poupelain foundation, the government of Occitanie, INRAe and the Institut Agro de Montpellier.

ACKNOWLEDGMENT

Authors thanks: Thierry Lacombe, Hernan Ojeda, Mélanie Veyret, Shanaelle Mochée, Cécile Marchal, Sandrine Dedet, Marc Farnos, Angélique Adivèze and Ricardo Tello for advices and valuable technical help at several stages of the experiments. Part of this work was done in the frame of the INRA metaprogram ACCAF-LACCAGE (www6.inrae.fr/laccage).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.01175/full#supplementary-material>

SUPPLEMENTARY TABLE 1 | List of the genotypes of the 2 subsets of genotypes.

SUPPLEMENTARY TABLE 2 | Sum of the GDD (growing degree days) in base 10 and means of the average of the maximum temperatures during the 4 months of sampling outdoors (varieties) or in greenhouse (microvines).

SUPPLEMENTARY TABLE 3 | Mean values and statistics of the fruit parameters measured for the 6 *V. vinifera* varieties in 2016 and 2017 at green and physiological fruit ripe stage.

SUPPLEMENTARY TABLE 4 | Mean values and statistics of the fruit parameters measured for the 6 *V. vinifera* microvines in 2016 and 2017 at green and physiological fruit ripe stage.

SUPPLEMENTARY FIGURE 5 | Potassium concentration as a function of sum of major osmotica (glucose + fructose + malate + tartrate) during ripening for all samples of this study (S5) and in variety (S6) and microvine (S7) subsets. Colored arrows show the evolution of K^+ concentration for 3 genotypes of each subset.

SUPPLEMENTARY FIGURE 6 | Magnesium concentrations as a function of sum of major osmotica (glucose + fructose + malate + tartrate) during ripening for all samples of this study (S8) and in variety (S9) and microvine (S10) subsets. Colored arrows show the evolution of Mg^{2+} concentration for 3 genotypes of each subset.

SUPPLEMENTARY FIGURE 7 | Calcium concentrations as a function of sum of major osmotica (glucose + fructose + malate + tartrate) during ripening for all

samples of this study (**S11**) and in variety (**S12**) and microvine (**S13**) subsets. Colored arrows show the evolution of Ca^{2+} concentration for 3 genotypes of each subset.

SUPPLEMENTARY FIGURE 8 | Ammonium concentrations as a function of sum of major osmotica (glucose + fructose + malate + tartrate) during ripening for all samples of this study (**S14**) and in variety (**S15**) and microvine (**S16**) subsets.

REFERENCES

- Arrizabalaga, M., Morales, F., Oyarzuna, M., Delrot, S., Gomès, E., Irigoyena, J. J., et al. (2018). Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. *Plant Sci.* 267, 74–83. doi: 10.1016/j.plantsci.2017.11.009
- Bashir, S., and Kaur, N. (2018). The biochemistry of grape berry development. *Int. J. Curr. Microbiol. Appl. Sci.* 7 (2), 1692–1699. doi: 10.20546/ijcmas.2018.702.204
- Bigard, A., Berhe, D. T., Maoddi, E., Sire, Y., Boursiquot, J. M., Ojeda, H., et al. (2018). *Vitis vinifera* L. fruit diversity to breed varieties anticipating climate changes. *Front. Plant Sci.* 9, 455. doi: 10.3389/fpls.2018.00455
- Bigard, A., Romieu, C., Sire, Y., Veyret, M., Ojeda, H., and Torregrosa, L. (2019). Grape ripening revisited through berry density sorting. *OenoOne* 4, 719–724. doi: 10.20870/oeno-one.2019.53.4.2224
- Bobeca, N., Poni, S., Hilbert, G., Renaud, C., Gomès, E., Delrot, S., et al. (2015). Differential responses of sugar, organic acids and anthocyanins to source-sink modulation in Cabernet Sauvignon and Sangiovese grapevines. *Front. Plant Sci.* 29, 382. doi: 10.3389/fpls.2015.00382
- Bonomelli, C., and Ruiz, R. (2010). Effects of foliar and soil calcium application on yield and quality of table grape cv. 'Thompson Seedless'. *J. Plant Nutr.* 33 (3), 299–314. doi: 10.1080/01904160903470364
- Boulton, R. (1980). The general relationship between potassium, sodium and pH in grape juice and wine. *Am. J. Enol. Vitic.* 31, 182–186.
- Boursiquot, J. M., Dessup, M., and Rennes, C. (1995). Distribution des principaux caractères phénologiques, agronomiques et technologiques chez *Vitis vinifera* L. *Vitis* 34, 31–35.
- Butrose, M. S., Hale, C. R., and Kiewer, W. M. (1971). Effect of temperature on the composition of Cabernet-Sauvignon berries. *Am. J. Enol. Vitic.* 22, 71–75.
- Butrose, M. S. (1969a). Fruitfulness in grapevine: effect of light intensity and temperature. *Bot. Gaz.* 130, 166–173. doi: 10.1086/336486
- Butrose, M. S. (1969b). Vegetative growth of grapevine varieties under controlled temperature and light intensity. *Vitis* 8, 280–285.
- Chaib, J., Torregrosa, L., Mackenzie, D., Corena, P., Bouquet, A., and Thomas, M. R. (2010). The microvine - a model system for rapid forward and reverse genetics of grapevines. *Plant J.* 62 (10), 1083–1092. doi: 10.1111/j.1365-3113.2010.04219
- Champagnol, F. (1984). *Éléments de Physiologie de la Vigne et de Viticulture Générale*, ed Dehan (Montpellier, France: Dehan).
- Chen, N., Wang, L. C., Fang, L. C., Liang, S. H., and Wu, B. H. (2015). Construction of a high-density genetic map and QTLs mapping for sugars and acids in grape berries. *BMC Plant Biol.* 15, 28. doi: 10.1186/s12870-015-0428-2
- Coetzee, Z. A., Walker, R. E., Liao, S., Barril, C., Deloire, A. J., Clarke, S. J., et al. (2019). Expression patterns of genes encoding sugar and potassium transport proteins are simultaneously upregulated or downregulated when Carbon and potassium availability is modified in Shiraz (*Vitis vinifera* L.) berries. *Plant Cell Physiol.* 60, 2331–2342. doi: 10.1093/pcp/pcz130
- Coombe, B. G. (1992). Research on Development and Ripening of the Grape Berry. *Am. J. Enol. Vitic.* 43, 101–110.
- Costantini, L., Malacarne, G., Lorenzi, S., Troggio, M., Mattivi, F., Moser, C., et al. (2015). New candidate genes for the fine regulation of the colour of grapes. *J. Exp. Bot.* 66, 4427–4440. doi: 10.1093/jxb/erv159
- Dai, Z. W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J. P., Bordenave, L., et al. (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. *Am. J. Enol. Vitic.* 62, 413–425. doi: 10.5344/ajev.2011.10116
- Doligez, A., Audiot, E., Baumes, R., and This, P. (2006). QTLs for muscat flavor and monoterpenic odorant content in grapevine (*Vitis vinifera* L.). *Mol. Breed.* 18, 109–125. doi: 10.1007/s11032-006-9016-3
- Doligez, A., Bertrand, Y., Farnos, M., Grolier, M., Romieu, C., Esnault, F., et al. (2013). New stable QTLs for berry weight do not colocalize with QTLs for seed traits in cultivated grapevine (*Vitis vinifera* L.). *BMC Plant Biol.* 13. doi: 10.1186/1471-2229-13-217
- Doneche, B., and Chardonnet, C. (1992). Evolution et localisation des principaux cations au cours du développement du raisin. *Vitis* 31 (4), 175–181. doi: 10.5073/vitis.1992.31.175-181
- Drappier, J., Thibon, C., Rabot, A., and Geny-Denis, L. (2017). Relationship between wine composition and temperature: impact on Bordeaux wine typicity in the context of global warming: review. *Crit. Rev. Food. Sci. Nutr.* 59 (1), 14–30. doi: 10.1080/10408398.2017.1355776
- Du Plessis, C. S. (1984). Optimum maturity and quality parameters in grapes: A Review. *S. Afr. J. Enol. Vitic.* 5, 35–42. doi: 10.21548/5-1-2367
- Duchêne, E., Dumas, V., Jaegli, N., and Merdinoglu, D. (2012). Deciphering the ability of different grapevine genotypes to accumulate sugar in berries. *Aus. J. Grape Wine Res.* 18, 319–328. doi: 10.1111/j.1755-0238.2012.00194.x
- Duchêne, E., Dumas, V., Jaegli, N., and Merdinoglu, D. (2013). Genetic variability of descriptors for grapevine berry acidity in Riesling, Gewürztraminer and their progeny. *Aus. J. Grape Wine Res.* 19, 91–99. doi: 10.1111/ajgw.12051
- Duchêne, E., Dumas, V., Butterlin, G., Jaegli, N., Rustenholz, C., Chauveau, A., et al. (2020). Genetic variations of acidity in grape berries are controlled by the interplay between organic acids and potassium. *Theor. Appl. Genet.* 133, 993–1008. doi: 10.1007/s00122-019-03524-9
- Dumas, V., Saurin, N., Destrac Irvine, A., Dedet, S., Veyret, M., Marchal, C., et al. (2020). Influence of grape juice extraction methods on basic analytical parameters. *Vitis* 59, 77–83. doi: 10.5073/vitis.2020.59.77-83
- Escudier, J. L., Cauchy, B., Lutin, F., and Moutounet, M. (2012). Wine acidification and wine stabilisation by subtractive technologies: comparison between ions exchange resins and membrane ion extraction. *Pub. Act. Vitic.* 129 (13–14), 324–341.
- Escudier, J. L., Payraud, R., Brienza, E., Moreau, S., Guyot, P., Samson, A., et al. (2016). Grape juice: Selection of grapevine species, juice making and stabilization. *BIOWeb Conf.* 7. doi: 10.1051/bioconf/20160701001
- Fernandez, L., Romieu, C., Moing, A., Bouquet, A., Maucourt, M., Thomas, M. R., et al. (2006). The grapevine fleshless berry mutation: A unique genotype to investigate differences between fleshy and non-fleshy fruit. *Plant Physiol.* 140, 537–547. doi: 10.1104/pp.105.067488
- Gascuel, Q., Diretto, G., Monforte, A. J., Fortes, A. M., and Granell, A. (2017). Use of natural diversity and biotechnology to increase the quality and nutritional content of tomato and grape. *Front. Plant Sci.* 8, 652. doi: 10.3389/fpls.2017.00652
- Glad, C., Regnard, J. L., Querou, Y., Brun, O., and Morot-Gaudry, J. F. (1992a). Phloem sap exudates as a criterion for sink strength appreciation in *Vitis vinifera* cv. pinot noir grapevines. *Vitis* 31, 131–138. doi: 10.5073/vitis.1992.31.131-138
- Glad, C., Regnard, J. L., Querou, Y., Brun, O., and Morot-Gaudry, J. F. (1992b). Flux and chemical composition of xylem exudates from chardonnay grapevines: temporal evolution and effect of recut. *Am. J. Enol. Vitic.* 43, 275–285.
- Greer, D. H., Weedon, M. M., and Weston, C. (2011). Reductions in biomass accumulation, photosynthesis in situ and net carbon balance are the costs of protecting *Vitis vinifera* 'Semillon' grapevines from heat stress with shade covering. *AoB Plants* 2011, plr023. doi: 10.1093/aobpla/plr023
- Greer, D. H. (2012). Modelling leaf photosynthetic and transpiration temperature-dependent responses in *Vitis vinifera* cv. Semillon grapevines growing in hot, irrigated vineyard conditions. *AoB Plants* 2012, pls009. doi: 10.1093/aobpla/pls009
- Hocking, B., Tyerman, S.D., Burton, R. A., and Gilliam, M. (2016). Fruit calcium: transport and physiology. *Front. Plant Sci.* 7, 569. doi: 10.3389/fpls.2016.00569

- Houel, C., Martin-Magniette, M.-L., Stéphane, N., Lacombe, T., Cunff, L., Torregrosa, L., et al. (2013). Genetic diversity of the berry size in grapevine (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 19, 208–220. doi: 10.1111/ajgw.12021
- Houel, C., Chatbanyong, R., Doligez, A., Rienth, M., Foria, S., Luchaire, N., et al. (2015). Identification of stable QTLs for vegetative and reproductive traits in the microvine (*Vitis vinifera* L.) using the 18K Infinium chip. *BMC Plant Biol.* 15, 205. doi: 10.1186/s12870-015-0588-0
- IOM - Institute of Medicine (2000). *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride* (Washington, DC: National Academy Press).
- IOM - Institute of Medicine (2004). *Dietary Reference Intakes: Water, Potassium, Sodium, Chloride, and Sulfate* (Washington, DC: National Academy Press).
- Jaime-Baro, A. L. (1973). Influence of different levels of potassium content on the quality of Rioja wines. *Semana Vitivinica*. 28, 647–651.
- Kliewer, W. M., and Lider, L. A. (1970). Effect of day temperature and light intensity on growth and composition of *Vitis vinifera* L. fruits. *J. Amer. Soc. Hortic. Sci.* 95, 766–769.
- Kliewer, W. M. (1965). Changes in the concentration of malates, tartrates, and total free acids in flowers and berries of *Vitis vinifera*. *Am. J. Enol. Vitic.* 16, 92–100.
- Kodur, S., Tisdall, J. M., Tang, C., Tang, T., and Walker, R. R. (2009). Accumulation of potassium in grapevine rootstocks (*Vitis*) as affected by dry matter partitioning, root traits and transpiration. *Aust. J. Grape Wine Res.* 16, 273–282. doi: 10.1111/j.1755-0238.2009.00088.x
- Kourakou, S. (1974). *Optimaler reifergrad der Traubenin bezug auf den gewünschten wein* (Fr). XIV OIV congress, 29/9–5/10, Bolzano. Italy.
- La Rosa, W. V. (1955). Maturity of grapes as related to pH at harvest. *Am. J. Enol. Vitic.* 6, 42–46.
- Lakso, A. N., and Kliewer, W. M. (1978). The influence of temperature on malic acid metabolism in grape berries. II. Temperature responses of net dark CO₂ fixation and malic acid pools. *Am. J. Enol. Vitic.* 29, 145–149.
- Liu, H., Wu, B., Fan, P., Xu, H., and Li, S. (2006). Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *J. Sci. Food Agric.* 86, 1526–1536. doi: 10.1002/jsfa.2541
- Liu, H., Wu, B., Fan, P., Xu, H., and Li, S. (2007). Inheritance of sugars and acids in berries of grape (*Vitis vinifera* L.). *Euphytica* 153, 99–107. doi: 10.1007/s10681-006-9246-9
- Lobos, G. A., and Hancock, J. F. (2015). Breeding blueberries for a changing global environment. *Front. Plant Sci.* 6, 782. doi: 10.3389/fpls.2015.00782
- Lobos, G. A., Camargo, V. A., Pozo, A., Araus, J. L., Ortiz, R., and Doonan, J. H. (2017). Editorial: plant phenotyping and phenomics for plant breeding. *Front. Plant Sci.* 8, 2181. doi: 10.3389/fpls.2017.02181
- Luby, J. J. (2009). Plant breeders' perspectives on improving yield and quality traits in horticultural food crops. *HortSci* 44, 20–22. doi: 10.21273/HORTSCI.44.1.20
- Luchaire, N., Rienth, M., Romieu, C., Nehe, A., Chatbanyong, R., Houel, C., et al. (2017). Microvine, a new model to study growth and developmental patterns in grapevine. *Am. J. Enol. Vitic.* 68, 283–292. doi: 10.5344/ajev.2017.16066
- Maathuis, F. J. M. (2009). Physiological functions of mineral macronutrients. *Curr. Op. Plant Biol.* 12, 250–258. doi: 10.1016/j.pbi.2009.04.003
- Matthews, M. A., Cheng, G., and Weinbaum, S. A. (1987). Changes in water potential and dermal extensibility during grape berry development. *J. Amer. Soc. Hortic. Sci.* 112, 314–319.
- Mejia, N., Gebauer, M., Munoz, L., Hewstone, N., Munoz, C., and Hinrichsen, P. (2007). Identification of QTL for seedlessness, berry size, and ripening date in a seedless x seedless table grape progeny. *Am. J. Enol. Vitic.* 58, 499–507.
- Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Res. Int.* 43 (7), 1844–1855. doi: 10.1016/j.foodres.2010.05.001
- Mpelasoka, B. S., Schachtman, D. P., Treeby, M. T., and Thomas, M. R. (2003). A review of potassium nutrition in grapevines with special emphasis on berry accumulation. *Aust. J. Grape Wine Res.* 9, 154–168. doi: 10.1111/j.1755-0238
- Mullins, M. G., Bouquet, A., and Williams, L. E. (1992). *Biology of Horticultural crops: Biology of the grapevine* (Cambridge: Cambridge University Press, United Kingdom).
- Nieves-Cordones, M., Andrianteranagna, M., Cuéllar, T., Chérel, I., Gibrat, R., Boeglin, M., et al. (2020). Characterization of the grapevine Shaker K⁺ channel VvK3.1 supports its function in massive potassium fluxes necessary for berry potassium loading and pulvinus-actuated leaf movements. *New Phytol.* 222, 286–300. doi: 10.1111/nph.15604
- Ojeda, H., Deloire, A., Carbonneau, A., Ageorges, A., and Romieu, C. (1999). Berry development of grapevines: Relations between the growth of berries and their DNA content indicate cell multiplication and enlargement. *Vitis* 38, 145–150. doi: 10.5073/vitis.1999.38.145-150
- Ojeda, H., Bigard, A., Escudier, J. L., Samson, A., Caillé, S., Romieu, C., et al. (2017). De la vigne au vin : des créations variétales adaptées au changement climatique et résistant aux maladies cryptogamiques 2/2 : Approche viticole pour des vins de type VDQA. *Rev. Des. Oenologues* 44, 22–27.
- Ollat, N., van Leeuwen, C., Destrac, A., Marguerit, E., Duchêne, E., Lebon, E., et al. (2015). Changement climatique : quels seront les déterminants du choix du matériel végétal ? *Rev. Des. Oenologues* 157, 1–4.
- Ollat, N., Marguerit, E., Lecourieux, F., Destrac-Irvine, A., Barrieu, F., Dai, Z., et al. (2018). Grapevine adaptation to abiotic stresses. *Acta Hortic.* 1248. doi: 10.17660/ActaHortic.2019.1248.68
- Possner, B. R. E., and Kliewer, W. M. (1985). The localisation of acids, sugars, potassium and calcium in developing grape berries. *Vitis* 24, 229–240.
- Preiner, D., Tupajic, P., Karoglan, J., Andabaka, Z., Markovic, Z., and Maletic, E. (2013). Organic acids profiles of the most important Dalmatian native grapevine (*V. vinifera* L.) cultivars. *J. Food Comp. Anal.* 32, 162–168. doi: 10.1016/j.jfca.2013.09.005
- R Core Team (2017). *R: A language and environment for statistical computing*. (Vienna: R Foundation for Statistical Computing).
- Ramesh-Kumar, A., Kumar, N., and Kavino, M. (2006). Role of potassium in fruit crops - a review. *Agric. Rev.* 27, 284–291.
- Rienth, M., Torregrosa, L., Gauthier, S., Ardisson, M., Brillouet, J.-L., and Romieu, C. (2016). Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels its transcriptome. *BMC Plant Biol.* 16, 164. doi: 10.1186/s12870-016-0850-0
- Ritschel, P., Garcia Maia, J., Almeida-Camargo, U., Celso Zanús, M., Teodoro de Souza, R., and Martins-Fajardo, T. V. (2014). BRS MAGNA - a novel grape cultivar for juice making, with wide climatic adaptation. *Crop Breed. Appl. Biotechnol.* 14. doi: 10.1590/1984-70332014v14n4c42
- Rogiers, S. Y., Coetzee, Z. A., Walker, R. R., Deloire, A., and Tyerman, S. D. (2017). Potassium in the grape (*Vitis vinifera* L.) berry: transport and function. *Front. Plant Sci.* 8, 1629. doi: 10.3389/fpls.2017.01629
- Romieu, C., Bigard, A., Breil, M., Shahood, R., Pellegrino, A., Doligez, A., et al. (2018). "New physiological and genetic approaches to breed grapevines challenging climate warming," in *XXII International Plant Molecular Biology Conference*, (Montpellier, France), 6–10.
- Salmon, J. M. (1996). Sluggish and stuck fermentations: Some actual trends on their physiological basis. *Vitic. Enol. Sci.* 51, 137–140.
- Seguin, B., Stevez, L., Herbin, C., and Rochard, J. (2004). Changements climatiques et perspectives pour la viticulture: conséquences potentielles d'une modification du climat. *Rev. Oenol.* 111, 59–60.
- Shahood, R. (2017). *La baie de vigne au sein d'une population asynchrone*. Doctoral dissertation. (Montpellier SupAgro: Montpellier, France).
- Shahood, R., Savoi, S., Bigard, A., Torregrosa, L., and Romieu, C. (2019). From average to individual berry, a paradigm shift for accurate analysis of water and primary metabolites accumulation into grapevine fruit. 21th Int. Symp. GiESCO June (Thessaloniki, Greece), 24–28.
- Shiraishi, M., Fujishima, H., and Chijiwa, H. (2010). Evaluation of table grape genetic resources for sugar, organic acid, and amino acid composition of berries. *Euphytica* 174, 1–13. doi: 10.1007/s10681-009-0084-4
- Simon, P. W. (2014). Progress toward increasing intake of dietary nutrients from vegetables and fruits: The case for a greater role for the horticultural sciences. *Hortic. Sci.* 49, 112–115. doi: 10.21273/HORTSCI.49.2.112
- Song, W., Yi, J., Kurniadinata, O. F., Wang, H., and Huang, X. (2018). Linking fruit Ca uptake capacity to fruit growth and pedicel anatomy, a cross-species study. *Front. Plant Sci.* 9, 575. doi: 10.3389/fpls.2018.00575
- Storey, R. (1987). Potassium localization in the grape berry pericarp by energy-dispersive X-ray microanalysis. *Am. J. Enol. Vitic.* 38, 301–309.
- Strik, B. C., and Bryla, D. R. (2015). Uptake and partitioning of nutrients in Blackberry and Raspberry and evaluating plant nutrient status for accurate assessment of fertilizer requirements. *Hort. Tech.* 25, 452–459. doi: 10.21273/HORTECH.25.4.452

- Sweetman, C., Sadras, V. O., Hancock, R. D., Soole, K. L., and Ford, C. M. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65, 5975–5988. doi: 10.1093/jxb/eru343
- Taghavi, T. S., Babalar, M., Ebadi, A., Ebrahimzadeh, H., and Asgari, M. A. (2004). Effects of nitrate to ammonium ratio on yield and nitrogen metabolism of Strawberry (*Fragaria x Ananassa* cv. Selva). *Int. J. Agric. Biol.* 6, 994–997.
- Taillandier, P., Ramon-Portugal, F., Fuster, A., and Strehaiano, P. (2007). Effect of ammonium concentration on alcoholic fermentation kinetics by wine yeasts for high sugar content. *Food Microbiol.* 24, 95–100. doi: 10.1016/j.fm.2006.04.002
- Teixeira, A., Eiras-Dias, J., Castellarin, S. D., and Geros, H. (2013). Berry phenolics of grapevine under challenging environments. *Int. J. Mol. Sci.* 14, 18711–18739. doi: 10.3390/ijms140918711
- Torregrosa, L., Pradal, M., Souquet, J. M., Rambert, M., Gunata, Z., and Tesniere, C. (2008). Manipulation of *VvAdh* to investigate its function in grapevine berry development. *Plant Sci.* 174, 149–155. doi: 10.1016/j.plantsci.2007.10.006
- Torregrosa, L., Bigard, A., Doligez, A., Lecourieux, D., Rienth, M., Luchaire, N., et al. (2017). Developmental, molecular and genetic studies on the grapevine response to temperature open breeding strategies for adaptation to warming. *OenoOne* 51, 155–165. doi: 10.20870/oeno-one.2016.0.0.1587
- Torregrosa, L., Rienth, M., Romieu, C., and Pellegrino, A. (2019). The microvigne, a model for grapevine physiology studies and genetics. *OenoOne* 53 (3). doi: 10.20870/oeno-one.2019.53.3.2409
- Van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, D., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy* 9 (9), 514. doi: 10.3390/agronomy9090514
- Vicente, A. R., Manganaris, G. A., Sozzi, G. O., and Crisosto, C. H. (2009). “Nutritional quality of fruits and vegetables,” in *Postharvest handling: A systems approach*, 2nd ed. Eds. W. J. Florkowski, R. L. Shewfelt, B. Brueckner and S. E. Prussia (Netherlands: Elsevier Inc. Academic Press.), 59–93.
- Villete, J., Cuélar, T., Verdeil, J.-L., Delrot, S., and Gaillard, I. (2020). Grapevine Potassium Nutrition and Fruit Quality in the Context of Climate Change. *Front. Plant Sci.* 11, 123. doi: 10.3389/fpls.2020.00123
- Wall, M. (2006). Ascorbic acid, vitamin A, and mineral composition of banana (*Musa* sp.) and papaya (*Carica papaya*) cultivars grown in Hawaii. *J. Food Comp. Anal.* 19, 434–445. doi: 10.1016/j.jfca.2006.01.002
- Webb, L. B., Whetton, P. H., and Barlow, E. W. R. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. *Aust. J. Grape Wine Res.* 13, 165–175. doi: 10.1111/j.1755-0238.2007.tb00247.x
- Wolkovich, E. M., García de Cortázar-Atauri, I., Morales-Castilla, I., Nicholas, K. A., and Lacombe, T. (2018). From Pinot to Xinomavro in the world's future wine-growing regions. *Nat. Clim. Change* 8, 29–37. doi: 10.1038/s41558-017-0016-6
- Xu, H., Liu, G., Yan, B., Duan, W., Wang, L., and Li, S. (2014). Comparison of investigation methods of heat injury in grapevine (*Vitis*) and assessment to heat tolerance in different cultivars and species. *BMC Plant Biol.* 14:156. doi: 10.1186/1471-2229-14-156
- Yinshan, G., Zaozhu, N., Kai, S., Jia, Z., Zhihua, R., Yuhui, Z., et al. (2017). Composition and content analysis of sugars and organic acids for 45 grape cultivars from northeast region of China. *Pak. J. Bot.* 49, 155–160.
- Zhang, X. Y., Wang, X. L., Wang, X. F., Xia, G. H., Pan, Q. H., Fan, R. C., et al. (2006). A shift of phloem unloading from symplasmic to apoplasmic pathway is involved in developmental onset of ripening in grape berry. *Plant Physiol.* 142, 220–232. doi: 10.1104/pp.106.081430

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Bigard, Romieu, Sire and Torregrosa. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Lars Hendrik Wegner,
Foshan University, China
Virginia Ferrari,
National Institute for Agricultural
Research (INIA), Uruguay

***Correspondence:**

Sahap Kaan Kurtural
skkurtural@ucdavis.edu

†ORCID:

Johann Martínez-Lüscher
orcid.org/0000-0002-3077-1346
Luca Brillante
orcid.org/0000-0002-5747-6312
Sahap Kaan Kurtural
orcid.org/0000-0001-9578-831X

***Present address:**

Johann Martínez-Lüscher,
SemiosBio Technologies, Vancouver,
BC, Canada
Luca Brillante,
Department of Viticulture and Enology,
California State University Fresno,
Fresno, CA, United States

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 01 July 2020

Accepted: 20 October 2020

Published: 10 November 2020

Citation:

Martínez-Lüscher J, Chen CCL,
Brillante L and Kurtural SK (2020)
Mitigating Heat Wave and Exposure
Damage to “Cabernet Sauvignon”
Wine Grape With Partial Shading
Under Two Irrigation Amounts.
Front. Plant Sci. 11:579192.
doi: 10.3389/fpls.2020.579192

Mitigating Heat Wave and Exposure Damage to “Cabernet Sauvignon” Wine Grape With Partial Shading Under Two Irrigation Amounts

Johann Martínez-Lüscher^{†‡}, Christopher Cody Lee Chen, Luca Brillante^{†‡} and Sahap Kaan Kurtural^{†*}

Department of Viticulture and Enology, University of California, Davis, Davis, CA, United States

Rising temperatures in most agricultural regions of the world are associated with a higher incidence of extreme weather events such as heat waves. We performed an experiment to mitigate the impact of heat waves and exposure of berries in grapevine (*Vitis vinifera* cv. “Cabernet Sauvignon”) with untreated vines (Exposed) or with fruit-zone partial shading (Shaded) under 40 and 80% replacement of crop evapotranspiration (ET_c) with sustained deficit irrigation in a factorially arranged experiment. The trial was performed in a vineyard with vertically shoot positioned trellis with a row orientation that concentrated solar radiation exposure on the southwest aspect of the fruit zone. Leaf stomatal conductance (g_s) and net carbon assimilation (A_N) were significantly lower in shaded leaves under partial fruit-zone shading that resulted in lower pruning mass for Shaded treatments. Stem water potential (Ψ_{stem}) responded to a large extent to increased irrigation. However, grapevines with partial fruit-zone shading had transiently better water status under 40% ET_c . Cluster maximum temperatures were 3.9°C greater in Exposed grapevines. Exposed clusters had transiently lower acidity and higher pH. However, Exposed clusters on 40% ET_c had higher total soluble solids (TSS). The experimental vineyard suffered a 4-day heat wave 21 days before harvest, resulting in 25% of the clusters being damaged in Exposed treatment, regardless of irrigation amount. Furthermore, berries in Exposed treatments suffered a great loss of anthocyanins and flavonols even if they were not damaged by direct solar exposure. The pre-planting decision of using a vertically shoot positioned trellis that concentrated solar radiation on the Southwest aspect offered mild protection in a hot climate region with a sunny growing season with extreme heat events during the execution of study. The extreme conditions under which this study was conducted are not unusual, and have become more expected. Our work provided evidence of the vulnerability of grape berry to heat waves and exposure during heat wave events and possible protection methods to mitigate these effects *in situ* in context of climate change.

Keywords: climate change, water stress, shade nets, flavonoids, heat wave, irrigation, fruit exposure, anthocyanin degradation

INTRODUCTION

The commercial success of a grape growing region is based on a fine-tuned match between the climate and cultivar and rootstock selection (Ollat et al., 2016), and it has to be combined with an adequate market demand. By the middle of twenty-first century, climatic conditions are expected to change potentially affecting key physiological and production parameters (Hannah et al., 2013; Fraga et al., 2016). The increase in atmospheric CO₂ and other greenhouse gases most certainly will increase the temperature of the planet ranging from 1.5 to 4.5°C (IPCC, 2013). Furthermore, the incidence of extreme events, such as heat waves, is increasing with an associated risk for crops (Fischer and Schär, 2010; Smith, 2011; Deryng et al., 2014; Martínez-Lüscher et al., 2017b). Higher temperatures are associated with greater rates evaporation of water and therefore, higher global precipitation. However, these are unevenly distributed. In fact, most regions where grapevines are grown are forecasted to experience a reduction in cloud coverage and rainfall and an increase in solar radiation reaching the earth's surface (Trenberth and Fasullo, 2009).

Grapevine is a rather resilient perennial crop, tolerating long periods of drought and extreme temperatures. However, as in many other fleshy fruits, grape berry is sensitive to exposure to solar radiation, causing damage on the surface of the fruit or even fruit abortion (Tinyane et al., 2018; Torres et al., 2020). Fruit exposure to solar radiation was highlighted for decades as a key factor to enhance ripening of fruits and their composition and is a very relevant concept for cultural practices in grapes (Jackson and Lombard, 1993; Cook et al., 2015). Fruit zone leaf removal in dense canopies can promote ripening and synthesis of flavonoids such as anthocyanins and flavonols (Pastore et al., 2013). Under controlled conditions, a combination of visible and UV radiation may upregulate structural and regulatory genes responsible for the synthesis of anthocyanins and flavonols (Azuma et al., 2012). These photomorphogenic effects are mediated by photoreceptors, phytochromes and cryptochromes and are responsive to changes in radiation spectra (González et al., 2015; Matus, 2016). However, solar radiation, especially infrared, transmits thermal energy to exposed objects with an intrinsic increase in their temperature. High temperature may play repressive role in the synthesis of anthocyanins, eventually inducing their degradation (Mori et al., 2007; Martínez-Lüscher et al., 2017b; Torres et al., 2020). In addition, high temperatures may be responsible for a desynchronization between the accumulation of sugars and anthocyanins leading to lower anthocyanin contents at harvest (Sadras and Moran, 2012).

In semiarid climates, where growing season rainfall is not enough to sustain production, grapevines are grown with supplemental irrigation. Mild water deficits may enhance grape ripening, through the re-concentration of the berry contents or improved grape composition through the synthesis of stress-related metabolites (Chaves et al., 2010; Kuhn et al., 2014). However, excessive water deficits in hot climates may lead to deleterious effect on fruit quality (Brillante et al., 2017; Martínez-Lüscher et al., 2017a; Yu and Kurtural, 2020; Yu et al., 2020). High temperatures may exacerbate water

deficits by increasing vapor pressure deficit, thus increasing evapotranspiration. Furthermore, an optimal water status during heat events may increase stomatal conductance helping to reduce canopy temperature (Drake et al., 2018). As a consequence, water requirements for a plant under heat stress may be increased (Restaino et al., 2016). Failure to replace enough crop evapotranspiration demand under heat waves may lead to substantial reductions in yields and ultimately crop failure (Terry and Kurtural, 2011; Daryanto et al., 2016). In grapevine, in-season yield loss may be mediated through reductions in berry size, at first, and a severe shriveling and bunch stem necrosis if high water deficits persist (Krasnow et al., 2010; Hall et al., 2011; Cook et al., 2015; Yu and Kurtural, 2020). Furthermore, in hot climates, post-veraison water deficits led to a diminution of anthocyanin content (Martínez-Lüscher et al., 2017a), modulation of anthocyanin profile toward dihydroxylated anthocyanidins (Yu et al., 2016) with rapid degradation of proanthocyanins of grape berry and wine (Yu et al., 2020).

In our previous research, qualitative and quantitative differences in fruit-zone microclimate were induced by means of color shade nets (Martínez-Lüscher et al., 2017b). We designed an experiment aiming to test the effect of partial solar radiation reduction and irrigation amounts and their interaction on gas exchange, stem water potential, berry temperature, must composition, and skin anthocyanins and flavonols. Specifically, the study aimed to evaluate the vulnerability of “Cabernet Sauvignon” grape berry to heat waves and exposure of clusters to solar radiation; and whether increased water application would palliate either high berry temperatures or deleterious effects on grape composition.

MATERIALS AND METHODS

Experimental Site and Plant Material

The experiment was conducted at the University of California Davis, Oakville Experimental Vineyard (38.428, -122.409; Oakville, CA) during the 2017 growing season. Eight-year old *Vitis vinifera* “Cabernet Sauvignon” Clone FPS08 grapevines grafted on 110 Richter (*Vitis berlandieri* × *Vitis rupestris*) rootstock were used. Plants were trained to bilateral cordons 0.90 m above vineyard floor (cordon height) and shoots were vertically shoot-positioned on 30-single bud spurs. Vine and row spacing was 2.0 m × 2.4 m, respectively, and rows were oriented Northwest to Southeast. The plants were drip-irrigated with 2 pressure compensating emitters per plant delivering 1.9 L/h each. Air temperatures and reference crop evapotranspiration (ET₀) from 1 March to 31 October for the year of study and the previous 10 years were obtained from the California Irrigation Management Information System network station installed on site. Clear sky days were calculated using the station radiometer and accounting for days with at least 75% to the total radiation ever recorded for that day of the year as reported elsewhere (Martínez-Lüscher et al., 2017b).

Experimental Design and Treatment Application

The treatments were arranged factorially in a randomized complete block design combining two sustained deficit irrigation amounts, which were 40 and 80% replacement of crop evapotranspiration (ET_c), and the absence/presence of shade nets (Exposed and Shaded). Fractions of ET_c were calculated weekly from the product of ET_o and crop coefficient (K_c) as in Williams and Ayars (Williams and Ayars, 2005). The 80% ET_c was achieved by adding two more emitters (1.9 L/h each) in the corresponding locations on a blind irrigation hose. All irrigations began on 20 May, on the day of 50% flowering and no special measures were taken prior to heat events as the intrinsic increase in ET_o of high temperatures was calculated and applied at the end of each week. The amount of irrigation applied with the corresponding K_c is reported in **Table 1**. The Shade netting that allowed 40% of solar radiation to pass through (60% shading) (Black-40; Ginegar, Kibbutz, Israel) was applied on 15 June, 26 days after flowering (DAF), at 100% fruit set. The shade nets applied as follows: the nets were cut into 6 m (long) \times 1 m (wide) strips (**Supplementary Figure S1A**) and hung onto the Southeast and Northwest sides of the canopy at 0.95–1.95 m above the vineyard floor, 0.25 m above the second catch wire as indicated in **Supplementary Figure S1B**.

Fruit Zone Microclimate

Spectral radiation in the fruiting zone were quantified using a spectrometer with a cosine-corrected head (Black Comet-SR, StellarNet; Tampa, FL, United States) for each of the experimental units around 1500 h, which corresponded the hottest moment of

the day (**Figure 1**). One measurement was taken pointing at the sun and 3 additional measurements were taken at 120° rotational intervals to estimate the light coming from every direction. Cluster temperatures were measured as follows. Portable infrared thermometers (Model 2956; Spectrum Technologies; Aurora, IL, United States) were used on berries following onset of anthocyanin accumulation (67 and 114 DAF, respectively) to measure diurnal shifts in cluster temperature. Two clusters with no leaves shading on either aspect of the canopy were chosen and marked in each experimental unit. Three measurements per cluster were averaged every 2 h from sunset until the moment in time when cluster temperature equilibrated with ambient temperature.

Mid-Day Stem Water Potential

Stem water potential (Ψ_{stem}) was measured from one fully expanded leaf per vine on the Northeast aspect of the canopy in each plant in the study, 9 times from bud break to harvest. One hour prior to measurement leaves were covered with a re-sealable zip-top foil bag and allowed to equilibrate with stem water potential. Between 1300 and 1400 h local time leaves were excised with a razor blade and measured for water potential with a portable pressure chamber (model 616, PMS Instrument Company, Albany, OR, United States).

Leaf Gas-Exchange

Leaf gas exchange was measured between 1130 and 1430 h local time 4 times (51, 82, 107, and 113 DAF) throughout the season, using a portable infrared gas analyzer CIRAS-3 (PP Systems, Amesbury, MA), featuring a broad-leaf chamber with

TABLE 1 | Timing of irrigation amounts for the two treatments applied and parameters used to calculate crop evapotranspiration.

Period accounted	Irrigation (DAF ^a)	ET_o^b (mm)	Precipitation (mm)	K_c^c	40% of ET_c^d (mm)	80% of ET_c (mm)
5/13 to 5/19	0	34.1	0	0.21	2.9	5.8
5/20 to 5/26	7	32.9	0	0.32	4.2	8.4
5/27 to 6/2	14	35.2	0	0.39	5.5	11.0
6/3 to 6/9	21	31.9	0	0.43	5.4	10.8
6/10 to 6/16	28	39.7	0	0.46	7.3	14.6
6/17 to 6/23	35	45.6	0	0.57	10.4	20.8
6/24 to 6/30	42	38.1	0	0.57	8.7	17.4
7/1 to 7/7	49	42.5	0	0.52	8.8	17.7
7/8 to 7/14	56	42.0	0	0.57	9.6	19.2
7/15 to 7/21	63	44.0	0	0.52	9.2	18.3
7/22 to 7/28	70	41.3	0	0.54	8.9	17.8
7/29 to 8/4	77	35.9	0	0.51	7.3	14.7
8/5 to 8/11	84	33.5	0	0.51	6.8	13.7
8/12 to 8/18	91	32.5	0	0.51	6.6	13.3
8/19 to 8/25	98	31.1	0	0.51	6.3	12.7
8/26 to 9/1	105	27.2	0	0.51	5.5	11.1
9/2 to 9/8	112	27.6	0	0.51	5.6	11.3
9/9 to 9/15	119	26.4	0	0.51	5.4	10.8
9/16 to 9/22	126	31.8	0	0.51	6.5	13.0
Total		673.3			131.1	262.2

^aDAF, Days after flowering. ^b ET_o , Reference crop evapotranspiration. ^c K_c , Crop coefficient. ^d ET_c , Estimated crop evapotranspiration ($ET_o \times K_c$) calculated as reported by Williams and Ayars (2005).

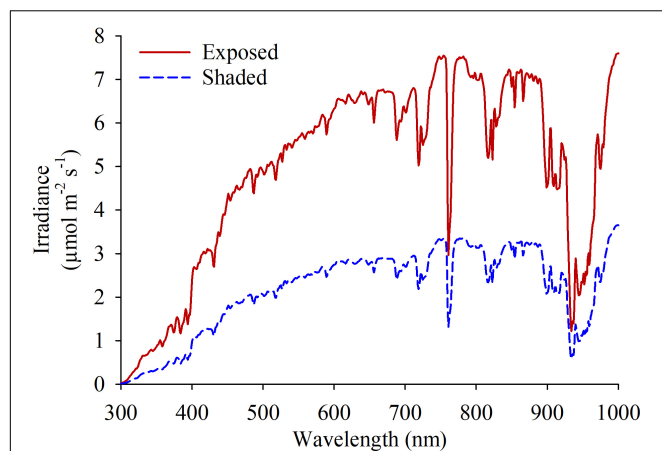


FIGURE 1 | Spectral irradiance measured at the fruit-zone of grapevines fully exposed and shaded at the fruit-zone by Black-40 nets.

4.5 cm² window. Chamber conditions were set up at 40% relative humidity, a CO₂ concentration of 400 μmol·mol⁻¹ and using a flow to the chamber of 300 mL·min⁻¹. In each experimental unit, one sun-exposed leaf per plant were measured right above the level of nets. In each experimental unit with shade nets, one additional leaf per plant in the lower part of the canopy was measured keeping the shade nets over the cuvette.

Chemicals and Chromatography Standards

All solvents were of HPLC grade. Chemicals and procedures followed previously described work (Martínez-Lüscher et al., 2019a). Acetonitrile, formic acid, hydrochloric acid and methanol were purchased from Fisher Scientific (Santa Clara, CA). Standards for Malvidin 3-*o*-glucoside, Myricetin-3-*o*-glucoside, Quercetin 3-*o*-glucoside, Quercetin 3-*o*-glucuronide, Quercetin 3-*o*-galactoside, Kaempferol 3-*o*-glucoside, Isorhamnetin 3-*o*-glucoside, and Syringetin 3-*o*-glucoside, obtained from Sigma-Aldrich (St. Louis, MO, United States).

Sample Collection and Processing

The berries were collected from bunch-closure to harvest. A total of seventy-five berries were collected from each experimental unit on six dates, on both sides of the canopy in equivalent numbers, avoiding severely dehydrated or raisined berries as reported previously (Martínez-Lüscher et al., 2017b; Torres et al., 2020). Each set of seventy-five berries were weighed for individual berry mass calculations. Twenty randomly chosen berries, separate from the 75-berries chosen used in berry composition in section “Berry Composition” (10 from either side of canopy), were weighed and frozen in a -20°C freezer for a minimum of 1 day then individually peeled by hand using a scalpel. Skins were stored in a -220°C freezer and freeze-dried (Centrivap, Labconco, Kansas City, MO, United States). Dried skins were pulverized in a ball mill (MM400, Retsch, Mammelen, Germany). A solution of MeOH:H₂O:7 M HCl (70:29:1) was added to 50 mg of freeze dried, pulverized skin

to quantify flavonols and anthocyanins and allowed to extract overnight at 4°C. Following extraction, samples were centrifuged at 14,000 rpm for 10 min and supernatants filtered (0.45 μm; VWR, Seattle, WA, United States) into HPLC vials and analyzed.

Berry Composition

The 75 berries collected were crushed by hand and filtered to obtain must. A digital refractometer (Palette PR-32, Atago, Tokyo, Japan) was then used to measure total soluble solids (TSS) of filtered juice. Using an autotitrator (862 Compact Titrator, Herisau, Switzerland), pH and titratable acidity (TA) were measured. NaOH was used to titrate up to pH 8.2. The TA was expressed as g·L⁻¹ equivalents of tartaric acid.

Reversed-Phase High Performance Liquid Chromatography

An HPLC-DAD (1260 series, Agilent, Santa Clara, CA) equipped with a degasser, quaternary pump, thermostatted column compartment and an auto-injector connected to a diode array detector was used to analyze the anthocyanins and flavonols. Mobile phase elution gradient, flavonol and anthocyanin quantification followed previously established procedures (Martínez-Lüscher et al., 2019a) with a reversed phase C18 column LiChrosphere® 100, 250 × 4 mm with a 5 μm particle size and a 4 mm guard column of the same material (Agilent Technologies, Santa Clara, CA, United States). The mobile phase flow rate was 0.5 mL min⁻¹, and two mobile phases were used, which included solvent A = 5.5% aqueous formic acid; solvent B = 5.5% formic acid in acetonitrile. The HPLC flow gradient started with 91.5% A with 8.5% B, 87% A with 13% B at 25 min, 82% A with 18% B at 35 min, 62% A with 38% B at 70 min, 50% A with 50% B at 70.01 min, 30% A with 70% B at 75 min, 91.5% A with 8.5% B from 75.01 min to 91 min. The column temperature was maintained at 25°C. Detection of flavonols and anthocyanins was carried out by the diode array detector at 365 and 520 nm, respectively. A computer workstation with Agilent OpenLAB (Chemstation edition, version A.02.10) was used for chromatographic analysis.

Standards for identification of Malvidin 3-*o*-glucoside, Myricetin-3-*o*-glucoside, Quercetin 3-*o*-glucoside, Quercetin 3-*o*-glucuronide, Quercetin 3-*o*-galactoside, Kaempferol 3-*o*-glucoside, Isorhamnetin 3-*o*-glucoside, and Syringetin 3-*o*-glucoside, purchased from Sigma-Aldrich (St. Louis, MO, United States). Malvidin-3-*o*-glucoside and Quercetin-3-*o*-glucoside were used as qualitative standards for anthocyanins and flavonols at 520 and 365 nm, respectively. Other compounds were identified using past literature using mass spectrometry (Castillo-Muñoz et al., 2009; Martínez-Lüscher et al., 2014). Individual anthocyanins and flavonols were grouped by substituents in the 3', 4', and 5' positions of the flavonoid B-ring.

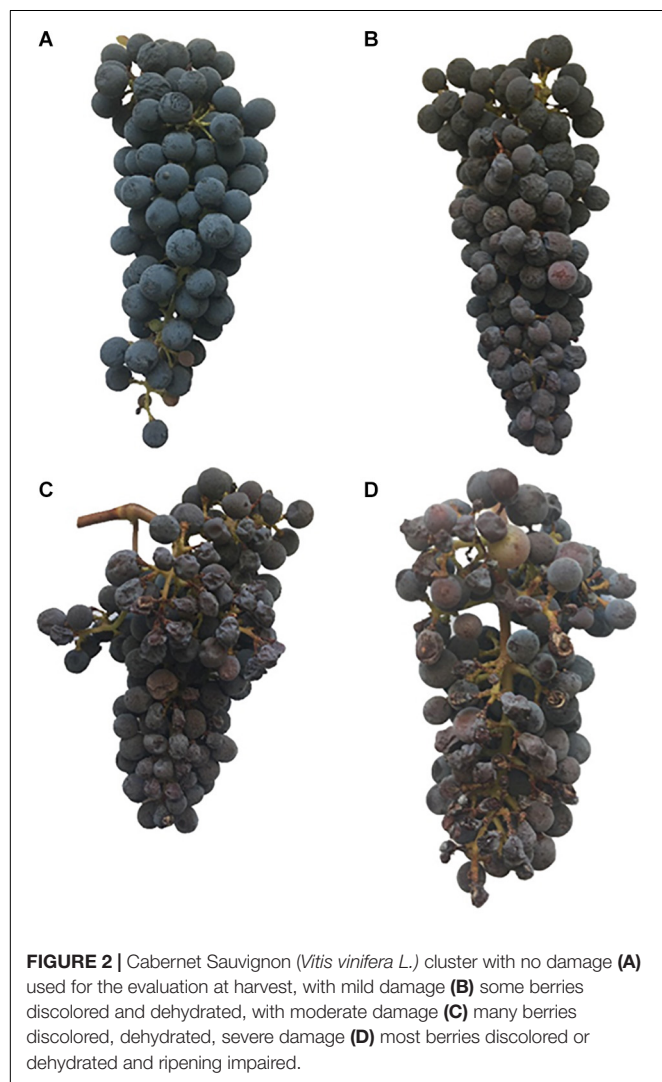
Yield Components

At harvest, clusters were removed, counted, and weighed for each plant in the experiment. At harvest, visible damage to each cluster due to excess solar radiation was quantified on a scale of no damage, mild damage, moderate damage, and severe damage

following the subjective criteria presented in **Figure 2**. In severely damaged clusters, all the grapes in the exposed side of the cluster where completely dehydrated. We measured dormant pruning weights on a top-loading scale, after pruning the grapevines to one bud spurs on 13 February 2018.

Statistical Analysis

Statistical analysis of data were conducted using SAS v.9.4 (SAS Institute Cary, NC). Data were tested for normality using Shapiro-Wilk's test and were subjected Levene's test (Levene, 1960) to ascertain the data met the assumptions of analysis of variance. Percentage of fruit damage data were log-transformed prior to statistical analysis according to results of the Shapiro-Wilk's test, but non-transformed means are presented to aid in discussion of the corresponding figure. The data were then subjected to a two-way analysis of variance (ANOVA). When the results of ANOVA were significant at $p < 0.05$ data were then subjected to *post hoc* Tukey's HSD test.



RESULTS

Environmental Conditions and Heat Waves

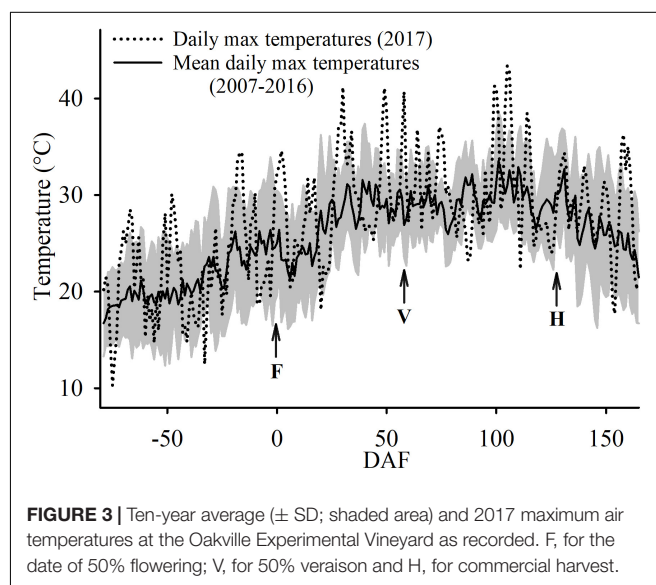
During execution of the trial, 80% of the days had no cloud cover and were sunny. The conditions of no cloud cover and sunny days was similar to the conditions during the last 10 years at the study site. However, the growing season in which the experiment was conducted was slightly warmer. The average daily maximum temperature was 1.5°C warmer 2017 (28.2°C) when compared to the last 10 years' average, at 26.7°C. Furthermore, the experimental year had several heat wave events where maximum air temperatures above 40°C that were recorded on 18 June (29 DAF), 7 July (48 DAF), 16 July (57 DAF), 26 August (98 DAF), 27 August (99 DAF), 1 September (104 DAF), and on 2 September (105 DAF) (**Figure 3**). In fact, on 2 September the maximum air temperature was 43.4°C which was the absolute maximum temperature recorded at the study site since 2006.

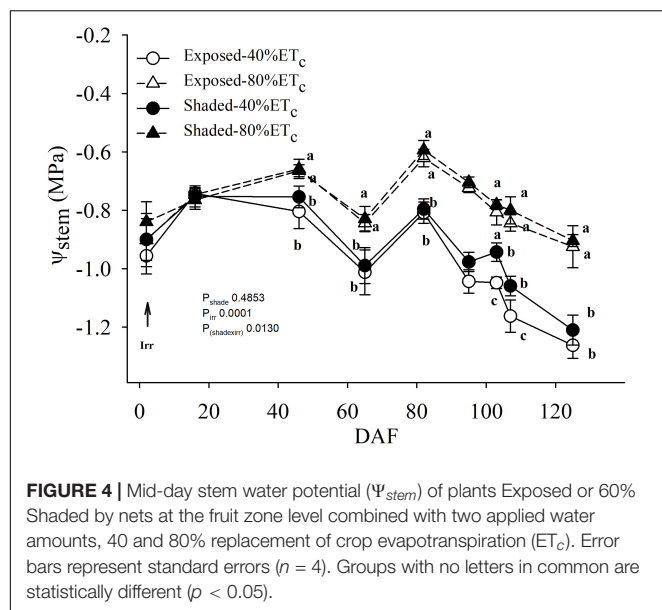
Plant Water Status

Irrigation was not initiated until 20 May 2017 (**Table 1** and **Figure 4**). There was no effect of partial shading on stem water potential throughout the monitoring period (**Figure 4**). The applied water amounts for the 40%ET_c treatment were half of the 80%ET_c treatment as planned (**Table 1**). The stem water potential of 80% ET_c treatments started to be significantly different from 40% ET_c on 65 DAF. The differences of stem water potentials between the 40 and 80% ET_c treatments increased throughout the season, culminating at harvest, when 40% ET_c had *ca.* -1.23 MPa and 80% ET_c had -0.90 MPa.

Leaf Gas Exchange and Microclimate

Net carbon assimilation (A_N) was reduced in leaves under the partial shading (**Figure 5A**). This was associated with a strong





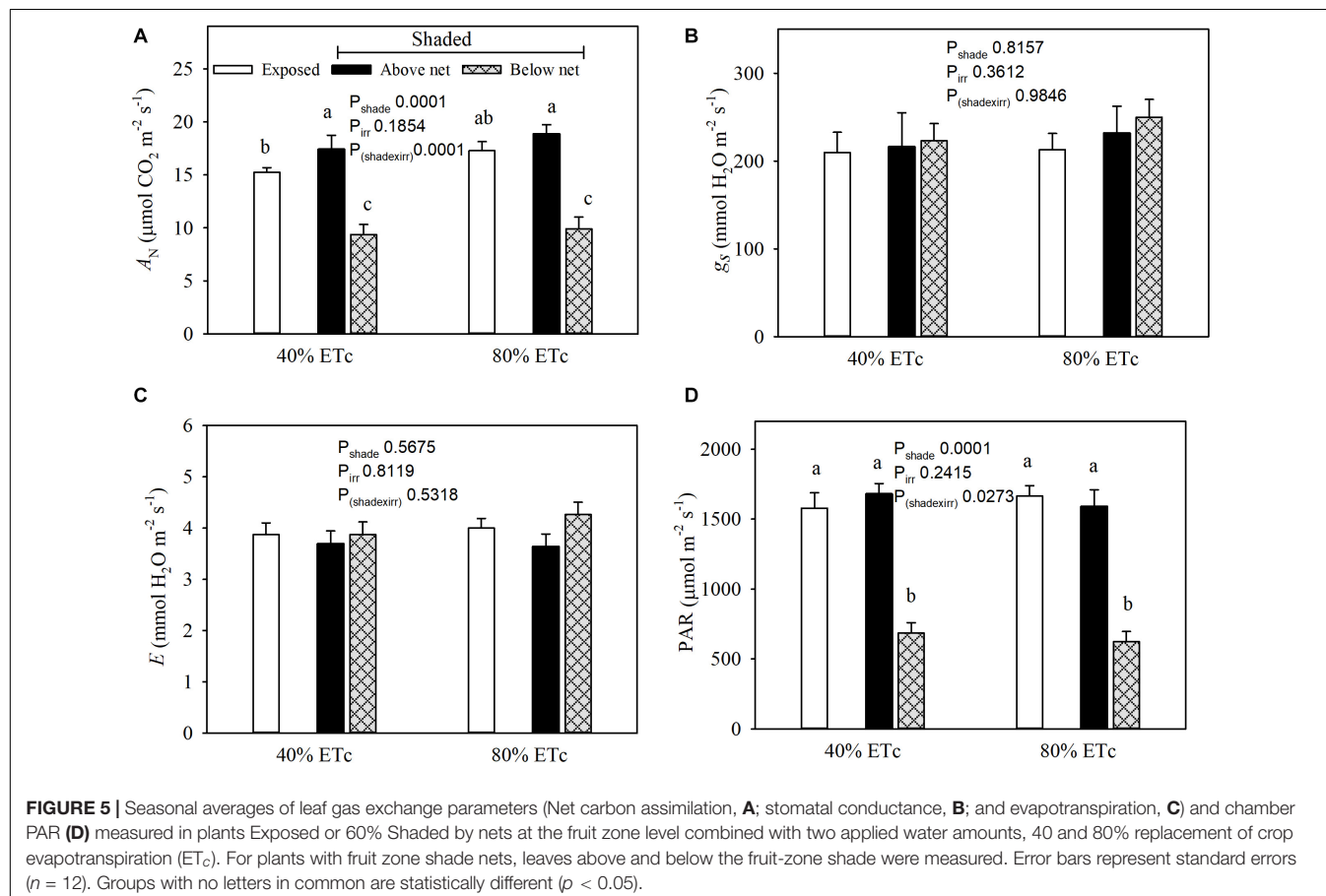
decrease in incident PAR of nearly 60% (Figure 5D). Conversely, the leaves above the nets with 80% ET_c had greater A_{Nt} than Exposed-40% ET_c . The 80% ET_c treatment did not display

higher stomatal conductance (g_s) or leaf evapotranspiration (E) (Figures 5B,C) under our conditions.

Cluster Temperature

We initially determined the cluster temperatures at 65 DAF, during immediate pre-veraison (Figures 6A,B). The air temperature reached a maximum of 33.5°C. Under these conditions, Exposed-80% ET_c Northeast-facing clusters started to warm up significantly above air temperature (up to 8°C with Exposed 80%- ET_c at 0930 h) and reached 29.6°C, which was 3.5°C higher than the partially Shaded clusters (Figure 6A). However, after 1100 h solar radiation no longer reached Northeast-facing clusters and cluster temperatures tended to equilibrate with air temperature. Conversely, Southwest-facing clusters at 65 DAF started to be fully exposed to solar radiation after 1300 h and cluster temperatures started to separate among Exposed and Shaded clusters (Figure 6B). However, significant differences were not observed until 1730 h, when Exposed-40% ET_c and Exposed-80% ET_c reached 45.4°C, which was approximately 4.5°C greater than the Shaded clusters.

At 114 DAF, when the second cluster temperature measurements were collected; clusters were more fully colored, and the air temperature reached 38°C. This resulted in cluster temperatures reaching 33.2°C at 1130 h in Northeast-facing Exposed clusters. These clusters were 3.85°C hotter than the



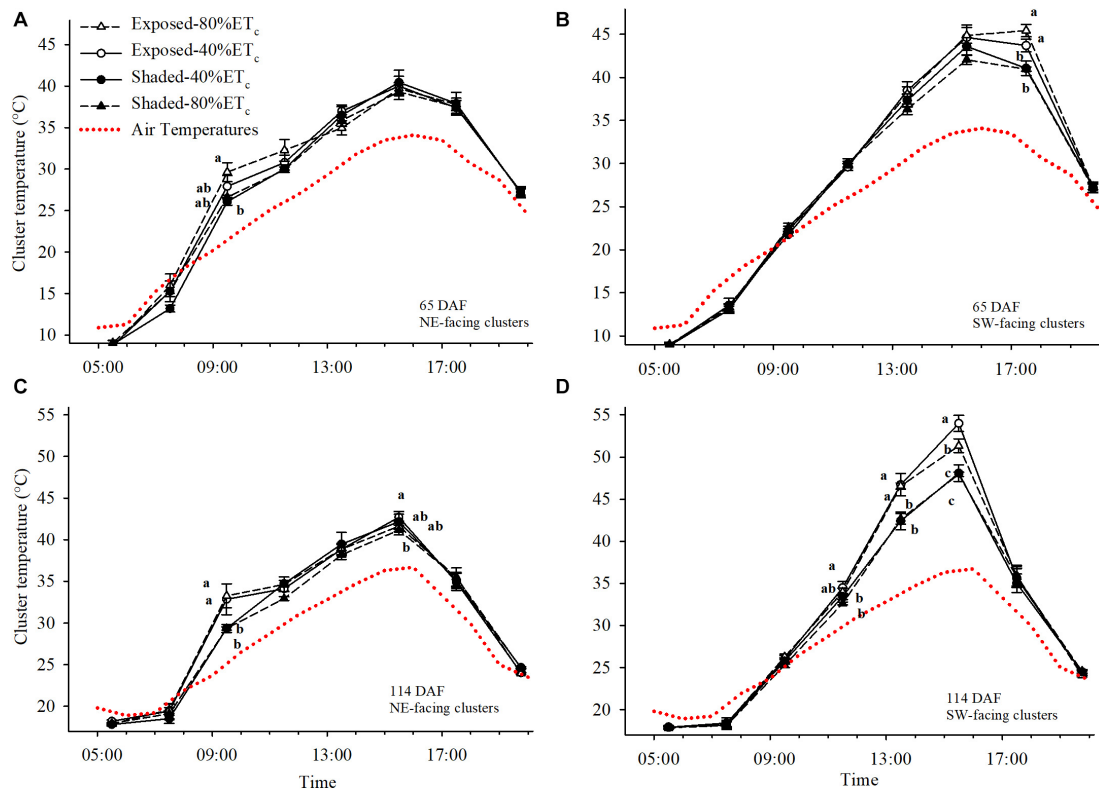


FIGURE 6 | Cluster and air temperatures measured on 24 July (A,B) and 11 September (C,D) in plants Exposed or Shaded by nets at the fruit zone level combined with two applied water amounts, 40 and 80% replacement of crop evapotranspiration (ET_c). Error bars represent standard errors ($n = 4$). Groups with no letters in common are statistically different ($p < 0.05$). The Northwest and Southwest aspects of the canopy were assessed.

Shaded (Figure 6C). Differences were more pronounced in the Southwest-facing Exposed clusters that were 4°C warmer than the Shaded clusters at 1330 h. Furthermore, cluster temperature of Exposed-40%ET_c clusters were nearly 6°C higher than the Shaded clusters at 1530 h (Figure 6D). Increasing the irrigation amount to 80%ET_c provided some relief to Exposed clusters, albeit the cluster temperatures of Exposed-80%ET_c were 9°C greater than the ambient air temperature.

Berry Fresh Mass and Must Composition

Significant differences in berry fresh mass (BFM) were intermittent as 80% ET_c tended to have greater BFM throughout the growing season (Figure 7A). For instance, the BFM of Exposed-80%ET_c BFM was greater than the rest of the treatments at 65 DAF. At 115 DAF, BFM was higher in 80% ET_c treatments regardless of the presence of Shading. However, at harvest (128 DAF), only the BFM of Shaded-80%ET_c was higher than the rest of the treatments. TSS were lower with Shaded-80%ET_c at the two last berry samplings (Figure 7B). The Exposed-40%ET_c had the greatest TSS (27.2°Brix) and Shaded-80%ET_c the lowest TSS (24.7°Brix) at harvest. Similar intermittent significant differences were observed for pH and titratable acidity (TA) as BFM. The Exposed-40%ET_c had higher pH (Figure 7C) and lower TA (Figure 7D) than Shaded treatments at 86 DAF. We observed a similar trend

at 102 and 115 DAF, where TA and pH were significantly different, respectively.

Berry Skin Anthocyanins and Flavonols

Berry skin anthocyanins were initially greater in Exposed-40%ET_c at 86 DAF (Figure 8A). However, as all treatments reached their maximum anthocyanin concentration, it started to decrease in the Exposed-40%ET_c treatment. By harvest (128 DAF), Exposed treatments, regardless of irrigation treatment had lower anthocyanins than the Shaded treatments. Shading and irrigation appeared to have an additive effect on the anthocyanin profile. This effect revealed itself as a decreases in the proportion of petunidin (Figure 8B), and cyanidin (Figure 8E) in favor of malvidin (Figure 8F). This effect was more pronounced especially toward harvest. Furthermore, the proportion of peonidin was only lower in the Shaded-40%ET_c for the last 3 sampling points (Figure 8D).

Berry skin flavonol concentration was more than 2 times greater in the Exposed berries at 86 and 102 DAF (Figure 9A). However, this difference decreased as flavonol concentration increased in the Shaded berries until 115 DAF. Meanwhile the Exposed berries reached their maximum flavonol concentration at 86 DAF. Flavonol profile was in most cases affected by the presence or absence of Shading rather than additional irrigation amounts. The proportion of myricetin and isorhamnetin was

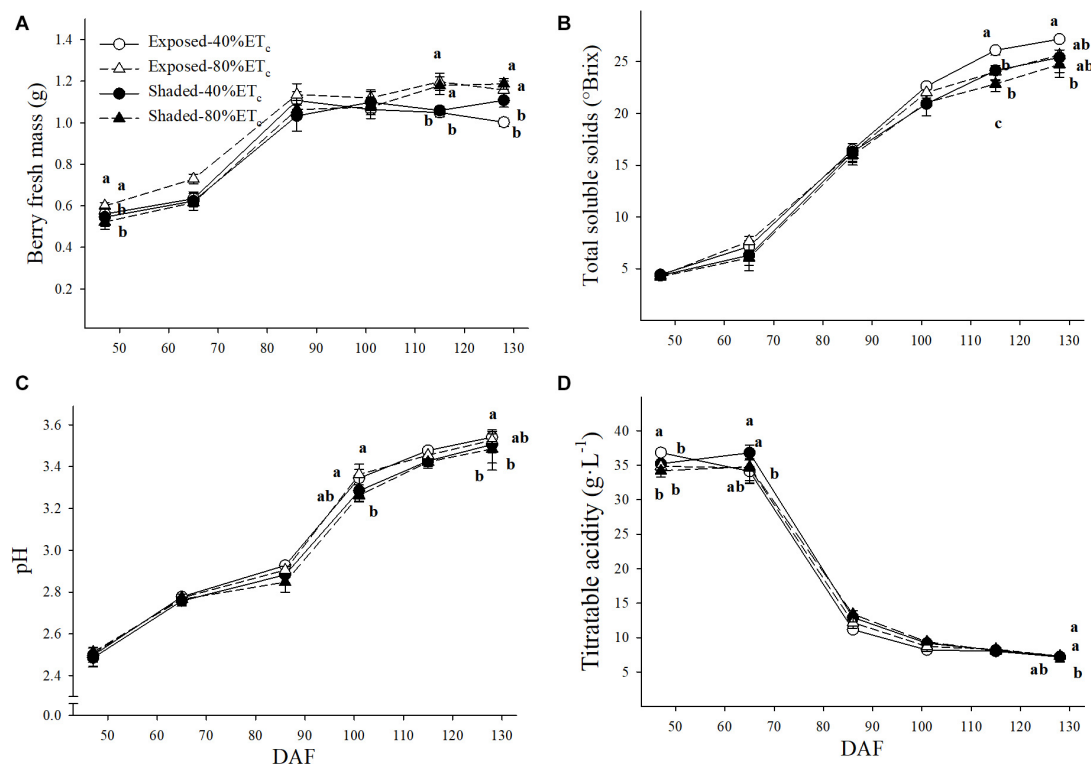


FIGURE 7 | Berry fresh mass (A), must total soluble solids (B), pH (C) and titratable acidity (D) in plants Exposed or Shaded 60% by nets at the fruit zone level combined with two applied water amounts, 40 and 80% replacement of crop evapotranspiration (ET_c). Error bars represent standard errors ($n = 4$). Groups with no letters in common are statistically different ($p < 0.05$).

greater in the Shaded treatments for the last three berry sampling points (Figures 9B,E). Likewise, this corresponded to decreases in the proportion of quercetin (Figure 9C). The proportion of kaempferol was lower in Shaded treatments and this difference continued to increase through harvest regardless of irrigation amount (Figure 9D).

During the most severe heat wave event of the year (103–106 DAF), anthocyanin loss occurred both in Exposed and Shaded berries. However, the loss was more pronounced in the Exposed berries (Table 2). We measured significant changes in the anthocyanin composition of berries which revealed themselves as reductions in the proportion of delphinidins, mainly in favor of malvidin in the Exposed treatment. Conversely, this modulation of anthocyanin profile was not observed in the Shaded berries. Likewise, flavonols in the Exposed treatment experienced a loss, as well. However the flavonol losses were less severe than the anthocyanins. During this most severe heat wave event, we measured a modulation of the flavonol profile, as well. After the heat wave, the proportion of kaempferol and laricitin were greater in detriment of myricetin.

Yield Components and Heat Wave Damage at Harvest

Harvest commenced at 128 DAF. The cluster weight (Table 3) was slightly greater with the 80% ET_c . However, this did not result

in higher yield per grapevine. We measured a 15% decrease of dormant pruning mass in the Shaded treatment, and this resulted in a greater yield-to-pruning mass ratio. However, the yield-to-pruning mass ratio was not statistically different.

The Southwest-facing clusters in the Exposed treatments showed evident signs of damage consisting in desiccated and aborted berries, affecting the appearance of clusters to a different extent (Supplementary Figure S2). In fact, the greater part of the berry abortion happened immediately after a heat wave with temperatures of 43.4°C and 42.8°C on 104 and 105 DAF, respectively. The proportion of damaged clusters was greater in the Exposed treatments when compared to Shaded (24 vs. 2%; Figure 10). Out of this damage, more than a third was severe; indicating that all the visible portions of the grape cluster, directly exposed were completely damaged. The 40% ET_c treatments had slightly higher proportion of damaged clusters than 80% ET_c but these were not significantly different. First signs of berry damage were observed 89 DAF on 17 August as a mild discoloration of the grape skins most oriented toward the sun only in well-exposed clusters on the Southwest aspect of the canopy. This damage progressed into flesh tissue death and berry mummification over the course of weeks (Supplementary Figures S2E,F). However, a heat shock phenomenon occurred the days of the heat wave, where sun-facing grapes within a cluster would discolor within hours and mummify over 1 or 2 days (Supplementary Figures S2A,B,G). A third phenomenon,

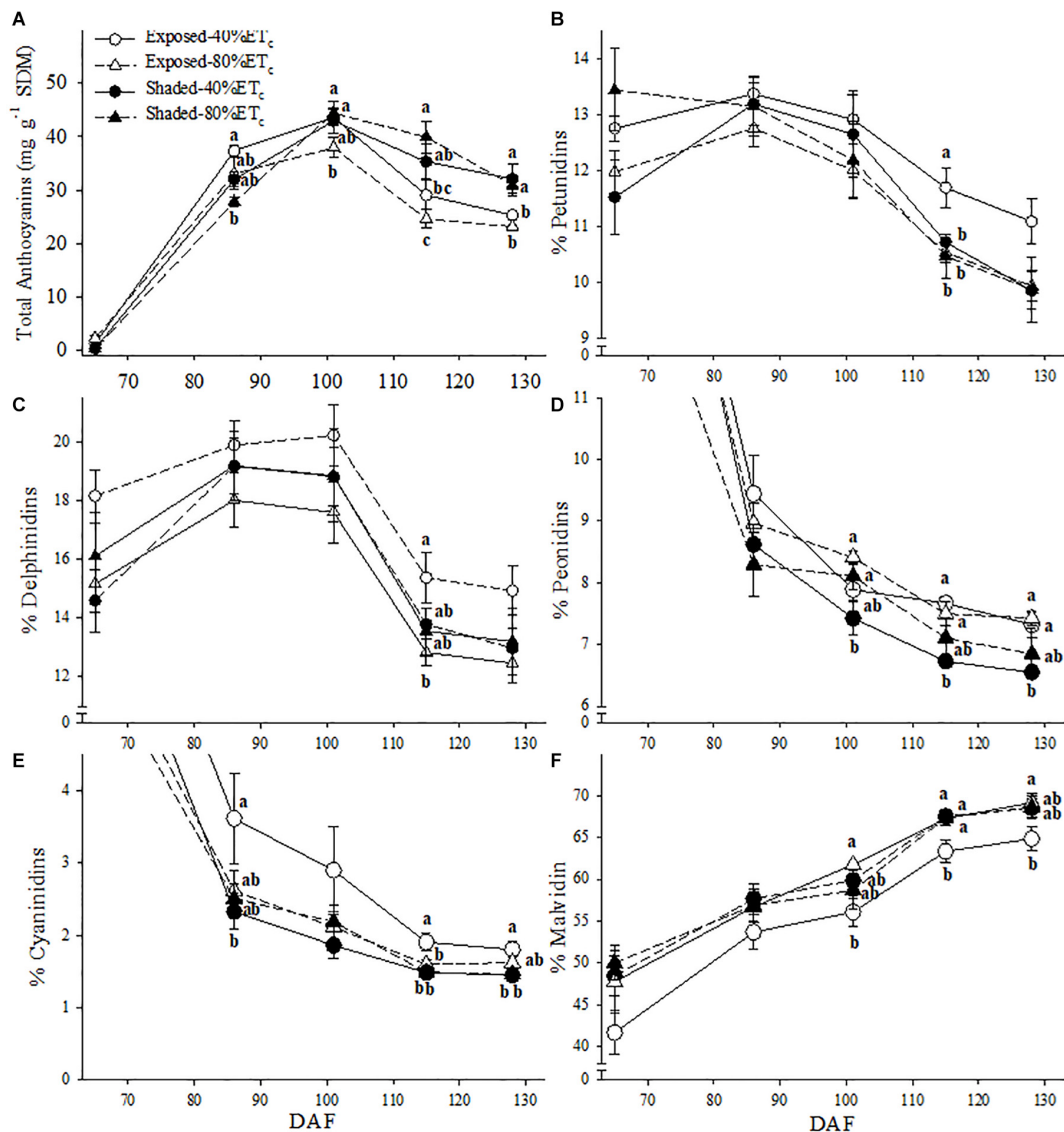


FIGURE 8 | Berry skin anthocyanin content (A) and the relative abundance petunidin (B), delphinidin (C) peonidin (D) cyanidin (E) and malvidin (F) of in plants Exposed or Shaded 60% by nets at the fruit zone level combined with two applied water amounts, 40 and 80% replacement of crop evapotranspiration (ET_c). Error bars represent standard errors ($n = 4$). Groups with no letters in common are statistically different ($p < 0.05$).

appeared by harvest where whole clusters were found to be completely mummified regardless of their exposure. Previously, this phenomenon was referred to as bunch stem necrosis (Krasnow et al., 2010).

DISCUSSION

Environmental Conditions and Heat Waves

During the execution of this study, a 4-day heat wave affected the study site from 31 of August to the 3 of September (103–106 DAF). Although other high heat events occurred that

year and the preceding years (Koch et al., 2012; Martínez-Lüscher et al., 2017b) at the study site, it was undeniable that this 4-day period with temperatures above 37°C with a maximum temperature of 43.4°C conditioned our results. These temperatures have not been observed at the study site since 2006. Therefore, results can be interpreted in the context of dealing with extreme climatic events that may become more frequent in many viticultural regions in the context of climate change predictions (Dosio et al., 2018). In regions that are accustomed to growing wine grapes under such high temperatures (Kurtural et al., 2013; Martínez-Lüscher et al., 2017a) events such as the one witnessed here are buffered with the use of different trellising systems. However, with the pre-planting decision of a vertical shoot positioning training system

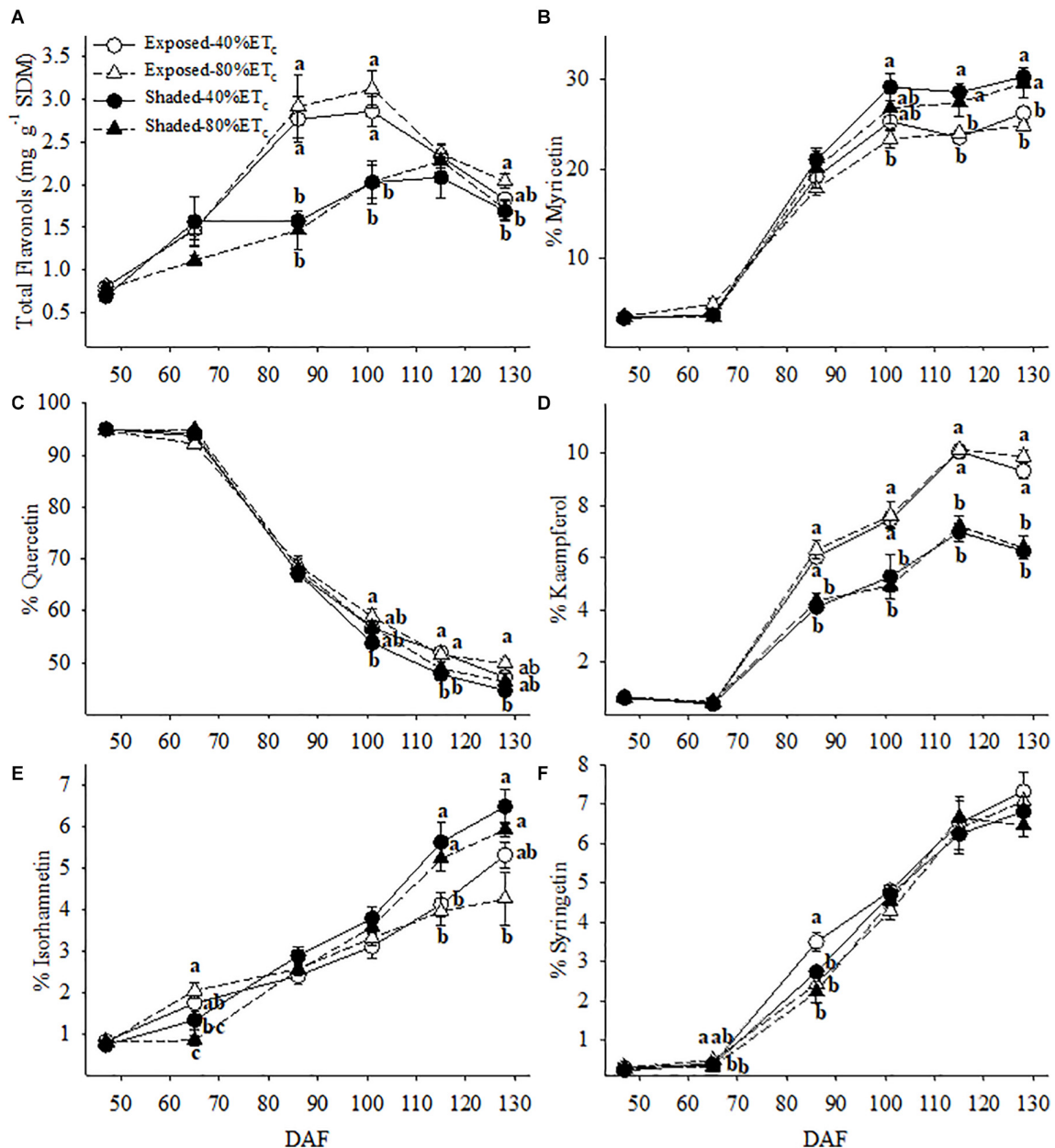


FIGURE 9 | Berry skin flavonol content (A) and the relative abundance of myricetin (B), quercetin (C), kaempferol (D), isorhamnetin (E) and syringetin (F) in plants Exposed or Shaded 60% by nets at the fruit zone level combined with two applied water amounts, 40 and 80% replacement of crop evapotranspiration (ET_c). Error bars represent standard errors ($n = 4$). Groups with no letters in common are statistically different ($p < 0.05$).

in coastal, but hot climate areas exacerbated the heat wave conditions even further with the monitored effects to cascading to plant primary metabolism.

Plant Primary Metabolism

Stem water potential (Ψ_{stem}) was greatly affected by doubling the amount of water applied. Customarily, vineyard operators would apply 40–50% of estimated ET_c in the study area (Torres et al., 2020). Contrary to our expectations, 80%ET_c did not incur higher stomatal conductance, transpiration or carbon

assimilation for the instances measured. The relatively frequent (once a week) replacement of ET_c fractions could have allowed maintaining stomatal conductance, and ultimately yields, while water stored in the soil profile and stem water potential decreases through the season. However, it should not be discarded that in this study, more frequent gas exchange measurements, at a different time of the day and further from the irrigation day may have resulted in differences in stomatal conductance according to the amounts of water applied. Shade nets reduced the solar radiation by 40% that reached the sides of the first

TABLE 2 | Effects of a 4-day-long heat event with a maximum temperature of 43.4°C on anthocyanins and flavonols.

	Exposed			Shaded			% Change ^a		
	Before	After	p-value	Before	After	p-value	Exposed	Shaded	p-value
TA ^b (mg g ⁻¹ SDM ^c)	40.8	28.6	*** ^d	43.7	34.9	**	-29.8	-20.2	n.s.
TA (mg per berry)	2.28	1.43	***	2.24	1.74	**	-37.1	-22.0	*
TA (mg g ⁻¹ BFM ^e)	2.09	1.30	***	2.06	1.58	***	-37.8	-23.2	*
3'4'-OH									
% Cyanidins	2.51	2.15	n.s.	2.02	2.02	n.s.	-0.4	0.0	n.s.
% Peonidins	8.15	8.08	n.s.	7.77	7.95	n.s.	-0.1	0.2	n.s.
3'4'5'-OH									
% Delphinidins	18.91	16.44	*	18.83	17.45	n.s.	-2.5	-1.4	n.s.
% Petunidins	12.46	11.90	n.s.	11.17	12.04	n.s.	-0.6	0.9	n.s.
% Malvidins	57.97	61.43	n.s.	60.21	60.54	n.s.	3.5	0.3	*
TF ^f (mg g ⁻¹ SDM)	3.05	2.52	*	2.10	2.21	n.s.	-17.5	5.1	n.s.
TF (mg per berry)	0.17	0.13	**	0.11	0.11	n.s.	-26.1	2.2	n.s.
TF (mg g ⁻¹ BFM)	0.16	0.11	***	0.10	0.10	n.s.	-26.7	0.7	n.s.
4'-OH									
% Kaempferol	7.37	9.84	***	4.93	6.90	n.s.	2.5	2.0	n.s.
3'4'-OH									
% Quercetin	56.60	56.93	n.s.	53.50	56.84	n.s.	0.3	3.3	n.s.
% Isorhamnetin	2.10	2.29	n.s.	2.38	2.59	n.s.	0.2	0.2	n.s.
3'4'5'-OH									
% Myricetin	25.87	22.12	***	30.34	24.51	n.s.	-3.7	-5.8	n.s.
% Laricitin	2.56	3.04	**	3.19	3.33	n.s.	0.5	0.1	n.s.
% Syringetin	5.49	5.79	n.s.	5.66	5.84	n.s.	0.3	0.2	n.s.

Total anthocyanins, total flavonols and the relative abundance of each group on 30 August (before the heat wave and 102 DAF) and 3 September (after the heat event and 106 DAF) in plants exposed or 60% shaded by nets at the fruit zone level. Two applied water amounts were pooled together ($n = 8$). ^aPercent of change of each variable before and after the heat wave to compare the loss of flavonoids in Exposed and Shaded berries. ^bTA, Total anthocyanins. ^cSDM, Skin dry mass. ^dAnalysis of variance p-values stand for "n.s." for $p > 0.05$, "***" for $p < 0.05$, "****" for $p < 0.01$ and "*****" for $p < 0.001$. ^eBFM, Berry fresh mass. ^fTF, Total flavonols.

TABLE 3 | Effects of partial solar radiation exclusion by Black-40 nets and replacement of 40% or 80% of estimated crop evapotranspiration replacement by irrigation on components of yield Cabernet Sauvignon grapevines.

	Cluster no.	Cluster weight (g)	Yield (kg/plant)	Dormant pruning mass (kg/plant)	Ravaz index ^a (kg/kg)
Exposed-40%ET _c	60.6 ± 3.8	102.1 ± 2.4	6.2 ± 0.5	1.49 ± 0.1	4.2 ± 0.1
Exposed-80%ET _c	59.3 ± 1.3	120.5 ± 6.3	7.2 ± 0.5	1.47 ± 0.0	5.0 ± 0.3
Shaded-40%ET _c	57.1 ± 3.8	106.5 ± 7.1	6.1 ± 0.7	1.20 ± 0.1	5.3 ± 0.7
Shaded-80%ET _c	55.8 ± 0.9	113.8 ± 6.4	6.3 ± 0.3	1.30 ± 0.1	5.2 ± 0.4
P(shade)	n.s. ^b	n.s.	n.s.	*	n.s.
P(irr)	n.s.	*	n.s.	n.s.	n.s.
P(Shade × irr)	n.s.	n.s.	n.s.	n.s.	n.s.

^aRavaz index = Ratio of fruit weight (kg) to pruning weight (kg). ^bTwo-way ANOVA p-values of main factors and their interaction: as "n.s." for $p > 0.05$, "***".

0.25 m of the canopy (composed of shoots 1.4 m or longer) and this reduced carbon assimilation in those shaded leaves, but not the rest. The lower carbon assimilation measured in Shaded treatments resulted in lower pruning mass (Greer and Weedon, 2013). Plant organ mass (in our case dormant shoot mass) is an important indicator of photomorphogenesis. Its cell division and expansion may be altered by sun exposure through photomorphogenic effects at low rates (Robson et al., 2015). In addition, shade nets led to slightly better water status under 40% ET_c, which could be indicative of a slightly lower water consumption by plants with nets covering the leaves around the fruit-zone. In studies where shading nets were placed above the

canopy, soluble solids may be reduced through lower carbon assimilation rates when shriveling berries were not accounted (Greer and Weedon, 2013).

The application of 80% ET_c of water slightly increased berry and cluster mass. In fact, increases in berry size by both shade nets double irrigation were coupled to a reduction in TSS. Decreases in berry size and/or increases in berry TSS were observed in response to solar exposure by fruit-zone leaf removal (Poni et al., 2009). Solar overexposure may exacerbated transpiration loss in the fruit-zone, which has showed to affect cell expansion during leaf growth (Devi et al., 2015). However, in the last weeks of fruit development, higher dehydration/cell

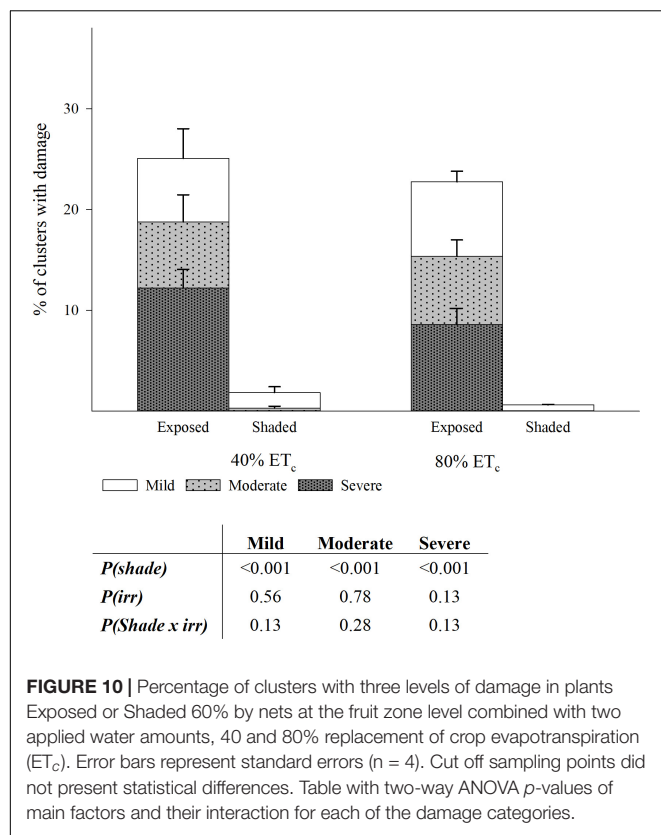


FIGURE 10 | Percentage of clusters with three levels of damage in plants Exposed or Shaded 60% by nets at the fruit zone level combined with two applied water amounts, 40 and 80% replacement of crop evapotranspiration (ET_c). Error bars represent standard errors ($n = 4$). Cut off sampling points did not present statistical differences. Table with two-way ANOVA p -values of main factors and their interaction for each of the damage categories.

death may better explain the lower berry weight of Exposed-40% ET_c (Bonada et al., 2013; Caravia et al., 2016). Changes in berry size are often one of the first events in a concomitant effect, in which berry soluble solids are concentrated, enhancing other processes intertwined to hexose signaling, transport and metabolism (Dai et al., 2014; Rienth et al., 2016). The Exposed-40% ET_c berries, had higher TSS (+ 2°Brix) than the other treatments. However, berry acidity was mildly impacted and no significant effects were evident at harvest. Having similar titratable acidity values in berries exposed to higher temperatures was a result that was somewhat unexpected as warmer berry temperatures are associated with a higher respiration rate of malic acid (Sweetman et al., 2014). Contrarily to experiments in which ambient temperature was manipulated (e.g., open top chambers) that have a very homogeneous effect on fruit metabolism, overexposure is an extreme—although greatly patchy—factor depending on the position of each cluster and how these are covered by leaves and the time of the day. Therefore, the spatiotemporal variation in cluster temperature differences among treatments can be anything from similar (e.g., at night or clusters occluded by leaves) to + 12°C (i.e., at 1530 h in Southwest exposed clusters). This finding provided evidence that the greater berry temperature experienced by berries of Exposed vines—only experienced by some clusters on the Southwest aspect for a period of less than 4 h—has a milder impact on overall grape must pH and TA (Zarrouk et al., 2016) than persistent changes in ambient temperature

reported elsewhere (Sweetman et al., 2014; Rienth et al., 2016; Torres et al., 2020).

Plant Secondary Metabolites

Berry skin anthocyanins were initially greater in Exposed-40% ET_c 86 DAF but higher in the two shaded treatments (regardless of irrigation) during the last weeks prior to harvest. Whereas part of these results responded to a shift in berry development, mediated by the higher TSS of Exposed-40% ET_c grapevines, the lower concentration of anthocyanin of Exposed grapevines at harvest responded to degradation. This result is corroborated by Torres et al. (2020) where optimal solar radiation exposure for Cabernet Sauvignon grape berry is in fact, less than 20% of the global radiation reaching the cluster. Furthermore, the first visual symptoms of berry color blanching were observed on 17 August, not long after veraison and during the first two heat waves of the year (Figure 3). At 102 DAF, before the 4-day heat wave, anthocyanin concentration was at its maximum. However, when anthocyanins were measured 4 days after this heat wave, anthocyanin concentration decreased at variable rates in each, and all treatments (Table 2). Doubling the irrigation amount did not change anthocyanin degradation rates in any case, thus only the factor of Shading was presented. Studies dealing with the effects of light and temperature on anthocyanins have proposed reduced synthesis (Yamane et al., 2006), chemical (Mori et al., 2007) and biological degradation (Sarni-Manchado et al., 1997) of these. For instance, anthocyanin degradation rates were 20–23% in Shaded treatments, which was interpreted as an effect of ambient temperatures above 40°C on berries at a stage (102 DAF) that synthesis was most certainly stopped (Castellarin et al., 2007). Exposed grapes also experienced extreme berry temperatures beyond 50°C for a few hours, and nearly doubled anthocyanin degradation rates (30–38%). Isothermal degradation kinetics studies with fruit extracts indicated half-life values of few days at 50°C (Cemeroğlu et al., 1994), which were enough to corroborate to a great extent the anthocyanin degradation rates in the present study.

Malvidin was the most abundant anthocyanin in all samples and its proportion increased in detriment of the other 4 anthocyanins as ripening progressed. Changes in anthocyanin profile have been reported before and attributed to differential synthesis concomitant to changes in expression of flavonoid hydroxylases (Castellarin et al., 2006; Martínez-Lüscher et al., 2014). A differential degradation of each of the anthocyanins could also explain this result based on the different antioxidant capacity of each compound (Arroyo-Currás et al., 2016; Csepregi and Hideg, 2018). However, the increase in the proportion of malvidin increased at a constant pace, regardless of net synthesis or degradation (i.e., before or after 102 DAF) and (Torres et al., 2020) reported similar findings in Cabernet Sauvignon to further corroborate these results.

Inhibition of flavonol synthesis is not as sensitive to temperature as anthocyanin synthesis (Mori et al., 2005). In contrast, flavonol synthesis is mainly regulated by the exposure to UV-B radiation (Martínez-Lüscher et al., 2014). Flavonols accumulate in the outer layers of plant tissues screening a great part of solar UV radiation. The involvement of flavonols in the

signaling and alleviation of oxidative stress was suggested (Agati et al., 2013; Watkins et al., 2017). However, our results do not provide strong evidence that flavonols may help to cope with high temperatures witnessed during heat waves. Therefore, when grape berries under field conditions are exposed to solar radiation with a subsequent increase in fruit temperature, there was a net synthesis flavonols up to mid/high levels of exposure (~48% canopy porosity). However, their concentration then rapidly decrease as berries become overexposed with the heat waves (Torres et al., 2020). Our results presented herein corroborated this finding, where grapes from Exposed and Shaded plants reached similar concentrations of flavonols at harvest through two different levels of exposure. The behavior of berries were statistically separated as such in the Exposed thorough higher rates of biosynthesis; and then degradation, and in the Shaded, through lower rates of biosynthesis. Furthermore, Exposed berries only displayed a 17–27% decrease in flavonols. The berries in the Shaded treatments did not experience a decrease in flavonols during the heat wave, providing evidence that flavonols have a slightly higher stability than anthocyanins under high temperature. This differential net synthesis/degradation left a footprint in flavonol profile, increasing the proportion of kaempferol and quercetin, in detriment of myricetin and isorhamnetin as reported previously (Pastore et al., 2017; Martínez-Lüscher et al., 2019a; Torres et al., 2020).

Heat Wave and Exposure Damage

In addition to the changes in chemical composition in the remaining fruit, a substantial part of the berries were completely mummified (at least 10%), and therefore, not sampled. During commercial harvest either through hand picking or with mechanical picking with on-board sorting, clusters and berries may be sorted and the grower may discard up to a 25% of the yield of this trial to optimize quality as desiccated berries may influence wine characteristics (Bonada et al., 2013). At the tissue level, the process of cell death has been characterized under elevated temperature and irrigation deficits (Bonada et al., 2013). It seems plausible that in our work, tissue temperatures of at least 50°C (berry temperature measurements while air temperature was 43.4°C were up to 54°C; data not shown) were enough to induce cell death, either progressively (**Supplementary Figures S2E,F**), or suddenly (**Supplementary Figure S2G**). However, to the best of our knowledge the estimation of certain temperature or irradiation thresholds for berry sunburn remain unexplored. In addition, cultural and environmental factors, featuring row orientation, trellis and training system, irrigation, and pre-exposure, may affect the incidence of sunburn for a given air temperature and irradiation (Webb et al., 2010; Zarrouk et al., 2016; Torres et al., 2020). Irrigating prior to an extreme heat event is meant by vineyard operators to increase leaf transpiration, leading to a cooling off effect at the fruit zone similar to overhead irrigation with sprinklers or misting system (Kliwer and Schultz, 1973). In our results, we had slightly higher berry temperatures in Exposed–40%ET_c than in Exposed–80%ET_c during the warmest part of the day on 114 DAF, but this was not associated to a reduced incidence of sunburn, less anthocyanin degradation or plant fitness. As both irrigation amounts tested in this study had

similarly high transpiration rates, it still remains to be tested whether a under low transpiration rates, misting could have ameliorated berry temperatures. Before these results may be extrapolated, it must be noted that vertically shoot positioned trellis systems, compared to sprawling training systems, have few leaf layers around the fruit zone, and thus, increases in transpiration may not influence berry temperature as much. Therefore, the potential benefit of irrigation prior to extreme heat events should not be completely discarded.

CONCLUSION

After a great number of studies—mostly performed in cooler climates—reporting the beneficial effects of solar radiation and water deficits for fruit ripening and especially flavonoids biosynthesis, new research focus is needed about the detrimental effects of the extreme climatic events such as drought and heat waves. Partial shading can have a positive role in the retention of grape berry skin flavonoids in a scenario of high temperatures and reduced cloud-cover. Conversely, solar exposure did not have a great effect on must acidity as controlled increases in air temperature reported elsewhere. Direct exposure to solar radiation was a necessary cooperater together with extreme heat events to produce fruit damage as practically all the damage recorded in this study was found in Southwest aspects of canopies without shade nets. Doubling irrigation amount had some mild effects lowering berry temperature and avoiding berry dehydration during the last part of ripening. Although doubling the amount of irrigation used may not be justified by this little gain, some additional irrigation prior to the heat events may be of practical use in rain-fed vineyards or with minimal irrigation in a case such as this. This study provided evidence of the necessity of field studies performed during extreme weather events, such as heat waves, to help complement the information gained under constant elevated temperature for the adaptation of cropping systems to climate change.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SK acquired the funding. CC collected the field data. JM-L and LB oversaw the field and lab data collection. JM-L wrote the first version of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

A graduate stipend was provided to CC by the Department of Viticulture and Enology at UC Davis, Horticulture and

Agronomy Graduate Group at UC Davis, and American Society for Enology and Viticulture.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.579192/full#supplementary-material>

REFERENCES

- Agati, G., Brunetti, C., Di Ferdinando, M., Ferrini, F., Pollastri, S., and Tattini, M. (2013). Functional roles of flavonoids in photoprotection: new evidence, lessons from the past. *Plant Physiol. Biochem.* 72, 35–45. doi: 10.1016/j.plaphy.2013.03.014
- Arroyo-Currás, N., Rosas-García, V. M., and Videa, M. (2016). Substituent inductive effects on the electrochemical oxidation of flavonoids studied by square wave voltammetry and Ab initio calculations. *Molecules* 21:1422. doi: 10.3390/molecules21111422
- Azuma, A., Yakushiji, H., Koshita, Y., and Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta* 236, 1067–1080. doi: 10.1007/s00425-012-1650-x
- Bonada, M., Sadras, V., Moran, M., and Fuentes, S. (2013). Elevated temperature and water stress accelerate mesocarp cell death and shrivelling, and decouple sensory traits in Shiraz berries. *Irrig. Sci.* 31, 1317–1331. doi: 10.1007/s00271-013-0407-z
- Brillante, L., Martínez-Lüscher, J., Yu, R., Plank, C. M., Sanchez, L., Bates, T. L., et al. (2017). Assessing spatial variability of grape skin flavonoids at the vineyard scale based on plant water status mapping. *J. Agric. Food Chem.* 65, 5255–5265. doi: 10.1021/acs.jafc.7b01749
- Caravia, L., Collins, C., Petrie, P. R., and Tyerman, S. D. (2016). Application of shade treatments during Shiraz berry ripening to reduce the impact of high temperature. *Aust. J. Grape Wine Res.* 22, 422–437. doi: 10.1111/ajgw.12248
- Castellarin, S. D., Di Gaspero, G., Marconi, R., Nonis, A., Peterlunger, E., Paillard, S., et al. (2006). Colour variation in red grapevines (*Vitis vinifera* L.): genomic organisation, expression of flavonoid 3'-hydroxylase, flavonoid 3',5'-hydroxylase genes and related metabolite profiling of red cyanidin-/blue delphinidin-based anthocyanins in berry skin. *BMC Genomics* 7:12. doi: 10.1186/1471-2164-7-12
- Castellarin, S. D., Matthews, M. A., Di Gaspero, G., and Gambetta, G. A. (2007). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101–112. doi: 10.1007/s00425-007-0598-8
- Castillo-Muñoz, N., Fernández-González, M., Gómez-Alonso, S., García-Romero, E., and Hermosín-Gutiérrez, I. (2009). Red-color related phenolic composition of Garnacha Tintorera (*Vitis vinifera* L.) grapes and red wines. *J. Agric. Food Chem.* 57, 7883–7891. doi: 10.1021/jf9002736
- Cemeroglu, B., Velioglu, S., and Isik, S. (1994). Degradation kinetics of anthocyanins in sour cherry juice and concentrate. *J. Food Sci.* 59, 1216–1218. doi: 10.1111/j.1365-2621.1994.tb14680.x
- Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., et al. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.* 105, 661–676. doi: 10.1093/aob/mcq030
- Cook, M. G., Zhang, Y., Nelson, C. J., Gambetta, G., Kennedy, J. A., and Kurtural, S. K. (2015). Anthocyanin composition of merlot is ameliorated by light microclimate and irrigation in central California. *Am. J. Enol. Vitic.* 66, 266–278. doi: 10.5344/ajev.2015.15006
- Csepregi, K., and Hideg, É. (2018). Phenolic compound diversity explored in the context of photo-oxidative stress protection. *Phytochem. Anal.* 29, 129–136. doi: 10.1002/pca.2720
- Dai, Z. W., Meddar, M., Renaud, C., Merlin, I., Hilbert, G., Delrot, S., et al. (2014). Long-term in vitro culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. *J. Exp. Bot.* 65, 4665–4677. doi: 10.1093/jxb/ert489
- Daryanto, S., Wang, L., and Jacinthe, P.-A. (2016). Global synthesis of drought effects on maize and wheat production. *PLoS One* 11:e0156362. doi: 10.1371/journal.pone.0156362
- Deryng, D., Conway, D., Ramankutty, N., Price, J., and Warren, R. (2014). Global crop yield response to extreme heat stress under multiple climate change futures. *Environ. Res. Lett.* 9:34011. doi: 10.1088/1748-9326/9/3/034011
- Devi, M. J., Taliercio, E. W., and Sinclair, T. R. (2015). Leaf expansion of soybean subjected to high and low atmospheric vapour pressure deficits. *J. Exp. Bot.* 66, 1845–1850. doi: 10.1093/jxb/eru520
- Dosio, A., Mentaschi, L., Fischer, E. M., and Wyser, K. (2018). Extreme heat waves under 1.5°C and 2°C global warming. *Environ. Res. Lett.* 13:54006. doi: 10.1088/1748-9326/aab827
- Drake, J. E., Tjoelker, M. G., Vårhammar, A., Medlyn, B. E., Reich, P. B., Leigh, A., et al. (2018). Trees tolerate an extreme heatwave via sustained transpirational cooling and increased leaf thermal tolerance. *Glob. Chang. Biol.* 24, 2390–2402. doi: 10.1111/gcb.14037
- Fischer, E. M., and Schär, C. (2010). Consistent geographical patterns of changes in high-impact European heatwaves. *Nat. Geosci.* 3, 398–403. doi: 10.1038/ngeo866
- Fraga, H., García de Cortázar Atauri, I., Malheiro, A. C., and Santos, J. A. (2016). Modelling climate change impacts on viticultural yield, phenology and stress conditions in Europe. *Glob. Chang. Biol.* 22, 3774–3788. doi: 10.1111/gcb.13382
- González, C. V., Fanzone, M. L., Cortés, L. E., Bottini, R., Lijavetzky, D. C., Ballaré, C. L., et al. (2015). Fruit-localized photoreceptors increase phenolic compounds in berry skins of field-grown *Vitis vinifera* L. cv. *Malbec*. *Phytochemistry* 110, 46–57. doi: 10.1016/j.phytochem.2014.11.018
- Greer, D. H., and Weedon, M. M. (2013). The impact of high temperatures on *Vitis vinifera* cv. Semillon grapevine performance and berry ripening. *Front. Plant Sci.* 4:491. doi: 10.3389/fpls.2013.00491
- Hall, G. E., Bondada, B. R., and Keller, M. (2011). Loss of rachis cell viability is associated with ripening disorders in grapes. *J. Exp. Bot.* 62, 1145–1153. doi: 10.1093/jxb/erq355
- Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., and Tabor, G. (2013). Climate change, wine, and conservation. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6907–6912. doi: 10.1073/pnas.1210127110
- IPCC (2013). "Climate change 2013: the physical science basis," in *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, eds T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, et al. (Cambridge: Cambridge University Press), doi: 10.1017/CBO9781107415324
- Jackson, D. I., and Lombard, P. B. (1993). Environmental and management practices affecting grape composition and wine quality. - A Review. *Am. J. Enol. Vitic.* 44, 409–430.
- Kliewer, W. M., and Schultz, H. B. (1973). Effect of sprinkler cooling of grapevines on fruit growth and composition. *Am. J. Enol. Vitic.* 24, 17–26.
- Koch, A., Ebeler, S. E., Williams, L. E., and Matthews, M. A. (2012). Fruit ripening in *Vitis vinifera*: light intensity before and not during ripening determines the concentration of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon berries. *Physiol. Plant.* 145, 275–285. doi: 10.1111/j.1399-3054.2012.01572.x
- Krasnow, M., Matthews, M., Smith, R., Benz, J., Weber, E., and Shackel, K. (2010). Distinctive symptoms differentiate four common types of berry shrivel disorder in grape. *Calif. Agric.* 64, 155–159. doi: 10.3733/ca.v064n03p155

- Kuhn, N., Guan, L., Dai, Z. W., Wu, B. H., Lauvergeat, V., Gomès, E., et al. (2014). Berry ripening: recently heard through the grapevine. *J. Exp. Bot.* 65, 4543–4559. doi: 10.1093/jxb/ert395
- Kurtural, S. K., Wessner, L. F., and Dervishian, G. (2013). Vegetative compensation response of a procumbent grapevine (*Vitis vinifera* cv. Syrah) cultivar under mechanical canopy management. *HortScience* 48, 576–583. doi: 10.21273/HORTSCI.48.5.576
- Levene, H. (1960). “Robust tests for equality of variances,” in *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, eds I. Olkin and H. Hotelling (Stanford: Stanford University Press), 278–292.
- Martínez-Lüscher, J., Brillante, L., and Kurtural, S. K. (2019a). Flavonol profile is a reliable indicator to assess canopy architecture and the exposure of red wine grapes to solar radiation. *Front. Plant Sci.* 10:10. doi: 10.3389/fpls.2019.00010
- Martínez-Lüscher, J., Plank, C. M., Brillante, L., Cooper, M. L., Smith, R. J., Al-Rwahnih, M., et al. (2019b). Grapevine red blotch virus may reduce carbon translocation leading to impaired grape berry ripening. *J. Agric. Food Chem.* 67, 2437–2448. doi: 10.1021/acs.jafc.8b05555
- Martínez-Lüscher, J., Brillante, L., Nelson, C. C., Al-Kereamy, A. M., Zhuang, S., and Kurtural, S. K. (2017a). Precipitation before bud break and irrigation affect the response of grapevine ‘Zinfandel’ yields and berry skin phenolic composition to training systems. *Sci. Hortic.* 222, 153–161. doi: 10.1016/j.scienta.2017.05.011
- Martínez-Lüscher, J., Chen, C. C. L., Brillante, L., and Kurtural, S. K. (2017b). Partial solar radiation exclusion with color shade nets reduces the degradation of organic acids and flavonoids of grape berry (*Vitis vinifera* L.). *J. Agric. Food Chem.* 65, 10693–10702. doi: 10.1021/acs.jafc.7b04163
- Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascual, I., and Gomès, E. (2014). Ultraviolet-B radiation and water deficit interact to alter flavonol and anthocyanin profile in grapevine berries through transcriptomic regulation. *Plant Cell Physiol.* 55, 1925–1936. doi: 10.1093/pcp/pcu121
- Matus, J. T. (2016). Transcriptomic and metabolomic networks in the grape berry illustrate that it takes more than flavonoids to fight against ultraviolet radiation. *Front. Plant Sci.* 7:1337. doi: 10.3389/fpls.2016.01337
- Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1935–1945. doi: 10.1093/jxb/erm055
- Mori, M., Yoshida, K., Ishigaki, Y., Matsunaga, T., Nikaido, O., Kameda, K., et al. (2005). UV-B protective effect of a polyacylated anthocyanin, HBA, in flower petals of the blue morning glory, *Ipomoea tricolor* cv. Heavenly Blue. *Bioorg. Med. Chem.* 13, 2015–2020. doi: 10.1016/j.bmc.2005.01.011
- Ollat, N., Touzard, J.-M., and van Leeuwen, C. (2016). Climate change impacts and adaptations: new challenges for the wine industry. *J. Wine Econ.* 11, 139–149. doi: 10.1017/jwe.2016.3
- Pastore, C., Allegro, G., Valentini, G., Muzzi, E., and Filippetti, I. (2017). Anthocyanin and flavonol composition response to veraison leaf removal on cabernet sauvignon, nero d’avola, raboso piave and sangiovese *Vitis vinifera* L. cultivars. *Sci. Hortic. (Amsterdam)*. 218, 147–155. doi: 10.1016/j.scienta.2017.01.048
- Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, M., Tornielli, G. B., and Filippetti, I. (2013). Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. *BMC Plant Biol.* 13:30. doi: 10.1186/1471-2229-13-30
- Poni, S., Bernizzoni, F., Civardi, S., and Libelli, N. (2009). Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red *Vitis vinifera* L. cultivars. *Aust. J. Grape Wine Res.* 15, 185–193. doi: 10.1111/j.1755-0238.2008.00044.x
- Restaino, C. M., Peterson, D. L., and Littell, J. (2016). Increased water deficit decreases Douglas fir growth throughout western US forests. *Proc. Natl. Acad. Sci. U.S.A.* 113, 9557–9562. doi: 10.1073/pnas.1602384113
- Rienh, M., Torregrosa, L., Sarah, G., Ardisson, M., Brillouet, J.-M., and Romieu, C. (2016). Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome. *BMC Plant Biol.* 16:164. doi: 10.1186/s12870-016-0850-0
- Robson, M. T., Klem, K., Urban, O., and Jansen, K. M. A. (2015). Re-interpreting plant morphological responses to UV-B radiation. *Plant. Cell Environ.* 38, 856–866. doi: 10.1111/pce.12374
- Sadras, V. O., and Moran, M. A. (2012). Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. *Aust. J. Grape Wine Res.* 18, 115–122. doi: 10.1111/j.1755-0238.2012.00180.x
- Sarni-Manchado, P., Cheynier, V., and Moutounet, M. (1997). Reactions of polyphenoloxidase generated caffeic acid o-quinone with malvidin 3-O-glucoside. *Phytochemistry* 45, 1365–1369. doi: 10.1016/s0031-9422(97)00190-8
- Smith, M. D. (2011). The ecological role of climate extremes: current understanding and future prospects. *J. Ecol.* 99, 651–655. doi: 10.1111/j.1365-2745.2011.01833.x
- Sweetman, C., Sadras, V. O., Hancock, R. D., Soole, K. L., and Ford, C. M. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65, 5975–5988. doi: 10.1093/jxb/eru343
- Terry, D. B. D. B., and Kurtural, S. K. K. (2011). Achieving vine balance of Syrah with mechanical canopy management and regulated deficit irrigation. *Am. J. Enol. Vitic.* 62, 426–437. doi: 10.5344/ajev.2011.11022
- Tinyane, P. P., Soundy, P., and Sivakumar, D. (2018). Growing ‘Hass’ avocado fruit under different coloured shade netting improves the marketable yield and affects fruit ripening. *Sci. Hortic.* 230, 43–49. doi: 10.1016/j.scienta.2017.11.020
- Torres, N., Martínez-Lüscher, J., Porte, E., and Kurtural, S. K. (2020). Optimal ranges and thresholds of grape berry solar radiation for flavonoid biosynthesis in warm climates. *Front. Plant Sci.* 11:931. doi: 10.3389/fpls.2020.00931
- Trenberth, K. E., and Fasullo, J. T. (2009). Global warming due to increasing absorbed solar radiation. *Geophys. Res. Lett.* 36:L07706. doi: 10.1029/2009gl037527
- Watkins, J. M., Chapman, J. M., and Muday, G. K. (2017). Absciscic acid-induced reactive oxygen species are modulated by flavonols to control stomata aperture. *Plant Physiol.* 175, 1807–1825. doi: 10.1104/pp.17.01010
- Webb, L., Whiting, J., Watt, A., Hill, T., Wigg, F., Dunn, G., et al. (2010). Managing grapevines through severe heat: a survey of growers after the 2009 summer heatwave in south-eastern Australia. *J. Wine Res.* 21, 147–165. doi: 10.1080/09571264.2010.530106
- Williams, L. E., and Ayars, J. E. (2005). Grapevine water use and the crop coefficient are linear functions of the shaded area measured beneath the canopy. *Agric. For. Meteorol.* 132, 201–211. doi: 10.1016/j.agrformet.2005.07.010
- Yamane, T., Jeong, S. T., Goto-Yamamoto, N., Koshita, Y., and Kobayashi, S. (2006). Effects of temperature on anthocyanin biosynthesis in grape berry skins. *Am. J. Enol. Vitic.* 57, 54–59.
- Yu, R., Brillante, L., Martínez-Lüscher, J., and Kurtural, S. K. (2020). Spatial variability of soil and plant water status and their cascading effects on grapevine physiology are linked to berry and wine chemistry. *Front. Plant Sci.* 11:790. doi: 10.3389/fpls.2020.00790
- Yu, R., Cook, M. G., Yacco, R. S., Watrelot, A. A., Gambetta, G., Kennedy, J. A., et al. (2016). Effects of leaf removal and applied water on flavonoid accumulation in grapevine (*Vitis vinifera* L. cv. Merlot) berry in a hot climate. *J. Agric. Food Chem.* 64, 8118–8127. doi: 10.1021/acs.jafc.6b03748
- Yu, R., and Kurtural, S. K. (2020). Proximal sensing of soil electrical conductivity provides a link to soil-plant water relationships and supports the identification of plant water status zones in vineyards. *Front. Plant Sci.* 11:244. doi: 10.3389/fpls.2020.00244
- Zarrouk, O., Brunetti, C., Egipto, R., Pinheiro, C., Genebra, T., Gori, A., et al. (2016). Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy. *Front. Plant Sci.* 7:1640. doi: 10.3389/fpls.2016.01640

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Martínez-Lüscher, Chen, Brillante and Kurtural. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



High Temperature and Elevated Carbon Dioxide Modify Berry Composition of Different Clones of Grapevine (*Vitis vinifera* L.) cv. Tempranillo

Marta Arrizabalaga-Arriazu^{1,2}, Eric Gomès², Fermín Morales³, Juan José Irigoyen¹, Inmaculada Pascual¹ and Ghislaine Hilbert^{2*}

OPEN ACCESS

Edited by:

Tommaso Frioni,
Catholic University of the Sacred
Heart, Piacenza, Italy

Reviewed by:

Diego S. Intrigliolo,
Center for Edaphology and Applied
Biology of Segura, Spanish National
Research Council, Spain
Xuequn Pang,
South China Agricultural University,
China

*Correspondence:

Ghislaine Hilbert
ghislaine.hilbert@inrae.fr

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 07 September 2020

Accepted: 06 November 2020

Published: 01 December 2020

Citation:

Arrizabalaga-Arriazu M, Gomès E,
Morales F, Irigoyen JJ, Pascual I and
Hilbert G (2020) High Temperature
and Elevated Carbon Dioxide Modify
Berry Composition of Different Clones
of Grapevine (*Vitis vinifera* L.) cv.
Tempranillo.
Front. Plant Sci. 11:603687.
doi: 10.3389/fpls.2020.603687

¹ Universidad de Navarra, Faculty of Sciences, Plant Stress Physiology Group, Associated Unit to CSIC (EEAD, Zaragoza, and ICVV, Logroño), Pamplona, Spain, ² EGFV, Univ. Bordeaux, Bordeaux Sciences Agro, INRAE, ISVV, Villenave d'Omon, France, ³ Instituto de Agrobiotecnología (IdAB), Consejo Superior de Investigaciones Científicas (CSIC)-Gobierno de Navarra, Pamplona, Spain

Tempranillo is a grapevine (*Vitis vinifera* L.) variety extensively used for world wine production which is expected to be affected by environmental parameters modified by ongoing global climate changes, i.e., increases in average air temperature and rise of atmospheric CO₂ levels. Apart from determining their effects on grape development and biochemical characteristics, this paper considers the intravarietal diversity of the cultivar Tempranillo as a tool to develop future adaptive strategies to face the impact of climate change on grapevine. Fruit-bearing cuttings of five clones (RJ43, CL306, T3, VN31, and 1084) were grown in temperature gradient greenhouses (TGGs), from fruit set to maturity, under two temperature regimes (ambient temperature vs. ambient temperature plus 4°C) and two CO₂ levels (ambient, ca. 400 ppm, vs. elevated, 700 ppm). Treatments were applied separately or in combination. The analyses carried out included berry phenological development, the evolution in the concentration of must compounds (organic acids, sugars, and amino acids), and total skin anthocyanins. Elevated temperature hastened berry ripening, sugar accumulation, and malic acid breakdown, especially when combined with high CO₂. Climate change conditions reduced the amino acid content 2 weeks after mid-veraison and seemed to delay amino acid maturity. Elevated CO₂ reduced the decoupling effect of temperature on the anthocyanin to sugar ratio. The impact of these factors, taken individually or combined, was dependent on the clone analyzed, thus indicating certain intravarietal variability in the response of Tempranillo to these climate change-related factors.

Keywords: climate change, grapevine (*Vitis vinifera*), genetic variability, sugars, malic acid, amino acids, anthocyanins

INTRODUCTION

Grapevine is one of the most prominent crops in agriculture given the cultural and economic importance of grape and wine production. Among the grape varieties cultivated worldwide, Tempranillo ranked #3 in 2017 with 231,000 ha, behind Cabernet Sauvignon and Kyoho (OIV, 2017), and it is one of the most important red grape varieties grown in Spain. This cultivar is characterized by subtle aroma, producing wines with fruity and spicy flavors, low acidity, and low tannins. However, wine organoleptic characteristics are highly determined by the characteristics of the grapes used for its production. Therefore, changes in grapevine growing conditions that affect berry composition are also likely to impact the wine produced. Grape quality is a complex trait that mainly refers to berry composition, including sugars, organic acids (malic and tartaric acid), amino acids, and a wide range of secondary metabolites such as phenolic compounds, aromas, and aroma precursors (Conde et al., 2007). Among the factors that affect berry content at harvest, climate parameters, and notably temperature, play a prominent role.

The Intergovernmental Panel on Climate Change (IPCC) has pointed out the ineluctable increase in the temperature worldwide and has identified climate change as an important threat to global food supply. Indeed, greenhouse gas (GHG) emissions have increased greatly during the last decades, affecting the equilibrium of biogeochemical cycles and, hence, the composition of the atmosphere (IPCC, 2013). Some of the consequences of the rise in the atmospheric concentration of GHG are at global climate level, as high levels of GHG block heat loss of the planet, thus contributing to the so-called “greenhouse effect” and to global warming. Another effect of the anthropogenic GHG emissions is the increase in the concentration of CO₂ in the atmosphere (IPCC, 2014). The IPCC has carried out different reports from scientific knowledges on climate change to determine the magnitude of these changes according to different scenarios. Some of the predictions for global mean temperature in 2100 show an increment between 2.2 ± 0.5 and $3.7 \pm 0.7^\circ\text{C}$, and the estimations for future atmospheric CO₂ concentration are between 669.7 and 935.9 ppm (scenarios RCP 6.0 and RCP 8.5 of the IPCC) (IPCC, 2013).

Research on the response of grapevine to the abovementioned environmental factors has concluded that high temperature affects the phenology of grapevine, as well as grape berry development and ripening, hastening the latter (Jones and Davis, 2000; Duchêne and Schneider, 2005; Webb et al., 2007; Keller, 2010; Martínez-Lüscher et al., 2016a) and affecting both primary and secondary metabolisms. Berry sugar accumulation is altered under climate change conditions (Duchêne and Schneider, 2005; Kuhn et al., 2014), while malic acid degradation is enhanced by high temperatures (Orduña, 2010; Carbonell-Bejerano et al., 2013), and by its combination with elevated CO₂ (Salazar Parra et al., 2010; Martínez-Lüscher et al., 2016b). Secondary metabolism is also sensitive to high temperatures, particularly the flavonoid and aroma precursor pathways, as evidenced

by transcriptomic, proteomic, and metabolomic approaches (Spayd et al., 2002; Tarara et al., 2008; Azuma et al., 2012; Rienth et al., 2014; Martínez-Lüscher et al., 2016a; Lecourieux et al., 2017, 2020; Arrizabalaga et al., 2018), thus affecting the balance of berry quality-related compounds at ripeness (Sadras and Moran, 2012; Kuhn et al., 2014). Whereas increased temperature has been consistently shown to reduce anthocyanin levels (Spayd et al., 2002; Mori et al., 2007; Tarara et al., 2008; Azuma et al., 2012), the effect of CO₂ on these compounds is more controversial, with some authors reporting no effect (Gonçalves et al., 2009), while others reported an increase in anthocyanin levels (Kizildeniz et al., 2015). Finally, the decoupling in the accumulation of anthocyanins and sugars, thus leading to an imbalance between these two compounds at maturity, has been also described as a consequence of elevated temperature (Sadras and Moran, 2012; Kuhn et al., 2014).

The studies on the impact of multiple stress factors associated with climate change on grape development and composition remain limited due to their complexity. However, in the future, plants will be exposed to multiple elements of environmental change simultaneously, their combined effects being not always additive. Indeed, the impact of an increase in atmospheric CO₂ concentration on plant carbon metabolism, notably on photosynthesis and respiration processes, has been previously reported (Dusenge et al., 2019). Zhao et al. (2019) also demonstrated that photosynthesis of *in vitro* grape plantlet was promoted by elevated CO₂ concentration. These modifications may directly affect primary and secondary plant metabolism, thus leading to substantial changes in fruit composition. However, Dusenge et al. (2019) showed that some effects of increased CO₂ concentration on carbon plant metabolism could be minimized by elevated temperatures and highlighted the interest to study these climatic parameters together, both separately and in combination.

Regarding grapevine, previous studies on the effect of elevated CO₂ and elevated temperature indicated that the combination of these two factors can affect plant phenology, thus hastening grape ripening (Salazar-Parra, 2011; Martínez-Lüscher et al., 2016b) and decreasing malic acid concentration (Leibar et al., 2016). Nevertheless, the authors reported also a decrease in grape total anthocyanin concentration at maturity. Therefore, in order to avoid detrimental wine traits, it is important to determine how grape characteristics will be affected by the abovementioned climate change-related factors, acting not only individually but also combined, and to investigate approaches to mitigate the undesired effects, ensuring the sustainability of this crop. Among the strategies proposed to adapt viticulture to a changing environment, the genetic improvement and adaptation of elite and autochthonous varieties are very relevant to keep their intrinsic varietal values and typicity (Cunha et al., 2009; Keller, 2010; Carbonell-Bejerano et al., 2019). Accordingly, the selection of grapevine varieties and clones within the same variety with longer ripening periods has been suggested as a valuable tool to exploit in order to find accessions keeping current traits under future climate conditions (Duchêne et al., 2010).

Certain varieties used for wine production are tightly linked to specific production areas. This is the case of Tempranillo in Spain, Merlot in France, Sangiovese in Italy, or Fernão Pires in Portugal (Sefc et al., 2003). Tempranillo requires warm, sunny days to reach full maturity but also cool nights to keep its natural acidity. Maturity occurs fairly early in comparison with Grenache, the variety commonly blended with Tempranillo. Nevertheless, the constant increase in temperature and CO₂ levels in the future could significantly shift the optimal maturation conditions in these areas, which would have a significant effect on berry quality. For these reasons, successfully exploiting the intravarietal diversity has the potential to face with the putative negative impacts of climate change, as it would allow to keep the culture of traditional varieties. The research done so far in Tempranillo includes the identification and characterization of a large number of clones (49 certified clones), which differ either in reproductive or vegetative traits (Ibáñez et al., 2015), making possible the research of clones better adapted to future climate conditions.

In previous studies, we have reported that Tempranillo clones differ in their response to elevated temperature in terms of sugar and anthocyanin accumulation (Arrizabalaga et al., 2018). We have also demonstrated that Tempranillo clones presented differences in their phenological development, notably in terms of vegetative production and carbon partitioning into organs, in response to elevated CO₂ and increased temperature (Arrizabalaga-Arriazu et al., 2020). However, plants showed signs of photosynthetic acclimation to CO₂, especially when they were exposed to elevated CO₂ combined with high temperature conditions, joining the idea that effects of increasing CO₂ concentration on plant carbon metabolism may be modulated by elevated temperatures as mentioned above. In this context, the objective of this work was to study the effects of increased temperature and rise in atmospheric CO₂, alone or in combination, on berry development and composition of five different clones of *Vitis vinifera* cv. Tempranillo exhibiting different lengths of their reproductive cycle. The study was focused on the evolution of must composition (malic acid, sugars, and amino acids) and skin total anthocyanins throughout the ripening period, aiming to assess whether the impact of temperature and CO₂ differs among different clones of Tempranillo. We hypothesized that clones within the same variety that show differences in phenological development may respond in a different manner to the abovementioned climate change-related factors, in terms of grape composition. Indeed, grape composition could be less affected by future climatic conditions in clones with longer reproductive cycle than in early ripening clones, since in the former the ripening would take place under cooler conditions. Few studies have assessed the performance of different clones of the same cultivar to climate conditions foreseen by the end of this century. The novelty of this work is the assessment of three-way interactions among Tempranillo clones, elevated temperature, and high atmospheric CO₂. The information obtained can be useful to evaluate whether genetic diversity can be an appropriate tool to design adaption strategies for viticulture in the future.

MATERIALS AND METHODS

Plant Material: Origin and Development

Dormant cuttings of five clones of grapevine (*V. vinifera* L.) cv. Tempranillo were obtained from the germplasm bank of three institutions: RJ43, CL306 (both clones widely cultivated in Spain), and T3 were obtained from Estación de Viticultura y Enología de Navarra (EVENA), located in Olite, Navarra (Spain); 1084 was obtained from the Institute of Sciences of Vine and Wine, located in “La Grajera,” La Rioja (Spain); and VN31 was facilitated by Vitis Navarra, located in Larraga, Navarra (Spain). The reproductive cycle length of these clones had been characterized previously, presenting differences among them: VN31 and 1084 have been described as long reproductive cycle accessions (Arrizabalaga et al., 2018; Vitis Navarra, 2019), CL306 and T3 have been defined as short cycle accessions (Rubio and Yuste, 2005; Vicente Castro, 2012; Cibrián et al., 2018), and RJ43 is considered to have an intermediate reproductive cycle length (EVENA, 2009).

Fruit-bearing cuttings were obtained as described in Arrizabalaga et al. (2018) with minor modifications. They were manipulated to develop a single berry bunch and they were grown at the same conditions as described in Arrizabalaga et al. (2018) from March to May 2016, when the fruit set took place. Then, plants were transferred to 13 L pots with 2:1 peat:perlite mixture (v/v) and moved afterward to temperature gradient greenhouses (TGGs) located at the campus of the University of Navarra (42°48'N, 1°40'W; Pamplona, Navarra, Spain). The irrigation, both before fruit set and in the TGGs, was carried out using the nutritive solution described by Ollat et al. (1998).

Treatments and Plant Growth in Temperature Gradient Greenhouses

Treatment application was conducted in TGGs, a research-oriented structure for plant growth with semicontrolled conditions, taking into consideration natural environmental conditions. Each TGG is divided into three temperature modules which create a gradient of temperature (from module 1 of ambient temperature to module 3 of ambient temperature + 4°C), as the air heats up when passing through them (Morales et al., 2014). The temperature records during the experiment are included in **Supplementary Figure 1**. In addition, CO₂ can be injected inside the TGGs, modifying the air CO₂ concentration.

An equal number of plants of each clone were placed in the first and the third module of four TGGs, leaving the central module free of plants. Half of the plants (those located in modules 1) grew at ambient temperature (*T*), corresponding to the ambient temperature outdoors, while the other half of the plants (those located in modules 3) grew under ambient plus 4°C warmer temperature (*T* + 4). Besides, the air CO₂ concentration was modified in two out of the four TGGs, resulting in half of the plants growing at current atmospheric CO₂ concentration (ca. 400 ppm; ACO₂) and the other half under increased air CO₂ concentration (700 ppm; ECO₂). Therefore, plants grew under four different CO₂ and temperature conditions:

(i) ambient temperature and ambient CO₂ (T/ACO_2), (ii) ambient temperature and elevated CO₂ (T/ECO_2), (iii) elevated temperature and ambient CO₂ ($T + 4/ACO_2$), and (iv) elevated temperature and elevated CO₂ ($T + 4/ECO_2$). The treatments were applied from fruit set (2016, May) to berry maturity (2016, September), which was considered to occur when the total soluble solid (TSS) content of the berries was ca. 22°Brix, each plant being measured individually (°Brix was determined on grape juice, after pressing entire grape berries, using a hand refractometer with temperature compensation). The number of plants per treatment and clone was 8.

Phenological Development and Berry Size

The length of the phenological development of grapes was determined for each plant by individually recording the dates corresponding to fruit set, mid-veraison (half of the berries in the bunch had started to change color), and maturity. The time intervals between fruit set and mid-veraison and between mid-veraison and maturity were calculated. Fruit set and mid-veraison were determined visually, and maturity was considered to be reached when the levels of TSS of two berries of each bunch were, at least, 22°Brix. This analysis was done periodically for each bunch every 2–3 days during the last weeks of development of the berries.

In order to carry out the different analyses of berries, five sampling points were determined: (i) onset of veraison, when berries had already softened and just began to color; (ii) mid-veraison (determined as described previously in this section), at this stage, berries with the same proportion of colored skin surface were sampled (ca. 50%); (iii) 1 week after mid-veraison; (iv) 2 weeks after mid-veraison; and (v) maturity, determined as described previously in this section. At the onset of veraison, three to four berries were sampled from each bunch, three berries at mid-veraison, 1 week after mid-veraison and 2 weeks after mid-veraison, and 10 berries at maturity. Each plant was sampled individually when reaching the desired stage. In order to avoid differences in berry composition due to their position in the bunch, all the berries were taken from the top and middle portion of the bunch, which allocate the highest number of berries. The diameter was measured with a caliper and berries were frozen in liquid nitrogen and stored at −80°C until analyses.

Berry Analyses Preparation

Analyses were carried out by doing pools of berries (3–10 berries per bunch, see above, from two or three different plants per pool, four pools per treatment and clone). Berries were manually peeled and separated into skin, pulp, and seeds. Fresh skins, pulps, and seeds were weighed and the data obtained were used to determine the relative skin mass (relation between skin fresh weight and total berry fresh weight, expressed as a percentage). The pulp was crushed to obtain the must, which was centrifuged and used for sugar, malic acid, and amino acid analyses. The skin was freeze-dried in an Alph1-4, lyophilizer (CHRIST, Osterode, Germany), weighed to calculate the water content, and ground

into powder using an MM200 ball grinder (Retsch, Haan, Germany) for carrying out the anthocyanin analyses.

Total Soluble Solids, Sugar, Malic Acid, and Amino Acid Profile Analyses

Total soluble solids content in the must was measured using a Handheld Digital Refractometer (RD110). Sugar (glucose and fructose) concentration was determined enzymatically using an automated absorbance microplate reader (Elx800UV, BioTek Instruments, Inc., Winooski, VT, United States) using the Glucose/Fructose kit from BIOSENTEC (Toulouse, France) according to the manufacturer. Malic acid was determined with a Bran and Luebbe TRAACS 800 autoanalyzer (Bran & Luebbe, Plaisir, France) as described in detail in Bobeica et al. (2015).

For the amino acid analysis, samples were derivatized with the AccQFluor Reagent (6-aminoquinolyl-*N*-hydroxy-succinimidyl-carbamate, Waters, Milford, MA, United States) (Cohen and Michaud, 1993) as described by Hilbert et al. (2015). The products of this reaction were analyzed with an UltiMate 3000 UHPLC system (Thermo Electron SAS, Waltham, MA, United States) equipped with an FLD-3000 Fluorescence Detector (Thermo Electron SAS, Waltham, MA, United States). Amino acids were separated using as eluents sodium acetate buffer (eluent A, 140 mM at pH 5.7), acetonitrile (eluent B), and water (eluent C) at 37°C and 0.5 ml min^{−1} through an AccQTag Ultra column, 2.1 mm × 100 mm, 1.7 μm (Waters, Milford, MA, United States) according to Habran et al. (2016). The concentration and identification of each compound was determined through a chromatographic analysis as described in Pereira et al. (2006), using an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Samples were loaded alternated with control samples as in Torres et al. (2017).

Total Skin Anthocyanin Analyses

Anthocyanin analyses were carried out according to Torres et al. (2017) and described in detail by Acevedo de la Cruz et al. (2012) and Hilbert et al. (2015) with minor changes. In brief, ground dried skins were treated with methanol containing 0.1% HCl (v/v), in order to extract the pigments, and filtered using a polypropylene syringe filter of 0.45 μm (Pall Gelman Corp., Ann Arbor, MI, United States). The obtained extracts were separated using a Synchronis C18, 2.1 mm × 100 mm, 1.7 μm column (Thermo Fisher Scientific, Waltham, MA, United States) and analyzed with an UltiMate 3000 UHPLC system (Thermo Electron SAS, Waltham, MA, United States) equipped with DAD-3000 diode array detector (Thermo Electron SAS, Waltham, MA, United States). The wavelength used for recording the chromatographic profiles was 520 nm and the standard was malvidin-3-*O*-glucoside (Extrasynthese, Genay, France). The peak areas of chromatograms were calculated using the Chromeleon software (version 7.1) (Thermo Electron SAS, Waltham, MA, United States). The concentration of total anthocyanins was calculated as the sum of the concentration of individual anthocyanins. In order to evaluate the impact of environmental factors on the balance between anthocyanins and

sugars, the ratio between the concentration of anthocyanins and the level of TSS was calculated at maturity.

Statistical Analysis

The data were statistically analyzed using R (3.5.1), carrying out a three-way ANOVA (clone, temperature, and CO₂ concentration) and a Fisher's least significant difference (LSD) was carried out as a *post hoc* test when statistically significant differences were found ($P < 0.05$).

RESULTS

Phenological Development

In general, elevated CO₂ had a higher impact on grape phenology in the period comprised between fruit set to mid-veraison, whereas ripening (mid-veraison to maturity) was more affected by elevated temperature (Figure 1A). The number of days elapsed between fruit set and mid-veraison was slightly, but significantly, shortened by ECO₂, and especially when it was combined with $T + 4$ (Figure 1A). However, this hastening effect of CO₂ was nullified between mid-veraison and maturity, whereas the increase of temperature reduced significantly this period in up to 5 days. Also, the duration from mid-veraison to maturity

was affected significantly by the clone identity, mainly because 1084 needed more time to reach maturity, regardless of the growing condition (Figure 1B). Although significant interactions among factors were not detected, it is worth pointing out the significant difference in the elapsed days between mid-veraison and maturity of T3 plants grown under $T + 4$ /ACO₂ compared with T /ACO₂, as maturity was reached 12 days earlier when $T + 4$ was applied.

Berry Characteristics

Berry diameter differed significantly among clones at the onset of veraison, mid-veraison, and maturity (Table 1). It was also modified by temperature, decreasing under $T + 4$ 2 weeks after mid-veraison and at maturity. By contrast, the CO₂ level did not markedly affect this berry characteristic. The only noteworthy interaction among the parameters was at mid-veraison, when the effect of ECO₂ was different depending on the temperature regime and the clone (triple interaction).

In general, berries from all the studied clones presented similar relative skin mass throughout the experiment except at maturity, when 1084 had significantly lower values than CL306, T3, and VN31 (Table 2). Relative skin masses were higher 1 and 2 weeks after mid-veraison in grapes developed under $T + 4$ compared with those grown at T . Grapes under

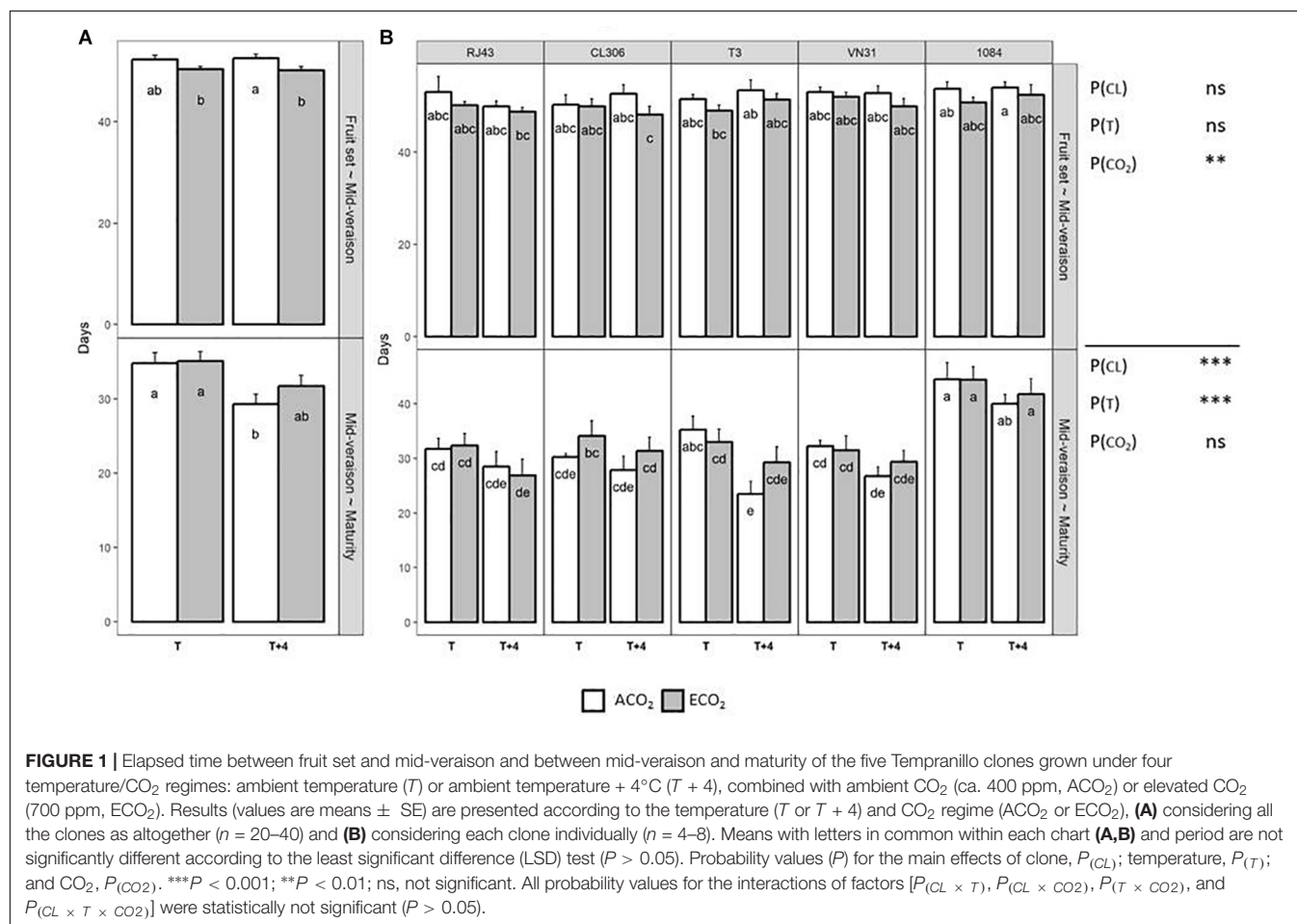


TABLE 1 | Grape berry diameter of the five Tempranillo clones (RJ43, CL306, T3, VN31, and 1084) grown under four temperature/CO₂ regimes: ambient temperature (T) or ambient temperature + 4°C (T + 4), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂).

			Berry diameter (mm)				
			Onset of veraison	Mid-veraison	1 week after mid-veraison	2 weeks after mid-veraison	Maturity
RJ43			10.51 ± 0.18ab	10.78 ± 0.23bc	11.36 ± 0.21b	13.19 ± 1.09a	12.71 ± 0.17ab
CL306			10.11 ± 0.16b	10.57 ± 0.13c	11.36 ± 0.19b	12.07 ± 0.19a	12.33 ± 0.20b
T3			10.86 ± 0.20a	11.11 ± 0.19b	11.63 ± 0.23ab	12.40 ± 0.22a	13.11 ± 0.17a
VN31			10.77 ± 0.15a	11.19 ± 0.13ab	13.00 ± 1.25a	13.81 ± 1.07a	13.07 ± 0.16a
1084			10.86 ± 0.23a	11.60 ± 0.19a	12.10 ± 0.20ab	12.16 ± 0.30a	12.88 ± 0.25a
T			10.75 ± 0.12a	11.18 ± 0.11a	11.85 ± 0.13a	13.39 ± 0.60a	13.14 ± 0.13a
T + 4			10.49 ± 0.13a	10.92 ± 0.13a	11.93 ± 0.52a	12.07 ± 0.15b	12.50 ± 0.10b
ACO ₂			10.60 ± 0.10a	11.07 ± 0.12a	12.16 ± 0.51a	12.42 ± 0.11a	12.68 ± 0.13a
ECO ₂			10.64 ± 0.14a	11.03 ± 0.13a	11.62 ± 0.15a	13.03 ± 0.63a	12.96 ± 0.13a
RJ43	T	ACO ₂	10.93 ± 0.49abc	11.18 ± 0.47abcd	11.55 ± 0.24b	12.70 ± 0.15bc	13.01 ± 0.52bcde
		ECO ₂	10.39 ± 0.36bcd	10.44 ± 0.43def	11.45 ± 0.53b	16.43 ± 4.35ab	12.82 ± 0.13cdef
	T + 4	ACO ₂	10.50 ± 0.24bc	10.18 ± 0.21ef	10.73 ± 0.44b	11.63 ± 0.18c	12.56 ± 0.42defg
		ECO ₂	10.21 ± 0.33cd	11.32 ± 0.53abcd	11.70 ± 0.37b	12.01 ± 0.11c	12.46 ± 0.24defg
CL306	T	ACO ₂	10.03 ± 0.18cd	10.52 ± 0.20def	11.35 ± 0.17b	12.11 ± 0.25c	11.84 ± 0.56g
		ECO ₂	10.32 ± 0.34cd	11.04 ± 0.07abcde	11.93 ± 0.22b	12.31 ± 0.37c	12.95 ± 0.27bcdef
	T + 4	ACO ₂	10.68 ± 0.18abc	10.71 ± 0.26cdef	11.44 ± 0.54b	12.69 ± 0.16bc	12.52 ± 0.34defg
		ECO ₂	9.42 ± 0.20d	10.02 ± 0.22f	10.71 ± 0.31b	11.18 ± 0.26c	12.03 ± 0.19fg
T3	T	ACO ₂	10.72 ± 0.33abc	11.16 ± 0.04abcd	12.09 ± 0.09b	12.45 ± 0.19bc	13.22 ± 0.14abcd
		ECO ₂	11.59 ± 0.24a	11.87 ± 0.09a	12.46 ± 0.28b	13.30 ± 0.23abc	13.97 ± 0.23a
	T + 4	ACO ₂	10.29 ± 0.34cd	10.74 ± 0.56cdef	11.17 ± 0.47b	11.95 ± 0.58c	12.43 ± 0.14defg
		ECO ₂	10.83 ± 0.45abc	10.68 ± 0.35cdef	10.82 ± 0.44b	11.91 ± 0.34c	12.81 ± 0.20cdef
VN31	T	ACO ₂	10.94 ± 0.31abc	11.25 ± 0.19abcd	11.97 ± 0.57b	12.67 ± 0.08bc	13.57 ± 0.13abc
		ECO ₂	10.90 ± 0.35abc	11.57 ± 0.23abc	11.77 ± 0.75b	16.79 ± 4.33a	13.21 ± 0.25abcd
	T + 4	ACO ₂	10.68 ± 0.29abc	11.16 ± 0.30abcd	16.81 ± 4.86b	13.04 ± 0.42abc	12.54 ± 0.25defg
		ECO ₂	10.55 ± 0.32bc	10.79 ± 0.21cdef	11.47 ± 0.39b	12.76 ± 0.29bc	12.97 ± 0.44bcdef
1084	T	ACO ₂	10.87 ± 0.24abc	11.93 ± 0.33a	12.32 ± 0.54b	12.63 ± 0.60bc	13.02 ± 0.38bcde
		ECO ₂	10.80 ± 0.53abc	10.87 ± 0.41bcdef	11.58 ± 0.50b	12.47 ± 0.22bc	13.84 ± 0.45ab
	T + 4	ACO ₂	10.38 ± 0.52bcd	11.86 ± 0.18a	12.15 ± 0.35a	12.36 ± 0.15c	12.12 ± 0.33efg
		ECO ₂	11.38 ± 0.54ab	11.76 ± 0.41ab	12.35 ± 0.18b	11.19 ± 0.95c	12.57 ± 0.52defg
P _(CL)			*	***	ns	ns	*
P _(T)			ns	ns	ns	*	***
P _(CO2)			ns	ns	ns	ns	ns
P _(CL × T)			ns	ns	ns	ns	ns
P _(CL × CO2)			ns	ns	ns	ns	ns
P _(T × CO2)			ns	ns	ns	ns	ns
P _(CL × T × CO2)			ns	**	ns	ns	ns

Results (values are means ± SE) are shown according to clone identity (n = 16), temperature regime (n = 40), CO₂ concentration (n = 40), and the combined factors (n = 4). Means with letters in common within the same stage and factor (clone, temperature, CO₂, or their interactions) are not significantly different according to the least significant difference (LSD) test (P > 0.05). Probability values (P) for the main effects of clone, P_(CL); temperature, P_(T); CO₂, P_(CO2); and their interactions, P_(CL × T), P_(CL × CO2), P_(T × CO2), and P_(CL × T × CO2). ***P < 0.001; **P < 0.01; *P < 0.05; ns, not significant.

ECO₂ had a lower relative skin mass than ACO₂ at the onset of veraison, but higher 1 and 2 weeks after mid-veraison. At maturity, the T3 clone was the most affected one by the increase in temperature of T + 4, especially when combined with ACO₂.

Malic Acid

The evolution of malic acid concentration throughout the ripening process was not affected by the clone identity, decreasing in a similar manner in all of them until maturity (Figure 2A).

However, at maturity, the 1084 accession had the lowest malic acid levels and CL306 the highest (Figure 2B). Considering all the clones as a whole, T + 4 decreased significantly malic acid from mid-veraison onward with respect to T, while ECO₂ raised acid malic significantly at the onset of veraison and reduced it at maturity compared with ACO₂ (Figure 2A). For all the clones studied, grapes developed under current situation (T/ACO₂) presented significantly higher levels of malic acid at maturity than those developed under climate change conditions (T + 4/ECO₂) (Figure 2B). In the case of 1084, this difference was observed with

TABLE 2 | Relative skin mass (%) in grape berries of the five Tempranillo clones (RJ43, CL306, T3, VN31, and 1084) grown under four temperature/CO₂ regimes: ambient temperature (*T*) or ambient temperature + 4°C (*T* + 4), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂).

			Relative skin mass (%)				
			Onset of veraison	Mid-veraison	1 week after mid-veraison	2 weeks after mid-veraison	Maturity
RJ43			16.76 ± 1.30a	15.60 ± 0.89a	14.02 ± 0.57a	15.02 ± 0.76a	16.50 ± 0.68ab
CL306			15.24 ± 0.66a	14.27 ± 0.40ab	14.73 ± 0.88a	17.02 ± 1.57a	17.51 ± 0.76a
T3			14.68 ± 0.64a	13.76 ± 0.70ab	14.20 ± 0.63a	14.83 ± 1.03a	16.88 ± 0.84a
VN31			15.43 ± 0.53a	13.90 ± 0.75ab	14.79 ± 0.55a	15.62 ± 0.90a	17.72 ± 0.87a
1084			14.57 ± 0.58a	13.24 ± 0.44b	14.35 ± 0.60a	14.81 ± 1.61a	14.41 ± 0.78b
<i>T</i>			15.48 ± 0.40a	14.36 ± 0.37a	13.85 ± 0.31b	13.72 ± 0.32b	16.03 ± 0.52a
<i>T</i> + 4			15.19 ± 0.59a	13.94 ± 0.48a	14.98 ± 0.47a	17.31 ± 1.01a	17.22 ± 0.51a
ACO ₂			16.56 ± 0.57a	13.74 ± 0.46a	13.67 ± 0.42b	14.35 ± 0.77b	16.81 ± 0.55a
ECO ₂			14.11 ± 0.33b	14.57 ± 0.39a	15.17 ± 0.37a	16.56 ± 0.75a	16.48 ± 0.5a
RJ43	<i>T</i>	ACO ₂	17.79 ± 0.71ab	15.33 ± 0.91abc	12.24 ± 0.35c	12.33 ± 0.57c	15.80 ± 1.19bcdef
		ECO ₂	13.75 ± 1.16bcd	15.51 ± 2.80abc	14.52 ± 0.79abc	14.63 ± 0.86bc	16.04 ± 1.72abcdef
	<i>T</i> + 4	ACO ₂	20.68 ± 4.63a	16.15 ± 2.06a	12.66 ± 0.18bc	15.87 ± 1.39bc	17.12 ± 1.93abcdef
		ECO ₂	14.81 ± 0.89bcd	15.41 ± 1.64abc	16.66 ± 1.27a	17.26 ± 2.08abc	17.02 ± 0.80abcdef
CL306	<i>T</i>	ACO ₂	16.64 ± 1.35abc	12.91 ± 0.41abc	13.90 ± 0.29abc	14.07 ± 1.08bc	18.16 ± 2.76abcd
		ECO ₂	14.48 ± 0.44bcd	15.75 ± 0.86ab	12.55 ± 0.74bc	15.70 ± 0.98bc	17.84 ± 1.15abcd
	<i>T</i> + 4	ACO ₂	15.04 ± 1.59bcd	13.94 ± 0.74abc	16.64 ± 3.13a	15.30 ± 1.59bc	18.60 ± 1.64abc
		ECO ₂	14.79 ± 1.80bcd	14.46 ± 0.63abc	15.84 ± 1.35ab	22.58 ± 5.00a	15.60 ± 0.71bcdef
T3	<i>T</i>	ACO ₂	17.56 ± 1.15abc	15.18 ± 1.05abc	14.48 ± 1.75abc	13.10 ± 0.82bc	14.21 ± 1.50def
		ECO ₂	12.05 ± 0.81d	13.18 ± 0.74abc	13.26 ± 0.65abc	13.29 ± 1.53bc	15.10 ± 0.29cdef
	<i>T</i> + 4	ACO ₂	15.17 ± 0.80bcd	12.13 ± 2.36bc	12.47 ± 0.60bc	15.82 ± 3.01bc	20.05 ± 0.91a
		ECO ₂	13.94 ± 0.52bcd	14.54 ± 0.83abc	16.58 ± 0.90a	17.88 ± 2.06abc	18.16 ± 1.95abcd
VN31	<i>T</i>	ACO ₂	17.32 ± 1.59abc	14.92 ± 1.19abc	14.36 ± 0.85abc	13.66 ± 0.93bc	15.99 ± 1.79abcdef
		ECO ₂	14.85 ± 0.75bcd	13.44 ± 0.50abc	14.78 ± 0.53abc	15.82 ± 0.84bc	19.61 ± 1.34ab
	<i>T</i> + 4	ACO ₂	15.43 ± 0.41bcd	11.81 ± 2.22c	13.36 ± 1.07abc	14.09 ± 2.50bc	17.34 ± 2.17abcde
		ECO ₂	14.10 ± 0.65bcd	15.44 ± 1.42abc	16.65 ± 1.42a	19.57 ± 0.95ab	17.94 ± 1.80abcd
1084	<i>T</i>	ACO ₂	15.59 ± 0.67bcd	13.07 ± 0.38abc	12.93 ± 1.14bc	12.34 ± 0.35c	14.10 ± 1.16def
		ECO ₂	14.78 ± 1.10bcd	14.35 ± 1.08abc	15.50 ± 1.45abc	12.75 ± 0.50bc	12.03 ± 1.08f
	<i>T</i> + 4	ACO ₂	14.38 ± 0.92bcd	11.97 ± 0.91bc	13.66 ± 0.95abc	17.12 ± 6.61abc	17.07 ± 1.73abcdef
		ECO ₂	13.55 ± 1.85cd	13.57 ± 0.82abc	15.31 ± 1.14abc	17.02 ± 0.96abc	13.24 ± 0.94ef
<i>P</i> _(CL)			ns	ns	ns	ns	*
<i>P</i> _(T)			ns	ns	*	**	ns
<i>P</i> _(CO₂)			***	ns	**	ns	ns

Results (values are means ± SE) are shown according to the clone (*n* = 16), temperature (*n* = 40), CO₂ concentration (*n* = 40), and the combined factors (*n* = 4). Means with letters in common within the same stage and factor (clone, temperature, CO₂, or their interactions) are not significantly different according to the LSD test (*P* > 0.05). Probability values (*P*) for the main effects of clone, *P*_(CL); temperature, *P*_(T); and CO₂, *P*_(CO₂). ****P* < 0.001; ***P* < 0.01; **P* < 0.05; ns, not significant. All probability values for the interactions of factors [*P*_(CL × T), *P*_(CL × CO₂), *P*_(T × CO₂), and *P*_(CL × T × CO₂)] were statistically not significant (*P* > 0.05).

plants grown at *T* + 4, regardless of the CO₂ regime. Globally, there were no significant interactions among factors.

Sugars and Total Soluble Solids

In general, the level of sugars (glucose and fructose) depended significantly on the clone from mid-veraison onward, being strongly affected by this factor 2 weeks after mid-veraison and at maturity (Figure 3A). Notably, 2 weeks after mid-veraison, the most contrasted clones were 1084 and CL306, with the lowest and highest sugar levels, respectively (Figure 3B). The *T* + 4 treatment increased significantly the sugar concentration 2 weeks after mid-veraison compared with *T*, whereas the atmospheric CO₂ level did not have any effect on this parameter. Nonetheless, there were no significant interactions among the

factors considered. The trends observed for total soluble solids at maturity (Supplementary Figure 2) were quite similar to those observed for the sum of glucose and fructose at this stage (Figure 3), clone 1084 being the one with the lowest TSS levels.

Amino Acids

In general, total amino acid concentration reached the highest values 2 weeks after mid-veraison in clones RJ43, CL306, and VN31, whereas the concentration of amino acid continued increasing until maturity in T3 and 1084 (Figure 4A and Supplementary Tables 1A–E). Amino acid levels were similar among clones throughout berry development, except 2 weeks after mid-veraison, when 1084 had lower levels in comparison with the other clones. At this stage, and considering all the clones,

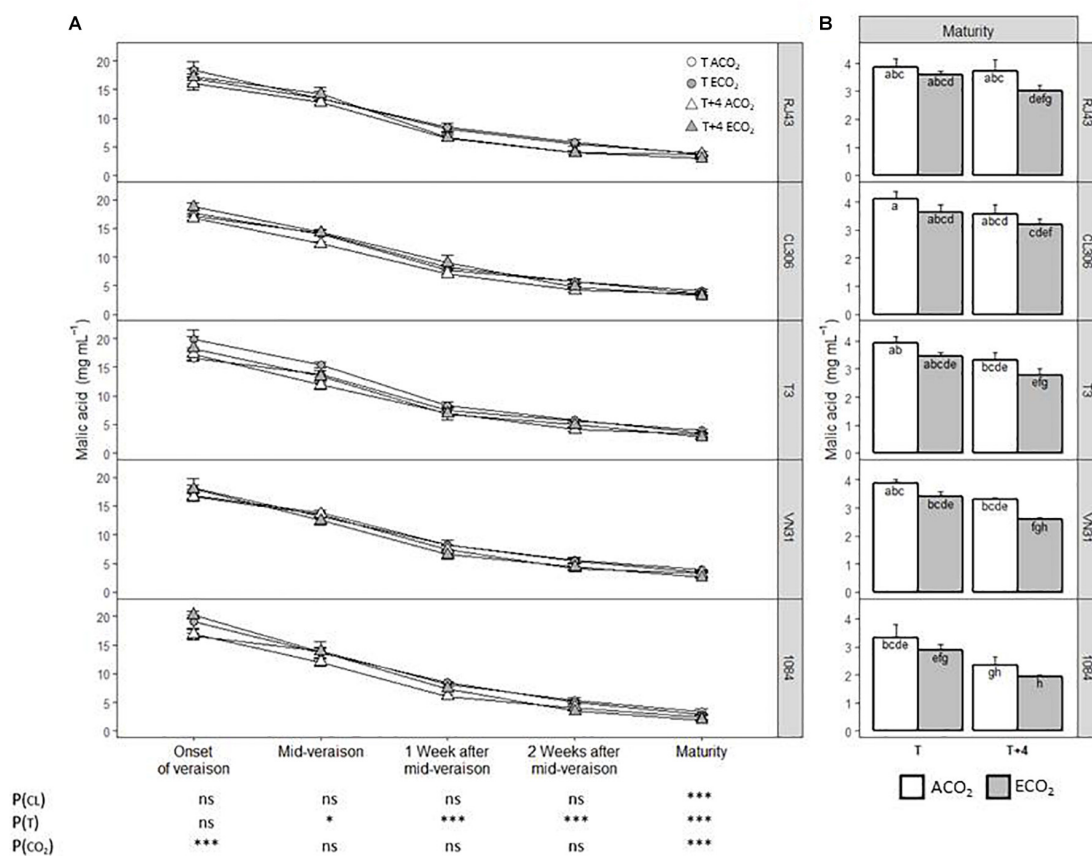


FIGURE 2 | Malic acid concentration of the five Tempranillo clones grown under four temperature/CO₂ regimes: ambient temperature (T) or ambient temperature + 4°C (T + 4), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂). Data (values are means ± SE, n = 4) are presented according to temperature (T or T + 4) and CO₂ regime (ACO₂ or ECO₂) (A) throughout ripening and (B) at maturity. Data are presented according to the temperature (T or T + 4) and CO₂ regime (ACO₂ or ECO₂) and considering each clone individually. Means with letters in common are not significantly different according to the LSD test ($P > 0.05$). Probability values (P) for the main effects of clone, $P_{(CL)}$; temperature, $P_{(T)}$; and CO₂, $P_{(CO_2)}$. *** $P < 0.001$; * $P < 0.05$; ns, not significant. All probability values for the interactions of factors [$P_{(CL \times T)}$, $P_{(CL \times CO_2)}$, $P_{(T \times CO_2)}$, and $P_{(CL \times T \times CO_2)}$] were statistically not significant ($P > 0.05$).

the T + 4 treatment reduced total amino acid concentration compared with the T treatment (from 15.9 ± 1.33 to $12.3 \pm 1.18 \mu\text{mol mL}^{-1}$, respectively, **Supplementary Table 1D**). Also, ECO₂ diminished the amino acid levels with respect to ACO₂ (from 17.1 ± 1.48 to $11.2 \pm 0.82 \mu\text{mol mL}^{-1}$, respectively) 2 weeks after mid-veraison (**Supplementary Table 1D**). At maturity, there were no significant effects of temperature and CO₂ (neither individually nor combined). However, T + 4 tended to reduce the concentration of total amino acids (especially under ACO₂) in all the clones and ECO₂ tended to reduce the amino acid levels of CL306, T3, and VN31 at ambient temperature (**Figure 4B** and **Supplementary Table 1E**).

The relative abundance of the different amino acids varied among clones. Specifically, 1 and 2 weeks after mid-veraison and at maturity, the pyruvate and aspartate derivatives were more abundant in 1084 at the expense of α -ketoglutarate derivatives (except GABA and arginine, which increased) (**Figure 4C** and **Supplementary Tables 1D,E**). Considering all the clones as a whole, ECO₂ significantly reduced the proportion of α -ketoglutarate derivatives (although arginine and GABA tended

to increase in the later stages of the ripening period at ECO₂), increasing the abundance of those originated from aspartate and pyruvate (2 weeks after mid-veraison and at maturity, respectively). Even though there were no significant interactions, there were two exceptions to this effect at maturity, as ECO₂ increased α -ketoglutarate derivatives in the grapes of clones RJ43 and 1084 exposed to T and T + 4, respectively. In addition, the rise in the relative abundance of pyruvate derivatives observed in the ECO₂ treatment at maturity was globally stronger at T + 4 (**Figure 4C** and **Supplementary Table 1E**). Moreover, at the onset of veraison, ECO₂ strongly increased the relative abundance of phenylalanine in all clones and more specifically in CL306 and 1084 when combined with elevated temperature.

Total Skin Anthocyanins

Anthocyanin levels did not differ significantly in the early ripening period among clones, but 2 weeks after mid-veraison and at maturity, the concentration of total anthocyanins was lower in 1084, whereas RJ43 showed the highest values (**Figures 5A,B**). T + 4 had a significant enhancing effect

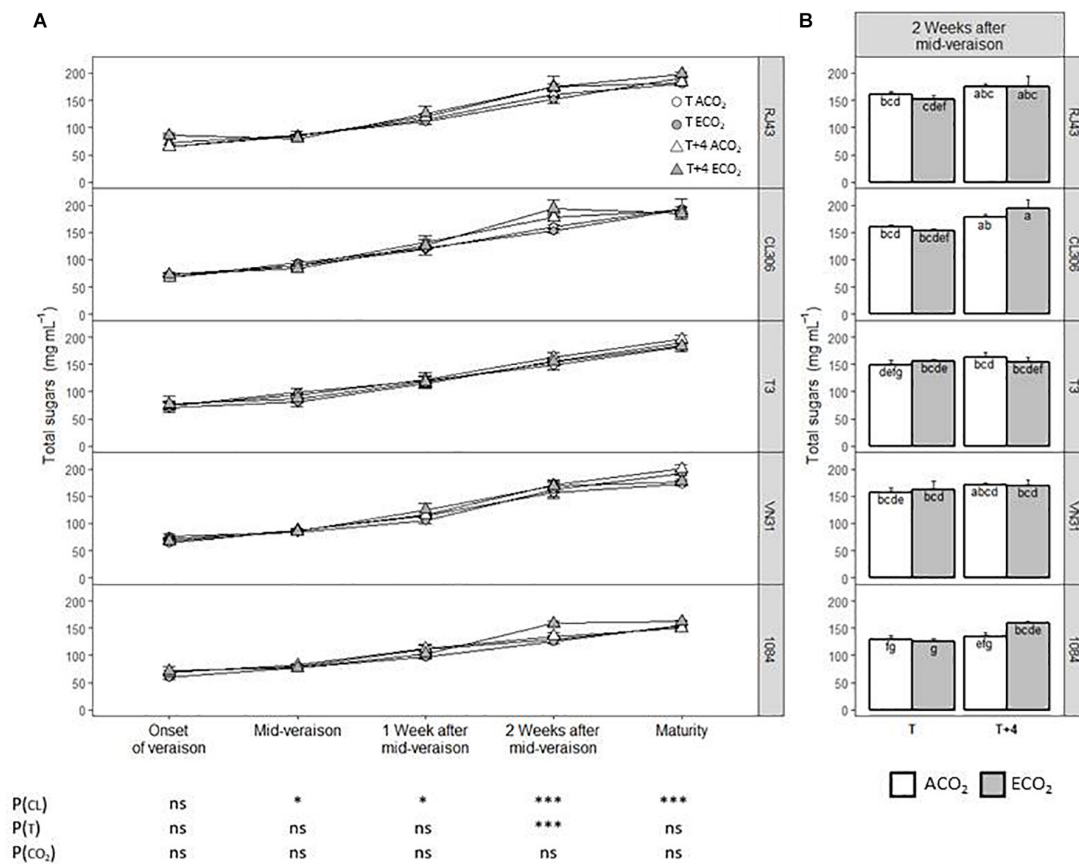


FIGURE 3 | Sugar concentration in berries of the five Tempranillo clones grown under four temperature/CO₂ regimes: ambient temperature (*T*) or ambient temperature + 4°C (*T* + 4), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂). Data are presented according to temperature (*T* or *T* + 4) and CO₂ regime (ACO₂ or ECO₂) and considering each clone individually (values are means ± SE, *n* = 4): **(A)** throughout ripening and **(B)** 2 weeks after mid-veraison. Means with letters in common are not significantly different according to LSD test (*P* > 0.05). Probability values (*P*) for the main effects of clone, *P*_(CL); temperature, *P*_(T); and CO₂, *P*_(CO₂). ****P* < 0.001; **P* < 0.05; ns, not significant. All probability values for the interactions of factors [*P*_(CL × T), *P*_(CL × CO₂), *P*_(T × CO₂), and *P*_(CL × T × CO₂)] were statistically not significant (*P* > 0.05).

on anthocyanins at the onset of veraison and 2 weeks after mid-veraison, regardless of the clone and CO₂ level, this effect disappearing at maturity. ECO₂ increased anthocyanin concentration at the onset of veraison and mid-veraison but reduced the levels 2 weeks after mid-veraison (Figure 5A). At maturity, although there were no significant interactions between factors, the *T* + 4/ECO₂ treatment seemed to have different effects depending on the clone. Whereas, in RJ43, the grapes exposed to *T* + 4/ECO₂ (climate change conditions) had significantly lower anthocyanin levels than those exposed to *T*/ACO₂ (current conditions), in CL306, the concentration of skin anthocyanins increased with climate change conditions (*T* + 4/ECO₂) (Figure 5B).

Total Anthocyanins to TSS Ratio

Clones showed different anthocyanin to TSS ratios at maturity regardless of the temperature and CO₂ regime, RJ43 and T3 having the highest values (Figure 6A). Considering the clones altogether, a significant interaction between temperature and CO₂ was observed (Figure 6B). Thus, the significant decrease of

the ratio between anthocyanins and TSS under ACO₂ induced by *T* + 4, with respect to *T*, disappeared under ECO₂. When the temperature and CO₂ effects were analyzed for each clone independently, the growing conditions showed slightly different effects on the anthocyanin to TSS ratio (Figure 6C). In RJ43 and VN31, the impact of *T* + 4 on the ratio was more evident under ACO₂ conditions. ECO₂ strongly increased the ratio in CL306 plants at *T* + 4, while it did not have any effect at *T*. Finally, neither temperature nor CO₂ had a marked impact on the relationship between anthocyanins and TSS in T3 and 1084.

DISCUSSION

Performance of Clones

Clones showed different characteristics in all the studied parameters. The accession that differed the most from the others studied was 1084. It had an extremely long berry ripening period associated with a lower sugar accumulation rate, not even reaching the optimum sugar levels for wine production. The

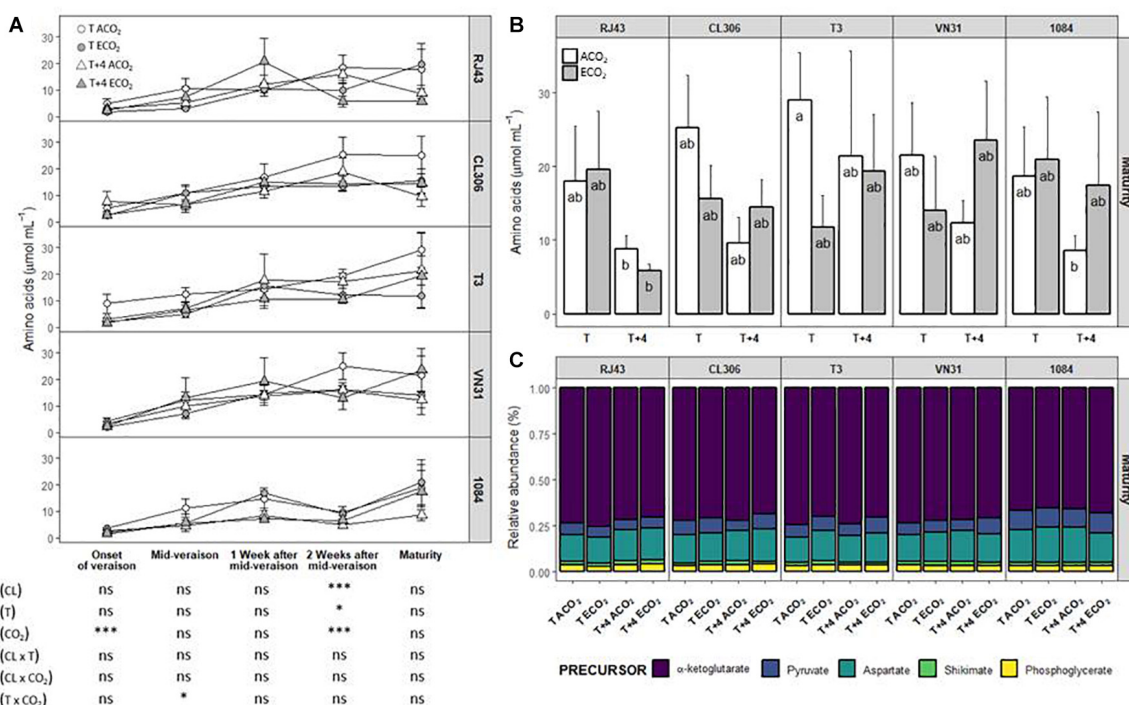


FIGURE 4 | Amino acid concentration in berries of the five Tempranillo clones grown under four temperature/CO₂ regimes: ambient temperature (T) or ambient temperature + 4°C (T + 4), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂). Data are presented according to temperature (T or T + 4) and CO₂ regime (ACO₂ or ECO₂) and considering each clone individually (values are means ± SE, n = 3–4). **(A)** Throughout ripening and **(B)** at maturity. Relative abundance of amino acids **(C)** grouped by their precursor. Means with letters in common are not significantly different according to the LSD test ($P > 0.05$). Probability values (P) for the main effects of clone, $P_{(CL)}$; temperature, $P_{(T)}$; and CO₂, $P_{(CO_2)}$. *** $P < 0.001$; * $P < 0.05$; ns, not significant. All probability values for the interactions of factors [$P_{(CL \times T)}$, $P_{(CL \times CO_2)}$, $P_{(T \times CO_2)}$, and $P_{(CL \times T \times CO_2)}$] were statistically not significant ($P > 0.05$).

1084 accession also had berries with low relative skin mass and presented the lowest values of malic acid at maturity. Despite T3, VN31, and RJ43 having similar berry diameter to 1084 (indicating similar size), their malic acid values were higher than in 1084. This result suggests that the low concentration of malic acid in 1084 was not associated with a dilution effect due to high berry size. After veraison, malate is released from the vacuole and becomes available for catabolism through involvement in gluconeogenesis, respiration through the tricarboxylic acid (TCA) cycle, amino acid interconversions, and the production of secondary compounds such as anthocyanins and flavonols (Ruffner and Kliever, 1975; Ruffner et al., 1976; Ruffner, 1982; Famiani et al., 2000; Ollat et al., 2002; Sweetman et al., 2009). Probably, the longer ripening period of the 1084 accession, already seen in previous experiments (Arrizabalaga et al., 2018), contributed to higher malate breakdown, thus reaching a lower malic acid concentration at maturity. Interestingly, clones differed in the moment to reach the amino acidic maturity, earlier in RJ43, CL306, and VN31 than in T3 and 1084. The amino acid profile at maturity was also different among clones, 1084 showing a reduced proportion of α -ketoglutarate and increased abundance of pyruvate and aspartate derivatives. The higher concentration of isoleucine, leucine, and valine (aromatic precursors) in 1084 might have a positive effect on the final wine aroma (Valdés et al., 2019).

The 1084 accession also presented a lower concentration of skin anthocyanins, compared with the other clones. The results are in agreement with our previous work (Arrizabalaga et al., 2018) and may be related to the slow sugar accumulation rate observed in 1084. Dai et al. (2014), using an experimental system allowing the long-term *in vitro* culture of grape berries, reported an induction in total anthocyanins by rising sugar concentrations in the culture medium, as well as a negative correlation between phenylalanine and total anthocyanin levels. In the present study, phenylalanine was similar in 1084, and even higher 2 weeks after mid-veraison, compared with the other clones. Therefore, the lower anthocyanin accumulation may be a consequence of a limitation in the biosynthetic enzymatic activity rather than to a limitation of its precursor, as suggested by Dai et al. (2014). Also, it is possible that α -ketoglutarate availability is lower in 1084; thus, biosynthetic steps that use α -ketoglutarate as reducing power for anthocyanin accumulation (i.e., hydroxylases) become limiting. Indeed, α -ketoglutarate level was reported to be one of the metabolic drivers of anthocyanin accumulation in grape cells (Soubeyrand et al., 2018). In opposition to the results obtained by Roby and Matthews (2008), the anthocyanin content in berries was not systematically positively correlated to the relative skin mass, except for 1084. These discrepancies may be related to the fact that in our case anthocyanins were measured in dry skin and Roby and Matthews (2008) did it in the whole berry. In

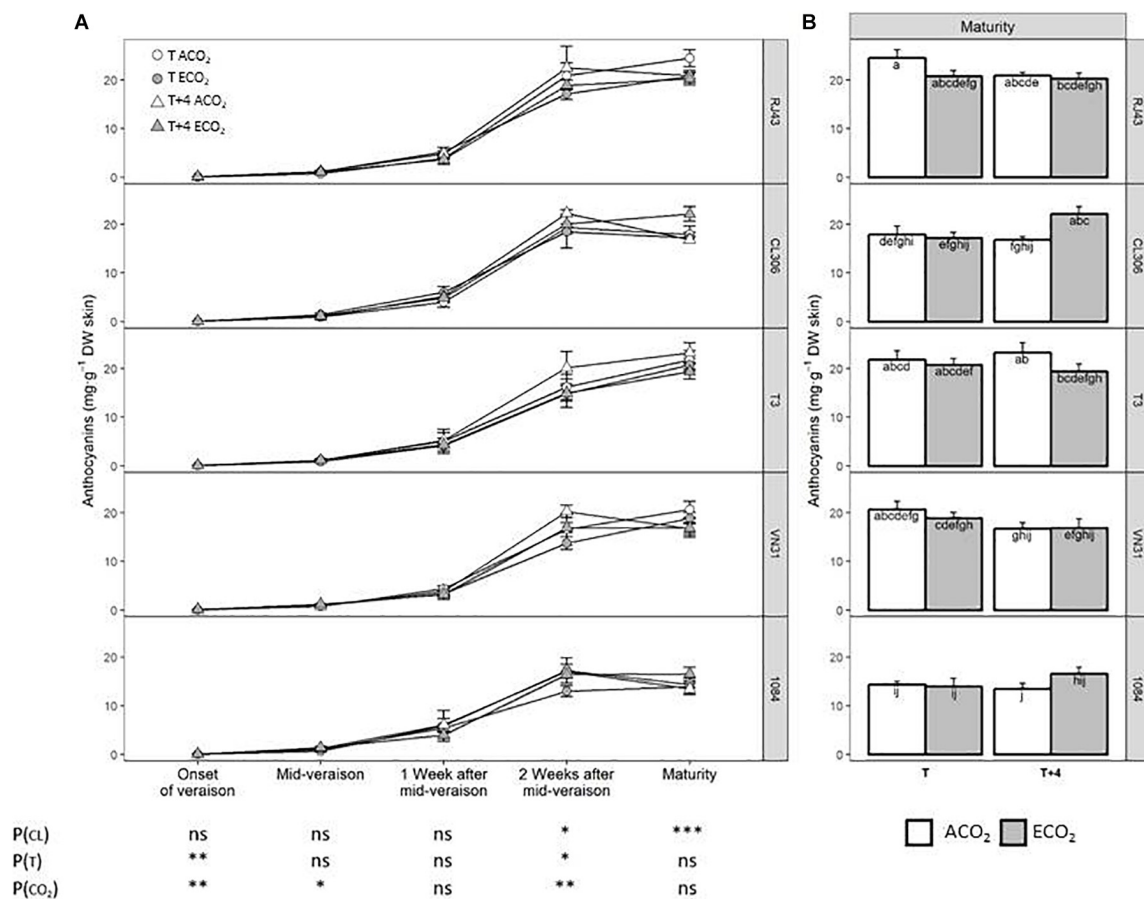


FIGURE 5 | Total anthocyanin concentration in berries of the five Tempranillo clones grown under four temperature/CO₂ regimes: ambient temperature (T) or ambient temperature + 4°C ($T + 4$), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂). Data are presented according to temperature (T or $T + 4$) and CO₂ regime (ACO₂ or ECO₂) and considering each clone individually (values are means \pm SE, $n = 4$). **(A)** Throughout ripening and **(B)** at maturity. Means with letters in common are not significantly different ($P > 0.05$) according to the LSD test. Probability values (P) for the main effects of clone, $P_{(CL)}$; temperature, $P_{(T)}$; and CO₂, $P_{(CO_2)}$. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant. All probability values for the interactions of factors [$P_{(CL \times T)}$, $P_{(CL \times CO_2)}$, $P_{(T \times CO_2)}$, and $P_{(CL \times T \times CO_2)}$] were statistically not significant ($P > 0.05$).

addition, both 1084 and T3 had some of the largest diameters. However, the relative skin mass of this two clones differed significantly, T3 having a higher value and 1084 the lowest. These results indicate a lack of effect of berry size on the relative skin mass already observed by Barbagallo et al. (2011). These berry parameters are important as they are considered to determine the solutes extraction during the maceration process (Walker et al., 2005; Matthews and Nuzzo, 2007; Roby and Matthews, 2008; Barbagallo et al., 2011).

Global Response of Tempranillo Clones to Elevated Temperature

The increment of temperature shortened the elapsed time between mid-veraison and maturity and reduced the size of berries at the end of the ripening process. These results agree with previous studies, in which berries stopped increasing their volume after being heat-treated at mid-veraison and mid-ripening (Kliewer, 1977; Roby and Matthews, 2008; Keller,

2010; Orduña, 2010) and high temperature accelerated the ripening process (Jones and Davis, 2000; Duchêne and Schneider, 2005; Keller, 2010; Arrizabalaga et al., 2018). Given that maturity was determined by the level of TSS (ca. 22°Brix), the reduction of the time to reach this stage in the $T + 4$ treatment indicates a faster and more efficient accumulation of sugars under these conditions. In the present study, the lower berry size in the $T + 4$ may have contributed to the higher concentration of sugars observed. However, the effect of high temperatures on sugar accumulation varies among studies and conditions. Whereas elevated temperature has been reported to enhance sugar accumulation (Jones et al., 2005; Mosedale et al., 2016), in other cases, berry sugar content at harvest was not affected (Lecourieux et al., 2017), and sugar accumulation was stopped (Roby and Matthews, 2008; Greer and Weston, 2010), or even decreased (Carbonell-Bejerano et al., 2013; Greer and Weedon, 2013; Kuhn et al., 2014). These apparently contradictory results may be due to differences in the experimental procedures, which included more

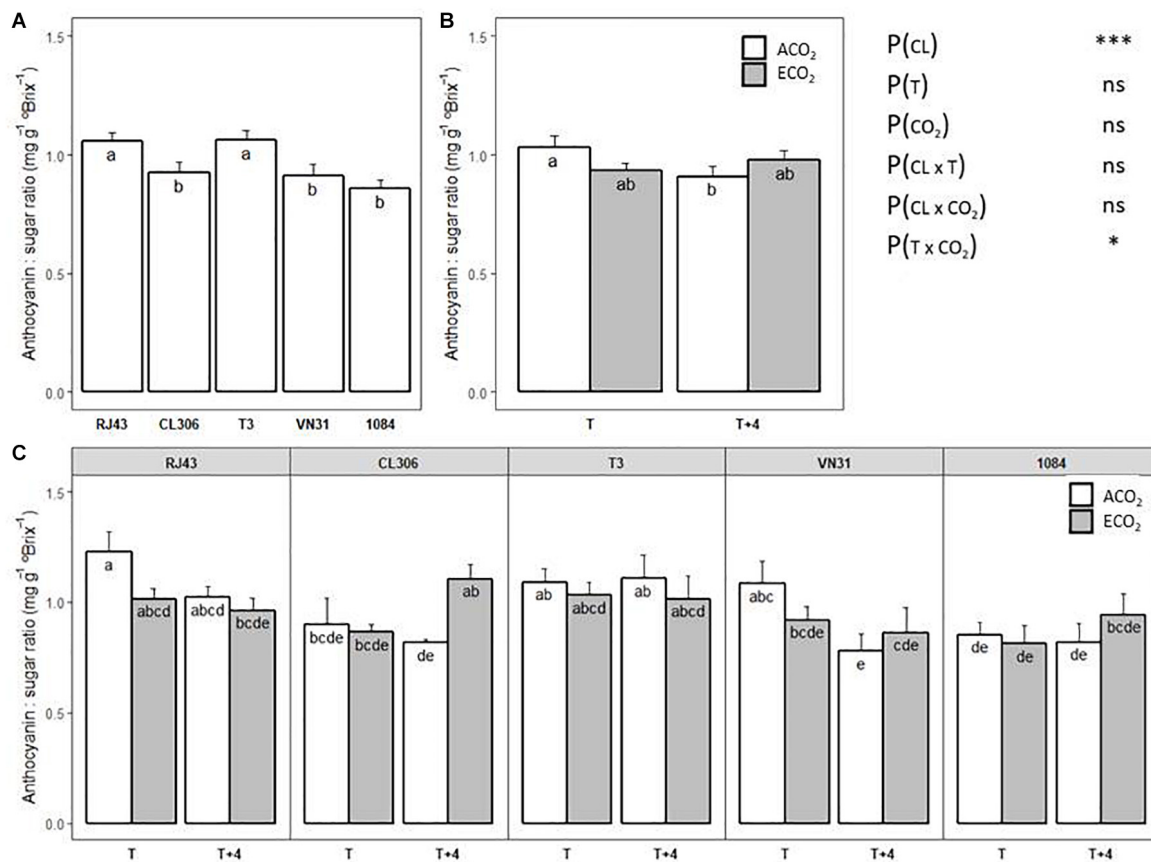


FIGURE 6 | Anthocyanin to TSS ratio at maturity of the five Tempranillo clones grown under four temperature/CO₂ regimes: ambient temperature (*T*) or ambient temperature + 4°C (*T* + 4), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂). Results (values are means ± SE) are presented according to: **(A)** clone identity (*n* = 15–16); **(B)** the temperature (*T* or *T* + 4) and CO₂ regime (ACO₂ or ECO₂) (*n* = 19–20); and **(C)** clone identity, temperature, and CO₂ regime (*n* = 3–4). Means with letters in common within each chart **(A–C)** are not significantly different according to the LSD test (*P* > 0.05). Probability values (*P*) for the main effects of clone, *P*(CL); temperature, *P*(T); and CO₂, *P*(CO₂). ****P* < 0.001; **P* < 0.05; ns, not significant. All probability values for the interactions of factors [*P*(CL × T), *P*(CL × CO₂), *P*(T × CO₂), and *P*(CL × T × CO₂)] were statistically not significant (*P* > 0.05).

extreme temperatures than in the present one, thus reducing photosynthesis and limiting the supply of sugar to the berries (Greer and Weedon, 2013).

It is well established that warm temperatures promote the decrease of organic acid levels in grape berries after mid-veraison, by accelerating malate degradation (Orduña, 2010; Carbonell-Bejerano et al., 2013; Sweetman et al., 2014; Torres et al., 2016). Malic acid respiration is favored by heat, and the genes involved in its transmembrane transport display a marked regulation by temperature (Rienth et al., 2016). In addition, the enhancement of the anaplerotic capacity of the TCA cycle for amino acid biosynthesis by elevated temperatures has also been suggested (Sweetman et al., 2014; Lecourieux et al., 2017). In our work, although malic acid degradation was promoted by *T* + 4 from mid-veraison onward, the concentration of total amino acids tended to decrease in this treatment compared with *T*, and changes in the proportion α-ketoglutarate, pyruvate, or aspartate amino acid derivatives were not so obvious. These results may indicate that under the temperature conditions assayed, 4°C of difference between *T* and *T* + 4 vs. increases

of 8 (Lecourieux et al., 2017) and 10°C (Sweetman et al., 2014) in the mentioned studies, the anaplerotic capacity of the TCA cycle for amino acid biosynthesis may not be markedly increased. Other pathways such as gluconeogenesis may have played a more important role in malate degradation, thus contributing to the differences in sugar accumulation observed between temperature regimes. Despite the limited changes in the amino acid profile, an increased proportion of proline and arginine was observed under high temperature, as previously reported by other authors (Lecourieux et al., 2017; Torres et al., 2017). Proline has a protective role in plants against abiotic stresses, including elevated temperature, whereas arginine is an important source of nitrogen during winemaking (Garde-Cerdán et al., 2011), despite being a precursor of putrescine, a compound with negative effects on human health (Guo et al., 2015).

Considering the clones altogether, high temperature significantly increased the concentration of anthocyanins 2 weeks after mid-veraison, but it did not affect the final anthocyanin levels at maturity. These results do not agree with previous studies that demonstrate that high temperature during ripening

had a negative impact on anthocyanin biosynthesis in berries by acting on the correspondent enzymes and transcription factors (Yamane et al., 2006; Mori et al., 2007; Rienth et al., 2014, 2016; Lecourieux et al., 2017). In those experiments, the expression of genes related to flavonoid biosynthesis in grape skins was found to be repressed by high temperatures, especially genes coding for the key enzyme of the phenylpropanoid pathway, the phenylalanine-ammonia-lyase (PAL) or *MYB* transcription factors, which control anthocyanin biosynthesis. However, the repression of *VvMYBA1* by high temperature described by Yamane et al. (2006) was not confirmed by other authors (Lijavetzky et al., 2012). Besides, in addition to a lower anthocyanin biosynthesis, some authors have reported increases in anthocyanin degradation due to high temperature (Mori et al., 2007). Accordingly, the increase in anthocyanin concentration induced by $T + 4$ observed 2 weeks after mid-veraison in our study was not detected at maturity ($P(T) > 0.05$), which might be caused by an earlier degradation of anthocyanins under elevated temperature.

Anthocyanin levels have also been reported to be differentially affected by temperature in different cultivars (Downey et al., 2006). Total anthocyanins decreased with high temperature in Merlot (Spayd et al., 2002; Tarara et al., 2008), Malbec (de Rosas et al., 2017), Pione (Azuma et al., 2012), Cabernet Sauvignon (Mori et al., 2007; Lecourieux et al., 2017; Wu et al., 2019), and Muscat Hamburg (Carbonell-Bejerano et al., 2013), while the final concentration of anthocyanins increased in Merlot by reducing the day temperature oscillations (Cohen et al., 2008). In the case of Tempranillo, Kizildeniz et al. (2018), in a 3-year experiment under similar conditions to this study, did not observe significant effects of high temperature on final anthocyanin levels. Also, differences in the response of anthocyanins to temperature were detected among different clones of Tempranillo, not all the clones being equally affected (Torres et al., 2017; Arrizabalaga et al., 2018). Furthermore, some authors also reported that the stage of berry growth at which heat treatment was applied could modulate the plant response in terms of anthocyanin content (Lecourieux et al., 2017; Gouot et al., 2019a,b).

Global Response of Tempranillo Clones to Elevated CO₂

High atmospheric CO₂ concentration slightly hastened grape phenology, but only from fruit set to mid-veraison, as reported by Martínez-Lüscher et al. (2016a) for red and white Tempranillo. Regarding fruit composition, the increase in malic acid concentration of grapes exposed to ECO₂ at the onset of veraison may indicate an enhancement of the organic acid biosynthesis at early berry development stages. These differences disappeared in later stages, when the degradation of malic acid took place, and the grapes ripened under ECO₂ conditions reaching even lower malic acid levels at maturity than those grown at ACO₂, possibly because of an accelerated breakdown. Similarly, Bindi et al. (2001), in a field experiment using a free air CO₂ enrichment (FACE) facility, observed that organic acid components were positively affected by increases in CO₂ concentration at the

middle of the ripening season, an effect that almost completely vanished at maturity. On the other hand, ECO₂ significantly decreased the amino acid concentration at the onset of veraison and 2 weeks after mid-veraison. Elevated CO₂ may induce a priority in the storage of nitrogen-free compounds (i.e., carbohydrates) compared with nitrogen-containing compounds, such as amino acids and proteins (Myers et al., 2014). In our case, the concentration of sugars in the berries was not significantly altered by ECO₂ at these stages, so the decrease in amino acids could be a consequence of the inhibition of N assimilation as reported in different plant species (Serret et al., 2018). Similarly, in a previous study with the same clones and under similar growth conditions, berries exposed to ECO₂ showed lower N concentration in the berries (Arrizabalaga-Arriazu et al., 2020). The differences observed up to 2 weeks after veraison in the present study decreased at maturity, when the ECO₂ treatment showed only a slightly lower total amino acid concentration. At this stage, even though the relative abundance of some individual amino acids was significantly affected by ECO₂ conditions, the proportions of amino acid families according to their precursor were little modified, except a slightly reduced proportion of α -ketoglutarate derivatives, at the expense of those originated from pyruvate. The significant decrease in the concentration of glutamine under ECO₂ may involve a limitation for yeast growth during fermentation since glutamine, together with arginine, is one of the mayor sources of N for yeast (Zoecklein et al., 1999).

Taking into consideration the clones altogether, plants grown at ECO₂ presented an increased concentration of total grape skin anthocyanins at the beginning of the ripening period, which may indicate a slight hastening in the synthesis of these compounds in this treatment. In contrast, 2 weeks after mid-veraison, ECO₂ plants had lower anthocyanin concentration than ACO₂, these differences disappearing at maturity. These results do not agree with the observations of other authors, who reported no effect (Gonçalves et al., 2009) or a rising effect of CO₂ on anthocyanins at maturity (Kizildeniz et al., 2015). In the same way, studies on the effects of elevated CO₂ on the anthocyanin biosynthetic pathway in different plant species report contradictory results. For example, in strawberry and table grapes, elevated CO₂ levels decreased anthocyanin content by decreasing the expression of genes involved in the phenylpropanoid metabolism, especially the one coding the PAL enzyme (Sanchez-Ballesta et al., 2007; Li et al., 2019). In contrast, increased anthocyanin content and stimulated enzyme activity have been observed in ginger and *Labisia pumila* under elevated CO₂ (Ghasemzadeh et al., 2012; Jaafar et al., 2012).

Response of Clones to Combined Elevated Temperature and CO₂

The analysis of the combined effects of high temperature and elevated CO₂ on the parameters analyzed indicates low interactive effects of these environmental factors on sugar accumulation and, thus, in the length of the ripening period, as ECO₂ did not modify the hastening effect induced by $T + 4$. Conversely, the increase in temperature and CO₂ showed additive effects enhancing malic acid degradation, a phenomenon

especially marked in the 1084 accession (probably due to its longer exposure to these conditions), but less evident in RJ43 and CL306. Such reduction of malic acid content and its impact on must acidity should certainly affect wine production, not only for its contribution to sourness and organoleptic properties but also because of its influence on wine microbiological stability. In addition, it might make the winemaking process more expensive in the future due to the need of acidifying the must for achieving a proper fermentation (Keller, 2010). The combined application of elevated temperature and high CO₂ did not seem to have a marked impact on the total level of amino acids at maturity, although a tendency to decrease the amino acid concentrations was observed as reported by Martínez-Lüscher et al. (2016a). This effect was more evident 2 weeks after mid-veraison, $T + 4$ and ECO₂ significantly reducing their concentration. These results suggest an impact of climate change delaying the amino acidic maturity of grapes, which seemed to be reached some weeks earlier in the T/ACO_2 treatment. Amino acids (excluding proline and hydroxyproline) are very important components of the yeast assimilable nitrogen (YAN), thus having implication for must fermentation. A decrease of total amino acids level could involve a lower N availability during the fermentation process. In the same way, a reduction in the concentration of shikimate and phosphoglycerate derivatives may have implications in the organoleptic properties of the wine produced from these grapes, since the shikimate route is involved in the biosynthesis of aromatic amino acids (tyrosine and phenylalanine) and the phenylpropanoid pathway (phenylalanine).

As for amino acids, combined elevated temperature and CO₂ did not significantly impact anthocyanin concentration at maturity when considering all the clones as a whole. However, the observed increase due to high temperature 2 weeks after veraison was offset by combination with elevated CO₂. Interestingly, a significant interaction between temperature and CO₂ was observed for the relationship between anthocyanin and sugar levels: the temperature-induced decrease in this ratio under ACO₂ was not observed under ECO₂. This result suggests that in a future climate change scenario, elevated CO₂ may, at least partially, mitigate the negative impact of high temperature on the imbalance between sugars and anthocyanins in ripe berries.

In addition, regarding the anthocyanin content, there was a wide range of responses of the clones studied to climate change conditions. Total anthocyanin concentration at maturity was impacted by combined elevated temperature and CO₂, showing a decrease in RJ43, T3, and VN31 (stronger in the former), despite their difference in the reproductive cycle length (defined as intermediate, short, and long, respectively). Conversely, combined elevated temperature and CO₂ increased anthocyanin content in CL306 (characterized as a short reproductive cycle clone) and in 1084 (long reproductive cycle). Moreover, while in RJ43 and VN31 climate change conditions ($T + 4/ECO_2$) markedly reduced the anthocyanin to sugar ratio with respect to the current conditions (T/ACO_2) (differences statistically significant in RJ43), the balance between these two compounds was less affected in CL306, T3, and 1084. Grapevine is characterized by a pronounced sensitivity toward the environment, and the metabolic composition of

the berries has been reported to show a broad phenotypic plasticity, offering advantages such as the range of different wines that can be produced from the same cultivar and the adaptation of existing cultivars to different growing regions (Keller, 2010). The present results suggest that clones of the same variety may perform differently under climate change conditions, some, such as RJ43, showing greater variation and others, such as 1084, offering more consistency. Unfortunately, we cannot directly associate these different performances with the length of their grape development period as we hypothesized originally, at least as far as anthocyanins and anthocyanin to sugar ratio are concerned. In this line, although 1084, with the longest length of the reproductive cycle, was one of the least affected in terms of anthocyanin and sugar balance, other clones that exhibited an early maturity (CL306 and T3) were not so affected either. A recent study has demonstrated differences in the berry phenotypic plasticity between grapevine varieties in response to changes in environmental conditions, Cabernet Sauvignon being less dependent on growth conditions, thus showing a limited transcriptomic plasticity associated with epigenetic regulation (Dal Santo et al., 2018). These authors also concluded that within-cultivar diversity may modulate gene expression in response to environmental cues. We suggest that clones within the same variety can also exhibit different degrees of phenotypic plasticity for grape composition and, consequently, different capacities of adaptation to climate change conditions. Recently, Tortosa et al. (2019) have reported that some clones of Tempranillo variety can perform better water use efficiency than others depending of the water availability conditions. Although the results obtained need to be considered in the light of the limitations of the study (i.e., 1 year experiment, potted plants, and controlled conditions), and they should be validated with studies in the field, the differences observed point toward the usefulness of the exploitation of the grapevine genotypic diversity in order to optimize the genotype–environment interaction and the adaptation of traditional varieties to the foreseen climate scenarios.

CONCLUSION

The projected increases in atmospheric CO₂ concentration and in average air temperature advanced grape maturity, reducing the elapsed time between fruit set and mid-veraison and between mid-veraison and maturity, respectively. High temperature hastened berry ripening, sugar accumulation, and malic acid breakdown, especially when combined with elevated CO₂. In contrast, climate change conditions seemed to delay amino acidic maturity. Even though the increase of temperature and high CO₂ concentration (both individually and combined) did not affect anthocyanin concentration, the clones studied showed different values for this parameter. The reduction of the ratio between anthocyanins and TSS under $T + 4$ conditions was partially mitigated by ECO₂. Additionally, the study reveals the existence of a differential response of Tempranillo clones to the projected future temperature and CO₂ levels in terms of grape composition.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

FM, GH, IP, and JI: conceptualization. GH and IP: methodology. MA-A: investigation. JI: resources, project administration, and funding acquisition. GH, IP, and MA-A: data curation and writing – original draft preparation. EG, FM, GH, IP, JI, and MA-A: writing – review and editing. GH, IP, and JI: supervision. All authors approved the manuscript for publication.

FUNDING

This work was supported by the Ministerio de Economía y Competitividad of Spain (AGL2014-56075-C2-1-R), Fundación Universitaria de Navarra (2018), European Union (Erasmus+ grant to MA-A), Aquitaine Regional Council (AquiMOB grant to MA-A), and Asociación de Amigos de la Universidad de Navarra (doctoral grant to MA-A). This work also supported by the metaprogramme Adaptation of Agriculture and Forests to Climate Change (AAFCC) of the French National Institute for Agriculture,

Food and Environment (INRAE), in the frame of LACCAGE2-21 project.

ACKNOWLEDGMENTS

Special thanks to M. Oyarzun, A. Urdiain, H. Santesteban, C. Renaud, and C. Bonnet for their excellent technical assistance, and E. García-Escudero, J. M. Martínez-Zapater, E. Baroja (ICVV), J. F. Cibrain (EVENA), and R. García (Vitis Navarra) for the selection of clones and for providing the plant material to do the experiments.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.603687/full#supplementary-material>

Supplementary Figure 1 | Daily minimum and maximum temperatures recorded in the modules of ambient temperature (T) and ambient temperature + 4°C (T + 4) of the TGGs during the experiment.

Supplementary Figure 2 | Total soluble solids concentration at maturity in berries of the five Tempranillo clones grown under four temperature/CO₂ regimes: ambient temperature (T) or ambient temperature + 4°C (T + 4), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂). Data are presented according to the temperature (T or T + 4) and CO₂ regime (ACO₂ or ECO₂) and considering each clone individually (values are means ± SE, n = 4). Means with letters in common are not significantly different according to LSD test (P > 0.05).

REFERENCES

- Acevedo de la Cruz, A., Hilbert, G., Rivière, C., Mengin, V., Ollat, N., Bordenave, L., et al. (2012). Anthocyanin identification and composition of wild *Vitis* spp. accessions by using LC-MS and LC-NMR. *Analyt. Chim. Acta* 732, 145–152. doi: 10.1016/j.aca.2011.11.060
- Arrizabalaga, M., Morales, F., Oyarzun, M., Delrot, S., Gomès, E., Irigoyen, J. J., et al. (2018). Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. *Plant Sci.* 267, 74–83. doi: 10.1016/j.plantsci.2017.11.009
- Arrizabalaga-Arriazu, M., Morales, F., Irigoyen, J. J., Hilbert, G., and Pascual, I. (2020). Growth performance and carbon partitioning of grapevine Tempranillo clones under simulated climate change scenarios: elevated CO₂ and temperature. *J. Plant Physiol.* 252:153226. doi: 10.1016/j.jplph.2020.153226
- Azuma, A., Yakushiji, H., Koshita, Y., and Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta* 236, 1067–1080. doi: 10.1007/s00425-012-1650-x
- Barbagallo, M. G., Guidoni, S., and Hunter, J. J. (2011). Berry size and qualitative characteristics of *Vitis vinifera* L. cv. Syrah. *S. Afr. J. Enol. Viticult.* 32, 129–136. doi: 10.21548/32-1-1372
- Bindi, M., Fibbi, L., and Miglietta, F. (2001). Free Air CO₂ Enrichment (FACE) of grapevine (*Vitis vinifera* L.): II. Growth and quality of grape and wine in response to elevated CO₂ concentrations. *Eur. J. Agron.* 14, 145–155. doi: 10.1016/S1161-0301(00)00093-9
- Bobeca, N., Poni, S., Hilbert, G., Renaud, C., Gomès, E., Delrot, S., et al. (2015). Differential responses of sugar, organic acids and anthocyanins to source-sink modulation in Cabernet Sauvignon and Sangiovese grapevines. *Front. Plant Sci.* 6:382. doi: 10.3389/fpls.2015.00382
- Carbonell-Bejerano, P., Royo, C., Mauri, N., Ibáñez, J., and Zapater, J. M. M. (2019). “Somatic variation and cultivar innovation in grapevine,” in *Advances in Grape and Wine Biotechnology*, eds A. Morata and I. Loira (London: IntechOpen), 1–22. doi: 10.5772/intechopen.86443
- Carbonell-Bejerano, P., Santa María, E., Torres-Pérez, R., Royo, C., Lijavetzky, D., Bravo, G., et al. (2013). Thermotolerance responses in ripening berries of *Vitis vinifera* L. cv Muscat Hamburg. *Plant Cell Physiol.* 54, 1200–1216. doi: 10.1093/pcp/ptc071
- Cibrián, F., Jimeno, K., Sagüés, A., Rodríguez, M., Abad, J., Martínez, M. C., et al. (2018). TempraNA?: tempranillos con matrícula. *Navarra Agraria* 229, 12–20.
- Cohen, S. A., and Michaud, D. P. (1993). Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Anal. Biochem.* 211, 279–287. doi: 10.1006/abio.1993.1270
- Cohen, S. D., Tarara, J. M., and Kennedy, J. A. (2008). Assessing the impact of temperature on grape phenolic metabolism. *Analyt. Chim. Acta* 621, 57–67. doi: 10.1016/j.aca.2007.11.029
- Conde, C., Silva, P., Fontes, N., Dias, A., Tavares, R., Sousa, M., et al. (2007). Biochemical changes throughout grape berry development and fruit and wine quality. *Food* 1, 1–22.
- Cunha, J., Santos, M. T., Carneiro, L. C., Feveireiro, P., and Eiras-Dias, J. E. (2009). Portuguese traditional grapevine cultivars and wild vines (*Vitis vinifera* L.) share morphological and genetic traits. *Genet. Resour. Crop Evol.* 56, 975–989. doi: 10.1007/s10722-009-9416-4
- Dai, Z. W., Meddar, M., Renaud, C., Merlin, I., Hilbert, G., Delrot, S., et al. (2014). Long-term in vitro culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. *J. Exp. Bot.* 65, 4665–4677. doi: 10.1093/jxb/ert489

- Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., et al. (2018). Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G × E) on the berry transcriptome. *Plant J.* 93, 1143–1159. doi: 10.1111/tpj.13834
- de Rosas, I., Ponce, M. T., Malovini, E., Deis, L., Cavanaro, B., and Cavanaro, P. (2017). Loss of anthocyanins and modification of the anthocyanin profiles in grape berries of Malbec and Bonarda grown under high temperature conditions. *Plant Sci.* 258, 137–145. doi: 10.1016/j.plantsci.2017.01.015
- Downey, M. O., Dokoozlian, N. K., and Krstic, M. P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *Am. J. Enol. Viticult.* 57, 257–268.
- Duchêne, E., Huard, F., Dumas, V., Schneider, C., and Merdinoglu, D. (2010). The challenge of adapting grapevine varieties to climate change. *Clim. Res.* 41, 193–204. doi: 10.3354/cr00850
- Duchêne, E., and Schneider, C. (2005). Grapevine and climatic changes: a glance at the situation in Alsace. *Agron. Sustain. Dev.* 25, 93–99. doi: 10.1051/agro:2004057
- Dusenge, M. E., Duarte, A. G., and Way, D. A. (2019). Plant carbon metabolism and climate change: elevated CO₂ and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytol.* 221, 32–49. doi: 10.1111/nph.15283
- EVENA (2009). *Evaluación de Clones Comerciales de seis Variedades de vid en Navarra. 1995-2005*. Pamplona: Desarrollo Rural y Medio Ambiente Gobierno de Navarra.
- Famiani, F., Walker, R. P., Tecs, L., Chen, Z., Proietti, P., and Leegood, R. C. (2000). An immunohistochemical study of the compartmentation of metabolism during the development of grape (*Vitis vinifera* L.) berries. *J. Exper. Bot.* 51, 675–683. doi: 10.1093/jxb/51.345.675
- Garde-Cerdán, T., Lorenzo, C., Martínez-Gil, A. M., Lara, J. F., Pardo, F., and Salinas, M. R. (2011). “Evolution of nitrogen compounds during grape ripening from organic and non-organic monastrell-nitrogen consumption and volatile formation in alcoholic fermentation,” in *Research in Organic Farming*, ed. R. Nekkoul (London: IntechOpen).
- Ghasemzadeh, A., Jaafar, H. Z. E., Karimi, E., and Ibrahim, M. H. (2012). Combined effect of CO₂ enrichment and foliar application of salicylic acid on the production and antioxidant activities of anthocyanin, flavonoids and isoflavonoids from ginger. *BMC Complement. Altern. Med.* 12:229. doi: 10.1186/1472-6882-12-229
- Gonçalves, B., Falco, V., Moutinho-Pereira, J., Bacelar, E., Peixoto, F., and Correia, C. (2009). Effects of elevated CO₂ on grapevine (*Vitis vinifera* L.): volatile composition, phenolic content, and in vitro antioxidant activity of red wine. *J. Agric. Food Chem.* 57, 265–273. doi: 10.1021/jf8020199
- Gouot, J. C., Smith, J., Holzapfel, B., and Barril, C. (2019a). Single and cumulative effects of whole-vine heat events on Shiraz berry composition. *OENO One* 53, 171–187. doi: 10.20870/oeno-one.2019.53.2.2392
- Gouot, J. C., Smith, J. P., Holzapfel, B. P., Walker, A. R., and Barril, C. (2019b). Grape berry flavonoids: a review of their biochemical responses to high and extreme high temperatures. *J. Exp. Bot.* 70, 397–423. doi: 10.1093/jxb/ery392
- Greer, D. H., and Weedon, M. M. (2013). The impact of high temperatures on *Vitis vinifera* cv. Semillon grapevine performance and berry ripening. *Front. Plant Sci.* 4:491. doi: 10.3389/fpls.2013.00491
- Greer, D. H., and Weston, C. (2010). Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Funct. Plant Biol.* 37:206. doi: 10.1071/fp09209
- Guo, Y. Y., Yang, Y. P., Peng, Q., and Han, Y. (2015). Biogenic amines in wine: a review. *Intern. J. Food Sci. Technol.* 50, 1523–1532. doi: 10.1111/ijfs.12833
- Habran, A., Commisso, M., Helwi, P., Hilbert, G., Negri, S., Ollat, N., et al. (2016). Roostocks/Scion/Nitrogen interactions affect secondary metabolism in the grape berry. *Front. Plant Sci.* 7:1134. doi: 10.3389/fpls.2016.01134
- Hilbert, G., Tamsamani, H., Bordenave, L., Pedrot, E., Chaher, N., Cluzet, S., et al. (2015). Flavonol profiles in berries of wild *Vitis* accessions using liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance spectrometry. *Food Chem.* 169, 49–58. doi: 10.1016/j.foodchem.2014.07.079
- Ibáñez, J., Carreño, J., Yuste, J., and Martínez-Zapater, J. M. (2015). “Grapevine breeding and clonal selection programmes in Spain,” in *Grapevine Breeding Programs for the Wine Industry*, ed. A. Reynolds (Amsterdam: Elsevier Ltd), 183–209. doi: 10.1016/B978-1-78242-075-0.00009-0
- IPCC (2013). “Climate change 2013: the physical science basis,” in *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, eds T. Stocker, D. Qin, G. Plattner, M. Tignor, S. Allen, J. Boschung, et al. (New York, NY: Cambridge University Press).
- IPCC (2014). “Climate change 2014: mitigation of climate change,” in *Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, eds O. Edenhofer, Y. Pichs-Madruga, E. Sokona, S. Farahani, K. Kadner, I. Seyboth, et al. (New York, NY: Cambridge University Press), doi: 10.1017/CBO9781107415416
- Jaafar, H. Z. E., Ibrahim, M. H., and Karimi, E. (2012). Phenolics and flavonoids compounds, phenylalanine ammonia lyase and antioxidant activity responses to elevated CO₂ in *Labisia pumila* (Myrsinaceae). *Molecules* 17, 6331–6347. doi: 10.3390/molecules17066331
- Jones, G. V., and Davis, R. E. (2000). Climate influences on grapevine phenology grape composition, and wine production and quality for Bordeaux, France. *Am. J. Enol. Viticult.* 51, 249–261.
- Jones, G. V., White, M. A., Cooper, O. R., and Storchmann, K. (2005). Climate change and global wine quality. *Clim. Chang.* 73, 319–343. doi: 10.1007/s10584-005-4704-4702
- Keller, M. (2010). Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. *Austr. J. Grape Wine Res.* 16, 56–69. doi: 10.1111/j.1755-0238.2009.00077.x
- Kizildeniz, T., Mekni, I., Santesteban, H., Pascual, I., Morales, F., and Irigoyen, J. J. (2015). Effects of climate change including elevated CO₂ concentration, temperature and water deficit on growth, water status, and yield quality of grapevine (*Vitis vinifera* L.) cultivars. *Agric. Water Manag.* 159, 155–164. doi: 10.1016/J.AGWAT.2015.06.015
- Kizildeniz, T., Pascual, I., Irigoyen, J. J., and Morales, F. (2018). Using fruit-bearing cuttings of grapevine and temperature gradient greenhouses to evaluate effects of climate change (elevated CO₂ and temperature, and water deficit) on the cv. red and white Tempranillo. Yield and must quality in three consec. *Agric. Water Manag.* 202, 299–310. doi: 10.1016/j.agwat.2017.12.001
- Kliever, W. M. (1977). Effect of high temperatures during the bloom-Set period on fruit-Set, ovule fertility, and berry growth of several grape cultivars. *Am. J. Enol. Vitic.* 28, 215–222.
- Kuhn, N., Guan, L., Dai, Z. W., Wu, B.-H., Lauvergeat, V., Gomès, E., et al. (2014). Berry ripening: recently heard through the grapevine. *J. Exp. Bot.* 65, 4543–4559. doi: 10.1093/jxb/ert395
- Lecourieux, D., Kappel, C., Claverol, S., Pieri, P., Feil, R., Lunn, J., et al. (2020). Proteomic and metabolomic profiling underlines the stage- and time-dependent effects of high temperature on grape berry metabolism. *J. Integrat. Plant Biol.* 62, 1132–1158. doi: 10.1111/jipb.12894
- Lecourieux, F., Kappel, C., Pieri, P., Charon, J., Pillet, J., Hilbert, G., et al. (2017). Dissecting the biochemical and transcriptomic effects of a locally applied heat treatment on developing Cabernet Sauvignon grape berries. *Front. Plant Sci.* 8:53. doi: 10.3389/fpls.2017.00053
- Leibar, U., Pascual, I., Morales, F., Aizpurua, A., and Unamunzaga, O. (2016). Grape yield and quality responses to simulated year 2100 expected climatic conditions under different soil textures. *J. Sci. Food Agric.* 97, 2633–2640. doi: 10.1002/jsfa.8086
- Li, D., Zhang, X., Li, L., Aghdam, M. S., Wei, X., Liu, J., et al. (2019). Elevated CO₂ delayed the chlorophyll degradation and anthocyanin accumulation in postharvest strawberry fruit. *Food Chem.* 285, 163–170. doi: 10.1016/j.foodchem.2019.01.150
- Lijavetzky, D., Carbonell-Bejerano, P., Grimplet, J., Bravo, G., Flores, P., Fenoll, J., et al. (2012). Berry flesh and skin ripening features in *Vitis vinifera* as assessed by transcriptional profiling. *PLoS One* 7:e39547. doi: 10.1371/journal.pone.0039547
- Martínez-Lüscher, J., Kizildeniz, T., Vuëtiæ, V., Dai, Z., Luedeling, E., van Leeuwen, C., et al. (2016a). Sensitivity of grapevine phenology to water availability, temperature and CO₂ concentration. *Front. Environ. Sci.* 4:48. doi: 10.3389/fenvs.2016.00048
- Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascual, I., and Gomès, E. (2016b). Ultraviolet-B alleviates the uncoupling effect of elevated CO₂ and increased temperature on grape berry (*Vitis vinifera* cv. Tempranillo)

- anthocyanin and sugar accumulation. *Austr. J. Grape Wine Res.* 22, 87–95. doi: 10.1111/ajgw.12213
- Matthews, M. A., and Nuzzo, V. (2007). Berry size and yield paradigms on grapes and wines quality. *Acta Hort.* 754, 423–436. doi: 10.17660/ActaHortic.2007.754.56
- Morales, F., Pascual, I., Sánchez-Díaz, M., Aguirreolea, J., Irigoyen, J. J., Goicoechea, N., et al. (2014). Methodological advances: using greenhouses to simulate climate change scenarios. *Plant Sci.* 226, 30–40. doi: 10.1016/j.plantsci.2014.03.018
- Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1935–1945. doi: 10.1093/jxb/erm055
- Mosedale, J. R., Abernethy, K. E., Smart, R. E., Wilson, R. J., and Maclean, I. M. D. (2016). Climate change impacts and adaptive strategies: lessons from the grapevine. *Glob. Chang. Biol.* 22, 3814–3828. doi: 10.1111/gcb.13406
- Myers, S. S., Zanobetti, A., Kloog, I., Huybers, P., Leakey, A. D. B., Bloom, A. J., et al. (2014). Increasing CO₂ threatens human nutrition. *Nature* 510, 139–142. doi: 10.1038/nature13179
- OIV (2017). *2017 World Viticulture Situation: OIV Statistical Report on World Vitiviniculture*. Paris: OIV.
- Ollat, N., Carde, J.-P., Gaudillière, J.-P., Barrieu, F., Diakou-Verdin, P., and Moing, A. (2002). Grape berry development: a review. *OENO One* 36, 109–131.
- Ollat, N., Geny, L., and Soyer, J.-P. (1998). Grapevine fruiting cuttings: validation of an experimental system to study grapevine physiology. I. Main vegetative characteristics. *J. Sci. Vigne Vin* 32, 1–9. doi: 10.20870/oeno-one.1998.32.1.1061
- Orduña, R. M. D. (2010). Climate change associated effects on grape and wine quality and production. *Food Res. Intern.* 43, 1844–1855. doi: 10.1016/j.foodres.2010.05.001
- Pereira, G. E., Gaudillière, J. P., Pieri, P., Hilbert, G., Maucourt, M., Deborde, C., et al. (2006). Microclimate influence on mineral and metabolic profiles of grape berries. *J. Agric. Food Chem.* 54, 6765–6775. doi: 10.1021/jf061013k
- Rienth, M., Torregrosa, L., Luchaire, N., Chatbanyong, R., Lecourieux, D., Kelly, M. T., et al. (2014). Day and night heat stress trigger different transcriptomic responses in green and ripening grapevine (*Vitis vinifera*) fruit. *BMC Plant Biol.* 14:108. doi: 10.1186/1471-2229-14-108
- Rienth, M., Torregrosa, L., Sarah, G., Ardisson, M., Brillouet, J., and Romieu, C. (2016). Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome. *BMC Plant Biol.* 16:164. doi: 10.1186/s12870-016-0850-0
- Roby, G., and Matthews, M. A. (2008). Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. *Austr. J. Grape Wine Res.* 10, 74–82. doi: 10.1111/j.1755-0238.2004.tb00009.x
- Rubio, J. A., and Yuste, J. (2005). Diferencias de clones de Tempranillo seleccionados en sus zonas de origen. *Vida Rural* 207, 38–44.
- Ruffner, H. P. (1982). Metabolism of tartaric and malic acids in *Vitis*: a review-Part B. *Vitis* 21, 65.
- Ruffner, H. P., Hawker, J. S., and Hale, C. R. (1976). Temperature and enzymic control of malate metabolism in berries of *Vitis vinifera*. *Phytochemistry* 15, 1877–1880. doi: 10.1016/S0031-9422(00)88835-88834
- Ruffner, H. P., and Kliewer, W. M. (1975). Phosphoenolpyruvate carboxykinase activity in grape berries. *Plant Physiol.* 56, 67–71. doi: 10.1104/pp.56.1.67
- Sadras, V. O., and Moran, M. A. (2012). Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. *Austr. J. Grape Wine Res.* 18, 115–122. doi: 10.1111/j.1755-0238.2012.00180.x
- Salazar Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J. J., and Morales, F. (2010). Effects of climate change scenarios on Tempranillo grapevine (*Vitis vinifera* L.) ripening: response to a combination of elevated CO₂ and temperature, and moderate drought. *Plant Soil* 337, 179–191. doi: 10.1007/s11104-010-0514-z
- Salazar-Parra, C. (2011). *Vid y Cambio Climático. Estudio del Proceso de Maduración de la baya en Esquejes Frutíferos de Tempranillo en Respuesta a la Interacción de CO₂ Elevado, Estrés hídrico y Temperatura Elevada*. Ph. D. Thesis, University of Navarra, Spain.
- Sanchez-Ballesta, M. T., Romero, I., Jiménez, J. B., Orea, J. M., González-Ureña, Á., Escribano, M. I., et al. (2007). Involvement of the phenylpropanoid pathway in the response of table grapes to low temperature and high CO₂ levels. *Postharvest Biol. Technol.* 46, 29–35. doi: 10.1016/j.postharvbio.2007.04.001
- Sefc, K. M., Steinkellner, H., Lefort, F., Botta, R., Da Câmara Machado, A., Borrego, J., et al. (2003). Evaluation of the genetic contribution of local wild vines to European grapevine cultivars. *Am. J. Enol. Viticult.* 54, 15–21.
- Serret, M. D., Yousfi, S., Vicente, R., Piñero, M. C., Otálora-Alcón, G., Del Amor, F. M., et al. (2018). Interactive effects of CO₂ concentration and water regime on stable isotope signatures, nitrogen assimilation and growth in sweet pepper. *Front. Plant Sci.* 8:2180. doi: 10.3389/fpls.2017.02180
- Soubeyrand, E., Colombié, S., Beauvoit, B., Dai, Z., Cluzet, S., Hilbert, G., et al. (2018). Constraint-based modeling highlights cell energy, redox status and α -ketoglutarate availability as metabolic drivers for anthocyanin accumulation in grape cells under nitrogen limitation. *Front. Plant Sci.* 9:421. doi: 10.3389/fpls.2018.00421
- Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. C. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Viticult.* 53, 171–182.
- Sweetman, C., Deluc, L. G., Cramer, G. R., Ford, C. M., and Soole, K. L. (2009). Regulation of malate metabolism in grape berry and other developing fruits. *Phytochemistry* 70, 1329–1344. doi: 10.1016/j.phytochem.2009.08.006
- Sweetman, C., Sadras, V. O., Hancock, R. D., Soole, K. L., and Ford, C. M. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65, 5975–5988. doi: 10.1093/jxb/eru343
- Tarara, J. M. M., Lee, J., Spayd, S. E. E., and Scagel, C. F. F. (2008). Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in merlot grapes. *Am. J. Enol. Vitic.* 59, 235–247.
- Torres, N., Goicoechea, N., Morales, F., and Antolín, M. C. (2016). Berry quality and antioxidant properties in *Vitis vinifera* cv. Tempranillo as affected by clonal variability, mycorrhizal inoculation and temperature. *Crop Past. Sci.* 67, 961–977. doi: 10.1071/cp16038
- Torres, N., Hilbert, G., Luquin, J., Goicoechea, N., and Antolín, M. C. (2017). Flavonoid and amino acid profiling on *Vitis vinifera* L. cv Tempranillo subjected to deficit irrigation under elevated temperatures. *J. Food Compos. Anal.* 62, 51–62. doi: 10.1016/j.jfca.2017.05.001
- Tortosa, I., Escalona, J. M., Douthe, C., Pou, A., García-Escudero, E., Toro, G., et al. (2019). The intra-cultivar variability on water use efficiency at different water status as a target selection in grapevine: Influence of ambient and genotype. *Agric. Water Manag.* 223:105764. doi: 10.1016/j.agwat.2019.05.032
- Valdés, M. E., Talaverano, M. I., Moreno, D., Prieto, M. H., Mancha, L. A., Uriarte, D., et al. (2019). Effect of the timing of water deficit on the must amino acid profile of Tempranillo grapes grown under the semiarid conditions of SW Spain. *Food Chem.* 292, 24–31. doi: 10.1016/j.foodchem.2019.04.046
- Vicente Castro, A. (2012). Respuesta Agronómica y Cualitativa de 4 Clones Certificados de *Vitis vinifera* L. cv. Tempranillo en la DO Arlanza. Available online at: <http://uvadoc.uva.es/handle/10324/1677> (accessed September 1, 2020).
- Vitis Navarra (2019). *Tempranillos Para el S. XXI. Recuperando el Origen*. Available online at: <http://www.vitisnavarra.com/clones-exclusivos> (accessed May 29, 2019).
- Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., Kerridge, G. H., Rühl, E. H., and Nicholas, P. R. (2005). Shiraz berry size in relation to seed number and implications for juice and wine composition. *Austr. J. Grape Wine Res.* 11, 2–8. doi: 10.1111/j.1755-0238.2005.tb00273.x
- Webb, L. B., Whetton, P. H., and Barlow, E. W. R. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. *Austr. J. Grape Wine Res.* 13, 165–175. doi: 10.1111/j.1755-0238.2007.tb00247.x
- Wu, J., Drappier, J., Hilbert, G., Guillaumie, S., Dai, Z., Geny, L., et al. (2019). The effects of a moderate grape temperature increase on berry secondary metabolites. *OENO One* 53, 321–333. doi: 10.20870/oeno-one.2019.53.2.2434
- Yamane, T., Jeong, S. T., Goto-Yamamoto, N., Koshita, Y., and Kobayashi, S. (2006). Effects of temperature on anthocyanin biosynthesis in grape berry skins. *Am. J. Enol. Viticult.* 57, 54–59.
- Zhao, X., Li, W. F., Wang, Y., Ma, Z. H., Yang, S. J., Zhou, Q., et al. (2019). Elevated CO₂ concentration promotes photosynthesis of grape (*Vitis vinifera* L. cv. 'Pinot noir') plantlet in vitro by regulating RbcS and Rca revealed by proteomic and transcriptomic profiles. *BMC Plant Biol.* 19:42. doi: 10.1186/s12870-019-1644-y

Zoecklein, B. W., Fugelsang, K. C., Gump, B. H., and Nury, F. S. (1999). *Wine Analysis and Production*. New York, NY: Kluwer Academic Publishers.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Arrizabalaga-Arriazu, Gomès, Morales, Irigoyen, Pascual and Hilbert. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Temperature Shift Between Vineyards Modulates Berry Phenology and Primary Metabolism in a Varietal Collection of Wine Grapevine

Kelem Gashu¹, Noga Sikron Persi¹, Elyashiv Drori^{2,3}, Eran Harcavi⁴, Nurit Agam¹, Amnon Bustan^{5*} and Aaron Fait^{1*}

¹ French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Beersheba, Israel, ² Department of Chemical Engineering, Ariel University, Ariel, Israel, ³ The Grape and Wine Research Center, Eastern Regional R&D Center, Ariel, Israel, ⁴ Ministry of Agriculture and Rural Development, Agricultural Extension Service – Shaham, Beit Dagan, Israel, ⁵ Ramat Negev Desert Agro-Research Center, Ramat Negev Works Ltd., Haluza, Israel

OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Antonio Chalfun-Junior,
Universidade Federal de Lavras, Brazil
Bruno Holzapfel,
New South Wales Department
of Primary Industries, Australia
Gastón Gutiérrez Gamboa,
Independent Researcher, Talca, Chile

*Correspondence:

Aaron Fait
fait@bgu.ac.il
Amnon Bustan
amnonbustan@gmail.com

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 29 July 2020

Accepted: 16 October 2020

Published: 17 December 2020

Citation:

Gashu K, Sikron Persi N, Drori E,
Harcavi E, Agam N, Bustan A and
Fait A (2020) Temperature Shift
Between Vineyards Modulates Berry
Phenology and Primary Metabolism
in a Varietal Collection of Wine
Grapevine.
Front. Plant Sci. 11:588739.
doi: 10.3389/fpls.2020.588739

Global climate change and the expected increase in temperature are altering the relationship between geography and grapevine (*V. vinifera*) varietal performance, and the implications of which are yet to be fully understood. We investigated berry phenology and biochemistry of 30 cultivars, 20 red and 10 white, across three seasons (2017–2019) in response to a consistent average temperature difference of 1.5°C during the growing season between two experimental sites. The experiments were conducted at Ramat Negev (RN) and Ramon (MR) vineyards, located in the Negev desert, Israel. A significant interaction between vineyard location, season, and variety affected phenology and berry indices. The warmer RN site was generally associated with an advanced phenological course for the white cultivars, which reached harvest up to 2 weeks earlier than at the MR site. The white cultivars also showed stronger correlation between non-consecutive phenological stages than did the red ones. In contrast, harvest time of red cultivars considerably varied according to seasons and sites. Warmer conditions extended fruit developmental phases, causing berry shriveling and cluster collapse in a few cultivars such as Pinot Noir, Ruby Cabernet, and Tempranillo. Analyses of organic acid content suggested differences between red and white cultivars in the content of malate, tartrate, and citrate in response to the temperature difference between sites. However, generally, cultivars at lower temperatures exhibited lower concentrations of pulp organic acids at véraison, but acid degradation until harvest was reduced, compared to the significant pace of acid decline at the warmer site. Sugars showed the greatest differences between sites in both white and red berries at véraison, but differences were seasonal dependent. At harvest, cultivars of both groups exhibited significant variation in hexose/sucrose ratio, and the averages of which varied from 1.6 to 2.9. Hexose/sucrose ratio was significantly higher among the red cultivars at the warmer RN, while this tendency was very slight among white cultivars. White cultivars seem to

harbor a considerable degree of resilience due to a combination of earlier and shorter ripening phase, which avoids most of the summer heat. Taken together, our study demonstrates that the extensive genetic capacity of *V. vinifera* bears significant potential and plasticity to withstand the temperature increase associated with climate change.

Keywords: arid viticulture, climate change, organic acids, phenological phase, primary metabolism, sugars, *Vitis vinifera*

INTRODUCTION

Most of the world's viticulture regions are confined to specific geographic niches. Few climatic indices have been employed as metrics to define the boundaries of these regions. However, the recent climate changes considerably threaten the validity of these boundaries, undermining the equilibrium between climate, soil, and variety (Moriondo et al., 2013; Wolkovich et al., 2018; Van Leeuwen et al., 2019). Particularly, the prevalence of recurring years with air temperatures higher than the long-term (30 years) average is disrupting the conservative relationships between geography and viticulture, resulting in remarkable changes in the presently known world wine industry (Fraga et al., 2016; Wolkovich et al., 2018), yet to be fully estimated. In addition, substantial effects on yield and quality along with increases in demand are expected to expand and generate a gradual shift of wine production from traditional regions to newly suitable areas (Hannah et al., 2013; Santillán et al., 2019; Morales-Castilla et al., 2020; Santos et al., 2020).

Recurrent high temperatures tend to diminish wine grape-berry quality traits such as sugars, acids, and phenylpropanoids. Therefore, and in spite of considerable diverse varietal sensitivity to temperature regimes (Gladstones, 1992), warmer regions are predicted to experience the greatest decline in quality and potentially in yield (Moriondo et al., 2013). For example, a recent study, conducted on land suitability for 11 popular cultivars using long-term records, found that a 2°C rise in air temperature might result in 24–56% loss of viticulture area within current wine-growing regions (Morales-Castilla et al., 2020). While efforts have been put to identify the most suitable climate zone for each cultivar (Hall and Jones, 2010; Jones et al., 2012) and to decipher the effect of heat stress on grapes (Jones et al., 2005; Sadras and Moran, 2013), a substantial gap of knowledge exists regarding possible implications of the 2°C rise predicted by climate models on grapevine varietal response, vine and berry phenology, and berry metabolism.

Temperature is known to affect grapevine phenology (Jones et al., 2005; Martínez-Lüscher et al., 2016a; van Leeuwen and Darriet, 2016). For example, accelerated phenological events due to high temperature can shift berry ripening into the warmest part of the season (Webb et al., 2007; Duchêne et al., 2010; Sadras and Moran, 2013; Ruml et al., 2016) and shorten the intervals between phenological phases (Webb et al., 2007; Bock et al., 2011; Tomasi et al., 2011). During fruit ripening, high temperatures reduce the accumulation of anthocyanins (Bergqvist et al., 2001; Pastore et al., 2017; Ramos and Martínez de Toda, 2020) and

enhance catabolism of the main organic acids (Lakso and Kliever, 1975; Sweetman et al., 2014; Rienth et al., 2016) causing a loss of acidity. The effect on berry sugar content remains unclear (Keller, 2010; Reshef et al., 2017). Beyond these general statements, cultivars may differ significantly in many aspects that determine fruit quality, including the timing of bud break, bloom, and véraison, as well as fruit development and ripening processes, when responding to identical sets of environmental conditions (Jones and Davis, 2000; Wolkovich et al., 2018). This broad genetic diversity encompassed by *Vitis vinifera* (Anderson and Aryal, 2013; Moriondo et al., 2013; Real et al., 2015) bears the capacity to provide varieties that can produce high-quality wines also in warm climates (Wolkovich et al., 2018).

Although the grapevine's phenology responses to climate change have been studied (Duchêne et al., 2010; Parker et al., 2011; De Cortázar-Atauri et al., 2017; Alikadic et al., 2019), a satisfactory understanding of how different wine grape cultivars may respond to a temperature shift is yet beyond reach. Moreover, studies employing artificial warming experiments to examine the effect of temperature on phenology and berry chemical compositions (Cleland et al., 2012; Sadras and Moran, 2013) might fail to provide reliable predictions (Wolkovich et al., 2012).

With the objective to explore the effects of environmental and varietal components modulating berry phenology and metabolism, we tested the effect of a consistent difference of 1.5°C in air temperature on the development and berry indices of 30 wine-grape varieties, 20 red and 10 white, grafted on the same rootstock, grown in vineyard conditions at two distinct arid topo-climatic regions. The advantages of field trials in arid regions are as follows: (i) reliable control of water input, as no rainfall occurs during fruit maturation; (ii) low air humidity and thus low risks of pathogenic hazards (Carroll and Wilcox, 2003; Eastburn et al., 2011); and (iii) a relatively low intra- and inter-seasonal variability. Moreover, significantly large gaps between daily minimum and maximum temperatures (yet within the range for viticulture), abundant clear-sky conditions, and sufficient exposure to sunlight provide suitable conditions for quality fruit development (Bernardo et al., 2018; Ohana-Levi et al., 2020). To our knowledge, this is one of the very few studies of its kind (Tomasi et al., 2011; Ruml et al., 2016).

MATERIALS AND METHODS

Plant Material and Experimental Site

The experiments were conducted during three consecutive seasons, from 2017 to 2019, in two vineyards in the Negev

Abbreviations: BB, bud break; FS, fruit set; Vér, véraison; Har, harvest; OA, organic acids; HSR, hexoses-to-sucrose ratio; MR, Ramon; RN, Ramat Negev.

Highlands in Israel (**Figure 1A**); Ramon (MR) vineyard (**Figure 1B**) vineyard (30°38'48.6"N 34°47'24.5"E, 850 m asl) and the Ramat Negev (RN) vineyard at the Desert Agro-Research Center (30°58'43.4"N 34°42'31.6"E, 300 m asl). The two locations are 53-km distant. The average annual precipitation are 105 and 80 mm at MR and RN, respectively, occurring only in the winter (typically November through April), with considerable year-to-year fluctuations. Both vineyards shared the same experimental setup, comprising 30 wine grape (*Vitis vinifera* L.) cultivars (10 white and 20 red; **Figure 1C**), grafted on 140 RU rootstock. Both vineyards were planted in 2012 in a randomized block design with four replicates of eight–nine vines each (30 cultivars × 2 locations × 4 biological replicates). Each cultivar was represented in each of four independent replicate blocks by at least eight vines (32–36 vines per cultivar, in each vineyard). Phenological assessments and sampling for biochemical analyses were conducted on each cultivar using each of the four replicate blocks (independent biological replicate) in each vineyard, as further detailed below.

The space between rows and between vines was 3 and 1.5 m, respectively. In order to reduce the variation between the vineyards the same rootstock, trellising technique (vertical shoot position, VSP), orientation (north-south), cultural practices, and irrigation systems were used. The soils at both sites are sandy loam. Drip-irrigation systems, mulched with white plastic sheets, as commonly used in the region, supplied about 500 mm each year, from bloom to harvest. Irrigation rate was adjusted weekly according to the current evapotranspiration and crop coefficients. Deficit irrigation was exercised to control vine vigor until véraison (crop coefficient of 0.35) and to impose a moderate water stress during fruit ripening (crop coefficient of 0.25). Yield was adjusted to 10–15 Mg ha⁻¹ using appropriate winter pruning, branch, and cluster thinning during the season. The vegetative growth and canopy size were controlled in the VSP design by pruning branches at 2.2 m aboveground. Fertilizer was supplied through the irrigation system, and pest management was carried out according to the common regional recommendations.

Meteorological Data Measurement

Hourly meteorological data (i.e., incoming solar irradiance, air temperature, relative humidity, and wind speed and direction) were extracted from standard meteorological stations (Meteotech Ltd., Israel) located at the Desert Agro-Research Center, 500 m distant from RN vineyard, and at MR vineyard, during 2017–2019 and 2018–2019 seasons, respectively. The incoming solar irradiance, air temperature, relative humidity, and wind speed and direction were measured continuously at 0.1 Hz using a portable meteorological station (WS501-UMB, Lufft, Fellbach, Germany) set 2 m above the canopy, and 15-min averages were logged by a data logger (CR200, Campbell Scientific, Utah, United States). During the growing season of 2017 (23 May–29 August), this meteorological station was set at MR site, at 2 m above the canopy, and provided 15-min averages of the meteorological conditions within the vineyard.

The Huglin index (HI; Huglin, 1978), a degree-day index used to estimate grapevine thermal exposure during its phenological course (Fraga et al., 2012; Lorenzo et al., 2013;

Sánchez et al., 2019), was fitted to the local earlier phenological course and was computed from March to August, instead of the standard April to October grapevine growing season (north hemisphere), using the following equation:

$$HI = \sum_{\text{March}}^{\text{August}} \left(\frac{(\text{MDT} - 10) + (\text{Tmax} - 10)}{2} k \right), \quad (1)$$

where MDT is the mean daily temperature, Tmax is maximum daily temperature, and *k* is day length coefficient (1.02 to 1.06).

Phenological Data Collection

The stage of grapevine crop development (E-L scale; Coombe, 1995) was determined weekly on eight–nine vines in each of four replicates of a given cultivar at each location. The timing of four phenological events and the duration of the intervals between them was recorded yearly during 2017–2019 seasons at both vineyards, at the cultivar and replicate levels. These events included the following: (1) date of bud break (BB), at E-L 4 (Coombe, 1995); (2) initial fruit set (FS; E-L 27); (3) véraison (Vér; E-L 35); and (4) harvest (Har; E-L 38). The difference in the durations of similar phenological intervals between MR and RN vineyards was calculated and defined as the phenological shift.

Berry Sampling and Metabolite Extraction

During each season, berries were sampled at véraison and at harvest for metabolite extraction and berry indices. At véraison, each cultivar's replicate was sampled when berries reached about 50% color change or softening (estimated weekly in eight tagged representative clusters per replicate), in red or white cultivars, respectively. Toward harvest, berries were sampled at each cultivar's replicate approaching a specific°Brix level, i.e., 23 ± 1 and 20 ± 1°. Brix, for red and white cultivars, respectively. For each cultivar, samples were collected from four biological replicates at each location, as follows. In each sampling, at least 30 berries per replicate were pooled from five different vines in each block (six berries per vine were sampled from the top, middle, and bottom of the bunch), on the east side of the vine, and immediately snap-frozen in liquid nitrogen. Berries were sectioned while still frozen, skin and pulp carefully separated, and seeds were removed. The pulp was kept at –80°C until further analysis.

Pulp Organic Acid and Sugar Analysis

Pulp samples were lyophilized and ground under liquid nitrogen using a Retsch-mill (Retsch, Haan, Germany) with prechilled holders and grinding beads. For metabolite extraction, 20 mg of frozen pulp powder was weighed and extracted in a 1-ml pre-cooled methanol/chloroform/water extraction solution (2.5:1:1 v/v) as described in Hochberg et al. (2013); Degu et al. (2014). Then, 120 µl (véraison) and 100 µl (harvest) of extracts were dried using Concentrator Plus (Eppendorf, Hamburg, Germany) and derivatized exactly as described in Hochberg et al. (2015) with sorbitol as the internal standard. Extracts were injected into the GC-MS for organic acid and



FIGURE 1 | Geographic location of experimental vineyards **(A)**. Aerial view of Ramon vineyard. The experimental area is marked with dashed lines **(B)**. List of cultivars used in the experiment and their origin **(C)**. Names with red and white color denote red and white cultivars, respectively. Cultivar names are composed by abbreviations in the bracket. *Country of origin not defined.

sugars analysis. Malate, tartrate, citrate, glucose, fructose, and sucrose were quantified using a calibration curve of standards (Sigma-Aldrich, MO, United States) as described previously by Reshef et al. (2017). The GC-MS conditions were exactly as described previously by Reshef et al. (2019).

Analyses were conducted in two consecutive seasons. Since the metabolic response of each cultivar was not always the same between two seasons, a third season (2019) was used as validation and analyzed in bulks of the four replicates \times cultivar \times location.

Qualitative and Quantitative Data Analysis-Mass Hunter Workstation Software (Agilent Technologies, Santa Clara, CA, United States) was used for integration of peak area and data analysis. Metabolite annotation was performed based on spectral searching supported by the National Institute of Standards and Technology (NIST, Gaithersburg, MA, United States) against RI libraries from the Max Planck Institute for Plant Physiology (Golm, Germany) and finally normalized by the internal standard sorbitol 6C¹³ (Cortecnet Corporation, Mill Valley, CA, United States) and pulp dry weight.

Statistical Analysis

Statistical analysis was performed using software “R” version 3.6.0 (R Development Core Team, 2017) and JMP®, version 13 (SAS institute Inc., Cary, NC, 1989–2007). One-way analysis of variance (ANOVA) was used to assess the genetic variability for each parameter between the seasons within the same location using the built-in *aov* function. The differences between locations for each cultivar were tested using *t.test* and *Wilcox.test* functions according to the distribution of the data. Histograms were created using *hist* function in “ggplot2” package. The wind rose graphs were created using “open air” package (Carslaw and Ropkins, 2012). Clustered heatmaps were created using *Complexheatmap* (Gu et al., 2016). Hierarchical clustering of samples was calculated by Euclidean distances and the Ward.D2 clustering method in functions *get_dist* and *hclust*, built-in “dendextend,” and “factoextra” packages (Galili, 2015). Correlation analysis was performed using *ggscatter* function built in “ggpubr” package. A three-way factorial analysis was performed using JMP®, version 13 (SAS institute Inc., Cary, NC, 1989–2007), to assess the interaction effects between cultivar, location, and growing seasons. Principal component analyses were plotted using the software “Metaboanalyst” version 4.0 (Chong et al., 2018).

RESULTS

Climatic Conditions in the Vineyards

The two vineyards differed in their climatic conditions (Figures 2, 3). MR vineyard experienced slightly higher incoming solar irradiance, lower temperature, both maximum and minimum, and as a result also slightly higher relative humidity. Wind speed in both vineyards is within the same magnitude range, but a slight difference in wind direction exists—the prevailing direction in MR is west-northwest, while in RN it is north-northwest. In both sites, the wind originates from the sea breeze from the Mediterranean Sea and peaks in the afternoon. The average HI computed from the meteorological data measured in each season categorized RN and MR vineyards as hot (HI > 3,000°C units) and warm (HI > 2400°C units) regions, respectively (Figure 3). These differences stem from a consistent 1.5°C difference in the daily mean temperature measured throughout the three seasons (Figure 3). Having said that, 2018’s temperature regime, especially during the spring, was warmer in both vineyards compared to 2017 and 2019 (Figures 2D–F).

The Interaction Between Climate and Season Strongly Affected the Timing of Phenological Stages in Red and White Grapevine Cultivars

The timing of phenological events was strongly affected by cultivar, site, year, and by the interaction between these factors (Supplementary Tables 3, 4). Generally, bud break (BB), fruit set (FS), and véraison (Vér) occurred earlier in the warmer RN than in MR (Figure 4 and Table 1). On average, the harvest date of the white cultivars shifted by 6–14 days from RN to MR. However, among the red cultivars, the harvest date varied more between seasons than between locations (Table 1).

In 2018, significant phenological shifts were evident compared to 2017 and 2019 regardless of the vineyard location (Figure 5 and Table 1). These inter-seasonal differences were mainly attributed to an earlier BB in 2018 (Table 1), due to exceptionally high spring temperatures (Figure 2E). Within each seasonal cluster, cultivars were grouped by ‘location’ (Figure 5). As expected, cultivars generally considered early- and late-maturing were separated within each location cluster. For example, early maturing white cultivars, such as Chardonnay, Pinot Gris, Gewurztraminer, and Muscat Blanc, were grouped away from the late-maturing cultivars Chenin Blanc, Colombard, Riesling, and Muscat Alexandria. Similarly, among red cultivars, Pinot Noir and Ruby Cabernet grouped together within each cluster, displaying a similar phenological pattern across seasons, with earlier véraison and harvest dates. Note that the coefficient of variation of red cultivars in RN was greater than in MR, showing greater plasticity in phenology between seasons, compared with the white cultivars that displayed greater variation at MR vineyard (Figure 5 and Supplementary Figure 1 and Supplementary Table 1).

For each cultivar and location, the onset dates of the four phenological stages were correlated with each other over the 3 years of experiment (Figure 6); the onset of each stage was strongly correlated with the preceding one (Figure 6 and Supplementary Table 2). Thus, bud break and fruit set displayed particularly strong correlations in both white and red cultivars: early or late bud break onset was corresponded by early or late onset of fruit set, respectively (Figures 6A,B). In a similar way, the onsets of fruit set and véraison were strongly correlated, although this relationship was weaker among the red cultivars (Figures 6G,H). In contrast, the linkage between the onsets of the consecutive véraison and harvest stages was much less pronounced, particularly among the red cultivars (Figures 6L,M). Exceptions to the strong linkage between consecutive stages were Tempranillo, Tinta Cao, and Touriga Nacional, among the red cultivars, and Sauvignon Blanc and Semillon—among the white ones (Supplementary Table 2).

The correlations between onsets of the non-consecutive stages were generally fainter, but considerably stronger among the white cultivars. The onsets of bud break and harvest, the most departed stages, were weakly correlated among the white cultivars (Figure 6E) and quite blurry among the red ones (Figure 6F). Later in the season, the correlation between the onsets of bud break and véraison remained strong among the white cultivars

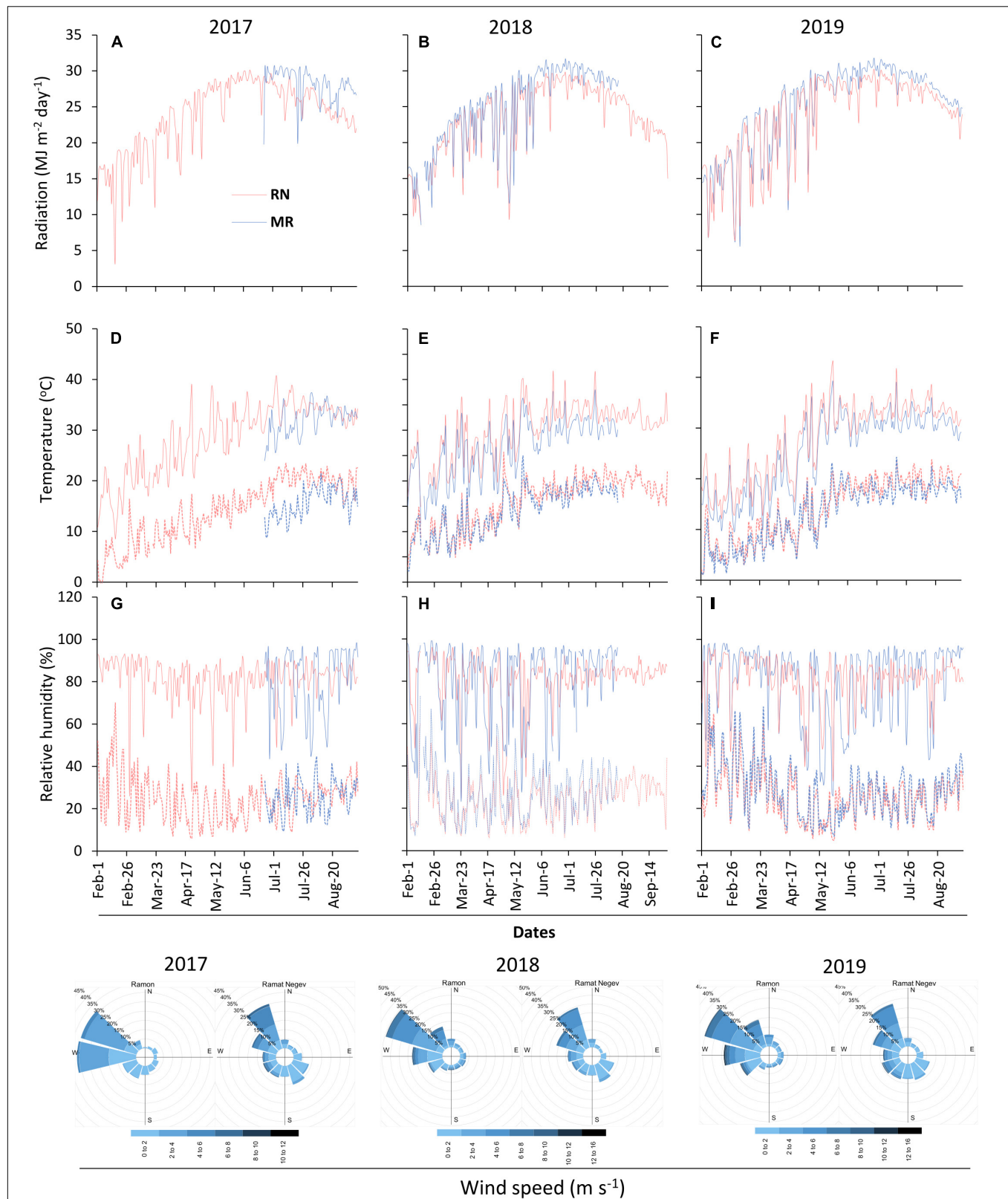


FIGURE 2 | Meteorological conditions at the experimental sites. Radiation (A–C), temperature (D–F), relative humidity (G–I), and wind speed were continuously measured at 2 m above canopy during 2017–2019 seasons. The solid and dotted lines in the temperature and relative humidity graphs denote maximum and minimum measurements, respectively. RN, Ramat Negev; MR, Ramon. The 2017 data at Ramon vineyard are from 23rd May 2017 until harvest end.

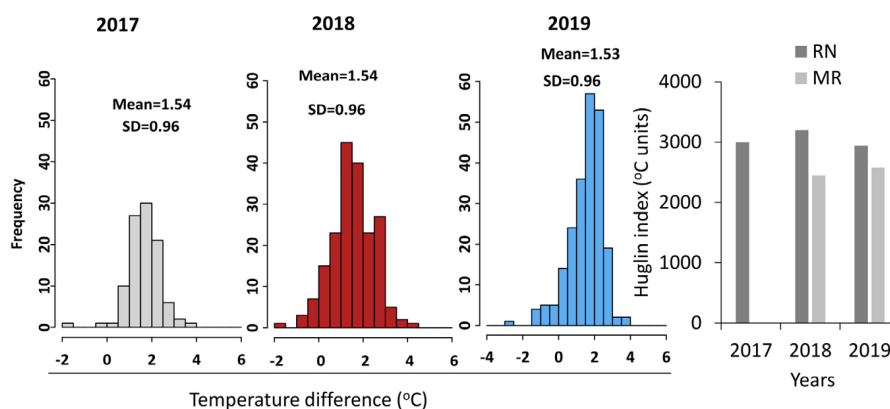


FIGURE 3 | Temperature and Hugin index differences between the sites. Histograms show the frequency distribution of mean daily temperature difference between Ramat Negev (RN) and Ramon (MR) (Ramat Negev-Ramon) vineyards in 2017 (gray), 2018 (red), and 2019 (blue) seasons. SD, standard deviation. The bar graph represents the Hugin index (HI) calculated from March to August. The temperature differences between the vineyards in the 2017 season were performed using the temperature data measured from 23rd May until the last harvest date 29th August. The 2017 HI of MR was not presented as the data were not available throughout the season.

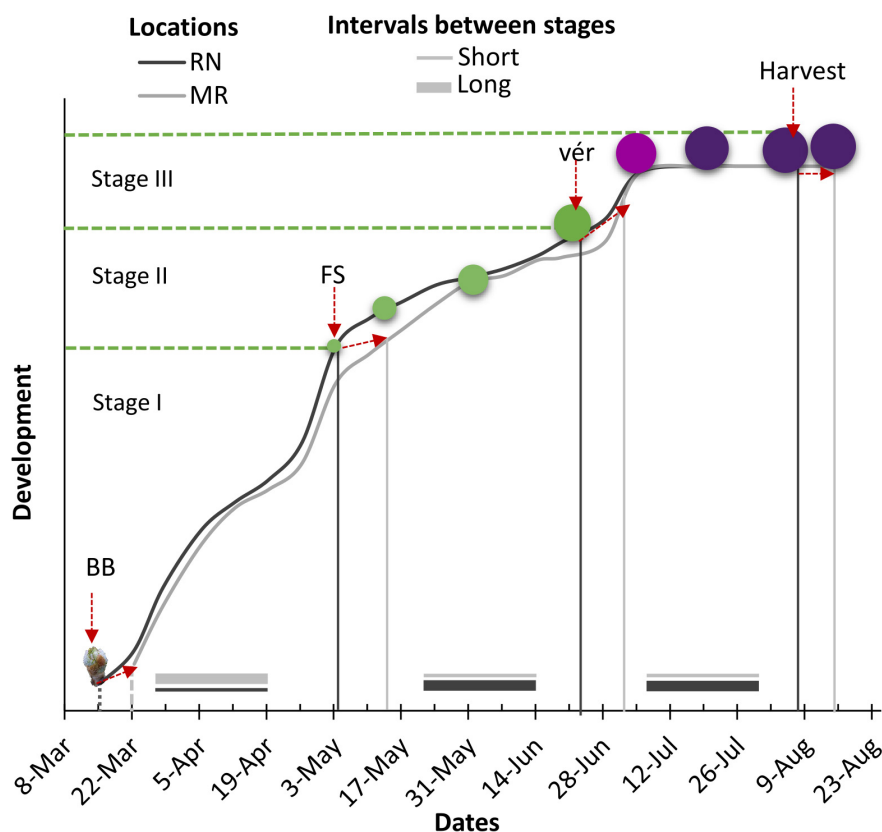


FIGURE 4 | Schematic presentation of the effect of location on timing of major phenological stages and the intervals between stages for white and red cultivars. RN, Ramat Negev; MR, Ramon; BB, bud break; FS, fruit set; VÉR, véraison. Stage I: vegetative growth, stage II: fruit cell enlargement and fruit hardening, stage III: fruit ripening.

(Figure 6C), but began to fold in the red group (Figure 6D). Nevertheless, the frailest relationships occurred between the onsets of fruit set and harvest, which were still positive and valid ($p = 1.6e-12$) among the white cultivars (Figure 6I), but weak

in the red group (Figure 6J). This dissection of the phenological course points to the fruit ripening phase, between véraison and harvest, as the main source of variation between cultivars, particularly in the red ones (Figure 6M).

TABLE 1 | The average occurrence of phenological phases (expressed in dates) and the length between phenological phases (expressed in days) in red and white cultivars grown at Ramon (MR) and Ramat Negev (RN) vineyards from 2017 to 2019.

Cultivar	Years	Location	The timing of phenological events (dates)				The intervals between phenological phases (days)			
			Bud break (BB)	Fruit set (FS)	Véraison (Vér)	Harvest (Har)	BB to FS	FS to Vér	Vér to Har	
Red	2017	RN	22-Mar ± 0.3 ^{B*}	04-May ± 0.3 ^{B*}	24-Jun ± 0.7 ^{B*}	09-Aug ± 1.3 ^B	43.2 ± 0.4 ^B	50.5 ± 0.7 ^{B*}	46.5 ± 1.0 ^{A*}	
	2018		11-Mar ± 0.3 ^{A*}	25-Apr ± 0.4 ^{A*}	14-Jun ± 0.6 ^{A*}	24-Jul ± 1.2 ^{A*}	45.3 ± 0.4 ^A	49.6 ± 0.5 ^B	40.2 ± 1.0 ^B	
	2019		30-Mar	13-May ± 0.2 ^{C*}	04-Jul ± 0.4 ^{C*}	17-Aug ± 1.1 ^C	44	52.7 ± 0.4 ^{A*}	43.8 ± 1.0 ^{A*}	
	2017	MR	31-Mar ± 0.4 ^B	20-May ± 0.6 ^B	06-Jul ± 0.5 ^B	12-Aug ± 0.8 ^B	50.7 ± 0.5 ^{A*}	46.5 ± 0.6 ^B	35.9 ± 0.7 ^B	
	2018		16-Mar ± 0.4 ^A	02-May ± 0.5 ^A	20-Jun ± 0.4 ^A	01-Aug ± 0.9 ^A	47.3 ± 0.4 ^{B*}	48.9 ± 0.5 ^A	41.8 ± 0.8 ^A	
White	2019	RN	07-Apr	20-May ± 0.2 ^B	06-Jul ± 0.4 ^B	16-Aug ± 0.7 ^C	43	46.7 ± 0.3 ^B	40.9 ± 0.6 ^A	
	2017		20-Mar ± 0.7 ^{B*}	02-May ± 0.6 ^{B*}	24-Jun ± 1.1 ^{B*}	21-Jul ± 2.0 ^{B*}	43.6 ± 0.4	52.7 ± 0.7 ^{A*}	27.6 ± 1.6 ^{A*}	
	2018		08-Mar ± 0.6 ^{A*}	22-Apr ± 0.8 ^{A*}	12-Jun ± 0.9 ^{A*}	11-Jul ± 1.2 ^A	44.7 ± 0.5	48.4 ± 0.5 ^B	28.7 ± 0.6 ^{A*}	
	2019	MR	28-Mar	10-May ± 0.5 ^{C*}	29-Jun ± 0.5 ^{C*}	27-Jul ± 1.2 ^{B*}	43	50.0 ± 0.4 ^{B*}	28.0 ± 0.9 ^A	
	2017		29-Mar ± 0.4 ^B	17-May ± 0.8 ^B	07-Jul ± 4.1 ^C	04-Aug ± 1.1 ^B	49.3 ± 0.6 [*]	50.5 ± 0.7 ^A	22.7 ± 0.9 ^B	
	2018		15-Mar ± 0.6 ^A	30-Apr ± 0.8 ^A	18-Jun ± 0.6 ^A	15-Jul ± 0.8 ^A	46.3 ± 0.5 [*]	49.1 ± 0.6 ^A	27.0 ± 0.5 ^A	
	2019		04-Apr	20-May ± 0.4 ^B	5-Jul ± 0.3 ^B	2-Aug ± 1.3 ^B	46	46.0 ± 0.3 ^B	27.5 ± 1.3 ^A	

Data are the overall mean value ± SE of red ($n = 4$ replicate × 20 cultivars) and white ($n = 4$ replicates × 10 cultivars) cultivars in 2017, 2018, and 2019 season. *Indicates a significant difference between locations within the same season. a, b, and c indicate significant differences between the seasons at Ramon vineyard. A, B, and C indicate significant differences between seasons at Ramat Negev vineyard. BB and BB to FS in 2019 were not recorded in varietal resolution.

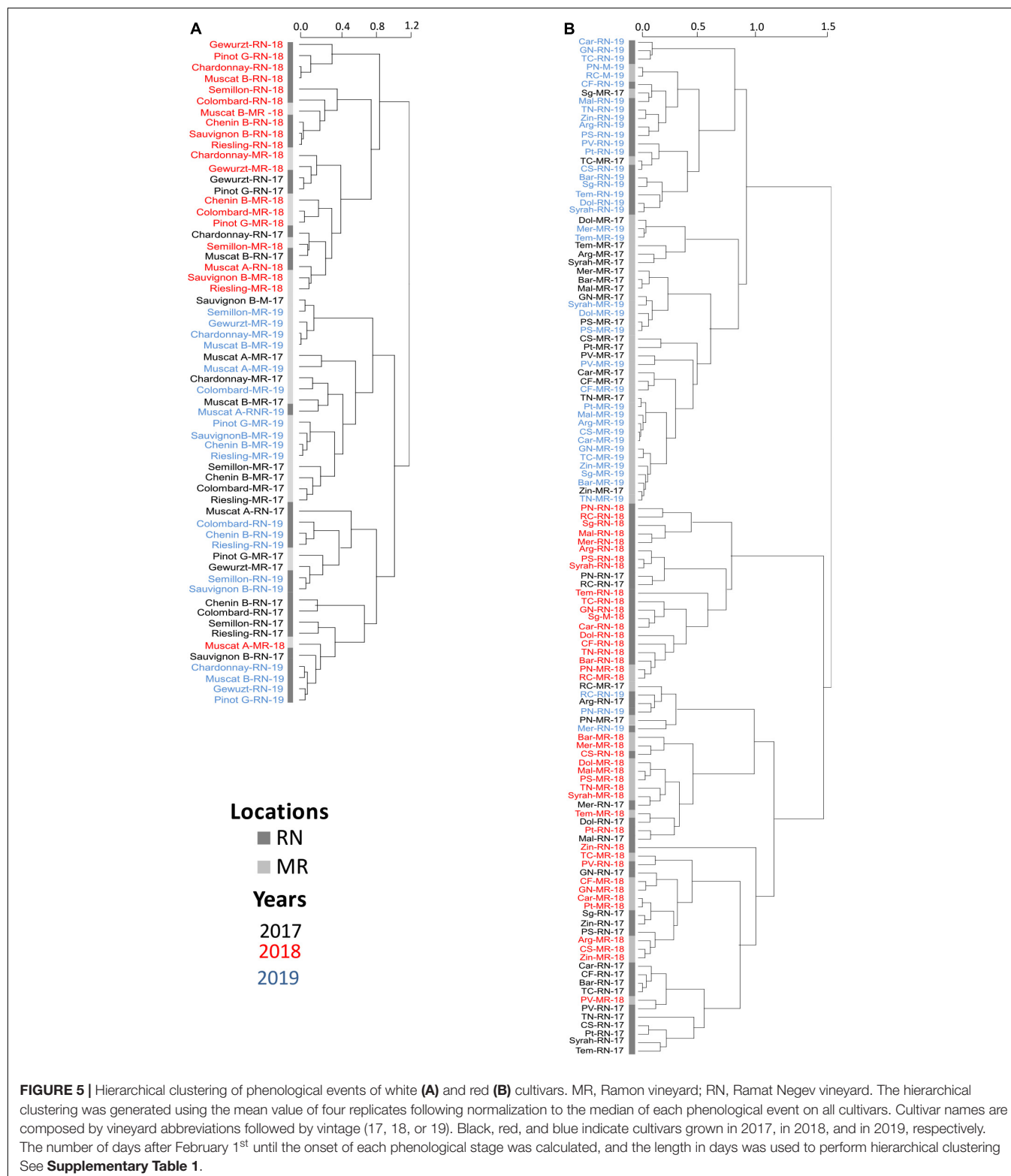
Varietal-Specific Differences in the Duration of Phenological Phases Reflect Genotype vs. Environment Interaction in Response to the Temperature Shift Between Locations

Statistical analysis of the duration of the phenological phases revealed significant effects of cultivar (C), location (L), year (Y), and the interactions among them for all phases except for the period from bud break to fruit set, which was not affected by the C × Y interaction (Supplementary Tables 3, 4). In order to evaluate the effect of the location climate on the duration of phenological phases, we introduced the phenological shift. This measure was calculated by subtracting the number of days of a given phenological phase in RN from that in MR (Figure 7).

The vegetative phase, from bud break to fruit set, varied considerably among cultivars, with short periods of 40 days (Muscat Blanc, Petit Verdot, Pinot Noir, and Tinta Cao) compared to much longer ones of 57 days (Pinotage) (Table 2). The phenological shift of the vegetative phase was consistently positive and longer at MR vineyard (Figure 7); however, it was strongly season dependent, as indicated by the significant L × Y interaction (Supplementary Tables 3, 4). Comparing seasons 2017 and 2018 (BB onset on 2019 was not recorded in varietal resolution), the overall mean phenological shift among white and red cultivars was three and six-fold greater in 2017, respectively, compared to 2018.

Among the white cultivars, Sauvignon Blanc, Chenin Blanc, Muscat of Alexandria, and Muscat Blanc displayed strong phenological shifts only in 2017 (6–10 days; Figure 7). In contrast, Pinot Gris, Semillon, Gewurztraminer, Riesling, and Colombard exhibited consistent moderate shifts (1–4 days), while Chardonnay showed large shifts in both years (Figure 7). Among the red cultivars, Tinta Cao, Pinotage, Petit Verdot, Carignan, Cabernet Sauvignon, Cabernet Franc, and Barbera displayed large shifts in 2017, but negligible ones in 2018. Merlot and Tempranillo were severely affected in both years, contrary to Pinot Noir, Ruby Cabernet, and Syrah that were unaffected. Most of the other red cultivars exhibited mild to moderate shifts, with considerable differences between seasons (Figure 7).

In contrast to the vegetative phase, phenological shifts of the fruit growth phase (FS-Vér) were primarily negative; this phase was shorter at MR vineyard (2017 and 2019 seasons; $P < 0.001$), with very few exceptions among cultivars (no significant shifts were recorded in 2018, Figure 7 and Table 1). Among white cultivars, Chenin Blanc, Semillon, and Riesling displayed the longest phase (57–58 days) at RN in 2017, and Gewurztraminer showed the shortest period in 2018 at MR (Table 2). Among the red cultivars, Tempranillo exhibited the shortest FS-Vér (35 days) in 2017, and Tinta Cao the longest (62 days) FS-Vér in 2018 at MR vineyard (Table 2). Gewurztraminer and Pinot Gris among the white cultivars and Ruby Cabernet, Pinotage, and Malbec among the red cultivars exhibited significantly contrasting trends of phenological shifts between the seasons (Figure 7). Pinotage, Carignan, and Cabernet Sauvignon displayed the strongest shifts in FS-Vér among the red cultivars.



The duration of Vér-Har phase varied from 23 to 29 days in the white and from 36 to 47 days in the red cultivars (Table 1). The end of this phase was defined upon obtaining target Brix values, $20 \pm 1\%$ and $23 \pm 1\%$ for white and red cultivars, respectively.

Noteworthy, however, is the failure of several cultivars to meet this threshold, which depended on the location and season. Cultivars with particular susceptibility were Pinot Noir, Barbera, Dolcetto, Tempranillo, and Zinfandel; the Brix of which failed to

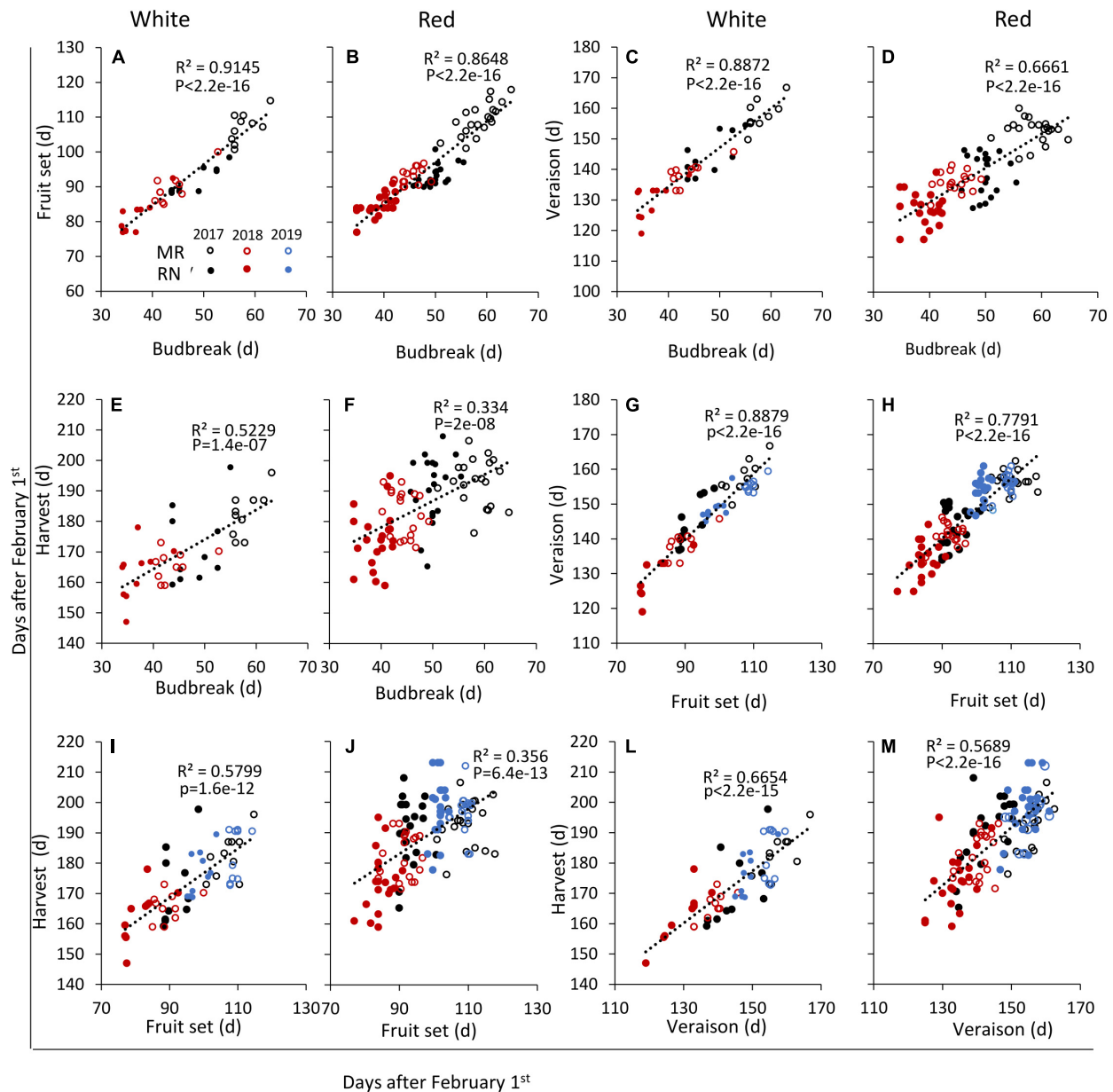
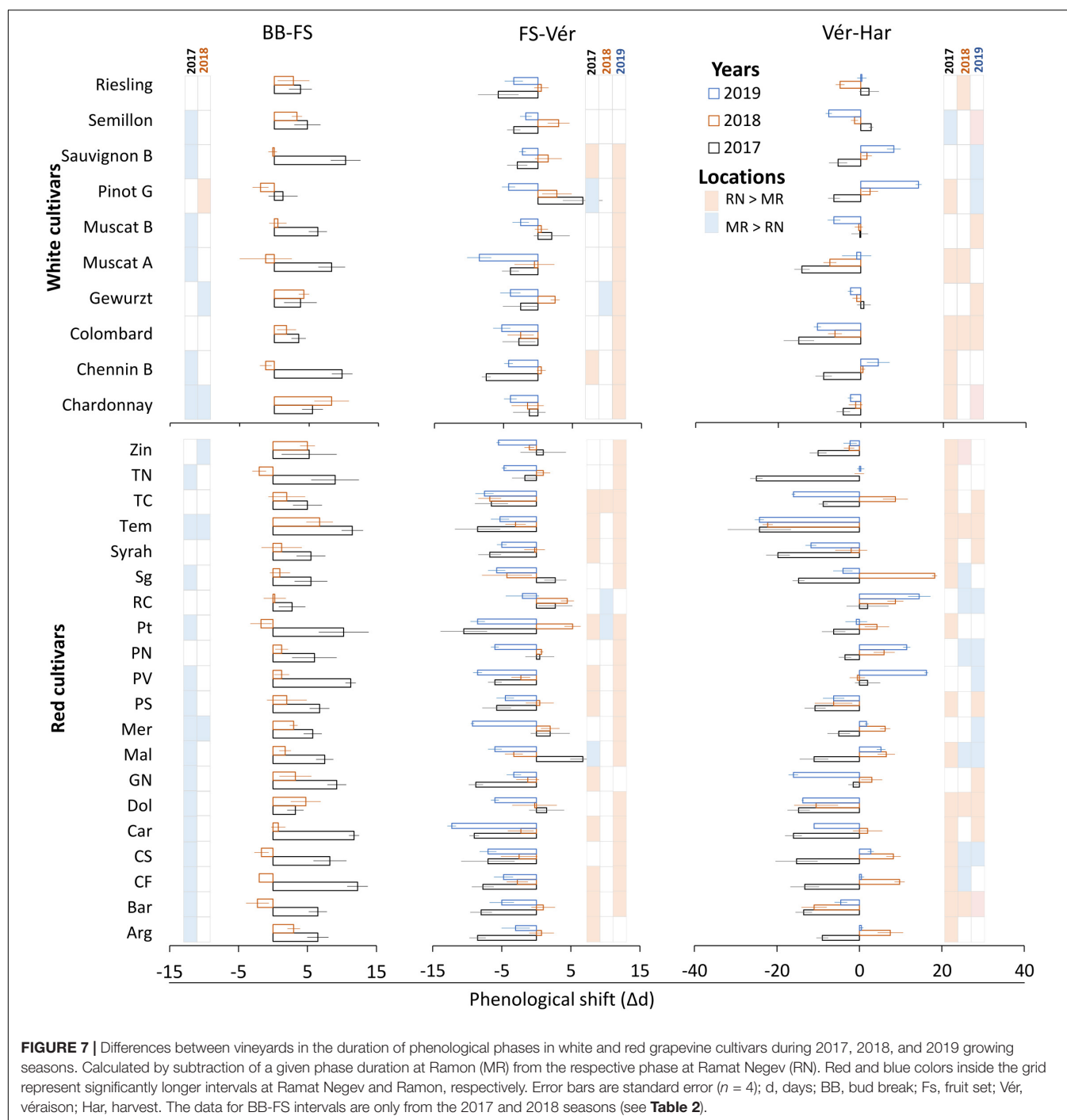


FIGURE 6 | Linear regressions between the timing of various pairs of phenological events in white and red wine grapevine cultivars, respectively, as follows: fruit set to budbreak, (A,B); véraison to budbreak, (C,D); harvest to budbreak, (E,F); véraison to fruit set, (G,H); harvest to fruit set, (I,J); and, harvest to véraison, (L,M). In each season, data are the average values of four biological replicates (each consisting of eight–nine plants) at Ramon (MR) and Ramat Negev (RN) vineyards. (d), days after February 1st until the onset of each phenological event. Open and close circles denote Ramon and Ramat Negev vineyard, respectively. Black, red, and blue indicate cultivars grown in 2017, in 2018, and in 2019, respectively. See **Supplementary Table 2**.

increase beyond a certain value, or furthermore, most of the fruit shriveled before reaching harvest. Similar to fruit development, the phenological shift of the Vér-Har phase was mostly negative, indicating an extension of this period in the warmer RN compared to MR, with considerable differences between seasons (Table 2). Among the white cultivars, Colombard and Muscat of Alexandria displayed the strongest shifts (up to 15 days); Chardonnay, Gewurztraminer, Muscat Blanc, Semillon, and Riesling hardly responded; Semillon, Pinot Gris, and Chenin

Blanc showed inconsistent phenological shifts that varied between years (Figure 7). Chardonnay, Gewurztraminer, Muscat Blanc, and Semillon had the shortest ripening periods (18 to 27 days, depending on the season), whereas Chenin Blanc, Colombard, Muscat Alexandria, and Riesling displayed much longer ripening periods (26 to 45 days) (Table 2). In an effort to identify sensitive cultivars to seasonal variation, we calculated the coefficient of variance (CV) for each cultivar separately at each site across three seasons (Supplementary Figure 2). The CV



can provide insights into the effect of environmental variability on cultivar sensitivity. Here, the higher the CV, the greater the sensitivity of a given cultivar for the respective trait in response to seasonal variation (Reed et al., 2002). The CV analysis for Vér-Har phases revealed that Muscat Blanc and Pinot Gris in MR and Muscat Alexandria and Semillon in RN were the most responsive cultivars to seasonal differences (**Supplementary Figure 2**).

The negative phenological shift of the Vér-Har phase was, on average, much stronger among the red cultivars, and

considerable variability was monitored between cultivars and seasons (**Figure 7**). Tempranillo, Dolcetto, Syrah, and Barbera displayed consistent and strong negative phenological shifts, whereas Ruby Cabernet, Pinot Noir, and Petit Verdot showed positive shifts. Zinfandel, Pinotage, and Merlot were hardly influenced by the location, as indicated by very small shifts. In contrast, quite many red cultivars exhibited seasonal variability in the direction and strength of the phenological shift (**Figure 7**). CV analysis among the red cultivars revealed that Argaman and

TABLE 2 | The duration of intervals between phenological events in red and white cultivars grown at Ramon (MR) and Ramat Negev (RN) vineyards during 2017, 2018, and 2019 seasons.

Cultivar	Bud break to fruit set interval				Fruit set to véraison interval						Véraison to harvest					
	2017		2018		2017		2018		2019		2017		2018		2019	
	MR	RN	MR	RN	MR	RN	MR	RN	MR	RN	MR	RN	MR	RN	MR	RN
Red																
Arg	54.5*	48.0	50.3	47.3	41.5	50.0 ^A	45.8	45.0 ^B	46.3	49.3 ^{AB}	26.5 ^C	35.5*	46.5 ^a	39.0	42.3 ^b	41.8
Bar	47.5*	41.0 ^B	43.8	46.0 ^A	50.8	58.8 ^A	53.3	52.3 ^B	50.0	55.0 ^{AB}	35.3 ^b	48.8 ^a	27.5 ^c	38.5 ^B	41.0 ^a	45.5 ^{AB}
CF	55.3 ^a	43.0 ^B	47.8 ^b	48.0 ^A	51.3	59.0*	53.3	56.0	51.0	55.8*	34.3 ^b	47.5 ^a	41.0 ^a	31.3 ^B	34.5 ^b	34.0 ^B
CS	51.3	43.0 ^B	49.5	48.8 ^A	42.3	49.3	46.3	48.8	45.5	52.5*	39.0 ^c	54.3 ^a	45.8 ^a	37.5 ^B	42.8 ^b	40.0 ^B
Car	54.3 ^a	42.5 ^B	50.0 ^b	49.3 ^A	48.0	57.0 ^a	48.5	49.3 ^B	45.5	57.8 ^a	39.5 ^c	55.5 ^a	48.8 ^a	46.8 ^B	43.0 ^b	54.0 ^a
Dol	48.8	44.0	48.8	44.0	42.0	42.0 ^B	43.3	43.5 ^B	45.8	51.8 ^a	31.3	46.0*	36.0	46.5*	36.3	50.0*
GN	54.5 ^a	45.3	49.8 ^b	46.5	48.3	57.0 ^a	52.5	53.8 ^B	49.8	53.0 ^B	35.5 ^b	37.0 ^B	43.5 ^a	40.5 ^B	42.0 ^a	58.0 ^a
Mal	49.5*	42.0	47.3	45.5	50.0*	43.3 ^B	47.8	51.0 ^A	46.8	52.8 ^a	35.3 ^b	46.3 ^a	34.8 ^{ab}	28.3 ^C	43.0 ^a	37.8 ^B
Mer	50.0 ^a	44.3	46.3 ^{ab}	43.3	49.0 ^a	47.0 ^B	50.8 ^a	48.8 ^{AB}	43.8 ^b	53.0 ^a	34.3	39.3 ^A	32.5	26.3 ^B	29.3*	27.5 ^B
PS	49.8*	43.0	49.3	47.3	43.5	49.3*	46.0	43.5	44.3	48.8*	38.0	48.8*	34.0	40.3	39.8	46.0*
PV	50.8 ^a	39.5 ^B	46.0 ^b	44.8 ^A	52.5 ^b	58.5*	56.3 ^a	58.5	50.5 ^b	59.0*	45.3 ^b	43.3 ^A	45.8 ^b	46.3 ^A	52.3 ^a	36.0 ^B
PN	45.5	39.5	43.5	42.3	45.3 ^{ab}	44.8 ^B	47.8 ^a	48.0 ^A	43.5 ^b	49.5 ^a	27.3 ^b	30.8 ^B	42.0 ^a	36.0 ^A	46.8 ^a	35.3 ^A
Pt	56.5 ^a	46.3	47.5 ^b	49.3	40.8 ^c	51.3 ^a	49.8 ^a	44.5 ^B	45.5 ^b	54.0 ^a	43.5	49.8 ^a	46.8	42.5 ^B	43.3	44.0 ^{AB}
RC	45.0	42.3	43.0	42.8	47.0	44.3	48.8*	44.3	45.0	47.0	38.5	36.5	44.0*	35.3	45.5*	31.0
Sg	50.0 ^a	44.5	45.0 ^b	44.0	51.5	48.8 ^B	49.5	51.8 ^a	47.8	53.5 ^a	35.0 ^c	49.8 ^a	53.8 ^a	49.5 ^B	44.3 ^b	48.3 ^A
Syrah	50.8	45.3	49.8	48.5	44.0	50.8*	46.5	46.8	46.5	51.5*	28.0 ^b	47.8 ^a	34.5 ^{ab}	36.5 ^B	37.5 ^a	49.3 ^a
Temp	53.0*	41.5	49.0*	42.3	35.8 ^b	44.3*	42.0 ^a	45.0	41.8 ^a	47.0*	28.5 ^b	52.8*	42.8 ^a	65.0*	30.8 ^b	55.0*
TC	49.3	44.3 ^B	50.5	48.5 ^A	53.5	60.0 ^a	50.0	56.8 ^B	48.8	56.3 ^B	39.0 ^b	47.8 ^B	53.8 ^a	45.0 ^B	41.0 ^b	57.0 ^a
TN	49.8 ^a	39.3	42.3 ^b	43.7	45.0 ^b	47.7 ^B	50.8 ^a	50.0 ^{AB}	48.3 ^{ab}	53.0 ^a	42.8 ^a	68.0 ^a	37.8 ^b	38.0 ^B	41.0 ^{ab}	40.7 ^B
Zin	47.5	42.3	47.3*	42.3	48.5	47.5 ^C	48.0	49.0 ^B	47.5	53.0 ^a	41.3	51.3 ^a	44.8	47.3 ^B	41.3	43.5 ^C
White																
Chardonnay	50.0*	44.5	50.8*	42.5	49.0	50.3	45.3	45.5	47.0	51.0*	17.5 ^b	21.8 ^B	25.0 ^a	26.3 ^A	18.5 ^b	21.0 ^B
WhiteChenin B	55.0 ^a	45.3 ^B	47.5 ^b	48.8 ^A	49.8 ^a	57.3 ^a	50.5 ^a	50.0 ^B	45.5 ^b	49.8 ^B	25.8 ^b	34.8*	33.3 ^a	32.8	35.5 ^a	31.3
Colombard	48.8	45.3	46.5	44.8	49.0 ^{ab}	51.8	52.3 ^a	52.5	45.5 ^b	50.8*	28.8	43.8 ^a	27.5	33.8 ^B	25.3	35.8 ^B
Gewurzt	48.3	44.5	47.0*	42.8	46.0	48.5 ^A	44.0*	41.5 ^B	45.0	49.0 ^a	23.3 ^{ab}	22.5 ^B	26.0 ^a	27.0 ^A	21.5 ^b	24.0 ^{AB}
Muscat A	51.8*	43.5	47.3	48.5	52.0 ^a	56.0 ^A	45.8 ^{ab}	46.3 ^B	45.3 ^b	53.8 ^a	30.5 ^{ab}	44.8 ^a	24.5 ^b	32.0 ^B	31.0 ^a	32.0 ^B
Muscat B	46.0*	39.8 ^B	43.3	42.8 ^A	53.0 ^a	51.0	48.0 ^b	47.5	47.5 ^b	50.0*	21.5 ^{ab}	21.8	26.0 ^a	26.5	17.5 ^b	24.0*
Pinot G	44.8	43.5 ^A	43.5*	40.3 ^B	54.8*	48.3 ^B	52.3	49.5 ^{AB}	47.5	51.8 ^a	17.5 ^c	24.0 ^B	30.3 ^b	28.0 ^A	36.0 ^a	22.0 ^B
Semillon	50.3 ^a	45.5	44.5 ^b	46.5	54.3 ^a	57.8 ^A	52.5 ^a	49.5 ^B	46.5 ^b	48.3 ^B	17.5 ^{ab}	15.0 ^B	23.5 ^a	25.0 ^A	18.3 ^b	26.0 ^a
Sauvignon B	52.8*	42.5	47.3	44.5	44.5 ^b	47.5 ^a	49.8 ^a	49.0 ^A	43.3 ^b	45.5 ^B	18.0 ^c	23.5 ^B	25.3 ^b	23.8 ^B	37.3 ^a	29.3 ^A
Riesling	45.8	42.0 ^B	45.5	45.8 ^A	52.5	58.3 ^A	50.0	49.5 ^B	46.8	50.3 ^B	26.3 ^b	24.3 ^B	28.3 ^b	33.3 ^a	34.5 ^a	34.3 ^A

*indicates significant differences between locations within the same season. a, b, and c indicate significant differences between the seasons at Ramon vineyard. A, B, and C indicate significant differences between seasons at Ramat Negev vineyard. Data are the mean value of four replicates (n = 4). The data of bud break to fruit set are from 2017 and 2018.

Pinot Noir in MR and Grenache Noir, Malbec, Merlot, Cabernet Sauvignon, and Cabernet Franc in RN were the most sensitive to differences between seasons (Supplementary Figure 2).

The Greatest Differences Between Vineyard Locations in Fruit Organic Acids Were Observed at Véraison and Their Level of Variance Differed Between Seasons

The change in malate, tartrate, and citrate was seasonal and cultivar dependent (Figures 8, 9). The greatest differences were observed in 2017, when, at véraison, higher OA were measured at RN compared to MR, but no marked differences between locations were scored at harvest; the higher content of malate in white berries at RN was an exception among OA (Figure 8). In

2018 and 2019 seasons, OA in white berries, at both véraison and harvest, were not affected by location, excluding citrate at harvest in 2019, which was higher at RN (Figure 8K). In contrast, malate and tartrate levels were considerably high at MR compared to RN in 2018 in red berries at véraison, but no marked differences between locations were identified at harvest in all seasons. At harvest, tartrate (in 2018) and citrate (in 2019) in red berries were exceptionally high at RN (Figure 8). Hierarchical clustering of 2017 and 2018 data highlighted the segregation not only between the seasons but also between vineyards, particularly for white cultivars (Figures 9A,B). In 2017, white cultivars at RN separated from MR, with the exception of Muscat Alexandria, Riesling, and Semillon, cultivars with low acid accumulation at véraison and similar to the levels measured at MR vineyard (Figure 9A). Red cultivars exhibited considerable plasticity at véraison in their OA concentrations that varied substantially between seasons, with the

exception of Grenache Noir, Sangiovese, Merlot, Petit Verdot, Cabernet Franc, and Dolcetto, whereas location-clusters were clearly discerned within each season-cluster (Figure 9B).

The pace of OA decrease from véraison to harvest considerably varied between vineyards and between seasons in each vineyard (Figure 9 and Supplementary Table 5). For example, in 2017, the average OA reduction in red berries was almost twice greater at the warmer RN compared to MR (Supplementary Table 5). OA reduction was significantly moderate in the white cultivars compared to the reds at RN. Comparing vineyards, the overall OA reduction among white cultivars at RN was greater than at MR. On the contrary, in 2018, the reduction of malate and tartrate in red berries was smaller at RN than at MR, whereas no differences between locations were observed in the white cultivars. Generally, cultivars grown at MR exhibited lower pulp OA concentration at véraison and reached harvest with minimum loss of acidity (Figure 9 and Supplementary Table 5). Moreover, white cultivars appeared to be rigid in their OA degradation compared to the red ones.

Pulp Sugar Differences Were Predominantly Expressed in Hexose/Sucrose Ratio and Were Largely Affected by Cultivar and Location

Statistical analysis of pulp sugars at véraison revealed significant location effects (Supplementary Figure 1), but the effect of cultivar differed between white and red berries; sugars did not differ significantly between white cultivars with exception of sucrose in 2018. The mean pulp sugars of all cultivars ranged from 32 to 54 mg g⁻¹ DW, with considerable variation among cultivars and locations (Figure 10 and Supplementary Table 8). The greatest differences in pulp sugars of white cultivars were observed between locations in 2017; across all white cultivars, the average fructose and sucrose concentrations were significantly higher at MR, in contrast with glucose, which was higher at RN (Figure 10C). Notably, in 2018 and 2019 seasons, pulp sugars of white cultivars at véraison did not differ between locations, excluding glucose in 2019, which was higher at RN (Figure 10C). Hexose/sucrose ratio was significantly higher at RN vineyard in 2017 and 2018 seasons (Figure 10G) with significant cultivar × location interaction effect in 2018 (Supplementary Material 1).

Among the red cultivars at véraison, fructose was significantly affected by cultivar and location interaction ($P < 0.0001$) in 2017 (Supplementary Material 1), while sucrose showed this course both in 2017 and 2018 seasons. Fructose concentration was significantly higher at RN only in 2018 (Figure 10B), while glucose exhibited this trend throughout all three seasons (Figure 10D). In contrast, sucrose was significantly lower at RN in 2017 and 2019 (Figure 10F). Hexose/sucrose ratio at the red cultivars' véraison was predominantly affected by cultivar and location (Supplementary Material 1). Noteworthy, this ratio was significantly higher at warmer RN than cooler MR in all seasons (Figure 10H).

Among white cultivars at harvest, the two-way ANOVA in each season revealed no significant effect of cultivars on

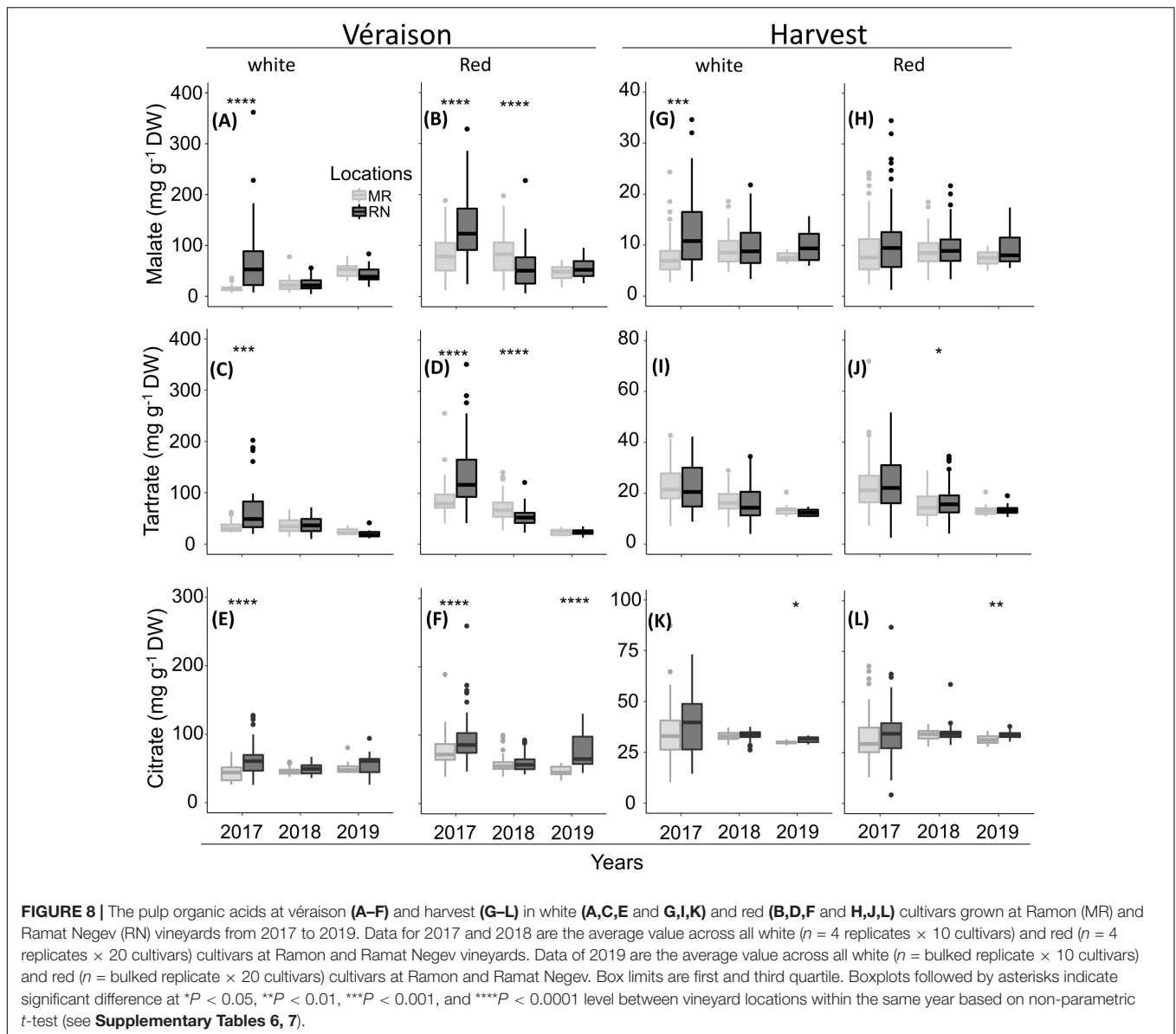
pulp sugars except for sucrose in 2018, which was significantly affected by cultivars and location interaction (Supplementary Material 1). Fructose was not affected by location in all seasons (Figure 11A). However, the location had a significant effect on sugars, particularly in 2018. The average sucrose (in 2018) and glucose (in 2019) across all cultivars were significantly higher at MR and RN, respectively (Figures 11C,E), whereas hexose/sucrose ratio was significantly high only in 2018 (Figure 11G). Among white cultivars, only Chenin Blanc and Pinot Gris showed a significant difference between locations (Figure 11I).

In the red cultivars, pulp hexoses were significantly higher at RN in 2017 and 2019, but not in 2018 (Figures 11B,D), while sucrose was lower at RN in 2018 (Figure 11F). Overall, pulp sugars at harvest tended to be lower in 2018 in both red and white cultivars, compared to the two other years. Only sucrose showed significant varietal differences ($P < 0.0001$), whereas fructose and glucose were significantly affected by location (Supplementary Material 1), being consistently lower at MR vineyard (Figures 11B,D). Subsequently, the mean hexose/sucrose ratio ranged from 1.88 to 2.25, exhibiting highly significant varietal differences ($p < 0.0001$). In addition, differences between locations were highly significant ($p < 0.0001$), with higher ratios at RN (Figure 11H). Cultivars Petit Verdot, Merlot, and Grenache Noir displayed opposite conduct, as well as higher mean hexose/sucrose ratio (Figure 11J). In 2019, hexose/sucrose ratios were remarkably extreme to both ends compared to the former two years.

Principal Component Analysis Highlights Responsive and Non-responsive Cultivars to Location and Season Differences

PCs were analyzed and plotted using OA and main sugar harvest data of 2017 and 2018. A two-way ANOVA (Supplementary Material 2) was performed using PCA scores for each cultivar. The analysis resulted in four groups of cultivars: (i) cultivars not affected by location and season (non-responsive cultivars) (Supplementary Figure 3A), (ii) cultivars affected by season and location (Figure 12B), (iii) cultivars affected by location and season interaction (Figure 12C), and (iv) cultivars affected by season only (Supplementary Figure 3D). Then, four sets were plotted on PCs (Figures 12A–D).

In the non-responsive cultivars' PCA (Figure 12A), cultivars were separated both on PC1 and PC2, due to the positive contribution of sugars (Supplementary Material 2) on PC1 and the inverse contribution of malate and citrate on PC2 (Supplementary Material 2). In PCA of cultivars affected by location and season (Figure 12B), samples were separated both on PCs, due to the positive contribution of tartrate, citrate, fructose, and sucrose on PC1 (Supplementary Material 2) and due to the inverse contribution of malate on PC2. In PCA of cultivars affected by location and season interaction (Figure 12C), 2017 and 2018 samples were separated on PC1, explaining 59.4% of the total variance due to the contribution of citrate, fructose, and sucrose (Supplementary



Material 2). Malate was the major negative contributor to PC2. In season-responsive cultivars' PCA (**Figure 12D**), PC1 represented 59.4% of the total variance and separated 217 samples from 2018 mainly due to the positive contribution of citrate, tartrate, fructose, and sucrose (**Supplementary Material 2**). Malate was the major positive contributor to PC2 (**Supplementary Material 2**).

DISCUSSION

One of the most striking patterns of phenological changes over the past two decades due to the rising temperature is the earlier onset of phenological events (Jones and Davis, 2000; Duchêne et al., 2010; van Leeuwen and Darriet, 2016; Piao et al., 2019). Mimicking this shift by setting experimental plots

in two vineyards, differing in their mean daily temperature by 1.5°C, we showed earlier onset of bud break, fruit set, and véraison at the warmer RN vineyard with greater variations between the seasons in the timing of harvest than in the cooler MR vineyard (**Supplementary Figure 1**). Such changes might influence the véraison-harvest time-window, imposing significant consequences on berry ripening and engustment and, subsequently, on wine quality (Morales-Castilla et al., 2020), hence defining the suitability of a given cultivar to a certain region. For instance, the date of harvest among white varieties was considerably earlier in warmer RN vineyard, while red cultivars were more affected by the season, and within each group, a gradient between early and late-ripening varieties was recorded (**Figure 5**). Rapid phenological progress in warmer climates might provide better yield quality for many white cultivars (e.g., Chenin Blanc), shifting bloom and véraison

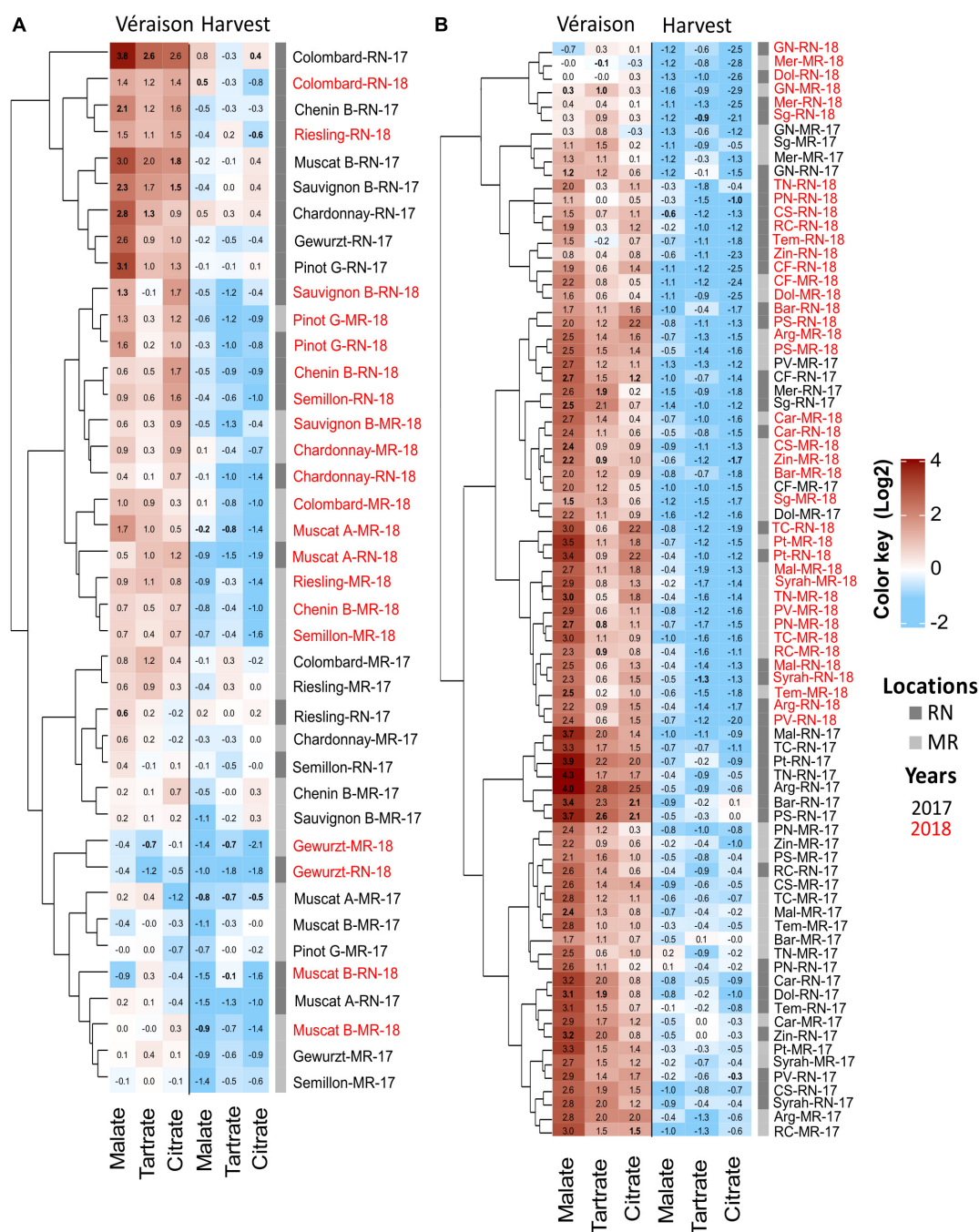
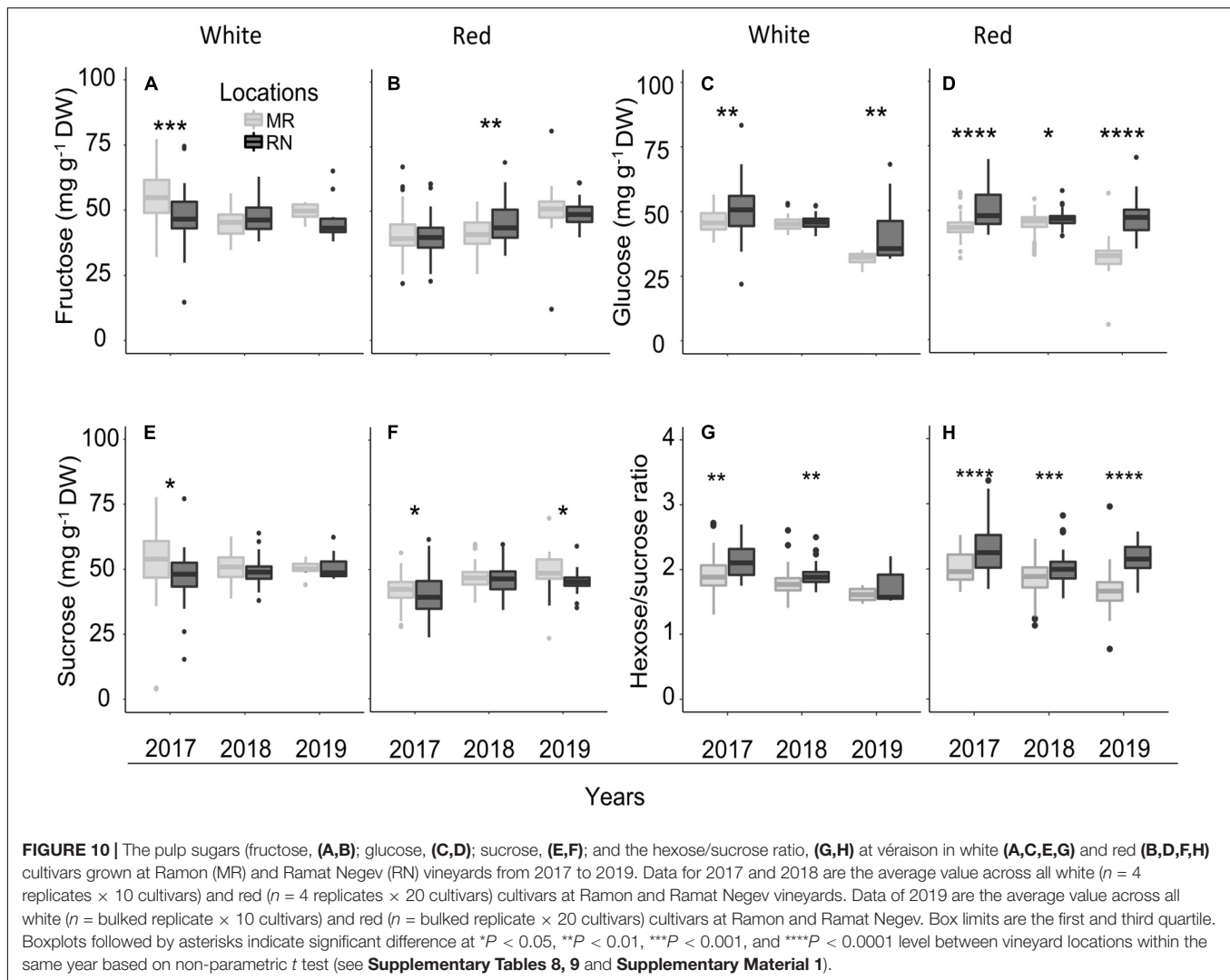


FIGURE 9 | Heatmap of pulp organic acids in white (A) and red (B) grapes. The heatmap was generated using the mean value of four biological replicates following normalization to the median of each metabolite on all cultivars and log2 transformation. Cultivar names are composed by vineyard abbreviations (MR and RN) followed by vintage (17 or 18). Cultivar names with black and red color indicate samples collected in 2017 and 2018, respectively. MR, Ramon; RN, Ramat Negev. Red and blue rectangles represent an increase and decrease of metabolite relative to the median (see **Supplementary Material 1** and **Supplementary Tables 6, 7**).

earlier to cooler months, thus shortening the exposure of the fruit-ripening phase to prevent potentially detrimental heat effects during the summer. The situation was more complex, however, with the red cultivars, most of which displayed a longer duration of the fruit-ripening phase, required to reach a

higher Brix threshold and simultaneously accomplish the desired engustment. In temperate climates, a shift to earlier fruit set and development might bring the fruit-ripening phase directly into the warmest summer period (Sadras and Moran, 2013), which is clearly disadvantageous.



The relationships between the seasonal course and berry phenology is a key element determining fruit quality in a given year. For instance, vintage was shown to be a predominant factor affecting grape and wine composition of Cabernet Sauvignon and Shiraz berry (Antalick et al., 2020); in Merlot, the metabolic response to post-véraison water deficit was consistently affected by interseason weather variability (Herrera et al., 2017). In the present study, interseasonal variability had a significant effect on the timing of phenological events; 2018 data were clearly different from those of 2017 and 2019 (Figure 5), mainly due to (i) earlier bud break, an outcome of a warmer winter and earlier spring and (ii) the correlation shown between the onsets of bud break, véraison, and harvest (Figure 6). These results differ from Ruml et al. (2016), who conducted a long-term study of 20 cultivars in Serbia and reported that shifts of berry ripening into warmer conditions resulted from earlier bloom and véraison rather than from the onset of bud break. Nevertheless, the onset of bud break appears exceedingly critical to the time course of berry development and ripening, particularly in arid regions, where year-to-year variations in the winter-spring interphase are

very common. Interestingly, the analysis of coefficient of variance revealed resilient cultivars to the seasonal variation, including Tempranillo and Tinta Cao at MR, Petit Verdot at RN, and Semillon at both sites (Supplementary Figure 1).

The Duration of Berry Development and Ripening Phases Were Extended at the Warmer RN Vineyard

Despite extensive research on grapevine phenology, only a few studies have focused on the interrelations between the onsets of phenological phases (Jones and Davis, 2000; Gladstones, 2011; Tomasi et al., 2011; Bock et al., 2014; Ruml et al., 2016). Climate conditions might lead to substantial asynchrony during development. For instance, heat treatments right after fruit set (at the fruit herbaceous stage) delayed the onset of véraison (Lecourieux et al., 2017). Furthermore, weather events and characteristics of each phenological phase have important consequences on berry development. Hence, the duration of each phase should be considered (Jones and Davis, 2000). An example

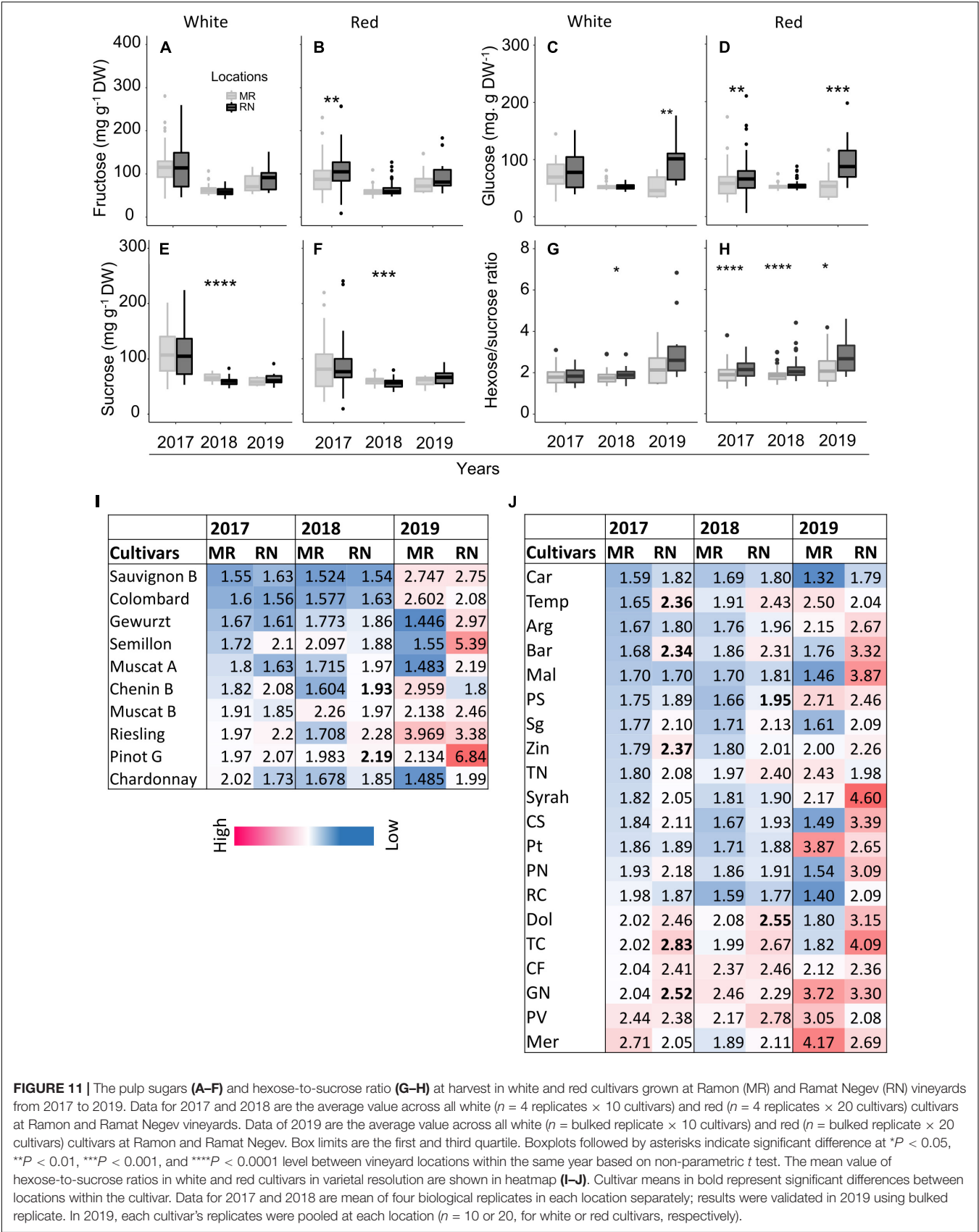


FIGURE 11 | The pulp sugars (A–F) and hexose-to-sucrose ratio (G–H) at harvest in white and red cultivars grown at Ramon (MR) and Ramat Negev (RN) vineyards from 2017 to 2019. Data for 2017 and 2018 are the average value across all white ($n = 4$ replicates \times 10 cultivars) and red ($n = 4$ replicates \times 20 cultivars) cultivars at Ramon and Ramat Negev vineyards. Data of 2019 are the average value across all white ($n =$ bulked replicate \times 10 cultivars) and red ($n =$ bulked replicate \times 20 cultivars) cultivars at Ramon and Ramat Negev. Box limits are the first and third quartile. Boxplots followed by asterisks indicate significant difference at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$ level between vineyard locations within the same year based on non-parametric t test. The mean value of hexose-to-sucrose ratios in white and red cultivars in varietal resolution are shown in heatmap (I–J). Cultivar means in bold represent significant differences between locations within the cultivar. Data for 2017 and 2018 are mean of four biological replicates in each location separately; results were validated in 2019 using bulked replicate. In 2019, each cultivar's replicates were pooled at each location ($n = 10$ or 20, for white or red cultivars, respectively).

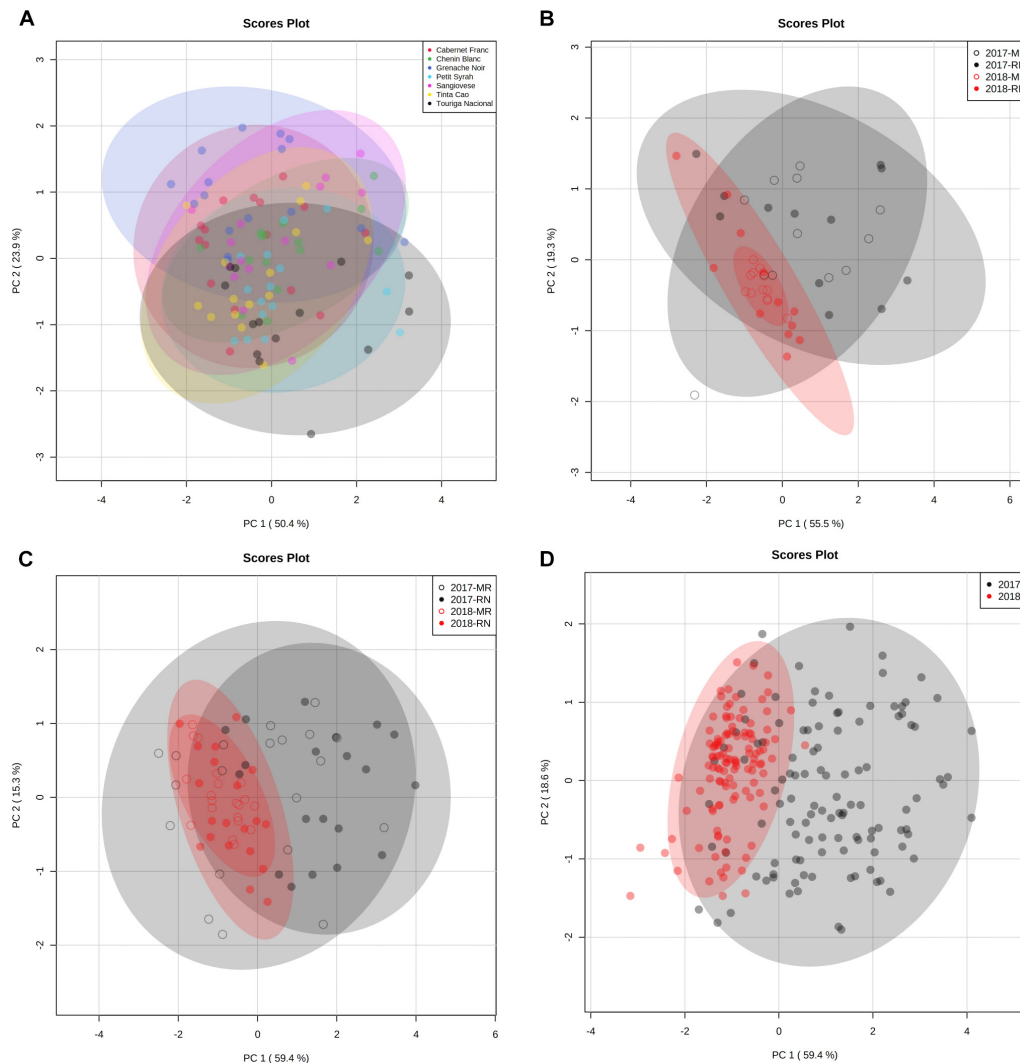


FIGURE 12 | Principal component analysis (PCA) of grapevine cultivars based on berry organic acids and sugar data at harvest in 2017 and 2018 seasons. PCA was first plotted for each cultivar (data are not shown) and a two-way ANOVA model (**Supplementary Material 2**) was performed using PCA scores for each cultivar separately. The analysis resulted in the identification of four subsets of cultivars that were used in separate PC plots. The ellipse indicates 95% confidence region based Hotelling's T^2 test. **(A)** PCA of cultivars that were not affected by location nor by season. The set includes in the red Cabernet Franc, Grenache Noir, Petit Syrah, Sangiovese, Tinta Cao, and Touriga Nacional and in the white Chenin Blanc. **(B)** PCA of cultivars affected by location and season. The set includes in the red Ruby Cabernet and in the white Muscat Alexandria and Colombard. **(C)** PCA of cultivars affected by the interaction of location and season. The set includes in the red Barbera, Dolcetto, Petit Verdot, and Pinot Noir and in the white Riesling. **(D)** PCA of cultivars affected by season. The set includes in the red Argaman, Cabernet Sauvignon, Carignan, Malbec, Merlot, Pinotage, Syrah, Tempranillo, and Zinfandel and in the white Chardonnay, Gewurztraminer, Muscat Blanc, Pinot Gris, Semillon, and Sauvignon Blanc. The PCs were generated using the raw data of four biological replicate in 2017, 2018, and 2019 seasons following log transformation and Pareto scaling. RN, Ramat Negev; MR, Ramon.

of the climate effect on phenological intervals is the vegetative phase from bud break to fruit set, which is susceptible in the temperate regions to frost and hailstorm (Davenport et al., 2008) and to heatwaves in Mediterranean regions (Webb et al., 2010; Arrizabalaga-Arriazu et al., 2020). The longer the period, the higher the chances to incur into environmental constraints. This interval is usually shorter under high temperatures due to a rapid phenological pace (Tomasi et al., 2011). Therefore, a shorter vegetative phase was expected at the warmer RN vineyard. This was confirmed for all white and red cultivars, with exception of

Colombard and Riesling among whites and Pinot Noir, Ruby Cabernet, Syrah, and Tinta Cao, which were not affected by the location (Figure 7).

A Prolonged Pre-véraison Interval Can Expose the Cluster to Recurrent Heatwaves

Gladstones (2011) emphasized the susceptibility of grapevine berries to excessively high temperatures during the fruit growth

phase, from fruit set to véraison. Direct exposure of clusters to sunlight was shown to reduce methoxyypyrazine accumulation by 21–44% (Ryona et al., 2008). Excessive heat decreased malate and increased concentrations of amino acids (Gouot et al., 2019), many of which participate in wine aroma biosynthesis (Garde-Cerdán and Ancín-Azpilicueta, 2008; Gutiérrez-Gamboa et al., 2020). Considering the intermittent heat waves that characterize the spring in arid regions (April–May, northern hemisphere), the relative duration of this phase is assumed to significantly affect berry quality traits (Gouot et al., 2019). The longer the phase, the higher the risk of high temperature events to imbalance the accumulation of precursors for aroma and quality-related compounds, consequently affecting the final wine quality. In the present study, several cultivars displayed significantly shorter fruit growth phase, among which were Dolcetto, Petit Syrah, Pinot Noir, and Tempranillo within the red cultivars and Sauvignon Blanc among the white cultivars (Table 2). The majority of the red cultivars examined exhibited considerable extension of the fruit growth phase at the warmer RN site, with only few inconsistent exceptions. Among the white cultivars, Chardonnay, Muscat Blanc, Sauvignon Blanc, and Semillon displayed relatively small phenological shifts in this stage (Figure 7 and Table 2). A relatively rigid duration, manifested by a small phenological shift between locations, may indicate a degree of genetic resilience. Nevertheless, the direct contribution of shorter or rigid duration of the fruit growth phase to the final berry or wine quality strongly depends on the consecutive fruit-ripening phase and requires further research. In addition, it is possible to suggest that the difference in hydric behavior between cultivars may provide an explanation for the differences in berry ripening and, furthermore, in the tendency for premature dehydration (Gutiérrez-Gamboa et al., 2019). For instance, Chardonnay was reported as an anisohydric variety, while Sauvignon Blanc as an isohydric variety (Gutiérrez-Gamboa et al., 2019). Having that said, further investigation is needed on the physiology of the different varieties to draw solid conclusions.

Post-véraison at the Warmer RN Vineyard Might Lead to Metabolic Disorders

The fruit-ripening phase, from véraison to harvest, determines the sugar/acid balance and engustment in the developing berry (Van Leeuwen et al., 2019; Morales-Castilla et al., 2020). Opposite to milder climates as Bordeaux, where longer and warmer growing seasons provide greater ripening potential (Jones and Davis, 2000), an extended fruit-ripening phase under the much higher temperature regime characterizing arid regions might lead to disorder in sugar accumulation, phenylpropanoid degradation, and sunburns (Greer and Weedon, 2013; Pastore et al., 2017). Under temperate climate regions, warmer seasons were associated with a shortened ripening phase (Tomasi et al., 2011; Alikadic et al., 2019). In the present study, the fruit-ripening phase significantly extended at the warmer RN vineyard (Table 2). This discrepancy can be easily explained in terms of an optimum temperature curve that the complex fruit-ripening

process obeys. Accordingly, berry ripening is hastened by increasing temperatures up to a maximum threshold, above which temperature becomes stressful and ripening is delayed or even prevented. Thus, supraoptimal temperatures during July–August in arid regions might slow down or even restrain carbon assimilation and sugar translocation rates (Greer and Weston, 2010; Mira de Orduña, 2010), problems that hardly occur in temperate regions. Here emerges a significant advantage of MR vineyard, where the temperature regime is relatively milder than at RN (Figure 3) and, in most of the cases, the fruit-ripening phase was shorter (Table 2 and Figure 7).

Having said that, considerable differences in the duration and in the phenological shift of the fruit-ripening phase occurred between the white and the red groups, as well as between individual cultivars within each group. White cultivars had significantly shorter fruit-ripening phase, ranging from 22 to 30 days, compared to 36–47 days in the red cultivars (Table 2). Thus, most of the white cultivars reached harvest during July, avoiding considerable portions of the mid-summer heat, with Chardonnay, Gewurztraminer, Muscat Blanc, and Semillon displaying particularly shorter ripening phases (Table 2). Yet, shorter ripening periods do not guarantee high fruit or wine quality, as the development of berry engustment may require adequate time. Harvest of the red cultivars usually occurred during August, with large differences between cultivars. While no specific red cultivars showed ripening phases adequately short to avoid the mid-summer heat, several cultivars (Petit Verdot, Malbec, Cabernet Franc, Cabernet Sauvignon, and Petit Syrah) exhibited relatively small or sometimes even opposite phenological shift, as opposed to the hyper-sensitive Tempranillo, which consistently displayed the largest shift (Figure 7). Red cultivars displaying high-temperature resilience are suitable candidates for the warmer edge of viticulture regions, as long as other conditions essential to ensure productivity and quality are satisfied. In contrast, several red cultivars such as Barbera, Dolcetto, Pinot Noir, Ruby Cabernet, Tempranillo, and Zinfandel often failed to reach the desired Brix threshold or engustment. This interruption of the ripening process, often accompanied by berry shriveling and collapse of the cluster (data not shown), was more frequent at RN, but did not occur every year among all cultivars mentioned.

Correlations between the onsets of phenological phases may be useful for the prediction of the harvest date, provided a stable phenological course. Practically, attempts to predict harvest date according to bud break onset were not successful in temperate regions (Jones and Davis, 2000; Tomasi et al., 2011). Furthermore, in the unpredictable course of winter and spring weather in arid regions, the relationships between the onsets of bud break and harvest were very poor, particularly among the red cultivars (Figure 6F). The predominant source of variation was clearly identified in the fruit-ripening phase (Figure 6M), suggesting that this is where red cultivars' suitability to arid regions should be evaluated.

Organic Acids and Sugars

Temperatures alter malate content in a developmental manner (Sweetman et al., 2014; Rienth et al., 2016). During pre-véraison,

malate content accumulates with increasing temperature, while an inverse relation is found during ripening (Famiani et al., 2014; Sweetman et al., 2014). In the present study, contrasting results between seasons were shown for red cultivars, while white grape berry acids showed differences between locations only in 2017. These results suggest that additional factors are involved in regulating malate homeostasis in the fruit.

During ripening, major OA levels in the berry are known to decrease at a pace dependent on the genotype and the environment (Liu et al., 2006; Bigard et al., 2018). Several studies have shown a positive relationship between the loss of malate and elevated temperature (Sweetman et al., 2014; Rienth et al., 2016; Lecourieux et al., 2017). In line with what has been shown previously, the pace of OA degradation was more pronounced in warmer RN and during the hottest vintage 2017. In addition, OA in berries of the white cultivars, mostly malate, tended to be higher when the harvest date, which was determined by total soluble solid (TSS), was earlier, suggesting that the early ripening of white cultivars might better fit in hot climates. Having that said, OA concentration in berries of both white and red cultivars at harvest was predominately affected by cultivar or by the interaction of cultivar and location.

Lecourieux et al. (2017) have shown that pre-véraison heat treatment slows down sugar accumulation, due to down-regulation of sugar transporter genes that resulted in a delay of véraison onset. In the present study, this phenomenon of delayed véraison onset was absent in almost all cultivars, white cultivars in particular, most of which escape the extremely high temperatures of July–August. Still, pulp sugar composition at véraison was subject to significant varietal influences. The differences between sites in hexoses at véraison were seasonal dependent, whereas sucrose was higher in content at cooler MR site at véraison (in 2017 and 2019) and harvest (in 2018). Sucrose, in particular, displayed strong interactions between cultivar and location, confirming the significant plasticity harbored in *V. vinifera* concerning sugar metabolism toward véraison (Ollat et al., 2018). In addition, the overall, pulp sugars at harvest tended to be lower in 2018 (a year with warmer spring season) in both red and white cultivars, compared to the two other years. It is conceivable that sugar transport/accumulation is modulated already early in the season as it was shown in Merlot and Cabernet Sauvignon vines by Herrera et al. (2017); Lecourieux et al. (2017). In a different study, it was shown that early drought led to an increase in anthocyanin accumulation during ripening (Castellarin et al., 2007). We can hypothesize that early heatwaves could impose enhanced sugar catabolism in the berries toward the secondary compounds and anticipate ripening.

Hexose/sucrose ratio (HSR) is an indicator of the conversion and use of the translocated sugar, sucrose, for the metabolism of developing organs. HSR is particularly useful evaluating the performance of hexose-accumulating organs, such as the pulp of a ripening grape berry. Contrasting results are found in the literature in respect to sugar accumulation and temperature in Tempranillo berries. For instance, under controlled environment, heat treatment (28°C/18°C day/night) hastened sugar accumulation rate and significantly shortened the ripening length (Martínez-Lüscher et al., 2016b). In other

studies, heat was reported to slow berry ripening in Semillon (Greer and Weedon, 2013) and in Muscat Hamburg (Carbonell-Bejerano et al., 2013). However, most of the studies investigating temperature effects on berry ripening focused on a single or very few cultivars, which may explain the unequivocal results. Furthermore, the temperature ranges studied there were much lower than those characterizing the exceptional viticulture regions explored in the present study. Our results suggest that the enzymatic apparatus responsible for sugars metabolism in the ripening fruit is highly sensitive to the temperature regime, and moreover, it may substantially differ from one cultivar to another (Basson et al., 2010; Božović et al., 2019). The higher HSR at the warmer site suggests that the stage of sucrose conversion to hexoses is thermophilic in most cultivars, but not in all. In contrast, the extension of the ripening phase and the consequent delay of harvest in many red cultivars may suggest that the foliar photosynthetic activity is damaged or inhibited under high temperature regime (Haldimann and Feller, 2004). Additionally, sucrose translocation may be significantly slowed down under high temperatures (Julius et al., 2017). The extension of the ripening phase may, in turn, lead to prolonged exposure of the berries to potentially harmful heat stress and, eventually, to berry shriveling and cluster collapse, the severity of which depends on many other berry traits (e.g., skin properties, water relations, etc.).

CONCLUSION

The present study offers a unique large-scale varietal perspective of the consequences that an apparently small difference in the seasonal mean daily temperature, about 1.5°C, may induce on wine grapevine performance and berry primary metabolism. Considerable topographic gaps over small geographic distances may bring about significantly different temperature regimes on a calendric scale, eventually creating distinct terroirs within a superficially homogeneous viticulture climate region. Despite earlier onset of phenological events, and in contrast to accelerated vegetative development, berry ripening was significantly slower at warmer RN. In sensitive varieties, berries' Brix failed to increase adequately, probably due to slower sucrose influx. In addition, the organic acids were rapidly degraded and HSR increased. Subsequently, harvest was delayed and was accompanied by low fruit quality indices.

Beyond the clear common responses to high temperature of grapevines and berry development that emerge from the present study, significant differences have occurred between the white and red groups of cultivars, as well as among cultivars within each group. Earliness seems an advantage for the white cultivars, with a much shorter ripening phase and hence avoidance of the warmest part of the season. The warmer site conditions have challenged most of the red cultivars, some of which even failed to reach adequate quality standards of ripening. Others, in contrast, exhibited impressive resilience to high temperature. Beyond cultural practices, such as shading nets and modified trellising, a careful selection of cultivars, well adapted to warm conditions, should be the utmost tool of the wine industry to meet the global climate challenge. Further research is required,

however, to unravel the particular traits that make a cultivar suitable to warm conditions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

EH, AB, AF, and ED conceived and planned the study. KG, NS, and AB collected the berry samples in the field. AB collected the meteorological data. KG analyzed the meteorological data, prepared the berry samples for extraction, performed the sample extraction and data analysis and analysis using the GC-MS device, and wrote the body of the manuscript with AF and AB. All authors reviewed and approved the manuscript.

REFERENCES

- Alikadic, A., Pertot, I., Eccel, E., Dolci, C., Zarbo, C., and Caffarra, A. (2019). The impact of climate change on grapevine phenology and the influence of altitude: A regional study. *Agric. For. Meteorol.* 271, 73–82. doi: 10.1016/j.agrformet.2019.02.030
- Anderson, K., and Aryal, N. R. W. (2013). *Which winegrape varieties are grown where: A global Empirical picture*. Australia: University of Adelaide Press.
- Antalick, L. M., Šuklje, K., and Deloire, J. W. (2020). Cultivar, site or harvest date: the gordian knot of wine terroir. *Metabolomics* 16:52.
- Arrizabalaga-Arriazu, M., Morales, F., Irigoyen, J. J., Hilbert, G., and Pascual, I. (2020). Growth performance and carbon partitioning of grapevine tempranillo clones under simulated climate change scenarios: elevated CO₂ and temperature. *J. Plant Physiol.* 252:153226. doi: 10.1016/j.jplph.2020.153226
- Bergqvist, J., Dokoozlian, N., and Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Vitic.* 52, 1–7. doi: 10.1130/spe234-p1
- Basson, C. E., Groenewald, J. H., Kossmann, J., Cronjé, C., and Bauer, R. (2010). Sugar and acid-related quality attributes and enzyme activities in strawberry fruits: Invertase is the main sucrose hydrolysing enzyme. *Food Chem.* 121, 1156–1162. doi: 10.1016/j.foodchem.2010.01.064
- Bernardo, S., Dinis, L. T., Machado, N., and Moutinho-Pereira, J. (2018). Grapevine abiotic stress assessment and search for sustainable adaptation strategies in Mediterranean-like climates. *Rev. Agron. Sustain. Dev.* 38:66.
- Bigard, A., Berhe, D. T., Maoddi, E., Sire, Y., Boursiquot, J.-M., Ojeda, H., et al. (2018). Vitis vinifera L. Fruit Diversity to Breed Varieties Anticipating Climate Changes. *Front. Plant Sci.* 9, 1–16. doi: 10.1080/15538362.2020.1833809
- Bock, A., Sparks, T., Estrella, N., and Menzel, A. (2011). Changes in the phenology and composition of wine from Franconia, Germany. *Clim. Res.* 50, 69–81. doi: 10.3354/cr01048
- Božović, P., Rogiers, S., and Deloire, A. (2019). Hexose efflux from the peeled grape berry. *Oeno One* 53, 249–260.
- Bock, A., Sparks, T. H., Estrella, N., Jee, N., Casebow, A., Schunk, C., et al. (2014). Changes in first flowering dates and flowering duration of 232 plant species on the island of Guernsey. *Glob. Chang. Biol.* 20, 3508–3519. doi: 10.1111/gcb.12579
- Carbonell-Bejerano, P., Santa María, E., Torres-Pérez, R., Royo, C., Lijavetzky, D., Bravo, G., et al. (2013). Thermotolerance responses in ripening berries of vitis vinifera L. cv muscat hamburg. *Plant Cell Physiol.* 54, 1200–1216. doi: 10.1093/pcp/pct071

FUNDING

This project was partially supported by the Chief Scientist Fund of the Israeli Ministry of Agriculture and the Israeli Wine Grapevine Board (Grant No. 29-01-0007).

ACKNOWLEDGMENTS

We would like to thank Destayehu Semaneh, Chao Song, Alon Shlisser, Dong Shuo, Khadijah Ayarnah, Yaara Zohar, Dr. Noam Reshef, Dr. Moses Kwame, Mais Dzharafarov, Angelica Shapiro, Tania Acuna, Maria Dolores, Millena Oliveira, BichThao Nguyen, and David for their support in the field and lab.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.588739/full#supplementary-material>

- Carroll, J. E., and Wilcox, W. F. (2003). Effects of humidity on the development of grapevine powdery mildew. *Phytopathology* 93, 1137–1144. doi: 10.1094/phyto.2003.93.9.1137
- Carlsaw, D. C., and Ropkins, K. (2012). Openair - An R package for air quality data analysis. *Environ. Model. Softw.* 27–28, 52–61. doi: 10.1016/j.envsoft.2011.09.008
- Castellarin, S. D., Matthews, M. A., Di Gasparo, G., and Gambetta, G. A. (2007). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101–112. doi: 10.1007/s00425-007-0598-8
- Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., et al. (2018). MetaboAnalyst 4.0: Towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res.* 46, W486–W494.
- Cleland, E. E., Allen, J. M., Crimmins, T. M., Dunne, J. A., Pau, S., Travers, S. E., et al. (2012). Phenological tracking enables positive species responses to climate change. *Ecology* 93, 1765–1771. doi: 10.1890/11-1912.1
- Coombe, B. G. (1995). Growth Stages of the Grapevine: Adoption of a system for identifying grapevine growth stages. *Aust. J. Grape Wine Res.* 1, 104–110. doi: 10.1111/j.1755-0238.1995.tb00086.x
- Davenport, J. R., Keller, M., and Mills, L. J. (2008). How cold can you go? Frost and winter protection for grape. *HortScience* 43, 1966–1969. doi: 10.21273/hortsci.43.7.1966
- De Cortázar-Atauri, I. G., Duchêne, É., Destrac-Irvine, A., Barbeau, G., De Rességuier, L., Lacombe, T., et al. (2017). Grapevine phenology in France: From past observations to future evolutions in the context of climate change. *Oeno One* 51, 115–126. doi: 10.20870/oeno-one.2016.0.0.1622
- Degu, A., Hochberg, U., Sikron, N., Venturini, L., Buson, G., Ghan, R., et al. (2014). Metabolite and transcript profiling of berry skin during fruit development elucidates differential regulation between Cabernet Sauvignon and Shiraz cultivars at branching points in the polyphenol pathway. *BMC Plant Biol.* 14:1–20.
- Duchêne, E., Huard, F., Dumas, V., Schneider, C., and Merdinoglu, D. (2010). The challenge of adapting grapevine varieties to climate change. *Clim. Res.* 41, 193–204. doi: 10.3354/cr00850
- Eastburn, D. M., McElrone, A. J., and Bilgin, D. D. (2011). Influence of atmospheric and climatic change on plant-pathogen interactions. *Plant Pathol.* 60, 54–69. doi: 10.1111/j.1365-3059.2010.02402.x
- Famiani, F., Farinelli, D., Palliotti, A., Moscatello, S., Battistelli, A., and Walker, R. P. (2014). Is stored malate the quantitatively most important substrate utilised by respiration and ethanolic fermentation in grape berry pericarp

- during ripening? *Plant Physiol. Biochem.* 76, 52–57. doi: 10.1016/j.plaphy.2013.12.017
- Fraga, H., Santos, J. A., Malheiro, A. C., and Moutinho-Pereira, J. (2012). Climate change projections for the portuguese viticulture using a multi-model ensemble. *Cienc. Tec. Vit.* 27, 39–48.
- Fraga, H., Santos, J. A., Moutinho-Pereira, J., Carlos, C., Silvestre, J., Eiras-Dias, J., et al. (2016). Statistical modelling of grapevine phenology in Portuguese wine regions: Observed trends and climate change projections. *J. Agric. Sci.* 154, 795–811. doi: 10.1017/s0021859615000933
- Galili, T. (2015). dendextend: An R package for visualizing, adjusting and comparing trees of hierarchical clustering. *Bioinformatics* 31, 3718–3720. doi: 10.1093/bioinformatics/btv428
- Garde-Cerdán, T., and Ancín-Azpilicueta, C. (2008). Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. *LWT Food Sci. Technol.* 41, 501–510. doi: 10.1016/j.lwt.2007.03.018
- Gladstones, J. (1992). *Viticulture and environment*. Adelaide: Winetitles.
- Gladstones, J. (2011). *Wine, terroir and climate change*. Kent Town, South Australia: Wakefield Press.
- Gouot, J. C., Smith, J. P., Holzapfel, B. P., and Barril, C. (2019). Impact of short temperature exposure of *Vitis vinifera* L. cv. Shiraz grapevine bunches on berry development, primary metabolism and tannin accumulation. *Environ. Exp. Bot.* 168:103866. doi: 10.1016/j.envexpbot.2019.103866
- Greer, D. H., and Weedon, M. M. (2013). The impact of high temperatures on *Vitis vinifera* cv. Semillon grapevine performance and berry ripening. *Front. Plant Sci.* 4:1–9. doi: 10.1080/15538362.2020.1804516
- Greer, D. H., and Weston, C. (2010). Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Funct. Plant Biol.* 37, 206–214. doi: 10.1071/fp09209
- Gu, Z., Eils, R., and Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 32, 2847–2849. doi: 10.1093/bioinformatics/btw313
- Gutiérrez-Gamboa, G., Garde-Cerdán, T., Rubio-Bretón, P., and Pérez-Álvarez, E. P. (2020). Study of must and wine amino acids composition after seaweed applications to Tempranillo blanco grapevines. *Food Chem.* 308:125605. doi: 10.1016/j.foodchem.2019.125605
- Gutiérrez-Gamboa, G., Pérez-Donoso, A. G., Pou-Mir, A., Acevedo-Opazo, C., and Valdés-Gómez, H. (2019). Hydric behaviour and gas exchange in different grapevine varieties (*Vitis vinifera* L.) from the Maule Valley (Chile). *South Afr. J. Enol. Vitic.* 40, 1–11.
- Haldimann, P., and Feller, U. (2004). Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant, Cell Environ.* 27, 1169–1183. doi: 10.1111/j.1365-3040.2004.01222.x
- Hall, A., and Jones, G. V. (2010). Spatial analysis of climate in winegrape-growing regions in Australia. *Aust. J. Grape Wine Res.* 16, 389–404. doi: 10.1111/j.1755-0238.2010.00100.x
- Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., et al. (2013). Climate change, wine, and conservation. *Natl. Acad. Sci. U S A* 110, 6907–6912.
- Herrera, J. C., Hochberg, U., Degu, A., Sabbatini, P., Lazarovitch, N., Castellarin, S. D., et al. (2017). Grape metabolic response to postveraison water deficit Is affected by interseason weather variability. *J. Agric. Food Chem.* 65, 5868–5878. doi: 10.1021/acs.jafc.7b01466
- Hochberg, U., Batushansky, A., Degu, A., Rachmilevitch, S., and Fait, A. (2015). Metabolic and physiological responses of shiraz and cabernet sauvignon (*Vitis vinifera* L.) to near optimal temperatures of 25 and 35 °C. *Int. J. Mol. Sci.* 16, 24276–24294. doi: 10.3390/ijms161024276
- Hochberg, U., Degu, A., Fait, A., and Rachmilevitch, S. (2013). Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. *Physiol. Plant* 147, 443–452. doi: 10.1111/j.1399-3054.2012.01671.x
- Huglin, M. (1978). Nouveau Mode d'évaluation des possibilites héliothermiques d'un milieu viticole. *Proc. Symp. Int. Sur. L'Ecol. De La Vigne.* 64, 89–98.
- Jones, G. V., and Davis, R. E. (2000). Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux. *France. Am. J. Enol. Vitic.* 51, 249–261.
- Jones, G. V., White, M. A., Cooper, O. R., and Storchmann, K. (2005). Climate change and global wine quality. *Clim. Change* 73, 319–343. doi: 10.1007/s10584-005-4704-2
- Jones, G. V., Reid, R., and Vilks, A. (2012). Climate, Grapes, and Wine: Structure and suitability in a variable and changing Climate. *Geogr. Wine* 2012, 109–133. doi: 10.1007/978-94-007-0464-0_7
- Julius, B. T., Leach, K. A., Tran, T. M., Mertz, R. A., and Braun, D. M. (2017). Sugar transporters in plants: New insights and discoveries. *Plant Cell Physiol.* 58, 1442–1460. doi: 10.1093/pcp/pcx090
- Keller, M. (2010). Managing grapevines to optimise fruit development in a challenging environment: A climate change primer for viticulturists. *Aust. J. Grape Wine Res.* 16, 56–69. doi: 10.1111/j.1755-0238.2009.00077.x
- Lakso, A. N., and Kliewer, W. M. (1975). The influence of temperature on malic acid metabolism in grape berries. *Plant Physiol.* 56, 370–372. doi: 10.1104/pp.56.3.370
- Lecourieux, F., Kappel, C., Pieri, P., Charon, J., Pillet, J., Hilbert, G., et al. (2017). Dissecting the biochemical and transcriptomic effects of a locally applied heat treatment on developing Cabernet Sauvignon grape berries. *Front. Plant Sci.* 8:53.
- Liu, H.-F., Wu, B.-H., and Fan, P.-G. (2006). Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *J. Sci. Food Agr.* 1243, 1237–1243.
- Lorenzo, M. N., Taboada, J. J., Lorenzo, J. F., and Ramos, A. M. (2013). Influence of climate on grape production and wine quality in the Rías Baixas, north-western Spain. *Reg. Environ. Chang* 13, 887–896. doi: 10.1007/s10113-012-0387-1
- Martinez-Lüscher, J., Kizildeniz, T., Vučetić, V., Dai, Z., Luedeling, E., van Leeuwen, C., et al. (2016a). Sensitivity of Grapevine Phenology to Water Availability, Temperature and CO₂ Concentration. *Front. Environ. Sci.* 4, 1–14.
- Martinez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreola, J., Pascual, I., and Gomès, E. (2016b). Ultraviolet-B alleviates the uncoupling effect of elevated CO₂ and increased temperature on grape berry (*Vitis vinifera* cv. Tempranillo) anthocyanin and sugar accumulation. *Aust. J. Grape Wine Res.* 22, 87–95. doi: 10.1111/ajgw.12213
- Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Res. Int.* 43, 1844–1855. doi: 10.1016/j.foodres.2010.05.001
- Morales-Castilla, I., Cook, B. I., Lacombe, T., Parker, A., Leeuwen, C., Van, Nicholas, K. A., et al. (2020). Diversity buffers winegrowing regions from climate change losses. *Proc. Natl. Acad. Sci. U S A* 117, 2864–2869. doi: 10.1073/pnas.1906731117
- Moriondo, M., Jones, G. V., Bois, B., Dibari, C., Ferrise, R., Trombi, G., et al. (2013). Projected shifts of wine regions in response to climate change. *Clim. Change* 119, 825–839. doi: 10.1007/s10584-013-0739-y
- Ohana-Levi, N., Munitz, S., Ben-Gal, A., Schwartz, A., Peeters, A., and Netzer, Y. (2020). Multiseasonal grapevine water consumption – Drivers and forecasting. *Agric. For. Meteorol.* 280:107796. doi: 10.1016/j.agrformet.2019.107796
- Ollat, N., Cookson, S. J., Destrac-Irvine, A., Lauvergeat, V., Ouaked-Lecourieux, F., Marguerit, E., et al. (2018). Grapevine adaptation to abiotic stress: An overview. *Acta Hort.* 1248, 497–512.
- Parker, A. K., De Cortázar-Atauri, I. G., Van Leeuwen, C., and Chuine, I. (2011). General phenological model to characterise the timing of flowering and veraison of *Vitis vinifera* L. *Aust. J. Grape Wine Res.* 17, 206–216. doi: 10.1111/j.1755-0238.2011.00140.x
- Pastore, C., Santo, S. D., Zenoni, S., Movahed, N., Allegro, G., Valentini, G., et al. (2017). Whole plant temperature manipulation affects flavonoid metabolism and the transcriptome of grapevine berries. *Front. Plant Sci.* 8, 1–16.
- Piao, S., Liu, Q., Chen, A., Janssens, I. A., Fu, Y., Dai, J., et al. (2019). Plant phenology and global climate change: Current progresses and challenges. *Chang. Biol.* 25, 1922–1940. doi: 10.1111/gcb.14619
- R Development Core Team (2017). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: <https://www.r-project.org/>
- Ramos, M. C., and Martínez de Toda, F. (2020). Variability in the potential effects of climate change on phenology and on grape composition of Tempranillo

- in three zones of the Rioja DOCa (Spain). *Eur. J. Agron.* 115:126014. doi: 10.1016/j.eja.2020.126014
- Real, A. C., Borges, J., Cabral, J. S., and Jones, G. V. (2015). Partitioning the grapevine growing season in the Douro Valley of Portugal: accumulated heat better than calendar dates. *Int. J. Biometeorol.* 59, 1045–1059. doi: 10.1007/s00484-014-0918-1
- Reed, G. F., Lynn, F., and Meade, B. D. (2002). Use of Coefficient of Variation in Assessing Variability of Quantitative Assays. *Ntibod. Mediat. Immun.* 9, 1235–1239. doi: 10.1128/CDLI.9.6.1235
- Reshef, N., Fait, A., and Agam, N. (2019). Grape berry position affects the diurnal dynamics of its metabolic profile. *Plant Cell Environ.* 42, 1897–1912. doi: 10.1111/pce.13522
- Reshef, N., Walbaum, N., Agam, N., and Fait, A. (2017). Sunlight modulates fruit metabolic profile and shapes the spatial pattern of compound accumulation within the grape cluster. *Front. Plant Sci.* 8, 1–20.
- Rienth, M., Torregrosa, L., Sarah, G., Ardisson, M., Brillouet, J. M., and Romieu, C. (2016). Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome. *BMC Plant Biol.* 16, 1–23.
- Ruml, M., Korac, N., Vujadinovic, M., Vukovic, A., and Ivanišević, D. (2016). Response of grapevine phenology to recent temperature change and variability in the wine-producing area of Sremski Karlovci, Serbia. *J. Agric. Sci.* 154, 186–206. doi: 10.1017/s0021859615000453
- Ryona, I., Pan, B. S., Intrigliolo, D. S., Lakso, A. N., and Sacks, G. L. (2008). Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. Cv. Cabernet Franc). *J. Agric. Food Chem.* 56, 10838–10846. doi: 10.1021/jf801877y
- Sadras, V. O., and Moran, M. A. (2013). Nonlinear effects of elevated temperature on grapevine phenology. *Agric. For. Meteorol.* 173, 107–115. doi: 10.1016/j.agrformet.2012.10.003
- Sánchez, Y., Martínez-Graña, A. M., Santos-Francés, F., and Yenes, M. (2019). Index for the calculation of future wine areas according to climate change application to the protected designation of origin “Sierra de Salamanca” (Spain). *Ecol. Indic.* 107:105646. doi: 10.1016/j.ecolind.2019.105646
- Santillán, D., Iglesias, A., La Jeunesse, I., Garrote, L., and Sotes, V. (2019). Vineyards in transition: A global assessment of the adaptation needs of grape producing regions under climate change. *Sci. Total Environ.* 657, 839–852. doi: 10.1016/j.scitotenv.2018.12.079
- Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L. T., Correia, C., et al. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Appl. Sci.* 10, 1–28.
- Sweetman, C., Sadras, V. O., Hancock, R. D., Soole, K. L., and Ford, C. M. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65, 5975–5988. doi: 10.1093/jxb/er u343
- Tomasi, D., Jones, G. V., Giust, M., Lovat, L., and Gaiotti, F. (2011). Grapevine phenology and climate change: Relationships and trends in the Veneto Region of Italy for 1964–2009. *Am. J. Enol. Vitic.* 62, 329–339. doi: 10.5344/ajev.2011.10108
- van Leeuwen, C., and Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *J. Wine Econ.* 11, 150–167. doi: 10.1017/jwe.2015.21
- Van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy* 9, 1–20.
- Webb, L. B., Whetton, P. H., and Barlow, E. W. R. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. *Aust. J. Grape Wine Res.* 13, 165–175. doi: 10.1111/j.1755-0238.2007.tb00247.x
- Webb, L., Whiting, J., Watt, A., Hill, T., Wigg, F., Dunn, G., et al. (2010). Managing grapevines through severe heat: A survey of growers after the 2009 summer heatwave in south-eastern Australia. *J. Wine Res.* 21, 147–165. doi: 10.1080/09571264.2010.530106
- Wolkovich, E. M., Cook, B. I., Allen, J. M., Crimmins, T. M., Betancourt, J. L., Travers, S. E., et al. (2012). Warming experiments underpredict plant phenological responses to climate change. *Nature* 485, 494–497. doi: 10.1038/nature11014
- Wolkovich, E. M., García De Cortázar-Atauri, I., Morales-Castilla, I., Nicholas, K. A., and Lacombe, T. (2018). From Pinot to Xinomavro in the world's future wine-growing regions. *Nat. Clim. Chang.* 8, 29–37. doi: 10.1038/s41558-017-0016-6

Conflict of Interest: AB was employed by Ramat Negev Desert Agro-Desert Research Center, a public research station, which operates under the financial umbrella of the company Ramat Negev Works Ltd., which belongs to the public entity Ramat Negev Regional Authority.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Gashu, Sikron Persi, Drori, Harcavi, Agam, Bustan and Fait. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Sunburn in Grapes: A Review

Joanna M. Gambetta^{1*†}, Bruno P. Holzapfel², Manfred Stoll³ and Matthias Friedel^{3*}

¹School of Agricultural and Wine Sciences, National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, NSW, Australia, ²Department of Primary Industries, National Wine and Grape Industry Centre, Wagga Wagga, NSW, Australia, ³Department of General and Organic Viticulture, Hochschule Geisenheim University, Geisenheim, Germany

OPEN ACCESS

Edited by:

Chris Winefield,
Lincoln University, New Zealand

Reviewed by:

Tse-Min Lee,
National Sun Yat-sen University,
Taiwan
Hojeung Lee,
Korea University, South Korea

*Correspondence:

Joanna M. Gambetta
joanna.gambetta@sa.gov.au
Matthias Friedel
matthias.friedel@hs-gm.de

[†]Present address:

Joanna M. Gambetta,
South Australian Research and
Development Institute, Adelaide, SA,
Australia

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 10 September 2020

Accepted: 04 December 2020

Published: 08 January 2021

Citation:

Gambetta JM, Holzapfel BP,
Stoll M and Friedel M (2021) Sunburn
in Grapes: A Review.
Front. Plant Sci. 11:604691.
doi: 10.3389/fpls.2020.604691

Sunburn is a physiological disorder that affects the visual and organoleptic properties of grapes. The appearance of brown and necrotic spots severely affects the commercial value of the fruit, and in extreme cases, significantly decreases yield. Depending on the severity of the damage and the driving factors, sunburn on grapes can be classified as sunburn browning (SB) or as sunburn necrosis (SN). Sunburn results from a combination of excessive photosynthetically active radiation (PAR) and UV radiation and temperature that can be exacerbated by other stress factors such as water deficit. Fruit respond to these by activating antioxidant defense mechanisms, *de novo* synthesis of optical screening compounds and heat-shock proteins as well as through morphological adaptation. This review summarizes the current knowledge on sunburn in grapes and compares it with relevant literature on other fruits. It also discusses the different factors affecting the appearance and degree of sunburn, as well as the biochemical response of grapes to this phenomenon and different potential mitigation strategies. This review proposes further directions for research into sunburn in grapes.

Keywords: sunburn browning, sunburn necrosis, ROS, photooxidation, antioxidants, mitigation

INTRODUCTION

Sunburn occurs in the field as the result of a combination of high-light intensities, high temperature, and UV radiation (Rustioni et al., 2014). Incidence and severity of the damage depend on a complex interplay of these factors together with the biochemical, physiological, and morphological condition of the berry, all of which are a function of the phenological stage, cultivar and adaptation to meteorological conditions. Symptoms range from the appearance of brown or necrotic spots on the epidermis of grapes to the complete desiccation of the berries. Sunburn represents a serious defect in table grapes, as browning strongly decreases the market value of the crop (United States Department of Agriculture, 1999; Suehiro et al., 2014), and causes significant losses in quality and yield of wine grapes (**Figure 1**). In Australia, sunburn affects 5–15% of the total wine grape production (Greer et al., 2006), and observations in Chile indicate that up to 40% of bunches can show sunburn damage in sensitive varieties like Muscat (Calderon-Orellana et al., 2018). In other crops such as blueberries (10% value loss in both Washington and Oregon in 2015; Yang et al., 2019), apples (10–50% crop losses



FIGURE 1 | Sunburn necrosis (SN) of *Bacchus*, a highly susceptible grape variety in the field after bunch zone defoliation.

reported in South Africa; Wand et al., 2006), pomegranates (30% crop loss; Melgarejo et al., 2004), and red bell peppers (12–36% loss; Barber and Sharpe, 1971; Rylski and Spigelman, 1986) the economic damage caused by sunburn can sometimes be more severe than in grapevines. Depending on the severity of the damage, grapes for wine production in Australia can be downgraded from an A-grade quality to a C- or D-grade with a consequent economic loss of ~50% of the crop's value (Gambetta et al., 2019a). In European viticultural regions, sunburn symptoms occur less frequently and do not necessarily lead to a downgrading of the fruit. Nevertheless, historical records show an increasing frequency of years with significant sunburn damages for German wine-producing regions (1892, 1930, 1947, 1966, 1973, 1998, 2007, 2012, and 2019; Zschokke, 1930; Mohr and Düring, 2000; Schultz, 2007; Stoll and Schultz, 2013, 2020). In France, this phenomenon has been mainly attributed to the higher frequency and intensity of heatwaves, in particular those experienced in 1994, 1998, 2003, 2015, and more recently, 2019 (INRA, 2003; Aubert, 2015; Tupinier, 2019). In Champagne, 5–15% of yield was lost for the years 1994 and 1998 due to sunburn (Mohr and Düring, 2000).

Given the projected increase in air temperatures, the higher frequency and intensity of heatwaves and the phenomenon of global brightening (Wild, 2016), sunburn damage to grapes will inevitably increase in the coming decades. This urges a better understanding and classification of this phenomenon, as well as the reconsideration of canopy management and trellis systems, row orientation, and other preventive measures to protect future berry crops from sunburn. The aim of this review is to provide an accurate description of sunburn, suggest a standard terminology, and give an overview of the factors causing sunburn in grapes and influencing its incidence and severity. The main physiological and chemical changes resulting from grape exposure to high-light and heat stress along with their consequences for grape quality will be discussed together with applicable protective measures. Further fields of research will be identified based on the current state of research.

DESCRIPTION OF THE PHENOMENON

Sunburn damages berry epidermal tissue at several levels. At the epicuticular level, sunburn causes degradation of the crystalline structure of the waxes into amorphous masses, which leads to a higher water permeability and dehydration, as well as to changes to its visual appearance (Bondada and Keller, 2012). At the epidermal level, it leads to the destruction of chlorophyll (and loss of green coloration) and causes a loss of cell compartmentalization, which exposes polyphenolic compounds to polyphenol oxidases (POX). The oxidation of polyphenols leads to the typical browning of the skin (Olivares-Soto et al., 2020). Oxidation has been observed even in the sub-epidermal layers of the fruit where damage has been reported as far as the seeds (Zschokke, 1930). Similarly, Greer and La Borde (2006) observed that brown sunburn lesions increased in size and depth over time, although they did not report the final depth of browning. This brown coloration has also been attributed to cell death in the epidermal layers of the exocarp (Greer et al., 2006; Nuzzo et al., 2009; Bondada and Keller, 2012) as evidenced by a higher electrical conductivity (and electrolyte leakage) in the peels of affected fruit (Schrader et al., 2001).

Considering the toll sunburn has on grapevine yield and quality, it is surprising that no consistent description of the phenomenon has been adopted in viticulture yet. Consequently, the phenomena described as severe sunburn damage in Chilean vineyards (Calderon-Orellana et al., 2018) might not even be recognized as sunburn under central European conditions, where the term sunburn includes some degree of shriveling. The only reports differentiating sunburn phenomena in grapes we are aware of were made by Krasnow et al. (2010), reporting sunburn browning (SB), sunburn cracking, and poor color development of red varieties as symptoms, and 80 years earlier by Zschokke (1930). Zschokke (1930) reported different levels of sunburn damage: sunburn spots on the berry skin, complete or partial shriveling of berries, and damages to the rachis and consequent shriveling of entire sections of the bunch. He also reported poor color development of red varieties attained by sunburn. This stands in contrast to other horticultural crops like apples, where the symptoms and driving factors of three different types of sunburn phenomena – SB, sunburn necrosis (SN), and photooxidative sunburn (PS) – have been accurately described (Racskó and Schrader, 2012).

Sunburn browning is the result of a combination of both high light and high temperature, and is observed mainly after véraison (Schrader et al., 2001). It is considered a sub-lethal form of damage that causes the appearance of yellow, brown, or bronze spots on the sun-exposed side of the fruit (Figures 2A–C; Schrader et al., 2009). In white grapes, sunburn causes brown lesions on the surface of the berry, and in red berries, SB affects anthocyanin biosynthesis and manifests as poor color development and bleached spots (Greer et al., 2006; Bondada and Keller, 2012; Bondada, 2019).

Sunburn necrosis is mainly a function of high temperature, and requires significantly higher temperature levels than those necessary for SB to occur. SN can be considered a lethal

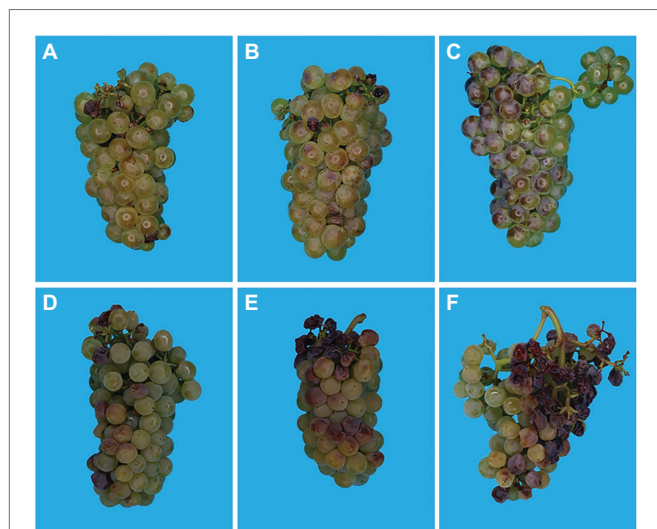


FIGURE 2 | Images of Chardonnay bunches with increasing degrees of sunburn browning (SB; **A–C**, 0–51%) and SN (**D–F**, 12–32%) damage. Pictures were taken at harvest (~22°Brix) in Orange, Australia.



FIGURE 3 | Rachis damage caused by SN in Riesling. 47% of berries were damaged due to a sunburn event occurring on July 25, 2019. Picture was taken on September 30, 2019, at 19.5°Brix in Geisenheim, Germany.

damage appreciated by the appearance of dark brown or black necrotic spots on the fruit's surface, where severe cases can lead to berry cracking and shriveling (**Figures 2D–F**; Barber and Sharpe, 1971; Schrader et al., 2003; Krasnow et al., 2010). SN causes serious changes in the cuticular, epidermal, and sub-epidermal tissues ultimately destroying the integrity of cell membranes (Schrader et al., 2001). Pre-véraison SN leads to shriveling of entire berries, affects parts of the rachis and even entire bunches (**Figure 3**), and leads to considerable yield losses.

Photooxidative sunburn is caused exclusively by an excessive amount of photosynthetically active radiation (PAR; Felicetti and Schrader, 2009) and manifests as bleached

pigments and, in severe cases, necrosis. There are no records of PS in grapes in the field to date.

ENVIRONMENTAL FACTORS AFFECTING SUNBURN DEVELOPMENT

Light as an Inducing Factor

Solar radiation can be divided into UV (UV-A, 400–315 nm and UV-B, 315–280 nm), visible (400–780 nm), which includes PAR (400–700 nm), and infrared radiation (IR, >780 nm). The intensity of these depends on altitude, latitude, season, time of day, and cloud coverage (McKenzie et al., 2003). Light acts both as a source of heat (section Ambient and Fruit Surface Temperature) and as the driver of photochemical and oxidative reactions in the berry, where photooxidation plays a central role in the development of SB symptoms. Regardless of the temperature, neither SN nor SB is observed in well-shaded bunches in the field (Rustioni et al., 2014).

An excessive amount of light promotes the production of triplet chlorophyll ($^3\text{Chl}^*$) and reactive oxygen species [ROS; singlet oxygen ($^1\text{O}_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\text{HO}\cdot$)], all promoters of oxidative stress in the plant's and fruit's photosystems. Of these, $\text{HO}\cdot$ is the ROS with the shortest half-life and highest phytotoxicity. It can be generated from H_2O_2 in the Fe-S center of photosystem I (PSI) through a process termed the Fenton reaction, which is catalyzed by metal ions such as Fe^{2+} , and peroxidases. Although ROS are normally present in non-stressed cells, stress conditions lead to a drastic increase of these highly reactive molecules and a reduction of photosynthetic CO_2 fixation, leading to excess excitation energy captured by PSI and PSII (measured as the maximum quantum yield of chlorophyll fluorescence, F_v/F_m ; Mittler et al., 2004; Glenn and Yuri, 2013). Stress conditions like high temperature or drought have been associated with increased ROS production (Carvalho et al., 2016). The plant can then either tolerate and adapt to the new levels of ROS or suffer some form of damage.

Photosynthetically active radiation and UV are the two main components of light involved in sunburn development. Exposure to high PAR levels decreases F_v/F_m of the exposed tissue, and as a consequence, non-photochemical quenching (NPQ) of PSII increases in an attempt to protect the photosystems. If PAR overexposure continues, NPQ becomes photoinhibited and sunburn damage ensues (Glenn and Yuri, 2013; Rustioni et al., 2015). UV is a high-energy form of radiation, which induces mutations if absorbed by DNA, inhibits electron transport, and collapses membrane integrity (Jenkins, 2009). Response to UV depends on the dose, duration, and wavelength the organ is exposed to. High fluence rates combined with short wavelengths cause stress responses and lead to necrosis whilst low rates initiate regulatory responses that promote the production of photoprotective compounds (Kolb et al., 2003; Jenkins, 2009; Pastore et al., 2013). Despite having relatively low average temperatures, areas like New Zealand and Chile report high incidences of sunburn in grapes and apples, most probably due to their high UV index (Hofmann et al., 2006; Schrader et al., 2008). Locations in the

southern hemisphere receive on average 12–15% more UV radiation than similar locations in the northern hemisphere with this difference increasing as latitude decreases (Gregar et al., 2012). Studies on the effect of PAR and UV have demonstrated that the interaction between them results in greater changes in F_v/F_m and fruit composition when compared to each separate factor alone. The UV \times PAR interaction plays a key role in the initiation of sunburn damage, although PAR plays a greater role in the degradation of the berry's photosystems (Glenn and Yuri, 2013; Joubert et al., 2016). An influence of IR-radiation on the development of sunburn has not been reported in fruits yet.

Ambient and Fruit Surface Temperature

Temperature is a major source of abiotic stress that affects many physiological responses at the plant and fruit level. Although there is no specific molecule that acts as a thermosensor, fruits possess a diverse intracellular signaling mechanism that is activated in response to heat. Thermal stress has amongst its main targets the photosynthetic apparatus, which consequently undergoes a series of reversible changes to cope with heat, although when the heat is excessive, the photosystems can be severely and irreversibly damaged (Araújo et al., 2018). High temperature induces an imbalance between light energy absorption and usage impairing electron transport activity. Consequently, fruit respiratory mechanisms are altered and the higher level of anaerobic respiration caused by higher temperatures induces the accumulation of ROS (Jiang et al., 2015). Chloroplasts themselves can be damaged or degraded by heat stress (Hu et al., 2020). Thermal stress can cause membrane destabilization, protein denaturation, and berry pericarp cell death. Experiments have demonstrated that high temperatures bring cell death forward in Shiraz by ~9 days (Bonada et al., 2013). Furthermore, elevated heat alters the regulation of major metabolic pathways and the expression of genes involved in all levels of plant physiology (Mittler et al., 2012). In grapevine, heat events ($>30^\circ\text{C}$) have deep consequences for berry growth and composition (Hale and Buttrick, 1974; Pastore et al., 2017).

Although a purely PS has been induced in grapes under laboratory conditions (Rustioni et al., 2020), the prevailing type of damage in vineyards results from the combination of high light and high temperatures. Very little to no damage occurred when greenhouse-grown berries were exposed to high light intensities at low-moderate temperatures ($25\text{--}30^\circ\text{C}$). However, when the temperature was increased to 38°C , damage of ripe Semillon berries was observed even at low light intensities, and was devastating at high light intensities with 94% of bunches affected (Hulands et al., 2014).

Berry temperature is a function of air temperature and radiative heat transfer – there is a linear relationship between temperature and light absorbed by the berry tissue (Hulands et al., 2014) which makes it very difficult to separate the effect of these two factors, especially when conducting vineyard studies. Direct exposure to the sun increases fruit surface temperature (FST) by as much as $12\text{--}15^\circ\text{C}$ above air temperature on the berry's sun-exposed side (Smart and Sinclair, 1976; Spayd et al., 2002). Consequently, FST can vary widely according to bunch location in the canopy and level of solar exposure

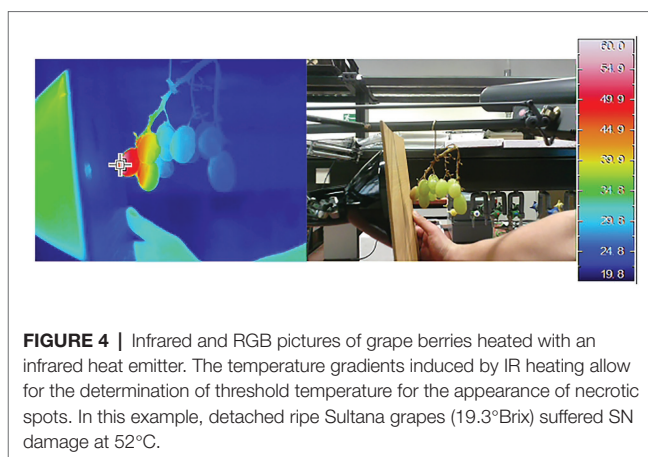


FIGURE 4 | Infrared and RGB pictures of grape berries heated with an infrared heat emitter. The temperature gradients induced by IR heating allow for the determination of threshold temperature for the appearance of necrotic spots. In this example, detached ripe Sultana grapes (19.3°Brix) suffered SN damage at 52°C .

(Spayd et al., 2002). FST is also modulated by wind velocity, berry color, and bunch compactness (Dry, 2009). In the field, exposed dark berries can have temperatures up to 5°C higher than white berries (Spayd et al., 2002). Sunburn of different crops has been observed between the thresholds of $45\text{--}49^\circ\text{C}$ (Schrader et al., 2008; Genovese et al., 2010; Yang, 2018), values that are rarely reached in the field without radiative heat transfer. This implies that FST is more relevant for sunburn induction than ambient temperature. FST also modulates the type of damage observed; when FST of apples reaches $52 \pm 1^\circ\text{C}$ SN occurs within 10 min whilst SB occurs when FST of sun-exposed apples reaches $46\text{--}49^\circ\text{C}$ for an hour (Schrader et al., 2003). Own experiments have shown the occurrence of SN in detached white table grape berries after 15 min of exposure to 52°C in the absence of solar radiation (Figure 4).

BIOCHEMICAL RESPONSE OF GRAPES TO LIGHT AND HEAT STRESS

Grapes regulate a number of physiological and biochemical processes as a response to a higher light and temperature environment to minimize damage to their photosynthetic system. Plants need to maintain fruit photosynthesis, which is important for fruit development, in particular in green berries. Protection from direct and ROS-mediated damage is achieved by dissipating the excess energy as heat through NPQ (Müller et al., 2001) and oxidative damage is alleviated *via* antioxidant enzymes, soluble antioxidants, and ROS scavengers (Figure 5; Carvalho et al., 2016).

Enzymatic Activity and Antioxidants

As a consequence of photooxidative and thermal stress, the activity of a suite of ROS-scavenging enzymes [e.g., ascorbate peroxidase (APX), ascorbate-glutathione cycle enzymes, superoxide dismutase (SOD), and catalase] increases, the production of antioxidant metabolites (e.g., ascorbate, glutathione, and α -tocopherol) is up-regulated and their reduction state increased (Thompson et al., 1987; Ma and Cheng, 2003; Jenkins, 2009). Ascorbate and glutathione are key water-soluble antioxidants located in the chloroplasts and the main objective of the ascorbate-glutathione cycle is to detoxify

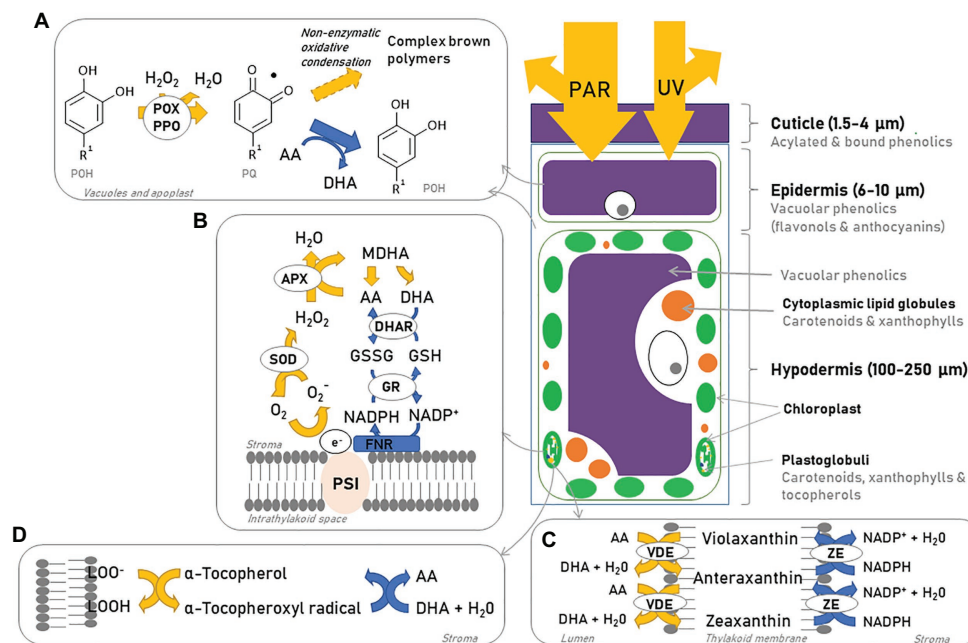


FIGURE 5 | Epidermal cell, photoprotection, and reactive oxygen species (ROS) scavenging mechanisms. As photosynthetically active radiation (PAR) and UV light reach the berry, part of these forms of radiation are reflected by the cuticle. Vacuolar phenolics (**A**) act as a screen helping to reduce the amount of incident light further penetrating the cell and help mitigate part of the ROS formed through the formation of oxidized phenolic forms and complex brown polymers (if ascorbic acid is absent). If light penetrates further into the hypodermis, the chloroplasts and mitochondria become the main target of radiation. The water-water cycle (**B**), non-photochemical quenching (NPQ; **C**), and tocopherol (**D**) are used to remove ROS and prevent damage to the photosystems. AA, ascorbic acid; DHA, dehydroascorbate; MDHA, monodehydroascorbate; DHAR, dehydroascorbate reductase; APX, ascorbate peroxidase; SOD, superoxide dismutase; GSSG, glutathione disulfide; GSH, glutathione; GR, glutathione reductase; PSI, photosystem I; POX, polyphenol oxidase; PPO, polyphenol peroxidase; POH, polyphenol; PQ, oxidized phenol; VDE, violaxanthin de-epoxidase; ZE, zeaxanthin epoxidase. Based on Solovchenko and Merzlyak (2008).

ROS via photoreduction of H₂O₂ into water and oxygen (Figure 5B; Ma and Cheng, 2003). The upregulation of the ascorbate-glutathione cycle is synchronized with the xanthophyll cycle (section Carotenoids) – the de-epoxidation of violaxanthin uses reduced ascorbate as reductant (Figures 5C, 6), which then regenerates via the ascorbate-glutathione cycle. Ascorbate deficiency can limit the de-epoxidation of violaxanthin and lower NPQ by limiting violaxanthin de-epoxygenase (VDE) activity (Müller-Moulé et al., 2002; Ma and Cheng, 2003). Ascorbate also plays a role in the Mehler-peroxidase reaction (also known as the water-water cycle) used by PSI to reduce ROS (Figure 5B). Therefore, the Mehler-peroxidase reaction competes with VDE for ascorbate but might also be involved in creating a sufficient pH gradient to activate VDE (Müller-Moulé et al., 2002). α -Tocopherol is a hydrophobic antioxidant associated with membranes. It quenches ¹O₂ and reacts with superoxide and lipid peroxy radicals to form tocopherol semiquinone and prevent lipid peroxidation (Figure 5C). Tocopherol semiquinones can be reduced by ascorbate, which is oxidized to dehydroascorbic acid (DHA) and later regenerated in the presence of glutathione (Thompson et al., 1987; Havaux, 2014).

Once oxidation processes have been initiated, ascorbate can suppress the complete oxidation of phenolic compounds by POX and polyphenol peroxidase (PPO) that lead to enzymatic browning. The blackening of the epidermis after high light exposure results from the polymerization of vacuolar

phenolics as the result of the penetration of H₂O₂ into vacuoles of epidermal cells and the activity of POX and PPO. However, POX can help scavenge H₂O₂ by using flavonols as electron donors (Figure 5A; Yamasaki et al., 1997). When this reaction is coupled to the ascorbate-glutathione cycle, ascorbate reduces the primary oxidized product of phenolics to their parent compounds and produces water and DHA, thus inhibiting the formation of degradation products and more O₂^{•−} and H₂O₂ (Yamasaki et al., 1997; Makris and Rossiter, 2002; Hernández et al., 2009). In the absence of ascorbate, polymerization products of flavonoids and other polyphenols may be irreversibly generated.

Pigments and Photoprotective Compounds

Plants possess multiple photoreceptors that are responsible for the activation of various signal transduction cascades that regulate light-dependent responses and related gene expression. These include the phytochrome superfamily, which consists of photoreceptors absorbing red/far-red light, cryptochromes (blue, green, and UV-A), phototropins (UV-A/blue-light), and UV-B photoreceptors (photoreceptor UV RESISTANCE LOCUS 8, UVR-8). After exposure to PAR or UV, these receptors up-regulate the expression of genes coding for photoprotective molecules such as carotenoids and flavonoids to protect the berry's DNA and photosynthetic apparatus from further damage.

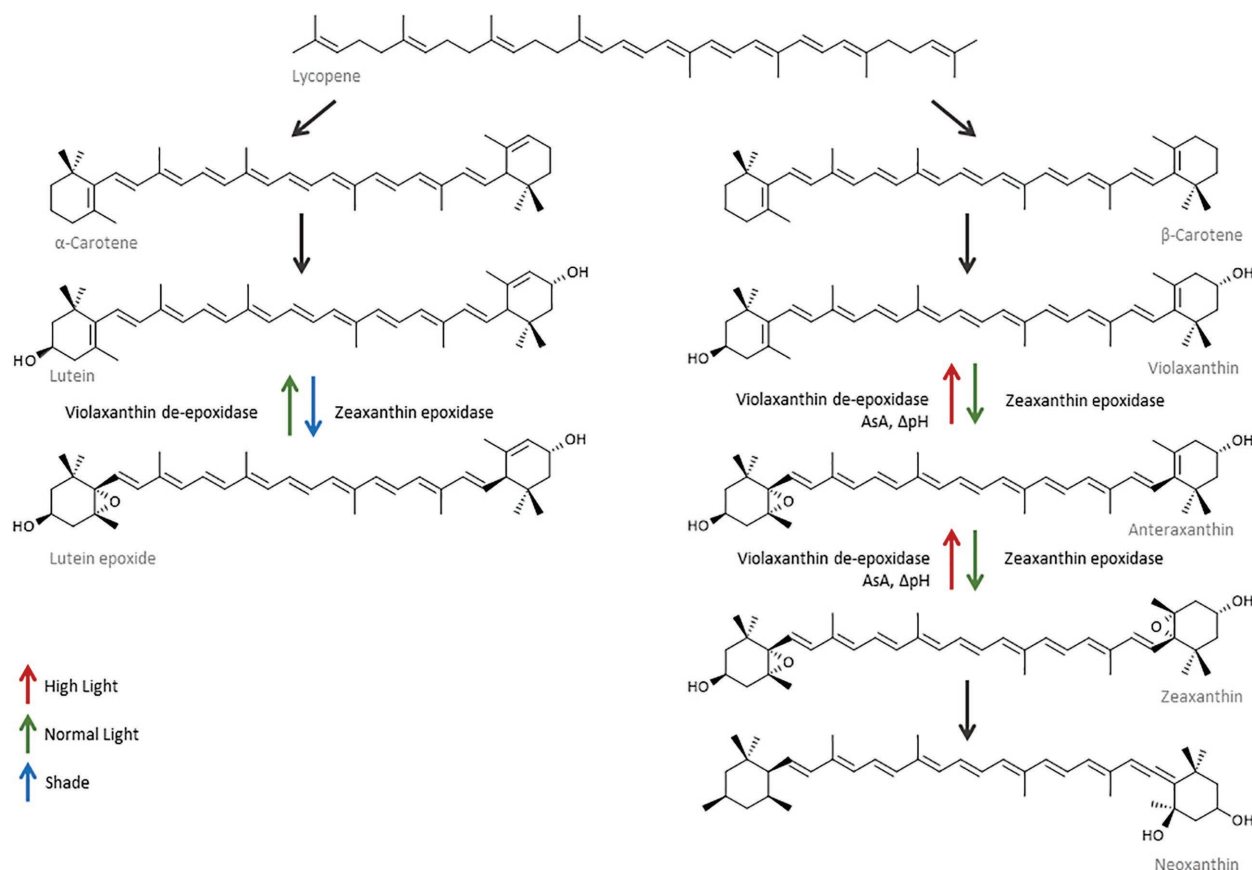


FIGURE 6 | Carotenoid synthesis is up-regulated in response to changes in the light environment. As a consequence of higher light, α - and β -carotene are synthesized from lycopene and used to produce more lutein and violaxanthin. The violaxanthin cycle is rapidly induced in response to high light, and violaxanthin is epoxidized first to anteraxanthin and then to zeaxanthin. Violaxanthin de-epoxidase (VDE) requires ascorbate (AsA) and a pH gradient to catalyze the reaction. In the absence of ascorbate, zeaxanthin is converted to neoxanthin. The lutein epoxide cycle converts lutein epoxide into lutein and is induced when tissues move from a shade to normal light situation or under prolonged high light stress.

Carotenoids

Carotenoid accumulation plays an important role in the photoprotection of grape berries; they are efficient antioxidants capable of scavenging $^1\text{O}_2^*$ and peroxy radicals, quenching $^3\text{Chl}^*$ generated during photooxidation processes, and possess the ability to screen light in the blue-green (450–570 nm) and UV part of the spectrum. They are also capable of modifying membrane fluidity, thereby increasing its thermostability and protecting it from lipid peroxidation (Solovchenko and Merzlyak, 2008; Joubert et al., 2016). The xanthophyll cycle is one of the most important antioxidant systems in grapes and constitutes one of the main modes of action of NPQ (Figures 5C, 6). It is a rapidly induced and rapidly reversible mechanism. In the green berry stage, the activation and interconversion of the xanthophylls violaxanthin (V), anteraxanthin (A), and zeaxanthin (Z) under excessive light conditions takes only minutes and helps quench $^1\text{O}_2$ and dissipate excess excitation energy of $^3\text{Chl}^*$ as heat. Consequently, V is first de-epoxidized to A and then to Z in a reaction mediated by VDE and catalyzed by ascorbate. A second

xanthophyll cycle constituted by lutein and lutein epoxide works in a similar way to regulate NPQ, but has a slower relaxation rate and is thought to aid in situations of prolonged stress (Figure 6; Joubert et al., 2016). At noon, almost all the xanthophyll cycle pool in sun-exposed peel is present as A + Z indicating that the xanthophyll cycle is operating at full capacity and that the pool size may become limiting in a higher stress situation, for example, at elevated temperatures and/or if the stress continues over a sustained period of time (Ma and Cheng, 2003). Zeaxanthin and lutein may also have a direct role in the protection of the thylakoid membrane, acting as antioxidants against lipid peroxidation by ROS (Müller et al., 2001). β -Carotene acts as a direct precursor to V, but also as an accessory pigment located in P680 reaction centers, where it protects the photosynthetic apparatus by scavenging $^1\text{O}_2^*$ and quenching $^3\text{Chl}^*$ (Felicetti and Schrader, 2009). Neoxanthin has also been implicated in energy-dependent quenching (Müller et al., 2001). Whether xanthophylls are directly or indirectly involved in the de-excitation of $^3\text{Chl}^*$, is still unknown.

Phenolic Compounds

Phenolic compounds include the flavonoids (flavonols, flavan-3-ols, and anthocyanins) and the non-flavonoids (stilbenes, hydroxycinnamic acids, and hydroxybenzoic acids and their derivatives). Their accumulation in the berry skin is strongly regulated by changes in the fruit environment (González et al., 2015). Phenolic compounds accumulate in the berry upper epidermis as well as in the hypodermis and cuticle, where they are used by plant tissues as photoprotectants due to their capacity to absorb and screen PAR and UV light, thereby constituting the plant's first line of defense against photo stress. They scavenge harmful singlet oxygen and H_2O_2 , inhibit ROS formation, and quench free radical reaction cascades in lipid peroxidation (Kolb et al., 2003). Polyphenols can also inhibit the Fenton reaction by complexing metals such as ferrous iron (Son and Lee, 2008; Chang et al., 2017).

Flavonols are mainly constituted by quercetin, myricetin, and kaempferol; with lower percentages of laricitrin, isorhamnetin, and syringetin; their profile varying amongst genotypes and grape color. They are present in berries as mono-, di-, and tri-hydroxylated forms and are only accumulated as glycosides. Flavonols have a high extinction coefficient at wavelengths characteristic of UV (Kolb et al., 2003) and their synthesis is strongly and rapidly induced by solar radiation – upon 8 h light exposure, the expression of flavonol synthase (*VvFLS1*) and flavonol glycosyltransferase (*VvGT5* and *VvGT6*) genes increased four-fold on a bunch level (Friedel et al., 2016). Oxidation and polymerization modifies the biological properties of polyphenols, and their polymerized and oxidized forms may further screen light in the PAR range, offering additional protection to chloroplasts (Rustioni, 2017). The antioxidant activity of polyphenols increases with their degree of polymerization up to a mean degree of polymerization of about 10 (Zhou et al., 2014). Polyphenol quinones have also been cited to modulate lipoxygenase activity, preventing membrane damage (Hernández et al., 2009; Ferrandino and Lovisolo, 2014).

Anthocyanins are synthesized in the skin from véraison onwards and include cyanidin, peonidin, delphinidin, petunidin, and malvidin derived pigments. They are involved in the protection against damage by high fluxes of visible radiation. Their maximum absorption range is located in the green range (500–600 nm), which is close to the solar energy peak and coincides with the gap between Chl and carotenoid absorption bands in which light penetrates deeply into plant tissue (Merzlyak and Chivkunova, 2000). A high anthocyanin content increases resistance to Chl photobleaching, as anthocyanins show a higher photostability than Chl (Solovchenko and Merzlyak, 2008).

Heat Shock Factor

The synthesis of heat shock factor (HSF) and heat shock proteins (HSPs) is considered the first line of defense against thermal stress. They help protect cell membranes from heat damage and lipid peroxidation, and maintain structural and functional proteins' quality and folding by protecting them from denaturation (Araújo et al., 2018; Hu et al., 2020). Small HSP (smHSP) proteins predominate during heat stress, their levels increase 2000-fold upon heat stress and both smHSP and

HSP70 concentrations are positively correlated with sun exposure. The total amount of HSP proteins produced seems to be cultivar related and decreases with grape maturity (Ritenour et al., 2001; Guillaumie et al., 2011).

Aroma Compounds

Like polyphenols, some volatile compounds such as the terpenes have been recognized as having antioxidant capacity and are capable of quenching excess energy. It is hypothesized that under high-temperature conditions, terpenes act as thermoprotective molecules that stabilize chloroplast membranes (Loreto and Schnitzler, 2010; Joubert et al., 2016).

Adaptation

Biochemical Adaptation

Grapes have the capacity to adapt to changes in microclimatic conditions and thus increase their resistance to sunburn (von Babo, 1840). Acclimation responses depend on the type, dose and duration of the light and thermal stress (Figure 6), to which plants respond by activating stress-signaling pathways that generate, amongst other metabolites, ROS and H_2O_2 . At low doses, $^1O_2^*$ and H_2O_2 act as signal transduction molecules and trigger protective mechanisms, while high doses of ROS cause necrosis and cell death (Gechev et al., 2006; Pourcel et al., 2007). Due to their instability, ROS cannot diffuse through membranes (Yamasaki et al., 1997) and must be detoxified *in situ*. Accordingly, the acquisition of photo- and thermo-tolerance seems to be a highly localized process. In apples, fruit rotation has been shown to drastically increase the appearance of sunburn symptoms as shaded fruits are more sensitive to photoinhibition and have lower F_v/F_m than sun-exposed fruit (Wünsche et al., 2001; Li and Cheng, 2008). Shaded and sun-exposed sides in apples show pronounced differences in skin composition, mainly in the accumulation of phenolics, carotenoids, and anthocyanins, but also chlorophylls, HSPs, and antioxidant enzymes (Ritenour et al., 2001; Merzlyak et al., 2002; Ma and Cheng, 2003). Within the grape cluster, a similar localized accumulation pattern of photoprotectants has been observed: their accumulation varies within a cluster and even within individual berries (Friedel et al., 2012; Pieri et al., 2016).

Light

Excessive light induces metabolic responses including the accumulation of antioxidants and of enzymes controlling their redox state (Rustioni et al., 2020). Light exposed berries accumulate higher amounts of ascorbate during berry development when compared to shaded berries (Debolt et al., 2007), and the capability to increase carotenoid concentration in response to light exposure appears as a major photoadaptation mechanism that distinguishes sunburn-susceptible cultivars from more resistant ones (Merzlyak et al., 2002). qRT-PCR analysis of sunburn affected peels of apples showed the upregulation of the genes phytoene synthase (PSY) and phytoene desaturase whilst lycopene β -cyclase and lycopene ϵ -cyclase remained unchanged. PSY converts geranylgeranyl diphosphate into phytoene as the first step of carotenoid biosynthesis, and these

genes have been shown to be generally up-regulated by light (Liu et al., 2018). Total carotenoids concentration and the xanthophyll cycle pool are larger in exposed fruit than in shaded grapes when measured before véraison, although some of these differences disappear by harvest (Hickey et al., 2018; Gambetta et al., 2019b). Düring and Davtyan (2002) showed that the relative importance of xanthophyll cycle carotenoids increases during adaptation to high light conditions along with an elevated NPQ.

Results on the effect of sunburn on carotenoid concentration so far have been contrasting due to differences in experimental conditions, ripening stage, and cultivar, but especially, from the choice of sample location in the canopy. Previously acclimated fruit (sun-exposed) appear to react very differently to shaded fruit in these experiments. Some authors report an overall degradation of these compounds as a result of sunburn damage, leading to lower concentrations of Chl, β -carotene, lutein, neoxanthin, and V + A + Z in the peel of injured fruit when compared to non-sunburnt fruit (Torres et al., 2006; Li and Cheng, 2009). Conversely, Felicetti and Schrader (2009) demonstrated a slight increase of V + A + Z and a marked increase in β -carotene in affected fruit, although these results depended on the season. Most authors agree however that the ratio of carotenoids/Chl, V + A + Z/Chl, and Chl a/Chl b increase as a result of the preferential destruction of Chl with sunburn (Ma and Cheng, 2003; Felicetti and Schrader, 2009; Torres et al., 2013) as carotenoids have been reported to be more photostable than Chl, in particular, Chl b (Merzlyak et al., 2002; Felicetti and Schrader, 2009).

Higher light exposure also increases the total amount of flavonoids present in the berry (Pastore et al., 2013; Kok and Bal, 2018; Würz et al., 2018; Brandt et al., 2019; Hickey and Wolf, 2019). UV-B radiation upregulates genes responsible for the synthesis of a range of phenolic compounds including phenylalanine ammonia-lyase (PAL), flavonoid-3'-hydroxylase (F3'H), flavonoid-3',5'-hydroxylase, flavonol synthase (FLS), MYB transcription factor, and UDP-glucosyl transferases (UFGT; Stracke et al., 2010; Czempl et al., 2012; Pastore et al., 2013). The concentration of quercetin and kaempferol glycosides were up to 10 times higher in sun-exposed Merlot and Pinot Noir berries than in shaded ones (Price et al., 1995; Spayd et al., 2002). UV-B exposure also favors the production of flavonoids with hydroxyl groups on ring B of the flavonoid skeleton, e.g., quercetin glycoside over kaempferol glycoside, thus increasing the potential antioxidant activity of the organ. When exposed to light, a cascade of reactions triggers the synthesis of flavonols and sinapyl derivatives. Responsiveness to light induction differs amongst flavonoid classes, with flavonol glycosides being the most responsive ones (Koyama et al., 2012; Reshef et al., 2018). However, adaptive responses to light depend heavily on the stage of ripening (as further discussed in section Developmental Stage). Flavonol production, and the expression of the genes that mediate their synthesis (*VvMYB1* and *VvFLS1*) peak between flowering and fruit set and decline after véraison, with a later peak at maturity (Downey et al., 2003; Czempl et al., 2012). After véraison, the expression of anthocyanin-specific genes (*MYBA* and *UFGT*) increases as does anthocyanin accumulation (Czempl et al.,

2012). However, higher light exposure after véraison reduces the expression of genes directly involved in anthocyanin synthesis and transport such as *UFGT* (Pastore et al., 2013).

Temperature

Exposure of tissues to sub-lethal temperatures confers increased transient thermotolerance that protects the plant from a second exposure to lethal temperatures that lead to SN. Thermotolerance is acquired through the accumulation of HSPs, genes encoding detoxification enzymes (e.g., glutathione S-transferase, glutathione reductase, SOD, CAT, peroxidase, and APX), antioxidants (GSH and ascorbate), and regulatory proteins (Lim et al., 2006; Wang and Li, 2006). In experiments on apples conditioned at 38°C, an inverse relationship between the appearance of sunburn symptoms and duration of conditioning was observed, with conditioned apples presenting symptoms at slightly higher temperatures than non-conditioned ones. Conditioning results in less H₂O₂ produced, as observed in leaves of whole vines conditioned at 38°C for 12 h and then exposed to 47°C during 2 h (Wang et al., 2009). This thermotolerance, however, is only temporary and degrades under continued stress or if the temperature increases above lethal thresholds (Figure 7; Naschitz et al., 2015).

When plants are subjected to multiple sources of stress simultaneously, antagonistic effects on gene expression are usually observed. Experiments contrasting the effect of high temperature, high light, and combined high light and temperature, have demonstrated that it is this last condition that affects the size of the carotenoid pool the most (Li and Cheng, 2009). As such, high temperatures might slow down or even reverse biochemical acclimation responses by negatively impacting the berry's antioxidant response. At temperatures above 35°C significantly more H₂O₂ is produced whilst the APX pathway, NPQ, APX, and SOD are inhibited (Rocheta et al., 2014). High temperatures affect carotenoid and chlorophyll biosynthesis and degradation by impairing the expression of genes in the carotenoid pathway and increasing the activity of chlorophyllases, peroxidases, and lipoxygenases (Shi et al., 2014). Although sub-lethal temperature increases have a limited impact on flavonoid synthesis (Pastore et al., 2013), temperatures over 30°C decrease overall flavonoid concentration. When the temperature rises above 35°C, enzymatic activity in the flavonoid biosynthetic pathway is impaired and degradation by PPO and POX increases, compromising flavonoid final concentrations (Mori et al., 2007; Mohaved et al., 2016). Anthocyanin accumulation is inhibited at even lower temperatures than flavonols, with the highest accumulation reported at 25°C (Mori et al., 2007). Experiments on apples have demonstrated a significant loss of anthocyanin content in sunburnt apples (~63% loss) together with a reduced level of expression of *MdANR* and *MdFLS* (Liu et al., 2018). Exposure to higher light and temperature also modifies the proportion of non-acylated anthocyanins and the level of B-ring hydroxylation and thus the ratio between di- and tri-hydroxylated forms. Tri-substituted anthocyanins have been reported to be more stable at high temperatures and more effective at scavenging free radicals than di-substituted ones, and are more abundant in berries

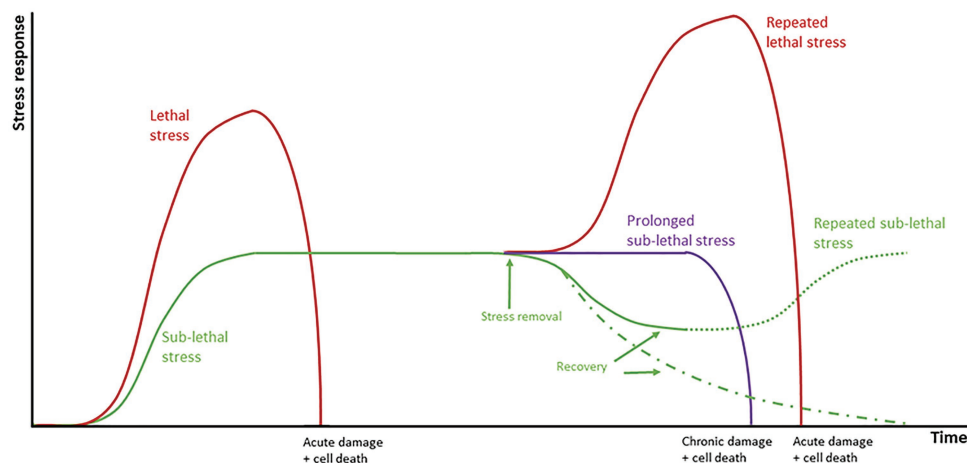


FIGURE 7 | Schematic representation of the responses of grapes to abiotic stress. Plants are initially in a basal state when stress is applied. Stress can be divided into lethal stress (red lines) which lead to acute damage and cell death; and sub-lethal stress (green line) which leads to the activation of a series of stress response mechanisms. Prolonged stress (purple line) leads ultimately to chronic damage and cell death. If sufficient recovery time is allowed, fruit returns to the original basal state (green dotted and dashed lines).

ripened under high-temperature conditions (Mori et al., 2007; Cohen et al., 2012; Koyama et al., 2012) although a field study by Pastore et al. (2013) does not support this theory. Likewise, acylated anthocyanins have been referred to as being more thermostable than their non-acylated counterparts (Tarara and Spayd, 2005) and their relative contribution to the anthocyanin profile is higher in grapes suffering heat stress (Mori et al., 2007).

Recovery Periods

Recovery periods are associated with the detoxification and activation of repair mechanisms and are critical to the ability of an organ to adapt to abiotic stress. When allowed recovery periods, the capacity of plants to adapt to different stresses is enhanced when compared to continuous periods of stress (Figure 6). In an experiment comparing constant doses of UV-B (6 h at 0.04 mW cm⁻²) and pulsed doses (6 × 1 h intervals interspersed with 30 min recovery periods), *Arabidopsis* plants allowed recovery periods produced more photoprotectants; 27% more total flavonols and sinapyl derivatives, 38% more kaempferols, and 90% more quercetins (Höhl et al., 2019). The authors also demonstrated that the amount by which these compounds increase depends on the duration of the recovery periods, with shorter recovery periods showing almost no differences when compared to plants treated continuously. Kaempferols, quercetins, and sinapyl derivatives required different amounts of recovery time to be expressed, with kaempferol requiring the least (~30 min) and sinapyl derivatives the longest (1.5 h) amounts of time. Similarly, sufficient recovery during low light periods and overnight permitted apples to better withstand sunburn, however, if full recovery did not occur, the damage was accumulated (Glenn and Yuri, 2013). The duration of these recovery periods, and whether a plant is able to recover at all, are contingent on the intensity of the applied stress. When exposed to 25 and 35°C for 5 h, plants

allowed a 1 day recovery period recovered their initial photosynthesis rates. However, when the temperature was increased to 40°C, it took plants 2–4 days to recover their initial levels, and when the temperature was increased to 45°C basal levels were not reattained even after 4 days of recovery. It takes temperatures higher than 35°C to cause significant changes to the NPQ capacity of the fruit, however, NPQ returns to basal levels rapidly when sufficient recovery time is allowed, but repeated stress means that this recovery time is prolonged and that irreversible damage can occur (Luo et al., 2011).

Morphological Adaptation – Waxes and Epidermis Thickness

Epicuticular waxes protect the berry against light and heat stress. Although their main function is as transport barriers, they also play a role in protection against PAR and UV radiation by scattering, reflection, and even absorption, thus reducing exposure levels in the underlying tissues (Figure 8). The capacity of this layer to scatter light is dependent on the size, distribution, and orientation of the wax crystals. Plate-like wax crystals reflect and scatter a higher proportion of light than amorphous waxes (Jenks and Ashworth, 1999), while still allowing for transpiration (Muganu et al., 2011). Plate-like wax structures prevail in light-exposed grape berries of several varieties, while berries grown in the shade of the canopy have a higher proportion of amorphous waxes (Muganu et al., 2011). As sunburn symptoms appear, these waxes lose their crystalline structure and become relatively amorphous (Figure 8; Greer et al., 2006).

Sun-exposed berries have a thicker layer of epicuticular wax and overall thicker cell walls than shaded ones (Rosenquist and Morrison, 1989; Muganu et al., 2011; Verdenal et al., 2019), which relates to a higher capacity to reflect light (20–80% of incoming radiation when compared to shaded plants that only reflect 10%; Jenks and Ashworth, 1999).

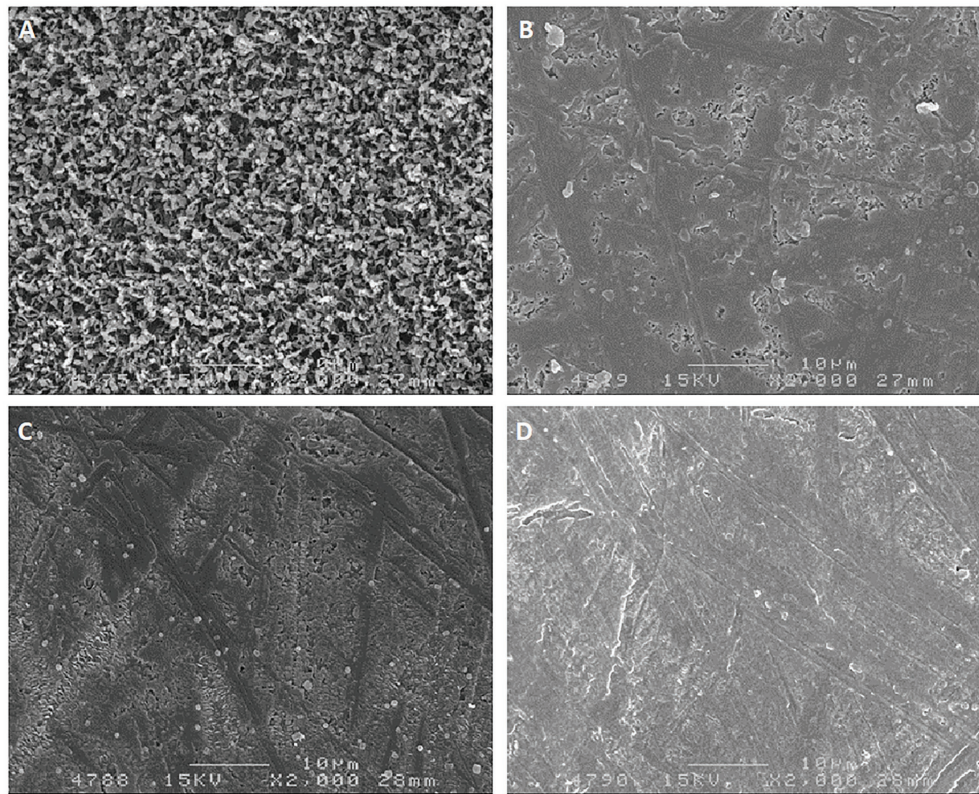


FIGURE 8 | Scanning electron micrographs ($\times 2000$ magnification) of epicuticular waxes of Chardonnay grapes. **(A)** Control grapes with no sunburn; **(B)** slight sunburn; **(C)** moderate sunburn; **(D)** severe sunburn (originally from Greer et al., 2006; reprinted with permission from Vitis).

A thicker epidermis also translates into more epidermal layers and increased capacity for anthocyanin and flavonol storage (McDonald et al., 1998; Pastore et al., 2013). Higher accumulation of polyphenols in the cuticle in response to light exposure modifies the cuticle's optical properties, converting it into a non-uniform filter that absorbs in the UV region (Solovchenko, 2010).

Higher light and thermal stress have also been observed to up-regulate genes involved in lignin precursor synthesis and lignin's biosynthetic pathway (Cabane et al., 2012; Pastore et al., 2013; Zenoni et al., 2017; Verdenal et al., 2019). Consequently, the peel of sunburnt apples contains higher amounts of lignin than shaded and healthy, sun-exposed organs. Lignification is a mechanism used by plants to increase their resistance to stress, however, the possibility that this increase is also a consequence of cell damage and polyphenol oxidation, cannot be ruled out (Torres et al., 2020).

Biochemical Changes Associated With Sunburn Browning and Sunburn Necrosis Damage

When the combined capacity of ROS scavenging systems is exceeded and the damage incurred by ROS is not repaired between exposure times, thermal, and photooxidative damage and sunburn occur (Glenn and Yuri, 2013). While mild damage can be manifested as growth impairment and damage to the photosystems, chloroplasts or mitochondria, increasing ROS

levels lead to pigment destruction (SB), lipid peroxidation, cellular membrane oxidative damage, and ultimately programmed cell death or necrosis (SN; Wang et al., 2009; Araújo et al., 2018).

In both grapes (Zschokke, 1930; Rustioni et al., 2014) and apples (Merzlyak et al., 2002) sunburn occurrence is accompanied by a loss of carotenoids and chlorophyll. While the total concentration of antioxidant enzymes and their products increase in response to sunburn, the ratios of reduced ascorbate/total ascorbate and reduced glutathione/total glutathione decrease linearly as sunlight and thermal-induced stress continue (Torres et al., 2006; Chen et al., 2008). During the photodestruction of the photosynthetic pigments, the antioxidant defense of the cells seems to be overwhelmed, and complete depletion of antioxidants (ascorbate and glutathione) ensues (Rustioni et al., 2020). When antioxidants are depleted, phenols oxidized to quinones by enzymatic (PPO and POX) or non-enzymatic reactions (ROS, autoxidation) can no longer be reduced and may polymerize to brown or black pigments (SB), possibly including non-phenolic substrates. The nature of this process and its end products have not yet been fully elucidated (Pourcel et al., 2007).

Under prolonged or extreme exposure to oxidative stress, irreversible damage occurs to the epidermal and sub-epidermal cells, which ultimately leads to thylakoid membrane destruction, cell death, and SN (Thompson et al., 1987). While relative

electrolyte conductivity of cell membranes is not affected in SB fruit, it increases significantly in SN fruit, indicating the destruction of membrane integrity. This is likely caused by the initiation of lipid peroxidation, which finally leads to cell decompartmentalization and exposure of anthocyanins to ROS and consequent bleaching (Edgley et al., 2019). In addition, polyphenols are exposed to PPO and POX activity (Pourcel et al., 2007), leading to the formation of brown pigments as observed in SN. Skin cracking in grapes is accelerated by Fenton reaction catalysts (Chang et al., 2017), indicating that HO \cdot may be involved in the skin cracking phenomena accompanying SN development.

FACTORS AFFECTING SUSCEPTIBILITY

Biotic Factors

Cultivar

The ability to tolerate light and heat stress varies greatly amongst individual grapevine cultivars (Silvestre et al., 2019). There is evidence from apples, but not from grapes, that sunburn susceptibility of individual cultivars may be related to fruit composition. In apples, anthocyanin accumulation increases the tolerance to light-induced photodegradation of Chl (Merzlyak and Chivkunova, 2000) and light-induced heat stress (Li and Cheng, 2009). Anthocyanin-deficient apple cultivars susceptible to sunburn accumulate lower amounts of carotenoids upon light exposure and show a higher level of Chl degradation than more tolerant cultivars (Merzlyak et al., 2002). Although morphological adaptation to high light and heat stress does occur in grapes (Rosenquist and Morrison, 1989; Mugaru et al., 2011; Verdenal et al., 2019), the morphological properties of apple cultivars were not related to their sunburn susceptibility (Racskó et al., 2005). Similarly, the extremely sunburn-sensitive grape cultivar Bacchus had similar cuticular, epidermis, and hypodermis thickness as the rather tolerant cultivar Müller-Thurgau (Alleweldt et al., 1981), rendering it likely that it is the composition of the berry skin rather than its morphology that confers cultivar resistance against sunburn. From a physical perspective, anthocyanin-containing fruit reach higher temperatures upon illumination than fruit lacking anthocyanins due to a lower albedo (Smart and Sinclair, 1976), possibly counteracting the photoprotective effects of anthocyanins. Cultivar susceptibility is also modulated by bunch morphology as tight clusters can reach higher temperatures above ambient than looser ones and large berries might reach higher temperatures than smaller ones (Smart and Sinclair, 1976).

Rustioni et al. (2015) compared the sunburn susceptibility of 20 white cultivars by exposing detached berries to artificial lighting (LED) after epicuticular wax removal. These authors classified white cultivars on a scale ranging from highly susceptible (e.g., Cornichon blanc, Riesling, Muscat of Alexandria) to tolerant (e.g., Moscato Giallo, Chardonnay, Sauvignon Blanc), based on their ability to protect Chl from photodegradation. More recently, Silvestre et al. (2019) evaluated the incidence of sunburn in 189 grapevine varieties following a heatwave in August 2018 in Alentejo (Portugal). Amongst red varieties,

Alicante Bouchet, Petit Verdot, Dolcetto, Syrah, and Malbec were the cultivars that sustained the most damage whilst Touriga Franca, Touriga Nacional, Grenache, Cabernet Franc, and Cinsaut were classified as tolerant to sunburn. The only international white variety that sustained severe damage was Alvarinho, and in general, white cultivars seemed to be less affected by sunburn than reds, possibly due to different vineyard management approaches. Webb et al. (2010) found no difference in sunburn incidence between red and white cultivars; and reported the most severe damage for Viognier, Pinot Noir, Semillon, and Shiraz; while Grenache, Pinot Gris, and Sauvignon Blanc were the least affected. These rankings of susceptibility under field conditions disagree with the browning index proposed by Rustioni et al. (2015) for some varieties. These discrepancies might be explained by the different approaches taken by the authors, i.e., surveying damage in the field and exposing formerly shaded, detached berries to high-light conditions in the lab. Additionally, the comparison of these results is complicated by a lack of common scale for sunburn damage determination and by the high influence of meta-data, such as cumulative temperatures, water status, UV-B radiation irrigation, and cultural practice.

Likewise, sunburn susceptibility in table grape varieties appears to be unrelated to berry color, with varieties like Calmeria (green berries) and red globe (red berries) being classified as highly susceptible whilst Italia (golden berries) and Flame seedless (red berries) have a low susceptibility (Hannah et al., 2002). Breeding strategies for table grape varieties have developed in different directions than wine grapes, as different characteristics (i.e., visual attributes and sugar loading capacity) have been prioritized for each of these crops. Amongst the characteristics prized in table grapes is their ability to maintain turgor, cultivar selection has thus made them less susceptible to shrivel than wine grapes (Hannah et al., 2002).

Developmental Stage

Contrasting findings have been reported regarding the influence of developmental stages on sunburn susceptibility. Hulands et al. (2014) reported that grape berry susceptibility to sunburn seems to be lowest at the early stages of berry development, and increasing thereafter. They found no significant effects of a high light/high temperature treatment on berry composition and sunburn incidence when Semillon berries were treated early (berry size ~7 mm), whereas the same conditions were found to significantly affect sunburn damage at later stages of development (Hulands et al., 2013, 2014). These findings are supported by Webb et al. (2010), who reported low sunburn damage in pre-véraison grapes and the highest damage during véraison. In contrast, Gouot et al. (2019a,b) have reported higher thermal susceptibility earlier in the season, with tissue necrosis occurring from FST 44.8°C at EL-31 (pea size, Coombe, 1995) and only from 50°C after véraison in Shiraz berries. This is consistent with results from Müller-Thurgau (1883), who reported damage thresholds of 43°C for pre-véraison berries and 55°C for ripening berries of different cultivars. Further, pre-véraison SN symptoms appear in a matter of hours after treatment (Zschokke, 1930).

while SN occurring during ripening leads to much slower shriveling and longer delay times (up to 5 days) for the appearance of symptoms (Nuzzo et al., 2009). Post-véraison SN often also leads to a lower loss in yield when compared to pre-véraison SN.

The varying susceptibility during berry development may relate to a very high ratio of photoprotective pigments to chlorophylls during and shortly after flowering, which gradually decreases during berry development. The concentration of many berry skin pigments and antioxidants on a surface area basis seems to be at a maximum (as is the capacity to up-regulate their biosynthesis) shortly after flowering and decreases thereafter. This has been shown for Chl a and b, a variety of carotenoids including those from the xanthophyll cycle, and berry skin phenolic compounds. The ratio between NPQ and electron transport rate in Kerner and Portugieser also seemed to be at a maximum shortly after flowering (Düring and Davtyan, 2002). During the early stages of development, chloroplasts are still active and berry behavior is more akin to that of leaves, which have developed a series of photoprotective mechanisms to protect the photosynthetic apparatus; a capacity that is progressively lost as berries develop (Joubert et al., 2016). Downey et al. (2004) showed that Chl concentration in berry skins of Shiraz decreased constantly after flowering, accompanied by a decrease in berry skin flavonol and tannin concentration, as well as *FLS* expression. Only after véraison, *FLS* expression and flavonol concentration reaches levels comparable to the flowering stage (Downey et al., 2003). Similarly, carotenoid concentration and waxes (on a surface area basis), as well as the activity of several antioxidant enzymes of grape berries seems to decrease from pea-size towards ripening (Kwasniewski et al., 2010; Muganu et al., 2011; Joubert et al., 2016). These observations might explain why early defoliations (around flowering) have been shown to be more efficient at decreasing susceptibility to sunburn when compared to defoliations performed at pea size and véraison (Gambetta et al., 2019b; Verdenal et al., 2019). At véraison, sunburn protection in grape berries appears to change from a chloroplast-based defense strategy mediated by carotenoids to a strategy based on the accumulation of phenolics, as well as ascorbate (Melino et al., 2009) and GSH (Adams and Liyanage, 1993) in their respective reduced forms. Grape susceptibility to sunburn is thus likely to peak around véraison, when the concentrations of anthocyanins and/or flavonols, ascorbate and GSH, as well as the Car/Chl ratio are comparatively low. Véraison also coincides with the initiation of the second phase of berry expansion that is likely accompanied by ROS-mediated cell wall softening. A study on loquats subjected to high-light and high-temperature regimes at different points in ripening (green, color-changing, and yellow) have also demonstrated differences in the level of expression of the main ROS scavenging enzymes between different ripening stages. Loquats appear to be particularly susceptible to sunburn when changing color from green to yellow (a developmental stage similar to véraison in grapes), with glutathione peroxidase levels at their lowest during color change and dehydroascorbate reductase expression decreasing as the fruit ripened (Jiang et al., 2015).

Abiotic Factors

Water Status and Transpiration

A sufficient water supply promotes canopy transpiration throughout much of the day, lowering the temperature and increasing the relative humidity (RH) in the bunch zone. Consequently, lower canopy transpiration under drought stress might increase FST and sunburn risk (Tarara and Spayd, 2005). Berry transpiration directly reduces FST, making it a potentially important contributor to sunburn protection. Müller-Thurgau (1883) sought to demonstrate this in an early experiment: when he heated berries in dry air (high transpiration), sunburn symptoms appeared at an air temperature of 44°C, while berries heated in water-saturated air (no transpiration) showed symptoms at 41.5°C. However, berry transpiration correlates linearly with VPD, as grape berries lack the ability to regulate transpiration (and thus, FST) actively (Zhang and Keller, 2015). Further, berries cut from drought-stressed vines transpired similar amounts of water as those cut from well-watered vines (Dimopoulos et al., 2020). Therefore, it is unlikely that water status influences sunburn incidence *via* berry transpiration.

Drought stress promotes ROS production in plants by increased electron leakage from PSII to the Mehler reaction and increased photorespiration. In most species, ROS homeostasis under drought is maintained by an increase in antioxidative defense (e.g., SOD, APX, GR) but when the capacity to scavenge ROS, is overwhelmed during prolonged or severe drought stress, oxidative damage occurs, ultimately leading to cell death (Cruz de Carvalho, 2008). In grape berries, limited water supply increases the incidence of cell death when compared to the effects of high light and temperatures on their own (Carvalho et al., 2016). However, drought stress priming has also been shown to promote resistance to heat stress *via* cross-priming reactions in wheat (Wang et al., 2015), and cross-talk between the response to both stresses has been reported in grapevines (Rocheta et al., 2014). This is not surprising, as the antioxidative systems stimulated by drought stress are general ROS defense mechanisms. It was recently demonstrated that grapes from drought-stressed vines also accumulate higher amounts of epicuticular wax than grapes from non-stressed vines (Dimopoulos et al., 2020), potentially increasing resistance to high-light conditions.

Finally, drought stress leads to reduced vigor and smaller canopies which increase bunch exposure and the potential damage by sunburn inducing conditions. Fruit from vigor-constrained drought-stressed canopies are, however, better acclimated to light and heat, and are therefore less sensitive than fruit from dense canopies that are suddenly exposed by cultural practices like leaf removal or hedging.

Wind

Sunburn appears to occur less frequently under windy conditions, mostly due to its cooling effect *via* forced convection, but also to increased berry transpiration at higher wind velocities. FST on the “hot spot” of a fully irradiated ripe berry is 5°C lower when wind velocity increases from 0.5 to 2.0 m·s⁻¹ (Smart and Sinclair, 1976). As direct sunlight elevates berry temperatures above air temperature, forced convection inevitably cools down sun-exposed berries. Although some authors have held that

windy conditions might play a role in sunburn phenomena by substantially increasing berry transpiration, ultimately leading to a hydraulic failure (Schultz, 2007), there is no experimental evidence for this hypothesis. In general, as wind velocity increases, sunburn incidence decreases (Racskó and Schrader, 2012).

Management Practices and Vineyard Layout

Many viticultural management practices directly affect fruit sunlight exposure and therefore, sunburn incidence. An additional consideration is the crop load, closely related to pruning level and the number of buds retained, as it also influences bunch exposure. Worse sunburn damage has been observed when canopies are small and crop loads high (Dry, 2009).

Leaf Removal

Practices such as defoliation are intended to improve aeration, spray penetration, and berry coloration (in red varieties) and decrease disease pressure, but when performed inadequately can lead to a higher canopy porosity increasing the percentage of sunburn. Commonly performed in cool and moderate climates where fruit maturation can be difficult or disease pressure high, the increase in heatwave frequency has made this practice problematic in hot or Mediterranean climates.

Early defoliations (around flowering) have been shown to decrease susceptibility to sunburn when compared to defoliations performed at véraison by promoting a higher accumulation of photoprotectants when compared to defoliations performed at véraison or to non-defoliated controls (Pastore et al., 2013; Young et al., 2016; Brandt et al., 2019; Gambetta et al., 2019a; Verdenal et al., 2019). A study of the transcriptome of Sangiovese berries defoliated at different developmental stages (pre-bloom and véraison), showed that such treatments, when performed early, up-regulated genes related to the synthesis of HSPs and to the phenylpropanoid/flavonoid pathway that controls flavonol glycosylation (Pastore et al., 2013; Zenoni et al., 2017). Conversely, when defoliation was performed at véraison, the affected genes belonged exclusively to the response to stress category, indicating that leaf removal at this stage induces berry stress responses rather than adaptation mechanisms (Pastore et al., 2013).

Row Orientation

Row orientation is an often-underestimated driver of sunburn, even in hot climates like Australia that have experienced substantial sunburn damage for decades. In many viticultural regions, the prevailing row orientation is N-S, which is intended to equally distribute radiation on both sides. However, while the light is indeed distributed equally between both sides of the canopy, berry temperatures differ massively between canopy sides, as E facing fruit is sun-exposed during the cool morning hours, while W facing fruit is sun-exposed during the daily maximum temperatures. At the same time, transpiration of the plant is reduced to a minimum even under well-watered conditions. In a study on Merlot grapes, west exposed berries spent an average of 70.5 h at temperatures above 35°C and 2.7 h above 40°C whilst east exposed bunches only spent

5.4 and 0 h at each of those temperatures. These differences led to sunburn symptoms being observed only on west exposed clusters (Spayd et al., 2002). Other row orientations than N-S have an unequal light distribution between canopy sides but show lower maximum bunch temperatures. In the Southern hemisphere, bunches located on the western side of an N-S oriented row spend the longest time at critical temperatures when compared to other orientations (E-W, NW-SE, NE-SW) and sides of the canopy, followed by berries on the north side of E-W rows (Dry, 2009). Bunches from the sun-exposed side of E-W oriented canopies in Germany have the highest mean temperatures and are sun-exposed during most of the day, but are shaded when ambient temperatures reach a maximum in the afternoon. Comparison to vines in N-S oriented rows within the same experiment demonstrated a higher sunburn incidence on bunches located on the W side of N-S oriented rows than on those from either side of E-W oriented rows (**Figure 9**). This is in accordance with an Australian survey conducted after the 2008 heatwave, which found the highest sunburn incidence occurred in N-S oriented vineyards, in which the median damage was twice as high as in E-W oriented vineyards (Webb et al., 2010). While this might be easily explained by the temperature regime, it is also worth noting that bunches on the sun-exposed side of E-W seemed to be better adapted to high light conditions, showing higher concentrations of flavonols compared to the W side of N-S oriented canopies, as they received a higher amount of radiation during the day (Friedel et al., 2016). Thus, E-W and NW-SE orientations have been recommended as a better alternative to lower FST in vineyards located in the Southern hemisphere (Dry, 2009; Webb et al., 2010). Light distribution can be further modified by row width.

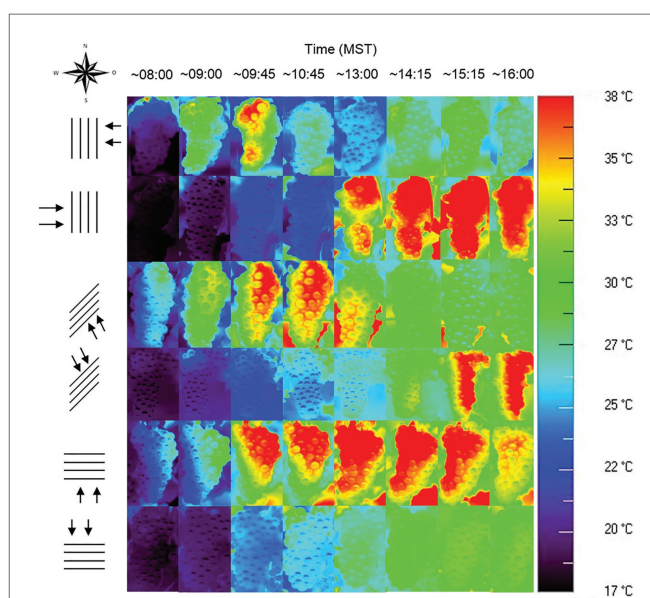


FIGURE 9 | Thermal images of Riesling bunches on the two canopy sides of N-S, NE-SW, and E-W row orientations during the course of the day, taken on August 26, 2012, in Geisenheim, Germany.

Narrower rows and higher canopy height create shading from neighboring plants and have been observed to decrease sunburn incidence (Danenberg, 2019).

Trellis and Training System

Many of the training systems that are utilized in traditional southern European and middle-eastern viticulture were developed to provide a certain degree of shelter to the grapes (e.g., gobelet, pergola). In contrast, traditional training systems in central Europe were usually designed to provide higher fruit exposure. Consequently, trellis systems that are designed to increase fruit exposure such as vertical shoot positioned (VSP), also risk overexposure of clusters (Dry, 2009). Although VSP is a popular system in many viticultural areas because of ease of mechanization, it can also increase the potential for sunburn damage. This seems to be aggravated by high bunch and berry weights normally occurring in strongly pruned systems. Alternative trellising systems such as single high-wire cordon (sprawl); head-training; tendone; pergola; Geneva Double Curtain; closing Y-shaped trellis have been proposed as suitable alternatives since they maintain bunches under a diffuse light regime and decrease direct radiation (Palliotti et al., 2014). Minimal pruning systems employed in the hottest winegrowing regions, normally also offer sufficient shelter to protect grapes from sunburn.

Soil and Irrigation Management

Depending on the type of soil, vineyard floor management can be an additional factor contributing to sunburn development. Bare soils reflect more light and heat than cultivated ones, especially when dealing with reflective soils such as pale-colored sands and shale (Webb et al., 2010). However, the use of cover crops can exacerbate water stress and have negative effects on canopy size by competing with vines for water. Studies have assessed the possibility of using organic (e.g., compost, bark, or straw) or synthetic (e.g., black polyethylene or geotextile) mulches instead. Less damage was observed in 2009 in Australia in vineyards with mulch and/or mown sward than in vineyards with bare soils (Dry, 2009; Webb et al., 2010).

Increasing irrigation to fill the profile has also been recommended in order to maintain the existing canopy and avoid leaf scorching and consequent fruit overexposure in the advent of heatwaves. However, a large grower survey conducted in Australia did not find any significant impacts of irrigation on sunburn appearance (Webb et al., 2010), although the authors strongly suggested irrigation as a means to prevent sunburn by maintaining canopy vitality as discussed in section Water Status and Transpiration.

STRATEGIES OF SUNBURN PROTECTION

A number of active sunburn protection strategies are currently available on the market, including the use of netting, particle-film forming products, antitranspirants, and hydrocooling. These can be deployed as needed to mitigate damage by heatwaves or to adapt established vineyards to changing

climatic conditions. Once a sunburn event has occurred, it might still be helpful to apply protective measures to prevent the spread of sunburn symptoms, especially if adverse meteorological conditions persist. This might reduce damage to berries in the cluster interior that are suddenly exposed to sunlight by the shriveling of exterior berries and might also prevent damage to the rachis.

Netting

The most efficient way to protect grapes against sunburn seems to be the use of nets, a technique that reduces sunburn effectively in table grapes, apples, and other crops. Commercial nets range in light transmission between 20 and 70% (Briassoulis et al., 2007) and are characterized in terms of their shading factor, which depends on the net color, mesh size, and texture (Castellano et al., 2008). Depending on the type and color of netting, reductions in sunlight intensity of 4–9% (PAR), 25–29% (UV), and 5% (IR) have been measured, reducing FST by 7°C and substantially decreasing sunburn incidence (Olivares-Soto et al., 2020). Lobos et al. (2015) observed a 36% reduction in sunburn severity (termed “berry dehydration”) and FST by 7°C when using 35% shading nets, and Oliveira et al. (2014), observed a 50% decrease of shriveled berries under bunch-zone netting. While yield increased in their trial, pH and anthocyanin concentration were significantly lower in berries grown under shade nets (Oliveira et al., 2014). Contrarily, Martínez-Lüscher et al. (2017) found a significant increase in anthocyanins in netted Cabernet Sauvignon grapes when compared to the non-netted control. As berry temperature, PAR and UV radiation are simultaneously reduced by netting, this strategy seems equally effective against SB and SN.

The choice of net color seems to be as important as the type of net. Nets of different colors (e.g., red, blue, pearl, etc.) also known as photo-selective nets, scatter light, alter spectral composition and absorb different spectral bands, thus affecting grape composition and shoot and fruit growth. Peaks in the absorption spectra of cryptochromes and phytochromes have been observed in the blue and red wavelength regions and irradiation at these wavelengths have been observed to increase phenolic compounds (González et al., 2015). Green and red netting transmit 3% more green and red light respectively, and blue nets have on average a 10% higher transmittance in the blue region than black nets (Martínez-Lüscher et al., 2017; Olivares-Soto et al., 2020). When compared to pearl-colored nets, red nets were more effective at reducing sunburn incidence in apples. They provided higher protection from UV-A, and by significantly decreasing the blue/red and blue/far-red ratios, promoted a higher synthesis of anthocyanins whilst pearl-colored nets decreased their synthesis (Olivares-Soto et al., 2020). Black nets have been proven to be more effective to reduce sunburn than white nets as they provide the highest reduction in light transmission and FST whilst not modifying the spectral quality of radiation (Martínez-Lüscher et al., 2017; Manja and Aoun, 2019). Black nets also preserved total anthocyanins more, and anthocyanins and flavonols exhibited higher hydroxylation levels than those under other net colors (blue, pearl, aluminet; Martínez-Lüscher et al., 2017).

Particle Film Forming and Antitranspirant Products

Chemical reflectants such as kaolin and calcium carbonate (CaCO_3) have been trialed with success in different fruit crops. Kaolin [$\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$] is an inert white clay that can reflect UV and IR and reduce FST (Rodríguez et al., 2019). Application of kaolin reduced FST by 1°C and sunburn severity by 12.5%, while fruit quality remained unchanged or even increased (Brillante et al., 2016). CaCO_3 acts in a similar way to kaolin. In Red Roomy grapes sunburn incidence was reduced from 14.8–15% (control) to 1.7–2% when a 2% CaCO_3 solution was applied (Ahmed et al., 2013). Results from trials on grapes, as well as on pomegranate fruit treated with kaolin have shown an increase in total polyphenols, anthocyanin, and ascorbate content (Dinis et al., 2016; Sharma et al., 2018). The application of particle films only marginally decreases FST but increases the reflection of radiation. Hence, this strategy appears to be more effective against SB than SN.

An alternative to particle film-forming products are pine resin-based products which possess antitranspirant properties. Results about the effectiveness of these products in viticulture are so far inconclusive. While Fahey and Rogiers (2019) showed that pinolene application was successful in lowering fruit transpiration, Rodríguez et al. (2019) showed that FST and sunburn actually increase due to a lack of transpiration. Further, Brillante et al. (2016) observed a decrease in fruit quality and consumer preference for the wines made with these products.

Other forms of transpiration regulation include the use of abscisic acid (S-ABA). S-ABA is a growth regulator that controls stomatal closure, transpiration, and the plant's response to water stress. Foliar application of S-ABA has been trialed on apples in Japan and South Africa. Similar to pine-based products, results are inconclusive (Iams et al., 2009; Zenoni et al., 2017).

Evaporative Cooling

This method consists of wetting the fruit and/or the canopy with overhead sprinklers or micro-sprinklers above or under the canopy in order to reduce FST and thus SN. Yang (2018) provided a detailed model for the activation of micro-sprinklers in northern highbush blueberries to avoid sunburn damage. Greenspan (2009) reported that under-canopy and over-canopy cooling using micro-sprinklers reduced FST by 5°C and almost 12°C , respectively, compared to control vines, reducing sunburn, and berry dehydration.

Bagging

Fruit bagging is often used to produce high-quality table grapes, enabling a good and homogenous coloration, aromatic quality, and protection against grape berry moth and sunburn (Karajeh, 2018). Paper bags have been cited as being as effective as dark nets in reducing sunburn (Tsai et al., 2013); they reduce the temperature inside the bag and block direct sunlight, which makes them effective against both SB and SN. The efficiency of bags depends on the color and material, as several options exist.

CONSEQUENCES FOR FRUIT QUALITY AND WINEMAKING

Whereas SN leads to shriveled berries and mostly impacts yields, SB affects berry composition with a consequent detrimental effect on wine quality. It is often unclear, however, whether the negative impact on wine sensory characteristics results from the sunburnt berries themselves or if it is simply a consequence of fruit overexposure to heat and sunlight. A study by Bondada and Keller (2012) on Cabernet Sauvignon berries showed lower TSS, tartaric, and malic acid levels in berries affected by sunburn when compared to healthy berries. The observed lower levels of tartaric and malic acid, however, were probably due to temperature-induced degradation rather than sunburn itself (Tarara and Spayd, 2005; Pastore et al., 2013; Brandt et al., 2019). The effect of SB on TSS is not clear, with multiple studies reporting inconsistent results across vintages or no effect at all on this parameter (Spayd et al., 2002; Greer and La Borde, 2006). This seems logical, as SB is mostly a skin phenomenon with little to no effect on the pulp. Nevertheless, uneven ripening is a disorder associated with sunburn in practice (Figure 10). Temperatures over 30°C

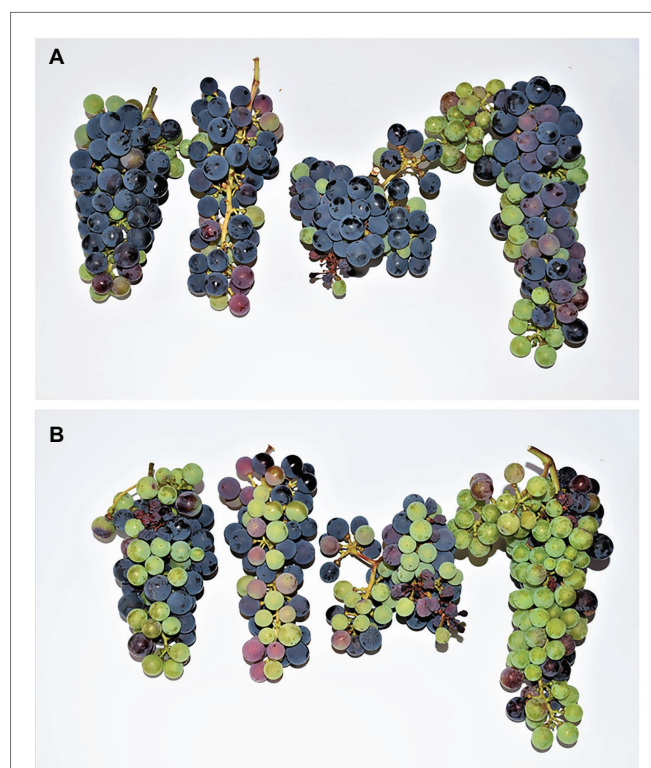


FIGURE 10 | Uneven berry development induced by light and heat overexposure in Cabernet Sauvignon with minimal sunburn damage (0–3%). **(A)** Eastern side of four bunches showing normal development. **(B)** Western side of the same bunches, showing delayed color change, smaller berries (mean: 1.04 vs. 1.29 g), and a delayed sugar accumulation (mean: 8.7 vs. 15.2°Brix). Images were taken on August 24, 2020, in Geisenheim, Germany, after a pre-véraison heatwave that occurred from August 7, 2020 to August 12, 2020.

overall flavonoid content, especially anthocyanin concentration (Pastore et al., 2013), a phenomenon likely related to oxidative stress (Mori et al., 2007). SB and SN lead to a further decrease of anthocyanin concentration, compromising wine color.

The response of aroma compounds in relation to sunburn damage has not been studied. However, studies under less stressful conditions than those leading to sunburn have demonstrated that light exposure modulates the synthesis of many compounds including the aroma compounds. Under moderate climatic conditions; increased PAR and UV radiation increased the final concentrations of terpenes; including linalool, citronellol, nerol and geraniol, and C_{13} -norisoprenoids; including TDN, 3-oxo- α -ionol, β -ionone, and β -damascenone; whilst increased UV radiation decreased the amount of ethyl esters of fatty acids in Pinot Noir wine (Marais et al., 1992; Schüttler et al., 2015; Song et al., 2015; Friedel et al., 2016; Sasaki et al., 2016; Young et al., 2016; Gambetta et al., 2017). In varieties like Riesling that are prone to accumulate TDN, it is reasonable to infer that this compound could increase to values above the perception threshold, negatively impacting the aroma quality of the wine. The effect of higher temperatures depends on the aroma class in consideration; C_{13} -norisoprenoid concentration is higher in grapes from warmer climates, although extreme temperature ($>35^{\circ}\text{C}$) appears to induce their degradation (Asproudi et al., 2016; Gambetta et al., 2017). Likewise, between 20 and 40°C , the concentration of terpenes increases whilst temperatures favoring sunburn development (above 40°C) inhibit the enzymes in the mevalonate pathway, reducing terpene synthesis while also increasing their degradation (Loreto and Schnitzler, 2010). Bagged fruit and fruit shaded in boxes retained a higher amount of aromatic compounds like monoterpenes and C_6 alcohols in hot climates (Bureau et al., 1998; Scafidi et al., 2013), indicating a degradation of aromatic quality under light and heat stress (Scafidi et al., 2013). Such conditions also impact on wine proteins and increase the tendency to form haze (Meier et al., 2016).

There are very few reports of the consequences of sunburn on wine quality. SB has been linked to undesirable phenolic characters (in particular in regards to white berries), a general loss of flavor and increased bitterness and browning of white wines (Allan, 2003; Dry, 2009). Likewise, Greer and La Borde (2006) reported increased brown coloration and bitterness in Chardonnay wines produced with sunburnt berries, and lower overall quality as reported by a sensory panel. These wines had more intense peaks at 440 nm suggesting a higher content of polyphenols that could be responsible for the increased bitterness. Red wine quality is intimately related to color, and as the appearance of sunburn symptoms requires berries to spend a certain amount of time above critical temperature thresholds ($30\text{--}35^{\circ}\text{C}$), the consequent degradation of anthocyanins leads to a loss of coloration and ultimately color bleaching, decreasing overall wine quality (Kliewer and Torres, 1972).

Necrotic SN berries remain on the vine if harvest machines are adjusted correctly or can be removed by automated sorting tables employing airflow or density sorting processes (Lafontaine and Freund, 2013). They may, however, be problematic when present in fermentations on the skins. The modification of

winemaking techniques such as lower pressing intensity and limited phenol extraction through shorter skin contact together with careful fining could be envisageable to limit the negative effects of sunburn on wine composition. More work on this topic is necessary.

FUTURE PERSPECTIVES

Great progress has been made in our understanding of sunburn in the past decade thanks to advancements in both analytical and molecular technologies. However, most of this knowledge has been generated on apples. Although apples and grapes share many common stress responses, differences in composition, physical properties, management and growth conditions, as well as their ability to adapt to stress make it difficult to extrapolate all findings from apples to grapes. Consequently, additional research is needed about sunburn in both wine and table grapes.

To yield comparable experimental results, future research should use a clear nomenclature of sunburn type, report severity and incidence of the damage. Access to accurate metadata, such as developmental stage, vineyard layout, fruit exposure and climatic conditions preceding the event, plant material, and cultural practice would be ideal. Information on vineyard layout and site characteristics would aid with the interpretation of SN and SB data collected in field surveys and would make large amounts of data accessible for research. Also, if provided correct metadata, an objective classification of the sunburn susceptibility of different grape varieties would be possible. This could guide producers' choice of planting material and management practices. The comparison of susceptible and tolerant varieties on a compositional and morphological level might help to identify traits conferring tolerance to high light and temperature, which would also be of use in phenotyping new tolerant varieties and clones. If the susceptibility of a given cultivar and developmental stage and the duration of adaptation were known, this information could be combined with accurate berry FST models to predict sunburn events. In addition, modeling approaches on the canopy level could provide a better insight for mitigation strategies of sunburn protection considering plant architecture and training systems in vineyards.

Studies investigating sunburn susceptibility at different stages of berry development have produced conflicting results so far. It remains unclear whether these different results originate from the methodology used, the prevailing type of sunburn (which is often not reported), or from cultivar-specific differences. If experimental plants grown under standardized conditions were exposed to combined heat and light stress at different developmental stages, response surfaces for SB and SN could be produced with a limited set of experiments in controlled environments. This would greatly advance the current understanding of SB and SN thresholds and their physiological background. Although recent progress has allowed to discriminate between short, medium, and long term adaptation to stress, it remains unclear how long it takes the berry to become

fully adapted to light and heat stress, or which conditions favor specific adaptation strategies.

Finally, although the consequences of SB and SN on the visual appearance and yield of grapes are increasingly well understood, there is very little insight into their effects on wine composition and quality, as are the oenological measures that have the best potential to alleviate sunburn-related problems. Whether it is a reduction of pressing intensity or changes in type and dosage of fining agents, understanding the best ways to manage affected fruit will help winemakers reduce economical losses at the winery as global temperatures and the incidence of sunburn rise.

CONCLUSION

Sunburn is mainly a consequence of photooxidative damage that is exacerbated by thermal stress. When faced with light and/or heat stress, the berry activates a cascade of reactions aimed at protecting its photosynthetic apparatus by compensating the accumulation of toxic ROS species. This is accomplished through an increased production of antioxidants, HSPs, carotenoids, and polyphenols. It is worth noting that research on the antioxidative apparatus of fruit is far from complete, and the relative contribution of different antioxidative defense pathways is not yet fully understood. Furthermore, these responses vary with the developmental stage of the fruit, the degree of acclimation and interaction with other environmental and biological factors. When the capacity of the berries to detoxify ROS is overwhelmed, permanent changes in the visual appearance and composition of the peel occur. Under sub-lethal conditions, SB occurs while lethal conditions lead to cell death accompanied by necrosis.

As temperature and drought increase with climate change, the frequency of sunburn is set to increase. Furthermore, this problem is not restricted to a particular region but is a worldwide phenomenon that leads to non-negligible economical losses and as such, merits the study of prevention and correction measures, at the vineyard and winery level.

REFERENCES

- Adams, D. O., and Liyanage, C. (1993). Glutathione increases in grape berries at the onset of ripening. *Am. J. Enol. Vitic.* 44, 333–338.
- Ahmed, F. F., Abdel Aal, A. M. K., El-Sayed, M. A., and Sayed, H. R. (2013). Protecting red roomy grapevines growing under Minia region conditions from sunburn damage. *Stem Cell* 4, 15–20.
- Allan, W. (2003). *Winegrape assessment in the vineyard and at the winery*. Broadview, Australia: Winetitles Media.
- Alleweldt, G., Engel, M., and Gebbing, H. (1981). Histologische Untersuchungen an Weinbeeren. *Vitis* 20, 1–7.
- Araújo, M., Santos, C., and Dias, M. C. (2018). “Can Young olive plants overcome heat shock?” in *Theory and practice of climate adaptation*. eds. F. Alves, W. L. Filho and U. Azeiteiro (Cham, Switzerland: Springer), 193–203.
- Asproudi, A., Petrozziello, M., Cavalletto, S., and Guidoni, S. (2016). Grape aroma precursors in cv. Nebbiolo as affected by vine microclimate. *Food Chem.* 211, 947–956. doi: 10.1016/j.foodchem.2016.05.070
- Aubert, I. (2015). Coup de sec sur les vignes. Available at: www.mon-viti.com (Accessed June 18, 2020).
- Barber, H. N., and Sharpe, P. J. H. (1971). Genetics and physiology of sunscald of fruits. *Agric. Meteorol.* 8, 175–191. doi: 10.1016/0002-1571(71)90107-5

The best prevention measures are those that achieve a reduction of both intercepted light (PAR and UV) and FST. Preventive measures in the vineyard include seasonal practices such as timing and intensity of leaf removal and hedging, irrigation including evaporative cooling and application of reflectants or nets, and long-term adaptation range from cultivars selection to structural adaptation in the vineyard such as training systems or row orientation. Information is particularly lacking on the organoleptic consequences of producing wine with sunburnt berries, and if there are any tolerance/rejection thresholds that should be considered for this type of damage. Further study could help clarify these aspects as well as develop effective corrective measure at the winery.

AUTHOR CONTRIBUTIONS

JG and MF initiated and designed the overall concept and wrote the manuscript. All authors revised the manuscript, approved the final version and approved it for publication.

FUNDING

The work was supported as preliminary studies through Forschungsring des deutschen Weinbaus (FDW). We acknowledge funding by the Open Access publication funds of Geisenheim University. JG would like to thank the Faculty of Science, Charles Sturt University for their financial support.

ACKNOWLEDGMENTS

We would like to thank Suzy Rogiers and Vitis for the pictures on cuticle damage and Prof. Joachim Schmid (Geisenheim University) for pictures of sunburn damage in the vineyard.

- Bonada, M., Sadras, V. O., and Fuentes, S. (2013). Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of shiraz and chardonnay and its relationship with berry shrivel. *Aust. J. Grape Wine Res.* 19, 87–94. doi: 10.1111/ajgw.12010
- Bondada, B. R. (2019). “Sustaining grape production under challenging climate circumstances” in *Proceedings of the 21st GIESCO International Meeting*; June 23–28, 2019; Thessaloniki, 57–60.
- Bondada, B. R., and Keller, M. (2012). Not all shrivels are created equal - morpho-anatomical and compositional characteristics differ among different shrivel types that develop during ripening of grape (*Vitis vinifera* L.) berries; June 23–28, 2019. *Am. J. Plant Sci.* 03, 879–898. doi: 10.4236/ajps.2012.37105
- Brandt, M., Scheidweiler, M., Rauhut, D., Patz, C. D., Will, F., Zorn, H., et al. (2019). The influence of temperature and solar radiation on phenols in berry skin and maturity parameters of *Vitis vinifera* L. cv. Riesling. *OENO One* 53, 261–276. doi: 10.20870/oeno-one.2019.53.2.2424
- Briassoulis, D., Mistriotis, A., and Eleftherakis, D. (2007). Mechanical behaviour and properties of agricultural nets. Part II: analysis of the performance of the main categories of agricultural nets. *Polym. Test.* 26, 970–984. doi: 10.1016/j.polymertesting.2007.06.010
- Brillante, L., Belfiore, N., Gaiotti, F., Lovat, L., Sansone, L., Poni, S., et al. (2016). Comparing kaolin and pinolene to improve sustainable grapevine

- production during drought. *PLoS One* 11:e0156631. doi: 10.1371/journal.pone.0156631
- Bureau, S. M., Razungles, A. J., Baumes, R. L., and Bayonove, C. L. (1998). Effect of vine or bunch shading on the carotenoid composition in *Vitis vinifera* L. berries. I. Syrah grapes. *Wein-Wissenschaft* 53, 64–71.
- Cabane, M., Afif, D., and Hawkins, S. (2012). “Lignins and abiotic stresses” in *Advances in botanical research*. eds. L. Jouanin and C. Lapiere (Netherlands: Elsevier), 219–262.
- Calderon-Orellana, A., Serra Stepke, I., and Puentes, P. (2018). Golpe de sol en uva para vino en el valle de Itata: más de lo esperado. *Boletín Vinos del Sur* 2, 3–4.
- Carvalho, L. C., Coito, J. L., Gonçalves, E. F., Chaves, M. M., and Amâncio, S. (2016). Differential physiological response of the grapevine varieties Touriga Nacional and Trincadeira to combined heat, drought and light stresses. *Plant Biol.* 18, 101–111. doi: 10.1111/plb.12410
- Castellano, S., Mugnozza, G. S., Russo, G., Briassoulis, D., Mistrisiotis, A., Hemming, S., et al. (2008). Plastic nets in agriculture: a general review of types and applications. *Appl. Eng. Agric.* 24, 799–808. doi: 10.13031/2013.25368
- Chang, B. W., Zhang, Y., and Keller, M. (2017). “Berry Splitting Resistance of Different Grape Cultivars” in *Proceedings of the 20th GIESCO International Meeting*; November 5–9, 2017; Mendoza, 370–374.
- Chen, L. S., Li, P., and Cheng, L. (2008). Effects of high temperature coupled with high light on the balance between photooxidation and photoprotection in the sun-exposed peel of apple. *Planta* 228, 745–756. doi: 10.1007/s00425-008-0776-3
- Cohen, S. D., Tarara, J. M., Gambetta, G. A., Matthews, M. A., and Kennedy, J. A. (2012). Impact of diurnal temperature variation on grape berry development, proanthocyanidin accumulation, and the expression of flavonoid pathway genes. *J. Exp. Bot.* 63, 2655–2665. doi: 10.1093/jxb/err449
- Coombe, B. G. (1995). Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. *Aust. J. Grape Wine Res.* 1, 104–110. doi: 10.1111/j.1755-0238.1995.tb00086.x
- Cruz de Carvalho, M. H. (2008). Drought stress and reactive oxygen species. *Plant Signal. Behav.* 3, 156–165.
- Czemmel, S., Heppel, S. C., and Bogs, J. (2012). R2R3 MYB transcription factors: key regulators of the flavonoid biosynthetic pathway in grapevine. *Protoplasma* 249, 109–118. doi: 10.1007/s00709-012-0380-z
- Danenberg, E. (2019). How is climate change affecting Australian vineyards and what are growers doing to respond? *Australian and New Zealand Grapegrower and Winemaker* (February), 18–21.
- Debolt, S., Melino, V., and Ford, C. M. (2007). Ascorbate as a biosynthetic precursor in plants. *Ann. Bot.* 99, 3–8. doi: 10.1093/aob/mcl236
- Dimopoulos, N., Tindjau, R., Wong, D. C. J., Matzat, T., Haslam, T., Song, C., et al. (2020). Drought stress modulates cuticular wax composition of the grape berry. *J. Exp. Bot.* 71, 3126–3141. doi: 10.1093/jxb/era046
- Dinis, L. T., Bernardo, S., Conde, A., Pimentel, D., Ferreira, H., Félix, L., et al. (2016). Kaolin exogenous application boosts antioxidant capacity and phenolic content in berries and leaves of grapevine under summer stress. *J. Plant Physiol.* 191, 45–53. doi: 10.1016/j.jplph.2015.12.005
- Downey, M. O., Harvey, J. S., and Robinson, S. P. (2003). Synthesis of flavonols and expression of flavonol synthase genes in the developing grape berries of shiraz and chardonnay (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 9, 110–121. doi: 10.1111/j.1755-0238.2003.tb00261.x
- Downey, M. O., Harvey, J. S., and Robinson, S. P. (2004). The effect of bunch shading on berry development and flavonoid accumulation in shiraz grapes. *Aust. J. Grape Wine Res.* 10, 55–73. doi: 10.1111/j.1755-0238.2004.tb00008.x
- Dry, P. (2009). *Bunch exposure management*. Adelaide: Australian Wine Research Institute.
- Düring, H., and Davtyan, A. (2002). Developmental changes of primary processes of photosynthesis in sun- and shade-adapted berries of two grapevine cultivars. *Vitis* 41, 63–67.
- Edgley, M., Close, D. C., Measham, P. F., and Nichols, D. S. (2019). Physiochemistry of blackberries (*Rubus* L. subgenus *Rubus Watson*) affected by red drupelet reversion. *Postharvest Biol. Technol.* 153, 183–190. doi: 10.1016/j.postharvbio.2019.04.012
- Fahey, D. J., and Rogiers, S. Y. (2019). Di-1-p-menthene reduces grape leaf and bunch transpiration. *Aust. J. Grape Wine Res.* 25, 134–141. doi: 10.1111/ajgw.12371
- Felicitetti, D., and Schrader, L. E. (2009). Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and carotenoids. *Plant Sci.* 176, 78–83. doi: 10.1016/j.plantsci.2008.09.013
- Ferrandino, A., and Lovisolo, C. (2014). Abiotic stress effects on grapevine (*Vitis vinifera* L.): focus on abscisic acid-mediated consequences on secondary metabolism and berry quality. *Environ. Exp. Bot.* 103, 138–147. doi: 10.1016/j.envexpbot.2013.10.012
- Friedel, M., Frotscher, J., Nitsch, M., Hofmann, M., Bogs, J., Stoll, M., et al. (2016). Light promotes expression of monoterpene and flavonol metabolic genes and enhances flavour of winegrape berries (*Vitis vinifera* L. cv. Riesling). *Aust. J. Grape Wine Res.* 22, 409–421. doi: 10.1111/ajgw.12229
- Friedel, M., Weber, M., Zacharias, J., Patz, C. D., and Stoll, M. (2012). “Impact of microclimate on berry quality parameters of white Riesling (*Vitis vinifera* L.)” in *IXE Congres des Terroirs Vitivinicoles*. ed. B. Bois (Epernay, France: Comité Interprofessionnel du vin de Champagne), 3–9.
- Gambetta, J. M., Cozzolino, D., Bastian, S. E. P., and Jeffery, D. W. (2017). Exploring the effects of geographical origin on the chemical composition and quality grading of *Vitis vinifera* L. cv. Chardonnay grapes. *Molecules* 22:218. doi: 10.3390/molecules22020218
- Gambetta, J. M., Holzapfel, B. P., and Schmidtke, L. (2019a). What is the best time to remove leaves to minimise sunburn? *Grapegrower and Winemaker* (661), 28–30.
- Gambetta, J. M., Romat, V., Holzapfel, B. P., and Schmidtke, L. (2019b). “Assessment of sunburn damage in Chardonnay grapes in relation to leaf removal timing.” in *Australian Wine Industry Technical Conference, Adelaide*; June 26–29, 2019.
- Gechev, T. S., Van Breusegem, F., Stone, J. M., Denev, I., and Laloi, C. (2006). Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *BioEssays* 28, 1091–1101. doi: 10.1002/bies.20493
- Genovese, M., Nuzzo, V., Schakel, M. A., and Matthews, M. A. (2010). Scottatura solari su vite, come e quando insorgono e come evitarle. *L'Informatore Agrario* 23, 45–53.
- Glenn, D. M., and Yuri, J. A. (2013). Photosynthetically active radiation (PAR) x ultraviolet radiation (UV) interact to initiate solar injury in apple. *Sci. Hortic.* 162, 117–124. doi: 10.1016/j.scienta.2013.07.037
- González, C. V., Fanzone, M. L., Cortés, L. E., Bottini, R., Lijavetzky, D. C., Ballare, C. L., et al. (2015). Fruit-localized photoreceptors increase phenolic compounds in berry skins of fieldgrown *Vitis vinifera* L. cv. Malbec. *Phytochemistry* 110, 46–57. doi: 10.1016/j.phytochem.2014.11.018
- Gouot, J. C., Smith, J. P., Holzapfel, B. P., and Barril, C. (2019a). Grape berry flavonoid responses to high bunch temperatures post véraison: effect of intensity and duration of exposure. *Molecules* 24:4341. doi: 10.3390/molecules24234341
- Gouot, J. C., Smith, J. P., Holzapfel, B. P., and Barril, C. (2019b). Impact of short temperature exposure of *Vitis vinifera* L. cv. Shiraz grapevine bunches on berry development, primary metabolism and tannin accumulation. *Environ. Exp. Bot.* 168:103866. doi: 10.1016/j.envexpbot.2019.103866
- Greenspan, M. D. (2009). Investigating low-volume approaches to vineyard cooling. *Wine Business Monthly*.
- Greer, D. H., and La Borde, D. (2006). Sunburn of grapes affects wine quality. *The Australian and New Zealand Grapegrower and Winemaker* 506, 21–23.
- Greer, D. H., Rogiers, S. Y., and Steel, C. C. (2006). Susceptibility of chardonnay grapes to sunburn. *Vitis* 45, 147–148.
- Gregan, S. M., Wargent, J. J., Liu, L., Shinkle, J., Hofmann, R., Winefield, C., et al. (2012). Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of sauvignon Blanc grapes. *Aust. J. Grape Wine Res.* 18, 227–238. doi: 10.1111/j.1755-0238.2012.00192.x
- Guillaumie, S., Fouquet, R., Kappel, C., Camps, C., Terrier, N., Moncomble, D., et al. (2011). Transcriptional analysis of late ripening stages of grapevine berry. *BMC Plant Biol.* 11:165. doi: 10.1186/1471-2229-11-165
- Hale, C. R., and Buttrose, M. S. (1974). Effect of temperature on ontogeny of berries of *Vitis vinifera* L. cv. Cabernet sauvignon. *J. Am. Soc. Hortic. Sci.* 99, 390–394.
- Hannah, R., Jaensch, D., and Moulds, G. (2002). *Production guidelines for Australian table grape varieties*. Available at: www.agriculture.vic.gov.au (Accessed June 18, 2020).
- Havaux, M. (2014). Carotenoid oxidation products as stress signals in plants. *Plant J.* 79, 597–606. doi: 10.1111/tpj.12386
- Hernández, I., Alegre, L., Van Breusegem, F., and Munné-Bosch, S. (2009). How relevant are flavonoids as antioxidants in plants? *Trends Plant Sci.* 14, 125–132. doi: 10.1016/j.tplants.2008.12.003
- Hickey, C. C., Kwasniewski, M. T., and Wolf, T. K. (2018). Leaf removal effects on cabernet franc and petit verdot: II. Grape carotenoids, phenolics, and wine sensory analysis. *Am. J. Enol. Vitic.* 69, 231–246. doi: 10.5344/ajev.2018.17107

- Hickey, C. C., and Wolf, T. K. (2019). Intensive fruit-zone leaf thinning increases *Vitis vinifera* L. 'cabernet sauvignon' berry temperature and berry phenolics without adversely affecting berry anthocyanins in Virginia. *HortScience* 54, 1181–1189. doi: 10.21273/HORTSCI13904-19
- Hofmann, R. W., Jordan, B. R., Winefield, C. S., Stilwell, S. A., Shinkle, J. R., and Wargent, J. J. (2006). *UV radiation in New Zealand: Implications for grape quality*. Auckland, New Zealand: National Institute of Water & Atmospheric Research.
- Höll, J., Lindner, S., Walter, H., Joshi, D., Poschet, G., Pfleger, S., et al. (2019). Impact of pulsed UV-B stress exposure on plant performance: how recovery periods stimulate secondary metabolism while reducing adaptive growth attenuation. *Plant, Cell Environ.* 42, 801–814. doi: 10.1111/pce.13409
- Hu, S., Ding, Y., and Zhu, C. (2020). Sensitivity and responses of chloroplasts to heat stress in plants. *Front. Plant Sci.* 11:375. doi: 10.3389/fpls.2020.00375
- Hulands, S., Greer, D. H., and Harper, J. D. I. (2013). The interactive effects of temperature and light intensity on *Vitis vinifera* cv. 'Semillon' grapevines. I. Berry growth and development. *Eur. J. Hortic. Sci.* 78, 175–184.
- Hulands, S., Greer, D. H., and Harper, J. D. I. (2014). The interactive effects of temperature and light intensity on *Vitis vinifera* cv. 'Semillon' grapevines. II. Berry ripening and susceptibility to sunburn at harvest. *Eur. J. Hortic. Sci.* 79, 1–7.
- Iamsub, K., Sekozawa, Y., Sugaya, S., Gemma, H., and Kamuro, Y. (2009). Alleviating sunburn injury in apple fruit using natural and fertilizer forms of S-abscisic acid and its underlying mechanism. *J. Food Agric. Environ.* 7, 446–452.
- INRA (2003). *Impacts de la canicule 2003 sur la vigne et le vin*. Available at: <https://www.futura-sciences.com/sciences/actualites/recherche-impacts-canicule-2003-vigne-vin-3418/> (Accessed June 18, 2020).
- Jenkins, G. I. (2009). Signal transduction in responses to UV-B radiation. *Annu. Rev. Plant Biol.* 60, 407–431. doi: 10.1146/annurev.arplant.59.032607.092953
- Jenks, M. A., and Ashworth, E. N. (1999). "Plant epicuticular waxes: function, production, and genetics" in *Horticultural reviews*. ed. J. Janick (Canada: John Wiley & Sons), 1–68.
- Jiang, J. M., Lin, Y. X., Chen, Y. Y., Deng, C. J., Gong, H. W., Xu, Q. Z., et al. (2015). Proteomics approach reveals mechanism underlying susceptibility of loquat fruit to sunburn during color changing period. *Food Chem.* 176, 388–395. doi: 10.1016/j.foodchem.2014.12.076
- Joubert, C., Young, P. R., Eyéghé-Bickong, H. A., and Vivier, M. A. (2016). Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions. *Front. Plant Sci.* 7:786. doi: 10.3389/fpls.2016.00786
- Karajeh, M. R. (2018). Pre-harvest bagging of grape clusters as a non-chemical physical control measure against certain pests and diseases of grapevines. *Org. Agric.* 8, 259–264. doi: 10.1007/s13165-017-0197-3
- Kliever, W. M., and Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. *Am. J. Enol. Vitic.* 23, 71–77.
- Kok, D., and Bal, E. (2018). Leaf removal treatments combined with kaolin particle film technique from different directions of grapevine's canopy affect the composition of phytochemicals of cv. Muscat Hamburg *V. vinifera* L. *Erwebs-Obstbau* 60, 39–45. doi: 10.1007/s10341-017-0337-7
- Kolb, C. A., Kopecký, J., Riederer, M., and Pfündel, E. E. (2003). UV screening by phenolics in berries of grapevine (*Vitis vinifera*). *Funct. Plant Biol.* 30, 1177–1186. doi: 10.1071/FP03076
- Koyama, K., Ikeda, H., Poudel, P. R., and Goto-Yamamoto, N. (2012). Light quality affects flavonoid biosynthesis in young berries of cabernet sauvignon grape. *Phytochemistry* 78, 54–64. doi: 10.1016/j.phytochem.2012.02.026
- Krasnow, M., Matthews, M. A., Smith, R. J., Benz, J., Weber, E., and Shackel, K. A. (2010). Distinctive symptoms differentiate four common types of berry shrivel disorder in grape. *Calif. Agric.* 64, 155–159. doi: 10.3733/ca.v064n03p155
- Kwasniewski, M. T., Vanden Heuvel, J. E., Pan, B. S., and Sacks, G. L. (2010). Timing of cluster light environment manipulation during grape development affects C13-norisoprenoid and carotenoid concentrations in Riesling. *J. Agric. Food Chem.* 58, 6841–6849. doi: 10.1021/jf904555p
- Lafontaine, M., and Freund, M. (2013). "Improving optical fruit sorting by non-destructive determination of quality parameters affecting wine quality" in *Optical characterization of materials*. eds. J. Bayerer, F. P. Leon and T. Langle (Germany: KIT Scientific Publishing), 115–126.
- Li, P., and Cheng, L. (2008). The shaded side of apple fruit becomes more sensitive to photoinhibition with fruit development. *Physiol. Plant.* 134, 282–292. doi: 10.1111/j.1399-3054.2008.01131.x
- Li, P., and Cheng, L. (2009). The elevated anthocyanin level in the shaded peel of 'Anjou' pear enhances its tolerance to high temperature under high light. *Plant Sci.* 177, 418–426. doi: 10.1016/j.plantsci.2009.07.005
- Lim, C. J., Yang, K. A., Hong, J. K., Choi, J. S., Yun, D. J., Hong, J. C., et al. (2006). Gene expression profiles during heat acclimation in *Arabidopsis thaliana* suspension-culture cells. *J. Plant Res.* 119, 373–383. doi: 10.1007/s10265-006-0285-z
- Liu, Y., Chen, N., Zuo, C., Wu, Y., Che, F., and Chen, B. (2018). The mechanism of color fading in sunburned apple peel. *Acta Physiol. Plant.* 41:2. doi: 10.1007/s11738-018-2792-7
- Lobos, G. A., Acevedo-Opazo, C., Guajardo-Monero, A., Valdés-Gómez, H., Taylor, J. A., and Laurie, F. (2015). Effects of kaolin-based particle film and fruit zone netting on cabernet sauvignon grapevine physiology and fruit quality. *OENO one* 49, 137–144. doi: 10.20870/oeno-one.2015.49.2.86
- Loreto, F., and Schnitzler, J. P. (2010). Abiotic stresses and induced BVOCs. *Trends Plant Sci.* 15, 154–166. doi: 10.1016/j.tplants.2009.12.006
- Luo, H. B., Ma, L., Xi, H. F., Duan, W., Li, S. H., Loescher, W., et al. (2011). Photosynthetic responses to heat treatments at different temperatures and following recovery in grapevine (*Vitis amurensis* L.) leaves. *PLoS One* 6:e23033. doi: 10.1371/journal.pone.0023033
- Ma, F., and Cheng, L. (2003). The sun-exposed peel of apple fruit has higher xanthophyll cycle-dependent thermal dissipation and antioxidants of the ascorbate–glutathione pathway than the shaded peel. *Plant Sci.* 165, 819–827. doi: 10.1016/S0168-9452(03)00277-2
- Makris, D. P., and Rossiter, J. T. (2002). An investigation on structural aspects influencing product formation in enzymic and chemical oxidation of quercetin and related flavonols. *Food Chem.* 77, 177–185. doi: 10.1016/S0308-8146(01)00333-8
- Manja, K., and Aoun, M. (2019). The use of nets for tree fruit crops and their impact on the production: A review. *Sci. Hortic.* 246, 110–122. doi: 10.1016/j.scienta.2018.10.050
- Marais, J., Versini, G., van Wyk, C. J., and Rapp, A. (1992). Effect of region on free and bound monoterpene and C13-norisoprenoid concentrations in Weisser Riesling wines. *S. Afr. J. Enol. Vitic.* 13, 71–77. doi: 10.21548/13-2-2177
- Martínez-Lüscher, J., Chen, C. L., Brillante, L., and Kurtural, S. K. (2017). Partial solar radiation exclusion with color shade nets reduces the degradation of organic acids and flavonoids of grape berry (*Vitis vinifera* L.). *J. Agric. Food Chem.* 65, 10693–10702. doi: 10.1021/acs.jafc.7b04163
- McDonald, M. S., Hughes, M., Burns, J., Lean, M. E., Matthews, D., and Crozier, A. (1998). Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. *J. Agric. Food Chem.* 46, 368–375. doi: 10.1021/jf970677e
- McKenzie, R., Smale, D., Bodeker, G., and Claude, H. (2003). Ozone profile differences between Europe and New Zealand: effects on surface UV irradiance and its estimation from satellite sensors. *J. Geophys. Res. [Atmos.]* 108. doi: 10.1029/2002JD002770
- Meier, M., Jaekels, N., Tenzer, S., Stoll, M., Decker, H., Fronk, P., et al. (2016). Impact of drought stress on concentration and composition of wine proteins in Riesling. *Eur. Food Res. Technol.* 242, 1883–1891. doi: 10.1007/s00217-016-2688-y
- Melgarejo, P., Martínez, J. J., Hernández, F., Martínez-Font, R., Barrows, P., and Erez, A. (2004). Kaolin treatment to reduce pomegranate sunburn. *Sci. Hortic.* 100, 349–353. doi: 10.1016/j.scienta.2003.09.006
- Melino, V. J., Soole, K. L., and Ford, C. M. (2009). Ascorbate metabolism and the developmental demand for tartaric and oxalic acids in ripening grape berries. *BMC Plant Biol.* 9:145. doi: 10.1186/1471-2229-9-145
- Merzlyak, M. N., and Chivkunova, O. B. (2000). Light-stress-induced pigment changes and evidence for anthocyanin photoprotection in apples. *J. Photochem. Photobiol. B* 55, 155–163. doi: 10.1016/S1011-1344(00)00042-7
- Merzlyak, M. N., Solovchenko, A. E., and Chivkunova, O. B. (2002). Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. *Plant Physiol. Biochem.* 40, 679–684. doi: 10.1016/S0981-9428(02)01408-0
- Mittler, R., Finka, A., and Goloubinoff, P. (2012). How do plants feel the heat? *Trends Biochem. Sci.* 37, 118–125. doi: 10.1016/j.tibs.2011.11.007
- Mittler, R., Vanderauwera, S., Gollery, M., and van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends Plant Sci.* 9, 490–498. doi: 10.1016/j.tplants.2004.08.009
- Mohaved, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., et al. (2016). The grapevine VviPrx31 peroxidase as a candidate gene involved

- in anthocyanin degradation in ripening berries under high temperature. *J. Plant Res.* 129, 513–526. doi: 10.1007/s10265-016-0786-3
- Mohr, H. D., and Düring, H. (2000). “Sonnenbrand bei Weinreben - eine Nachlese” in *Deutsches Weinbau-Jahrbuch*. eds. W. Madel and G. Schruft (Waldkirch: Waldkircher Verlag), 95–102.
- Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1932–1945. doi: 10.1093/jxb/erm055
- Mugan, M., Bellincontro, A., Barnaba, F. E., Paolocci, M., Bignami, C., Gambellini, G., et al. (2011). Influence of bunch position in the canopy on berry epicuticular wax during ripening and on weight loss during postharvest dehydration. *Am. J. Enol. Vitic.* 62, 91–98. doi: 10.5344/ajev.2010.10012
- Müller, P., Li, X. P., and Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant Physiol.* 125, 1558–1566. doi: 10.1104/pp.125.4.1558
- Müller-Moulé, P., Conklin, P. L., and Niyogi, K. K. (2002). Ascorbate deficiency can limit violaxanthin de-epoxidase activity in vivo. *Plant Physiol.* 128, 970–977. doi: 10.1104/pp.010924
- Müller-Thurgau, H. (1883). Über Beschädigung von Trauben durch Sonnenbrand. *Der Weinbau* 9, 143–145.
- Naschitz, S., Naor, A., Sax, Y., Shahak, Y., and Rabinowitch, H. D. (2015). Photo-oxidative sunscald of apple: effects of temperature and light on fruit peel photoinhibition, bleaching and short-term tolerance acquisition. *Sci. Hortic.* 197, 5–16. doi: 10.1016/j.scienta.2015.11.003
- Nuzzo, V., Genovese, M., Shackel, K., and Matthew, M. (2009). “Preliminary investigations on sunburn in Chardonnay grapevine variety” in *Proceedings of 16th International GIESCO Symposium*; July 12–15, 2009; California, 12–15.
- Olivares-Soto, H., Bastias, R. M., Calderón-Orellana, A., and López, M. D. (2020). Sunburn control by nets differentially affects the antioxidant properties of fruit peel in ‘gala’ and ‘Fuji’ apples. *Hortic. Environ. Biotechnol.* 61, 241–254. doi: 10.1007/s13580-020-00226-w
- Oliveira, M., Teles, J., Barbosa, P., Olazabal, F., and Queiroz, J. (2014). Shading of the fruit zone to reduce grape yield and quality losses caused by sunburn. *OENO One* 48, 179–187. doi: 10.20870/oeno-one.2014.48.3.1579
- Pallioti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., and Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. *Sci. Hortic.* 178, 43–54. doi: 10.1016/j.scienta.2014.07.039
- Pastore, C., dal Santo, S., Zenoni, S., Mohaved, N., Allegro, G., Valentini, G., et al. (2017). Whole plant temperature manipulation affects flavonoid metabolism and the transcriptome of grapevine berries. *Front. Plant Sci.* 8:929. doi: 10.3389/fpls.2017.00929
- Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, M., Toriellini, G. B., and Filippetti, I. (2013). Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. *BMC Plant Biol.* 13:30. doi: 10.1186/1471-2229-13-30
- Pieri, P., Zott, K., Gomès, E., and Hilbert, G. (2016). Nested effects of berry half, berry and bunch microclimate on biochemical composition in grape. *OENO One* 50, 23–33. doi: 10.20870/oeno-one.2016.50.3.52
- Pourcel, L., Routaboul, J. M., Cheynier, V., Lepiniec, L., and Debeaujon, I. (2007). Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends Plant Sci.* 12, 29–36. doi: 10.1016/j.tplants.2006.11.006
- Price, S. F., Breen, P. J., Valladao, M., and Watson, B. T. (1995). Cluster sun exposure and quercetin in pinot noir grapes and wines. *Am. J. Enol. Vitic.* 46, 187–194.
- Racskó, J., Nagy, J., Szabó, Z., Major, M., and Nyéki, J. (2005). The impact of location, row direction, plant density and rootstock on the sunburn damage of apple cultivars. *Int. J. Hortic. Sci. Technol.* 11, 19–30.
- Racskó, J., and Schrader, L. E. (2012). Sunburn of apple fruit: historical background, recent advances and future perspectives. *Crit. Rev. Plant Sci.* 31, 455–504. doi: 10.1080/07352689.2012.696453
- Reshef, N., Agam, N., and Fait, A. (2018). Grape berry acclimation to excessive solar irradiance leads to repartitioning between major flavonoid groups. *J. Agric. Food Chem.* 66, 3624–3636. doi: 10.1021/acs.jafc.7b04881
- Ritenour, M. A., Kochhar, S., Schrader, L. E., Hsu, T. P., and Ku, M. S. (2001). Characterization of heat shock protein expression in apple peel under field and laboratory conditions. *J. Am. Soc. Hortic. Sci.* 126, 564–570. doi: 10.21273/JASHS.126.5.564
- Rocheta, M., Becker, J. D., Coito, J. L., Carvalho, L., and Amâncio, S. (2014). Heat and water stress induce unique transcriptional signatures of heat-shock proteins and transcription factors in grapevine. *Funct. Integr. Genomics* 14, 135–148. doi: 10.1007/s10142-013-0338-z
- Rodriguez, J., Anorou, A., Jifon, J., and Simpson, C. (2019). Physiological effects of exogenously applied reflectants and anti-transpirants on leaf temperature and fruit sunburn in citrus. *Plan. Theory* 8:549. doi: 10.3390/plants8120549
- Rosenquist, J. K., and Morrison, J. C. (1989). Some factors affecting cuticle and wax accumulation on grape berries. *Am. J. Enol. Vitic.* 40, 241–244.
- Rustioni, L. (2017). Oxidized polymeric phenolics: could they be considered photoprotectors? *J. Agric. Food Chem.* 65, 7843–7846. doi: 10.1021/acs.jafc.7
- Rustioni, L., Fracassetti, D., Prinsi, B., Geuna, F., Ancelotti, A., Fauda, V., et al. (2020). Oxidations in white grape (*Vitis vinifera* L.) skins: comparison between ripening process and photooxidative sunburn symptoms. *Plant Physiol. Biochem.* 150, 270–278. doi: 10.1016/j.plaphy.2020.03.003
- Rustioni, L., Milani, C., Parisi, S., and Failla, O. (2015). Chlorophyll role in berry sunburn symptoms studied in different grape (*Vitis vinifera* L.) cultivars. *Sci. Hortic.* 185, 145–150. doi: 10.1016/j.scienta.2015.01.029
- Rustioni, L., Rocchi, L., Guffanti, E., Cola, G., and Failla, O. (2014). Characterization of grape (*Vitis vinifera* L.) berry sunburn symptoms by reflectance. *J. Agric. Food Chem.* 62, 3043–3046. doi: 10.1021/jf405772f
- Rylski, I., and Spigelman, M. (1986). Effect of shading on plant development, yield and fruit quality of sweet pepper grown under conditions of high temperature and radiation. *Sci. Hortic.* 29, 31–35.
- Sasaki, K., Takase, H., Matsuyama, S., Kobayashi, H., Matsuo, H., Ikoma, G., et al. (2016). Effect of light exposure on linalool biosynthesis and accumulation in grape berries. *Biosci. Biotechnol. Biochem.* 80, 2376–2382. doi: 10.1080/09168451.2016.1217148
- Scafidi, P., Pisciotta, A., Patti, D., Tamborra, P., Di Lorenzo, R., and Barbagallo, M. (2013). Effect of artificial shading on the tannin accumulation and aromatic composition of the Grillo cultivar (*Vitis vinifera* L.). *BMC Plant Biol.* 13:175. doi: 10.1186/1471-2229-13-175
- Schrader, L. E., Kahn, C., and Elfving, D. C. (2009). Sunburn browning decreases at-harvest internal fruit quality of apples (*Malus domestica* Borkh.). *Int. J. Fruit Sci.* 9, 425–437. doi: 10.1080/15538360903378781
- Schrader, L. E., Sun, J., Zhang, J., Felicetti, D., and Tian, J. (2008). Heat and light-induced apple skin disorders: causes and prevention. *Acta Hortic.* 772, 51–58. doi: 10.17660/ActaHortic.2008.772.5
- Schrader, L. E., Zhang, J., and Duplaga, W. K. (2001). Two types of sunburn in apple caused by high fruit surface (peel) temperature. *Plant Health Prog.* 2:3. doi: 10.1094/PHP-2001-1004-01-RS
- Schrader, L. E., Zhang, J., and Sun, J. (2003). Environmental stresses that cause sunburn of apple. *Acta Hortic.* 618, 397–405. doi: 10.17660/ActaHortic.2003.618.47
- Schultz, H. R. (2007). Sonnenbrand - was steckt dahinter? *Das deutsche Weinmagazin* 16, 30–31.
- Schüttler, A., Friedel, M., Jung, R., Rauhut, D., and Darriet, P. (2015). Characterizing aromatic typicality of Riesling wines: merging volatile compositional and sensory aspects. *Food Res. Int.* 69, 26–37. doi: 10.1016/j.foodres.2014.12.010
- Sharma, R. R., Datta, S. C., and Varghese, E. (2018). Effect of surround WP®, a kaolin-based particle film on sunburn, fruit cracking and postharvest quality of ‘Kandhari’ pomegranates. *Crop Prot.* 114, 18–22. doi: 10.1016/j.cropro.2018.08.009
- Shi, F., Zhan, W., Li, Y., and Wang, X. (2014). Temperature influences β -carotene production in recombinant *Saccharomyces cerevisiae* expressing carotenogenic genes from *Phaffia rhodozyma*. *World J. Microbiol. Biotechnol.* 30, 125–133. doi: 10.1007/s11274-013-1428-8
- Silvestre, J., Damásio, M., Egipto, R., Cunha, J., Brazão, J., Eiras-Dias, J., et al. (2019). “Tolerance to sunburn: A variable to consider in the context of climate change” in *Proceedings of the 21st GIESCO International Meeting*; June 23–28, 2019; Thessaloniki, 681–682.
- Smart, R. E., and Sinclair, T. R. (1976). Solar heating of grape berries and other spherical fruits. *Agric. Meteorol.* 17, 241–259.
- Solovchenko, A. E. (ed.) (2010). “Manifestations of the buildup of screening pigments in the optical properties of plants” in *Photoprotection in plants*. (Berlin, Heidelberg: Springer), 89–118.

- Solovchenko, A. E., and Merzlyak, M. N. (2008). Screening of visible and UV radiation as a photoprotective mechanism in plants. *Russ. J. Plant Physiol.* 55:719. doi: 10.1134/S1021443708060010
- Son, I. C., and Lee, C. H. (2008). The effects of bags with different light transmittance on the berry cracking of grape 'Kyoho'. *Hortic. Environ. Biotechnol.* 49, 98–103.
- Song, J., Smart, R., Wang, H., Damberg, B., Sparrow, A., and Qian, M. C. (2015). Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of *Vitis vinifera* L. cv. Pinot noir wine. *Food Chem.* 173, 424–431. doi: 10.1016/j.foodchem.2014.09.150
- Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. C. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* 53, 171–182.
- Stoll, M., and Schultz, H. R. (2013). *Deutsches Weinbau Jahrbuch Stuttgart*. Germany: Ulmer Verlag.
- Stoll, M., and Schultz, H. R. (2020). *Deutsches Weinbau Jahrbuch*. Stuttgart, Germany: Ulmer Verlag.
- Stracke, R., Favory, J. J., Gruber, H., Bartelniewoehner, L., Bartels, S., Binkert, M., et al. (2010). The *Arabidopsis* bZIP transcription factor HY5 regulates expression of the *PFG1/MYB12* gene in response to light and ultraviolet-B radiation. *Plant, Cell Environ.* 33, 88–103. doi: 10.1111/j.1365-3040.2009.02061.x
- Suehiro, Y., Mochida, K., Itamura, H., and Esumi, T. (2014). Skin browning and expression of *PPO*, *STS*, and *CHS* genes in the grape berries of 'Shine Muscat'. *J. Jpn. Soc. Hortic. Sci.* 83, 122–132. doi: 10.2503/jjshs1.CH-095
- Tarara, J. M., and Spayd, S. (2005). Tackling 'sunburn' in red wine grapes through temperature and sunlight exposure. *The Good Fruit Grower* 56, 40–41.
- Thompson, J. E., Legge, R. L., and Barber, R. F. (1987). The role of free radicals in senescence and wounding. *New Phytol.* 105, 317–344.
- Torres, C. A., Andrews, P. K., and Davies, N. M. (2006). Physiological and biochemical responses of fruit exocarp of tomato (*Lycopersicon esculentum* mill.) mutants to natural photo-oxidative conditions. *J. Exp. Bot.* 57, 1933–1947. doi: 10.1093/jxb/erj136
- Torres, C. A., Azocar, C., Ramos, P., Pérez-Díaz, R., Sepulveda, G., and Moya-León, M. A. (2020). Photooxidative stress activates a complex multigenic response integrating the phenylpropanoid pathway and ethylene, leading to lignin accumulation in apple (*Malus domestica* Borkh.) fruit. *Hortic. Res.* 7:22. doi: 10.1038/s41438-020-0244-1
- Torres, C. A., Sepulveda, A., Gonzalez-Talica, J., Yuri, R., and Razmilic, I. (2013). Fruit water relations and osmoregulation on apples (*Malus domestica* Borkh.) with different sun exposures and sun-injury levels on the tree. *Sci. Hort.* 161, 143–152. doi: 10.1016/j.scienta.2013.06.035
- Tsai, M. S., Lee, T. C., and Chang, P. T. (2013). Comparison of paper bags, calcium carbonate, and shade nets for sunscald protection in 'Murcott' tangerine fruit. *Hortic. Technol.* 23, 659–667. doi: 10.21273/HORTTECH.23.5.659
- Tupinier, C. (2019). *Il fait chaud, très chaud, les vignes font le dos rond, mais quels sont les véritables risques pour le vignoble?* Available at: www.bourgogneaujourd'hui.com (Accessed June 18, 2020).
- United States Department of Agriculture (1999). *United States standards for grades of table grapes (European or Vinifera type)*. Washington, DC, USA: United States Department of Agriculture.
- Verdenal, T., Zufferey, V., Dienes-Nagy, A., Bourdin, G., Gindro, K., Viret, O., et al. (2019). Timing and intensity of grapevine defoliation: an extensive overview on five cultivars in Switzerland. *Am. J. Enol. Vitic.* 70, 427–434. doi: 10.5344/ajev.2019.19002
- von Babo, L. (1840). *Der Weinbau nach der praktischen Reihenfolge der Arbeiten dargestellt*. Heidelberg: Akademische Verlagsbuchhandlung C.F. Winter.
- Wand, S., Theron, K., Ackerman, J., and Marais, S. (2006). Harvest and post-harvest apple fruit quality following applications of kaolin particle film in south African orchards. *Sci. Hort.* 107, 271–276. doi: 10.1016/j.scienta.2005.11.002
- Wang, L. J., and Li, S. H. (2006). Thermotolerance and related antioxidant enzyme activities induced by heat acclimation and salicylic acid in grape (*Vitis vinifera* L.) leaves. *Plant Growth Regul.* 48, 137–144. doi: 10.1007/s10725-005-6146-2
- Wang, L. J., Loeschner, W., Duan, W., Li, W. D., Yang, S. H., and Li, S. H. (2009). Heat acclimation induced acquired heat tolerance and cross adaptation in different grape cultivars: relationships to photosynthetic energy partitioning. *Funct. Plant Biol.* 36, 516–526. doi: 10.1071/FP090008
- Wang, X., Vignjevic, M., Liu, F., Jacobsen, S., Jiang, D., and Wollenweber, B. (2015). Drought priming at vegetative growth stages improves tolerance to drought and heat stresses occurring during grain filling in spring wheat. *Plant Growth Regul.* 75, 677–687. doi: 10.1007/s10725-014-9969-x
- Webb, L., Whiting, J., Watt, A., Hill, T., Wigg, F., Dunn, G., et al. (2010). Managing grapevines through severe heat: A survey of growers after the 2009 summer heatwave in South-Eastern Australia. *J. Wine Res.* 21, 147–165. doi: 10.1080/09571264.2010.530106
- Wild, M. (2016). Decadal changes in radiative fluxes at land and ocean surfaces and their relevance for global warming. *Wiley Interdiscip. Rev. Clim. Change* 7, 91–107. doi: 10.1002/wcc.372
- Wünsche, J. N., Greer, D. H., Palmer, J. W., Lang, A., and McGhie, T. (2001). "Sunburn - the cost of a high light environment." in *International Symposium on Orchard and Plantation Systems*. eds. J. W. Palmer and J. N. Wünsche. January 30–February 5, 2000 [Leuven, Belgium: International Society for Horticultural Science (ISHS)], 349–356.
- Würz, D. A., Allebrandt, R., Filho, J. L. M., Bem, B. P. d., Brighenti, A. F., Rufato, L., et al. (2018). Época de desfolha e sua influência no desempenho vitícola da uva 'sauvignon Blanc' em região de elevada altitude. *Revista de Ciências Agroveterinárias* 17, 91–99. doi: 10.5965/22381171712018091
- Yamasaki, H., Sakihama, Y., and Ikehara, N. (1997). Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H₂O₂. *Plant Physiol.* 115, 1405–1412. doi: 10.1104/pp.115.4.1405
- Yang, F. H. (2018). Predictions and practices for reducing heat damage in northern highbush blueberry (*Vaccinium corymbosum* L.). [dissertation/doctoral thesis]. Oregon State University.
- Yang, F. H., Bryla, D. R., and Strik, B. C. (2019). Critical temperatures and heating times for fruit damage in northern highbush blueberry. *HortScience* 54, 2231–2239. doi: 10.21273/hortsci14427-19
- Young, P. R., Eyeghe-Bickong, H. A., du Plessis, K., Alexandersson, E., Jacobson, D. A., Coetzee, Z., et al. (2016). Grapevine plasticity in response to an altered microclimate: sauvignon Blanc modulates specific metabolites in response to increased berry exposure. *Plant Physiol.* 170, 1235–1254. doi: 10.1104/pp.15.01775
- Zenoni, S., dal Santo, S., Tornielli, G. B., D'Inca, E., Filippetti, I., Allegro, G., et al. (2017). Transcriptional responses to pre-flowering leaf defoliation in grapevine berry from different growing sites, years, and genotypes. *Front. Plant Sci.* 8:630. doi: 10.3389/fpls.2017.00630
- Zhang, Y., and Keller, M. (2015). Grape berry transpiration is determined by vapor pressure deficit, cuticular conductance, and berry size. *Am. J. Enol. Vitic.* 66, 454–462. doi: 10.5344/ajev.2015.15038
- Zhou, H. C., Tam, N. F., Lin, Y. M., Ding, Z. H., Chai, W. M., et al. (2014). Relationships between degree of polymerization and antioxidant activities: a study on proanthocyanidins from the leaves of a medicinal mangrove plant *Ceriops tagal*. *PLoS One* 9:e107606. doi: 10.1371/journal.pone.0107606
- Zschokke, A. (1930). Sonnenbrand-, Hitztod- und Austrocknungsschäden an Reben. *Die Gartenbauwissenschaft* 4, 196–232.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Gambetta, Holzapfel, Stoll and Friedel. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Evaluating Strategies for Adaptation to Climate Change in Grapevine Production—A Systematic Review

Audrey Naulleau^{1*}, Christian Gary¹, Laurent Prévot² and Laure Hossard³

¹ ABSys, Univ Montpellier, INRAE, CIRAD, CIHEAM-IAMM, Institut Agro, Montpellier, France, ² LISAH, Univ Montpellier, INRAE, IRD, Institut Agro, Montpellier, France, ³ Innovation, Univ Montpellier, INRAE, CIRAD, Institut Agro, Montpellier, France

In many areas of the world, maintaining grapevine production will require adaptation to climate change. While rigorous evaluations of adaptation strategies provide decision makers with valuable insights, those that are published often overlook major constraints, ignore local adaptive capacity, and suffer from a compartmentalization of disciplines and scales. The objective of our study was to identify current knowledge of evaluation methods and their limitations, reported in the literature. We reviewed 111 papers that evaluate adaptation strategies in the main vineyards worldwide. Evaluation approaches are analyzed through key features (e.g., climate data sources, methodology, evaluation criteria) to discuss their ability to address climate change issues, and to identify promising outcomes for climate change adaptations. We highlight the fact that combining adaptation levers in the short and long term (location, vine training, irrigation, soil, and canopy management, etc.) enables local compromises to be reached between future water availability and grapevine productivity. The main findings of the paper are three-fold: (1) the evaluation of a combination of adaptation strategies provides better solutions for adapting to climate change; (2) multi-scale studies allow local constraints and opportunities to be considered; and (3) only a small number of studies have developed multi-scale and multi-lever approaches to quantify feasibility and effectiveness of adaptation. In addition, we found that climate data sources were not systematically clearly presented, and that climate uncertainty was hardly accounted for. Moreover, only a small number of studies have assessed the economic impacts of adaptation, especially at farm scale. We conclude that the development of methodologies to evaluate adaptation strategies, considering both complementary adaptations and scales, is essential if relevant information is to be provided to the decision-makers of the wine industry.

Keywords: viticulture, adaptation evaluation, drought, management practices, climate change, multi-scale, multi-criteria

OPEN ACCESS

Edited by:

Maria Paz Diago,
Institute of Vine and Wine Sciences
(ICVV), Spain

Reviewed by:

Diego S. Intrigliolo,
Spanish National Research
Council, Spain
Ravindra N. Chibbar,
University of Saskatchewan, Canada

*Correspondence:

Audrey Naulleau
aurely.naulleau@inrae.fr

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 18 September 2020

Accepted: 09 December 2020

Published: 14 January 2021

Citation:

Naulleau A, Gary C, Prévot L and
Hossard L (2021) Evaluating
Strategies for Adaptation to Climate
Change in Grapevine Production—A
Systematic Review.
Front. Plant Sci. 11:607859.
doi: 10.3389/fpls.2020.607859

INTRODUCTION

Climate change adaptation is a key to the future of agriculture, a particularly vulnerable economic sector that depends heavily on weather and climatic conditions. Climate change adaptation can broadly be defined as “the set of actions and processes that societies must take to limit the negative impacts of the changes and maximize their beneficial effect” (Carter, 1996). In the case of grape growing, the potential adaptation levers are numerous, encompassing both the temporality of

technical operations along the production chain—from plantation to annual crop management and winemaking, and their spatial variations due to the existing diversity of cropping systems and the close link between localization and technical adaptation (Viguie et al., 2014). It is thus interesting to understand how the research on grapevines examines management practices as well as socio-economic and cultural factors, to propose and evaluate strategies of adaptation to climate change.

It is essential that adaptation evaluation follow a comprehensive path to the understanding of climate change impacts. In the past three decades, an abundant literature—both scientific and technical—has been published on the impacts of climate change in viticulture (Mosedale et al., 2016). These impacts have been established from experiments in controlled conditions (Bindi et al., 1996; Moutinho-Pereira et al., 2009, 2010; Carvalho et al., 2016), the design of suitability maps based on bioclimatic indices (Fraga et al., 2012; Hannah et al., 2013), or crop modeling (Lobell et al., 2006; Moriondo et al., 2015). The major trends identified are: a 50% increase of biomass production in an elevated CO₂ environment (Bindi et al., 1996); a 3 to 4 days per decade advancement of the vegetative and reproductive cycle due to higher temperatures (Caffarra and Eccel, 2011); and a higher risk of water stress impacting yield in quantity and quality (Jones et al., 2005; Schultz, 2010; Mosedale et al., 2016; Van Leeuwen and Darriet, 2016). Among these three main factors (biomass increase, cycle advancement, and water stress), the latter is the most preoccupying, as water resources are particularly vulnerable in most grape-producing areas, which are in Mediterranean climates (Medrano et al., 2015).

Although vineyard water management has been a core subject of interest for decades with regard to controlling wine quality, today climate change and the resulting water scarcity threaten yield and wine quality on an unprecedented scale (IPCC et al., 2015). This has led to studies focusing on various but complementary scales. At field scale, irrigation is one of the most effective tools to limit adverse effects of water scarcity. Medrano et al. (2015) reviewed the different irrigation management techniques designed to enhance water use efficiency (e.g., deficit irrigation, partial root-zone drying, water re-use). They also explored soil and cover crop management as a way to maximize green water use. Palliotti et al. (2014) listed the impacts of various canopy management practices to delay the advancement of ripening due to temperature and to water deficit. The selection of drought-tolerant grape and rootstock varieties (Duchêne et al., 2010; Romero et al., 2018) has also been studied. At farm scale, the study of the socio-ecological system allows different types of adaptations like wine-making innovation, yield limitation, diversification and so on to be included (Nicholas and Durham, 2012; Lereboullet et al., 2013). At regional scale, the migration of viticulture production toward higher elevation and/or higher latitude regions is also considered as an adaptation strategy (Hannah et al., 2013; Delay et al., 2015). We can thus see that there are already many opportunities for implementing a wide diversity of adaptation levers to improve the management of viticulture under future climatic conditions.

These studies are however often scattered across disciplines and have little regard for the wide diversity of winegrowing systems or for the spatial heterogeneity of water resources and climate change impacts. The focal research question is now: how does the current body of literature on the evaluation of adaptation integrate the possible trade-off between adaptations, considering both time and space? To address this question, the present study investigates the current literature to determine the ways in which adaptation levers and scales can be integrated and evaluated, and in which integrative approaches may be further developed. Recently, Santos et al. (2020) provided an updated overview of adaptation levers in viticulture on the basis of results of relevant and illustrative research. The wide-ranging scope of their review does not allow an exhaustive compilation of previous studies. In this article, we propose an exclusive compilation of adaptation evaluation only. We aim to reach both researchers and policy makers by providing a comprehensive review of the current adaptation strategies and methodologies. We explicitly focus on the adaptation to water scarcity since: (1) water resources are projected to be strongly limited by an increase of water demand and a decrease of water availability under future climatic conditions (IPCC et al., 2015); (2) water availability and water management studies require spatial and temporal variations to be considered explicitly; and (3) we assume synergies and trade-offs to exist among the numerous adaptation levers proposed at different scales (water storage/competition, water use efficiency/water needs, etc.). Here we specifically discuss how current approaches and knowledge about adaptation could be integrated into locally specific adaptation evaluation in order to provide relevant information to decision-makers.

The present paper is structured as follows. In section Methods, we present the methodology we used to select and analyze the available publications. In section Adapting viticulture to future water scarcity, we synthesize the literature on adaptation strategies, highlighting the potential synergies and trade-offs when combining levers and scales. In section Evaluating climate change adaptation in viticulture, we detail the various methodologies proposed for assessing the impact of adaptation strategies. Section Discussion discusses possible future prospects.

METHODS

Article Selection and Analysis

In this review we applied systematic methods for document selection and inclusion, and we mixed qualitative and quantitative analyses. The literature search, conducted in June 2019 in the Clarivate Analytics' Web of Science (formerly operated by the Institute for Scientific Information) for the whole available period (1955–2019), included peer-reviewed papers, working papers, and conference presentations. The research equation was divided into three types of keywords delimited by the operand “AND” and applied on TOPIC. The first part of the equation referred to climate change “climat* NEAR chang* OR global* NEAR warm*,” the second part referred to wine-growing systems “wine* OR vine* OR grape* OR viti*,” and the third part referred to adaptation or water

management “water* OR adapt*.” The choice to put the operand “OR” between adaptation and water management allowed us to include studies focusing solely on water management as well as studies that considered water management practices among more general adaptive strategies. We did not specify the study scale as our objective was to compare adaptation studies at various scales, from the plant to the region. To reduce the risk of missing relevant papers, we verified that the most cited references in the collected articles were present in the search results. The initial search yielded 645 results, duplicates excluded.

Title and abstract were scanned for their relevance, articles requiring further consideration were shortlisted, and full papers were accessed. For this review, we excluded papers that did not match the following selection criteria: (1) focused on wine grape, not table grape, production; (2) construction or evaluation of adaptation strategies at the core of the study, not only in a discussion after an impact study; and (3) at least one adaptation to water scarcity was included. A total of 260 articles remained after this first selection and were read in full. Only the ones where adaptation was explicitly evaluated were included in the present study; in other words, adaptations were explicitly projected under future climatic conditions (data-based or not) and their impacts were either quantified or qualified regarding their feasibility evaluation. Our final dataset included 111 references (**Figure 1**; complete list in **Supplementary Table 1**).

First, we used the information from the Web of Science database to characterize the set of literature on adaptation for: authors, title, publication year, journal, web of science category, first author localization. We then described each of the 111 studies with categorical variables (**Table 1**). Analyses were performed with the R software version 3.5.1 (R Core Team, 2018), and diagrams with the “ggplot2” R package (Wickham, 2016), and the “VennDiagram” R package (Chen, 2018).

Second, we extracted two sets of variables. The first set of variables concerns the adaptations. Adaptations were first categorized according to their long- (LT) or short-term (ST) aspects. LT concerned site-specific planting choices that allow viticultural suitability to be increased, e.g., the environmental conditions in which grapevines could grow. ST concerned the flexible management that allows vine productivity to be adapted to the yearly specific climatic conditions. We then identified various adaptation categories according to their associated technical operations (e.g., fertilization, mulching, irrigation strategy, etc.). The context of the evaluation (studied area, variety, other crop considered), as well as the main impacts of the adaptation were also described. We also performed an in-depth quantitative analysis focusing on the impacts of adaptation levers on five main outputs from plant to region scale: grapevine water status, phenology, yield, berry quality (sugar content and acidity) and freshwater ecosystem (streamflow, pollution). These outputs were chosen because they were the main evaluation indicators. At least one of these outputs had been quantitatively evaluated in 43 studies, and the results of these studies were extracted. Each result [combination of an adaptation, an output and an experimental condition (year, site, simulation)] was expressed as the absolute and/or relative effects of adaptation

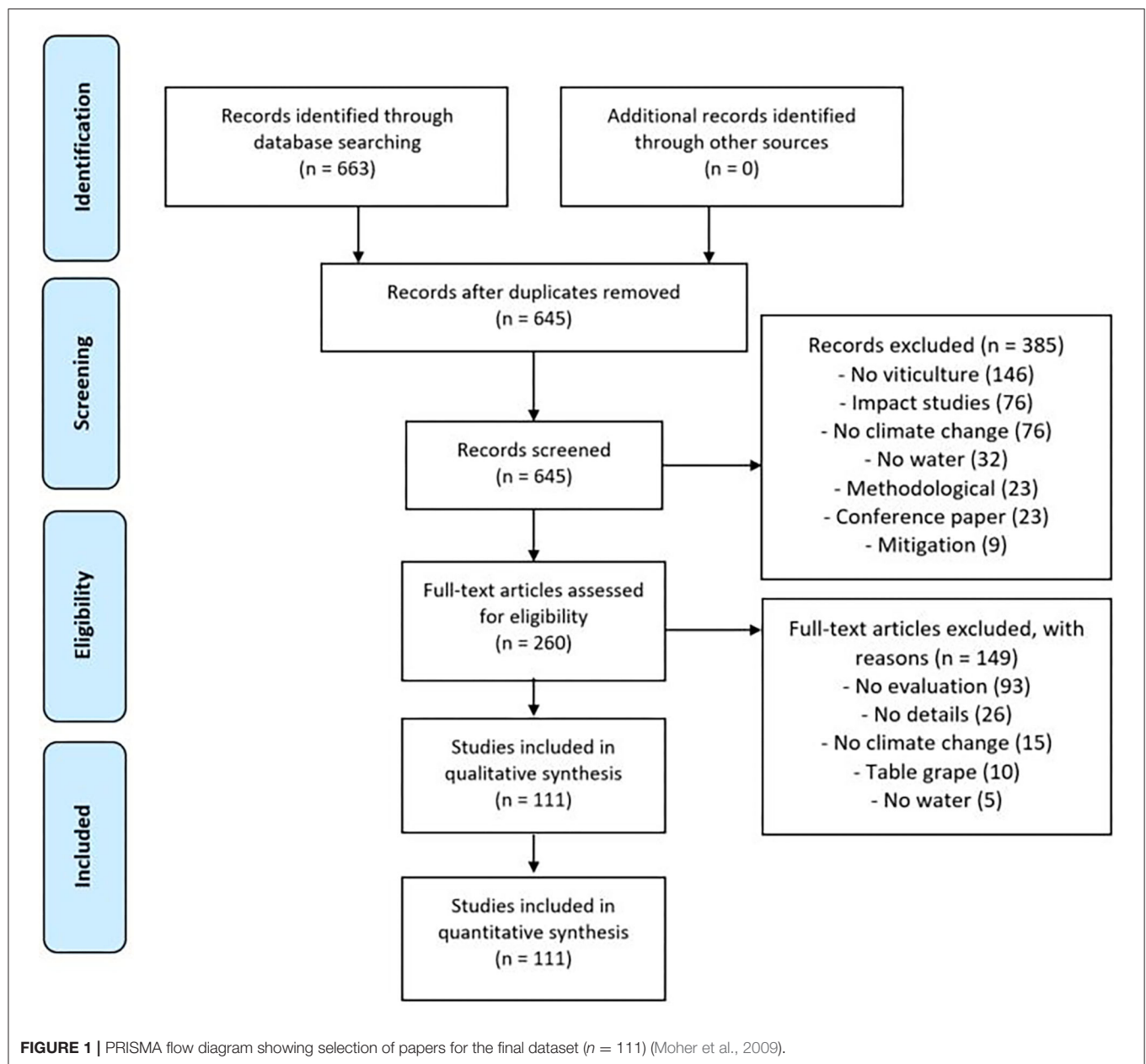
compared to the control. We classified results according to their “positive” or “negative” effects on the outputs with regard to climate change outcomes. Positive or negative effects were not systematically similar to an “increase” or a “decrease,” depending on the considered output. For example, positive effects on phenology is a delayed occurrence of phenological stages, as climate change tends to accelerate the phenological cycle. The positive effects on water status and yield are the reduction of water stress and the increase of yield, respectively. The positive effects on berry quality are a decrease of sugar content and an increase of acidity. Negative effects on freshwater ecosystems are the reduction of streamflow and the increase of pollution. Non-significant results are classified as “neutral”. Section Adapting viticulture to future water scarcity presents the results, describing how the combination of adaptation levers at long and short term allows a better adaptation to climate change.

The second set of variables concerns the evaluation methods. We excluded the 18 review papers from our analysis (18 out of the 111 papers), as we did not consider reviewing as a way to evaluate an adaptation. Four types of information were collected: the scientific approach, the climate data source, the study scale, and the evaluation criteria. First, the scientific approach is characterized according to the three categories described in Carter (1996): experimentation, impact projections (i.e., modeling) and expert judgement. Second, the performance of an adaptation under future climatic conditions depends largely on the data used to define those conditions. Climate data sources are classified by Carter (1996) into three categories: synthetic scenarios that consist of current meteorological data adjusted systematically (e.g., +2°, −10% annual precipitation, etc.); analog scenarios based on the identification of current climatic regimes that may occur in the future (i.e., perception); and data from climate models. Third, Neethling et al. (2019) have demonstrated the importance of scales to assess expected impacts, understand uncertainty, and frame sustainable responses over space and time. Herein, we classify the articles according to the scales of the studied processes: the plant scale corresponding to the eco-physiological processes (e.g., gas exchange, photosynthesis, water status); the field scale corresponding to the agronomic processes (e.g., soil properties, yield, berry composition); the farm scale corresponding to socio-economic processes (e.g., income, cost, labor); and the region scale corresponding to agro-eco-environmental processes (e.g., streamflow, wine market, regulation). At each scale, the evaluation criteria, i.e., the specific measured, simulated, or observed outputs of the studies are listed.

Overview of the Final Selection of Articles

In our database, the first journal article to focus on adaptation of grapevine to climate change was published in 2006 (Belliveau et al., 2006), 10 years after the first impact study of climate change in viticulture (Bindi et al., 1996). The number of papers has increased steeply since 2016 (**Figure 2A**).

Over the whole set of articles, authors from the Mediterranean area, i.e., Spain, Portugal, Italy, and France, accounted for 58% of the articles, followed by other major viticulture regions



in the world, mainly Australia, USA, Canada, Germany, and Chile (**Figure 2B**). Most of the studies concern one region (87 papers) while a few others are comparative studies between countries [Australia and France (Lereboullet et al., 2013), Germany and Argentina (Uliarte et al., 2013), France, Italy, and Germany (Battaglini et al., 2009)] or a worldwide analysis (Hannah et al., 2013). The two journals that publish the most on the adaptation of viticulture to climate change are grapevine-specialized journals, namely *Australian Journal of Grape and Wine Research* and *Oeno One* (11 and 8 papers, respectively). *Agricultural Water Management*, *Scientia Horticulturae*, and *Regional Environmental Change* published 6 papers each.

ADAPTING VITICULTURE TO FUTURE WATER SCARCITY

In the reviewed scientific literature, short-term (ST) and long-term (LT) adaptations, implemented, respectively, during the grapevine growing season and at vineyard plantation, were evaluated. Long-term adaptations concern: Site selection (LT1), consisting in the relocation of vineyards; Plant material (LT2), consisting in the implementation or creation of adapted grapevine cultivars and rootstock; Vineyard design (LT3), which implies changes in density, row orientation, training system; and Farm strategy (LT4), which includes wine-market orientation and diversification. Short-term adaptations concern: Irrigation

TABLE 1 | List of the recorded information of the final dataset ($n = 111$).

Variable	Category
On adaptation	
Crop	Grapevine and others crops (including forestry)
Variety	Grape variety (e.g., Shiraz, Tempranillo)
Adaptation	Deficit irrigation, drought tolerant variety, etc. (complete list in Figure 3)
Studied area	One or several countries, worldwide (if concerns all the main viticultural areas)
On methodology	
Scientific approach	Experimental, modeling, expert judgement
Climate data source	Meteorological data, perceptions, climate model
Study scale	Plant, Field, Farm, Region
Evaluation criteria	Physiological, agronomical, economic, environmental (detailed list in Supplementary Table 2)

(ST1); Soil management (ST2) concerning both soil surface (cover crop, mulching, tillage, etc.) and fertilization management; Canopy management (ST3); and Harvest and post-harvest management (ST4).

Figure 3 represents the occurrence of each adaptation in the studied dataset. One study could be counted several times as it examined more than one adaptation. Long-term and short-term adaptations were studied almost equally with 93 occurrences of LT adaptations, and 117 occurrences for ST adaptations. We recorded 32 individual levers limiting adverse effects of climate change on water resources in the vineyard. Irrigation was the most cited adaptation (55 studies), with a wide diversity of individual levers: on irrigation strategies (deficit irrigation, partial root drying irrigation, water spraying) and water sources (water re-use, water reservoir). The plant material ranked 2nd (41 studies), and could be classified in three types of adaptation lever: drought-tolerant rootstocks; late-ripening varieties; and drought-tolerant varieties. Last of all came canopy management, soil management, vineyard design, and site selection, which received an intermediate amount of attention (19 to 32 studies), whereas farm strategy and harvest management were given significantly less attention (<10 studies each).

In the selected literature, 60% of the articles considered only one adaptation lever, 20% considered an association or comparison of two levers, and 7% considered three levers. The 17 remaining articles proposed a combination of several levers (up to 14), but in these articles, their evaluation was only qualitative.

Combination of Long-Term Adaptations to Increase Viticultural Suitability (LT) Site Selection (LT1)

Viticultural suitability has been examined mostly under future climatic conditions (Fraga et al., 2012; Hannah et al., 2013; Moriondo et al., 2013). Suitability maps provide spatial representations of bioclimatic indices for describing changes in the suitability of land for viticulture (Mosedale et al., 2016).

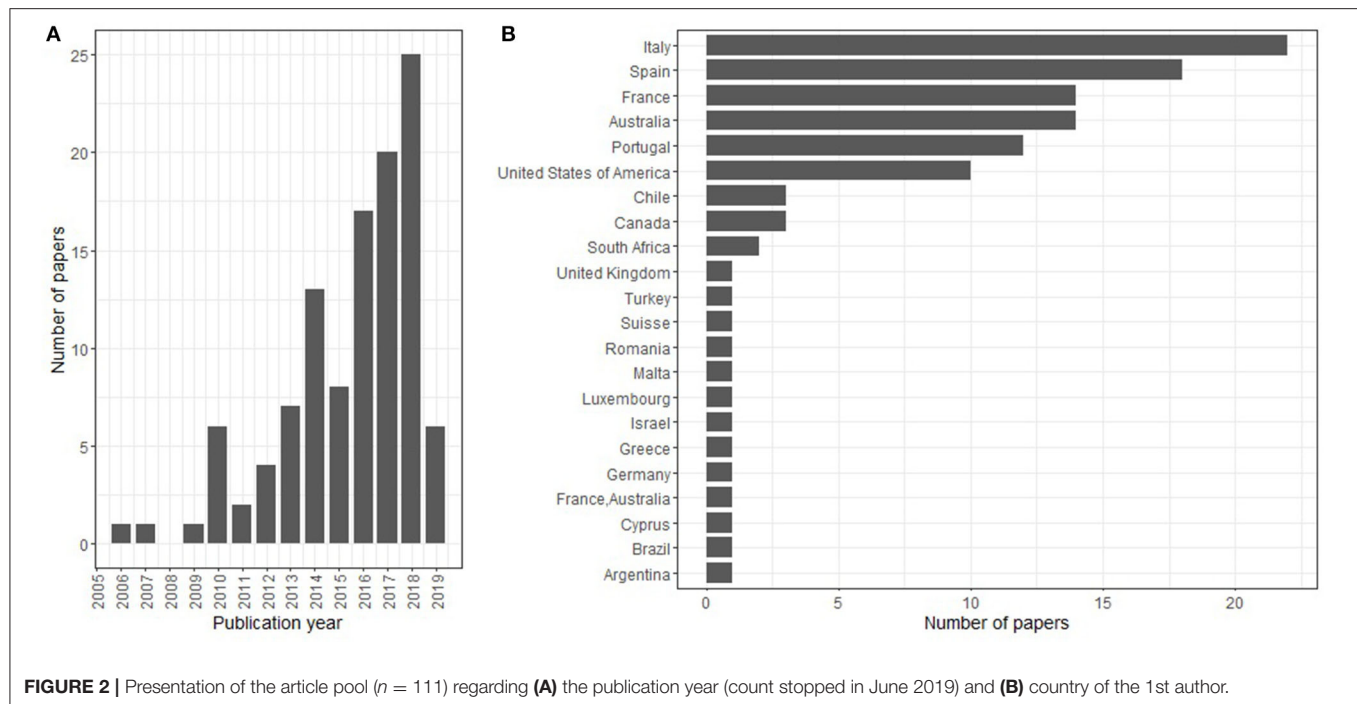
Viticultural suitability is predicted to decrease in main wine-producing areas (25–73%), leading to a reconfiguration of vineyard locations worldwide. New areas are expected to become suitable, multiplying by a factor of 2 to 3 the wine-growing areas in Northern Europe, New Zealand and Western North America (Hannah et al., 2013). New suitable areas concern higher altitudes, as well as latitudes where annual precipitations are higher and grapevines suffer less from high temperatures.

Such alarming conclusions are however controversial within the scientific community (Van Leeuwen et al., 2013). The main limitation of these studies is the fact that they are based solely on bioclimatic indices (temperature and precipitation), without considering (1) specifically local conditions, (2) competition between various land uses, and (3) winegrower adaptive capacity to limit migration. First, local conditions [e.g., soil available water capacity (SAWC), irrigation water availability, sun exposure] are not integrated into suitability mapping studies. Second, as lands that have recently become suitable for grapevine are currently—or will become—suitable for other crops, conflicts may rise around agricultural land use and conservation policies (Hannah et al., 2013; Fuhrer et al., 2014). Third, Delay et al. (2015) demonstrated the role of stakeholders' organization for the maintenance of viticulture in unsuitable areas, with the example of the role of cooperatives, not only in conserving production levels but also in respecting the emblematic viticultural landscape structure. In case of migration, the future of abandoned vineyard areas is still an unknown. Whereas other crops could not be considered without irrigation, forestry (pine/eucalyptus) appears as a solution (Carvalho et al., 2016). Otherwise, stakeholders predict a return to shrublands with consequences on the local economy, tourism, and fire risk (García-Ruiz et al., 2011).

Plant Material Adapted to Site Selection (LT2 * LT1)

As the climate warms up, the phenological stages are advanced, generating concerns in the spring season when grapevines becomes more exposed to late frosts, and in summer when climatic conditions during berry ripening are less favorable (e.g., high night temperatures and water deficit). The existing phenological diversity among grapevine cultivars offers an opportunity for climate change adaptation, to limit the loss of suitable areas for grapevine (Wolkovich et al., 2017). However, suitability maps do not integrate this phenological diversity into their indicators, nor do they integrate the PDO delimitation, which restricts the implementation of specific cultivars.

The IPCC has emphasized that Mediterranean climate areas are more likely to face an increase of drought and a reduction of renewable surface water and groundwater resources in the future (IPCC et al., 2015). Accordingly, plant material (cultivar and rootstock) should be selected for their drought tolerance. There are many studies comparing the behaviors of various grapevine genotypes under water-restricted conditions (e.g., Tomás et al., 2014; Vaz et al., 2016). Empirical knowledge of winegrowers is reported by Lereboullet et al. (2013) in Australia: “in 2011, many producers were starting to plant alternative Mediterranean varieties such as Grenache, Tempranillo or Mourvedre that offer a better resilience to water stress than Shiraz.” However, the understanding of the genetic factors relevant to water stress



tolerance is still limited, and quantification of yield response to water scarcity for various cultivars and in interaction with other climate variables remains difficult. An attempt to model these factors was undertaken in Tuscany. The authors found that the combination of partial uphill relocation, combined with the expansion of a drought-tolerant variety leads to a higher economic efficiency than each adaptation separately (Zhu et al., 2016). These results are however based on a major assumption that the yield of the drought-tolerant variety would not be affected by the climate change.

Duchene (2016) and Medrano et al. (2015) also highlighted the fact that rootstock-scion interaction plays a fundamental role in water use efficiency. Rootstocks have long been an unexplored field of research that is now increasingly being investigated for two reasons: its effect on root development and density, and therefore on the capacity to extract water from soil and to detect drought; and its effect on scion vigor, which influences light interception, carbon assimilation and consequently yields. Serra et al. (2014) and Zhang et al. (2016) reviewed and classified the current knowledge about the drought resistance of various rootstocks. Surprisingly, no reference to the grafting techniques (methods, period, height) have been mentioned in the selected literature, although it is a determinant of the plant's rootedness and the regulation of water flow (De Micco et al., 2018).

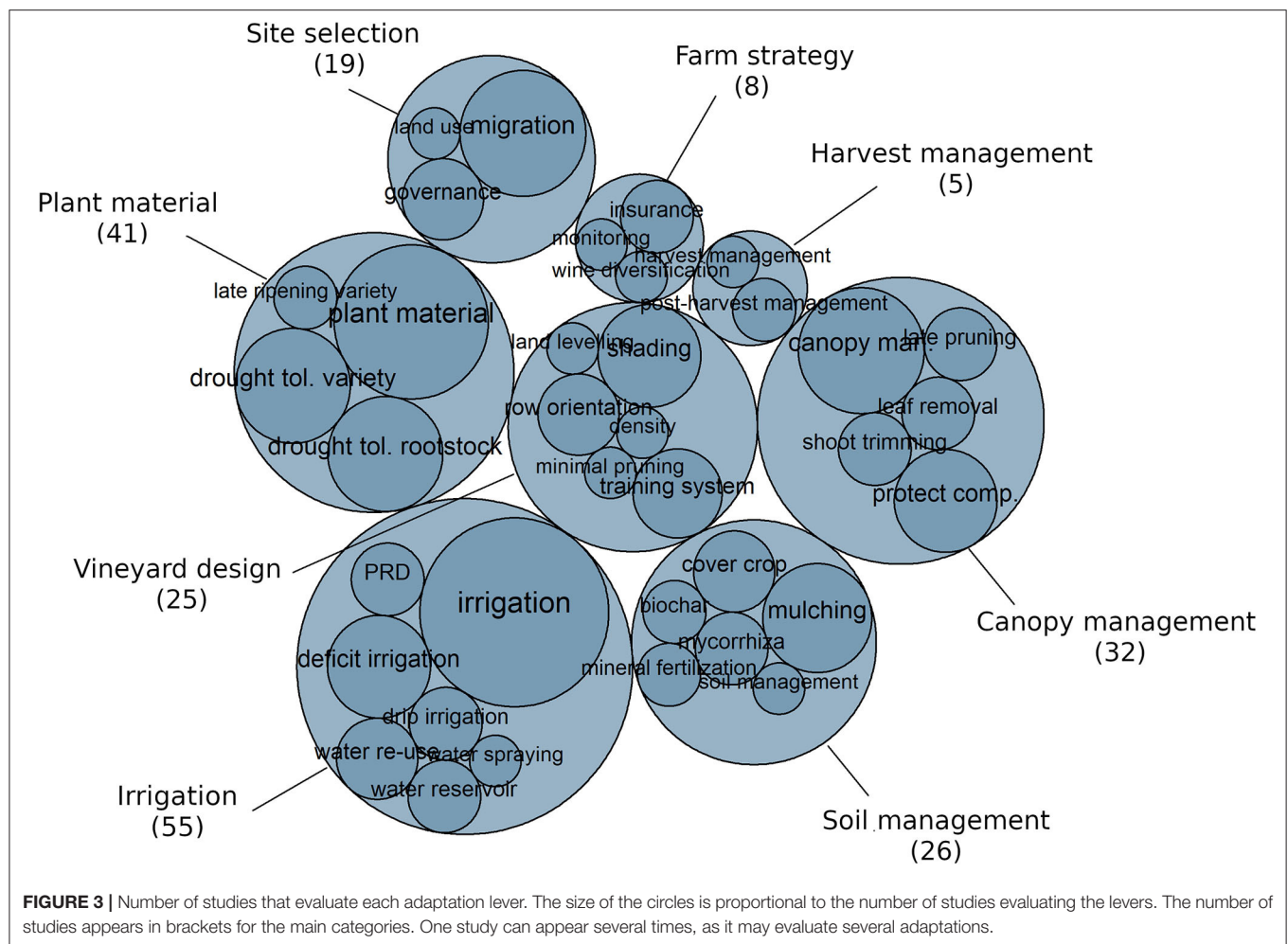
Vineyard Design (LT3)

Plantation density has a direct effect on a vineyard's water consumption. The objective is to increase drought resistance by reducing the competition between vines. Two studies based on a water balance model highlighted the potential of low-density systems as an adaptation to future water scarcity. Pieri et al. (2012) tested two planting densities (3,000 and 9,000 plants/ha)

in the five main viticultural regions of France, for 3 vine cultivars that differ in terms of their phenological timing. They found that reduced planting density allows grapevine water status to be maintained within moderate limits, even under future climatic conditions. Van Leeuwen et al. (2019) went further by evaluating the economic effects of a density reduction. When density was reduced by 50%, r_{water} deficit was also halved, leading to higher yield at plant scale but lower yield at field scale, offset by lower costs (e.g., pruning and trellising, labor, chemicals, etc.). This demonstrated the economic viability of low-density.

Hunter et al. (2016) studied the impact of row orientation on microclimatic conditions (temperature, wind) and vine physiological status. They highlighted a lower water stress for east-west orientation, which may be induced by row orientation. In Australia, Galbreath (2014) likewise showed that east-west row orientation limited canopy temperature increase. Row orientation, as well as drainage terraces, also have an effect on water balance by reducing runoff. A study in Spain showed that drainage terraces could be expected to limit runoff volumes of between 19 and 50% at the 2050 horizon, thus favoring infiltration and limiting soil losses (Concepción Ramos, 2016).

The vine training system determines above all the light interception and bunch sun exposure, and thus the completion of berry ripening. Palliotti et al. (2014) identified adapted training systems allowing for an optimal bunch microclimate under future climatic conditions. However, it is difficult to state which training system is better adapted to drought. The only reference to drought is to a lower leaf-to-air vapor pressure deficit. It is sometimes argued that goblet pruned vines are more drought resistant (Van Leeuwen and Destrac-Irvine, 2017). We note a lack of comparison of the water use efficiency of different training systems, including traditional forms like goblet systems



(Medrano et al., 2015). In Central Europe, under relatively cool climates, light pruning systems such as semi minimal pruning are promoted as an adaptation to climate change, as they present higher yields with lower alcohol degrees than vertical-shoot positioning systems (Clingeffer, 2010; Molitor et al., 2019). However, the large water requirements of such systems would not be adapted to rainfed systems under semi-arid climate.

Shading systems are proposed as adaptation to climate change, designed to limit the effects of high temperatures and to limit evapotranspiration. Experiments with shade (e.g., natural with agroforestry systems, artificial with nets, shading panels, or photovoltaic panels) concentrate mainly on the effect of shade on the canopy temperatures. Overhead shade seems to be the most efficient way to decrease temperatures and water stress, as compared to full canopy shade, bunch shade, soil shade, and side-canopy shade (Caravia et al., 2016). More studies on the relationship between timing and duration of shading, whole-vine and specific canopy portion shading, and analysis of technical feasibility of canopy shading (i.e., suitability of training systems, mechanization of net setting and removal, cost/benefit ratio, etc.) are needed (Palliotti et al., 2014).

Farm Strategy in Relation to Planting Choices (LT4 * LT1 * LT2 * LT3)

Like any economic activity, wine growing needs to be viable. On the one hand, adaptation strategies should be acceptable to the producers: cost/benefit ratio, working conditions (mechanization) and labor availability. Yet no quantitative evaluations of adaptation on farm systems have been found in literature. On the other hand, adaptation strategies should also be suited to consumers' preferences. As Belliveau et al. (2006) have shown in Canada, planting new varieties can minimize market risks but increase climate risks; but it can also reduce climate risks and create marketing difficulties. These considerations are spatially and temporarily difficult to reconcile.

The long-term adaptation of viticulture to climate change is a result of current planting choices: where (low land, uphill)? What (cultivar, rootstock)? How (orientation, density, training system)? For which type of wine? While little attention seems to have been paid to the combined effect of site selection and cultivar choice, the evaluation overall of the combined effect of various practices remains poor. Moreover, the proposed long-term adaptations are rarely balanced by considering the final

TABLE 2 | Irrigation strategies (FI, Full Irrigation; DI, Deficit Irrigation; PRD, Partial Root Drying) and associated water requirements in different climate scenarios (SRES, Special Reports on Emission Scenarios; RCP, Representative Climate Pathway).

References	Location	Method	Irrigation strategy	Period	Climate scenario	Irrigation water requirement
Present						
dos Santos et al. (2007)	Southern Portugal	Field experiment	FI	2002		197 mm
			50% DI	2002		99 mm
			50% PRD	2002		99 mm
Savi et al. (2018)	Italy, NE	Field experiment	Summer supplemental irrigation	2015		20–40 mm
Wenter et al. (2018)	Northern Italy	Field experiment	FI	2014–2015		72–262 mm
			DI	2014–2015		36–131 mm
Trigo-Córdoba et al. (2015), Mirás-Avalos et al. (2016)	Galicia, Spain	Field experiment	DI	2012–2014		50–79 mm
Aparicio et al. (2019)	Malta	Cost-benefit analysis	DI	Present		60 mm
Gaudin and Gary (2012)	Southern France	WaLIS model	DI	1972–2010		0–90 mm
In combination						
Cirigliano et al. (2017)	Central Italy	Field experiment	DI	2011–2013		125–591 mm
			DI + compost	2011–2013		125–291 mm
Future						
Kapur et al. (2007)	Apulia, Italy	Water balance model	FI	1970	SRES A2	320 mm
			FI	2095	SRES A2	480 mm
Fraga et al. (2018)	Portugal	STICS model	DI	2041–2070	RCP 8.5	50–250 mm
Phogat et al. (2018)	Australia	Hydrus 1D model	DI	2004–2015		350 mm
				2020–2039	RCP 8.5	250–450 mm
				2040–2059	RCP 8.5	260–460 mm
				2060–2079	RCP 8.5	240–480 mm
				2080–2099	RCP 8.5	280–500 mm

production objectives and economic returns that are defined and expected at farm scale.

Combination of Short-Term Adaptations to Enhance Flexible Management (ST) Combining Irrigation With Water-Saving Soil Management Practices (ST1*ST2)

Irrigation is part of most adaptation strategies proposed by stakeholders. Examples can be found in the South of France (Lereboullet et al., 2013; Neethling et al., 2017), Australia (Lereboullet et al., 2013; Galbreath, 2014), the USA (Nicholas and Durham, 2012), Italy (Sacchelli et al., 2016), Canada (Belliveau et al., 2006), and Spain (Alonso and Liu, 2013). However, irrigation needs, coupled with their possible satisfaction, are still not explored in socio-ecological studies. The main question remains: how much water do we need, now and in the future.

Two types of methodologies to assess future irrigation needs exist in the literature: experimental approaches and modeling approaches. Medrano et al. (2015) reviewed in detail the different irrigation strategies and their effects on physiological and agronomic parameters in field experiments. They concluded that regulated deficit irrigation (RDI) at an early or late stage is crucial for the sustainability of vineyards. They also detailed water saving practices—both agronomic techniques and genetic improvements—to increase water use efficiency under current

climatic conditions. However, Bonada et al. (2018) showed that when dealing with climate change, elevated temperatures will increase water demand. Thus, the relationship between rainfall decrease and increase in irrigation needs is not straightforward. A modeling exercise by Fraga et al. (2018) highlighted that in some parts of Portugal required irrigation may exceed the reduction in precipitation, while irrigation could largely alleviate projected yield decreases. Based on the selected articles, we synthesized current and future irrigation needs according to the vineyard location, irrigation strategy and the different climate scenarios (Table 2).

Table 2 illustrates the small number of studies that quantify irrigation needs under future climatic conditions, especially those concerning grapevine deficit irrigation in Europe—currently mostly rainfed. Future needs tend to vary widely across regions and to be double current needs in European regions. Lower increases are forecast in Australia as the current requirements are already high.

In areas where future water requirements will exceed water availability, agronomic practices may decrease irrigation needs by increasing soil water capacity and/or decreasing water losses. Canopy shade cloth and soil plastic mulch result in a 50% reduction in water use without detrimental effects on plant physiology under irrigated vineyards in Chile, through a reduction of soil evaporation or of evaporative demand (Gil et al.,

2018). Transparent plastic covering (TPC) has been reported to increase water use efficiency in vineyards in Brazil, by creating higher humidity and lowering evapotranspiration as compared to open field conditions (da Silva et al., 2018). The use of organic matter as compost increases the soil water storage capacity and reduces irrigation needs (Cirigliano et al., 2017). Tomaz et al. (2017) showed that the presence of a cover crop under irrigated conditions forces the vine root system, mainly its thinner roots, to seek water in increasingly deeper soil.

Few combined adaptations under irrigated conditions have been reported, while a broader focus of attention has been given to precision scheduling and timing of irrigation supply. A wide diversity of equipment is explored: subsurface, drip, sprinkler, gravity, high-pressure system. Concerning the timing factor, tools for measuring the water status of grapevines are being developed to determine the frequency of irrigation through direct measurement of plant and fruit parameters (Scholasch and Rienth, 2019).

Enhancing Flexible Management Strategies in Rainfed System

Soil management (ST2)

Soil management is crucial to reduce water losses. As half of the water needed by grapevines is provided by rain during fall and winter in Mediterranean climates (Flexas et al., 2010), the soil has a decisive role in buffering the mismatch between water supply and demand. Two main aspects are considered in the literature: the soil structure (porosity, stoniness, deepness) impacting its available water capacity; and the soil's surface state, influencing infiltration and evapotranspiration.

In the selected literature, biochar is the most studied adaptation to improve soil structure. Biochar is a co-product of a thermochemical conversion of biomass, recognized to be a beneficial soil amendment which increases soil water retention (Amendola et al., 2017). Effects of biochar depend on its physical and structural elements, the rate of application, and the soil type (Baronti et al., 2014). While biochar is more efficient in sandy soils, the extent to which the soil's available water capacity could be improved in each production area, and whether it will be sufficient to counteract a decrease of rainfall during the vine cycle or not, is unknown. In any case, improving soil quality (organic matter and soil microbiology) will help to buffer the adverse effect of higher intra- and inter-annual climate variability.

The soil surface state largely influences the water balance (infiltration, runoff, soil evaporation). It is determined by soil type, technical operations (tillage, cover crop seedling, herbicide application) and rain intensity. Chrysargyris et al. (2018) found that no tillage compensated for the lack of irrigation, while slight tillage allowed for better rainfall infiltration. Cover crops are also promoted to enhance infiltration. The water competition induced by the transpiration of a cover crop could be limited by its partial or total destruction at the end of the rainy season. One potential adaptation measure to consider in further studies concerns mulches, that is, organic or inorganic products that may be placed on the soil surface. Mulches reduce soil compaction and retain soil moisture, regulate soil temperature and reduce evaporation. According to STICS simulations, mulches may

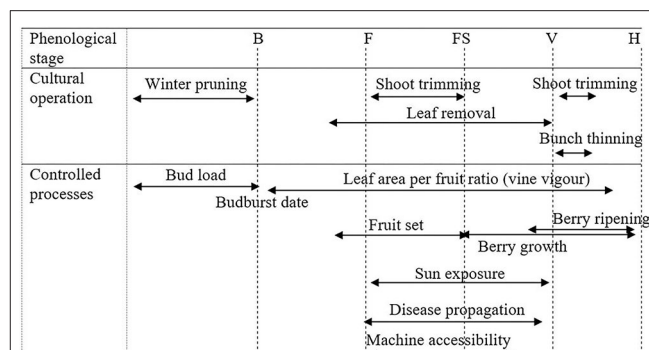


FIGURE 4 | Technical operations on canopy and controlled processes (B, Budburst; F, Flowering; FS, Fruit Set; V, Veraison; H, Harvest).

mitigate yield decreases by 10 to 25% in Alentejo vineyards in Portugal (Fraga and Santos, 2018).

Canopy management (ST3)

Canopy management determines water consumption by controlling the leaf area index and so the transpiration rate. The diversity of operations throughout the year (winter, before flowering, after flowering, until the last days before harvest) enables a wide range of processes (Figure 4) impacted by climate change (e.g., berry ripening, sun exposure) to be controlled. The expected results from applying these techniques are closely connected to the timing and intensity of the intervention, as well as to the vine's vigor, soil fertility and environmental factors, primarily rainfall (Palliotti et al., 2014).

Leaf removal after veraison is proposed as an adaptation to climate change, as it results in a reduction of sugar accumulation rates and a postponement of the harvest date without effecting yields (Poni et al., 2018). This has been shown by tests on irrigated Sangiovese vines in Italy (Valentini et al., 2019). Results are however more mitigated under rainfed conditions (Buesa et al., 2019), which highlights the importance of environmental context on the effect of such adaptation. Although late winter pruning helps to delay ripening (Petrie et al., 2017), excessive crop load, compared to soil resources, will ultimately have negative effects on yield and grape composition and cause a delayed ripening. Yet the boundary between an adequate and an excessive crop load is not clear-cut (Palliotti et al., 2014). Șerdinescu et al. (2014) recommended the reduction of bud load only in very dry conditions.

Anti-transpirants have been used to counteract drought as their application on leaves significantly reduces water loss and heat stress (Palliotti et al., 2013). Depending on the molecules, they act in two ways: a film polymer on the leaf surfaces (e.g., kaolin); or stomatal closing compounds. They also have positive effects on the control of sugar accumulation. Their effects on plant and fruit temperatures are more contrasted, due to their effects on stomatal aperture.

Harvest and post-harvest management (ST4)

As the climate is changing, with higher temperatures and higher water deficits that tend to advance harvesting and affect grape

composition, new harvesting management is needed. The main idea is to alter harvesting dates in accordance with temperatures (Alonso Ugaglia and Peres, 2017), but winegrowers have also envisaged other solutions. Neethling et al. (2017) identified that most adaptive responses occurred during harvest and winemaking. Harvesting with machines allows winegrowers to intervene rapidly (day and night), whereas manual harvesting systems are more restrictive. However, manual harvesting allows them to repeat the picking several times, and thus to select grape bunches that have reached their optimal maturity. Once the harvest is at the cellar, adaptations in the winemaking process are proposed. Dequin et al. (2017) recently reviewed winemaking practices adjusted to modified grape composition under climate change conditions (specific yeast strains with lower alcohol yield, membrane-based technologies to reduce the ethanol content and to increase the acidity, etc.).

Combination of Long-Term and Short-Term Adaptation

The analysis of individual adaptation levers allows for potential beneficial combinations of short- and long-term adaptation to be identified. The individual effects of adaptation levers on five main outputs (water status, phenology, yield, berry composition, and freshwater ecosystem) are synthesized in **Figure 5**. The sources of information (papers) are detailed in **Supplementary Figure 1**. The majority of the impacts of adaptations concerning water status and phenology showed an alleviation of water stress and a delay of phenology for all adaptations. However, the effects on yield of these different adaptations showed contradictory results. For instance, while vineyard design and canopy management adaptations had positive effects on grapevine water status, impacts on yield are in some cases deleterious. We noticed also that the effect of irrigation on yield, which is the most studied adaptation lever, was not significant in half of the cases, thus showing that the positive effect of irrigation on yield may depend on the year and the location. The low number of articles that evaluate impacts of adaptation levers at regional scale through their effects on freshwater ecosystems is worrying, especially as all the currently available results showed negative impacts. Results on soil management adaptations were mostly not significant on grapevine outputs, while they show positive effects on soil specific outputs (data not shown).

Figure 5 allows us to identify possible tradeoffs between short-term and long-term adaptations. For example, while vineyard design adaptation (LT3) can have negative effects on yield, it could be compensated by irrigation (ST1). Likewise, the negative effect of irrigation (ST1) on berry composition could be offset by adapted plant material (LT2, e.g., drought tolerant rootstock). In the selected literature, long- and short-term adaptations were combined in the majority of studies that involved the stakeholders (Nicholas and Durham, 2012; Lereboullet et al., 2013; Neethling et al., 2017). However, quantitative evaluations of these combinations are scarce. The combined effect of variety choice and irrigation treatment has been carefully studied by Carvalho et al. (2018). The same consideration appeared recently in rootstock selection: Romero et al. (2018) demonstrated

the compromise between rootstock selection and well-designed deficit irrigation strategies that allow long-term yield-quality-efficiency and returns for the grower. Vine training is also expected to influence water irrigation needs. For instance, Clingeffer (2010) found that minimal pruning, combined with PRD irrigation, significantly increases water use efficiency compared to spur pruned and controlled irrigation treatments.

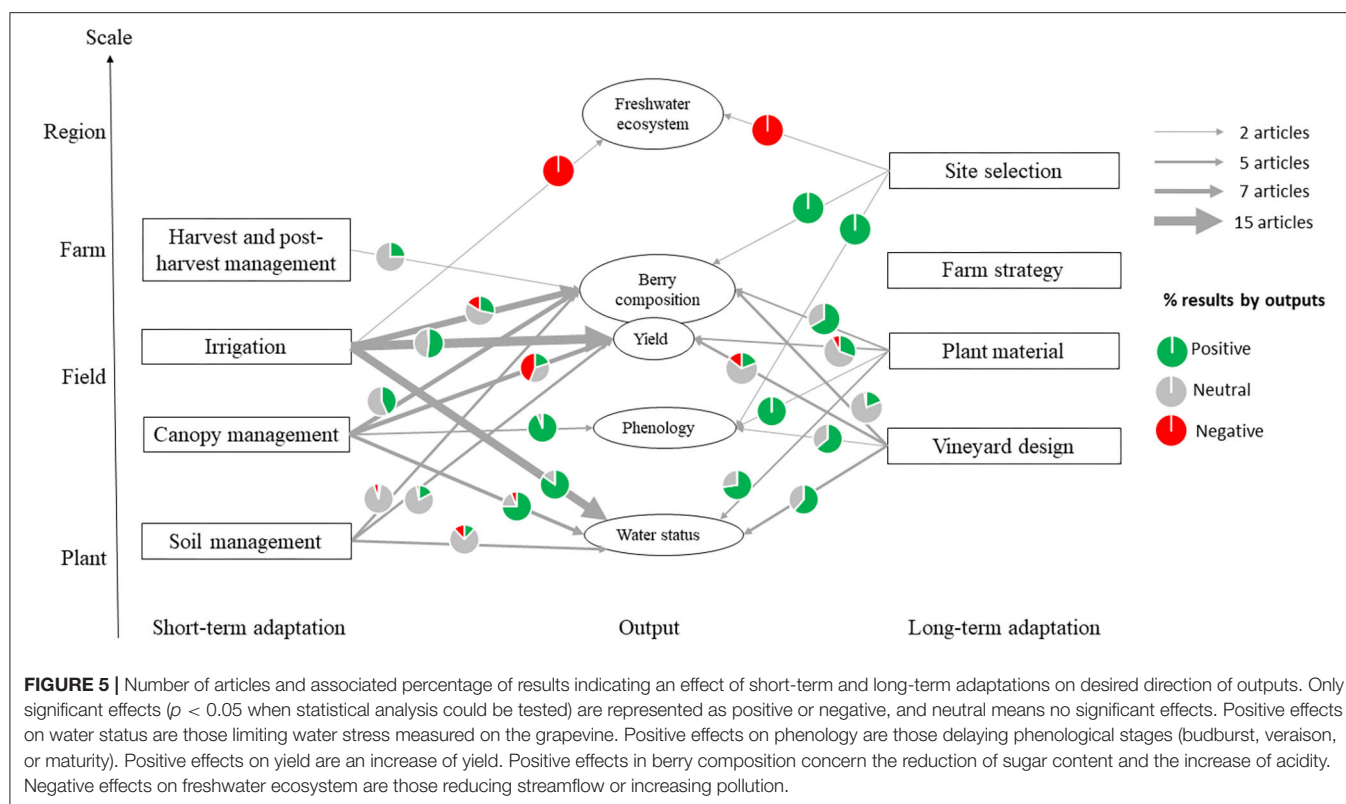
The integration of long-term considerations when evaluating short-term adaptation is crucial when dealing with economic and regulatory aspects. For instance, an analysis of technical feasibility and the economic cost of irrigation infrastructure for various localities and types of production is still lacking. Beyond irrigation needs, the irrigation decision is dependent of water resources (limited or not) and water pricing policy (Olen et al., 2016). We note the necessity to integrate the decision model into the development of irrigation-based adaptation strategies. In a trial, Trigo-Córdoba et al. (2015) estimated that irrigation is not economically viable under the current conditions of Galicia vineyards, considering both yield and quality, even though there is a physiological need for irrigation. In the vineyard of the “Old World,” irrigation was recently authorized (since 1996 in Portugal and since 2006 in France) and is still limited in “Protected Designation of Origin” areas, which can considerably change the feasibility of irrigation-based adaptations.

In addition to the economic aspect, some authors have looked at whether irrigation is an environmentally sustainable trend in semi-arid areas. One aspect is ecosystem protection, which was examined by Grantham et al. (2010), who evaluated the impact of small storage ponds on streamflow. They showed that strategic placement of storage ponds could reduce summer water withdrawals, thus protecting environmental flow. However, this could have an impact on winter flow. The development of high water-use efficiency systems in areas previously not irrigated still results in an increase of total water use. The second aspect deals with salinity problems, which appeared first in countries like Israel and Australia (Phogat et al., 2018). Model simulations indicate a steep increase of salinity in the root zone as rainfall-induced salt leaching declined significantly with climate change. The simulated seasonal average salinity increased three to four times compared to the baseline (Phogat et al., 2018). Adaptation strategies should include salinity tolerant rootstock, or the use of desalinated water (Aparicio et al., 2019).

EVALUATING CLIMATE CHANGE ADAPTATION IN VITICULTURE

Characterization of Climate Change

Characterizing future climatic conditions is the first step to evaluate an adaptation strategy, as its effectiveness will depend on local climatic conditions. Climate is a complex phenomenon involving many variables on different spatial and temporal scales. The ability to forecast climatic conditions is limited by the uncertainty about future greenhouse gas emissions and by the scientific uncertainty of their effects on climate and crops. The effects of combined climatic factors (e.g., higher CO₂ concentration with higher water deficit) need to be



considered simultaneously. In addition, spatial resolution of climate information is crucial to predict local phenomena. The performance of an adaptation under future climatic conditions therefore depends largely on the data used to define future climatic conditions.

First, future climatic conditions are described as a systematic adjustment of present meteorological data (e.g., a temperature increase of 2°C , a 50% reduction of rainfall, etc.) in 35 studies in the article pool (Table 3). Climatic conditions can be directly measured under controlled conditions. Controlled experiments evaluate the combined effects of different climatic changes, as for example the effect of water stress induced by deficit irrigation under elevated temperatures created with open top chambers (Torres et al., 2017; Bonada et al., 2018). Experiments that reproduce elevated CO_2 conditions remain rare and limited to climate change impact studies without the introduction of an adaptation (Bindi et al., 1996; Wohlfahrt et al., 2018).

Second, climate models (16 studies, Table 3) provide long and complete series of daily variations of a wide range of meteorological variables (CO_2 , temperature, rainfall, etc.) for the past and next centuries. Ongoing advances in modeling allow global climate models (GCM's) to be downscaled to regional climate models (RCM's) and their microclimatic versions (Quénol et al., 2017). However, the use of several models is still recommended to account for their intrinsic uncertainties. While changes in average daily climate parameters such as temperature or rainfall could be described by climate models, this approach still hardly represents extreme weather events and sub-daily variations (e.g., extreme temperature, heavy rains).

TABLE 3 | Number of studies that describe future climate according to meteorological data, climate model, or stakeholders' perception.

Climate data sources	Number of articles
Unspecified	27
Meteorological Data	35
Climate modeling	16
Perception	12
Meteorological + Perception	1
Climate modeling + Perception	2

The 18 review articles are excluded.

Third, stakeholders' perceptions and experiences are the basis of 12 studies (Table 3) to describe future climatic conditions. Future climatic conditions are the result of elicitation exercises which may be individual (Nicholas and Durham, 2012; Neethling et al., 2017; Bardsley et al., 2018) or collective (Lereboullet et al., 2013; Galbreath, 2014). They have the advantage of being locally adapted and of representing extreme events with their consequences. However, climate change may tend to be underestimated as the disruptive climatic conditions and new combinations of stresses, which may go far beyond local experiences, are hard to explore.

In a quarter of the selected studies, the climate evolution was not clearly specified (Table 3). The use of climate projection datasets is the only credible tool available for simulating the physical processes that determine climate change (Carter,

1996). However, it does not necessarily represent all the events proposed by stakeholders, notably those highlighted by Bardsley et al. (2018): extreme events (heat waves and heavy storms) and changes in natural resources (rainfall during the growing season and volumes of groundwater recharge). Data sources for future climatic conditions are poorly hybridized, despite the complementarity they offer. In our dataset, a single study coupled past evolution of meteorological data with winegrowers' perceptions (Lereboullet et al., 2013). Similarly, only two studies (Table 3) combined climate modeling at local scale with stakeholders' perceptions (Sacchelli et al., 2017; Tissot et al., 2017).

Approaches to Evaluate Adaptation Effects

Figure 6 illustrates the approaches used in our pool of articles to evaluate each category of adaptation. The number of studies that employed experimental and expert assessments is similar (34 and 35, respectively), whereas modeling approaches concern 21 studies. We did not find studies that used a combination of two approaches to evaluate an adaptation. It is noteworthy that all the adaptations were evaluated by experts, and that a few of them were also evaluated by modeling or experimental approaches (harvest management, farm strategy). By contrast, some specific adaptations (not detailed in the figure), such as biochar application and protective compound, were studied through experimentation only, and have never been reported by other types of study.

Experimental approaches have been widely used to understand vines' responses to changes in climatic conditions. Controlled conditions allow for the study of processes when one or several environmental factors are changed: CO₂ enrichment (Bindi et al., 1996), experimental drought (Medrano et al., 2003; Șerdinescu et al., 2014; Vaz et al., 2016; Cirigliano et al., 2017; Chrysargyris et al., 2018), or elevated temperatures (Bonada et al., 2018). The conditions of experimentation largely differ: from greenhouse conditions with fruit-bearing cutting under totally controlled conditions (Torres et al., 2017), to less controlled field experiments. Even if combinations of climatic factors are starting to be studied at plant scale, it is clearly difficult to extrapolate results at larger scales (e.g., field, region). The interactions between soil, climate, and cultural practices are difficult to identify fully. Moreover, the conditions of field experiments may not accurately reflect the overall production system constraints (vine age, cash flow, labor availability, water availability, etc.).

Unlike the experimental approach, which produces knowledge about the impact of environmental variables on a few processes only, models try to integrate that knowledge in order to predict the combined effects of climate change on the whole plant. Several approaches have been developed: empirical models, process-based models, suitability mapping, agent-based models, etc. We will not detail all existing models as they have been amply illustrated in a recent review by Moriondo et al. (2015). The aim of this section is to describe the types of models that are mostly used and how they are applied to evaluate combined adaptations. Suitability mapping has been used in four studies of our dataset, mainly to evaluate site selection and irrigation adaptations (Hannah et al., 2013; Fuhrer et al., 2014;

Teixeira et al., 2014; Resco et al., 2016). Empirical models have been used in one study to evaluate the effect of various cases of irrigation management under future climate change (Teixeira et al., 2014; Olen et al., 2016). However, empirical models show their limits when evaluating an adaptation under alternative management conditions and future climatic conditions on which experiments have not yet been run. Process-based models have also been used in 8 studies to evaluate adaptations dealing with irrigation (Grantham et al., 2010; Pieri et al., 2012; Fraga et al., 2018; Phogat et al., 2018), plant material (Pieri et al., 2012; Zhu et al., 2016), planting density (Van Leeuwen et al., 2019), site selection (Carvalho-Santos et al., 2016; Zhu et al., 2016) and mulching (Fraga and Santos, 2018). The development of models is limited by controversial effects of climate change on various processes, such as the effect of CO₂ on stomatal conductance. In addition, they poorly represent the perennial aspect of grapevines, as the multi-year succession of stresses and the age of the vine are not considered.

The first actors of adaptation are the decisions makers (policy makers and winegrowers). Yet both experimental and modeling approaches have rapidly derived into "top-down" approaches, moving from global climate model scenarios to impact studies, and then to assessments of adaptation. Hence, methodologies based on expert judgement have been implemented, resulting in qualitative or semi-quantitative results. Quantitative studies are mostly based on the dissemination of questionnaires in the vine industry. They allow for comparison of climate change adaptation under various macro-climatic conditions (Battaglini et al., 2009), and identify trade-offs, opportunities, and hurdles. Qualitative studies are more diverse (socio ecological studies, regional risk assessments, semi-structured interviews, etc.). These approaches deal with multiple scales and multiple adaptations, and consider a multitude of external factors. Two studies employed agent-based models to develop decision support systems that combine dynamic models with expert judgements (Delay et al., 2015; Tissot et al., 2017). These agent-based models are considered to be particularly appropriate tools for simulating complex interactions between ecological and social components (Tissot et al., 2017).

Evaluation Scales and Criteria

Among the selected articles, 33 studied plant scale, 32 studied field scale, 14 studied regional scale, and 14 studied farm scale (Figure 7). Most of them focused on one scale, and only 17 studies considered two or more scales simultaneously. It is noteworthy that 5 out of those 17 studies applied the expert judgement methodology (Battaglini et al., 2009; Lereboullet et al., 2013; Neethling et al., 2017; Tissot et al., 2017; Bardsley et al., 2018). Upscaling can be seen as "abrupt" in some studies (e.g., Hannah et al., 2013; Fraga et al., 2018). For example, moving from field to regional scale without considering the intermediary farm scale, implies that the constraints and opportunities of the farming system are not considered (farm delimitation, wine-making processes and sales, labor availability, etc.). In the same way, the scaling-up between plant and regional scale overlooks agronomic practices than can influence the performance of an adaptation.

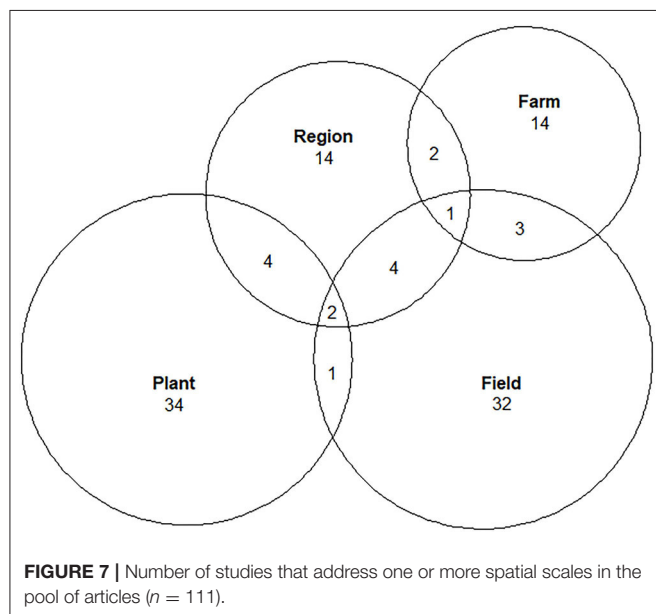
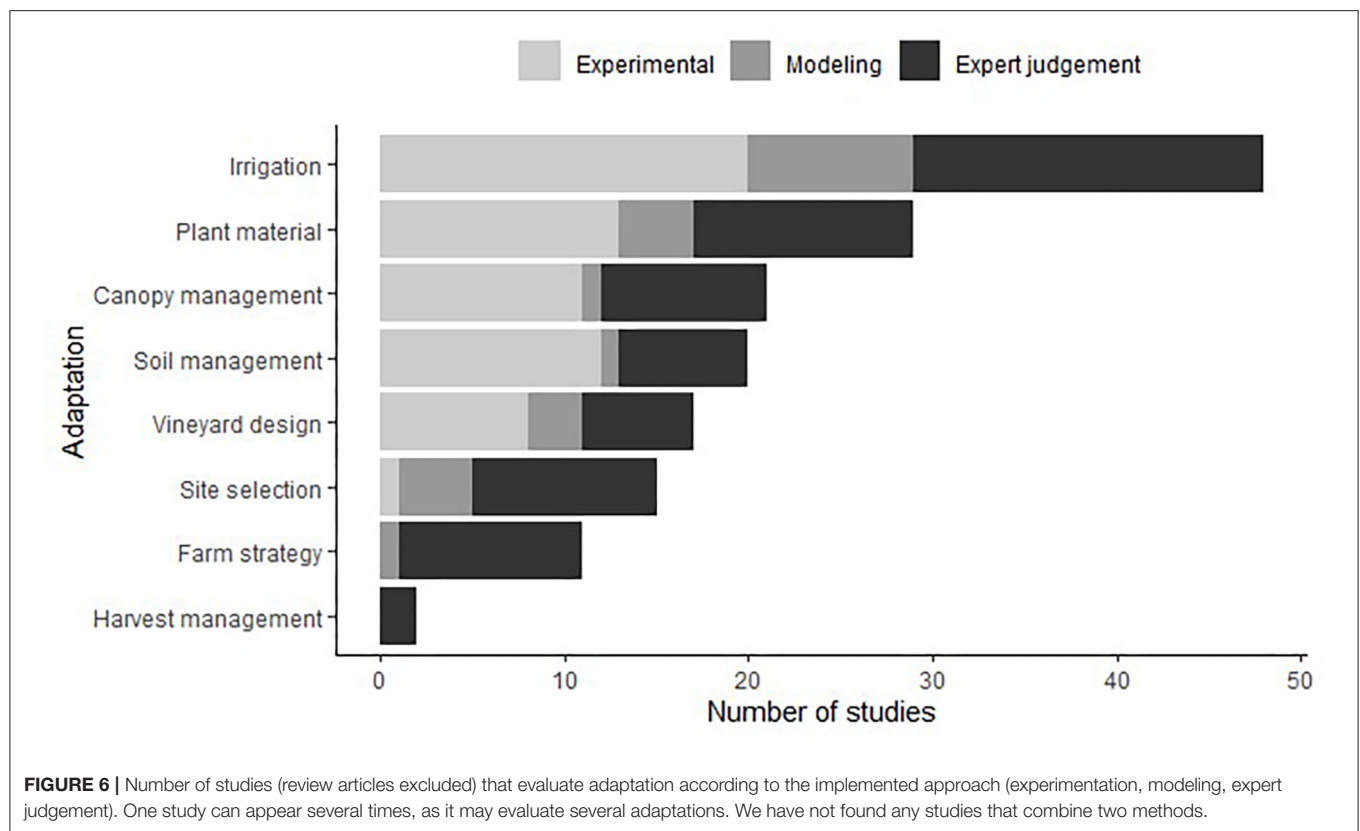


Figure 8 indicates the number of studies that quantified one or several indicators for each adaptation. We see a large number of indicators at plant and field scales, whereas farm and regional scales are studied less. Yield, berry composition and water status were the most studied indicators (31, 30, and 31

studies, respectively). Seven studies addressed regional scale in a quantitative way (reference in Suppl. Mat) and concern mainly irrigation and site selection adaptation. The lack of multi-year processes at plant and field scale is noteworthy (for example, the mortality rate).

DISCUSSION

Identifying the Site-Specific Trade-Off Between Adaptations

Most potential adaptations to water scarcity under future climate change have been evaluated individually. Our review suggests that the few existing studies dealing with combinations of adaptations help in identifying several compromises between these adaptations: the reduction of irrigation requirement through water-saving practices (Cirigliano et al., 2017; Chrysargyris et al., 2018; Gil et al., 2018; Romero et al., 2018; Torres et al., 2018); the benefits of cover crops despite water competition (Tomaz et al., 2017); the conservation of vineyard areas thanks to cultivar changes and new governance modalities (Galbreath, 2014; Delay et al., 2015; Zhu et al., 2016; Morales-Castilla et al., 2020); and the role of socio-economic conditions in promoting or regulating adaptations (Olen et al., 2016; Georgopoulou et al., 2017). It is noteworthy that since the systematic review ended, new developments have been published: Buesa et al. (2020) confirm the positive effects of east-west row orientation on yields; Morales-Castilla et al. (2020) quantified

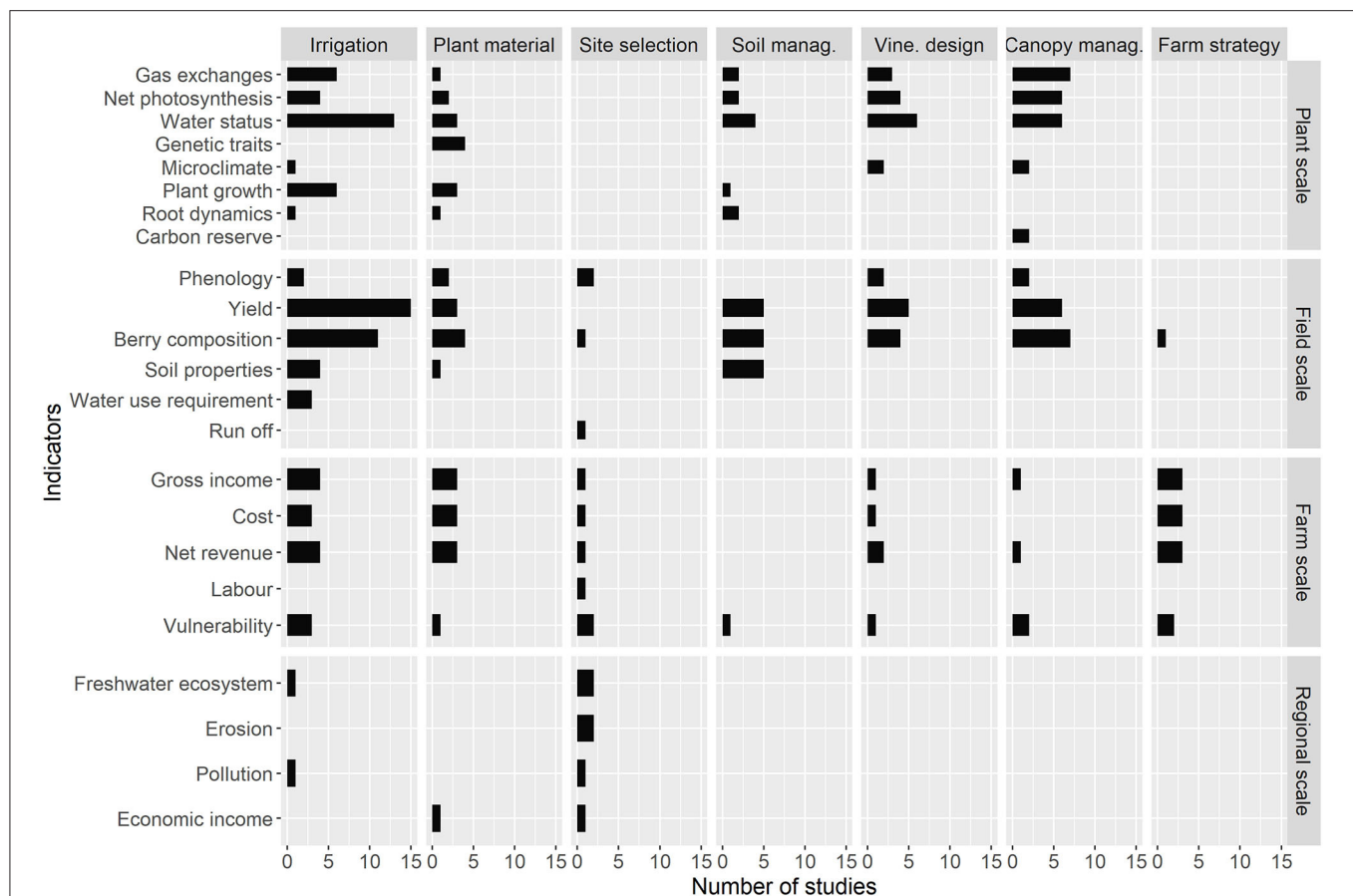


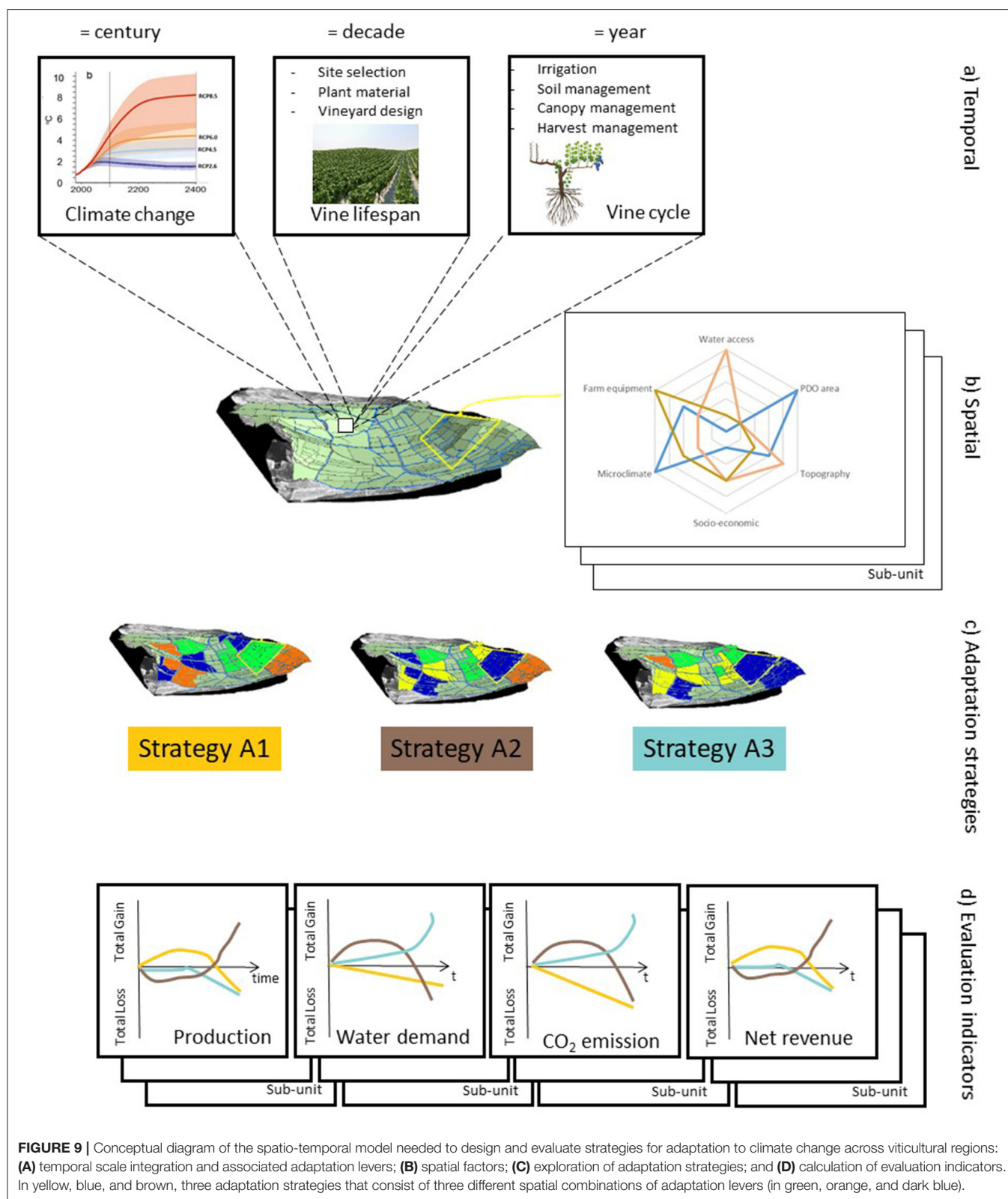
FIGURE 8 | Number of studies that evaluate adaptation according to their evaluation indicators, from plant scale to field, farm, and regional scale (from top to bottom). Only quantitative evaluations of adaptation are included (60 studies). Detailed table and references in **Supplementary Table 2**.

the reduction of suitable area lost thanks to late-ripening cultivar (from 56 to 24%); Phogat et al. (2020) went further in the estimation of future irrigation water requirement and demonstrate the importance of reducing evaporation loss; while López-Urrea et al. (2020) quantified the effect of organic and plastic mulch on evaporation. Our findings are in accordance with the climate adaptation wedges concept developed by Diffenbaugh et al. (2011). These authors illustrate the benefits of adding two adaptation strategies in limiting adverse effects of climate change in a changing context (population, development, etc.). For instance, the yield loss prevented by an adapted cultivar could be even greater when combined with an appropriate irrigation treatment.

In fact, many of the identified trade-offs occur at nested temporal and spatial resolutions (e.g., short-term vs. long-term effects of cover crop, irrigation practices vs. regional water availability, planting choice vs. local, or national governance) that have hardly been captured by previous evaluation studies. With regard to time scale, the spatial expansion of long-term adaptation (e.g., cultivars, planting density) is limited by the vineyard renewal, which is estimated at around 2 to 3% in

France (Agreste, 2018), whereas the adoption of short-term adaptations depends on the infra-annual organization of farm labor. Moreover, climatic changes could be described at a century scale (global warming) when dramatic events may occur at the scale of a few hours or days (heavy rain, heat waves). With regard to spatial scale, the close link between viticulture and *terroir* means that a wide range of spatial factors must be considered—soil, microclimate, and socio-economic (“Protected designation of Origin” areas, farm size, etc.)—when designing and evaluating adaptation strategies.

The design and implementation of effective combinations of adaptations require a quantification of the possible impacts of climate change, coupled with the sensitivity of those impacts to different adaptation activities (Diffenbaugh et al., 2011). Models may play a central role in managing various time steps and spatial units. Previous works dealing with adaptation have developed modeling tools with the aim of integrating climate projection into grapevine crop models (Moriondo et al., 2015). Models exist for some specific processes: WaLIS for water balance (Celette et al., 2010), VitiSim for carbon balance (Mirás-Avalos et al., 2018), NVINE for nitrogen cycle (Nendel and Kersebaum, 2004),



STICS for yield (Fraga et al., 2018), among others. The few studies that integrated decision-making into their models are based on agent-based modeling (Delay et al., 2015; Tissot et al.,

2017). Other decision models developed in viticulture could be adapted to climate change studies: VERDI (Ripoche et al., 2011), or DHIVINE (Martin-Clouaire et al., 2016). However, Corbeels

et al. (2018) recently challenged the ability of crop models driven by climate model projections to identify promising adaptation, given the large uncertainties of model predictions.

In addition, the contribution of stakeholders is important in characterizing and considering local constraints and opportunities. The example of co-design and evaluation studies oriented toward the reduction of pesticide use offers promising tools (Lafond and Métral, 2015; Thiollot-Scholtus and Bockstaller, 2015). In fact, strategies of adaptation to climate change with the participation of stakeholders have already been evaluated (Battaglini et al., 2009; Nicholas and Durham, 2012; Alonso and Liu, 2013; Lereboullet et al., 2013). However, the quantitative evaluation or comparison of co-designed strategies under future climatic conditions has not yet been developed. Further researches need to be conducted in order to combine the co-design of spatial adaptation strategies with their quantitative evaluation under future climatic conditions.

Insight for Developing a New Evaluation Framework

Based on the lessons learnt from reforestation studies (Cunningham et al., 2015), we propose a new framework of adaptation evaluation in four steps, considering different time and space scales, with a few to building spatially explicit strategies (Figure 9). A first step concerns the integration of three temporal scales (year, decade, century). A second step integrates spatial factors into the evaluation processes (water access, Protected Denomination of Origin areas, microclimate, etc.). A third step explores the spatialized adaptation strategies, considering a combination of adaptation in both time and space. A fourth step allows trade-offs to be identified by calculating multiple evaluation indicators over time.

The first step in evaluating local adaptation strategies may help researchers in considering the impact of climate change and of adaptation strategies over relevant times scales (year to century) (Figure 9A). Adequate models exist but they are far from being exhaustive (e.g., high temperature, CO₂ effects) and parameterized for various contexts [soil, climate, cultivar, etc.] (Moriondo et al., 2015). The existing models could be improved by conducting more focused research (experimental or on-farm), particularly in traditional grapevine systems (low density, traditional cultivars, and crop management). Other improvements lie in considering multi-year processes (e.g., mortality). Given the urgency of adaptation, expert opinion might also be used to develop and parameterize models when quantitative knowledge is unavailable. Close collaboration between researchers and winegrowers might help in designing better adaptation trials in order to fill knowledge gaps.

The second step consists in delimiting spatial sub-units that represent regions where the conditions of adaptation to climate change can be expected to be similar. The collection of data to parameterize models in each spatial sub-unit is laborious. The relevancy of required data (e.g., slope, soil type, water access, "Protected Designation of Origin" area) could be discussed with

experts or local stakeholders. To go further, models should be scaled up to larger sub-units (farm, small agricultural region, catchment, country, etc.) (Figure 9B). This scaling up process requires spatial and temporal modeling methods that predict the aggregated effects of adaptation.

The third step, the integration of a detailed understanding of the plant and field processes with regional-scale modeling is a key toward predicting the effects of the spatial distribution of adaptation levers while considering biophysical and socio-economic diversity. The use of large-scale spatial and temporal models makes possible the exploration of a large range of plausible adaptation strategies, including future climate evolution (e.g., more frequent droughts, higher temperatures), economic choices (e.g., expansion of PDO areas, marketing labels, water prices) and social changes (e.g., consumer preferences) (Figure 9C).

In the final step, such models may be used to quantify a large range of evaluation indicators (environmental, economic, agronomic, etc.) in order to reveal trade-offs and avoid potential deleterious adaptation strategies (e.g., unbalanced water demand and supply, yield reduction, climate change mitigation) (Figure 9D). Evaluation indicators should be calculated across time as a beneficial strategy could appear as a mal-adaptation under future climatic conditions or, on the contrary, an apparently disadvantageous strategy could appear beneficial in the near future. The development of indicators should meet the objectives of various local stakeholders (wine-growers, policy-makers, environmental defenders, etc.).

In conclusion, rigorous evaluation of adaptation strategies for climate change helps to identify site-specific adaptation trade-offs. We argue that the development of methodologies to evaluate adaptation strategies, considering both complementary adaptations and scales, is essential to propose relevant information to decision-makers in the winegrowing sector. The development of spatial and temporal evaluation tools based on mixed knowledge—local and scientific—about grapevine response to climatic conditions, is a key for deciding how to locally adapt viticulture to climate change.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AN lead the systematic bibliographic research (problematic, equation search, metadata extraction). Articles selection procedure and criteria of analysis have been defined by AN, CG, LP, and LH. LH ensured the consistency of the method with her experience in dealing with PRISMA flows diagram. AN has processed to the reading and selection. The final article pool has been discussed and validated by the AN, CG, LP, and LH. AN organized and wrote the article with frequent interactions with the CG, LP, and LH.

All authors contributed to the article and approved the submitted version.

FUNDING

This work was carried out with the financial support of the Occitanie Regional Council, the INRAE Department AgroEcoSystem and the LACCAGE 2.21 project funded by the meta-program Adaptation of Agriculture and Forests to Climate Change (AAFCC) of the French National Research Institute for Agriculture, Food, and Environment (INRAE).

REFERENCES

- Agreste (2018). *Étude sur l'âge Du Vignoble Et Des Vignesarrachées Dans le Bassin Viticolelanguedoc-Roussillon*. Toulouse: DRAAF Occitanie.
- Alonso Ugaglia, A., and Peres, S. (2017). Knowledge dynamics and climate change issues in the wine industry: a literature review. *J. Innov. Econ. Manage.* 24, 105–125. doi: 10.3917/jie.pr1.0016
- Alonso, A. D., and Liu, Y. (2013). Climate change in the wine sector of an ultra-peripheral european region: a case study. *Agroecol. Sustain. Food Syst.* 37, 291–315. doi: 10.1080/10440046.2012.712089
- Amendola, C., Montagnoli, A., Terzaghi, M., Trupiano, D., Oliva, F., Baronti, S., et al. (2017). Short-term effects of biochar on grapevine fine root dynamics and arbuscular mycorrhizae production. *Agric. Ecosyst. Environ.* 239, 236–245. doi: 10.1016/j.agee.2017.01.025
- Aparicio, J., Tenza-Abri, A. J., Borg, M., Galea, J., and Candela, L. (2019). Agricultural irrigation of vine crops from desalinated and brackish groundwater under an economic perspective. *A case study in Siggiewi, Malta. Sci. Total Environ.* 650, 734–740. doi: 10.1016/j.scitotenv.2018.09.059
- Bardsley, D. K., Palazzo, E., and Pütz, M. (2018). Regional path dependence and climate change adaptation: a case study from the McLaren Vale, South Australia. *J. Rural Stud.* 63, 24–33. doi: 10.1016/j.jrurstud.2018.08.015
- Baronti, S., Vaccari, F. P., Miglietta, F., Calzolari, C., Lugato, E., Orlandini, S., et al. (2014). Impact of biochar application on plant water relations in *Vitis vinifera* (L.). *Eur. J. Agron.* 53, 38–44. doi: 10.1016/j.eja.2013.11.003
- Battaglini, A., Barbeau, G., Bindi, M., and Badeck, F.-W. (2009). European winegrowers' perceptions of climate change impact and options for adaptation. *Reg. Environ. Change* 9, 61–73. doi: 10.1007/s10113-008-0053-9
- Belliveau, S., Smit, B., and Bradshaw, B. (2006). Multiple exposures and dynamic vulnerability: evidence from the grape industry in the Okanagan Valley, Canada. *Glob. Environ. Change* 16, 364–378. doi: 10.1016/j.gloenvcha.2006.03.003
- Bindi, M., Fibbi, L., Gozzini, B., Orlandini, S., and Miglietta, F. (1996). Modelling the impact of future climate scenarios on yield and yield variability of grapevine. *Clim. Res.* 7, 213–224. doi: 10.3354/cr007213
- Bonada, M., Buesa, I., Moran, M. A., and Sadras, V. O. (2018). Interactive effects of warming and water deficit on shiraz vine transpiration in the Barossa Valley, Australia. *OENE One* 52, 189–202. doi: 10.20870/oeno-one.2018.52.2.2141
- Buesa, I., Ballester, C., Mirás-Avalos, J. M., and Intrigliolo, D. S. (2020). Effects of leaning grapevine canopy to the west on water use efficiency and yield under Mediterranean conditions. *Agric. For. Meteorol.* 295:108166. doi: 10.1016/j.agrformet.2020.108166
- Buesa, I., Caccavello, G., Basile, B., Merli, M. C., Poni, S., Chirivella, C., et al. (2019). Delaying berry ripening of bobal and tempranillo grapevines by late leaf removal in a semi-arid and temperate-warm climate under different water regimes: late leaf removal effects in Bobal and tempranillo. *Aust. J. Grape. Wine Res.* 25, 70–82. doi: 10.1111/ajgw.12368
- Caffarra, A., and Eccel, E. (2011). Projecting the impacts of climate change on the phenology of grapevine in a mountain area. *Aust. J. Grape. Wine Res.* 17, 52–61. doi: 10.1111/j.1755-0238.2010.00118.x
- Caravia, L., Collins, C., Petrie, P. R., and Tyerman, S. D. (2016). Application of shade treatments during shiraz berry ripening to reduce the impact of high temperature: shade reduces impact of high temperature on shiraz. *Aust. J. Grape. Wine Res.* 22, 422–437. doi: 10.1111/ajgw.12248
- Carter, T. R. (1996). "Assessing climate change adaptations: the IPCC guidelines," in *Adapting to Climate Change: An International Perspective*, eds. J. B. Smith, N. Bhatti, G. V. Menzhulin, R. Benioff, M. Campos, B. Jallow (New York, NY: Springer New York), 27–43.
- Carvalho, L. C., Coito, J. L., Gonçalves, E. F., Lopes, C., and Amâncio, S. (2016). Differential physiological response of the grapevine varieties touriga nacional and trincadeira to combined heat, drought and light stresses. *Plant Biol.* 18 (Suppl. 1), 101–111. doi: 10.1111/plb.12410
- Carvalho, L. C., Coito, J. L., Gonçalves, E. F., Lopes, C., and Amâncio, S. (2018). Physiological and agronomical responses to environmental fluctuations of two Portuguese grapevine varieties during three field seasons. *Ciência Téc. Vitiv.* 33, 1–14. doi: 10.1051/ctv/20183301001
- Carvalho-Santos, C., Nunes, J. P., Monteiro, A. T., Hein, L., and Honrado, J. P. (2016). Assessing the effects of land cover and future climate conditions on the provision of hydrological services in a medium-sized watershed of Portugal: impacts of land cover and future climate on hydrological services. *Hydrol. Process* 30, 720–738. doi: 10.1002/hyp.10621
- Celette, F., Ripoche, A., and Gary, C. (2010). WaLIS—A simple model to simulate water partitioning in a crop association: the example of an intercropped vineyard. *Agric. Water Manage.* 97, 1749–1759. doi: 10.1016/j.agwat.2010.06.008
- Chen, H. (2018). *VennDiagram: Generate High-Resolution Venn and Euler Plots*. Available online at: <https://CRAN.R-project.org/package=VennDiagram>
- Chrysargyris, A., Xylia, P., Litskas, V., Mandoulaki, A., Antoniou, D., Boyias, T., et al. (2018). Drought stress and soil management practices in grapevines in cyprus under the threat of climate change. *J. Water Clim. Change* 9, 703–714. doi: 10.2166/wcc.2018.135
- Cirigliano, P., Vincenza Chiriaco, M., Nunez, A., Dal Monte, G., and Labagnara, T. (2017). Efecto combinado de la aplicación de riego y compost sobre la composición de la baya montepulciano en un entorno volcánico de la región de lacio (Italia central). *Ciencia Invest. Agraria* 44, 195–206. doi: 10.7764/rcia.v44i2.1691
- Clingeffer, P. R. (2010). Plant management research: status and what it can offer to address challenges and limitations. *Aust. J. Grape Wine Res.* 16, 25–32. doi: 10.1111/j.1755-0238.2009.00075.x
- Concepción Ramos, M. (2016). Soil losses in rainfed Mediterranean vineyards under climate change scenarios. The effects of drainage terraces. *AIMS Agric. Food* 1, 124–143. doi: 10.3934/agrfood.2016.2.124
- Corbeels, M., Berre, D., Rusinamhodzi, L., and Lopez-Ridaura, S. (2018). Can we use crop modelling for identifying climate change adaptation options? *Agric. For. Meteorol.* 256–257, 46–52. doi: 10.1016/j.agrformet.2018.02.026
- Cunningham, S. C., Mac Nally, R., Baker, P. J., Cavnano, T. R., Beringer, J., Thomson, J. R., et al. (2015). Balancing the environmental benefits of reforestation in agricultural regions. *Perspect. Plant Ecol. Evol. Syst.* 17, 301–317. doi: 10.1016/j.ppees.2015.06.001
- da Silva, J. R., Rodrigues, W. P., Ferreira, L. S., de Paula Bernado, W., Paixão, J. S., Patterson, A. E., et al. (2018). Deficit irrigation and transparent plastic covers can save water and improve grapevine cultivation in the tropics. *Agric. Water Manage.* 202, 66–80. doi: 10.1016/j.agwat.2018.02.013

ACKNOWLEDGMENTS

We thank Liz Carey Libbrecht for the English language editing of this paper.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.607859/full#supplementary-material>

- De Micco, V., Zalloni, E., Battipaglia, G., Erbaggio, A., Scognamiglio, P., Caputo, R., et al. (2018). Rootstock effect on tree-ring traits in grapevine under a climate change scenario. *IAWA J.* 39, 145–155. doi: 10.1163/22941932-20170199
- Delay, E., Piou, C., and Quenot, H. (2015). The mountain environment, a driver for adaptation to climate change. *Land Use Policy* 48, 51–62. doi: 10.1016/j.landusepol.2015.05.008
- Dequin, S., Escudier, J.-L., Bely, M., Noble, J., Albertin, W., Masneuf-Pomarède, I., et al. (2017). How to adapt winemaking practices to modified grape composition under climate change conditions. *OENO One* 51, 205–214. doi: 10.20870/oeno-one.2017.51.2.1584
- Diffenbaugh, N. S., White, M. A., Jones, G. V., and Ashfaq, M. (2011). Climate adaptation wedges: a case study of premium wine in the western United States. *Environ. Res. Lett.* 6:024024. doi: 10.1088/1748-9326/6/2/024024
- dos Santos, T. P., Lopes, C. M., Lucília Rodrigues, M., de Souza, C. R., Ricardo-da-Silva, J. M., Maroco, J. P., et al. (2007). Effects of deficit irrigation strategies on cluster microclimate for improving fruit composition of moscatel field-grown grapevines. *Sci. Hortic.* 112, 321–330. doi: 10.1016/j.scienta.2007.01.006
- Duchêne, E., Huard, F., Dumas, V., Schneider, C., and Merdinoglu, D. (2010). The challenge of adapting grapevine varieties to climate change. *Clim. Res.* 41, 193–204. doi: 10.3354/cr00850
- Duchene, E. (2016). How can grapevine genetics contribute to the adaptation to climate change? *OENO One* 50, 113–124. doi: 10.20870/oeno-one.2016.50.3.98
- Flexas, J., Galmés, J., Gallé, A., Gulías, J., Pou, A., Ribas-Carbo, M., et al. (2010). Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. *Aust. J. Grape. Wine Res.* 16, 106–121. doi: 10.1111/j.1755-0238.2009.00057.x
- Fraga, H., García de Cortázar Atauri, I., and Santos, J. A. (2018). Viticultural irrigation demands under climate change scenarios in Portugal. *Agric. Water Manage.* 196, 66–74. doi: 10.1016/j.agwat.2017.10.023
- Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., and Santos, J. A. (2012). An overview of climate change impacts on European viticulture. *Food Energy Secur.* 1, 94–110. doi: 10.1002/fes3.14
- Fraga, H., and Santos, J. A. (2018). Vineyard mulching as a climate change adaptation measure: future simulations for Alentejo, Portugal. *Agric. Syst.* 164, 107–115. doi: 10.1016/j.agry.2018.04.006
- Fuhrer, J., Smith, P., and Gobiet, A. (2014). Implications of climate change scenarios for agriculture in alpine regions — a case study in the swiss rhone catchment. *Sci. Total Environ.* 493, 1232–1241. doi: 10.1016/j.scitotenv.2013.06.038
- Galbreath, J. (2014). Climate change response: evidence from the margaret river wine region of Australia. *Bus. Strategy Environ.* 23, 89–104. doi: 10.1002/bse.1762
- García-Ruiz, J. M., López-Moreno, J. I., Vicente-Serrano, S. M., Lasanta-Martínez, T., and Begueria, S. (2011). Mediterranean water resources in a global change scenario. *Earth-Sci. Rev.* 105, 121–139. doi: 10.1016/j.earscirev.2011.01.006
- Gaudin, R., and Gary, C. (2012). Model-based evaluation of irrigation needs in mediterranean vineyards. *Irrigation Sci.* 30, 449–459. doi: 10.1007/s00271-012-0349-x
- Georgopoulou, E., Mirasgedis, S., Sarafidis, Y., Vitaliotou, M., Lalas, D. P., Theloudis, I., et al. (2017). Climate change impacts and adaptation options for the Greek agriculture in 2021–2050: a monetary assessment. *Clim. Risk Manage.* 16, 164–182. doi: 10.1016/j.crm.2017.02.002
- Gil, P. M., Lobos, P., Durán, K., Olguín, J., Cea, D., and Schaffer, B. (2018). Partial root-zone drying irrigation, shading, or mulching effects on water savings, productivity and quality of 'Syrah' grapevines. *Sci. Hortic.* 240, 478–483. doi: 10.1016/j.scienta.2018.06.050
- Grantham, T. E., Merenlender, A. M., and Resh, V. H. (2010). Climatic influences and anthropogenic stressors: an integrated framework for streamflow management in mediterranean-climate California, USA. *Freshw. Biol.* 55, 188–204. doi: 10.1111/j.1365-2427.2009.02379.x
- Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., et al. (2013). Climate change, wine, and conservation. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6907–6912. doi: 10.1073/pnas.1210127110
- Hunter, J. J., Volschenk, C. G., and Zorer, R. (2016). Vineyard row orientation of *Vitis vinifera* L. cv. Shiraz/101-14 Mgt: climatic profiles and vine physiological status. *Agric. For. Meteorol.* 228–229, 104–119. doi: 10.1016/j.agrformet.2016.06.013
- IPCC., Pachauri, R. K., and Mayer, L. (eds.). (2015). *Climate Change 2014: Synthesis Report*. Geneva: Intergovernmental Panel on Climate Change.
- Jones, G. V., White, M. A., Cooper, O. R., and Storchmann, K. (2005). Climate change and global wine quality. *Clim. Change* 73, 319–343. doi: 10.1007/s10584-005-4704-2
- Kapur, B., Steduto, P., and Todorovic, M. (2007). Prediction of climatic change for the next 100 years in the apulia region, Southern Italy. *Ital J. Agron.* 2:365. doi: 10.4081/ija.2007.365
- Lafond, D., and Métral, R. (2015). Concevoir en partenariat une ecoviticulture ECONoiquement viable et ECOlogiquement responsable par rapport aux pesticides (EcoViti). *Innov. Agron.* 46, 39–50. doi: 10.15454/1.4622667630938416E12
- Lereboullet, A.-L., Beltrando, G., and Bardsley, D. K. (2013). Socio-ecological adaptation to climate change: a comparative case study from the Mediterranean wine industry in France and Australia. *Agric. Ecosyst. Environ.* 164, 273–285. doi: 10.1016/j.agee.2012.10.008
- Lobell, D. B., Field, C. B., Cahill, K. N., and Bonfils, C. (2006). Impacts of future climate change on California perennial crop yields: model projections with climate and crop uncertainties. *Agric. For. Meteorol.* 141, 208–218. doi: 10.1016/j.agrformet.2006.10.006
- López-Urrea, R., Sánchez, J. M., Montoro, A., Mañas, F., and Intrigliolo, D. S. (2020). Effect of using pruning waste as an organic mulching on a drip-irrigated vineyard evapotranspiration under a semi-arid climate. *Agric. For. Meteorol.* 291:108064. doi: 10.1016/j.agrformet.2020.108064
- Martin-Clouaire, R., Rellier, J.-P., Paré, N., Voltz, M., and Biarnès, A. (2016). Modelling management practices in viticulture while considering resource limitations: the dhivine model. *PLoS ONE* 11:e0151952. doi: 10.1371/journal.pone.0151952
- Medrano, H., Escalona, J. M., Cifre, J., Bota, J., and Flexas, J. (2003). A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Func. Plant Biol.* 30, 607–619. doi: 10.1071/FP02110
- Medrano, H., Tomás, M., Martorell, S., Escalona, J.-M., Pou, A., Fuentes, S., et al. (2015). Improving water use efficiency of vineyards in semi-arid regions. A review. *Agron. Sust. Dev.* 35, 499–517. doi: 10.1007/s13593-014-0280-z
- Mirás-Avalos, J. M., Uriarte, D., Lakso, A. N., and Intrigliolo, D. S. (2018). Modeling grapevine performance with 'VitiSim', a weather-based carbon balance model: water status and climate change scenarios. *Sci. Hortic.* 240, 561–571. doi: 10.1016/j.scienta.2018.06.065
- Mirás-Avalos, J. M., Trigo-Córdoba, E., Bouzas-Cid, Y., and Orriols-Fernández, I. (2016). Irrigation effects on the performance of grapevine (*vitis vinifera* L.) cv. 'Albariño' under the humid climate of galicia. *OENO One* 50, 183–194. doi: 10.20870/oeno-one.2016.50.4.63
- Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS ONE* 6:e1000097. doi: 10.1371/journal.pmed1000097
- Molitor, D., Schultz, M., Mannes, R., Pallez-Barthel, M., Hoffmann, L., and Beyer, M. (2019). Semi-minimal pruned hedge: a potential climate change adaptation strategy in viticulture. *Agronomy* 9:173. doi: 10.3390/agronomy9040173
- Morales-Castilla, I., García de Cortázar-Atauri, I., Cook, B. I., Lacombe, T., Parker, A., Van Leeuwen, C., et al. (2020). Diversity buffers winegrowing regions from climate change losses. *Proc. Natl. Acad. Sci. U.S.A.* 117, 2864–2869. doi: 10.1073/pnas.1906731117
- Moriondo, M., Ferrise, R., Trombi, G., Brilli, L., Dibari, C., and Bindi, M. (2015). Modelling olive trees and grapevines in a changing climate. *Environ. Modell. Softw.* 72, 387–401. doi: 10.1016/j.envsoft.2014.12.016
- Moriondo, M., Jones, G. V., Bois, B., Dibari, C., Ferrise, R., Trombi, G., et al. (2013). Projected shifts of wine regions in response to climate change. *Clim. Change* 119, 825–839. doi: 10.1007/s10584-013-0739-y
- Mosedale, J. R., Abernethy, K. E., Smart, R. E., Wilson, R. J., and Maclean, I. M. D. (2016). Climate change impacts and adaptive strategies: lessons from the grapevine. *Glob. Chang. Biol.* 22, 3814–3828. doi: 10.1111/gcb.13406
- Moutinho-Pereira, J. M., Bacelar, E. A., Gonçalves, B., Ferreira, H. F., Coutinho, J. F., and Correia, C. M. (2010). Effects of open-top chambers on physiological and yield attributes of field grown grapevines. *Acta Physiol. Plantar.* 32, 395–403. doi: 10.1007/s11738-009-0417-x

- Moutinho-Pereira, J., Gonçalves, B., Bacelar, E., Cunha, J. B., Coutinho, J., and Correia, C. M. (2009). Effects of elevated CO₂ on grapevine (*Vitis vinifera* L.): physiological and yield attributes. *J. Grap. Res.* 48, 159–165. doi: 10.5073/vitis.2009.48.159-165
- Neethling, E., Barbeau, G., Coulon-Leroy, C., and Quénel, H. (2019). Spatial complexity and temporal dynamics in viticulture: a review of climate-driven scales. *Agric. For. Meteorol.* 276–277:107618. doi: 10.1016/j.agrformet.2019.107618
- Neethling, E., Petitjean, T., Quénel, H., and Barbeau, G. (2017). Assessing local climate vulnerability and winegrowers' adaptive processes in the context of climate change. *Mitig. Adapt. Strateg. Glob. Change* 22, 777–803. doi: 10.1007/s11027-015-9698-0
- Nendel, C., and Kersebaum, K. C. (2004). A simple model approach to simulate nitrogen dynamics in vineyard soils. *Ecol. Modell.* 177, 1–15. doi: 10.1016/j.ecolmodel.2004.01.014
- Nicholas, K. A., and Durham, W. H. (2012). Farm-scale adaptation and vulnerability to environmental stresses: insights from winegrowing in Northern California. *Glob. Environ. Change* 22, 483–494. doi: 10.1016/j.gloenvcha.2012.01.001
- Olen, B., Wu, J., and Langpap, C. (2016). Irrigation decisions for major west coast crops: water scarcity and climatic determinants. *Am. J. Agric. Econ.* 98, 254–275. doi: 10.1093/ajae/aav036
- Palliotti, A., Panara, F., Famiani, F., Sabbatini, P., Howell, G. S., Silvestroni, O., et al. (2013). Postveraison application of antitranspirant Di-1-*p*-menthene to control sugar accumulation in sangiovese grapevines. *Am. J. Enol. Vitic.* 64, 378–385. doi: 10.5344/ajev.2013.13015
- Palliotti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., and Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. *Sci. Hortic.* 178, 43–54. doi: 10.1016/j.scienta.2014.07.039
- Petrie, P. R., Brooke, S. J., Moran, M. A., and Sadras, V. O. (2017). Pruning after budburst to delay and spread grape maturity. *Aust. J. Grape Wine Res.* 23, 378–389. doi: 10.1111/ajgw.12303
- Phogat, V., Cox, J. W., Mallants, D., Petrie, P. R., Oliver, D. P., and Pitt, T. R. (2020). Historical and future trends in evapotranspiration components and irrigation requirement of winegrapes. *Austr. J. Grape Wine Res.* 26, 312–324. doi: 10.1111/ajgw.12446
- Phogat, V., Cox, J. W., and Šimunek, J. (2018). Identifying the future water and salinity risks to irrigated viticulture in the murray-darling basin, South Australia. *Agric. Water Manage.* 201, 107–117. doi: 10.1016/j.agwat.2018.01.025
- Pieri, P., Lebon, E., and Brisson, N. (2012). Climate change impact on french vineyards as predicted by models. *Acta Hortic.* 931, 29–37. doi: 10.17660/ActaHortic.2012.931.2
- Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T.-T., et al. (2018). Grapevine quality: a multiple choice issue. *Sci. Hortic.* 234, 445–462. doi: 10.1016/j.scienta.2017.12.035
- Quénel, H., Garcia de Cortazar Atauri, I., Bois, B., Sturman, A., Bonnardot, V., and Le Roux, R. (2017). Which climatic modeling to assess climate change impacts on vineyards? *OENO One* 51, 91–97. doi: 10.20870/oeno-one.2016.0.0.1869
- R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: <https://www.R-project.org/>
- Resco, P., Iglesias, A., Bardají, I., and Sotés, V. (2016). Exploring adaptation choices for grapevine regions in Spain. *Reg. Environ. Change* 16, 979–993. doi: 10.1007/s10113-015-0811-4
- Ripoche, A., Rellier, J.-P., Martin-Clouaire, R., Paré, N., Biarnès, A., and Gary, C. (2011). Modelling adaptive management of intercropping in vineyards to satisfy agronomic and environmental performances under mediterranean climate. *Environ. Modell. Softw.* 26, 1467–1480. doi: 10.1016/j.envsoft.2011.08.003
- Romero, P., Botia, P., and Navarro, J. M. (2018). Selecting rootstocks to improve vine performance and vineyard sustainability in deficit irrigated monastrell grapevines under semiarid conditions. *Agric. Water Manage.* 209, 73–93. doi: 10.1016/j.agwat.2018.07.012
- Sacchelli, S., Fabbri, S., Bertocci, M., Marone, E., Menghini, S., and Bernetti, I. (2017). A mix-method model for adaptation to climate change in the agricultural sector: a case study for Italian wine farms. *J. Clean. Prod.* 166, 891–900. doi: 10.1016/j.jclepro.2017.08.095
- Sacchelli, S., Fabbri, S., and Menghini, S. (2016). Climate change, wine and sustainability: a quantitative discourse analysis of the international scientific literature. *Agric. Agric. Sci. Proc.* 8, 167–175. doi: 10.1016/j.aaspro.2016.02.090
- Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L.-T., Correia, C., et al. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Appl. Sci.* 10:3092. doi: 10.3390/app10093092
- Savi, T., Petruzzellis, F., Martellos, S., Stenni, B., Dal Borgo, A., Zini, L., et al. (2018). Vineyard water relations in a karstic area: deep roots and irrigation management. *Agric. Ecosyst. Environ.* 263, 53–59. doi: 10.1016/j.agee.2018.05.009
- Scholasch, T., and Rienth, M. (2019). Review of water deficit mediated changes in vine and berry physiology; consequences for the optimization of irrigation strategies. *OENO One* 53, 423–444. doi: 10.20870/oeno-one.2019.53.3.2407
- Schultz, H. R. (2010). Climate change and viticulture: research needs for facing the future. *J. Wine Res.* 21, 113–116. doi: 10.1080/09571264.2010.530093
- Șerdinescu, A., Pircălabu, L., and Foteșcu, L. (2014). *Influence of Soil Maintenance Systems and Fruit Load on Grapes Quality Under Drought Conditions*. Scientific Papers - Series B, Horticulture, 201–204.
- Serra, I., Strever, A., Myburgh, P. A., and Deloire, A. (2014). Review: the interaction between rootstocks and cultivars (*Vitis vinifera* L.) to enhance drought tolerance in grapevine. *Aust. J. Grape Wine Res.* 20, 1–14. doi: 10.1111/ajgw.12054
- Teixeira, A. H. C., Tonietto, J., Pereira, G. E., Hernandez, F. B. T., Angelotti, F., Lopes, H. L., et al. (2014). Agro-climatic suitability delimitation for table and wine grape crops under irrigation conditions in northeastern Brazil. *Acta Hortic.* 1038, 277–286. doi: 10.17660/ActaHortic.2014.1038.33
- Thiollet-Scholtus, M., and Bockstaller, C. (2015). Using indicators to assess the environmental impacts of wine growing activity: the INDIGO® method. *Eur. J. Agron.* 62, 13–25. doi: 10.1016/j.eja.2014.09.001
- Tissot, C., Neethling, E., Rouan, M., Barbeau, G., Quenol, H., and Le Coq, C. (2017). Modeling environmental impacts on viticultural ecosystems: a first case study in a regulated wine producing area. *Int. J. Agric. Environ. Inf. Syst.* 8, 1–20. doi: 10.4018/IJAEIS.2017070101
- Tomás, M., Medrano, H., Escalona, J. M., Martorell, S., Pou, A., Ribas-Carbó, M., et al. (2014). Variability of water use efficiency in grapevines. *Environ. Exp. Bot.* 103, 148–157. doi: 10.1016/j.envexpbot.2013.09.003
- Tomaz, A., Pacheco, C. A., and Coletto Martinez, J. M. (2017). Influence of cover cropping on water uptake dynamics in an irrigated Mediterranean vineyard: cover cropping and water uptake dynamics. *Irrig. and Drain.* 66, 387–395. doi: 10.1002/ird.2115
- Torres, N., Goicoechea, N., and Carmen Antolín, M. (2018). Influence of irrigation strategy and mycorrhizal inoculation on fruit quality in different clones of tempranillo grown under elevated temperatures. *Agric. Water Manage.* 202, 285–298. doi: 10.1016/j.agwat.2017.12.004
- Torres, N., Hilbert, G., Luquin, J., Goicoechea, N., and Antolín, M. C. (2017). Flavonoid and amino acid profiling on *Vitis vinifera* L. cv tempranillo subjected to deficit irrigation under elevated temperatures. *J. Food Compos. Anal.* 62, 51–62. doi: 10.1016/j.jfca.2017.05.001
- Trigo-Córdoba, E., Bouzas-Cid, Y., Orriols-Fernández, I., and Mirás-Avalos, J. M. (2015). Effects of deficit irrigation on the performance of grapevine (*Vitis vinifera* L.) cv. 'Godello' and 'Trexadura' in Ribeiro, NW Spain. *Agric. Water Manage.* 161, 20–30. doi: 10.1016/j.agwat.2015.07.011
- Uliarte, E. M., Schultz, H. R., Frings, C., Pfister, M., Parera, C. A., and del Monte, R. F. (2013). Seasonal dynamics of CO₂ balance and water consumption of C3 and C4-type cover crops compared to bare soil in a suitability study for their use in vineyards in Germany and Argentina. *Agric. For. Meteorol.* 181, 1–16. doi: 10.1016/j.agrformet.2013.06.019
- Valentini, G., Allegro, G., Pastore, C., Colucci, E., and Filippetti, I. (2019). Post-veraison trimming slow down sugar accumulation without modifying phenolic ripening in sangiovese vines: post-veraison trimming of sangiovese vines. *J. Sci. Food Agric.* 99, 1358–1365. doi: 10.1002/jsfa.9311
- Van Leeuwen, C., and Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *J. Wine Econ.* 11, 150–167. doi: 10.1017/jwe.2015.21
- Van Leeuwen, C., and Destrac-Irvine, A. (2017). Modified grape composition under climate change conditions requires adaptations in the vineyard. *OENO One* 51, 147–154. doi: 10.20870/oeno-one.2017.51.2.1647

- Van Leeuwen, C., Pieri, P., Gowdy, M., Ollat, N., and Roby, J.-P. (2019). Reduced density is an environmental friendly and cost effective solution to increase resilience to drought in vineyards in a context of climate change. *OENO One* 53, 129–146. doi: 10.20870/oeno-one.2019.53.2.2420
- Van Leeuwen, C., Schultz, H. R., Garcia de Cortazar-Atauri, I., Duchene, E., Ollat, N., Pieri, P., et al. (2013). Why climate change will not dramatically decrease viticultural suitability in main wine-producing areas by 2050. *Proc. Natl. Acad. Sci. U.S.A.* 110, E3051–E3052. doi: 10.1073/pnas.1307927110
- Vaz, M., Coelho, R., Rato, A., Samara-Lima, R., Silva, L. L., Campostrini, E., et al. (2016). Adaptive strategies of two mediterranean grapevines varieties (aragonez syn. tempranillo and trincadeira) face drought: physiological and structural responses. *Theor. Exp. Plant Physiol.* 28, 205–220. doi: 10.1007/s40626-016-0074-6
- Viguie, V., Lecocq, F., and Touzard, J.-M. (2014). Viticulture and adaptation to climate change. *J. Int. Sci. Vigne Vin.* 7, 55–60.
- Wenter, A., Zanotelli, D., Montagnani, L., Tagliavini, M., and Andreotti, C. (2018). Effect of different timings and intensities of water stress on yield and berry composition of grapevine (cv. *Sauvignon blanc*) in a mountain environment. *Sci. Hortic.* 236, 137–145. doi: 10.1016/j.scienta.2018.03.037
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.
- Wohlfahrt, Y., Smith, J. P., Tittmann, S., Honermeier, B., and Stoll, M. (2018). Primary productivity and physiological responses of *vitis vinifera* L. cvs. under free air carbon dioxide enrichment (FACE). *Eur. J. Agron.* 101, 149–162. doi: 10.1016/j.eja.2018.09.005
- Wolkovich, E. M., Burge, D. O., Walker, M. A., and Nicholas, K. A. (2017). Phenological diversity provides opportunities for climate change adaptation in winegrapes. *J. Ecol.* 105, 905–912. doi: 10.1111/1365-2745.12786
- Zhang, L., Marguerit, E., Rossdeutsch, L., Ollat, N., and Gambetta, G. A. (2016). The influence of grapevine rootstocks on scion growth and drought resistance. *Theor. Exp. Plant Physiol.* 28, 143–157. doi: 10.1007/s40626-016-0070-x
- Zhu, X., Moriando, M., van Ierland, E. C., Trombi, G., and Bindi, M. (2016). A model-based assessment of adaptation options for chianti wine production in tuscany (Italy) under climate change. *Reg. Environ. Change* 16, 85–96. doi: 10.1007/s10113-014-0622-z

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Naulleau, Gary, Prévot and Hossard. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Impacts of Pre-bloom Leaf Removal on Wine Grape Production and Quality Parameters: A Systematic Review and Meta-Analysis

Joshua VanderWeide^{1,2}, Chris Gottschalk¹, Steven R. Schultze³, Esmaeil Nasrollahiazar^{1,4}, Stefano Poni⁵ and Paolo Sabbatini^{1*}

¹ Department of Horticulture, Michigan State University, East Lansing, MI, United States, ² Faculty of Land and Food Systems, Wine Research Center, The University of British Columbia, Vancouver, BC, Canada, ³ Department of Earth Sciences, University of South Alabama, Mobile, AL, United States, ⁴ Michigan State University Extension, East Lansing, MI, United States, ⁵ Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza, Italy

OPEN ACCESS

Edited by:

Claudio Bonghi,
University of Padua, Italy

Reviewed by:

Chiara Pastore,
University of Bologna, Italy
Maria Paz Diago,
Institute of Vine and Wine Sciences
(ICVV), Spain

*Correspondence:

Paolo Sabbatini
sabbatin@msu.edu

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 26 October 2020

Accepted: 21 December 2020

Published: 04 February 2021

Citation:

VanderWeide J, Gottschalk C,
Schultze SR, Nasrollahiazar E, Poni S
and Sabbatini P (2021) Impacts of
Pre-bloom Leaf Removal on Wine
Grape Production and Quality
Parameters: A Systematic Review and
Meta-Analysis.
Front. Plant Sci. 11:621585.
doi: 10.3389/fpls.2020.621585

Wine grape (*Vitis vinifera* L.) is the most widely cultivated fruit crop in the world. However, the climatic characteristics in some growing regions are suboptimal for grape production, including short season length and excess precipitation. Grape growers can utilize an array of methods to mitigate these issues, including “early leaf removal,” a management practice involving the removal of leaves from selected basal nodes along shoots around bloom. This meta-analysis reviews the extensive literature on this practice, with specific regards to application at “pre-bloom” (PB). One hundred seventy-five publications on the topic of “early leaf removal” were identified using key terms and subsequently narrowed via eight data curation steps. The comparison between treated (PB) and control plants in these studies revealed two important results. First, PB lowered bunch rot disease (−61%), partially through reducing the compactness of clusters. Second, PB promoted a significant increase in fruit total soluble solids (°Brix, +5.2%), which was related to the increase in the leaf-to-fruit ratio. Furthermore, cultivar and rootstock were found to have a large influence on the success of PB, while the contribution of climate was smaller. In conclusion, PB significantly lowers yield and bunch rot disease and increases °Brix, both of which improve grape and wine quality.

Keywords: bunch rot, canopy management, defoliation, fruit quality, grapevine, rootstock

HIGHLIGHT

- A meta-analysis of 59 publications revealed that the wine grape management practice “pre-bloom leaf removal” consistently decreased bunch rot disease, yield, and cluster compactness while improving fruit sugar concentrations.

INTRODUCTION

Grapevines are among the most intricately managed food crops due to their sensitivity to external and internal factors, such as the environment and source–sink relations (Kliewer and Dokoozlian, 2005). The interaction between internal and external factors has given rise to the notion of “terroir,” unique to viticulture and enology (Van Leeuwen, 2010). Several viticultural practices

are utilized to align vine growth, vine development, and fruit ripening (internal factors) with environment conditions (external factors). One such practice is “leaf removal,” otherwise referred to as “defoliation” or “leaf thinning.” Leaf removal is a technique that involves the removal of a select number of leaves that cover the fruiting region along shoots (Poni et al., 2006). This allows for a more open fruit-zone microclimate, which can lead to numerous production and fruit quality benefits.

Using the Eichhorn-Lorenz grape phenology scale as a reference (Coombe, 1995), the two most researched times of leaf removal application are (1) “early,” which includes application from “pre-bloom” (E-L 17, flower caps on) through “bloom” (E-L 23, flower caps off) and “fruit set” (E-L 27, berries >2 mm), as well as (2) “late,” which centers around “veraison” (E-L 35, berry ripening initiation).

The primary objective of early leaf removal practices is the mitigation of yield loss from cluster rot diseases, such as gray mold (*Botrytis cinerea*) and sour rot, particularly in compacted cluster varieties (Poni et al., 2017). In warm/hot, dry growing regions, gray mold is more prominent. Gray mold is a necrotrophic fungus ubiquitous to crops and particularly fruit production (Ky et al., 2012). It initially infects fruit from the surface, followed by degradation of subtending tissues, leading to a loss of yield while compromising quality-related metabolites, such as organic acids, phenolics, and volatiles. In cool/warm regions that receive high volumes of precipitation during the fruit ripening period, sour rot is the more problematic form of bunch rot disease. The bacteria and yeast comprising the sour rot complex convert the fruit sugars (glucose, fructose) into acetic acid and other metabolites, such as acetaldehyde, galacturonic acid, gluconic acid, ethanol, ethyl acetate, and glycerol (Zoecklein et al., 1995). Increases in the concentration of acetic acid engenders a noticeable “vinegar” flavor to wines made from these fruits, thus lowering quality and value.

The second major objective of early leaf removal is to enhance fruit and wine quality (Tardaguila et al., 2010; VanderWeide et al., 2018). Crop load regulation is required in specific regions to meet yield standards in some prominent production regions, such as DOCG in Italy or AOC in France. Additionally, in warm/hot, dry growing regions, the yield of highly fruitful cultivars must be reduced to maintain vine balance, and early leaf removal provides an effective tool to achieve targeted crop levels. This, in turn, leads to an improvement in both basic fruit quality components as well as total anthocyanins (Tardaguila et al., 2012; Poni and Gatti, 2017; Silvestroni et al., 2018). In addition to crop level, the capacity of a grapevine to produce “high-quality” fruit is related to seasonal accumulation of growing degree days (GDDs). Cool/warm regions are defined by low mean day temperatures, while the low GDDs experienced by vineyards in cool regions can also hinder the accumulation of hexoses in fruit (Liang et al., 2014).

Leaf removal at pre-bloom consistently induces a reduction in fruit set in both red and white cultivars (Poni et al., 2009; Sabbatini and Howell, 2010; Tardaguila et al., 2010; Molitor et al., 2011; Acimovic et al., 2016). Carbon deprivation from leaf removal at this stage impacts meiosis in inflorescence, reducing the flow of hexoses and decreasing flower fertility (Lebon et al.,

2004). The severity of leaf removal at either pre- or after-bloom greatly affects fruit set, as well as developmental processes throughout fruit ripening. Using Pinot noir (*Vitis vinifera* L.), Acimovic et al. (2016) evaluated the response of removing 4, 6, 8, or 10 leaves. They reported that the removal of six and eight leaves only induced the desired effect on reducing fruit set and improving fruit quality. Removal of 4 leaves had little to no effect, while 10 leaves induced a severe carbon stress on vines, decreasing yield below an economical viable threshold (Acimovic et al., 2016). This decrease in fruit set lowers the compactness of clusters, which has significant impact on gray mold (Gubler et al., 1991; Palliotti et al., 2011; Sivilotti et al., 2016) and sour rot (Zoecklein et al., 2000; Mosetti et al., 2016; Sivilotti et al., 2016).

An increase in total soluble solids (TSS) was observed in fruit subjected to pre-bloom leaf removal when compared to the undefoliated control (Poni et al., 2006; Zenoni et al., 2017), while some results were mixed between treatments and years (Acimovic et al., 2016). Mixed results were seen for alterations in pH and titratable acidity (Intrieri et al., 2008; Acimovic et al., 2016; Zenoni et al., 2017). Pre-bloom leaf removal's effect on total phenolics is inconsistent, with some studies observing a consistent increase compared to the control (Poni et al., 2006; Intrieri et al., 2008) and others reporting no differences (Talaverano et al., 2016). The majority of publications reported an increase in anthocyanins with pre-bloom leaf removal compared to the control (Poni et al., 2006; Lee and Skinkis, 2013; Pastore et al., 2013; Zenoni et al., 2017), while some results were mixed between years, treatments, or varieties (Tardaguila et al., 2010), and some reporting no differences in all years and treatments of experimentation (Lee and Skinkis, 2013; Acimovic et al., 2016; Sivilotti et al., 2016).

Previous reviews in viticulture have focused on grapevine management practices (Smart, 1985), with some devoting space to this practice (Poni et al., 2017). Still others have reviewed the practice of early leaf removal within a specific region (Verdenal et al., 2019) or with a particular focus on aroma biosynthesis (Wang et al., 2018; Alem et al., 2019). However, no review or meta-analysis has been published in the literature that approaches the impact of early leaf removal on major production and quality traits. The objectives of this meta-analysis were 2-fold. The first objective was to understand whether pre-bloom leaf removal has a consistent impact on production and fruit quality parameters regardless of differences in climate, cultivar, rootstock, vine age, or berry color. The second objective was to assess whether factors, such as climate, cultivar, rootstock, vine age, or berry color influence the success of pre-bloom leaf removal on production and fruit quality parameters. This meta-analysis seeks to confirm the collective hypotheses generated from publications in this field in order to direct future research.

MATERIALS AND METHODS

Data Collection

A literature review was performed to identify works published from January 1985 to May 2020 in peer-reviewed scientific journals and conference proceedings that focused on the topic of early leaf removal in grape. MS Thesis and Ph.D. Dissertations

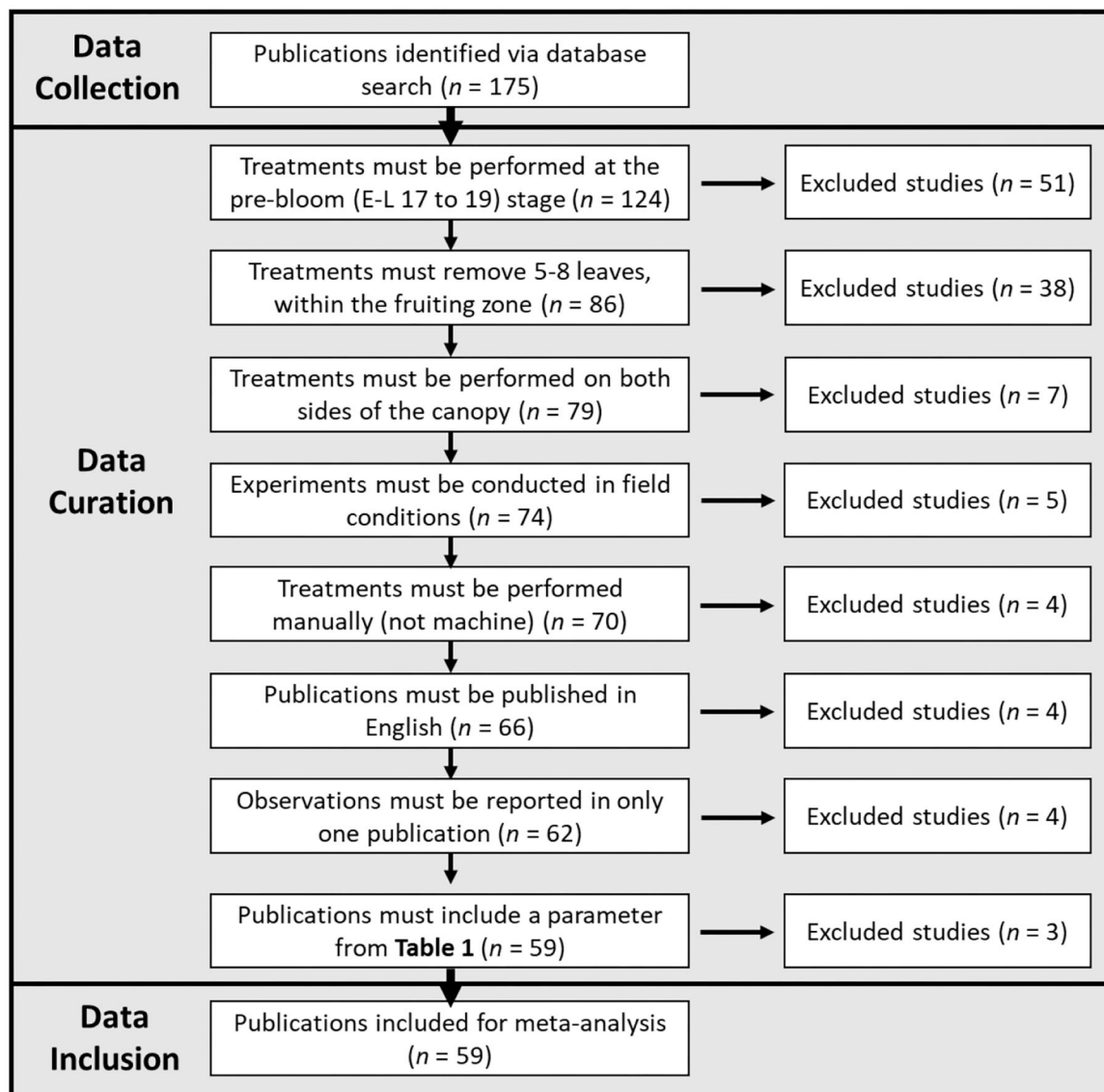


FIGURE 1 | Flowchart demonstrating the data collection, data curation, and data inclusion process utilized in this meta-analysis.

were not included. We used search terms of “defoliation grape” and “leaf removal grape” in Google Scholar and Web of Science to identify works for inclusion. A total of 175 publications were identified that involved the removal of leaves in grape.

Data Curation

Publications were maintained for further statistical analysis according to **Figure 1**.

The exclusion of publications to fit these seven criteria resulted in 59 studies (**Supplementary Figure 1**). In some cases, data from the same experiment (observation/s) were presented in multiple publications, and when this occurred, the duplicate/s of these data were eliminated from analysis. In cases where all desirable data from a study was present in a previous publication, the

more recent study was excluded. “Training system” and “Species” were originally considered as categorical variables; however, only two publications in our curated set included vines not trained to a vertical shoot positioning trellis system and two with vines that were not *vinifera* species, so they were maintained without further categorization. For each publication, in case that desired data were only present in figures, ImageJ software (Version 1.51e) was utilized to extract data points when the treatments from the respective publication were distinguishable. In the case of “yield,” “cluster compactness index,” “bunch rot incidence,” “bunch rot severity,” “total anthocyanins,” and “total phenolics,” unit representation of some parameters was heterogeneous between studies. When possible, data were converted to a common unit. For “yield,” “shoot number per vine” data were used to convert

“yield/shoot” to “yield/vine,” and “vine density” data were used to convert “yield/meter (row length)” to “yield/vine.” In the case of “total anthocyanins” and “total phenolics,” data were converted to “mg/100 g (fresh weight).” In the case that multiple acceptable units were presented in a publication, all were included. Such was the case only for “cluster compactness index” and “bunch rot incidence/severity.” In two instances, severe outliers that could be attributed to a miscalculation in the publication were deleted prior to analysis. This was the case for “total phenolics (mg/100 g) FW berry” (VanderWeide et al., 2018) and “total phenolics (AU)” (Frioni et al., 2018).

Climate Data

Thirty years climatological normals data were obtained from several meteorological agencies with long-term, monthly climate normals for temperature and precipitation (NCDC 2020, MeteoSwiss 2020, DataMeteo 2020, Agencia Estatal de Meteorología 2020, Hydrological and Meteorological Service of Montenegro 2020) for each location included in this study. In most cases, weather data were available for the study location. However, there were a few locations that did not have data, as the location was not located in a specific “town.” As such, the nearest station with similar conditions (elevation, windward/leeward dynamics) was used. The alternative stations were never more than 15 km away from the research location.

The climate data obtained allowed us to separate observations into four types: Climate 1 (hot), Climate 2 (warm/dry), Climate 3 (warm/wet), and Climate 4 (cool). The delineations between each cluster were based on average growing season temperature (GST) and average total precipitation. Climate 1 points included all study locations with average GSTs above 20°C. Climate 4 points included all study locations with average GSTs below 16°C. Climates 2 and 3 have temperatures between 16 and 20°C and are delineated by having more or <500 mm precipitation (the median for all location precipitations was 462 mm). It should be noted that these delineations serve as cutoffs for the data points we have acquired for this study. Temperature is based roughly on the breakdown of climatic classes by Jones (2007). The 500-mm precipitation cutoff for Climates 2 and 3 exists to differentiate between the largest pool of climates (38). This cutoff was deemed necessary because, if it did not exist, this study would consider Oslavia, Italy (17.9°C, 851 mm) the same climate classification as Erzincan, Turkey (17°C, 187 mm).

Statistical Analysis

Among the 59 publications used for analysis, few reported the standard error for all the parameters included in this study. Given this, the variable errors within each experiment were not accounted for. For all dependent variables, power was calculated using the G*Power Software (version 3.1.9.7). For dependent variables (**Supplementary Table 2, Figures 4–6**), an independent samples *t*-test ($p = 0.05$) was used to compare pre-bloom leaf removal treatments against the untreated control using IBM SPSS software (IBM, Armonk, NY, USA). When parameters were expressed as a percentage, the multiple acceptable units for each parameter were combined. In the case that multiple forms of a parameter existed in a publication (“cluster compactness index,”

“bunch rot incidence/severity”), both were included, and the data from the remaining parameters doubled. Factor analysis of mixed data (FAMD) was conducted using R version 3.6.2 (R Core Team, 2016). For FAMD, our data set contained multiple missing data points. To account for this, we utilized the missMDA R package by Josse and Husson (2016) that analyzes incomplete data sets for underlying data structures. We also performed an imputation of the missing data values and reanalyzed the data set using missMDA to confirm the data structure. **Figures 2A,B** were generated using Sigma Plot ver. 11.0 (Systat Software, Inc.) and R.

RESULTS AND DISCUSSION

Study Location and Number

Leaf removal (early and late) has been studied as an approach for mitigating major wine grape production issues for ~2 decades (**Figure 2**). The first studies on this topic were conducted in the late 1980s and early 1990s and focused on application at the fruit-set stage (E-L 27) (Coombe, 1995) when fruit are ~4–6 mm in diameter. In 1988, Bledsoe et al. were the first to show that leaf removal at fruit set could increase sugar concentrations (total soluble solids, TSS) in fruit while decreasing acidity in California’s dry climate (Bledsoe et al., 1988). Soon after, additional publications reported that this practice performed at the same timing greatly decreased the incidence of *Botrytis cinerea* (English et al., 1989; Gubler et al., 1991). Given that disease pressure is higher in more humid climates, researchers in these regions sought to understand whether performing this practice earlier (pre-bloom) to alter cluster architecture could further reduce bunch rot disease. This is reflected by the number of studies focusing on the pre-bloom timing occurring more recently in the last 10–15 years (**Figure 2**). With our data curation steps considered, Poni et al. were the first to characterize the response of pre-bloom leaf removal using the “Trebiano” cultivar in a peer-reviewed journal (Poni et al., 2006). They revealed that this practice significantly reduced bunch rot incidence and increased total soluble solids (TSS) concentrations in the fruit at harvest.

Leaf removal implemented prior to (or during) bloom has now been tested in many growing regions throughout the world (**Figure 3A**), with the majority of studies being conducted in the United States, Italy, and Spain (**Figures 3B,C**). Since the mid-2000s, multiple researchers have thoroughly tested this approach in growing regions, which are represented in **Figure 3**. These include the following: Ollauri (La Rioja) and Badajoz (Extremadura), Spain; Bologna, Perugia and Ragusa, Italy; Benton Harbor, Michigan; Northwest Oregon (Willamette Valley); and Shenandoah Valley, Virginia. With the exception of Badajoz (Climate 1) and Perugia (Climate 2), these growing regions share a similarity of producing wine grapes in an environment receiving low accumulation of heat units (GDD) and/or environments receiving high volumes of precipitation (Climates 1 and 3) (**Supplementary Table 1, Supplementary Figure 1**). This is reflective of two major objectives for performing pre-bloom leaf removal: reducing bunch rot disease and enhancing fruit ripening.

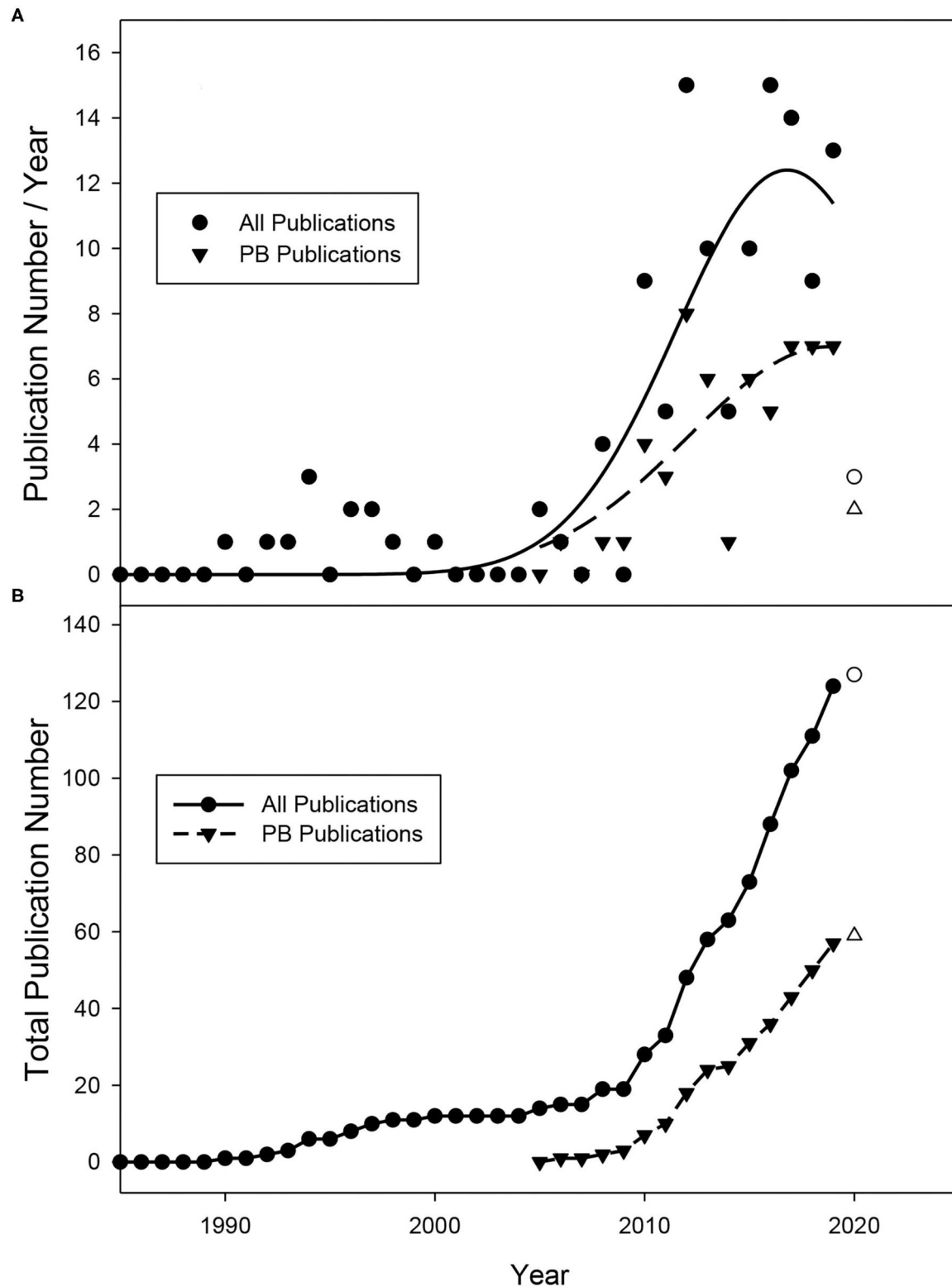
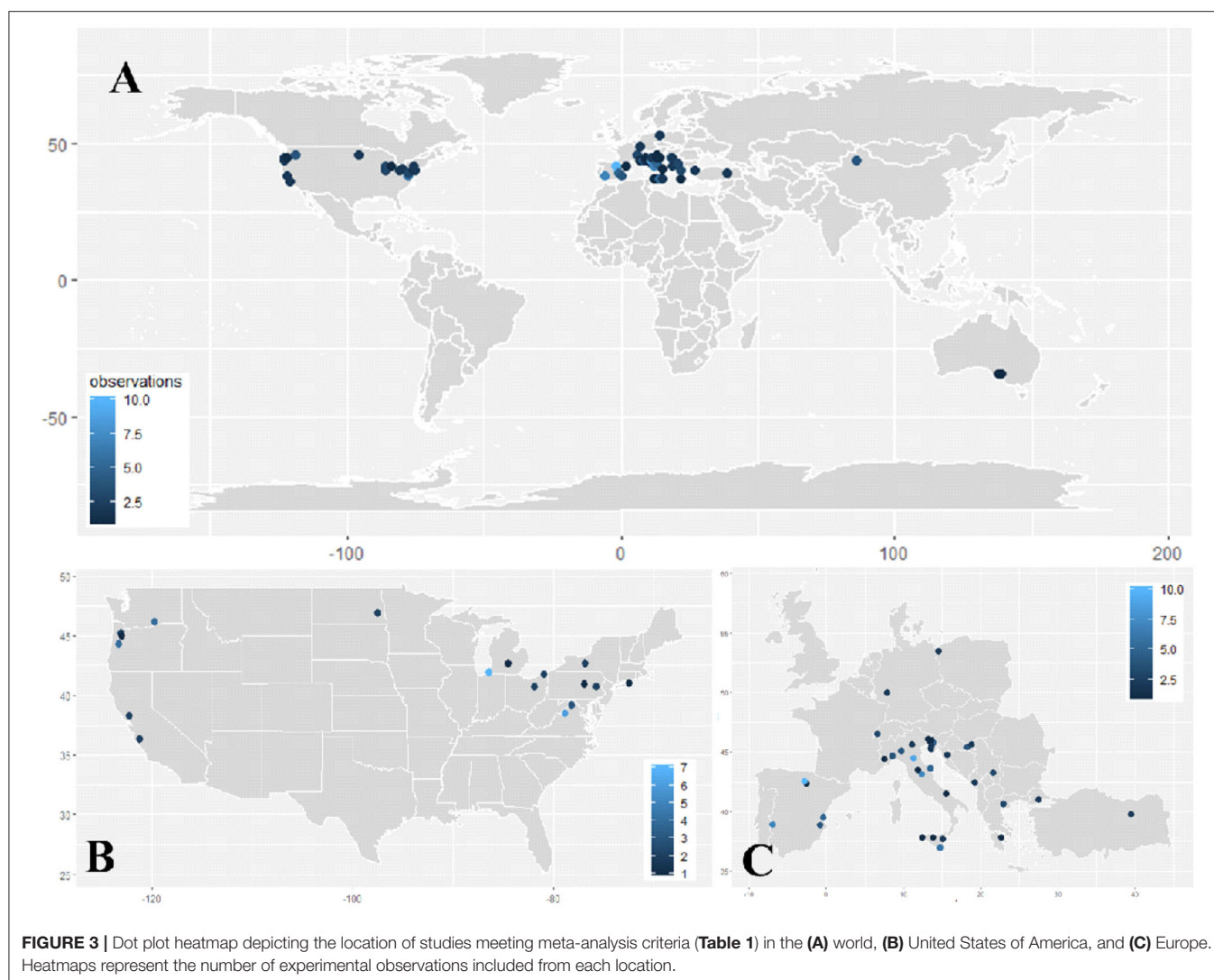


FIGURE 2 | (A) Publication number per year and **(B)** total publication number obtained from database searches between January 1985 and May 2019. No publications were identified prior to 1988. Data from 2020 (hollow circle) does not encompass the entire year (January–May) and is not included in regression analysis.

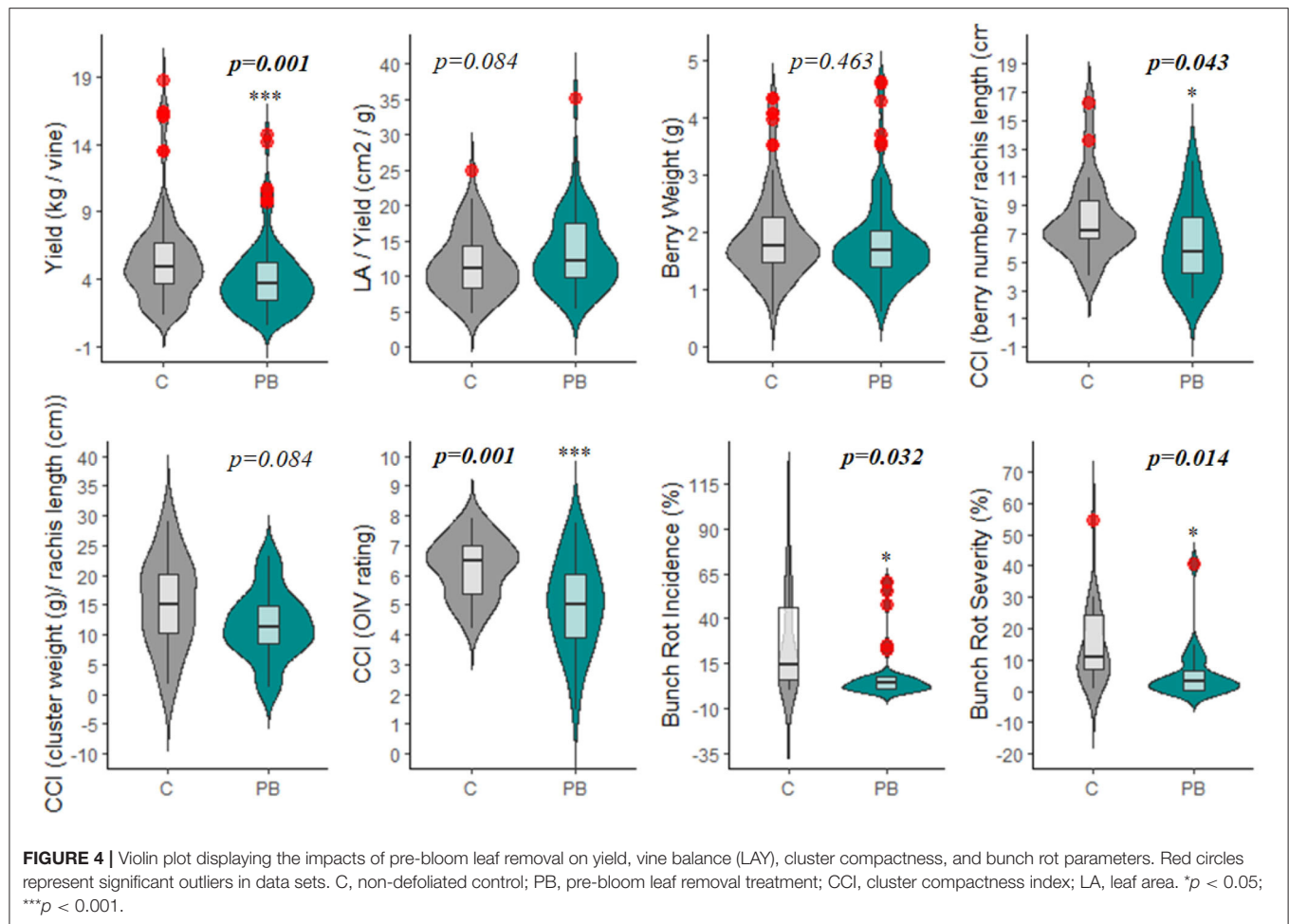


Effect of PB Leaf Removal on Production Parameters

The leaf area removed from plants with the PB treatment ranged from 30.7 to 96.0%, with an average of 61.6% (data not reported). Although there is a large variation in floret sensitivity to abscission among grape cultivars (Lebon et al., 2004), PB led to a significant reduction in yield per vine (**Figures 4, 6, Supplementary Table 2**). This is due to the decrease in fruit set that occurs when a large percentage of the carbohydrate source (leaves) is removed from the plant during the period of strong vegetative growth, drastically reducing the carbon portioning to the reproductive organs (Frioni et al., 2018). The decrease in fruit set corresponded to a significant reduction in yield (26%) in response to the PB treatment (**Figure 4**). In **Table 1**, yield is highlighted as a production parameter having one of the most consistent alterations by PB, at 80%. The similarity in yield reduction from a wide range of percentage of leaf area removed is due to translocation of carbohydrates from shoots having a surplus of carbohydrates to those with a deficit to support fruit set

(Frioni et al., 2019). This suggests that the leaf area of the whole vine is important for dictating fruit set and yield reduction and not just the leaf area of individual shoots.

Also relating to the reduction in fruit set, Cluster Compactness indices (CCI2, CCI3) were significantly decreased at a high rate of 68 and 82%, respectively (**Table 1**). Meanwhile, CCI1 reported only 50% of observations as significantly altered (**Table 1**). The differences observed in CCI parameters suggest varying sensitivities of the indices for detecting fruit-set alteration and, consequently, modifications of morphological characteristics of the clusters. Although CCI3 was the most sensitive among the indices at detecting modifications to cluster morphology, this method is highly subjective; fruit compactness is visually matched to a 6-point scale. Therefore, we suggest that CCI2 should be utilized in future studies that measure this parameter, as it is both a more sensitive metric than CCI1 and a more rapid approach. CCI describes the “openness” of the cluster, which is greatly enhanced as a result of floret abscission (Tello and Ibáñez, 2018). This decrease in compactness positively impacts the quality of



fruit, as an “open” cluster is more resistant to bunch rot disease (Table 1 and Figure 4) (Hed et al., 2009). Wind speed through the fruit zone is increased by three to four times after PB leaf removal (English et al., 1989). As a result, the evaporative potential of water from the fruit surface is higher, preventing water from collecting on the fruit surface (Acimovic et al., 2016). This is the reason for the consistent reduction (62 and 60%) in bunch rot incidence (BRI) and bunch rot severity (BRS), respectively. The identical rate of significant observations for both BRI and BRS highlight the viability of either parameter as a suitable index for estimating changes in bunch rot disease infection (Table 1 and Figures 4, 6). In addition to significantly decreasing BRI and BRS, PB leaf removal greatly narrowed the distribution of the data when compared to the non-defoliated control (C), the undefoliated treatment (Figure 4). This suggests that a threshold exists whereby continuing to decrease fruit set has no additional impact on lowering disease pressure.

Effect of PB Leaf Removal on Fruit Quality Parameters

Most studies focusing on pre-bloom leaf removal (PB) prioritize basic fruit quality components (TSS, pH, TA) over that of secondary metabolite parameters (ANT, PHE) (Table 1). TSS was

the only quality parameter that reported a significant change in response to PB treatments (Figure 5, Supplementary Table 2). This could be attributed to the significant decrease in yield or bunch rot disease (Figures 4, 6, Supplementary Table 2). However, the combination of multiple factors is likely to drive the increase in fruit sugar concentration at harvest reported by the studies. In this meta-analysis, TSS increase was not shown to be explicitly related to the yield reduction (Figure 7A), as has been suggested in some studies (Xi et al., 2018). Instead, decreased yield promotes a greater ratio between leaf area and yield (LAY), which has been used an index of vine balance, shown to be more related to fruit quality parameters than vine crop level in several previous studies (Kliwer and Dokoozlian, 2005; Pastore et al., 2011; Sivilotti et al., 2020). This is also the case here in our elaboration of data from the available literature (Figure 7A). It is worth noting that the increase in LAY is not solely due to the decrease in yield. Numerous studies show that removing leaves prior to bloom in the fruit zone leads to a stimulation of lateral leaf growth, leading the significantly larger leaf area in PB vines at harvest (Poni et al., 2006, 2009). Although LAY was increased by 12.7%, it was not significantly altered from C (Figures 4, 7A, Supplementary Table 2). This is likely related to the variability and inconsistency among the methods used to

TABLE 1 | Listing of parameters, variable type, and number of observations (comparing C and PB).

Acronym	Parameter	Variable	Total observations ^a	Significant observations ^b
DEPENDENT VARIABLES				
Yield	Yield (kg/vine)	Production	103	82 (80%)
LAY	Leaf area/Yield (cm ² /g)	Production	62	19 (31%)
BW	Berry weight (g)	Production	97	39 (40%)
CCI1	Cluster Compactness Index (berry number/cm ²)	Production	20	10 (50%)
CCI2	Cluster Compactness Index [berry weight (g/cm ²)]	Production	19	13 (68%)
CCI3	Cluster Compactness Index (OIV visual rating)	Production	33	27 (82%)
BRI	Bunch rot incidence (%)	Production	26	16 (62%)
BRS	Bunch rot severity (%)	Production	20	12 (60%)
TSS	Total soluble solids (°Brix)	Fruit quality	108	56 (52%)
pH	pH	Fruit quality	102	25 (25%)
TA	Titrateable acidity (g/L)	Fruit quality	105	34 (32%)
ANT1	Total anthocyanins (mg/100 g) FW skins	Fruit quality	14	7 (50%)
ANT2	Total anthocyanins (mg/100 g) FW berry	Fruit quality	73	44 (60%)
PHE1	Total phenolics (mg/100 g) FW skins	Fruit quality	15	8 (53%)
PHE2	Total phenolics (mg/100 g) FW berry	Fruit quality	53	34 (64%)
PHE3	Total phenolics (Absorbance Units)	Fruit quality	12	4 (33%)
CATEGORICAL VARIABLES				
BC	Berry color	–	136	–
CL	Climate	–	136	–
CUL	Cultivar	–	136	–
RS	Rootstock	–	123	–
VA	Vine age (years)	–	121	–

^aNumber of observations comparing between C and PB.

^bNumber of observations where PB was significantly larger or smaller ($p < 0.05$) than C.

calculate the leaf area partitioning of this metric. Additionally, the contribution of decreased BRI and BRS to increasing TSS is realized in this study (**Figure 7A**). However, it is challenging to explicitly link these parameters, as one form (sour rot) decreases sugar concentrations, while the other (gray mold) increases it (VanderWeide et al., 2020), and it was not possible to distinguish between both forms of bunch rot in this analysis.

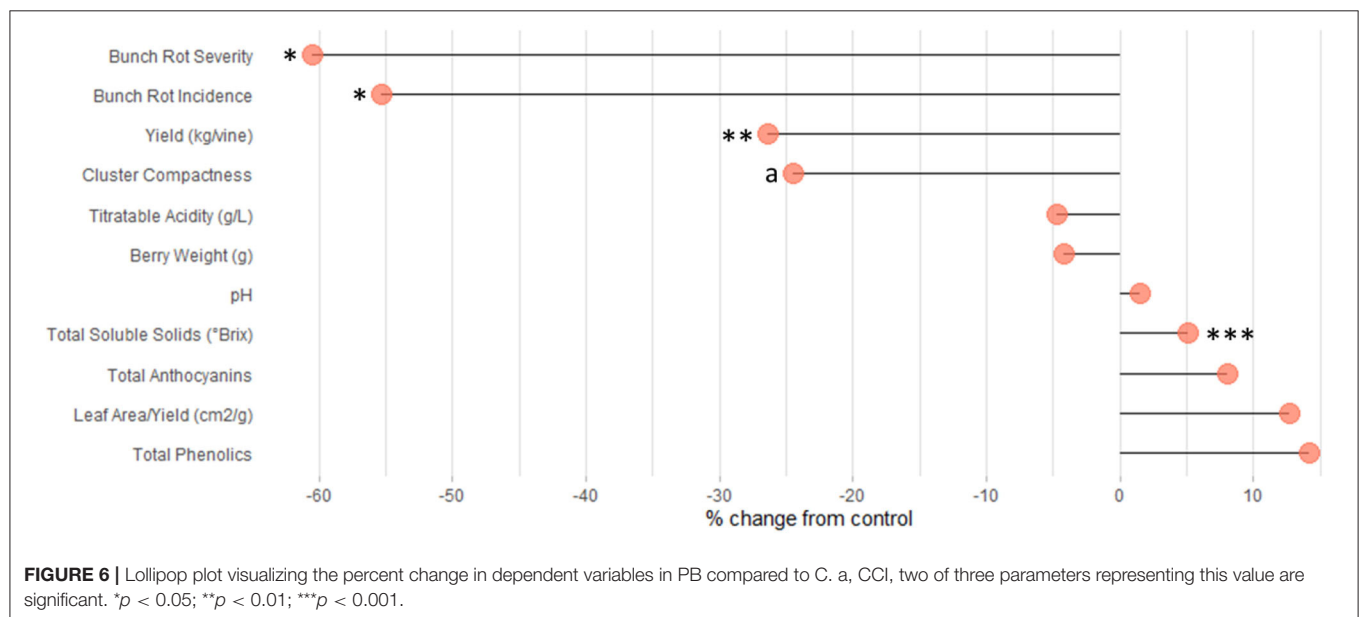
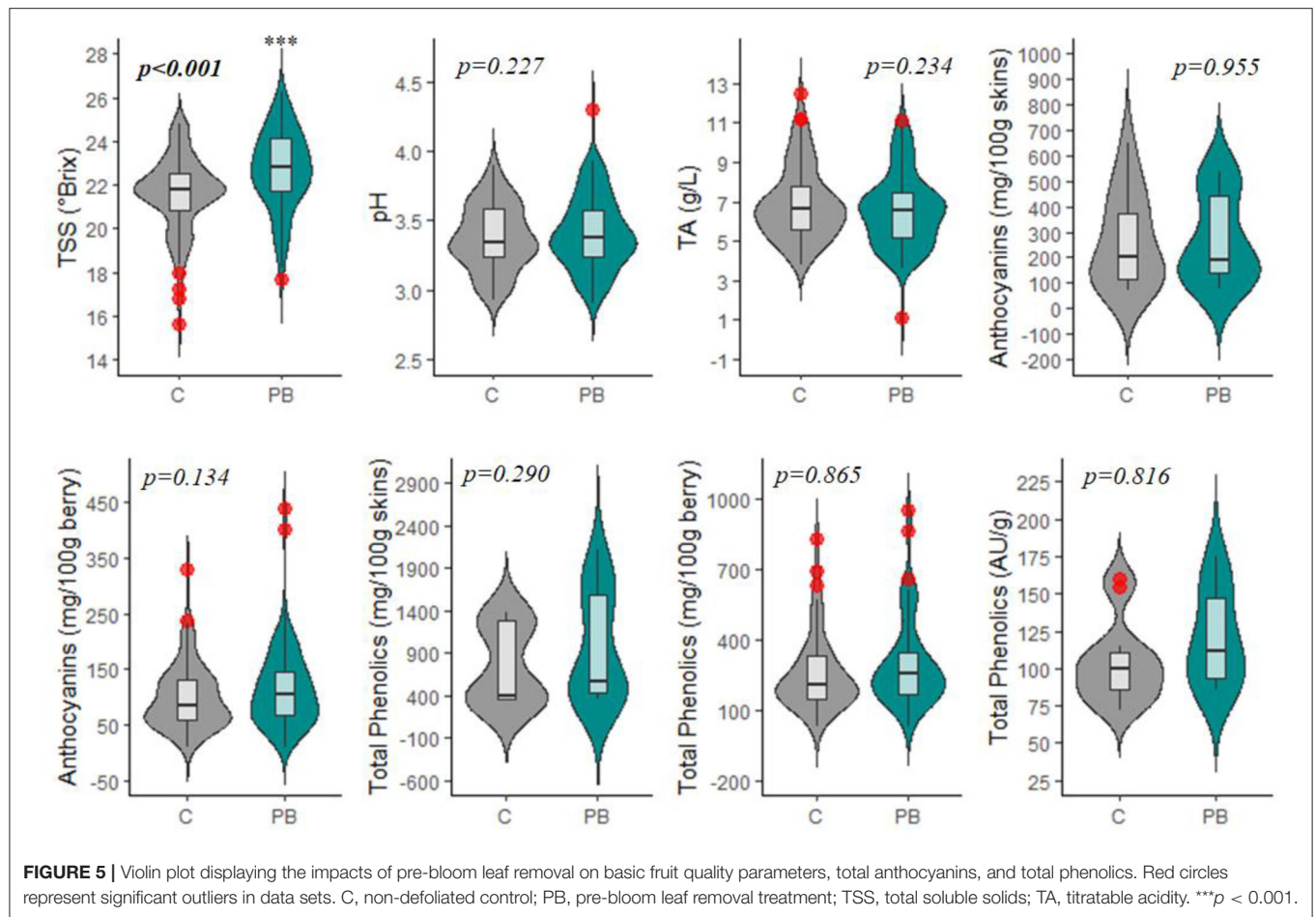
Interestingly, PB leaf removal altered secondary metabolites, namely, anthocyanins (ANT) and phenolics (PHE), to a greater percent than TSS (**Figure 6**) but were not significantly modulated from the C (**Figures 5, 6, Supplementary Table 2**). This is likely due to the large variability that exists in total anthocyanin and phenolic concentrations between cultivars (Mattivi et al., 2006), as well as the many different extraction protocols and chromatography/spectroscopy methods used for the quantification of the metabolites (De Beer et al., 2004). Specifically, ANT1, ANT2, PHE1, PHE2, and PHE3 had 9-, 41-, 4-, 72-, and 10-fold differences in concentrations between the smallest and largest data points, respectively.

In many studies, ANT and PHE concentrations were expressed in both mg/tissue and mg/berry. In the case of ANT and PHE, calculation on a mg/berry basis (ANT2, PHE2) resulted in a more consistent alteration following the PB treatment than measurement on a per-tissue basis (ANT1, PHE1) (**Table 1**). Grape phenolics are thought to be impacted by berry size;

however, this has not been firmly established (Walker et al., 2005; Ariani et al., 2016). Most phenolic compounds are located in the skin or seed tissues, and smaller berries have a greater ratio of skin and seeds to pulp and therefore will contribute more anthocyanins and phenolics per volume of fruit (Roby et al., 2004). However, berry weight (BW) was not decreased significantly in this experiment, suggesting that this slight increase in ANT and PHE in response to PB is due to an increased biosynthesis (Pastore et al., 2013) or, in the case of anthocyanins, increased skin thickness (Poni et al., 2009; Verdenal et al., 2019).

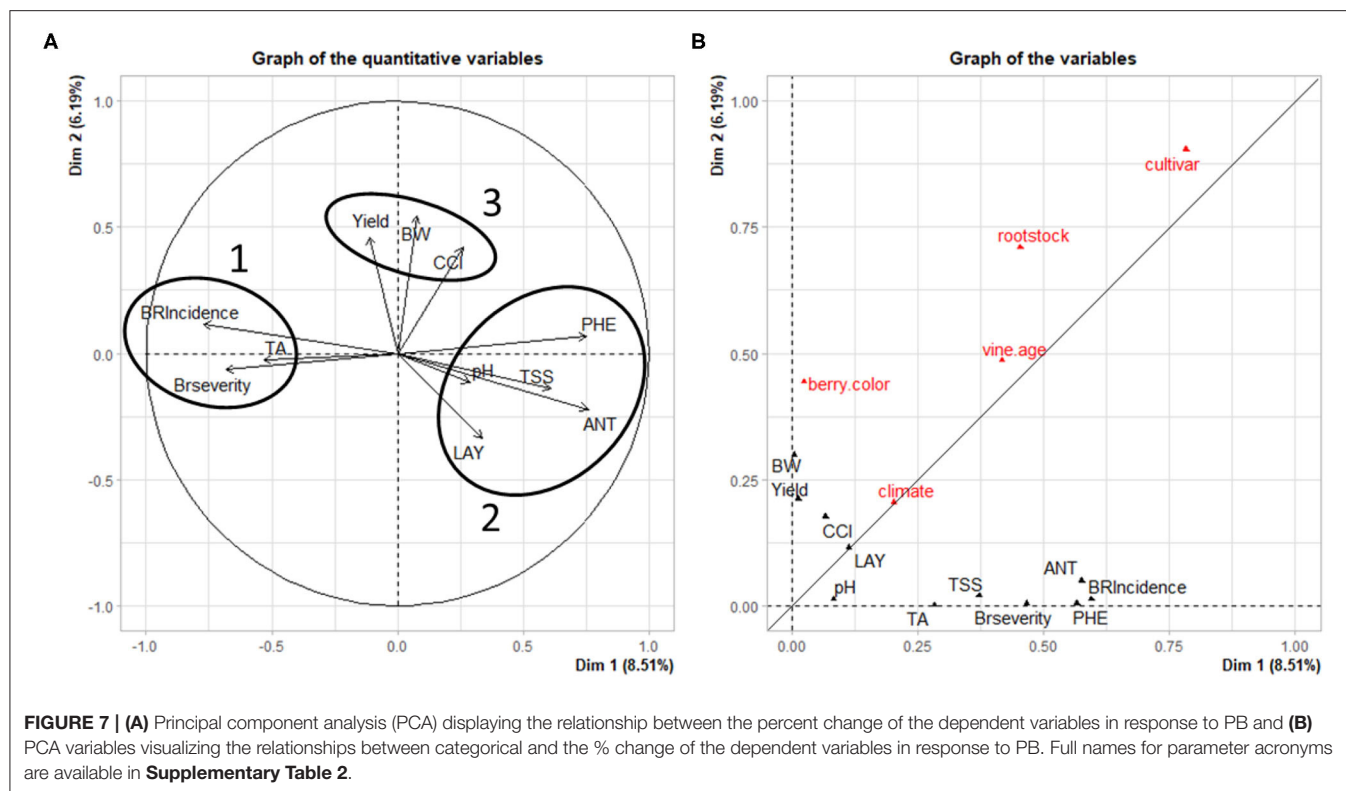
RELATIONSHIP BETWEEN CATEGORICAL AND DEPENDENT PARAMETERS

In **Figure 7A**, the principal component analysis (PCA) displays relationships among dependent variables analyzed in this work. Three distinct groups of variables are visible; two exhibit an inverse relationship to one another on dimension 1, while the third is along the positive axis of dimension 2. In group 1, bunch rot parameters (BRI, BRS) are closely aligned with TA. This could be due to cluster sour rot infection increasing acetic acid concentrations (Zoecklein et al., 1995), which would influence TA by increasing it. However, multiple studies included in this analysis did not distinguish between either form of bunch rot



disease (sour rot, gray mold), making this difficult to confirm. An additional explanation is that a higher TA, indicative of under-ripe fruit, is an artifact of fruit being harvested early

due to high presence of either sour rot or gray mold in fruit. This is backed up by the near-opposite relationship between groups 1 and 2.



Group 2 includes TSS and pH, which increase in ripening grapes, opposite to TA, which decreases. In addition to TSS and pH, group 2 also includes the other quality parameters: ANT and PHE. During the ripening process, sugars are understood to be a physiological “trigger” for the accumulation of ANT (Larronde et al., 1998; Lecourieux et al., 2014), which likely explains the grouping of these two parameters. This is not the case for most phenolics (PHE); however, anthocyanins comprise the majority of this group post-veraison, suggesting that PHE is reflective of ANT. Group 2 also indicates a relationship between fruit quality parameters and LAY. It is well-known that this ratio, often referred in viticulture as “vine balance” index, rather than the simple reduction of yield, influences fruit quality parameters (Kliewer and Dokoozlian, 2005; Parker et al., 2015). This is supported by yield in group 3, which, along with BW and CCI, were not advertently related with parameters from either group 1 or 2.

Regarding group 3, the positive relationship between BW (berry weight) and CCI (number of berries per cluster) on yield is unsurprising, as the number and size of individual berries directly influence yield. However, the lack of a strong relationship between CCI and BW with the other groups in **Figure 7A** is worth noting. Our previous research identified a significant negative relationship between cluster compactness and ANT concentration in “Merlot” berries (VanderWeide et al., 2018), while others have confirmed this with additional quality metabolites (Ziegler et al., 2020). Likewise, cluster compactness has been shown to correlate negatively with bunch rot parameters (Marois et al., 1986; Hed et al., 2009). This lack of a relationship

between CCI and either bunch rot or fruit quality parameters may be due to two reasons. First, and only regarding CCI and bunch rot disease, the presence of many observations deriving from warm and hot regions that display low bunch rot disease pressure may be skewing the data sets for BRI and BRS. Second, for both relationships, it may be that other factors have a greater influence on these parameters, such as the aforementioned one between LAY, TSS, and ANT, or an open cluster zone for bunch rot parameters, as is mentioned in the literature (English et al., 1989; VanderWeide et al., 2020). The underlying genetic and physiological mechanisms governing BW are complex (Dai et al., 2011), and PB did not cause a consistent modulation to them, different from other grapevine cultural practices (Gambetta et al., 2020). A reduction in BW by PB was reported only following the removal of 10 leaves from vines (Acimovic et al., 2016). At this threshold, the limitation of source availability was likely extended through the more active phase of cell division. Our analysis restricts studies to those that removed five to eight leaves. Additionally, BW was significantly increased and decreased from the control in different observations within this analysis, which likely explains why BW was not correlated to either bunch rot disease or fruit quality parameters.

The second component of **Figure 7** reveals the relationships among the categorical and dependent variables from each study. All categorical variables were similarly affected by both dimensions with the exceptions of berry color and rootstock, which were more closely aligned on dimension 2. Yield and BW were also oriented along dimension 2. With regards to yield and berry color, this is likely due to the different cropping (yield

adjustment) standards for white vs. red grapes, as red cultivars require greater GDD to reach harvest maturity and therefore require a more aggressive reduction in yield when compared to white cultivars, especially in cooler climates. Berry weight is also related to berry color, as red cultivars tend to have smaller berries than white cultivars. The primary roles of rootstock selection are to control water uptake and vine growth (Poni et al., 2017). The relationship between rootstock and these production parameters is intriguing, as there is no subsequent impact on quality parameters.

Surprisingly, Climate had the smallest effect among categorical variables on dependent variables. Meanwhile, Cultivar and Rootstock had the greatest influences on these variables. This is, in part, due to the fact that most cultivars and rootstocks are selected on a climate-specific basis (Keller, 2015), therefore mitigating differences in climate among growing regions. Additionally, red cultivars are known to possess higher concentrations of total phenolics than white cultivars, and white cultivars almost exclusively lack anthocyanin production (Mattivi et al., 2006). The lesser influence from climate may also come from scales of data between the climatological data and the leaf removal experiments. The climatology data were taken as 30-year climate norms for each site, while the studies were taken from certain years' worth of data. Higher resolution climate data—weather data taken for each year of study for all 59 studies—would likely increase the connection with climate. However, because of a lack of quality weather data in certain study areas, this was not possible. Future work with higher resolution data may yet reveal a stronger connection. This suggests the need for further investigation into our data set to more explicitly uncover the influence of climate and other categorical variables on the “success” of PB.

CONCLUSION

This meta-analysis was conducted using 59 publications that describe the response of grapevines to pre-bloom leaf removal: an important grapevine canopy management technique. The results of this work provide a clear physiological picture into the response of PB on both production and fruit quality parameters. Pre-bloom leaf removal applied early in the vine growth and developmental stages restricts carbohydrate availability to

inflorescence, which accelerates inflorescence abscission and causes a reduction in fruit set. This significantly decreases yield by 26%. Additionally, lowered fruit set significantly reduced CCI, which, in turn, led to a reduction in bunch rot incidence (BRI) and severity (BRS) by ~55–60%. Among fruit quality parameters, only °Brix was significantly increased by PB, likely influenced by both the decrease in yield and bunch rot disease. PCA indicated a strong relationship between the percent increase in vine balance (leaf-to-fruit ratio) and TSS in response to PB. This analysis also revealed a strong correlation between the percent increase in multiple fruit quality parameters, including TSS, pH, anthocyanins, and phenolics; the latter two are likely influenced by the higher TSS. Together, this study provides grape producers with a clear outline of the benefits of performing pre-bloom leaf removal to achieve high fruit quality in challenging growing climates.

DATA AVAILABILITY STATEMENT

The data in this article is available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JV and PS planned and organized the study. JV, PS, and EN curated the literature data. JV and CG elaborated the data, ran the statistical analysis, and arranged the tables and figures. JV, PS, and CG wrote the first draft of the manuscript. SP critically revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was financially supported by AgBio-Research at Michigan State University (Project GREEN) and the Michigan Craft Beverage Council.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.621585/full#supplementary-material>

REFERENCES

- Acimovic, D., Tozzini, L., Green, A., Sivilotti, P., and Sabbatini, P. (2016). Identification of a defoliation severity threshold for changing fruitset, bunch morphology and fruit composition in Pinot Noir. *Aust. J. Grape Wine Res.* 22, 399–408. doi: 10.1111/ajgw.12235
- Alem, H., Rigou, P., Schneider, R., Ojeda, H., and Torregrosa, L. (2019). Impact of agronomic practices on grape aroma composition: a review. *J. Sci. Food Agric.* 99, 975–985. doi: 10.1002/jsfa.9327
- Ariani, P., Regaiolo, A., Lovato, A., Giorgetti, A., Porceddu, A., Camiolo, S., et al. (2016). Genome-wide characterisation and expression profile of the grapevine ATL ubiquitin ligase family reveal biotic and abiotic stress-responsive and development-related members. *Sci. Rep.* 6:38260. doi: 10.1038/srep38260
- Bledsoe, A. M., Kliewer, W. M., and Marios, J. J. (1988). Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon blanc grapevines. *Control* 39, 49–54.
- Coombe, B. G. (1995). Adoption of a system for identifying grapevine growth stages. *Aust. J. Grape Wine Res.* 1, 104–110. doi: 10.1111/j.1755-0238.1995.tb00086.x
- Dai, Z. W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J. P., Bordenave, L., et al. (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. *Am. J. Enol. Vitic.* 62, 413–425. doi: 10.5344/ajev.2011.10116
- De Beer, D., Harbertson, J. F., Kilmartin, P. A., Roginsky, V., Barsukova, T., Adams, D. O., et al. (2004). Phenolics: a comparison of diverse analytical methods. *Am. J. Enol. Vitic.* 55, 389–400.

- English, J. T., Thomas C.S., Marois, J. J., and W.D., G. (1989). Microclimates of grapevine canopies associated with leaf removal and control of Botrytis bunch rot. *Am. Phytopathol. Soc.* 79, 395–401. doi: 10.1094/Phyto-79-395
- Frioni, T., Acimovic, D., Tombesi, S., Sivilotti, P., Palliotti, A., Poni, S., et al. (2018). Changes in within-shoot carbon partitioning in pinot noir grapevines subjected to early basal leaf removal. *Front. Plant Sci.* 9:1122. doi: 10.3389/fpls.2018.01122
- Frioni, T., Acimovic, D., VanderWeide, J., Tombesi, S., Palliotti, A., Gatti, M., et al. (2019). Whole-canopy source-sink balance at bloom dictates fruit set in cv. Pinot noir subjected to early leaf removal. *Am. J. Enol. Vitic.* 4:ajev.2019.19004. doi: 10.5344/ajev.2019.19004
- Gambetta, G. A., Herrera, J. C., Dayer, S., Feng, Q., Hochberg, U., and Castellarin, S. D. (2020). The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. *J. Exp. Bot.* 71, 4658–4676. doi: 10.1093/jxb/era245
- Gubler, W. D., Bettiga, L. J., and Heil, D. (1991). Comparisons of hand and machine leaf removal for the control of Botrytis bunch rot. *Am. J. Enol. Vitic.* 42, 233–236.
- Hed, B., Ngugi, H. K., and Travis, J. W. (2009). Relationship between cluster compactness and bunch rot in Vignoles grapes. *Plant Dis.* 93, 1195–1201. doi: 10.1094/PDIS-93-11-1195
- Intrieri, C., Filippetti, I., Allegro, G., Centinari, M., and Poni, S. (2008). Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 14, 25–32. doi: 10.1111/j.1755-0238.2008.00004.x
- Jones, G. V. (2007). “Climate change: observations, projections, and general implications for viticulture and wine production,” in *Climate and Viticulture Congress*, eds E. Essick, P. Griffin, B. Keefer, S. Miller, and K. Storchmann (Zaragoza: Cambridge University Press), 1–6.
- Josse, J., and Hussen, F. (2016). missMDA : a package for handling missing values in multivariate data analysis. *J. Stat. Softw.* 70, 1–31. doi: 10.18637/jss.v070.i01
- Keller, M. (2015). *The Science of the Grapevines Anatomy and Physiology*. Burlington, MA: Academic Press, 377.
- Kliwer, W. M., and Dokoozlian, N. K. (2005). Leaf area crop weight ratios of grapevines influence on fruit composition and wine quality. *Am. J. Enol. Vitic.* 2, 170–181.
- Ky, I., Lorrain, B., Jourdes, M., Pasquier, G., Fermaud, M., Gény, L., et al. (2012). Assessment of grey mould (*Botrytis cinerea*) impact on phenolic and sensory quality of Bordeaux grapes, musts and wines for two consecutive vintages. *Aust. J. Grape Wine Res.* 18, 215–226. doi: 10.1111/j.1755-0238.2012.00191.x
- Larronde, F., Krisa, S., Decendit, A., Chèze, C., Deffieux, G., and Mérillon, J. M. (1998). Regulation of polyphenol production in *Vitis vinifera* cell suspension cultures by sugars. *Plant Cell Rep.* 17, 946–950. doi: 10.1007/s002990050515
- Lebon, G., Duchene, E., Brun, O., Magne, C., and Clement, C. (2004). Flower abscission and inflorescence carbohydrates in sensitive and non-sensitive cultivars of grapevine. *Sex. Plant Reprod.* 17, 71–79. doi: 10.1007/s00497-004-0217-9
- Lecourieux, F., Kappel, C., Lecourieux, D., Serrano, A., Torres, E., Arce-Johnson, P., et al. (2014). An update on sugar transport and signalling in grapevine. *J. Exp. Bot.* 65, 821–832. doi: 10.1093/jxb/ert394
- Lee, J., and Skinkis, P. A. (2013). Oregon “Pinot noir” grape anthocyanin enhancement by early leaf removal. *Food Chem.* 139, 893–901. doi: 10.1016/j.foodchem.2013.02.022
- Liang, N. N., Zhu, B. Q., Han, S., Wang, J. H., Pan, Q. H., Reeves, M. J., et al. (2014). Regional characteristics of anthocyanin and flavonol compounds from grapes of four *Vitis vinifera* varieties in five wine regions of China. *Food Res. Int.* 64, 264–274. doi: 10.1016/j.foodres.2014.06.048
- Marois, J. J., Nelson, J. K., Morrison, J. C., Lile, L. S., and Bledsoe, A. M. (1986). The influence of berry contact within grape clusters on the development of *Botrytis cinerea* and epicuticular wax. *Am. J. Enol. Vitic.* 37, 293–296.
- Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., and Velasco, R. (2006). Metabolite profiling of grape: flavonols and anthocyanins. *J. Agric. Food Chem.* 54, 7692–7702. doi: 10.1021/jf061538c
- Molitor, D., Behr, M., Fischer, S., Hoffmann, L., and Evers, D. (2011). Timing of cluster-zone leaf removal and its impact on canopy morphology, cluster structure and bunch rot susceptibility of grapes. *J. Int. Sci. Vigne Vin* 45, 149–159. doi: 10.20870/oeno-one.2011.45.3.1495
- Mosetti, D., Herrera, J. C., Sabbatini, P., Green, A., Alberti, G., Peterlunger, E., et al. (2016). Impact of leaf removal after berry set on fruit composition and bunch rot in “Sauvignon blanc.” *Vitis J. Grapevine Res.* 55, 57–64. doi: 10.5073/vitis.2016.55.57-64
- Palliotti, A., Gatti, M., and Poni, S. (2011). Early leaf removal to improve vineyard efficiency: gas exchange, source-to-sink balance, and reserve storage responses. *Am. J. Enol. Vitic.* 62, 219–228. doi: 10.5344/ajev.2011.10094
- Parker, A. K., Hofmann, R. W., van Leeuwen, C., McLachlan, A. R. G., and Trought, M. C. T. (2015). Manipulating the leaf area to fruit mass ratio alters the synchrony of total soluble solids accumulation and titratable acidity of grape berries. *Aust. J. Grape Wine Res.* 21, 266–276. doi: 10.1111/ajgw.12132
- Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, M., Tornielli, G. B., and Filippetti, I. (2013). Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. *BMC Plant Biol.* 13:1. doi: 10.1186/1471-2229-13-30
- Pastore, C., Zenoni, S., Tornielli, G. B., Allegro, G., Dal Santo, S., Valentini, G., et al. (2011). Increasing the source/sink ratio in *Vitis vinifera* (cv Sangiovese) induces extensive transcriptome reprogramming and modifies berry ripening. *BMC Genomics* 12:631. doi: 10.1186/1471-2164-12-631
- Poni, S., Bernizzoni, F., Civardi, S., and Libelli, N. (2009). Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red *Vitis vinifera* L. cultivars. *Aust. J. Grape Wine Res.* 15, 185–193. doi: 10.1111/j.1755-0238.2008.00044.x
- Poni, S., Casalini, L., Bernizzoni, F., Civardi, S., and Intrieri, C. (2006). Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *Am. J. Enol. Vitic.* 57, 397–407.
- Poni, S., and Gatti, M. (2017). Affecting yield components and grape composition through manipulations of the source-sink balance. *Acta Hort.* 1188, 21–34. doi: 10.17660/ActaHortic.2017.1188.4
- Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T. T., et al. (2017). Grapevine quality: a multiple choice issue. *Sci. Hortic.* 234, 445–462. doi: 10.1016/j.scienta.2017.12.035
- R Core Team. (2016). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: <https://www.R-project.org/>
- Roby, G., Harbertson, J. F., Adams, D. a., and Matthews, M. a. (2004). Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. *Aust. J. Grape Wine Res.* 10, 100–107. doi: 10.1111/j.1755-0238.2004.tb00012.x
- Sabbatini, P., and Howell, G. (2010). Effects of early defoliation on yield, fruit composition, and harvest season cluster rot complex of grapevines. *HortScience* 45, 1804–1808. doi: 10.21273/HORTSCI.45.12.1804
- Silvestroni, O., Lanari, V., Lattanzi, T., Palliotti, A., Vanderweide, J., and Sabbatini, P. (2018). Canopy management strategies to control yield and grape composition of Montepulciano grapevines. *Aust. J. Grape Wine Res.* 25, 30–42. doi: 10.1111/ajgw.12367
- Sivilotti, P., Falchi, R., Vanderweide, J., Sabbatini, P., Bubola, M., Vanzo, A., et al. (2020). Yield reduction through cluster or selective berry thinning similarly modulates anthocyanins and proanthocyanidins composition in Refosco dal peduncolo rosso (*Vitis vinifera* L.) grapes. *Sci. Hortic.* 264:109166. doi: 10.1016/j.scienta.2019.109166
- Sivilotti, P., Herrera, J. C., Lisjak, K., Baša Cesnik, H., Sabbatini, P., Peterlunger, E., et al. (2016). Impact of leaf removal, applied before and after flowering, on anthocyanin, tannin, and methoxypprazine concentrations in ‘Merlot’ (*Vitis vinifera* L.) grapes and wines. *J. Agric. Food Chem.* 64, 4487–4496. doi: 10.1021/acs.jafc.6b01013
- Smart, R. E. (1985). Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review. *Am. J. Enol. Vitic.* 36, 230–239.
- Talaverano, M. I., Moreno, D., Rodríguez-Pulido, F. J., Valdés, M. E., Gamero, E., Jara-Palacios, M. J., et al. (2016). Effect of early leaf removal on *Vitis vinifera* L. cv. Tempranillo seeds during ripening based on chemical and image analysis. *Sci. Hortic.* 209, 148–155. doi: 10.1016/j.scienta.2016.06.013
- Tardaguila, J., Blanco, J. A., Poni, S., and Diago, M. P. (2012). Mechanical yield regulation in winegrapes: comparison of early defoliation and crop thinning. *Aust. J. Grape Wine Res.* 18, 344–352. doi: 10.1111/j.1755-0238.2012.00197.x
- Tardaguila, J., de Toda, F. M., Poni, S., and Diago, M. P. (2010). Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. *Am. J. Enol. Vitic.* 61, 372–381.

- Tello, J., and Ibáñez, J. (2018). What do we know about grapevine bunch compactness? A state-of-the-art review. *Aust. J. Grape Wine Res.* 24, 6–23. doi: 10.1111/ajgw.12310
- Van Leeuwen, C. (2010). “Terroir: the effect of the physical environment on vine growth, grape ripening and wine sensory attributes,” in *Managing Wine Quality: Viticulture and Wine Quality*, ed A. Reynolds (Oxford: Woodhead Publishing Ltd.) 273–315. doi: 10.1533/9781845699284.3.273
- VanderWeide, J., Frioni, T., Ma, Z., Stoll, M., Poni, S., and Sabbatini, P. (2020). Early leaf removal as a strategy to improve ripening and lower cluster rot in cool climate (*Vitis vinifera* L.) Pinot grigio. *Am. J. Enol. Vitic.* 71, 70–79. doi: 10.5344/ajev.2019.19042
- VanderWeide, J., Medina-Meza, I. G., Frioni, T., Sivilotti, P., Falchi, R., and Sabbatini, P. (2018). Enhancement of fruit technological maturity and alteration of the flavonoid metabolomic profile in Merlot (*Vitis vinifera* L.) by early mechanical leaf removal. *J. Agric. Food Chem.* 66, 9839–9849. doi: 10.1021/acs.jafc.8b02709
- Verdenal, T., Zufferey, V., Dienes-Nagy, A., Bourdin, G., Gindro, K., Viret, O., et al. (2019). Timing and intensity of grapevine defoliation: an extensive overview on five cultivars in Switzerland. *Am. J. Enol. Vitic.* 70, 427–434. doi: 10.5344/ajev.2019.19002
- Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., Kerridge, G. H., Rühl, E. H., and Nicholas, P. R. (2005). Shiraz berry size in relation to seed number and implications for juice and wine composition. *Aust. J. Grape Wine Res.* 11, 2–8. doi: 10.1111/j.1755-0238.2005.tb00273.x
- Wang, Y., He, L., Pan, Q., Duan, C., and Wang, J. (2018). Effects of basal defoliation on wine aromas: a meta-analysis. *Molecules* 23:779. doi: 10.3390/molecules23040779
- Xi, X. J., Zha, Q., Jiang, A. L., and Tian, Y. H. (2018). Stimulatory effect of bunch thinning on sugar accumulation and anthocyanin biosynthesis in Shenhua grape berry (*Vitis vinifera* × *V. labrusca*). *Aust. J. Grape Wine Res.* 24, 158–165. doi: 10.1111/ajgw.12323
- Zenoni, S., Dal Santo, S., Tornielli, G. B., D’Inca, E., Filippetti, I., Pastore, C., et al. (2017). Transcriptional responses to pre-flowering leaf defoliation in grapevine berry from different growing sites, years, and genotypes. *Front. Plant Sci.* 8:630. doi: 10.3389/fpls.2017.00630
- Ziegler, M., Wegmann-Herr, P., Schmarr, H. G., Gök, R., Winterhalter, P., and Fischer, U. (2020). Impact of rootstock, clonal selection, and berry size of *Vitis vinifera* sp. riesling on the formation of TDN, vitispiranes, and other volatile compounds. *J. Agric. Food Chem.* 68, 3834–3849. doi: 10.1021/acs.jafc.0c00049
- Zoecklein, B. W., Fugelsang, K. C., Gump, B. H., and Nury, F. S. (1995). *Volatile Acidity*. New York, NY: Chapman and Hall, 192–198.
- Zoecklein, B. W., Williams, J. M., and Duncan, S. E. (2000). Effect of sour rot on the composition of white riesling (*Vitis vinifera* L.) grapes. *Small Fruits Rev.* 1, 63–77. doi: 10.1300/J301v01n01_08

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 VanderWeide, Gottschalk, Schultze, Nasrollahiazar, Poni and Sabbatini. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Georgian Grapevine Cultivars: Ancient Biodiversity for Future Viticulture

Maryam Sargolzaei¹, Laura Rustioni², Gabriele Cola¹, Valentina Ricciardi¹, Piero A. Bianco¹, David Maghradze^{3,4}, Osvaldo Failla¹, Fabio Quaglino¹, Silvia L. Toffolatti^{1*} and Gabriella De Lorenzis^{1*}

OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Goran Zdunic,
Institute for Adriatic Crops and Karst
Reclamation, Croatia
Annarita Marrano,
University of California, Davis,
United States
Javier Tello,
Institute of Vine and Wine Sciences
(ICVV), Spain

*Correspondence:

Silvia L. Toffolatti
silvia.toffolatti@unimi.it
Gabriella De Lorenzis
gabriella.delorenzis@unimi.it

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 16 November 2020

Accepted: 13 January 2021

Published: 05 February 2021

Citation:

Sargolzaei M, Rustioni L, Cola G, Ricciardi V, Bianco PA, Maghradze D, Failla O, Quaglino F, Toffolatti SL and De Lorenzis G (2021) Georgian Grapevine Cultivars: Ancient Biodiversity for Future Viticulture. *Front. Plant Sci.* 12:630122. doi: 10.3389/fpls.2021.630122

¹ Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano, Milan, Italy, ² Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento – Centro Ecotekne, Lecce, Italy, ³ Faculty of Viticulture and Winemaking, Caucasus International University, Tbilisi, Georgia, ⁴ National Wine Agency of Georgia, Tbilisi, Georgia

Grapevine (*Vitis vinifera*) is one of the most widely cultivated plant species of agricultural interest, and is extensively appreciated for its fruits and the wines made from its fruits. Considering the high socio-economic impact of the wine sector all over the world, in recent years, there has been an increase in work aiming to investigate the biodiversity of grapevine germplasm available for breeding programs. Various studies have shed light on the genetic diversity characterizing the germplasm from the cradle of *V. vinifera* domestication in Georgia (South Caucasus). Georgian germplasm is placed in a distinct cluster from the European one and possesses a rich diversity for many different traits, including eno-carpological and phenological traits; resistance to pathogens, such as oomycetes and phytoplasmas; resistance to abiotic stresses, such as sunburn. The aim of this review is to assess the potential of Georgian cultivars as a source of useful traits for breeding programs. The unique genetic and phenotypic aspects of Georgian germplasm were unraveled, to better understand the diversity and quality of the genetic resources available to viticulturists, as valuable resources for the coming climate change scenario.

Keywords: *Vitis vinifera* L., genetic diversity, phenotypical characterization, resistance to diseases, climate change

GRAPEVINE: A HIGH SOCIO-ECONOMIC IMPACT CROP STRONGLY THREATENED BY CLIMATE CHANGE

The genus *Vitis* is present in 10 distribution areas, all in the northern hemisphere: five in North America, where 29 species have been described; four in Asia, with at least 11 species; and only one, *Vitis vinifera*, in a wide range that includes the Mediterranean, sub-Mediterranean, and Caucasian floristic regions with a spread toward the Pontic, Caspian, and Central Asiatic areas (Mullins et al., 1992). *V. vinifera* is one of the most widely cultivated plant species of agricultural interest and

the only species extensively used in the global wine industry, covering approximately 7.4 Mha in 2018, and producing more than 77.8 mt of grapes (wine, table and dried grapes) and a world wine trade worth around EUR 32 billion¹. Regions of its cultivation are located roughly between the 35th and 55th northern parallels and between the 25th and 35th southern parallels, in areas with average annual temperatures between 10 and 20°C. These environments are characterized by the alternation of a favorable growing season and an unfavorable cold one. However, the cold winters are not too intense (minimum temperatures range between −10 and 15°C) and the favorable season (average temperature higher than 10°C) is long enough (>200 days) for grapes to ripen (Gladstones, 1992).

Viticulture depends on environmental resources (i.e., climate and soil conditions) in terms of yields and quality (van Leeuwen and Darriet, 2016). The current climatic phase, characterized by the increase of average global temperature, has led to changes in the environmental conditions of agricultural areas that need to be tackled with suitable tools, in a context of adaptation and mitigation. Due to the socio-economic impact of the wine sector in Europe and around the world, over recent years, there has been an increase in work aiming to study the impact of climate change on viticulture (Hannah et al., 2013; Morales-Castilla et al., 2020).

Santos et al. (2020) proposed a list of measures to be adopted in viticulture to face with the climate change. The list divided the measures in two categories: the short-term adaptation strategies and the long-term adaptation strategies. The short-term strategies include crop cultural practices and techniques to delay ripening time, plant protection against extreme heat, irrigation, pest and disease control and soil management. Among the long-term strategies, there are: change in training systems, varietal/clonal and rootstock selection and vineyard relocation. Breeding programs for new varieties which will be better able to perform in the environmental conditions expected in the future could be one of the most promising solutions, although this strategy is included in the long-term ones. An appropriate cultivar selection reduces the inputs required for plant management, increasing the sustainability of production. Great sources of biodiversity in the *V. vinifera* species have been recently found in its domestication cradle, located in Georgia (South Caucasus) (Imazio et al., 2013) (Figure 1).

Georgia counts 48,000 hectares of vineyards and a production of wine and table grapes of 159,000 and 8,000 tons, respectively (see text footnote 1). In 2015, about 100 M liters of wine were produced, 80% of them obtained by white and 20% from red berry grapes. More than 90% of the 2015 production was supported by Kakheti region, producing mainly white and red wines from Rkatsiteli and Saperavi grapes, in the ratio 7:3.

The aim of this review is to assess the potential of Georgian cultivars as sources of useful traits for new breeding programs, aiming to face the future challenges that await viticulture worldwide. To do this, we reviewed the particular genetic and phenotypic aspects (such as berry traits and resistance to pathogens) of Georgian germplasm, in the hope of better understanding the diversity and quality of the genetic resources

available to viticulturists, coming directly from the origin of domestication.

SOUTH CAUCASUS, THE FIRST GRAPEVINE DOMESTICATION CENTER

Vitis vinifera is indigenous to Eurasia and it is suggested that the ancestors of the first *Vitis* genus appeared about 65 million years ago (Olmo et al., 1995). Nowadays, *V. vinifera* species includes both cultivated (*V. vinifera* subsp. *sativa*) and wild (*V. vinifera* subsp. *silvestris*) subspecies, the latter considered the progenitor of subspecies *sativa* (This et al., 2006). Its domestication process seems to be strongly linked to the alcoholic and gustative superiority of its fermented juice (the wine) in comparison to that of other fleshy fruits (fruit wines), although it is not well known which process predated the other (Terral et al., 2010). The main changes driving grapevine domestication were identified in the flower morphology (appearance of hermaphrodite flowers), larger berry size, higher berry sugar content, a wide range of berry color and aromatic content, characters which ensure yield, quality and a greater sugar content for a better fermentation (This et al., 2006). The major questions about grapevine domestication process are related to the number of events occurred, single event *versus* multiple events, and the geographical location where these events took place. For a vine domestication center to be born, different conditions need to occur. Among these, there is a strong awareness in practicing and developing viticulture by entire peasant villages. To bring out such a situation, many factors have to converge: territories with a (relatively) high population density, with stable settlements and in positions at crossroads of trade flows and cultural trends (Forni, 2012). It would be reasonable to expect that such situation could have occurred in several areas, differing in chronology and level of development. The most accredited hypothesis suggests that *V. vinifera* was domesticated from its wild form in the South Caucasus, between the Caspian and Black Seas, around 6,000–5,800 BC, and then spread throughout Europe and Mediterranean areas thanks to the spread of civilizations (McGovern et al., 2017). Recently, Zhou et al. (2019), proposing a four-state domestication process for grapevine, date the beginning of this process around 20,000 years ago, when South Caucasian human populations started to manage and harvest the local wild populations (Stage 1). In the same region around 8,000 years ago, the humans started with the conscious or unconscious selection of desirable phenotypical traits (Stage 2), although this transition is not well documented yet. Another force driving the transition from Stage 1 to Stage 2 is the bottleneck. Nevertheless, genetic evidences showed that grapevine did not experience a severe bottleneck (Myles et al., 2011; Zhou et al., 2017), making the conscious or unconscious selection as a unique force shaping the genetic diversity of grapevine during the domestication process. Stage 3 consists on spreading of newborn crop in new locations and the consequence of local domestication or introgression events. Reviewing the most comprehensive studies on grapevine genetic population, it turned out that an East-to-West grapevine gene flow after the first domestication process occurred, with some evidence

¹<http://www.oiv.int/>

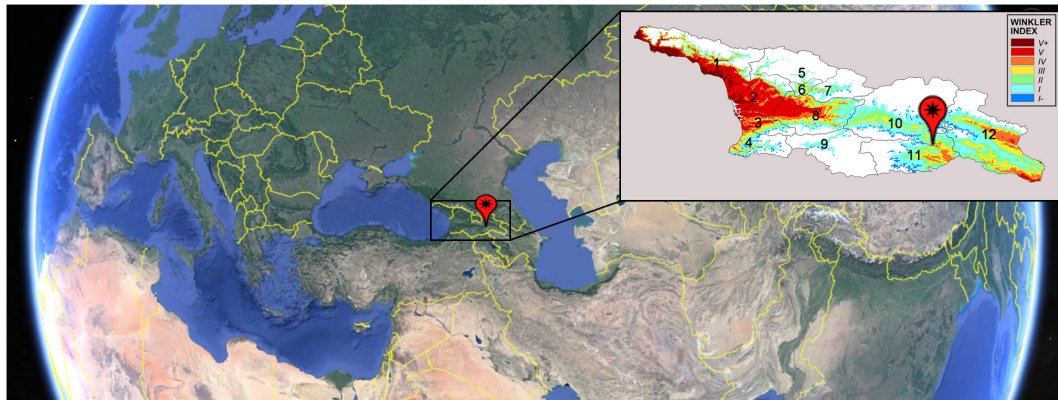


FIGURE 1 | Map of Georgia and location of 12 Georgian wine-growing regions: 1 – Abkhazeti; 2 – Samegrelo; 3 – Guria; 4 – Adjara; 5 – Svaneti; 6 – Lechkhumi; 7 – Racha; 8 – Imereti; 9 – South Kartli; 10 – Inner Kartli; 11 – Lower Kartli; 12 – Kakheti. Image is obtained by Google Earth. Pin indicates Tbilisi position. Map on the right reports the Winkler classification based on yearly average Winkler index calculated for the period 1994–2013 in Georgia (Caucasus). The analysis is limited to the areas below 1250 m above sea level. Description of Winkler indices: (I–) GDD (Growth Degree Days) < 850, viticultural climate is very cool, vinicultural aptitude is very early ripening grapes for fresh and fruity wines or sparkling wine bases. (II) GDD 850–1400, viticultural climate is cool, vinicultural aptitude is early ripening grapes for fresh and fruity wines or sparkling wine bases. (III) GDD 1400–1650, viticultural climate is temperate cool, vinicultural aptitude is early ripening grapes for wines to be aged. Medium ripening grapes for white or red wines ready to drink. (IV) GDD 1650–1950, viticultural climate is temperate, vinicultural aptitude is medium ripening grapes for white or red wines ready to be aged. (V) GDD 1950–2200, viticultural climate is temperate warm, vinicultural aptitude is late ripening grapes for white or red wines ready to be aged. (VI) GDD 2200–2700, viticultural climate is hot, vinicultural aptitude is late ripening grapes for bodied red wines to be aged. (V+) GDD > 2700, viticultural climate is very hot; vinicultural aptitude is very late ripening grapes for bodied red wines to be aged.

of putative secondary domestication centers along the main migration routes due to genetic relationships between wild and cultivated accessions, especially in the Mediterranean Basin and Central Asia (Grassi et al., 2003; Arroyo-García et al., 2006; Myles et al., 2011; Zhou et al., 2017; Riaz et al., 2018). The coexistence of wild populations together with domesticated ones is often and the bidirectional gene flow (wild-to-cultivated and cultivated-to-wild) has been well documented (De Andrés et al., 2012; Ekhvaia et al., 2014; Riaz et al., 2018; D’Onofrio, 2020; Maraš et al., 2020), supporting the occurrence of secondary domestication events from local wild populations or introgression events. These events, the geographical origin and human usage were found to strongly shape the genetic structure of grapevine germplasm (Bacilieri et al., 2013). Another aspect, although less investigated, is the role of wild *Vitis* species in the *sativa* domestication process. It seems that wild *Vitis* species have contributed to the current structure of grapevine germplasm (Zhou et al., 2019). The last stage proposed by Zhou et al. (2017) (Stage 4) takes into account the modern breeding programs, a relative recent event occurred over the last few hundred years and led to the birth of so-called anthropic crossings, with the aim of satisfying specific requirements.

GEORGIAN TERRITORY, CLIMATE, AND GRAPEVINE PRODUCTION

Georgia is a large basin of the mid latitudes, bordered by the Greater Caucasus in the North and the Lesser Caucasus in the South, and opening toward the Black Sea in the West and toward the Caspian depression in the East (Figure 1). Those geographical features strongly characterize the climate of its 12 wine-growing regions that, following the Köppen – Geiger

classification (Köppen and Geiger, 1936), are characterized by profoundly different climatic conditions, ranging from hot summer continental climates to warm summer continental or hemiboreal climates, that translate into different classes of the Winkler index (Figure 1).

In relation to the climatic conditions of each wine-growing region, the Georgian varietal assortment is strongly differentiated as well, being adapted to a very wide range of cold and summer stresses (Table 1). Worldwide wine-growing regions experiencing the same climatic conditions of Georgia may provide benefit by this so differentiated varietal spectrum.

It is interesting to highlight that, in 1994 Georgia faced an abrupt rise in temperatures, similarly to what happened in Western Europe in the late 1980s (Reid et al., 2016), with 1987 as the most likely year of change (Mariani et al., 2012). This delay could be explained by the progressive dilution of the Atlantic circulation signal as it moves into the European continent (Cola et al., 2017). The increase of temperature determined an advance in grapevine phenology, which was more significant at the higher altitudes, where more favorable thermal conditions were established. On the other hand, at lower altitudes the phenological advance was partially depleted by the increase of super-optimal thermal conditions (increasing the occurrence of stress conditions during ripening). For instance, in the case of the widely diffused cultivar Rkatsiteli, the average advance of veraison was less than 6 days for the 250–500 m asl elevation belt and around 18 days for the 750–1000 m one (Cola et al., 2017).

In parallel, it is worth noticing the high variability in the plant phenology among Georgian cultivars, both in the sprouting date and in the ripening period. A delayed budburst period could represent an avoidance mechanism against spring frosts. Considering Georgian cultivars, bud swelling of ‘Partala’

TABLE 1 | Georgian grape ranges in comparisons to the main abiotic stress.

Region	Risk of				Recommended varieties	
	Summer light-thermal stress ¹	Summer water stress ²	Winter frost ³	Spring frost ⁴	Colored varieties ⁵	White varieties ⁵
Abkhazeti	Very high	Very low – low	Very low	Very low – high	Amlakhu N, Kachichi N, Absuaj N, Lakoaj N, Ojaleshi N, Chkhaveri N, Amlakhu N	Avasikhva B, Aghbizh B, Akabuli B, Khapshira B, Khunaliji B, Tsolikouri B, Krakhuna B
Samegrelo	Very high	Very low – low	Low – very low	Low – high	Ojaleshi N, Chvitiluri N	Chechipeshi B
Guria	Medium	Very low	Low	Very low – high	Chkhaveri N, Jani N, Mtevandidi N, Skhilatubani N	Sakmiela B
Adjara	Very low – low	Very low – low	Low – very low	Very low – low	Mekrenchkhi N, Burdzghala N, Jineshi N, Satsuri N, Batomura N	Brola B, Khopaturi B, Klarjuli B, Kviristava B, Shavshura B
Svaneti	Very low (high)	Very low	Very low – high	Very low – high	Alexandrouli N, Mujuretili N, Orbeluri Ojaleshi N, Usakhelouri N, Rachuli Dzelshavi N	Tsulukidzis Tetra B, Tsolikouri B
Lechkhumi	High – very high	Very low – low	Very low	Low		
Racha	Very low – low – (high)	Very low – low	Very low – very high	Very low – very high		
Imereti	High – very high	Very low	Low	Very low – very high	Aladasturi N, Dzelshavi N, Otskhanuri Sapere N, Argvetuli Sapere N, Rko N, Adanasuri N, Ezvanura N, Dondghlabi Shavi N, Vani [or Vanura?] N, Chkhaveri N	Goruli Mtsvane B, Krakhuna B, Tsolikouri B, Tsitska B, Kvishkhuri (sin. Goruli Mtsvane) B, Dondghlabi B, Bazaleturi B, Kundza Tetri B, Tklapa B
South Kartli	Very low – very high	Low – medium	Very low – very high	Very low – very high	Tavkveri N, Asuretili Shavi N, Shavkapito N, Saperavi Budeshuriseburi N, Saperavi N, Dzelshavi N	Chinuri B, Goruli Mtsvane B, Rkatsiteli B, Budeshuri B, Jvari B, Adreuli B, Aragvispiruli B, Grdelmtevana B, Melikuda B, Chrola Kartlis B, Kharistvala B
Inner Kartli	High – very high	Medium	Very low –high	Very low –high		
Lower Kartli	Low – high	Medium	Very low	Very low – low		
Kakheti	Very high	Medium – high	Very low	Low – very low	Saperavi N, Saperavi Budeshuriseburi N, Tavkveri N, Budeshuri Tsiteli N, Ikaltos Tsiteli N	Rkatsiteli B, Kisi B, Mtsvane Kakhuri B, Khikhvi B, Muskaturi Rkatsiteli B, Chinuri B, Mtsvivani Kakhuri B, Sapena B, Kumsi Tetri B

¹ **Summer Stress** (1974–2013): The risk of summer stress is expressed as the percentage of the years of the reference period with at least 7 days with maximum temperature above the 35°C threshold. The classes are: very low (<3%), low (3–5%), medium (5–6.7), high (6.7–10%) and very high (>10%).

² **Water Shortage** (1974–2013) Calculated by means of a daily water balance. Average yearly sum of the stress level of stress days. The classes are: very low (0 day/year), low (0–15 days/year), medium (15–30 days/year), high (30–60 days/year), and very high (>60 days/year).

³ **Winter Frost** (1974–2013): The risk of winter frost is expressed as the percentage of the years of the reference period winter minimum temperature below the –15°C threshold. The classes are: very low (<3%), low (3–5%), medium (5–6.7), high (6.7–10%) and very high (>10%).

⁴ **Spring Frost** (1974–2013): The risk of spring frost is expressed as the percentage of the years of the reference period with spring minimum temperature below the –2°C threshold. The classes are: very low (<3%), low (3–5%), medium (5–6.7), high (6.7–10%), and very high (>10%).

⁵ N, noir; B, Blanc.

vines was recorded at the end of March, and, thus, a higher susceptibility to spring frost is expected when compared to the other cultivars that sprouted in April (Maghradze et al., 2014). Global warming generally resulted in the increase of cases of temperature above the optimal range (24–26°C) during summer and in particular during grape ripening (Cola et al., 2020). A delay in the maturation process, obtained through the selection of late-ripening cultivars, could ensure thermal conditions during ripening more suitable for berry metabolism. Maghradze et al. (2012) studied the phenology of Georgian

cultivars in northern Italy in comparison to Chardonnay and Cabernet Sauvignon grown in the same area, and they found a relatively late ripening with respect to the reference varieties: nevertheless, a very wide range of variability was observed. Similar results were found in other comparative evaluation carried out in Georgian ampelographic collections and are reported by Maghradze et al. (2014) and Rustioni et al. (2014). Some extreme cases are: early ripening cultivars – Kartuli Saadreo, Meskhuri Mtsvane, Buza, Budeshuri Tsiteli and Daisi; late ripening cultivars – Ojaleshi, Akomshtali, Kamuri,

Shavi, Tavkara, Khushia Shavi, Satsuravi, Maghlari Tvrina, Mtevandidi, Argvetula, Dziganidzis Shavi, Adanasuri, Mamukas Vazi, Otskhanuri Sapere, Gorula, Saperavi Meskhuri, Ghrubela and Shavtita. The same results were obtained when comparing the phenological timing of Georgian varieties internationally grown. The phenological model developed by Mariani et al. (2013) for Cabernet Sauvignon and Chardonnay and adapted to the Georgian varieties Saperavi, Rkatsiteli, Mtsvane Kakhuri (Cola et al., 2017) was applied to a long time series of daily temperature (Perugia–Italy. 1990–2019). **Figure 2** shows the late phenological timing of Georgian varieties (average values are shown).

THE AMPELOGRAPHIC COLLECTIONS OF GEORGIAN GERmplasm

To date, fifty grapevine varieties are recommended for cultivation in Georgia. Most of them (37) are wine grape cultivars, while the others (13) are generally used to produce grapes for fresh consumption. Predominantly, the Georgian vineyards are cultivated with autochthonous varieties; among the recommended wine grapes, 31 are local varieties and seven are international cultivars, while, considering the table grapes, nine of them have a local origin: four are traditional, autochthonous, Georgian cultivars, five are from local breeding outputs, and five are allochthonous varieties (Chkhartishvili and Maghradze, 2012). Beside the recommended autochthonous varieties, other cultivars enlarge the intraspecific biodiversity preserved in Georgia: Tsertsvadze (2012) described 48 grapevine native cultivars in the ‘Caucasus and Northern Black Sea Region Ampelography’ and further studies are in progress to continuously increase the number of recognized Georgian

cultivars preserving this important source of biodiversity. Based on information available, more than 700 Georgian accessions can be counted (**Supplementary Table 1**), most of them are germplasm accessions and the rest are classified as major (20) and minor (8) cultivars. These accessions are available in nine Georgian collections (**Table 2**) and other collections hold by foreign Institutions, such as Italy (443 accessions), Ukraine (309 accessions), Russia (191 accessions), Moldova (122 accessions), Uzbekistan (32 accessions), France (20 accessions), and Slovakia (7 accessions). Although the number of accessions is high, only a limited number of them were genotyped and phenotyped (see sections “Georgian Germplasm as a Source of Genetic Variability” and “The Phenotypical Characterization of Georgian Germplasm Collections”). This limited number of information makes a not so easy determination of the exact number of autochthonous Georgian varieties. Further efforts are needed to better understand the genetic diversity of this valuable germplasm and to identify synonyms, homonyms and misidentifications.

Georgian Germplasm as a Source of Genetic Variability

Historical information coupled with archeological and palaeobotanical findings pointed to Georgia as a cradle for grapevine domestication (Zohary and Hopf, 2000; McGovern, 2003; McGovern et al., 2017). Molecular analysis produced the same evidence. Genetic diversity of Georgian germplasm was investigated, by both nuclear SSR (simple sequence repeat) (Lauco et al., 2011; Imazio et al., 2013; Ekhvaia et al., 2014) and SNPs (single nucleotide polymorphisms) (De Lorenzis et al., 2015; Lauco et al., 2018) molecular markers, although a number of autochthonous cultivars, collected in local ampelographic collections, still remain to be studied (**Supplementary Table 1**).

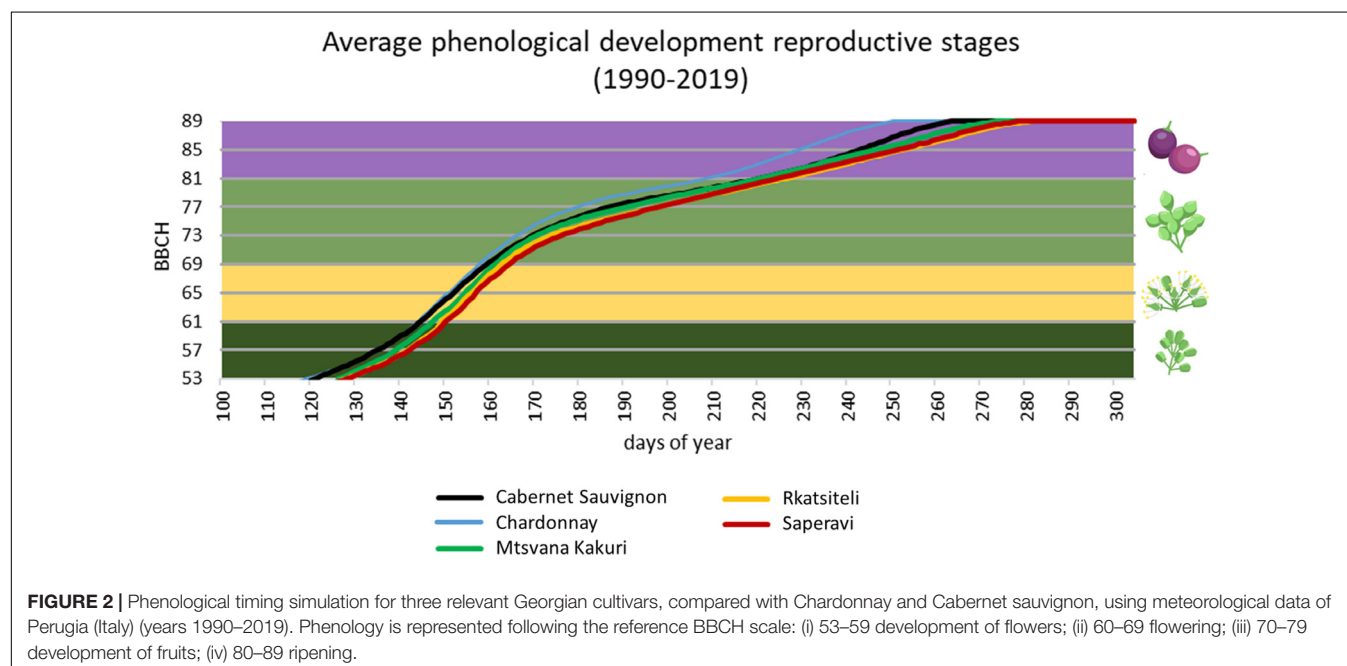


TABLE 2 | List of grapevine germplasm collections in Georgia.

Collection	Year of plantation	Total accessions	Old varieties	Foreign varieties	<i>Vitis vinifera</i> accessions	Rootstocks/non- <i>vinifera</i> species
Jighaura	2008	932	425	500	925	7
Mukhrani	2014	280	275	5	280	0
Skra 2	2008	330	330	0	330	0
Vachebi	2008	219	212	0	212	7
Telavi 2	2008	173	168	5	173	0
Shumi	2006	271	179	92	271	0
Kindzmarauli	2005	400	400	0	400	0
Telavi 1	1987	141	141	0	141	0
Skra 1	1972	75	38	37	75	0
Total		2821	2148	639	2807	14

Thanks to two European research programs, GrapeGen06 (2007–2010) (Laucou et al., 2011), first, and then COST Action FA1003 (2011–2014) (Failla, 2015), a strong and still active network of scientific collaborations has been developed between European and Georgian researchers, to genetically characterize and preserve the Georgian genetic resources of vines.

All the outcomes about the genetic characterization of Georgian germplasm reported the uniqueness and originality of this germplasm when compared to the European and Central Asian germplasm (Myles et al., 2011; Bacilieri et al., 2013; Imazio et al., 2013; Riaz et al., 2018; De Lorenzis et al., 2019). The Georgian cultivars showed the distinctive features of a domestication center, such as high levels of genetic diversity and heterozygosity, the presence of alleles absent or poorly represented in other countries, and differentiation from the European varieties, clustering in a well-separated branch (as reported in the **Figure 3**, where SSR and SNP genetic profiles of varieties from France, Georgia, Italy and Spain were re-elaborated to perform a discriminant analysis of principal component, using data published in De Lorenzis et al., 2015, 2019, Laucou et al., 2018, and Riaz et al., 2018). A differentiation inside the germplasm, based on the geographical origin of cultivars, was identified as well: the varieties putatively originated in Kartli and Kakheti (Eastern regions) differ from the ones originating in Abkhazeti, Samegrelo, Guria, Adjara, Imereti, Racha, and Lechkhumi (Western regions). The origin of this subdivision lies in the geographical subdivision of Georgia into two major parts, due to the Likhi Mountains running in a North-to-South direction across Georgia (Imazio et al., 2013; De Lorenzis et al., 2015), confirming that, despite long-standing cultivation, the Georgian cultivars maintain their originality.

Genetic variation provides the foundation for any breeding programs, and natural genetic diversity represented historically the major source of variability for crop improvement and adaptation to changing environmental conditions. Given the uniqueness of Georgian germplasm, its strong link with the regions of origin coupled with the evidence of this country being the center of domestication makes this germplasm very attractive for investigation from the perspectives of phenology, grape phenotype and resistance to biotic and abiotic stresses, as sources of new variability for future breeding programs.

The Phenotypical Characterization of Georgian Germplasm Collections

The collaboration among European and Asian researchers makes feasible the comparisons among ampelographic collections using common protocols. Among them, phenotyping was considered in the framework of the COST Action FA1003 (Rustioni et al., 2014), allowing the description of numerous autochthonous cultivars (Abashidze et al., 2015; Cornea and Savin, 2015; Goryslavets et al., 2015; Maghradze et al., 2015; Ujmajuridze and Mamasakhlisashvili, 2015). This work, finally, produced a general overview of the *V. vinifera* variability concerning eno-carpological traits (Rustioni et al., 2019). **Table 3** reports the distribution of the Georgian records with respect to the variability described for the *V. vinifera* species (data obtained by the reworking of the results published in Rustioni et al., 2014, 2019, and Abashidze et al., 2015). To emphasize some results, showing the differences among the two groups of data, **Figure 4** shows the frequency distribution of the Georgian records in comparison with the data collected for the entire *V. vinifera* species, concerning some specific traits (titratable acidity, percentage of skin, skin phenolic content). Briefly, the main differences highlighted in **Table 3** in terms of oenological applications are that Georgian grapes have, with respect to the *V. vinifera* species population, higher concentrations in both sugars and acids and thicker skins, ensuring acceptable amounts of phenolics despite the lower accumulation per unit of tissue. Details concerning the results of this comparison are discussed in Sections “Fruit Morphology and Technological Quality of Georgian Cultivars” and “Abiotic Stress Adaptations and Secondary Metabolisms.” It is worth noting that the phenotypic variability reported is due to the genotype, to the environmental growing conditions, and to their interactions. Thus, further studies will be necessary to discriminate these effects, highlighting the role of genotypes.

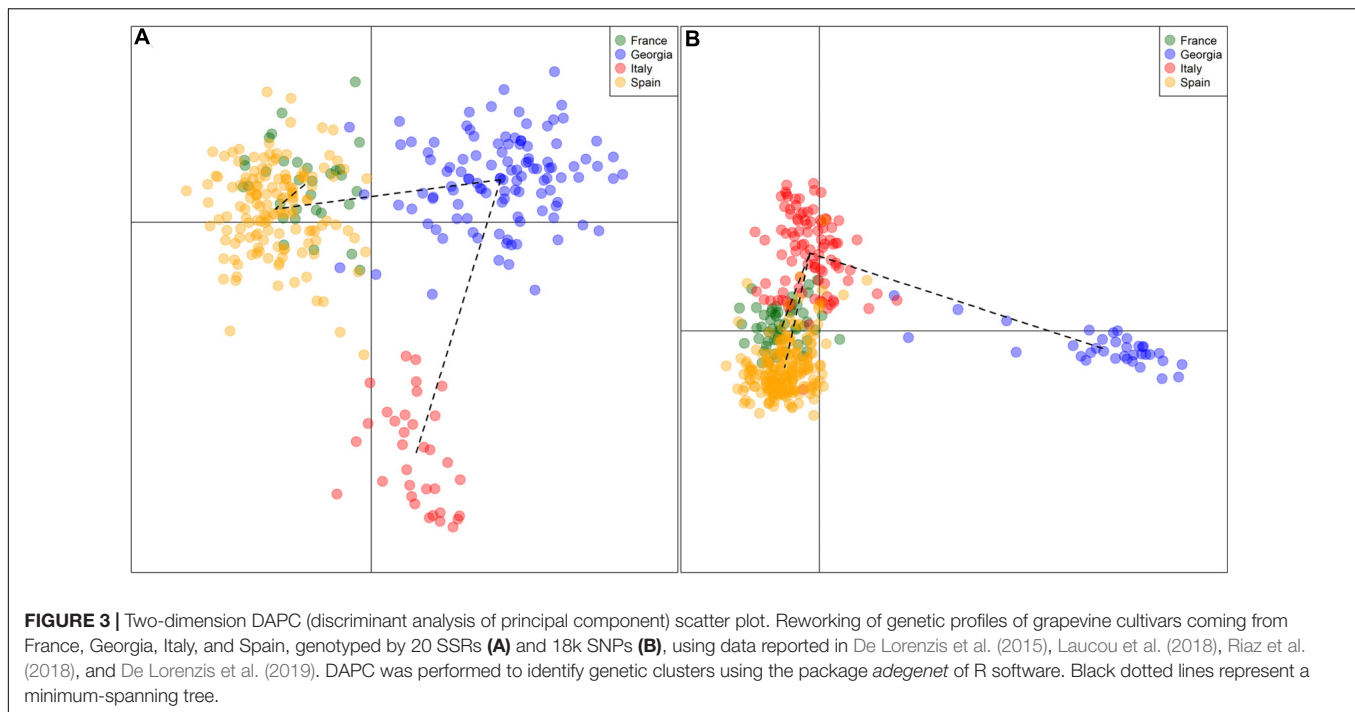
Fruit Morphology and Technological Quality of Georgian Cultivars

Despite the wide variability for berry shapes within the species, Georgian grapes generally have round or slightly elongated small berries (**Table 3**) (OIV, descriptor 223). This is probably due to the ancient traditions that, during millennia of winemaking activities

TABLE 3 | Physical dimensions and chemical components distribution of Georgian grapevine germplasm with respect to the variability described for the entire *V. vinifera* species (reworking of the results published in Rustioni et al., 2014, 2019 and Abashidze et al., 2015).

Variable	Sample number		Average			Minimum		Maximum		Quartiles					
	Georgia	Species	Georgia	Species	Significance of the difference ¹	Georgia	Species	Georgia	Species	25		50 – median		75	
										Georgia	Species	Georgia	Species	Georgia	Species
Berry length (mm)	303	22383	14.16	15.02	0.000	9.3	5	19	37	12.9	13	14	15	15.5	17
Berry width (mm)	303	22385	13.03	14.18	0.000	9	6	17.5	29	11.6	12	13	14	14.3	16
Length/width	303	22383	1.10	1.06	0.000	0.93	0.50	1.39	3.60	1.04	1.00	1.08	1.00	1.14	1.10
Bunch weight (g)	261	5737	184.46	247.76	0.000	26	10	641	1362	109	143	167	220	229	319
Sugar content (Brix)	336	2162	21.51	20.8	0.073	12	10.0	28	35.0	20	19.0	21	21.0	24	23.0
Titrateable acidity (g/l tartaric acid)	336	2161	7.01	6.3	0.005	3.5	0.8	13.2	22.7	6	4.7	6.8	6.0	7.8	7.4
Berry weight (g)	336	2404	2.20	2.4	0.037	0.8	0.6	4	10.1	1.7	1.6	2.2	2.2	2.6	2.8
% Skin (w/w)	334	2368	29.63	17.0	0.000	7	3.0	54	54.0	24	11.0	29	15.0	34	21.0
% Seed (w/w)	336	2355	4.24	4.0	0.047	2	0.0	10	17.0	3	3.0	4	4.0	5	5.0
Weight of 1 skin (g)	334	2369	0.64	0.4	0.000	0.1	0.1	1.3	1.9	0.5	0.2	0.6	0.3	0.8	0.5
Number of seeds/berry	336	2321	2.04	2.1	0.047	0.9	0.0	3.8	4.3	1.7	1.7	2	2.1	2.4	2.5
Weight of 1 seed (mg)	336	2293	44.17	41.0	0.000	20	10.0	90	160.0	40	30.0	40	40.0	50	50.0
Anthocyanins (mg/kg of grapes)	206	1141	756.55	710.1	0.699	50	50.0	3350	5350.0	350	200	600	550	950	1000
Anthocyanins (mg/berry)	204	1138	1.45	1.4	0.153	0.1	0.1	5	8.5	0.7	0.5	1.3	1.0	2	1.9
Anthocyanins (mg/g of skin)	204	1138	2.77	4.7	0.000	0.1	0.1	9.8	45.0	1.125	1.5	2.35	3.2	3.7	6.1
Skin phenolic (mg/kg of grapes)	336	1739	1182.23	1375.8	0.002	200	90.0	3780	6590.0	720	680	1030	1090	1590	1800
Skin phenolic (mg/berry)	336	1739	2.45	2.8	0.313	0.2	0.2	6.2	12.0	1.5	1.6	2.3	2.4	3.275	3.6
Skin phenolic (mg/g of skin)	334	1735	4.34	9.1	0.000	0.5	0.3	30.6	61.4	2.3	4.5	3.7	7.3	6	11.9
Seed phenolic (mg/kg of grapes)	335	1724	177.70	337.0	0.000	10	10.0	1050	4180.0	60	100	120	210	260	430
Seed phenolic (mg/berry)	316	1692	0.40	0.7	0.000	0.1	0.1	2.3	5.4	0.1	0.2	0.3	0.5	0.6	0.9
Seed phenolic (mg/g of seed)	324	1704	4.46	8.7	0.000	1	1.0	28	98.0	2	3.0	3	6.0	7	11.0
Seed phenolic (μg/seed)	335	1723	190.87	338.4	0.000	10	10.0	1350	5390	60	110	130	220	290	440
Skin phenolics (%)	335	1734	86.28	79.9	0.000	30	22.0	99	100.0	81	70.0	89	84.0	95	92.0
Seed phenolics (%)	335	1734	13.72	20.1	0.000	1	0.0	70	78.0	5	8.0	11	16.0	19	30.0
Total phenolics (mg/kg of grape)	335	1735	1360.60	1708.7	0.000	250	100.0	4200	9550.0	850	900	1200	1450	1750	2200
Total phenolics (mg/berry)	335	1737	2.83	3.4	0.004	0.3	0.3	6.9	12.3	1.9	2.1	2.7	3.0	3.7	4.3

¹ Significance between Georgian and species values has been evaluated by ANOVA performed in SPSS v.25 software.



(Chkhartishvili and Maghradze, 2012; McGovern et al., 2017), favored the selection of wine grapes over table grapes.

Considering technological maturity, Georgian records generally show higher concentrations in both sugars and acids than foreign varieties (Table 3). In the perspective of climate change, the high sugar content could represent a problem, due to the risk of further increases related to the higher temperatures during the anticipated ripening periods (Keller, 2010; Mira de Orduña, 2010; van Leeuwen and Destrac-Irvine, 2017). The increased sugar concentrations expected in hot ripening conditions may cause growth inhibition or lysis in the yeasts responsible for wine fermentation (Mira de Orduña, 2010). Furthermore, high sugar stress could modify the yeast metabolisms, increasing the accumulation of by-products (such as glycerol and acetic acid) that, together with the increased alcoholic content, affects the wine perceptions of consumers and, thus, it could modify the expected characteristics of traditional wines (Mira de Orduña, 2010). However, the deviation of Georgian data with respect to the species variability concerning the sugar content is rather limited (significance of the difference = 0.073), neither covering the maximum records of the one obtained when analyzing wider grapevine genetic pools (Table 3) (Rustioni et al., 2019). Furthermore, the expected sugar content increase is usually ascribed to the earlier ripening anticipated in climate changed conditions (Pallioti et al., 2014; Martínez-Moreno et al., 2019), and it is important to remind the prevalence of late ripening cultivars among the Georgian grapevines (Maghradze et al., 2012). Finally, the sugar content seems to be well counterbalanced by the acidity (Table 3). The distribution of Georgian grapes concerning the titratable acidity, showed a right shift (Figure 4A), demonstrating the ability of these cultivars to keep a high acidic concentration despite the

sweetness of the berries. This is a crucial point for viticulture adaptation to climate change, because high temperatures usually cause a decrease in acids, especially due to malic acid degradation (Keller, 2010; Mira de Orduña, 2010; van Leeuwen and Destrac-Irvine, 2017).

In the perspective of climate change adaptation, it is very interesting to note a particular feature of Georgian grapes concerning the proportions among skin, seeds and pulp, at the expense of the latter (Table 3). The Georgian records shown in Figure 4B, demonstrate an important shift toward thicker skins with respect to the general *V. vinifera* species. This is due to the lighter berries and heavier skins (Table 3). Considering the effect of climate change on the berries, a thicker skin could represent a more resistant barrier against the stressful environment. In fact, it has been shown that a possible adaptation to climate change could be related to berry skin thickening (van Leeuwen and Destrac-Irvine, 2017).

Abiotic Stress Adaptations and Secondary Metabolisms

Grape epicuticular waxes also have important protective roles against dehydration (Pangavhane et al., 1999; Di Matteo et al., 2000; Doymaz, 2006; Muganu et al., 2011) and pathogen infections (Marois et al., 1986; Rosenquist and Morrison, 1988; Percival et al., 1993). Furthermore, a study conducted on Georgian cultivars, suggested a possible eco-physiological role of epicuticular waxes in reducing heating stresses by an interaction with infrared radiation (Rustioni et al., 2012). However, a comparison among Georgian cultivars and grape varieties cultivated in other regions is not available and, thus, we should suppose that this mechanism is not exclusive for Georgian cultivars.

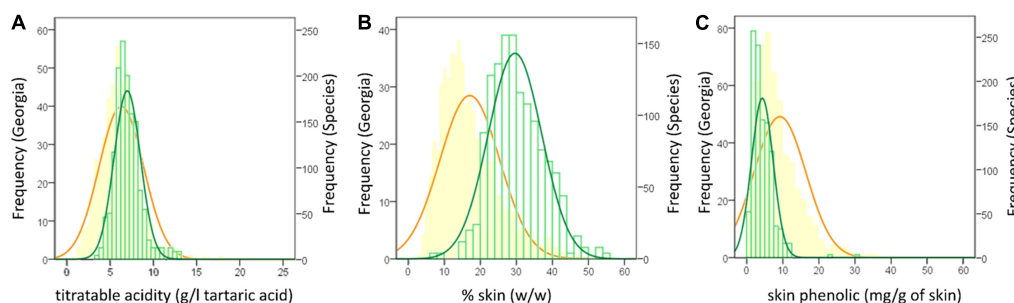


FIGURE 4 | Frequency distribution of the entire *V. vinifera* species (orange) in comparison with the Georgian (green) records concerning the traits: titratable acidity (A); % of skin (B) and skin phenolics (C) (reworking of the results published in Rustioni et al., 2014, 2019 and Abashidze et al., 2015).

Excesses of photosynthetically active radiation (PAR) could cause problems in grapes due to chlorophyll overexcitation (Rustioni et al., 2015; Rustioni, 2017). Rkatsiteli response to photo-oxidative sunburn was tested by Rustioni et al. (2015). It was considered among the “tolerant cultivars,” as it showed relatively low susceptibility to sunburn (recorded as browning appearance) at all the phenological periods studied. In particular, the correlation between chlorophyll contents and browning symptoms had a high R^2 (0.989), but the slope coefficient (60.2) together with the average Browning Intensity Index (27.5) indicated a light symptom appearance in Rkatsiteli grapes. In photo-oxidative sunburn, browning symptoms appear due to the reactive oxygen species (ROS) scavenging activity of phenolics through their oxidation and consequent polymerization that produce brown pigments (Felicetti and Schrader, 2008; Rustioni et al., 2015). Often, plants face stresses through secondary metabolites, and the crucial role of phenolics against photodamage is well known (Close and McArthur, 2002; Graham et al., 2004; Rustioni, 2017). However, if the substrate for these oxidative polymerizations (phenolics) are in low concentrations, the sunburn browning symptoms could appear less intense: this is likely in the case of Rkatsiteli. Abashidze et al. (2015) reported 404.7 ± 58.3 mg/kg of grapes as average skin phenolics for this cultivar, which falls in the first 10th percentile of the *V. vinifera* variability concerning this trait (Rustioni et al., 2019) (Table 3). Considering total phenolic compounds, Georgian cultivars appeared to accumulate low amounts of these molecules in skins (Figure 4C and Table 3), but the difference is still exacerbated by seed phenolics (Table 3). In fact, the average percentage of phenolics arising from seeds is much lower in data coming from Georgia (13.7% in Georgian records in comparison with 20.1% of species characteristics). Of course, considering the eco-physiological role of phenolics, this trait could be considered as a downside of Georgian cultivars. However, in a production perspective, it could be an important advantage.

Climate changes often produce disequilibria in the berry ripening processes, increasing the quantity of phenolic compounds (Keller, 2010; van Leeuwen and Destrac-Irvine, 2017) that, often, do not reach an optimal ripening quality. Unripe phenolics could strongly compromise the wine quality, being involved in the perception of bitterness and astringency

(Kontoudakis et al., 2011). Seed phenolics, due to their intrinsic characteristics, are often considered as an undesirable source of defects, so it is true that technologies have been developed to separate seeds to prevent phenolic extractions in wines (Canals et al., 2008) or to artificially ripen them under controlled conditions (Rustioni et al., 2018; VanderWeide et al., 2020). In this perspective, and considering that climate change is expected to make it harder to reach an equilibrated phenolic ripening in grapes, the lower phenolic concentration of Georgian cultivars (especially in the seeds) could be considered as a positive trait to deal with future difficult ripening conditions.

Another important class of phenolic molecules is represented by anthocyanin pigments. Among the 48 native Georgian grapevine varieties described by Tsertsvadze (2012), 21 of them are white grape cultivars, while the other 27 have pigmented berries (22 black, 2 red, 1 gray, and 2 pink). Among the native Georgian grape varieties described by Ketskhoveli et al. (1960), 245 of them are not pigmented (241 white and 4 yellow) grape cultivars, while the other 278 have pigmented berries (221 black, 27 red, 5 gray, and 25 pink). The reflectance spectra of 51 Georgian cultivars, together with other 69 accessions originated from other countries, were studied by Rustioni et al. (2013). Based on this first screening, some of these cultivars were selected to highlight dysfunctions in anthocyanin accumulation: Ubakluri, Ghrubela Kartlis, Rkatsiteli Vardisperi (and Marguli Sapere among the reference cultivars). Ubakluri shows a very light color, due to a very low pigment accumulation. Ghrubela Kartlis, due to the prevalence of peonidin-3-*O*-glucosides among the anthocyanins, has a gray appearance. Rkatsiteli Vardisperi, with the salmon pink color due to the high proportion of cyanidin-3-*O*-glucosides, is considered a berry color mutant resulting from a retro-transposon-induced mutation of the Rkatsiteli white-skinned cultivar (Rustioni et al., 2016; De Lorenzis et al., 2020). These color peculiarities could be interesting for future selections, especially considering the importance of appearance for table grape markets.

The environmental conditions (e.g., light and temperature) can affect the pigment accumulation in skins and the modulation of the anthocyanin biosynthetic pathway in berries could be considered as a grapevine eco-physiological adaptation mechanism (Keller, 2010; Rustioni et al., 2011; De Lorenzis et al., 2016). Considering anthocyanins (Table 3), Georgian

data generally show slightly higher contents of pigments with respect to the *V. vinifera* species average when expressed as mg/kg of grapes or mg/berry. However, this appears mainly due to the thick Georgian berry skins, and, thus, it is not due to a higher accumulation in this tissue, but to the higher quantity of pigmented tissue itself. In fact, when considering the anthocyanin accumulation in skins, the average Georgian record is 2.77 mg/g of skin, while the species average is nearly twice higher (4.7 mg/g of skin).

RESISTANCE TO GRAPEVINE FUNGAL DISEASES

The grapevine varieties cultivated worldwide belong to the Eurasian grapevine, *V. vinifera*, and are susceptible, at different levels, to several pathogens (fungi, bacteria, and viruses), while non-*vinifera* species, from North American and Asian, are resistant to fungi and tolerant to viruses and some bacteria (Oliver and Fuchs, 2011; Armijo et al., 2016). Amongst the various diseases which directly affect grapevines, powdery mildew (caused by the ascomycete *Erysiphe necator*) and downy mildew (caused by the oomycete *Plasmopara viticola*) are two of the most important (Bois et al., 2017). Disease management became an unavoidable task for European viticulture in the second half of the nineteenth century, when the two pathogens were introduced into Europe and the European grapevine growers were faced with their destructive effects (Töpfer et al., 2011). The *P. viticola* introduction was a probable consequence of the massive importation of American grapevine species to be used as rootstock for *V. vinifera* and contrast the destructive effects of phylloxera, caused by *Daktulosphaira vitifoliae*, on the Eurasian grapevine species (Granett et al., 2001; Gessler et al., 2011). The search for suitable tools for disease management rapidly became a priority for the viticulturists. The discovery of the efficacy of sulfur and copper in controlling the diseases was a key point, but great attention was also paid to the development of resistant cultivars. The American *Vitaceae* soon proved to be the best sources of resistance, due to co-evolution with the pathogens, and extensive breeding programs, based on interspecific crosses between American *Vitis* species (e.g., *Vitis riparia*, *Vitis rupestris*, *Vitis berlandieri* and *Vitis labrusca*) and *V. vinifera*, were undertaken at the beginning of the XX century (Gessler et al., 2011). Nevertheless, the interest in searching for resistant plants decreased over time, probably due to the discovery of new fungicides (Russell, 2005), that were widely employed for disease control, and the inheritance of the specific foxy off-flavors from the non-*vinifera* parent species.

Recently, public concern about sustainability in agriculture and new regulations on plant protection products have renewed the interest of growers in the cultivation of resistant varieties (Merdinoglu et al., 2018). In fact, although viticulture in the whole of the EU only occupies a low percentage of arable land, the industry is responsible for a high use of fungicides to fight downy mildew infections (Eurostat²). Furthermore, studies on the effects

of CO₂ and temperature on downy and powdery mildews showed that the disease incidence of downy mildew increases with rises in gas and temperature, while an increase in CO₂ did not influence powdery mildew incidence (Pugliese et al., 2011). In view of the coming climate change, that will potentially favor the pathogens' development, it is also important to search for new resistance genes, focusing on alternative species, such as *V. vinifera*, to the non-*vinifera* ones.

V. vinifera Resistant Cultivars Against *P. viticola*

The identification of *P. viticola* dates back to 1838, when Schweinitz, one of the founders of American mycology, collected the first samples from wild *Vitis* species in South Carolina (Gessler et al., 2011). In Europe, downy mildew was first reported during 1878 in Bordeaux and then it spread all over the old continent and beyond, reaching Australia and New Zealand between 1919 and 1926 (Emmett et al., 1992). All traditional European grapevine cultivars showed high susceptibility to the pathogen, leading to severe pandemics across Europe (Boso and Kassemeyer, 2008; Gessler et al., 2011). Today, the pathogen is found in warm and humid climates worldwide.

Symptoms of downy mildew (Figure 5) are observable on infected organs as yellowish oily lesions (sometimes red, in black cultivars) on the upper surface of the leaves (Figures 5A,B) followed by sporulation on the underside of the leaf (Figure 5C); malformations and necrosis on herbaceous shoots and inflorescences (Figures 5D,E); change of color to violet and withering on berries (Figure 5F), that detach from the rachis leaving a dry stem scar (Gessler et al., 2011). The disease negatively impacts grape production at both qualitative and quantitative levels: the loss of photosynthetic tissues limits the sugar amount in berries, that produce low quality wines; the shoot and bunch damage leads to poor yields. Severe infections, in the absence of disease control, can result in total loss of leaves and in some cases, total yield loss (Töpfer et al., 2011; Toffolatti et al., 2018).

Most of the *Vitis* taxa native to North America are to some extent resistant to *P. viticola* (Unger et al., 2007). The resistance response to *P. viticola* results in rapid plant cell death after pathogen recognition and local necrosis induction. This mechanism, known as the hypersensitive response (HR), is an actively triggered procedure initiated by fungal elicitors or other elicitors (Balint-Kurti, 2019) that leads to bursts of production of ROS and nitric oxide (NO). Consequently, the host cells collapse and shrink, hampering the fungal infection (Toffolatti et al., 2016). Cell death is visible to the naked eye as small necrotic spots on plant tissues.

The Georgian grapevine germplasm is characterized by very high genetic diversity, with cultivars differing from major European ones (Imazio et al., 2013). Considering that this high variability could also be a source of resistance to important pathogens, studies have been undertaken to assess the resistance levels of Georgian accessions to *P. viticola*. The first one, carried out by Bitsadze et al. (2015), showed that 20 accessions were characterized by medium to high levels of resistance to

²<http://ec.europa.eu/eurostat/de>

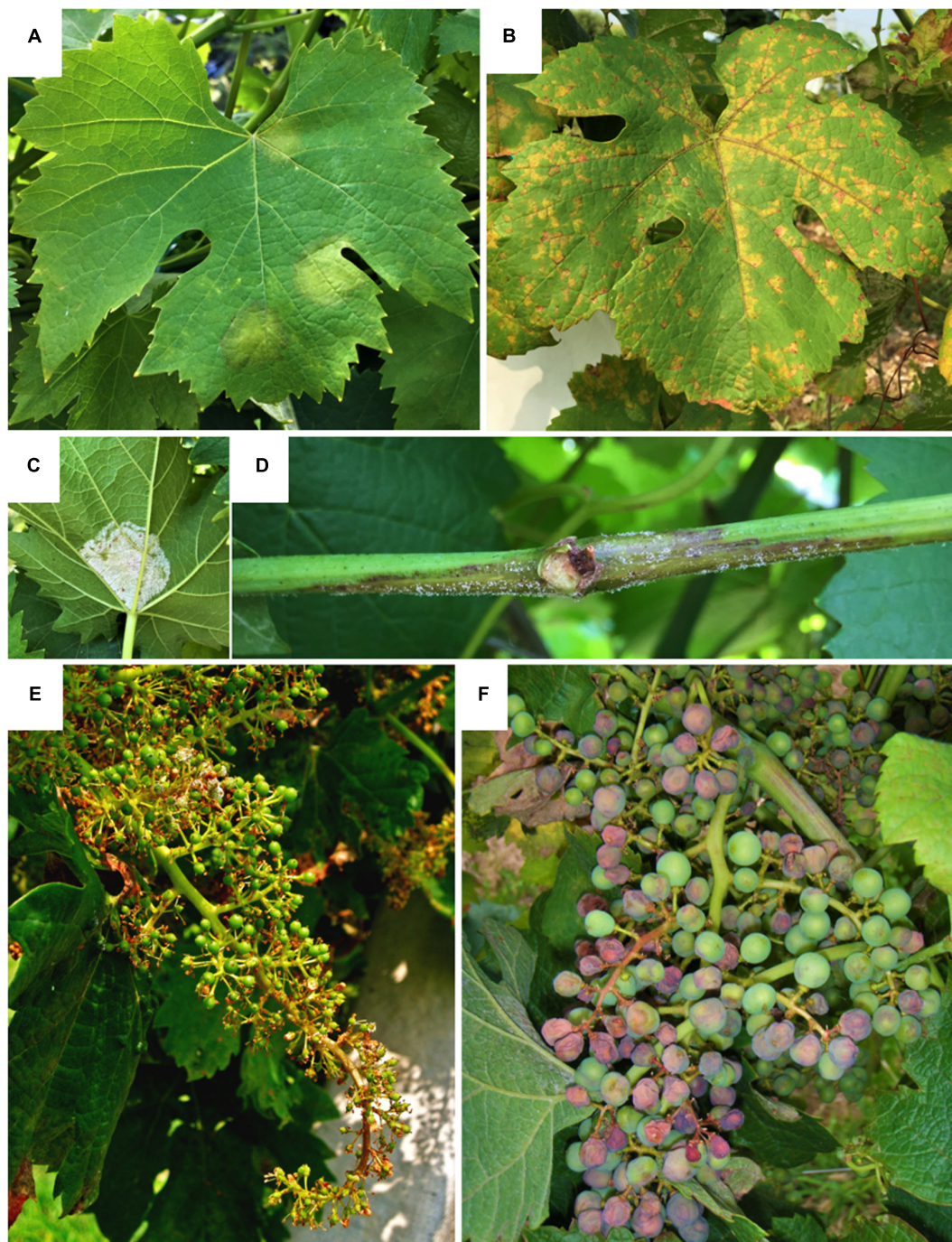


FIGURE 5 | Symptoms of grapevine downy mildew on leaves (A–C), shoot (D) and bunches (E,F). (A) Oilspot (yellow circular spots with an oily appearance) on the upper side of the leaf; (B) mosaic symptom (yellow spot restricted by veins to form yellow-to-brown small, angular spots in a mosaic pattern) on the upper side of the leaf; (C) sporulation (sporangiophores and sporangia appearing as a bright white, fluffy growth) on the undersides of leaves; (D) shoot covered by sporulation turning brown; (E) distorted bunch (U-shaped) turning necrotic; (F) shrinking berries turning violet.

downy mildew in a collection of 61 native Georgian varieties. Given the promising results, it appeared worthwhile to keep screening Georgian germplasm. In Toffolatti et al. (2016), a total of 93 accessions were studied over a period of 3 years in field surveys and in the laboratory. A small group of

varieties, including Kamuri Shavi, Mgaloblishvili and Ubakluri, showed low disease severity values, but only Mgaloblishvili showed a strong and constant phenotypical resistance against the pathogen. In **Supplementary Table 1**, a list of Georgian resistant varieties is reported. Indeed, recent studies on the transcriptome

of Mgaloblishvili showed that the cultivar possesses a unique response to *P. viticola* that is based on the overexpression of genes that are not modulated or downregulated in susceptible (Pinot Noir, a *V. vinifera* cv) and resistant (Bianca, interspecific hybrid) cultivars (Toffolatti et al., 2018). The resistance mechanism of Mgaloblishvili is based on the overexpression of genes encoding: (i) receptors for pathogen recognition (PAMP – Pathogen Associated Microbial Patterns-receptors) and for damage at the cell wall (DAMP – Damage Associated Microbial Patterns); (ii) an NB-LRR receptor of fungal effectors (named Lr10); (iii) ethylene signaling; (iv) synthesis of terpenes, such as valencene, and flavonoids; and (v) strengthening of cell walls. Besides genes involved in resistance, susceptibility genes were identified as well. Susceptibility genes are essential for plant-pathogen interaction and their disruption leads to resistance, as with *mlo* gene, whose knockdown is involved in resistance to *E. necator* (Pessina et al., 2016). The candidate gene related to susceptibility to *P. viticola* in *V. vinifera* encodes an LOB domain-containing (LBD) protein (Toffolatti et al., 2020) that has been previously found in the interaction between *Arabidopsis thaliana* and *Fusarium oxysporum* (Thatcher et al., 2012). The new genome editing tools, providing several protocols to introduce knockout on target sequences, makes the understanding of plant pathogen-resistance mechanism mediated by susceptibility genes a very attractive alternative for the development of durable disease-resistant varieties (Zaidi et al., 2018).

New Resistant Loci Associated With Resistance to *P. viticola* in *V. vinifera*

The investigation of the genetic basis of *P. viticola* resistance through QTL (Quantitative Trait Loci) analysis on a range of North American and Asian *Vitis* species has led to the identification of 28 resistance (R) loci (Figure 6). These R loci (designated Rpv for Resistance to *P. viticola*) confer different degrees of resistance to disease, ranging from partial to total resistance (Dry et al., 2019). The major loci on this list are: (i) Rpv1, identified in *Muscadinia rotundifolia*, that confers a not total resistance to *P. viticola* infection and is associated with a gene encoding a TIR-NB-LRR protein (MrRPV1) (Merdinoglu et al., 2003; Feechan et al., 2013); (ii) Rpv2, identified in *M. rotundifolia*, that confers total resistance to downy mildew and is associated to a cluster of TIR-NB-LRR genes (Dry et al., 2019); (iii) Rpv3, identified in *V. labrusca*, *Vitis lincecumii*, *V. riparia* and *V. rupestris*, that confers partial resistance to downy mildew (Bellin et al., 2009; Gaspero et al., 2011; Welter et al., 2017); (iv) Rpv8 and Rpv12, identified in *V. amurensis*, that confer a high resistance to *P. viticola* infection and are associated with the cluster of genes encoding NB-LRR proteins (Blasi et al., 2011; Venuti et al., 2013); (v) Rpv15, identified in *Vitis piasezkii*, that confers strong resistance to *P. viticola* infection (Dry et al., 2019). The other R loci are considered minor loci due to their ability to confer low degrees of resistance and they are only useful when combined with major R loci.

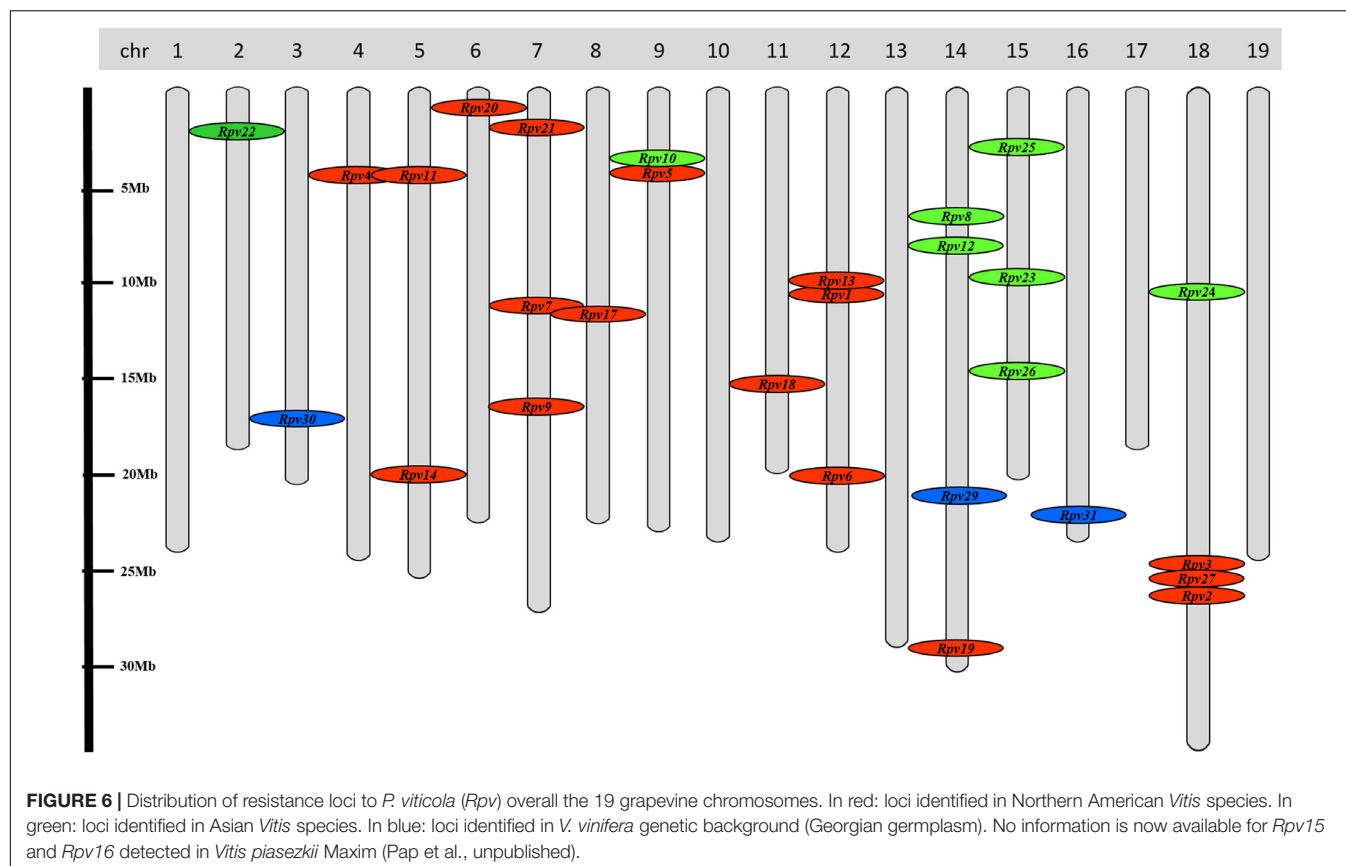
Very recently, new promising downy mildew R loci (Rpv29, Rpv30, and Rpv31) have been identified, through a GWAS (Genome Wide Association Study), in the genetic background of the Georgian *V. vinifera* germplasm (Figure 6) (Sargolzaei

et al., 2020). These new R loci, mapping on chromosome 14, 3 and 16 for Rpv29, 30 and 31, respectively, and conferring from high to very high resistance to downy mildew, seem to be associated with receptors of pathogen effectors, signaling mediated by protein ubiquitination and a cluster of Lr10-like (NB-LRR) effector receptors.

Low Susceptibility of Georgian Grapevine Cultivars to Phytoplasma-Associated Diseases

Flavescence dorée (FD) and Bois noir (BN) are the more important diseases of the grapevine yellows (GY) complex, responsible for severe yield losses in vineyards worldwide (Belli et al., 2010). FD and BN are associated with phytoplasmas, phloem-limited bacteria transmitted by insect vectors (Weintraub and Beanland, 2006). Even if their symptoms were indistinguishable (desiccation of inflorescences, berry shrivel, leaf discolorations, reduction of growth, and irregular ripening of wood), FD and BN are associated with phytoplasmas distinct at both genetic and ecological level (Belli et al., 2010). The FD phytoplasma is efficiently transmitted from grapevine to grapevine by the insect *Scaphoideus titanus*, which sustains its whole life cycle on *Vitis* spp. (Oliveira et al., 2019). Consequently, geographic areas hosting large vector populations and FD phytoplasma can be damaged by strong FD epidemics. Due to this aspect, FD phytoplasma is a quarantine pathogen, to be controlled through mandatory measures (Oliveira et al., 2019). On the other hand, BN phytoplasma (*Candidatus* Phytoplasma solani) (Quaglino et al., 2013) is occasionally transmitted to grapevine by the insect *Hyalesthes obsoletus*, a polyphagous vector living preferentially on *Urtica dioica* (nettle), *Convolvulus arvensis* (bindweed), and *Vitex agnus-castus* (chaste tree) (Langer and Maixner, 2004; Kosovac et al., 2016). The epidemiological cycle associated with BN is extremely complex and was recently discovered to include other highly polyphagous insect vectors and a very broad range of secondary wild hosts (Mori et al., 2015; Quaglino et al., 2019). Moreover, the typical management strategies for phytoplasma diseases, based on the control of the vector(s) with insecticides and the removal of infected plants, are not effective against BN. Thus, it is difficult to organize effective prevention and containment measures. An ambitious strategy is based on the selection of plant varieties as the source of resistance-genes for plant breeding programs (Bianco et al., 2019). Unfortunately, none of the *Vitis* species and *V. vinifera* varieties studied have been found to be resistant or tolerant to the GY phytoplasmas (Laimer et al., 2009).

Surveys conducted in vineyards of Khaketi and Shida Kartli regions in eastern Georgia highlighted a wide diffusion of BN, while FD was not reported (Quaglino et al., 2014). Moreover, most autochthonous Georgian grapevine cultivars were found to be only mildly symptomatic, maintaining complete berry production, while internationally known cultivars exhibited severe symptoms (Quaglino et al., 2016) (Figure 7). As largely reported for phytoplasma-associated diseases of stone fruits, symptom intensity observed in infected plants



can be influenced both by the virulence of the pathogen and the susceptibility level of the plant host (Kison and Seemüller, 2001; Seemüller and Schneider, 2007). Molecular characterization, supported by phylogenetic analyses, revealed that BN phytoplasma strains identified in Georgia constitute a bindweed-related population which is genetically distinct from the one found in central-western Europe. Interestingly, the presence of the same phytoplasma strain in grapevine cultivars showing a range of symptom intensity suggested a low susceptibility of Georgian local cultivars to BN (Quaglino et al., 2016) (**Supplementary Table 1**). Studies in progress are focusing on (i) identifying genetic traits associated with this low susceptibility to BN in the perspective of improving breeding programs to produce novel tolerant and/or resistant grapevine cultivars; (ii) investigating the susceptibility of Georgian grapevine cultivars to FD.

A BRIEF INTERLUDE ON THE STATUS OF GEORGIAN WILD COMPARTMENT

The *V. vinifera* subsp. *silvestris* is considered the progenitor of cultivated species. In the last two decades, an increase interest in preserving wild genetic resources has led to surveys on Georgian land aimed to localize and gather wild grapevine material. The plant material collected in these surveys is summarized in the **Supplementary**

Table 2. These accessions are now partially (more than 100) available in Georgian collections (Saguramo, Skra and other collections) and some other in USDA National Clonal Germplasm Repository of Davis (CA, United States) and in the collection of Milan University. This subspecies is seriously worldwide endangered by human activities, such as urbanization, forest cleaning and setting fires (Arnold et al., 2005). The Georgian one is not an exception. Indeed, very small wild populations have been identified overall the Georgian land (Ocete Rubio et al., 2012). Populations with both male and female individuals were detected, but in Zhinvali and Sabue populations no female individuals were identified. Generally, in the Georgian populations the number of male individuals is higher than the female ones (**Supplementary Table 2**). Most of the wild Georgian populations showed severe downy and powdery mildew symptoms, although three individuals showed high resistance to *P. viticola* infection (**Supplementary Table 2**) (Ocete Rubio et al., 2012; Bitsadze et al., 2015). Nevertheless, remarkable is the absence of symptoms caused by phylloxera in the populations sampled by Ocete Rubio et al. (2012). In the same populations, symptoms caused by two mites, *Colomerus vitis* and *Calepitrimerus vitis*, have been observed, although the damages were not serious and appeared to do not affect the viability of the plants.

From the genetic point of view, some of these accessions were genotyped by SSR and SNP molecular markers

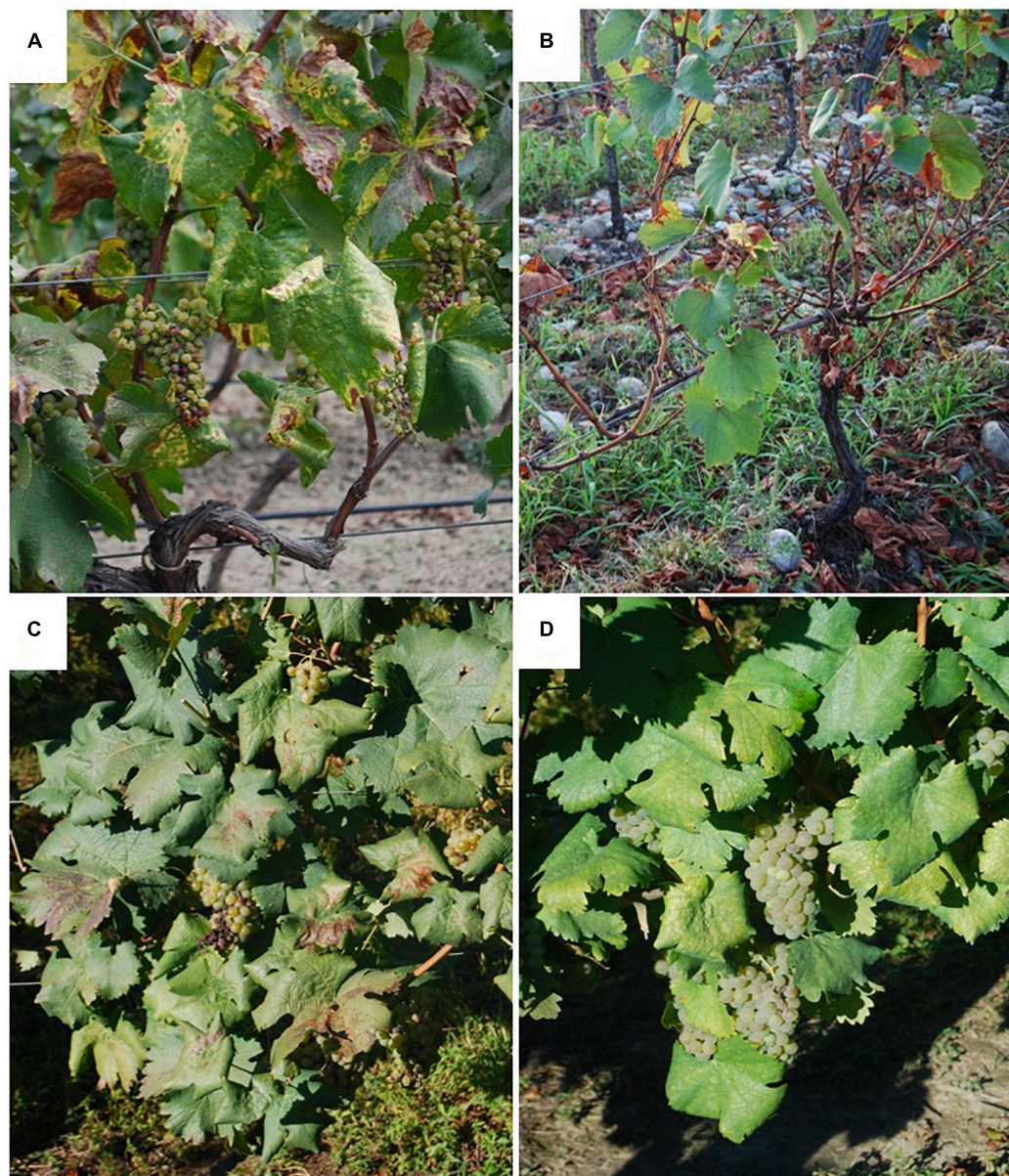


FIGURE 7 | Symptoms observed on '*Candidatus Phytoplasma solani*' infected grapevine cultivars in Georgia. Severe symptoms on international cultivar Chardonnay (A) and Georgian cultivar Kisi (B); moderate symptoms on Georgian cultivar Goruli Mtsvane (C); mild symptoms on Georgian cultivar Tsitska (D).

(**Supplementary Table 2**) (Imazio et al., 2013; Ekhvaia et al., 2014; De Lorenzis et al., 2015; Riaz et al., 2018). Results reported in Imazio et al. (2013) and De Lorenzis et al. (2015) clearly discriminated the wild individuals from the cultivated ones, two subspecies that diverged at least 22,000 years ago (Zhou et al., 2017; Liang et al., 2019). The Georgian accessions were differentiated by European wild accessions and cultivated accessions (Riaz et al., 2018). Interestingly, Ekhvaia et al. (2014) identified absence of genetic isolation among some of the analyzed wild populations due to gene flow among them.

At the phenotypical level, few information is available and further studies need to deeply investigate the enological potential

of this compartment. Nevertheless, preliminary results showed that musts obtained by Georgian wild grapes could be added to the must of traditional cultivars to improve the wine color (Maghradze et al., 2020).

WHAT'S NEXT?

Climate change will impact many aspects of human life, the environment, agriculture and food. Regarding viticulture, data available on climate change have already demonstrated impacts on wine growing areas, resulting in changes in grape chemical

composition as well as grape phenology. Because of their isolated geographical origin and huge genetic variability, the Georgian grapevine germplasm is of great interest as a worthwhile resource for breeding programs. The Georgian germplasm has distinguished itself by including cultivars characterized by late ripening, which could potentially reduce issues related to excessive temperatures in summertime, distinctive eno-carpological traits, which affect the grape and wine quality, specific response to abiotic stresses, such as sunburn, and resistance traits related to biotic stresses, such as *P. viticola* and phytoplasmas.

Given the reasons stated in this review, the screening and assessment of Georgian germplasm should be promoted at the phenotypical, agronomical, physiological and genetic level. A number of gaps has still to be filled, such as their attitude to abiotic (drought, salinity, iron chlorosis) and biotic stresses, as well as the whole genome analysis of the most performing Georgian cultivars, in order to identify the genetic regions related to such valuable traits. A step toward this direction has been performed by Tabidze et al. (2017), sequencing the whole genome of four major Georgian varieties (Chkhaveri, Saperavi, Meskhetian green, and Rkatsiteli) and releasing information useful to understand the complexity of grape genome and for further comparative analysis. Aside from traditional breeding programs, these invaluable resources could be exploited in breeding programs based on the use of New Breeding Technologies (NBTs), by means of genome editing applied to both resistance and, with even more practical advantages, susceptibility candidate genes to abiotic and biotic stresses. In this way, it will be possible to exploit the valuable traits carried by this unique source of genetic variability for new varieties able to meet the challenges awaiting viticulture in the era of climate change.

AUTHOR CONTRIBUTIONS

GDL and SLT conceived the work. MS, VR, and GDL wrote the introduction and genetic variability section. GC wrote the

climate section. LR wrote the section on phenotype. SLT wrote the section on resistance to fungal pathogens. FQ wrote the section on susceptibility to phytoplasma diseases. DM, OF, and PAB critically revised the manuscript. All the authors read and approved the final version of the manuscript.

FUNDING

This research was supported by University of Milan, DiSAA, Research Support Plan 2018, Linea 2 A, Project “Dal phenotyping al genome editing: strategie per limitare i danni da peronospora e legno nero in vite (ResVite)” and by the National Wine Agency of Georgia within the ‘Research Project for the Study of the Georgian Grapes and Wine Culture.’ University of Milan supported the article processing charges.

ACKNOWLEDGMENTS

We would like to thank Dr. Lesley Currah for supporting us on English language editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.630122/full#supplementary-material>

Supplementary Table 1 | List of Georgian autochthonous and breeding grapevine accessions based on Ketskheveli et al. (1960) and VIVC. Table includes information related to berry color, origin, usage, spreading, genotyping and resistance to plant disease. Synonyms, homonyms and misidentifications are not verified. SSR, simple sequence repeat; SNP, single nucleotide polymorphism; VIVC, Vitis International Variety Catalogue (<https://www.vivc.de/>).

Supplementary Table 2 | List of Georgian wild grapevine accessions. Table includes information related to flower sex, origin, genotyping and resistance to plant disease. SSR, simple sequence repeat; SNP, single nucleotide polymorphism; VIVC, Vitis International Variety Catalogue (<https://www.vivc.de/>). F, female; H, hermaphrodite; M, male; UNIMI, University of Milan.

REFERENCES

- Abashidze, E., Mdinardze, I., Chipashvili, R., Vashakidze, L., Maghradze, D., Rustioni, L., et al. (2015). Evaluation of eno-carpological traits in Georgian grapevine varieties from Skra germplasm repository. *Vitis* 54, 151–154.
- Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C., and Arce-Johnson, P. (2016). Grapevine pathogenic microorganisms: understanding infection strategies and host response scenarios. *Front. Plant Sci.* 7:382. doi: 10.3389/fpls.2016.00382
- Arnold, C., Schnitzler, A., Douard, A., Peter, R., and Gillet, F. (2005). Is there a future for wild grapevine (*Vitis vinifera* subsp. *silvestris*) in the Rhine Valley? *Biodivers. Conserv.* 14, 1507–1523. doi: 10.1007/s10531-004-9789-9
- Arroyo-García, R., Ruiz-García, L., Bolling, L., Ocete, R., López, M. A., Arnold, C., et al. (2006). Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphisms. *Mol. Ecol.* 15, 3707–3714. doi: 10.1111/j.1365-294x.2006.03049.x
- Bacilieri, R., Lacombe, T., Le Cunff, L., Di Vecchi-Staraz, M., Laucou, V., Genna, B., et al. (2013). Genetic structure in cultivated grapevines is linked to geography and human selection. *BMC Plant Biol.* 13:25. doi: 10.1186/1471-2229-13-25
- Balint-Kurti, P. (2019). The plant hypersensitive response: concepts, control and consequences. *Mol. Plant Pathol.* 20, 1163–1178. doi: 10.1111/mpp.12821
- Belli, G., Bianco, P. A., and Quaglino, F. (2010). Grapevine yellows in Italy: past, present and future. *J. Plant Pathol.* 92, 303–326.
- Bellin, D., Peressotti, E., Merdinoglu, D., Wiedemann-Merdinoglu, S., Adam-Blondon, A. F., Cipriani, G., et al. (2009). Resistance to plasmopara viticola in grapevine ‘Bianca’ is controlled by a major dominant gene causing localised necrosis at the infection site. *Theor. Appl. Genet.* 120, 163–176. doi: 10.1007/s00122-009-1167-2
- Bianco, P. A., Romanazzi, G., Mori, N., Myrie, W., and Bertaccini, A. (2019). “Integrated management of phytoplasma diseases,” in *Phytoplasmas: Plant Pathogenic Bacteria-II*, eds A. Bertaccini, P. G. Weintraub, G. P. Rao, and N. Mori (Singapore: Springer Singapore).
- Bitsadze, N., Aznarashvili, M., Vercesi, A., Chipashvili, R., Failla, O., and Maghradze, D. (2015). Screening of Georgian grapevine germplasm for susceptibility to downy mildew (*Plasmopara viticola*). *Vitis J. Grapevine Res.* 54, 193–196. doi: 10.17660/ActaHortic.2014.1032.25
- Blasi, P., Blanc, S., Prado, E., Rühl, E. H., Mestre, P., and Merdinoglu, D. (2011). Construction of a reference linkage map of *Vitis amurensis* and genetic mapping

- of Rpv8, a locus conferring resistance to grapevine downy mildew. *Theor. Appl. Genet.* 123, 43–53. doi: 10.1007/s00122-011-1565-0
- Bois, B., Zito, S., Calonne, A., and Ollat, N. (2017). Climate vs grapevine pests and diseases worldwide: the first results of a global survey. *J. Int. des Sci. la Vigne du Vin* 51, 133–139. doi: 10.20870/oeno-one.2016.0.0.1780
- Boso, S., and Kassemeyer, H. H. (2008). Different susceptibility of European grapevine cultivars for downy mildew. *Vitis J. Grapevine Res.* 47, 39–49.
- Canals, R., del Carmen, Llaudy, M., Canals, J. M., and Zamora, F. (2008). Influence of the elimination and addition of seeds on the colour, phenolic composition and astringency of red wine. *Eur. Food Res. Technol.* 226, 1183–1190. doi: 10.1007/s00217-007-0650-8
- Chkhartishvili, N., and Maghradze, D. (2012). Viticulture and winemaking in Georgia. *Am. J. Enol. Vitic.* 51, 169–239.
- Close, D. C., and McArthur, C. (2002). Rethinking the role of many plant phenolics – protection from photodamage not herbivores? *Oikos* 99, 166–172. doi: 10.1034/j.1600-0706.2002.990117.x
- Cola, G., Failla, O., Maghradze, D., Megrelidze, L., and Mariani, L. (2017). Grapevine phenology and climate change in Georgia. *Int. J. Biometeorol.* 61, 761–773. doi: 10.1007/s00484-016-1241-9
- Cola, G., Mariani, L., Maghradze, D., and Failla, O. (2020). Changes in thermal resources and limitations for Georgian viticulture. *Aust. J. Grape Wine Res.* 26, 29–40. doi: 10.1111/ajgw.12412
- Cornea, V., and Savin, G. (2015). Exploration and revaluation of old autochthonous varieties in the Republic of Moldova. *Vitis Geilweilerhof* 54, 115–119.
- De Andrés, M. T., Benito, A., Pérez-Rivera, G., Ocete, R., Lopez, M., Gaforio, L., et al. (2012). Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. *Mol. Ecol.* 21, 800–816. doi: 10.1111/j.1365-294x.2011.05395.x
- De Lorenzis, G., Chipashvili, R., Failla, O., and Maghradze, D. (2015). Study of genetic variability in *Vitis vinifera* L. germplasm by high-throughput Vitis18kSNP array: the case of Georgian genetic resources. *BMC Plant Biol.* 15:154. doi: 10.1186/s12870-015-0510-9
- De Lorenzis, G., Mercati, F., Bergamini, C., Cardone, M. F., Lupini, A., Mauceri, A., et al. (2019). SNP genotyping elucidates the genetic diversity of Magna Graecia grapevine germplasm and its historical origin and dissemination. *BMC Plant Biol.* 19:7. doi: 10.1186/s12870-018-1576-y
- De Lorenzis, G., Rustioni, L., Parisi, S. G., Zoli, F., and Brancadoro, L. (2016). Anthocyanin biosynthesis during berry development in corvina grape. *Sci. Hortic.* 212, 74–80. doi: 10.1016/j.scienta.2016.09.039
- De Lorenzis, G., Rustioni, L., Pozzi, C., and Failla, O. (2020). Disfunctions in the anthocyanin accumulation of *Vitis vinifera* L. varieties studied by a targeted resequencing approach. *J. Berry Res.* 10, 1–19. doi: 10.3233/jbr-190478
- Di Matteo, M., Cinquanta, L., Galiero, G., and Crescitelli, S. (2000). Effect of a novel physical pretreatment process on the drying kinetics of seedless grapes. *J. Food Eng.* 46, 83–89. doi: 10.1016/S0260-8774(00)00071-6
- D'Onofrio, C. (2020). Introgression among cultivated and wild grapevine in Tuscany. *Front. Plant Sci.* 11:202. doi: 10.3389/fpls.2020.00202
- Doymaz, İ. (2006). Drying kinetics of black grapes treated with different solutions. *J. Food Eng.* 76, 212–217. doi: 10.1016/j.jfoodeng.2005.05.009
- Dry, I., Riaz, S., Fuchs, M., Sosnowski, M., and Thomas, M. (2019). “Scion breeding for resistance to biotic stresses,” in *The Grape Genome*, eds D. Cantu and A. M. Walker (Berlin: Springer), 319–347. doi: 10.1007/978-3-030-18601-2_15
- Ekhvaia, J., Gurushidze, M., Blattner, F. R., and Akhalkatsi, M. (2014). Genetic diversity of *Vitis vinifera* in Georgia: relationships between local cultivars and wild grapevine, *V. vinifera* L. subsp. *sylvestris*. *Genet. Resour. Crop Evol.* 61, 1507–1521. doi: 10.1007/s10722-014-0125-2
- Emmett, R. W., Wicks, T. J., and Magarey, R. (1992). “Downy mildew of grapes,” in *Plant Diseases of International Importance*, eds J. Kumar, H. S. Chaube, U. S. Singh, and A. N. Mukhopadhyay (Prentice Hall, NJ: Englewood Cliffs), 90–128.
- Eurostat (2007). Available online at: <http://ec.europa.eu/eurostat/de>
- Failla, O. (2015). East-West collaboration for grapevine diversity exploration and mobilization of adaptive traits for breeding: a four years story. *Vitis Geilweilerhof* 54, 1–4.
- Feechan, A., Anderson, C., Torregrosa, L., Jermakow, A., Mestre, P., Wiedemann-Merdinoglu, S., et al. (2013). Genetic dissection of a TIR-NB-LRR locus from the wild North American grapevine species *Muscadinia rotundifolia* identifies paralogous genes conferring resistance to major fungal and oomycete pathogens in cultivated grapevine. *Plant J.* 76, 661–674. doi: 10.1111/tpj.12327
- Fellicetti, D., and Schrader, L. (2008). Photooxidative sunburn of apples: characterization of a third type of apple sunburn. *Int. J. Fruit Sci.* 8, 160–172. doi: 10.1080/15538360802526472
- Forni, G. (2012). “The origin of ‘old world’ viticulture,” in *Caucasus and Northern Black Sea Region*, eds D. Maghradze, L. Rustioni, A. Scienza, J. Turok, and O. Failla (Dresden: Julius Kuhn-Institut), 27–38.
- Gaspero, G., Copetti, D., Coleman, C., Castellarin, S. D., Eibach, R., Kozma, P., et al. (2011). Selective sweep at the Rpv3 locus during grapevine breeding for downy mildew resistance. *Theor. Appl. Genet.* 124, 277–286. doi: 10.1007/s00122-011-1703-8
- Gessler, C., Pertot, I., and Perazzolli, M. (2011). Plasmopara viticola : a review of knowledge on downy mildew of grapevine and effective disease management. *Phytopathol. Mediterr.* 50, 3–44.
- Gladstones, J. (1992). *Viticulture and Environment : A Study of the Effects of Environment on Grapegrowing and Wine Qualities, With Emphasis on Present and Future Areas for Growing Winegrapes in Australia*. Broadview, SA: Winetitles.
- Goryslavets, S., Bacilieri, R., Risovannaya, V., Memetova, E., and Laucou, V. (2015). Genetic diversity of ancient grape cultivars of the Crimea region. *Vitis J. Grapevine Res.* 54, 37–41.
- Graham, L. E., Kodner, R. B., Fisher, M. M., Graham, J. M., Wilcox, L. W., Hackney, J. M., et al. (2004). “9 – Early land plant adaptations to terrestrial stress: A focus on phenolics,” *The Evolution of Plant Physiology*, eds R. Alan, R. Hemsley, and I. Poole (Academic Press), 155–169. doi: 10.1016/B978-012339552-8/50010-X
- Granett, J., Walker, M. A., Kocsis, L., and Omer, A. D. (2001). Biology and management of Grape Phylloxera. *Annu. Rev. Entomol.* 46, 387–412.
- Grassi, F., Labra, M., Imazio, S., Spada, A., Sgorbati, S., Scienza, A., et al. (2003). Evidence of a secondary grapevine domestication centre detected by SSR analysis. *Theor. Appl. Genet.* 107, 1315–1320. doi: 10.1007/s00122-003-1321-1
- Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., et al. (2013). Climate change, wine, and conservation. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6907–6912. doi: 10.1073/pnas.1210127110
- Imazio, S., Maghradze, D., Lorenzis, G., Bacilieri, R., Laucou, V., This, P., et al. (2013). From the cradle of grapevine domestication: molecular overview and description of Georgian grapevine (*Vitis vinifera* L.) germplasm. *Tree Genet. Genomes* 9, 641–658. doi: 10.1007/s11295-013-0597-9
- Keller, M. (2010). Managing grapevines to optimize fruit development in a challenging environment: a climate change primer for viticulturists. *Environ. Sustain. Vitic. Pract. Pract.* 16, 259–292. doi: 10.1201/b18226
- Ketskshvili, N., Ramishvili, M., and Tabidze, D. (1960). “Ampelography of Georgia,” in *Georgian and Russian* (Tbilisi: Georgian Academy of Science), 20–439.
- Kison, H., and Seemuller, E. (2001). Differences in strain virulence of the European stone fruit yellows phytoplasma and susceptibility of stone fruit trees on various rootstocks to this pathogen. *J. Phytopathol.* 149, 533–541. doi: 10.1046/j.1439-0434.2001.00671.x
- Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., De Freitas, V., and Zamora, F. (2011). Influence of the heterogeneity of grape phenolic maturity on wine composition and quality. *Food Chem.* 124, 767–774. doi: 10.1016/j.foodchem.2010.06.093
- Köppen, W., and Geiger, G. (eds) (1936). *Handbuch der Klimatologie*. Stuttgart: Borntraeger.
- Kosovac, A., Radonjić, S., Hrnčić, S., Krstić, O., Toševski, I., and Jović, J. (2016). Molecular tracing of the transmission routes of bois noir in Mediterranean vineyards of Montenegro and experimental evidence for the epidemiological role of *Vitex agnus-castus* (Lamiaceae) and associated *Hyalosphaera obsoletus* (Cixiidae). *Plant Pathol.* 65, 285–298. doi: 10.1111/ppa.12409
- Laimer, M., Lemaire, O., Herrbach Herrbach, E., Goldschmidt, V., Minafra, A., Bianco, P. A., et al. (2009). Resistance to viruses, phytoplasmas and their vectors in the grapevine in Europe: a review. *J. Plant Pathol.* 91, 7–23.
- Langer, M., and Maixner, M. (2004). Molecular characterisation of grapevine yellows associated phytoplasmas of the stolbur-group based on RFLP-analysis of non-ribosomal DNA. *Vitis J. Grapevine Res.* 43, 191–199.
- Laucou, V., Lacombe, T., Dechesne, F., Siret, R., Bruno, J.-P., Dessup, M., et al. (2011). High throughput analysis of grape genetic diversity as a tool for

- germplasm collection management. *Theor. Appl. Genet.* 122, 1233–1245. doi: 10.1007/s00122-010-1527-y
- Laucou, V., Launay, A., Bacilieri, R., Lacombe, T., Adam-Blondon, A.-F., Bérard, A., et al. (2018). Extended diversity analysis of cultivated grapevine *Vitis vinifera* with 10K genome-wide SNPs. *PLoS One* 13:e0192540. doi: 10.1371/journal.pone.0192540
- Liang, Z., Duan, S., Sheng, J., Zhu, S., Ni, X., Shao, J., et al. (2019). Whole-genome resequencing of 472 *Vitis* accessions for grapevine diversity and demographic history analyses. *Nat. Commun.* 10, 1–12.
- Maghradze, D., Melyan, G., Salimov, V., Chipashvili, R., Iñiguez, M., Puras, P., et al. (2020). Wild grapevine (*Vitis sylvestris* C.C.Gmel.) wines from the Southern Caucasus region. *OENO One* 54, 809–822. doi: 10.20870/oeno-one.2020.54.3720
- Maghradze, D., Rustioni, L., Scienza, A., FailLa, and Os. (2012). Phenological diversity of georgian grapevine cultivars in Northern Italy. *J. Am. Pomol. Soc.* 66, 56–67.
- Maghradze, D., Salimov, V., Melyan, G., Musayev, M., Ocete, C. A., Chipashvili, R., et al. (2015). Sanitary status of the Eurasian wild grapevine in the South Caucasian region. *Vitis J. Grapevine Res.* 54, 203–205.
- Maghradze, D., Vashakidze, L., Abashidze, E., Chipashvili, R., Mdinaradze, I., Failla, O., et al. (2014). Multidisciplinary study of traditional grape cultivars from Kartli province of Georgia (the Caucasus region) and activities for their preservation. *Acta Hort.* 1032, 235–241. doi: 10.17660/actahortic.2014.1032.33
- Maraš, V., Tello, J., Gazivoda, A., Mugoša, M., Perišić, M., Raičević, J., et al. (2020). Population genetic analysis in old Montenegrin vineyards reveals ancient ways currently active to generate diversity in *Vitis vinifera*. *Sci. Rep.* 10:15000.
- Mariani, L., Alilla, R., Cola, G., Monte, G. D., Epifani, C., Puppi, G., et al. (2013). IPHEN—a real-time network for phenological monitoring and modelling in Italy. *Int. J. Biometeorol.* 57, 881–893. doi: 10.1007/s00484-012-0615-x
- Mariani, L., Parisi, S. G., Cola, G., and Failla, O. (2012). Climate change in Europe and effects on thermal resources for crops. *Int. J. Biometeorol.* 56, 1123–1134. doi: 10.1007/s00484-012-0528-8
- Marois, J. J., Nelson, J. K., Morrison, J. C., Lile, L. S., and Bledsoe, A. M. (1986). The influence of berry contact within grape clusters on the development of botrytis cinerea and epicuticular wax. *Am. J. Enol. Vitic.* 37, 293–296.
- Martínez-Moreno, A., Sanz, F., Yeves, A., Gil-Muñoz, R., Martínez, V., Intrigliolo, D. S., et al. (2019). Forcing bud growth by double-pruning as a technique to improve grape composition of *Vitis vinifera* L. cv. Tempranillo in a semi-arid Mediterranean climate. *Sci. Hortic.* 256:108614. doi: 10.1016/j.scienta.2019.108614
- McGovern, P., Jalabadze, M., Batiuk, S., Callahan, M. P., Smith, K. E., Hall, G. R., et al. (2017). Early Neolithic wine of Georgia in the South Caucasus. *Proc. Natl. Acad. Sci. U.S.A.* 114, 10309–10318. doi: 10.1073/pnas.1714728114
- McGovern, P. E. (2003). *Ancient Wine*. Princeton, NJ: University Press.
- Merdinoglu, D., Schneider, C., Prado, E., Wiedemann-Merdinoglu, S., and Mestre, P. (2018). Breeding for durable resistance to downy and powdery mildew in grapevine. *OENO One* 52, 203–209. doi: 10.20870/oeno-one.2018.52.3.2116
- Merdinoglu, D., Wiedeman-Merdinoglu, S., Coste, P., Dumas, V., Haetty, S., Butterlin, G., et al. (2003). Genetic analysis of downy mildew resistance derived from *Muscadinia rotundifolia*. *Acta Hort.* 603, 451–456. doi: 10.17660/actahortic.2003.603.57
- Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Res. Int.* 43, 1844–1855. doi: 10.1016/j.foodres.2010.05.001
- Morales-Castilla, I., de Cortázar-Atauri, I. G., Cook, B. I., Lacombe, T., Parker, A., van Leeuwen, C., et al. (2020). Diversity buffers winegrowing regions from climate change losses. *Proc. Natl. Acad. Sci. U.S.A.* 117, 2864–2869. doi: 10.1073/pnas.1906731117
- Mori, N., Quagliano, F., Tessari, F., Pozzebon, A., Bulgari, D., Casati, P., et al. (2015). Investigation on ‘bois noir’ epidemiology in north-eastern Italian vineyards through a multidisciplinary approach. *Ann. Appl. Biol.* 166, 75–89. doi: 10.1111/aab.12165
- Muganu, M., Bellincontro, A., Barnaba, Paolocci, M., Bignami, C., Scossa, et al. (2011). Influence of bunch position in the canopy on berry epicuticular wax during ripening and on weight loss in postharvest dehydration process. *Am. J. Enol. Vitic.* 62, 91–98. doi: 10.5344/ajev.2010.10012
- Mullins, M. G., Bouquet, A., and Williams, L. E. (1992). *Biology of the Grapevine*. Cambridge: Cambridge University Press.
- Myles, S., Boyko, A. R., Owens, C. L., Brown, P. J., Grassi, F., Aradhya, M. K., et al. (2011). Genetic structure and domestication history of the grape. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3530–3535. doi: 10.1073/pnas.1009363108
- Ocete Rubio, R., Ocete Rubio, E., Ocete Pérez, C., Ángeles Pérez, Izquierdo, M., Rustioni, L., et al. (2012). Ecological and sanitary characteristics of the Eurasian wild grapevine (*Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi) in Georgia (Caucasian region). *Plant Genet. Resour. Character. Util.* 10, 155–162. doi: 10.1017/S1479262112000160
- Oliveira, M. J. R. A., Roriz, M., Vasconcelos, M. W., Bertaccini, A., and Carvalho, S. M. P. (2019). Conventional and novel approaches for managing “flavescence dorée” in grapevine: knowledge gaps and future prospects. *Plant Pathol.* 68, 3–17. doi: 10.1111/ppa.12938
- Oliver, J. E., and Fuchs, M. (2011). Tolerance and resistance to viruses and their vectors in *Vitis* sp.: a virologist’s perspective of the literature. *Am. J. Enol. Vitic.* 62, 438–451. doi: 10.5344/ajev.2011.11036
- Olmo, H. P., McGovern, P. E., Fleming, S. J., and Katz, S. H. (1995). *The Origins and Ancient History of Wine*, Vol. 31. Abingdon: Routledge, 43.
- Palliotti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., and Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. *Sci. Hortic.* 178, 43–54. doi: 10.1016/j.scienta.2014.07.039
- Pangavhane, D. R., Sawhney, R. L., and Sarsavadi, P. N. (1999). Effect of various dipping pretreatment on drying kinetics of Thompson seedless grapes. *J. Food Eng.* 39, 211–216. doi: 10.1016/s0260-8774(98)00168-x
- Percival, D., Sullivan, J., and Fisher, K. (1993). Effect of cluster exposure, berry contact and cultivar on cuticular membrane formation and occurrence of bunch rot (*Botrytis cinerea* PERS.: FR.) with 3 *Vitis vinifera* L. cultivars. *Vitis* 32, 87–97.
- Pessina, S., Lenzi, L., Perazzolli, M., Campa, M., Dalla Costa, L., Urso, S., et al. (2016). Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. *Hortic. Res.* 3:16016. doi: 10.1038/hortres.2016.16
- Pugliese, M., Gullino, M. L., and Garibaldi, A. (2011). Effect of climate change on infection of grapevine by downy and powdery mildew under controlled environment. *Commun. Agric. Appl. Biol. Sci.* 76, 579–582.
- Quagliano, F., Maghradze, D., Casati, P., Chkhaidze, N., Lobjanidze, M., Ravasio, A., et al. (2016). Identification and characterization of new ‘*Candidatus* Phytoplasma solani’ strains associated with bois noir disease in *Vitis vinifera* L. cultivars showing a range of symptom severity in Georgia, the Caucasus Region. *Plant Dis.* 100, 904–915. doi: 10.1094/pdis-09-15-0978-re
- Quagliano, F., Maghradze, D., Chkhaidze, N., Casati, P., Failla, O., and Bianco, P. A. (2014). First report of ‘*Candidatus* Phytoplasma solani’ and ‘*Ca. P. convolvuli*’ associated with grapevine bois noir and bindweed yellows, respectively, in Georgia. *Plant Dis.* 98, 1151–1151. doi: 10.1094/pdis-01-14-0026-pdn
- Quagliano, F., Sanna, F., Moussa, A., Faccincani, M., Passera, A., Casati, P., et al. (2019). Identification and ecology of alternative insect vectors of ‘*Candidatus* Phytoplasma solani’ to grapevine. *Sci. Rep.* 9:19522.
- Quagliano, F., Zhao, Y., Casati, P., Bulgari, D., Bianco, P. A., Wei, W., et al. (2013). ‘*Candidatus* Phytoplasma solani’, a novel taxon associated with stolbur- and bois noir-related diseases of plants. *Int. J. Syst. Evol. Microbiol.* 63, 2879–2894. doi: 10.1099/ijs.0.044750-0
- Reid, P. C., Hari, R. E., Beaugrand, G., Livingstone, D. M., Marty, C., Straile, D., et al. (2016). Global impacts of the 1980s regime shift. *Glob. Chang. Biol.* 22, 682–703.
- Riaz, S., De Lorenzis, G., Velasco, D., Koehmstedt, A., Maghradze, D., Bobokashvili, Z., et al. (2018). Genetic diversity analysis of cultivated and wild grapevine (*Vitis vinifera* L.) accessions around the Mediterranean basin and Central Asia. *BMC Plant Biol.* 18:137. doi: 10.1186/s12870-018-1351-0
- Rosenquist, J. K., and Morrison, J. C. (1988). The development of the cuticle and epicuticular wax of the grape berry. *Vitis* 27, 63–70.
- Russell, P. (2005). A century of fungicide evolution. *J. Agric. Sci.* 143, 11–25. doi: 10.1017/s0021859605004971
- Rustioni, L. (2017). Oxidized polymeric phenolics: could they be considered photoprotectors? *J. Agric. Food Chem.* 65, 7843–7846. doi: 10.1021/acs.jafc.7b03704
- Rustioni, L., Basilico, R., Fiori, S., and Leoni, A. (2013). Grape colour phenotyping: development of a method based on the reflectance spectrum. *Phytochem. Anal.* 24, 453–459. doi: 10.1002/pca.2434

- Rustioni, L., Cola, G., Fiori, S., Failla, O., Bacilieri, R., Maul, E., et al. (2014). Application of standard methods for the grapevine (*Vitis vinifera* L.) phenotypic diversity exploration: phenological traits. *Acta Hortic.* 1032, 253–260. doi: 10.17660/ActaHortic.2014.1032.35
- Rustioni, L., Cola, G., Maghradze, D., Abashidze, E., Argiriou, A., Aroutiounian, R., et al. (2019). Description of the *Vitis vinifera* L. phenotypic variability in eno-carpological traits by a Euro-Asiatic collaborative network among ampelographic collections. *Vitis* 58, 37–46.
- Rustioni, L., Cola, G., VanderWeide, J., Murad, P., Failla, O., and Sabbatini, P. (2018). Utilization of a freeze-thaw treatment to enhance phenolic ripening and tannin oxidation of grape seeds in red (*Vitis vinifera* L.) cultivars. *Food Chem.* 259, 139–146. doi: 10.1016/j.foodchem.2018.03.120
- Rustioni, L., Lorenzis, G., De, and Monica, H. (2016). Plant Physiology and Biochemistry Pink berry grape (*Vitis vinifera* L.) characterization: reflectance spectroscopy, HPLC and molecular markers. *Plant Physiol. Biochem.* 98, 138–145. doi: 10.1016/j.plaphy.2015.11.018
- Rustioni, L., Maghradze, D., and Failla, O. (2012). Optical properties of berry epicuticular waxes in four Georgian Grape Cultivars (*Vitis vinifera* L.). *South Afric. J. Enol. Vitic.* 33, 138–143.
- Rustioni, L., Milani, C., Parisi, S., and Failla, O. (2015). Chlorophyll role in berry sunburn symptoms studied in different grape (*Vitis vinifera* L.) cultivars. *Sci. Hortic.* 185, 145–150. doi: 10.1016/j.scienta.2015.01.029
- Rustioni, L., Rossoni, M., Cola, G., Mariani, L., and Failla, O. (2011). Bunch exposure to direct solar radiation increases ortho-diphenol anthocyanins in Northern Italy climatic condition. *J. Int. DES Sci.* 45, 85–99. doi: 10.20870/oeno-one.2011.45.2.1489
- Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L.-T., Correia, C., et al. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Appl. Sci.* 10:3092. doi: 10.3390/app10093092
- Sargolzaei, M., Maddalena, G., Bitsadze, N., Maghradze, D., Bianco, P. A., Failla, O., et al. (2020). Rpv29, Rpv30 and Rpv31: three novel genomic loci associated with resistance to *Plasmopara viticola* in *Vitis vinifera*. *Front. Plant Sci.* 11:562432. doi: 10.3389/fpls.2020.562432
- Seemüller, E., and Schneider, B. (2007). Differences in virulence and genomic features of strains of ‘Candidatus Phytoplasma mali’, the apple proliferation agent. *Phytopathology* 97, 964–970. doi: 10.1094/PHYTO-97-8-0964
- Tabidze, V., Pipia, I., Gogniashvili, M., Kunelauri, N., Ujmajuridze, L., Pirtskhalava, M., et al. (2017). Whole genome comparative analysis of four Georgian grape cultivars. *Mol. Genet. Genomics* 292, 1377–1389. doi: 10.1007/s00438-017-1353-x
- Terral, J. F., Tabard, E., Bouby, L., Ivorra, S., Pastor, T., Figueiral, I., et al. (2010). Evolution and history of grapevine (*Vitis vinifera*) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. *Ann. Bot.* 105, 443–455. doi: 10.1093/aob/mcp298
- Thatcher, L. F., Powell, J. J., Aitken, E. A. B., Kazan, K., and Manners, J. M. (2012). The lateral organ boundaries domain transcription factor LBD20 functions in Fusarium wilt susceptibility and jasmonate signaling in *Arabidopsis*. *Plant Physiol.* 160, 407–418. doi: 10.1104/pp.112.199067
- This, P., Lacombe, T., and Thomas, M. R. (2006). Historical origins and genetic diversity of wine grapes. *Trends Genet.* 22, 511–519. doi: 10.1016/j.tig.2006.07.008
- Toffolatti, S. L., De Lorenzis, G., Brilli, M., Moser, M., Shariati, V., Tavakol, E., et al. (2020). Novel aspects on the interaction between grapevine and *Plasmopara viticola*: dual-RNA-Seq analysis highlights gene expression dynamics in the pathogen and the plant during the battle for infection. *Genes* 11:261. doi: 10.3390/genes11030261
- Toffolatti, S. L., De Lorenzis, G., Costa, A., Maddalena, G., Passera, A., Bonza, M. C., et al. (2018). Unique resistance traits against downy mildew from the center of origin of grapevine (*Vitis vinifera*). *Sci. Rep.* 8:12523. doi: 10.1038/s41598-018-30413-w
- Toffolatti, S. L., Maddalena, G., Salomoni, D., Maghradze, D., Bianco, P. A., and Failla, O. (2016). Evidence of resistance to the downy mildew agent *Plasmopara viticola* in the Georgian *Vitis vinifera* germplasm. *Vitis J. Grapevine Res.* 55, 121–128. doi: 10.5073/vitis.2016.55.121-128
- Töpfer, R., Hausmann, L., and Eibach, R. (2011). “Molecular breeding,” in *Genetics, Genomics and Breeding of Grapes*, eds A. F. Adam-Blondon, M. M. Zapater, and C. Kole (Enfield, NJ: Science Publishers), 160–185.
- Tsertsvadze, N. (2012). “Georgia: native varieties of grapevines,” in *Caucasus and Northern Black Sea Region Ampelography*, (Maghradze, Rustioni, Turok, Scienza, Failla eds) (Julius Kühn-Institut: Siebeldingen), 177–239.
- Ujmajuridze, L., and Mamasakhlisashvili, L. (2015). Agricultural and biological characteristics of Georgian grapevine varieties. *VITIS J. Grapevine Res.* 54, 163–164.
- Unger, S., Büche, C., Boso, S., and Kassemeyer, H.-H. (2007). The course of colonization of two different vitis genotypes by *Plasmopara viticola* indicates compatible and incompatible host-pathogen interactions. *Phytopathology* 97, 780–786. doi: 10.1094/phyto-97-7-0780
- van Leeuwen, C., and Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *J. Wine Econ.* 11, 150–167. doi: 10.1017/jwe.2015.21
- van Leeuwen, C., and Destrac-Irvine, A. (2017). Modified grape composition under climate change conditions requires adaptations in the vineyard. *OENO One* 51, 147–154. doi: 10.20870/oeno-one.2017.51.2.1647
- VanderWeide, J., Forte, A., Peterlunger, E., Sivillotti, P., Medina-Meza, I. G., Falchi, R., et al. (2020). Increase in seed tannin extractability and oxidation using a freeze-thaw treatment in cool-climate grown red (*Vitis vinifera* L.) cultivars. *Food Chem.* 308:125571. doi: 10.1016/j.foodchem.2019.125571
- Venuti, S., Copetti, D., Foria, S., Falginella, L., Hoffmann, S., Bellin, D., et al. (2013). Historical introgression of the Downy Mildew Resistance Gene Rpv12 from the Asian Species *Vitis amurensis* into Grapevine Varieties. *PLoS One* 8:e61228. doi: 10.1371/journal.pone.0061228
- Weintraub, P. G., and Beanland, L. (2006). Insect vectors of phytoplasmas. *Annu. Rev. Entomol.* 51, 91–111. doi: 10.1146/annurev.ento.51.110104.151039
- Welter, L. J., Tisch, C., Kortekamp, A., Topfer, R., and Zyprian, E. (2017). Powdery mildew responsive genes of resistant grapevine cultivar “regent”. *Vitis J. Grapevine Res.* 56, 181–188. doi: 10.5073/vitis.2017.56.181-8
- Zaidi, S. S.-A., Mukhtar, M. S., and Mansoor, S. (2018). Genome editing: targeting susceptibility genes for plant disease resistance. *Trends Biotechnol.* 36, 898–906. doi: 10.1016/j.tibtech.2018.04.005
- Zhou, Y., Massonnet, M., Sanjak, J. S., Cantu, D., and Gaut, B. S. (2017). Evolutionary genomics of grape (*Vitis vinifera* ssp. *vinifera*) domestication. *Proc. Natl. Acad. Sci. U.S.A.* 114, 11715–11720. doi: 10.1073/pnas.1709257114
- Zhou, Y., Muyle, A., and Gaut, B. S. (2019). “Evolutionary genomics and the domestication of grapes,” in *The Grape Genome*, eds D. Cantu and M. A. Walker (Cham: Springer Nature Switzerland), 39–55. doi: 10.1007/978-3-030-18601-2_3
- Zohary, D., and Hopf, M. (2000). *Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe, and the Nile Valley*. Oxford: Oxford University Press.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Sargolzaei, Rustioni, Cola, Ricciardi, Bianco, Maghradze, Failla, Quaglino, Toffolatti and De Lorenzis. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Molecular Tools for Adapting Viticulture to Climate Change

Éric Gomès¹, Pascale Maillot^{2,3} and Éric Duchêne^{2*}

¹ EGFV, University of Bordeaux – Bordeaux Sciences-Agro – INRAE, Villenave d'Ornon, France, ² SVQV, INRAE – University of Strasbourg, Colmar, France, ³ University of Haute Alsace, Mulhouse, France

OPEN ACCESS

Edited by:

Chris Winefield,
Lincoln University, New Zealand

Reviewed by:

Ksenija Taski-Ajdukovic,
Institute of Field and Vegetable Crops,
Serbia

Luisa C. Carvalho,
University of Lisbon, Portugal

*Correspondence:

Éric Duchêne
eric.duchene@inrae.fr

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 26 November 2020

Accepted: 19 January 2021

Published: 10 February 2021

Citation:

Gomès É, Maillot P and
Duchêne É (2021) Molecular Tools
for Adapting Viticulture to Climate
Change. *Front. Plant Sci.* 12:633846.
doi: 10.3389/fpls.2021.633846

Adaptation of viticulture to climate change includes exploration of new geographical areas, new training systems, new management practices, or new varieties, both for rootstocks and scions. Molecular tools can be defined as molecular approaches used to study DNAs, RNAs, and proteins in all living organisms. We present here the current knowledge about molecular tools and their potential usefulness in three aspects of grapevine adaptation to the ongoing climate change. (i) Molecular tools for understanding grapevine response to environmental stresses. A fine description of the regulation of gene expression is a powerful tool to understand the physiological mechanisms set up by the grapevine to respond to abiotic stress such as high temperatures or drought. The current knowledge on gene expression is continuously evolving with increasing evidence of the role of alternative splicing, small RNAs, long non-coding RNAs, DNA methylation, or chromatin activity. (ii) Genetics and genomics of grapevine stress tolerance. The description of the grapevine genome is more and more precise. The genetic variations among genotypes are now revealed with new technologies with the sequencing of very long DNA molecules. High throughput technologies for DNA sequencing also allow now the genetic characterization at the same time of hundreds of genotypes for thousands of points in the genome, which provides unprecedented datasets for genotype-phenotype associations studies. We review the current knowledge on the genetic determinism of traits for the adaptation to climate change. We focus on quantitative trait loci and molecular markers available for developmental stages, tolerance to water stress/water use efficiency, sugar content, acidity, and secondary metabolism of the berries. (iii) Controlling the genome and its expression to allow breeding of better-adapted genotypes. High-density DNA genotyping can be used to select genotypes with specific interesting alleles but genomic selection is also a powerful method able to take into account the genetic information along the whole genome to predict a phenotype. Modern technologies are also able to generate mutations that are possibly interesting for generating new phenotypes but the most promising one is the direct editing of the genome at a precise location.

Keywords: grapevine, climate change, adaptation, molecular tools, QTL, gene expression

INTRODUCTION

Expected Impacts of Climate Change

The increase of atmospheric CO₂ concentrations is the main trigger of the greenhouse effect that led to an increase in earth surface temperature (IPCC, 2013). As such, higher (CO₂) is beneficial to photosynthesis and consequently to plant growth. Indirectly, for an equivalent amount of carbon fixed, an elevated (CO₂) is associated with higher water use efficiency (WUE), i.e., lower transpiration of water through stomata (Schultz, 2000).

The past increase of temperatures already led to an advance of developmental changes, well documented all over the world. The tight relationship between temperatures and grapevine phenology allows predicting that this trend will continue (Duchêne et al., 2010; Morales-Castilla et al., 2020).

The first consequence of earlier dates of *véraison* is an increase in temperatures during the ripening period. The ripening period is indeed not only shifting toward the warmest period of summer, at least in the Northern hemisphere, but also temperatures are higher on the same calendar day (Molitor and Junk, 2019). The extent of the advances of budburst dates is still uncertain because they depend on the dates of dormancy release (Leolini et al., 2020), which are difficult to observe and therefore to model. Higher risks of spring frost after budburst should not be overlooked and could increase in vineyards in northern France (Sgubin et al., 2018).

The fulfillment of water needs results from the atmospheric water demand, the soil water availability, and the grapevine canopy architecture. Changes in precipitations in the future are not expected to be uniform, contrasts between wet and dry areas, wet and dry seasons should increase as well as the frequency of extreme precipitation events (IPCC, 2013). The last IPCC report predicts specific regional changes but does not confirm a general tendency of increased drought risks. The evolution of atmospheric water demand is a matter of debate. Using computational methods such as the Penman-Monteith-FAO equation, the evapotranspiration (ET) is believed to increase together with temperatures but trends for a decrease in pan evaporation were also reported (Roderick et al., 2009; Schultz, 2017). The opinion that the water deficit will increase in the future is nevertheless widely shared.

Climate change can have indirect effects on the grapevine by changing the existing equilibrium with pests and diseases. The capacity of the soils to provide nutrients such as nitrogen could also evolve: reduced soil humidity can not only induce water stress but also impair the mineralization of the soil organic matter, and consequently lower nitrogen availability on the top horizons (Curtin et al., 2012).

At last, more frequent extreme events (heavy rains, storms, hail, unexpected cold, or heat waves) can severely impair the long-term sustainability of grape production, but

such events are not predictable and technical solutions are difficult to implement.

Consequences on Yield and Grape and Wine Composition

Climate change can have direct effects on yield components: not only spring frosts can destroy young shoots but higher temperatures around budburst can lower the number of flowers per inflorescence (Petrie and Clingeleffer, 2005). As a consequence of elevated (CO₂), a higher plant vigor and biomass production in the future, as observed in field-conducted FACE (Free Air Carbon Enrichment) CO₂ enrichment experiments (Bindi et al., 2005; Wohlfahrt et al., 2018), can likely result in a higher number of inflorescences and flowers per shoot. However, drought during summer can reduce single berry weight in the current season but also lower the number of inflorescences per shoot in the following one (Matthews and Anderson, 1989).

The environmental conditions during ripening concentrate most of the interest, not only of the scientific community but also of producers and winemakers.

Temperatures during ripening are expected to increase, with negative effects on secondary metabolism, such as anthocyanin synthesis (Lecourieux et al., 2017) and faster degradation of malic acid (Lecourieux et al., 2017). Meanwhile, sugar concentrations have increased during the last decades very likely because of the shift of the ripening period toward longer days, and hence, higher global radiation interception. Sugar accumulation could however be limited in the future by reduced water availability, which can not only lower gas exchanges through stomata and photosynthesis activity, but also sometimes impair the ripening process.

Adaptation of viticulture to these changes includes exploration of new geographical areas, new training systems, new management practices, or new varieties, both for rootstocks and scions.

MOLECULAR TOOLS FOR UNDERSTANDING THE RESPONSE OF GRAPEVINE TO ENVIRONMENTAL CONDITIONS

Molecular tools can be defined as molecular approaches used to study DNAs, RNAs, and proteins in all living organisms, including grapevine.

A fine description of the regulation of gene expression is a powerful tool to understand the physiological mechanisms set up by the grapevine to respond to abiotic stress such as high temperatures or drought. The current knowledge on gene expression is continuously evolving with increasing evidence of the role of small RNAs, long non-coding RNAs (lncRNAs), DNA methylation or chromatin activity, and, more recently, of alternative transcription of pre-mRNAs.

In parallel, the description of the grapevine genome is more and more precise. After the first release of a whole-genome sequence for the PN40024 line (Jaillon et al., 2007), the

genetic variations among genotypes are now revealed by new technologies with very long reads of single DNA molecules (Chin et al., 2016). High throughput technologies for DNA sequencing also now allow the genetic characterization at the same time of hundreds of genotypes for thousands of points in the genome, which provides unprecedented datasets for genotype-phenotype associations studies.

At last, new methods for genome editing open the gate for efficient and stable genetic transformations of the grapevine.

Transcriptomics

Medium to high throughput transcriptome analysis in grape roots back to the Expressed Sequence Tags (ESTs) programs of the end of the 1990s and the beginning of the years 2000s, which provide the first probe set for the first-generation 3,200 unigenes microarrays used to study grape development (Terrier et al., 2006, 2011). The number of unigenes present on the microarrays rapidly expanded to 14,500 with the Operon (Camps et al., 2010) or Affymetrix (Deluc et al., 2009) grape arrays. Then, with the release of the 12X genome sequence of the PN40024 line, (nearly) genome-wide NimbleGen microarrays, with over 29,000 unigenes represented, were used to study grape transcriptome (Pastore et al., 2014). Full coverage of the grapevine transcriptome was finally achieved by the use of next-generation deep RNA-sequencing (RNA-seq; Zenoni et al., 2010), which provides greater flexibility than microarrays, allowing to work with genotypes distant to the grape reference genome, including non-*vinifera* *Vitis* species. Both genome-wide microarrays and RNA-seq have been used to characterize the response of grapevine to drought stress (Berdeja et al., 2015; Corso et al., 2015), UV-B/light intensities (Carbonell-Bejerano et al., 2014; du Plessis et al., 2017), and elevated temperature (Rienth et al., 2014, 2016; Lecourieux et al., 2017). Such high-throughput transcriptomics can highlight relevant candidate genes for future breeding programs tailored to produce new grape cultivars better adapted to anticipated climate change conditions, provided that two conditions are met. Firstly, it is paramount that transcriptomics is applied on an eco-physiologically sound and well-characterized experimental plot, with a precise quantitation of the applied stress factor and its physiological impact on the plants (Berdeja et al., 2015). Even the modalities of stress application can be of importance. For example, Rienth et al. (2014) recently demonstrated that the same elevation of temperature applied on grapevine plants during day or night periods led to distinct transcriptomic modulations, suggesting different acclimation responses (Rienth et al., 2014). Secondly, to get the most out of transcriptomic approaches, it is highly recommended to go beyond classical differentially expressed gene analysis and use powerful data mining and meta-analysis tools, such weighted gene co-expression network analysis, that allows identifying co-regulated gene modules and master “switch” genes that are most likely to be key for abiotic stress responses (Palumbo et al., 2014; Hopper et al., 2016; Cochetel et al., 2017). Last, but not least, to the best of our knowledge, all transcriptomic studies published so far on grapevine deal with the response to one single abiotic factor, often applied in controlled or semi-controlled conditions. This is in contradiction with the fact

that in the frame of the ongoing global climate change, several abiotic factors will be affected and will most certainly interact to affect grapevine physiology and grape ripening, as evidenced for UV-B and drought (Martinez-Lüscher et al., 2014; Martinez-Lüscher et al., 2015a), water availability and elevated temperature (Zarrouk et al., 2016), UV-B, temperature and ambient CO₂ levels (Martinez-Lüscher et al., 2015b; Martinez-Lüscher et al., 2016; Arrizabalaga-Arriazu et al., 2020). Future transcriptomic studies aiming to provide relevant molecular data to breed new cultivars better adapted to future climatic conditions will have to integrate stress combinations in their experimental design.

Proteomics

Thanks to continuous technological improvement, grapevine proteomic have evolved from 2D gel electrophoresis techniques to large-scale shotgun proteomics using iTRAQ labeling or more recently label-free quantification methods, using multiplexed hybrid mass spectrometers (Vincent et al., 2007; Cramer et al., 2013). Besides transcriptomic, proteomic studies can also provide relevant and complementary information on grapevine response to abiotic stimuli at the molecular level (George and Haynes, 2014; Cramer et al., 2017). Indeed, reports of parallel transcriptome and proteome analysis in response to environmental abiotic factors have shown that transcript levels were not always directly correlated to corresponding protein abundance in various tissues or organs, highlighting the multiple (i.e., transcriptional, translational, and post-translational) levels of gene regulation (Lan et al., 2012; Pan et al., 2012; Wu et al., 2014). This demonstrates the added value of proteomic approaches to decipher grapevine molecular response to climate change-related abiotic factors such as elevated temperature (Liu et al., 2014; Jiang et al., 2017; Lecourieux et al., 2020) or long-term drought stress (Krol and Weidner, 2017).

Transcriptome Complexification by Alternative Splicing

The full transcriptome includes messenger RNAs (mRNAs) carrying the coding sequences and “non-coding RNAs” (ncRNAs). Recently, alternative splicing (AS) has been shown to participate in the construction of the complete RNA landscape, by being able to generate multiple transcripts from a single multi-exon gene (Reddy et al., 2013). Besides the canonical isoform, a subset of alternative transcripts may arise by intron retention (IR), exon skipping (ES), or usage of alternative splice sites (5'- and 3'-ASS). This notwithstanding, not all alternative transcripts fulfill biological functions, since the use of alternative splice sites may introduce premature termination codons (PTCs) targeting transcripts to the cytoplasmic nonsense-mediated mRNA decay (NMD) pathway (Chaudhary et al., 2019). However, a significant proportion of non-canonical mRNAs are thought to serve in gene expression regulation while some others are likely to encode functional proteins. Like in other plants, AS is ubiquitous in grapevine (Vitulo et al., 2014) and numerous alternative isoforms have been identified and included in the *V. vinifera* reference genome annotation (Canaguier et al., 2017). Both constitutive and AS occur in the nucleus, mainly co-transcriptionally, and

are catalyzed by the spliceosome, a macromolecular complex regulated by splicing factors such as serine and arginine-rich (SR) proteins and heterogeneous ribonuclear proteins (hnRNPs) (Syed et al., 2012). Interestingly, SR proteins are themselves subjected to differential splicing, notably under stress conditions (Palusa et al., 2007).

Alternative splicing is regulated during plant growth and development, being highly sensitive to environmental signals. Among positive examples, many genes depending on the circadian clock are prone to AS, enabling the plant to rapidly modify its physiological activity in response to changing conditions during the 24-h cycle (Gil and Park, 2019). Light and temperature are the main stimuli modulating the circadian clock: heat stress induces the differential splicing of several core clock genes, the manipulation of which being of particular interest in the view of adaptation to climate warming (Gil and Park, 2019).

A better knowledge of the genetic determinism and AS regulation of phenological traits could also be very helpful for selecting climate-resilient varieties. Precisely, several genes determining the flowering time are submitted to splicing regulation, which modulates their functioning based on light and temperature conditions. For example, the flowering activator *CONSTANS* (*CO*) is affected by AS upon light fluctuations, producing a full-size functional protein isoform (*CO* α) and a C-terminally truncated isoform (*CO* β) acting as a competitive inhibitor of *CO* α (Park et al., 2019). Moreover, the flowering repressor *FLOWERING LOCUS M* (*FLM*) expresses multiple splicing variants, whose predominant isoforms *FLM* β (repressor) and *FLM* δ (activator) result from alternative usage of two mutually exclusive exons (Nibau et al., 2019). Differential splicing of *FLM* is controlled by temperature variation, preferentially releasing one or other of these two isoforms for fine-tuning the flowering time (Figure 1).

High light conditions, extreme temperatures, and water stress are powerful inducers of AS, a process therefore supposed to trigger plant adaptation to hard environmental conditions (Filichkin et al., 2018). Heat shock transcription factors (HSFs) conferring heat tolerance, are under the control of the DEHYDRATION-RESPONSIVE ELEMENT BINDING 2

(DREB2) transcription factor, which is differentially spliced in response to abiotic stress, the full-length functional transcript being only produced under stress conditions (Egawa et al., 2006). Moreover, converging evidence suggests that AS modulates the expression of genes of the abscisic acid (ABA) pathway, in response to abiotic stresses (Laloum et al., 2018). One example is provided by the differential splicing of the negative regulator *HAB1*, a PP2C protein able to dephosphorylate *OST1* involved in stomatal movement, leading to the on-off control of the plant response to ABA (Wang et al., 2015). In grapevine, application of a heat shock (35–45°C) greatly modified the leaf transcriptome, AS pattern, and proteome (Jiang et al., 2017). In particular, the transcription level of several SR proteins, as well as their phosphorylation status, a marker of functionality, significantly increased with temperature, showing that the whole splicing machinery was modulated (Jiang et al., 2017; Liu et al., 2019). Because transcription and translation are energy costly, the strong induction of AS under stress conditions is suspected to be a means for reducing the amount and diversity of translatable transcripts (Chaudhary et al., 2019). Intron-retaining transcripts are preferentially produced following abiotic stress application, and accumulate in the nucleus as non-mature isoforms, enabling rapid suspension of translational activity. By this way, nucleus-sequestered transcripts escape to NMD and remain available for further rapid processing and release to the cytoplasm, upon favorable conditions.

Although AS events may be conserved among species and genotypes, some studies have reported on differential AS behavior of distinct genotypes subjected to stress conditions. Two rice varieties, with contrasting levels of tolerance to water stress, showed extensive differential AS when submitted to drought conditions (Zhang and Xiao, 2018). AS divergence affected genes belonging to usual stress response pathways, as well as many spliceosome- and DNA damage repair-related genes that could also be involved in the adaptation to water stress, as suggested by their co-localization with drought-related quantitative trait loci (QTLs) (Zhang and Xiao, 2018). Among others, this strongly suggests that intraspecific genetic variation of components of the splicing machinery itself contributes to differential adaptability

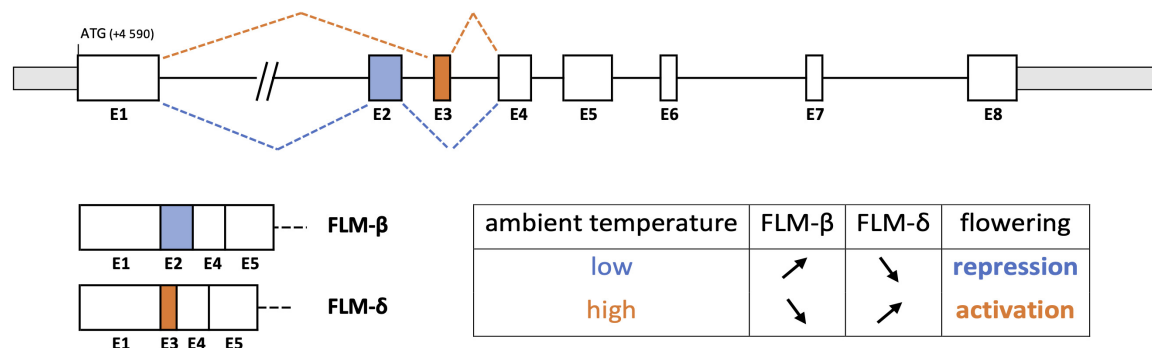


FIGURE 1 | Temperature-dependent alternative splicing of *FLOWERING LOCUS M* (*FLM*) in *Arabidopsis thaliana* (Capovilla et al., 2017). Alternative usage of exons 2 and 3, two mutually exclusive exons, acts as a thermosensitive regulator in the flowering time pathway. The *FLM* β variant isoform is down-regulated by increasing ambient temperature while the *FLM* δ variant is up-regulated, inducing flowering.

to climatic conditions. Similarly, in *Arabidopsis*, a very low overlap was found between AS patterns of different accessions submitted to temperature changes (Wang X. et al., 2019). DNA polymorphism was associated with AS pattern specificity, most probably accounting for genetic adaptation to distinct native environments. Characterizing genotype-dependent AS patterns in controlled stressful conditions could thus provide an opportunity to identify genes active in stress alleviation. Moreover, the characterization of specific alternative isoforms involved in phenological traits and the response to abiotic stresses should certainly help improving grapevine adaptability to future climate scenarios.

Regulation patterns of transcription intensity and AS, in response to developmental requirements and environmental cues, have most often been reported to overlap poorly, identifying AS as an important process acting independently in transcriptome reprogramming (Karlebach et al., 2020).

Regulation of Gene Expression: Non-coding RNAs and Micropeptides

There is increasing literature about the role of ncRNAs in the regulation of gene expression patterns in response to environmental conditions, including drought stress (Visentin et al., 2020) and more generally adaptation to climate change (Xu et al., 2019). Small ncRNAs include microRNAs (miRNAs, 21–24 nt) and small interfering RNAs (siRNAs), whereas lncRNAs are RNAs that are more than 200 nt long (Harris et al., 2017) and do not contain an open reading frame. Small RNAs are mobile in the plants and siRNA-dependent epigenetic modifications could be heritable (Pagliarani and Gambino, 2019). RNAs derived from tRNAs and rRNAs also seem to participate in the response to abiotic stress (Cao et al., 2016). siRNAs and lncRNAs also play a role in DNA methylation (Matzke et al., 2015; Tamiru et al., 2018). Additionally, AS is tightly linked to miRNA-mediated regulation of gene expression, in particular via inclusion/exclusion of miRNA target sequences in distinct transcript isoforms, enabling differential regulation by the corresponding small RNA (Yang et al., 2012).

For the grapevine, Belli Kullán et al. (2015) constructed an atlas of miRNAs expression using 70 libraries. They identified 110 already known miRNAs and 185 novel miRNAs. One of their main conclusions is that miRNAs profiling shapes organ identity and that they participate in hormonal regulation. In line with this idea, Carra et al. (2009) had previously identified siRNA 165 as targeting a cytokinin synthase gene, and Wang et al. (2017) VvmiR061 as regulating the gibberellin-signaling pathway. More recently, Rossmann et al. (2020) showed that miR396 participate in the genetic variations of inflorescence architecture in grapevine. Regarding abiotic stress for the grapevine, Leng et al. (2017) showed that miR398 upregulation enhanced the tolerance to oxidative stress and Sun et al. (2015) described the effects of cold on the pattern of miRNAs expression.

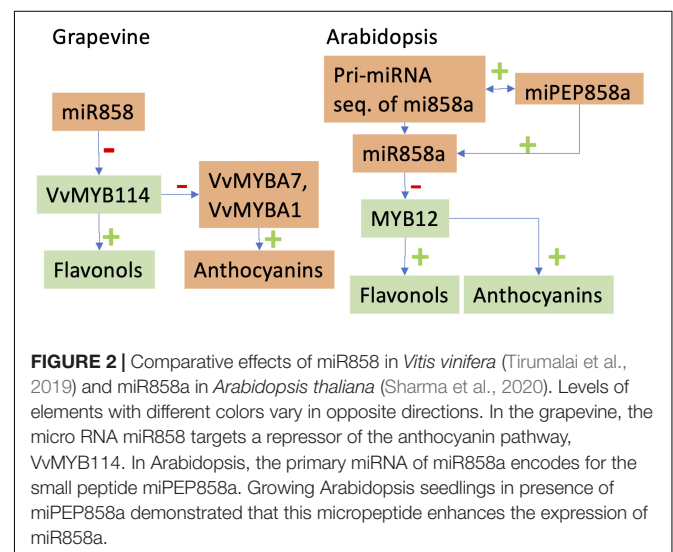
MicroRNAs profiles are different between irrigated/drought stress conditions but also depend on the grafting combinations (Pagliarani et al., 2017). Pantaleo et al. (2016) also showed the regulations of several miRNAs in response to water stress and to

virus infection. In both studies, the expected negative correlation between the abundance of miRNAs and their targeted genes was however not always observed. These results nevertheless open new perspectives for using miRNAs for controlling the genome expression toward a better adaptation to abiotic stress. We can also speculate that miRNAs could be used to control the secondary metabolism of grapevine berries. For example, it was shown that miR828 and miR858 regulate VvMYB114 to promote anthocyanin and flavonol accumulation in grapes (Figure 2; Tirumalai et al., 2019).

Long non-coding RNAs can play a role in the vernalization processes (Liu et al., 2018), in fruit ripening (Arrizabalaga et al., 2018) or in the response to fungal infections (Chen et al., 2018). lncRNAs were identified in the grapevine (Harris et al., 2017; Bhatia et al., 2019; Wang P. et al., 2019) where they participate in many biological functions via interactions with both coding and ncRNAs as well as with transcription factors. They can participate in the response to abiotic stress such as cold stress (Wang P. et al., 2019). To further increase the complexity of gene expression regulation, Chen et al. (2018) also highlighted the role of circular RNAs, related to transposons, in transcriptomic variations in maize leaves.

There is currently no specific knowledge on how to control gene expression in the context of grapevine adaptation to climate change. However, Castro et al. (2016) proved the concept of using miRNAs for genetic engineering by constructing an artificial miRNA precursor, whose corresponding miRNA was able to silent a GFP gene and methods are currently set up for inducing gene silencing by spraying small RNAs on plants (Dalakouras et al., 2016). Application of RNA molecules is even now suggested as a method to trigger RNA interference instead of using genetically modified (GM) organisms (Dalakouras et al., 2020).

Another emerging field is the role of non-conventional micropeptides in the control of biological processes (Lauressergues et al., 2015; Wang et al., 2020). Regarding the previously cited example of the effects of miR828 and miR858 on anthocyanin and flavonol synthesis in grapevine (Tirumalai et al., 2019),



Sharma et al. (2020) demonstrated that pri-miR858a of *Arabidopsis thaliana* encodes a small peptide, miPEP858a, which regulates the expression of miR858a and associated target genes (Figure 2). Chen et al. (2020) also shown that a miRNA-encoded small peptide, miPEP171d1, regulates the formation of adventitious roots. These results increase the complexity of mechanisms of the regulation of gene expression but provide us with tools to better control the phenotypes of grapevine under changing environmental conditions.

Epigenetics: DNA Methylation and Histone Modifications

The synthesis of an mRNA requires that the corresponding DNA is accessible to the transcriptional machinery. DNA in eukaryotes is wrapped on a structure named chromatin, made of an assembly of proteins called histones. DNA methylation of specific cytosines as well as post-translational modifications (PTMs) of histones, such as acetylation or phosphorylation, determine the accessibility of the genomic information to the transcriptional machinery and the ability to synthesize an mRNA (Gallusci et al., 2017).

DNA methylation and histones PTMs are powerful mechanisms to modulate the gene expression patterns and plant responses to stress (Fortes and Gallusci, 2017). The extent of the actual influence of DNA methylation on gene expression patterns and the level of independence between DNA methylation and genetic variations is however a matter of debate (Seymour and Becker, 2017). Epigenetic changes are part of the developmental program of plants (Gallusci et al., 2017; Shangguan et al., 2020), including sex determination (Latrasse et al., 2017), and can occur in response to changing environments (Fortes and Gallusci, 2017), even at a very small scale (Konate et al., 2020). Epigenetics can be considered as a source of adaptation in perennial species (Brautigam et al., 2013; Gallusci et al., 2017). The heritability and stability of epigenetic changes across generations may however be variable according to the loci (Tricker et al., 2013) or the presence of the initial stress (Tricker et al., 2013). For the grapevine, DNA methylation was shown to participate in the regulation of stilbene synthase genes (Kiselev et al., 2013) and of *VvUGT*, the gene coding for the anthocyanidin 3-*O*-glucosyltransferase which stabilizes anthocyanidins by glycosylation, allowing red grape varieties to accumulate anthocyanins during maturation (Jia et al., 2020). Histone modifications may also play a role in the regulation of the expression of *VvOMT3*, a gene coding for a methyltransferase (Battilana et al., 2017).

Different methylation patterns were described among grapevine clones of the same variety by methylation-sensitive amplified polymorphism (MSAP) (Ocana et al., 2013). DNA methylation is a dynamic process highly influenced by environmental conditions (Marfil et al., 2019). Methylation patterns (MSAP and methylation-sensitive genotyping by sequencing) in plants of Syrah could be associated with their geographical origin and to the pruning system (Xie et al., 2017). Varela et al. (2020) also showed the effects of the environment on the MSAP profiles but the three clones studied did not respond

in the same way, which suggests that epigenetic modifications also depend on genetic variations between clones.

These results raise the idea that environmental conditions can generate clonal variations. For poplar trees, there are indications that clonal history can shape the transcriptomic profiles after modifying the level of DNA methylation (Brautigam et al., 2013).

Recently, using bisulfite sequencing polymerase chain reaction, Jia et al. (2020) demonstrated that the DNA methylation level modulates AS of the *VvDFR* (dihydroflavonol-4-reductase), *VvCHS* (chalcone synthase), and *VvGST* (glutathione-S-transferase) genes in ripening Kyoho grapes by IR, altering berry anthocyanin content. Indeed, given the fact that AS proceeds co-transcriptionally, the chromatin state unsurprisingly interferes with splicing regulation (Rahhal and Seto, 2019). For instance, histone acetylation, by inducing chromatin decompaction, speeds up transcription elongation, enabling splicing factors recruitment only at the strongest splice sites and favoring ES. Also, H3K36 methylation, prevalent in actively transcribed gene regions, has been shown to mark genes with temperature-induced AS (Pajoro et al., 2017). It is worth noting that AS could also be implied in stress memories. Priming, which enables the development of a rapid and adequate response to stress after a first exposure, has long been known to be based on heritable chromatin modifications (Mauch-Mani et al., 2017). Interestingly, splicing memory, highlighted by de-repression of AS, has been observed in heat-primed plants after exposure to further lethal stress, suggesting another link between AS and epigenetic footprints (Ling et al., 2018).

If the hypothesis that environmental conditions induce epigenetic adaptations is validated, we can imagine that grapevine plants could be artificially “prepared” for new climatic conditions.

GENETICS AND GENOMICS

Tools and Methods

The complete sequence of the grapevine genome is available since 2007 after the sequencing and assembly of the nearly homozygous PN40024 line (Jaillon et al., 2007). This first release has been widely used in numerous studies and was improved on the one hand by reducing the number of pseudomolecules representing the chromosomes (Canaguier et al., 2017) and on the other hand by improving the predictions of genes structures, i.e., gene annotations, and the corresponding transcripts. The 12xV2 release of the PN40024 genome¹ comprises 19 pseudomolecules (for the 19 chromosomes) covering 458,641,822 bp and a pseudomolecule of 2,654,308 bp for all the non-anchored scaffolds. Three sources for gene annotations were used to propose a V3 set of annotations (Canaguier et al., 2017). A total of 42,414 gene structures were predicted but only 15,288 were present in the three annotations sources. Reliable gene annotations are necessary to predict the protein sequences, but also to allow precise quantification of gene expression with RNA sequencing techniques (RNA-seq).

¹https://urgi.versailles.inra.fr/jbrowse/gmod_jbrowse/

The sequence of the PN40024 line is the reference for identifying genetic variations between genotypes. Resequencing 47 genotypes allowed the design of a DNA chip able to reveal the polymorphisms at the level of a single nucleotide (single nucleotide polymorphism, SNP) at 18,071 positions of the genome. Laucou et al. (2018) used this DNA-chip to characterize 783 different genotypes from the germplasm of Vassal and proposed 118 full parentages and 490 parent-offspring duos. Short reads sequencing was also used to identify variations on the DNA from different clones of Nebbiolo (Gambino et al., 2017) and to characterize progenies by “Genotyping by sequencing” (GBS) (Tello et al., 2019). These high throughput technologies for DNA sequencing give access to a very detailed view of the genetic variability and proved also powerful to identify genes not represented in the reference genome (Da Silva et al., 2013) and to characterize “catastrophic” rearrangements among chromosomes (Carbonell-Bejerano et al., 2017). They however failed to describe the high heterozygosity of the grapevine genome. Single DNA molecule sequencing [Pacific Biosciences® Single-Molecule Real-Time (SMRT) technology] was used for the first time for the Cabernet-Sauvignon genome (Chin et al., 2016). The range of read length was 30–100 kb, giving access to the information on haplotypes, i.e., a precise description of the DNA sequence for each chromosome of the same pair. Gambino et al. (2017) reported that 4,900 new loci could be found in the Cabernet-Sauvignon sequence when compared to PN40024. The Pacific Biosciences® SMRT technology was also used to identify full-length cDNAs in the Cabernet-Sauvignon berry transcriptome, showing the extent of AS (Minio et al., 2019). Recently, a combination of long reads (Pacific Biosciences® SMRT) and short reads (Illumina HiSeq3000 and 2500), allowed the *de novo* phased assembly of the *Vitis riparia* cv. Gloire de Montpellier genome, with a 30× coverage, paving the way for future genome sequence-assisted grapevine rootstock breeding (Girrollet et al., 2019).

All these tools and methods are very useful to decipher the links between variations in DNA sequences and traits of interest, especially when considering adaptation to climate change.

Genetic Determinism of Traits for the Adaptation to Climate Change

Using new varieties or clones is a natural answer when speaking about adaptation to climate change. Present choices of genotypes are adapted to local environmental conditions, soil, meso-climate, microclimate, and to the profile of wine produced. The strategy for local adaptation in the future can be to try to maintain the type of wine that made the renown of the area; it can also consist in a shift, from white to red wines production for example.

If a change in terms of market is possible and accepted, it is likely that technical solutions for adaptation to climate change already exist for most of the grape-growing regions in the world: scion × rootstock × training system combinations are already used for dry and hot environments in the South of Spain, in Chile or Australia.

The specifications of an ideotype for a variety adapted to climate change can be divided into several chapters. With the aim that the ripening period avoids the warmest periods of summer,

a strategy can be to shift this period later in autumn by choosing late genotypes. We could however show that it will be more and more difficult to follow the pace of temperature increase, which shifts the ripening period earlier in summer while the “cool” period moves later in autumn (Duchêne et al., 2010). Another strategy, yet not tested, is to propose very early varieties, whose ripening would take place before the peak of temperatures in summer. In this case, their ripening period would shift toward spring with climate change, in a “self-adaptive” mode (Figure 3). This possibility is however limited by the date of budbreak, which cannot be too early to avoid risks of spring frosts.

The following challenge is also to maintain an economically sustainable yield, especially in the case of drought. New adapted varieties should have a high WUE, i.e., maximize the “crop per drop,” and should be able to maintain the ripening process of the grapes even in case of severe water stress. Keeping an active photosynthetic system under high temperatures or after heatwaves would also be a requested feature but the main challenge is to produce high-quality wines under warm conditions. High temperatures accelerate the degradation of malic acid, impair anthocyanin accumulation, and can be detrimental to aromas or aroma precursors synthesis. The ability to maintain a good acidity of the berries, color, and aromas even under high temperatures is a key expectation for a variety adapted to climate change.

Solutions provided by clonal diversity are the easiest to implement, as they do not require any change in the local legal rules. A lot of accessions are available. In Alsace for example, 1168 clones, representing nine varieties, are present in the INRAE germplasm collections. Contrasted behaviors of Tempranillo clones toward temperatures exist (Arrizabalaga et al., 2018) but

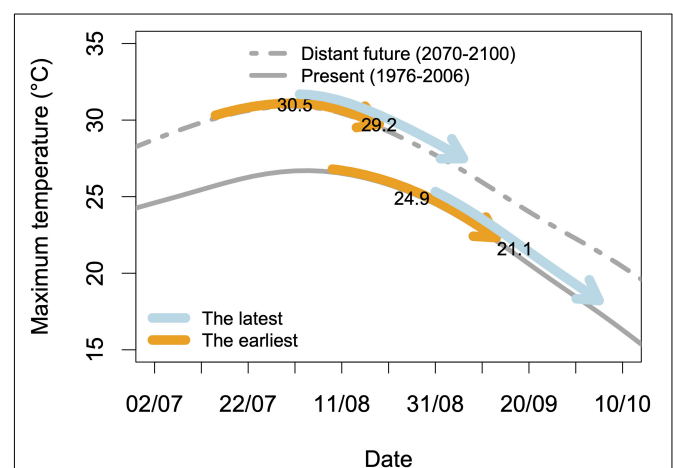


FIGURE 3 | Simulations of maximum temperatures during the ripening period for two virtual extreme genotypes and two climatic datasets. The arrows represent the ripening periods, i.e., 35 days starting at 50% véraison, for two virtual genotypes: the earliest and the latest that should be found in an infinite progeny from a Riesling × Gewürztraminer cross. Two climatic datasets are used: historical data from 1976 to 2006 and simulated data (A1B scenario) for Colmar (48°04'46.3"N 7°21'26.0"E). Details in Duchêne et al. (2010). The figures are the mean values of maximum temperatures during these periods.

the extent of clonal genetic variability useful for the adaptation to climate change might be limited.

Evaluating varieties already cultivated in warm and dry regions is another source of adaptation, but wine producers can be reluctant to adopt varieties previously cultivated elsewhere.

The third way is to create new varieties by breeding. A surprisingly high number of well-renowned cultivars are the progeny of crosses, including Cabernet-Sauvignon, Chardonnay, Merlot, or Syrah (Lacombe et al., 2013). The need for reducing the use of fungicides, but also the idea of adaptation to climate change, has recently stimulated “*de novo*” breeding programs, including in wine-producing areas with protected designation of origin. Molecular markers are key components in these modern approaches.

Whatever the trait of interest, the approach used to detect links between variations in the DNA sequence and values for this trait is the same. First, a population of variable genotypes is requested. It can be extracted from germplasm collections, or created by crossing two varieties (bi-parental cross), or several of them (di-allele cross). The genome of each individual from such a population will be characterized at several loci (points in the genome) by molecular markers. Such markers can be “Simple Sequence Repeat markers” (SSRs), “Single nucleotide polymorphisms,” insertions/deletions (indels), or insertions of retrotransposons. SSRs markers were extensively used for describing the genetic variability within collections (Lacombe et al., 2013), in progeny from crosses (Duchêne et al., 2012), or for clonal identification (Pelsy et al., 2010). SNPs are variations at a single base of the genome. Several methods can be used to characterize the nucleotide present at a precise position of the genome for a given genotype. These methods include direct sequencing of PCR fragments, hybridization on DNA chips, and GBS. GBS is currently one of the most efficient method and can provide thousands of markers for pools of genotypes in a single run (Tello et al., 2019).

Retrotransposons are mobile elements that expand in the genome with a copy paste mechanism and that can also be used as molecular markers (Castro et al., 2012; Villano et al., 2014). One of the most spectacular effects of a retrotransposon is the insertion of *Gret1* in the promoter region of a MYB factor that enables the synthesis of anthocyanins. When the insertion is homozygous, berries are white because anthocyanins cannot be synthesized (Kobayashi et al., 2004; Walker et al., 2007).

After the genomic features of the genotypes under study are obtained, the second step is to collect phenotypic information on these genotypes. When crossing two varieties generates the phenotypic variability, mathematical methods for searching loci with a quantitative effect (QTLs) rely on genetic maps that represent the genetic links between loci. The thousands of grapevine genotypes available are another source of variability. Because it is not possible to study at the same time all of them, specific panels, designed for association studies, are constituted (Nicolas et al., 2016). Using dense information on DNA variations among individuals from these panels, “Genome-wide association studies” (GWAS) can search for relationships between genomic and phenotypic data, locus

by locus (Nicolas et al., 2016; Guo et al., 2019; Liang et al., 2019). Finally, “genomic selection” methods try to fit mathematical models that use all the genetic information available to predict the value of a trait (Meuwissen et al., 2001; Fodor et al., 2014).

Molecular Markers for Developmental Stages

Quantitative trait locus detection was performed on several progenies and yielded several QTLs for budburst, flowering, and veraison. QTLs for budburst are rare (Duchene et al., 2012) and are difficult to detect because budbreak is the consequence of two phenomena: the date of dormancy release and the heat requirements between this date and actual leaf appearance. **Table 1** summarizes the QTLs detected for flowering time and veraison, including with GWAS (Laucou et al., 2018). Using the same type of data, Delfino et al. (2019) identified four veraison meta-QTLs located on linkage groups 1 and 2, and additional meta-QTLs on LG 14, 16, and 18.

The results from QTL studies show that it is possible to find some genetic explanations for the high range of phenological stages among grapevine varieties (Parker et al., 2013; Laucou et al., 2018). By combining specific alleles, it is possible to imagine and to try to create new genotypes with desired features (early or late *véraison* for example). Such genotypes are called “ideotypes.” Regarding adaptation to climate change, new genotypes created today will be cultivated 15–20 years ahead under different environmental conditions. Only a few traits, such as resistance to diseases or berry color, are stable under a changing environment. To predict behavior in the future, a modeling step is necessary. Mechanistic models can predict phenotypic values using environmental variables and genetic specific model parameters. This approach was used for maize (Reymond et al., 2003), peach (Quilot-Turion et al., 2016), tomato (Prudent et al., 2011), and cauliflower (Rosen et al., 2018).

Duchêne et al. (2010) provided an example of such an approach for the developmental stages of the grapevine. The use of heat summations between 15 February and budbreak, budbreak and flowering and flowering to *véraison* proved efficient to predict the dates of budbreak, flowering, and *véraison* for Riesling and Gewurztraminer (Duchêne et al., 2010). This model was used to give an estimate of the advance of phenological stages in the future. In a second step, independent QTLs were identified in the progeny of a Riesling × Gewurztraminer cross for the parameters of this model for grapevine phenology (Duchene et al., 2012). This allowed the construction of virtual genotypes: the earliest and the latest one that could be found in an infinite progeny by combining in a single genotype, on the one hand, all the alleles shortening the different phases, and on the other hand all the alleles with the opposite effects. Such virtual genotypes can be projected in the climate of the future and their interest compared (**Figure 3**). This result would not have been possible without molecular markers and the identification of QTLs. Moreover, breeding desired genotypes with marker-assisted selection (MAS) will use the same molecular information.

TABLE 1 | Main QTLs for developmental stages.

Chromosome	Flowering time or budbreak-flowering duration	Date of véraison, flowering-véraison duration, or budburst-véraison duration
1	Costantini et al., 2008; Fechter et al., 2014	Fechter et al., 2014
2	Costantini et al., 2008; Grzeskowiak et al., 2013	Costantini et al., 2008; Grzeskowiak et al., 2013
3		Laucou et al., 2018
4	Fechter et al., 2014	
6	Costantini et al., 2008	Costantini et al., 2008
7	Duchene et al., 2012; Grzeskowiak et al., 2013	
8	Fechter et al., 2014	
11	Fechter et al., 2014	Fechter et al., 2014
13		Fechter et al., 2014
14	Duchene et al., 2012; Fechter et al., 2014	
15		Grzeskowiak et al., 2013
16	Fechter et al., 2014	Duchene et al., 2012; Zyprian et al., 2016; Costantini et al., 2008
17	Fechter et al., 2014	Grzeskowiak et al., 2013
18		Duchêne et al., 2012; Zyprian et al., 2016
19	Fechter et al., 2014	

Molecular Markers for Water Use Efficiency

Increasing water stress is a major concern in the adaptation of viticulture to climate change. There is a large genetic variability of the responses to water shortage both for scions (Tomás et al., 2014) and rootstocks varieties (Serra et al., 2014 for a review).

Many traits and mechanisms are involved in the response of a rootstock × scion combination to the water demand/water availability ratio.

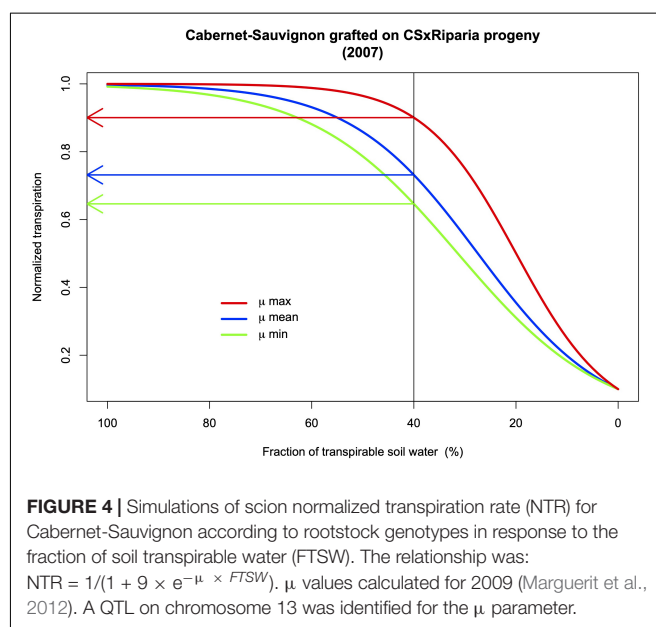
Considering rootstocks, they can differ by their capacity to extract water from the soil, which is primary linked to root biomass, but also to the hydraulic conductivity of the roots. The stomatal aperture is under the control of ABA, which is mainly synthesized by the roots in response to drought. ABA could also partly control the hydraulic conductance of the leaves (Simonneau et al., 2017). The genes responsible for the genetic variations of these traits are not yet precisely identified but the information provided by molecular markers is increasingly affordable.

Tandonnet et al. (2018) measured seven traits related to root architecture in the vineyard in the progeny of a Cabernet-Sauvignon × Riparia Gloire cross used as rootstocks for five scion varieties. They identified several significant QTLs on chromosomes 1, 2, and 5 for root biomass for example. Interestingly, a QTL for aerial biomass and QTLs for the aerial: root ratio were detected on different chromosomes (3 for the first trait; 6, 9, and 18 for the second). This means that it is likely possible to breed rootstocks with high root biomass, and a good water extraction capacity, while controlling aerial growth, the evaporative surface, and consequently water demand. The link between the response to drought stress and root/aerial biomass was not established in this study, but using the same progeny in a drought stress experiment with potted plants, Marguerit et al. (2012) identified several QTLs from the rootstock that control the transpiration rate by the scions. They also detected a QTL for a coefficient for the mathematical relationship between the changes in soil water availability and the transpiration rates

(Figure 4) that can be integrated into modeling simulation of ideotypes of rootstocks.

These results show that the control of the response to water stress depends on many genes from the rootstock and that the combination of alleles for the “ideal” rootstock adapted to drought is not straightforward. It however shows which traits are inter-dependent which is essential for preparing future studies but also for identifying targets for breeding programs.

The response of the scion to drought depends on the roots but genetic studies highlighted the complexity of the components of the aerial part. The study under well-watered and moderate stress conditions of the progeny from a Syrah × Grenache cross grown in pots on a phenotyping platform provided key results. Coupel-Ledru et al. (2014) identified in this experiment QTLs for leaf area, specific transpiration rate, specific hydraulic conductance,



or minimal daytime leaf water potential. These QTLs, spread over 10 chromosomes, were partly independent, showing that global behavior depends on many factors under genetic control. The same progeny was also used to demonstrate that nighttime transpiration was a major component of the genetic variability (Coupel-Ledru et al., 2016). Nighttime transpiration was partly due to incomplete stomatal closure at night (estimated to 70%) and to water loss through the cuticle (estimated to 30%). A genetic variability exists for both components. Stable QTLs for nighttime transpiration were identified on chromosomes 1, 4, and 13. More importantly, these QTLs did not colocalize with QTLs for daytime transpiration. This means that is possible to partly uncouple the overall capacity of photosynthesis (correlated to daytime transpiration) to overall water losses, which opens new perspectives to breeding programs. The availability of molecular tools for genetic studies was pivotal in this approach.

Molecular Markers for Stable Berry Quality

Possible effects on grape characteristics and modifications of the aroma profiles are the main concerns about climate change.

Increasing sugar content currently leads to high alcoholic contents of the wines, reducing their drinkability (Alston et al., 2011) and the consumers' willingness to pay (Tempere et al., 2019). The decoupling between sugar accumulation and anthocyanins synthesis is also a major concern (Martinez de Toda et al., 2014). For a given genotype, the final sugar content of the grape berries is determined by the leaf to fruit ratio (Duchêne et al., 2012) and by the photosynthetic conditions during ripening (solar radiation temperature, water availability, ...). Training systems and vineyard geographical position, as well as genetic diversity, can help to counterbalance the expected increase of sugar accumulation (van Leeuwen et al., 2019). The range of genetic variability for sugar content in germplasm collections, measured as total soluble contents (TSS in °Brix), can indeed reach 13.7–31.5°Brix (678–1784 mmol.L⁻¹ sugars) between different cultivars (Kliewer et al., 1967; Liu et al., 2006). It is however clear that the way the sampling date is chosen can have undesirable effects on the evaluation of genetic effects (Duchêne et al., 2012). To overcome this difficulty Bigard et al. (2018) proposed to collect berry samples when berry volume reaches a maximum, i.e., when phloem uploading ceases. They recorded variations from 813 to 1353 mmol.L⁻¹ of sugars among *V. vinifera* varieties, which confirms the reality of a genetic variability for sugar accumulation capacities at a precise physiological stage. QTLs for sugar content were described in different segregating progenies but their effects were weak (Chen et al., 2015; Houel et al., 2015) or observed only during one season (Yang et al., 2016). Ban et al. (2016) identified a QTL for TSS on chromosome 2 that explained more than 20% of the phenotypic variance over two seasons. However, TSS was significantly negatively correlated to harvest dates and the QTL detected might result from confusing effects. The data published on QTLs for sugar accumulation did not distinguish between the role of developmental stages, fruit load, and leaf area. Duchêne et al. (2012) demonstrated that the variability of TSS measured

on the same date in progeny from a cross between Riesling and Gewurztraminer was mainly explained by the dates of véraison and by the fruit to leaf ratio. By collecting berry samples after the same heat summation after the onset of ripening for each genotype and by correcting the measured values according to the fruit to leaf ratio, a QTL on chromosome 8 can be detected (Figure 5) whereas the likelihood of a QTL on chromosome 14, where was previously detected a QTL for flowering time (Duchene et al., 2012), is no longer significant. The allelic effect at the locus on chromosome 8 represents approximately 1°Brix, i.e., 0.7% v/v potential alcohol. This is not negligible but building ideotypes for controlling sugar accumulation taking into account the yield potential, the leaf area (plant vigor), the earliness at véraison and a supplementary QTL more closely linked to berry physiology might take too much effort when compared to changing training systems and management practices such as leaf removal.

Exploring a genetic context beyond the unique *V. vinifera* species can open new perspectives: some progenies from species such as *Vitis rotundifolia* exhibit a low ability to accumulate sugars (Salmon et al., 2018) and the underlying genetic architecture is under study (Torregrosa et al., 2017).

Acidity

Acidity is a major trait of grape berry quality driving the sensory properties of wines, their chemical and microbiological stability as well as aging potential. Grape acidity can be assessed by titratable acidity or pH. pH is determined by the content in organic acids, mainly malic acid and tartaric acid but also by cations, mainly potassium, that partly neutralize the organic acids (Boulton, 1980).

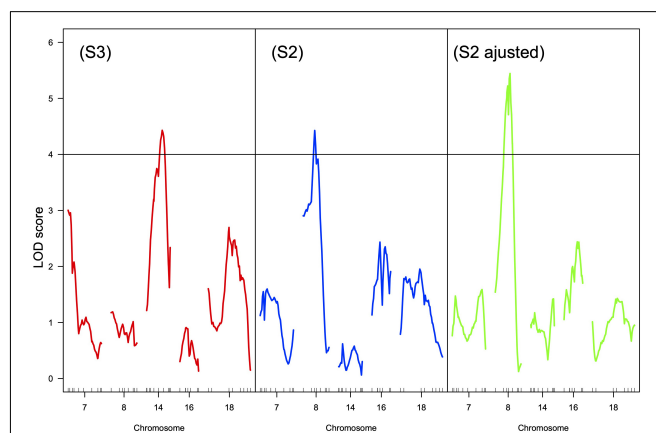


FIGURE 5 | LOD curves for the mean sugar content of the berries over 3 years in progeny from a Riesling × Gewurztraminer cross according to three sampling procedures: (S3) at harvest, same date for all the genotypes, (S2) 230 degree.days (base 10) after mid-véraison, (S2 adjusted) 230 degree.days (base 10) after mid-véraison but adjusted for the fruit to leaf ratio (Duchêne, 2015). The horizontal line is the genome wide LOD threshold at $p = 0.05$. Small vertical ticks on the X-axis represent the position of molecular markers on the genetic map. Chromosomes 7, 14, 16, and 18 are presented because QTLs for development stages were detected (Duchene et al., 2012).

The genotypes used, both for scions and rootstocks varieties, play a major role in the final acidity of wines, with pH varying at harvest from 2.91 (Duchêne et al., 2014) to 4.36 (Kliewer et al., 1967) in *V. vinifera* grapes. Phenology is a confusing factor when trying to compare genotypes. Comparing acidity parameters for different genotypes, even after the same number of days after véraison, can be biased because malic acid degradation depends on temperatures during ripening (Duchêne et al., 2014). The tartaric acid concentration of the berries is far less sensitive to high temperatures than the malic acid concentration (Kliewer and Torres, 1972). Indeed, the quantity of tartaric acid per berry is generally considered constant throughout berry ripening (DeBolt et al., 2008). Grapevine varieties with a high tartaric/malic ratio should be better adapted to warmer climatic conditions. There is a genetic variability for the tartaric/malic ratio in grapevine genotypes (Shiraishi, 1995; Duchêne et al., 2014; Bigard et al., 2018). QTLs for pH and tartaric acid concentration have already been found in segregating populations (Viana et al., 2013; Chen et al., 2015; Houel et al., 2015; Ban et al., 2016; Duchene et al., 2020). Diversity panels were also studied to detect QTLs for the concentration of malic acid and tartaric acid (Liang et al., 2019) or for wine acidity (Laucou et al., 2018). These results open the gate for breeding varieties able to keep a correct level of acidity in the warm conditions of the future. The links between genetic variations in (Mal), (Tart), or (Mal)/(Tart) and genetic variations of pH have however never formally been established. The missing element is likely (K^+). Indeed, Duchene et al. (2020) showed that malic acid concentrations, or the malic/tartaric acid ratios, were driven by strong QTLs on chromosomes 6 and 8, but were not associated with variations of pH. These variations of pH were explained by QTLs for the potassium-to-tartaric acid ratio, on chromosomes 10, 11, and 13.

(K^+) in grape juices also depends on the rootstock used, which could induce variations of pH between 3.76 and 4.27 in “Shiraz” grapes (Kodur et al., 2013). Genetic variations for (K^+) in leaves in hybrids from a rootstocks cross (Gong et al., 2014) open the possibility of breeding rootstocks for K^+ accumulation in scions.

Aromas and Aroma Precursors

Empirical knowledge often associates wine quality with cool temperatures. Indirect results are showing that increasing temperatures are generally unfavorable to wine quality (Tonietto and Carbonneau, 1998; Tonietto and Carbonneau, 2004; Jones et al., 2005; Moriondo et al., 2010), but experimental data supporting this idea are rare. Correlations have been detected between temperatures and the concentrations of methoxypyrazines (Falcao et al., 2007) or C13-norisoprenoids, which result from the breakdown of carotenoids (Marais et al., 1992). Water stress can also modify the aromatic profiles of wines. 3-sulfanyl hexanol (3-SH) concentrations, for example, were significantly higher in Riesling wines when vines were irrigated (Pons et al., 2017). Many studies also highlighted the role of light exposure on the secondary metabolism in grape berries (Kwasniewski et al., 2010; Friedel et al., 2016). Shading grapes can however induce confusing effects between light and temperature (Bureau et al., 2000). Understanding the effects of temperature, light, and water availability on the synthesis of aromas and aroma

precursors is a challenge for anticipating the effects of climate change and for proposing adaptation strategies.

Genetic approaches can show which genes are responsible for genetic variations.

Monoterpenols are 10-carbon molecules found in high concentration in berries of cultivars such as Gewurztraminer and varieties of the Muscat family. They are associated with floral aromas (Mateo and Jimenez, 2000). Duchene et al. (2009) and Battilana et al. (2011) demonstrated in three different progenies that a QTL for high terpenol synthesis colocalized with a gene coding for a 1-deoxy-D-xylulose 5-phosphate (DOXP) synthase; DOXP is the precursor of geranyl diphosphate (GPP), the substrate used by terpene synthases (VvTPS) to produce monoterpenols such as geraniol, linalool or α -terpineol. In aromatic genotypes, a mutation of a single base in the gene coding for the 1-deoxy-D-xylulose 5-phosphate synthase (DXS), is sufficient to enable a higher synthesis of DOXP, and further GPP, in aromatic cultivars (Battilana et al., 2011). These results were confirmed with genome wide association studies (Emanuelli et al., 2010; Laucou et al., 2018; Guo et al., 2019). A genetic approach also confirmed the role of a cytochrome P450 in the synthesis of carboxy-linalool, a precursor of wine-lactone (Ilc et al., 2017).

The pepper-like fragrance of methoxypyrazine is often not appreciated when concentrations are too high (Guillaumie et al., 2013). 2-methoxy-3-isobutylpyrazine (IBMP) is an example of methoxypyrazine, whose non-volatile precursor, 2-hydroxy-3-isobutylpyrazine, is methoxylated by an S-adenosyl-methionine-dependent O-methyltransferase, VvOMT3, to form IBMP. Guillaumie et al. (2013) detected a QTL for IBMP concentration in the progeny from a Cabernet-Sauvignon \times Riparia Gloire cross that colocalized with VvOMT3. Variations of the level of expression of VvOMT3 correlated with the level of IBMP synthesis.

Rotundone is the molecule responsible for the green peppery aroma in Shiraz grapes and wines (Siebert et al., 2008). Using a genomic approach, Drew et al. (2016) showed that variations at two amino acid positions in VvTPS24, a sesquiterpene synthase, were responsible for functional changes that allow the synthesis of α -guaiene, the precursor of rotundone. α -guaiene is then oxidized by the cytochrome P450 CYP71BE5 to form rotundone (Takase et al., 2016).

Knowing all the genes participating in aromas or aromas precursors synthesis is essential for more precise monitoring of mRNA synthesis according to environmental conditions or management practices.

Phenolic Compounds

Phenolic compounds are key components of wines: anthocyanins for berry color and condensed tannins for wine structure and astringency.

The decrease in anthocyanin content under high temperatures is well documented (Mori et al., 2007; Bonada et al., 2015; Lecourieux et al., 2017). Using empirical models linking berry composition and climatic data, Barnaud et al. (2014) forecasted a decrease of anthocyanins concentrations in the future for a given sugar level (22°Brix). Their simulation showed that this decrease could be higher for Cabernet-Sauvignon than

for Syrah. Experimental data also showed that the loss of grape color under high temperatures was lower in Cabernet-Sauvignon or Pinot noir than in Tokay grapes (Kliewer and Torres, 1972). High temperatures do not reduce the concentrations of all anthocyanins with the same intensity: di-hydroxylated anthocyanins are more affected than tri-hydroxylated anthocyanins (Lecourieux et al., 2017), malvidin-3-O-glucoside less than delphinidin-3-O-glucoside (Lecourieux et al., 2017). A study combining a bi-parental cross and a core collection confirmed that a locus on chromosome 2 is responsible for berry color (Fournier-Level et al., 2009) and that, within colored varieties, genetic polymorphisms in the same genomic region are associated with continuous variations of anthocyanin concentrations (Fournier-Level et al., 2009). Data from Lecourieux et al. (2017) suggest that the effects of high temperatures are all the more significant as the number of methyl groups is lower. In parallel, Fournier-Level et al. (2011) detected a link between genetic variations on chromosomes 1 and 2 with the levels of anthocyanin methylation in a Syrah \times Grenache progeny. They could associate two SNPs in a gene coding for an O-methyltransferase with the level of methylation. These results indicate that molecular markers can be used for breeding varieties with a high capacity to maintain their coloration under high temperatures. Costantini et al. (2015) also detected QTLs on 13 chromosomes that drive the anthocyanin profiles in a Syrah \times Pinot noir progeny.

Quantitative trait loci from segregating populations or diversity panels were also proposed for proanthocyanidins synthesis (Huang et al., 2012, 2014; Carrier et al., 2013). These molecules are however less sensitive to temperatures than anthocyanins (Pastore et al., 2017) and are not critical in the challenge of adaptation to climate change.

CONTROLLING THE GENOME AND ITS EXPRESSION

Obtaining new genotypes with specific characteristics was for centuries performed by choosing plants showing new and interesting phenotypes among hundreds (mass selection). The next step was to cross two plants and to select the best individuals within a progeny. These methods relied on the observations of the phenotypes of the plants. Molecular tools allow now choosing plants according to genetic information at the DNA level. Modern technologies are also able to generate random mutations that are possibly interesting but the most promising one is the direct editing of the genome at a precise location.

Breeding: Marker-Assisted Selection and Genomic Selection

The search for QTLs provides the breeder with statistical links between the presence of specific alleles at a given locus and the quantitative value of a trait. The strength of this relationship, the quantitative value of the variation due to allelic changes, the number of loci driving the trait of interest will determine whether the information can be used in breeding programs. For the grapevine, the generation of offspring from a bi-parental cross

is time-consuming (manual castration and manual pollination). The number of genotypes in such progenies is often too small to allow selecting plants for traits depending on several loci with weak effects. In practice, MAS is only used for traits depending on a few loci with strong effects. This is the case for resistance to diseases (Merdinoglu et al., 2018), for berry color (Yang et al., 2016), or for the ability to produce terpenols at high concentrations (Emanuelli et al., 2014). The ability to characterize thousands of SNPs in a genome for a reasonable cost is the basis of the “Genomic selection” method (Meuwissen et al., 2001). Instead of trying to predict a phenotype with a few points in the genome identified by QTL detection, mathematical methods are used to take into account the genetic information of all the SNPs. Genomic selection is routinely used for dairy cattle selection at the industrial level (Wiggans et al., 2017). The general principle of genomic selection is to build genomic prediction models with a training population and use them to predict phenotypic traits in a breeding population with genetic information only, in order to choose the individuals combining the most interesting features. The interest of genomic selection for grapevine breeding was first evaluated by simulations (Fodor et al., 2014), and the best predictions were obtained by combining GWAS and genomic selection. Good prediction accuracy were only calculated when the breeding population was not too distant from the training population. Working with actual data, Migicovsky et al. (2017) calculated genomic prediction accuracies for 32 traits, reaching 0.76 for berry length. Genomic selection is expected to be more efficient than MAS for complex traits depending on many loci with small effects. New approaches based on artificial intelligence and neural networks are also underway (Gonzalez-Camacho et al., 2016).

Creating Mutations

Targeting Induced Local Lesions in Genomes (TILLING) is a reverse genetics method that allows identification of mutations in genes of interest after inducing mutagenesis with a chemical mutagen. The following step is to establish links between mutations in a gene of interest and specific phenotypes to reveal the function of this gene (Henikoff et al., 2004).

Such an approach was attempted with the grapevine by the SVQV INRAE laboratory in Colmar using ethyl methanesulfonate (EMS) on the seeds collected on selfings of the PN40024 line, the nearly homozygous line that provided the grapevine reference genome (Jaillon et al., 2007). Several experiments led to the result that the sub-lethal EMS dose/treatment duration was 4 mM for 16 h. However, searching for mutations in 34 genes in 1,217 plants led to the conclusion that the number of mutations detected was too low to consider this population as a “tilling” population. Toxic effects of EMS certainly appeared before enough mutations were generated.

Genetic Engineering

Transgenesis allows adding or modifying unique traits in cultivars without, in theory, modifying their desirable characteristics. Like in other economically important crops, the production of GM grapevine plants has attracted a lot of attention since the early 1990s. Historically, the first successful

attempt to create GM grapevines was reported by Baribault et al. (1990) who used co-culture of shoot pieces with *Agrobacterium tumefaciens* to generate *in vitro* cultivated shoots expressing the GUS reporter gene. Severe limitations to this approach were noted, however: the obtained shoots consisted of a mosaic of wild-type and transgenic cells that failed to root and to regenerate plants. These issues were solved by the advent of embryogenic cell lines from various grape genotypes, which allowed regenerating “true” (non-mosaic) transgenic plant from single cells through somatic embryogenesis (Martinelli and Mandolino, 1994; Scorza et al., 1995; Mozsár et al., 1998). This paved the way to the obtention of the first generation of GMO grapevines, mostly tailored for pest resistance, by overexpressing defense-related genes. For example, the coding sequence of rice chitinase *RCC2* was introduced in the Japanese table grape Neo Muscat, under the control of the 35S promoter to breed resistance against *Uncinula necator* (Yamamoto et al., 2000). Coutos-Thévenot et al. (2001) transformed the rootstock 41B with a more elaborate construct bearing the grapevine stilbene synthase 1 *VST1* coding region under the control of the alfalfa, pathogen-inducible, PR10 promoter, conferring tolerance toward *Botrytis cinerea* to the transgenic plants. More recently, besides pest tolerance, new traits were gradually targeted for breeding through genetic transformation, including abiotic stress tolerance and fruit-related quality traits. Freezing tolerance was enhanced by overexpressing the cold-inducible *A. thaliana* Dehydration Response Element Binding (AtDREB1b) or the *V. Vinifera* C-Repeat Binding Protein 4 (VvCBF4) transcription factors in the table grape “Centennial Seedless” (Jin et al., 2008; Tillett et al., 2012). The aquaporin VvPIP2 was introduced in the cultivar “Brachetto” and expressed under the control of the 35S promoter by Perrone et al. (2012) in an attempt to produce grapevine plants more tolerant to drought stress. Finally, overexpression of the VvMYBA1 master regulator in both red (Shiraz) and white (Chardonnay) cultivars, led to enhanced production of acylated anthocyanin, through transcriptional up-regulation of the anthocyanin acyltransferase Vv3AT (Rinaldo et al., 2015) paving the way to transgenic grape with improved fruit quality.

Even though the above-mentioned production of transgenic grapevine was technically successful, little, if any, made it to production vineyards, mostly because of both consumers and growers’ reluctance to accept transgenic grapes, on grounds of health and environmental concerns, at least in Europe (Fuchs, 2008). Next-generation plasmid-free CRISPR/Cas9 genome edition technique may have the potential to overcome this reluctance to accept GM grapes, or more generally crops (Malnoy et al., 2016). Recently, a genome-wide survey of suitable sites for CRISPR/Cas9 genome editing has been conducted in grapevine (Wang et al., 2016) and successful attempts to actually generate genome-edited grapevine have been reported (Ren et al., 2016; Wang et al., 2016). Although the latter were just merely proof of concept attempts, Wan et al. (2020) reported this technology to generate grapevine plants with enhanced powdery mildew resistance through *Mlo* gene edition. The authors reported a 38.5% successful gene edition rate, a value lower to those previously reported in rice (84.3% on average) but comparable to those obtained in *Arabidopsis* (35.6% on average) (Ma

et al., 2015). The CRISPR/Cas9 technology was also used for creating plants expressing only one of the two main isoforms of the FLM gene involved in flowering regulation and was effective in producing early (FLM- δ expressing)- and late (FLM- β expressing)- flowering phenotypes (Figure 1; Capovilla et al., 2017). This demonstrates the crucial role of AS in determining phenological traits as well as the potentiality of genome editing for creating new varieties adapted to future climate change. Moreover, engineered CRISPR Artificial Splicing Factors have recently been shown effective for controlling AS in animal cell cultures, which constitutes a promising strategy to modify phenotypes by manipulating the transcriptome (Du et al., 2020). Thus, the technology has undoubtedly great potential for future grapevine, and more broadly plant breeding programs. Its actual use, however, will be largely dependent on local regulations. United States Department of Agriculture does not impose any GM restrictions on genome-edited plants if they are free of any foreign or transgenic DNA, thus there is a fair chance that CRISPR/Cas9 modified plant could be free of GM organism regulations, at least in the United States (Waltz, 2012; Jones, 2015). Conversely, in Europe and New Zealand, the current legal status of genome-edited plants classifies them as GM organisms, and the same regulations as for transgenic plants apply (Schmidt et al., 2020).

CONCLUSION

Molecular tools for describing genome sequences, genetic variations among varieties or clones, levels of gene transcription, and protein quantification have evolved exponentially during the last decades. The first release of a reliable grapevine sequence in 2007 required several years of sequencing with the Sanger technology before attempting a puzzling assembly, whereas a complete sequence of a heterozygous variety, build with long reads of DNA, takes now only a few weeks. GBS technology allows now characterizing hundreds of genotypes at thousands of points in a genome in a single run of sequencing, and transcriptomic as well as proteomic tools follow the same trend. There is still a lot to learn on the regulation of gene transcription and AS, on the mechanisms of interfering RNAs, DNA methylation, or chromatin activity but also on the mechanisms regulating protein synthesis and turnover.

Adaptation of grapevine to new environmental conditions will be all the more efficient as the physiological responses to drought, elevated temperatures, or combined stress on plant growth, development, and berry composition are precisely described. To achieve this goal, the first challenge is to characterize the levels of stress imposed in experiments in a way the results can be extrapolated in other environmental conditions and that they make sense in real vineyard conditions. The second challenge is to develop and to use methods able to integrate and interpret large datasets that include genomic sequences, transcriptomic, proteomic, and metabolomic data. This requires huge efforts toward integrated network analysis and “system biology.” The final goal is to build a corpus of knowledge that

includes the responses to quantified environmental variables and genetic variability.

Finally, this knowledge can help to construct adaptation strategies not only on the plant side for the control of gene expression, for breeding new varieties by hybridization or by genome editing technologies, but also on training systems and plant management techniques.

REFERENCES

- Alston, J. M., Fuller, K. B., Lapsley, J. T., and Soleas, G. (2011). Too much of a good thing? Causes and consequences of increases in sugar content of California wine grapes. *J. Wine Econ.* 6, 135–159. doi: 10.1017/s1931436100001565
- Arrizabalaga, M., Morales, F., Oyarzun, M., Delrot, S., Gomes, E., Irigoyen, J. J., et al. (2018). Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. *Plant Sci.* 267, 74–83. doi: 10.1016/j.plantsci.2017.11.009
- Arrizabalaga-Arriazu, M., Gomès, E., Morales, F., Irigoyen, J., Pascual, I., and Hilbert, G. (2020). High temperature and elevated carbon dioxide modify berry composition of different clones of grapevine (*Vitis vinifera* L.) cv. Tempranillo. *Front. Plant Sci.* 11:603687. doi: 10.3389/fpls.2020.603687
- Ban, Y., Mitani, N., Sato, A., Kono, A., and Hayashi, T. (2016). Genetic dissection of quantitative trait loci for berry traits in interspecific hybrid grape (*Vitis labruscana* × *Vitis vinifera*). *Euphytica* 211, 295–310. doi: 10.1007/s10681-016-1737-8
- Baribault, T., Skene, K., Cain, P., and Steele Scott, N. (1990). Transgenic grapevines: regeneration of shoots expressing β -glucuronidase. *J. Exp. Bot.* 41, 1045–1049. doi: 10.1093/jxb/41.8.1045
- Barnuud, N. N., Zerihun, A., Mpelasoka, F., Gibberd, M., and Bates, B. (2014). Responses of grape berry anthocyanin and titratable acidity to the projected climate change across the Western Australian wine regions. *Int. J. Biometeorol.* 58, 1279–1293. doi: 10.1007/s00484-013-0724-1
- Battilana, J., Dunlevy, J. D., and Boss, P. K. (2017). Histone modifications at the grapevine VvOMT3 locus, which encodes an enzyme responsible for methoxypyrazine production in the berry. *Funct. Plant Biol.* 44, 655–664. doi: 10.1071/fp16434
- Battilana, J., Emanuelli, F., Gambino, G., Griboaud, I., Gasperi, F., Boss, P. K., et al. (2011). Functional effect of grapevine 1-deoxy-D-xylulose 5-phosphate synthase substitution K284N on Muscat flavour formation. *J. Exp. Bot.* 62, 5497–5508. doi: 10.1093/jxb/err231
- Belli Kullán, J., Lopes Paim, D., Bertolini, E., Fasoli, M., Zenoni, S., et al. (2015). miRVine: a microRNA expression atlas of grapevine based on small RNA sequencing. *BMC Genomics* 16:393. doi: 10.1186/s12864-015-1610-5
- Berdeja, M., Nicolas, P., Kappel, C., Dai, Z., Hilbert, G., Peccoux, A., et al. (2015). Water limitation and rootstock genotype interact to alter grape berry metabolism through transcriptome reprogramming. *Hort. Res.* 2:15012.
- Bhatia, G., Sharma, S., Upadhyay, S. K., and Singh, K. (2019). Long non-coding RNAs coordinate developmental transitions and other key biological processes in grapevine. *Sci. Rep.* 9:3552.
- Bigard, A., Berhe, D. T., Maoddi, E., Sire, Y., Boursiquot, J. M., Ojeda, H., et al. (2018). *Vitis vinifera* L. fruit diversity to breed varieties anticipating climate changes. *Front. Plant Sci.* 9:455. doi: 10.3389/fpls.2018.00455
- Bindi, M., Fibbi, L., and Miglietta, F. (2005). Free air CO₂ enrichment (FACE) of grapevine (*Vitis vinifera* L.): II. Growth and quality of grape and wine in response to elevated CO₂. *Eur. J. Agron.* 14, 145–155. doi: 10.1016/s1161-0301(00)00093-9
- Bonada, M., Jeffery, D. W., Petrie, P. R., Moran, M. A., and Sadras, V. O. (2015). Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Aust. J. Grape Wine Res.* 21, 240–253. doi: 10.1111/ajgw.12142
- Boulton, R. (1980). The general relationship between potassium, sodium, and pH in grape, juice and wine. *Am. J. Enol. Vitic.* 31, 182–186.
- Brautigam, K., Vining, K. J., Lafon-Placette, C., Fossdal, C. G., Mirouze, M., Marcos, J. G., et al. (2013). Epigenetic regulation of adaptive responses of forest tree species to the environment. *Ecol. Evol.* 3, 399–415. doi: 10.1002/eece3.461

AUTHOR CONTRIBUTIONS

ÉD, ÉG, and PM carried out bibliographic searches, redacted the first draft on specific topics, and reviewed the whole manuscript. ÉD initiated and supervised the work. All authors contributed to the article and approved the submitted version.

- Bureau, S. M., Razungles, A. J., and Baumes, R. L. (2000). The aroma of muscat of frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. *J. Sci. Food Agric.* 80, 2012–2020. doi: 10.1002/1097-0010(200011)80:14<2012::aid-jsfa738>3.0.co;2-x
- Camps, C., Kappel, C., Lecomte, P., Leon, C., Gomes, E., Coutos-Thevenot, P., et al. (2010). A transcriptomic study of grapevine (*Vitis vinifera* cv. Cabernet-Sauvignon) interaction with the vascular ascomycete fungus *Eutypa lata*. *J. Exp. Bot.* 61, 1719–1737. doi: 10.1093/jxb/erq040
- Canaguier, A., Grimplet, J., Di Gaspero, G., Scalabrini, S., Duchene, E., Choise, N., et al. (2017). A new version of the grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). *Genom. Data* 14, 56–62. doi: 10.1016/j.gdata.2017.09.002
- Cao, L., Yu, N., Li, J., Qi, Z., Wang, D., and Chen, L. (2016). Heritability and reversibility of DNA methylation induced by *in vitro* grafting between *Brassica juncea* and *B. Oleracea*. *Sci. Rep.* 6:27233.
- Capovilla, G., Symeonidi, E., Wu, R., and Schmid, M. (2017). Contribution of major FLM isoforms to temperature-dependent flowering in *Arabidopsis thaliana*. *J. Exp. Bot.* 68, 5117–5127. doi: 10.1093/jxb/erx328
- Carbonell-Bejerano, P., Diago, M.-P., Martinez-Abaigar, J., Martinez-Zapater, J., Tardaguila, J., and Nunez-Olivera, E. (2014). Solar ultraviolet radiation is necessary to enhance grapevine fruit ripening transcriptional and phenolic responses. *BMC Plant Biol.* 14:183. doi: 10.1186/1471-2229-14-183
- Carbonell-Bejerano, P., Royo, C., Torres-Perez, R., Grimplet, J., Fernandez, L., Franco-Zorrilla, J. M., et al. (2017). Catastrophic unbalanced genome rearrangements cause somatic loss of berry color in grapevine. *Plant Physiol.* 175, 786–801.
- Carra, A., Mica, E., Gambino, G., Pindo, M., Moser, C., Pe, M. E., et al. (2009). Cloning and characterization of small non-coding RNAs from grape. *Plant J.* 59, 750–763. doi: 10.1111/j.1365-313x.2009.03906.x
- Carrier, G., Huang, Y. F., Le Cunff, L., Fournier-Level, A., Violet, S., Souquet, J. M., et al. (2013). Selection of candidate genes for grape proanthocyanidin pathway by an integrative approach. *Plant Physiol. Biochem.* 72, 87–95. doi: 10.1016/j.plaphy.2013.04.014
- Castro, A., Quiroz, D., Sanchez, E., Miccono Mde, L., Aguirre, C., Ramirez, A., et al. (2016). Synthesis of an artificial *Vitis vinifera* miRNA 319e using overlapping long primers and its application for gene silencing. *J. Biotechnol.* 233, 200–210. doi: 10.1016/j.jbiotec.2016.06.028
- Castro, I., D'onofrio, C., Martin, J. P., Ortiz, J. M., De Lorenzis, G., Ferreira, V., et al. (2012). Effectiveness of AFLPs and retrotransposon-based markers for the identification of Portuguese grapevine cultivars and clones. *Mol. Biotechnol.* 52, 26–39. doi: 10.1007/s12033-011-9470-y
- Chaudhary, S., Jabre, I., Reddy, A. S. N., Staiger, D., and Syed, N. H. (2019). Perspective on alternative splicing and proteome complexity in plants. *Trends Plant Sci.* 24, 496–506. doi: 10.1016/j.tplants.2019.02.006
- Chen, J., Wang, N., Fang, L. C., Liang, Z. C., Li, S. H., and Wu, B. H. (2015). Construction of a high-density genetic map and QTLs mapping for sugars and acids in grape berries. *BMC Plant Biol.* 15:28. doi: 10.1186/s12870-015-0428-2
- Chen, L., Zhang, P., Fan, Y., Lu, Q., Li, Q., Yan, J., et al. (2018). Circular RNAs mediated by transposons are associated with transcriptomic and phenotypic variation in maize. *New Phytol.* 217, 1292–1306. doi: 10.1111/nph.14901
- Chen, Q. J., Deng, B. H., Gao, J., Zhao, Z. Y., Chen, Z. L., Song, S. R., et al. (2020). A miRNA-encoded small peptide, vvi-miPEP171d1, regulates adventitious root formation. *Plant Physiol.* 183, 656–670. doi: 10.1104/pp.20.00197
- Chin, C. S., Peluso, P., Sedlazeck, F. J., Nattestad, M., Concepcion, G. T., Clum, A., et al. (2016). Phased diploid genome assembly with single-molecule real-time sequencing. *Nat. Methods* 13, 1050–1054. doi: 10.1038/nmeth.4035
- Cochetel, N., Escudie, F., Cookson, S. J., Dai, Z. W., Vivin, P., Bert, P. F., et al. (2017). Root transcriptomic responses of grafted grapevines to heterogeneous

- nitrogen availability depend on rootstock genotype. *J. Exp. Bot.* 68, 4339–4355. doi: 10.1093/jxb/erx224
- Corso, M., Vannozzi, A., Maza, E., Vitulo, N., Meggio, F., Pitacco, A., et al. (2015). Comprehensive transcript profiling of two grapevine rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance. *J. Exp. Bot.* 66, 5739–5752. doi: 10.1093/jxb/erv274
- Costantini, L., Battilana, J., Lamaj, F., Fanizza, G., and Grando, M. S. (2008). Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. *BMC Plant Biol.* 8:38. doi: 10.1186/1471-2229-8-38
- Costantini, L., Malacarne, G., Lorenzi, S., Troggio, M., Mattivi, F., Moser, C., et al. (2015). New candidate genes for the fine regulation of the colour of grapes. *J. Exp. Bot.* 66, 4427–4440. doi: 10.1093/jxb/erv159
- Coupel-Ledru, A., Lebon, E., Christophe, A., Doligez, A., Cabrera-Bosquet, L., Pechier, P., et al. (2014). Genetic variation in a grapevine progeny (*Vitis vinifera* L. cvs GrenacheSyrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought. *J. Exp. Bot.* 65, 6205–6218. doi: 10.1093/jxb/eru228
- Coupel-Ledru, A., Lebon, E., Christophe, A., Gallo, A., Gago, P., Pantin, F., et al. (2016). Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proc. Natl. Acad. Sci. U.S.A.* 113, 8963–8968. doi: 10.1073/pnas.1600826113
- Coutos-Thévenot, P., Poinssot, B., Bonomelli, A., Yean, H., Breda, C., Buffard, D., et al. (2001). *In vitro* tolerance to *Botrytis cinerea* of grapevine 41B rootstock in transgenic plants expressing the stilbene synthase VST1 under the control of a pathogen-inducible PR10 promoter. *J. Exp. Bot.* 52, 901–910. doi: 10.1093/jxb/52.358.901
- Cramer, G. R., Hopper, D. W., Quilici, D. R., Woolsey, R. J., Cushman, J. C., Vincent, D., et al. (2017). Early and late responses of grapevine (*Vitis vinifera* L.) to water deficit: a proteomics perspective. *Acta Hort.* 1157, 263–272. doi: 10.17660/actahortic.2017.1157.37
- Cramer, G. R., Van Sluyter, S., Hopper, D. W., Pascovici, D., Keighley, T., and Haynes, P. (2013). Proteomic analysis indicate massive changes in metabolism prior to the inhibition of growth and photosynthesis of grapevine (*Vitis vinifera* L.) in response to water deficit. *BMC Plant Biol.* 13:49. doi: 10.1186/1471-2229-13-49
- Curtin, D., Beare, M., and Hernandez-Ramirez, G. (2012). Temperature and moisture effects on microbial biomass and soil organic matter organic matter mineralization. *Soil Sci. Soc. Am. J.* 76, 2055–2067. doi: 10.2136/sssaj2012.0011
- Da Silva, C., Zamperin, G., Ferrarini, A., Minio, A., Dal Molin, A., Venturini, L., et al. (2013). The high polyphenol content of grapevine cultivar tannat berries is conferred primarily by genes that are not shared with the reference genome. *Plant Cell* 25, 4777–4788. doi: 10.1105/tpc.113.118810
- Dalakouras, A., Wassenegger, M., Dadami, E., Ganopoulos, I., Pappas, M. L., and Papadopoulou, K. (2020). Genetically modified organism-free RNA interference: exogenous application of RNA molecules in plants. *Plant Physiol.* 182, 38–50. doi: 10.1104/pp.19.00570
- Dalakouras, A., Wassenegger, M., Mcmillan, J. N., Cardoza, V., Maegele, I., Dadami, E., et al. (2016). Induction of silencing in plants by high-pressure spraying of *In vitro*-synthesized small RNAs. *Front. Plant Sci.* 7:1327. doi: 10.3389/fpls.2016.01327
- DeBolt, S., Ristic, R., Iland, P. G., and Ford, C. M. (2008). Altered light interception reduces grape berry weight and modulates organic acid biosynthesis during development. *HortScience* 43, 957–961. doi: 10.21273/hortsci.43.3.957
- Delfino, P., Zenoni, S., Imanifard, Z., Tornielli, G. B., and Bellin, D. (2019). Selection of candidate genes controlling veraison time in grapevine through integration of meta-QTL and transcriptomic data. *BMC Genomics* 20:739. doi: 10.1186/s12864-019-6124-0
- Deluc, L. G., Quilici, D. R., Decendit, A., Grimplet, J., Wheatley, M. D., Schlauch, K. A., et al. (2009). Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics* 10:212. doi: 10.1186/1471-2164-10-212
- Drew, D. P., Andersen, T. B., Sweetman, C., Moller, B. L., Ford, C., and Simonsen, H. T. (2016). Two key polymorphisms in a newly discovered allele of the *Vitis vinifera* TPS24 gene are responsible for the production of the rotundone precursor alpha-guaiene. *J. Exp. Bot.* 67, 799–808. doi: 10.1093/jxb/erv491
- Du, M., Jillette, N., Zhu, J. J., Li, S., and Cheng, A. W. (2020). CRISPR artificial splicing factors. *Nat. Commun.* 11:2973.
- du Plessis, K., Young, P. R., Eyeghe-Bickong, H. A., and Vivier, M. A. (2017). The transcriptional responses and metabolic consequences of acclimation to elevated light exposure in grapevine berries. *Front. Plant Sci.* 8:1261. doi: 10.3389/fpls.2017.01261
- Duchêne, E. (2015). *Une Exploration des Possibilités Génetiques Pour l'adaptation de la Vigne au Changement Climatique*. PhD thesis. Strasbourg: Université de Strasbourg.
- Duchêne, E., Butterlin, G., Claudel, P., Dumas, V., Jaegli, N., and Merdinoglu, D. (2009). A grapevine (*Vitis vinifera* L.) deoxy-D: -xylulose synthase gene colocalizes with a major quantitative trait loci for terpenol content. *Theor. Appl. Genet.* 118, 541–552. doi: 10.1007/s00122-008-0919-8
- Duchêne, E., Butterlin, G., Dumas, V., and Merdinoglu, D. (2012). Towards the adaptation of grapevine varieties to climate change: QTLs and candidate genes for developmental stages. *Theor. Appl. Genet.* 124, 623–635. doi: 10.1007/s00122-011-1734-1
- Duchêne, E., Dumas, V., Butterlin, G., Jaegli, N., Rustenholz, C., Chauveau, A., et al. (2020). Genetic variations of acidity in grape berries are controlled by the interplay between organic acids and potassium. *Theor. Appl. Genet.* 133, 993–1008. doi: 10.1007/s00122-019-03524-9
- Duchêne, E., Dumas, V., Jaegli, N., and Merdinoglu, D. (2012). Deciphering the ability of different grapevine genotypes to accumulate sugar in berries. *Aust. J. Grape Wine Res.* 18, 319–328. doi: 10.1111/j.1755-0238.2012.00194.x
- Duchêne, E., Dumas, V., Jaegli, N., and Merdinoglu, D. (2014). Genetic variability of descriptors for grapevine berry acidity in Riesling, Gewürztraminer and their progeny. *Aust. J. Grape Wine Res.* 20, 91–99. doi: 10.1111/ajgw.12051
- Duchêne, E., Huard, F., Dumas, V., Schneider, C., and Merdinoglu, D. (2010). The challenge of adapting grapevine varieties to climate change. *Clim. Res.* 41, 193–204. doi: 10.3354/cr00850
- Egawa, C., Kobayashi, F., Ishibashi, M., Nakamura, T., Nakamura, C., and Takumi, S. (2006). Differential regulation of transcript accumulation and alternative splicing of a DREB2 homolog under abiotic stress conditions in common wheat. *Genes Genet. Syst.* 81, 77–91. doi: 10.1266/ggs.81.77
- Emanuelli, F., Battilana, J., Costantini, L., Le Cunff, L., Boursiquot, J. M., This, P., et al. (2010). A candidate gene association study on muscat flavor in grapevine (*Vitis vinifera* L.). *BMC Plant Biol.* 10:241. doi: 10.1186/1471-2229-10-241
- Emanuelli, F., Sordo, M., Lorenzi, S., Battilana, J., and Grando, M. S. (2014). Development of user-friendly functional molecular markers for *VvDXS* gene conferring muscat flavor in grapevine. *Mol. Breed.* 33, 235–241. doi: 10.1007/s11032-013-9929-6
- Falcao, L. D., De Revel, G., Perello, M. C., Moutsiou, A., Zanusi, M. C., and Bordignon-Luiz, M. T. (2007). A survey of seasonal temperatures and vineyard altitude influences on 2-methoxy-3-isobutylpyrazine, C13-norisoprenoids, and the sensory profile of Brazilian Cabernet Sauvignon wines. *J. Agric. Food Chem.* 55, 3605–3612. doi: 10.1021/jf070185u
- Fechter, I., Hausmann, L., Zyprian, E., Daum, M., Holtgrawe, D., Weisshaar, B., et al. (2014). QTL analysis of flowering time and ripening traits suggests an impact of a genomic region on linkage group 1 in *Vitis*. *Theor. Appl. Genet.* 127, 1857–1872. doi: 10.1007/s00122-014-2310-2
- Filichkin, S. A., Hamilton, M., Dharmawardhana, P. D., Singh, S. K., Sullivan, C., Ben-Hur, A., et al. (2018). Abiotic stresses modulate landscape of poplar transcriptome via alternative splicing, differential intron retention, and isoform ratio switching. *Front. Plant Sci.* 9:5. doi: 10.3389/fpls.2018.00005
- Fodor, A., Segura, V., Denis, M., Neuenschwander, S., Fournier-Level, A., Chatelet, P., et al. (2014). Genome-wide prediction methods in highly diverse and heterozygous species: proof-of-concept through simulation in grapevine. *PLoS One* 9:e110436. doi: 10.1371/journal.pone.0110436
- Fortes, A. M., and Gallusci, P. (2017). Plant stress responses and phenotypic plasticity in the epigenomics era: perspectives on the grapevine scenario, a model for perennial crop plants. *Front. Plant Sci.* 8:82. doi: 10.3389/fpls.2017.00082
- Fournier-Level, A., Huguency, P., Verries, C., This, P., and Ageorges, A. (2011). Genetic mechanisms underlying the methylation level of anthocyanins in grape (*Vitis vinifera* L.). *BMC Plant Biol.* 11:179. doi: 10.1186/1471-2229-11-179
- Fournier-Level, A., Le Cunff, L., Gomez, C., Doligez, A., Ageorges, A., Roux, C., et al. (2009). Quantitative genetic bases of anthocyanin variation in grape (*Vitis vinifera* L. ssp. sativa) berry: a quantitative trait locus to quantitative trait

- nucleotide integrated study. *Genetics* 183, 1127–1139. doi: 10.1534/genetics.109.103929
- Friedel, M., Frotscher, J., Nitsch, M., Hofmann, M., Bogs, J., Stoll, M., et al. (2016). Light promotes expression of monoterpene and flavonol metabolic genes and enhances flavour of winegrape berries (*Vitis vinifera* L. cv. Riesling). *Aust. J. Grape Wine Res.* 22, 409–421. doi: 10.1111/ajgw.12229
- Fuchs, M. (2008). Les plantes transgéniques et la lutte contre les virus phytopathogènes: état de l'art et perspectives. *Virologie* 12, 27–37.
- Gallusci, P., Dai, Z., Genard, M., Gauffretau, A., Leblanc-Fournier, N., Richard-Molard, C., et al. (2017). Epigenetics for plant improvement: current knowledge and modeling avenues. *Trends Plant Sci.* 22, 610–623. doi: 10.1016/j.tplants.2017.04.009
- Gambino, G., Dal Molin, A., Boccacci, P., Minio, A., Chitarra, W., Avanzato, C. G., et al. (2017). Whole-genome sequencing and SNV genotyping of 'Nebbiolo' (*Vitis vinifera* L.) clones. *Sci. Rep.* 7:17294.
- George, I. S., and Haynes, P. A. (2014). Current perspectives in proteomic analysis of abiotic stress in grapevines. *Front. Plant Sci.* 5:686. doi: 10.3389/fpls.2014.00686
- Gil, K. E., and Park, C. M. (2019). Thermal adaptation and plasticity of the plant circadian clock. *New Phytol.* 221, 1215–1229. doi: 10.1111/nph.15518
- Girollet, N., Rubio, B., Lopez-Rocque, C., Valière, S., Ollat, N., and Bert, P.-F. (2019). De novo phased assembly of the *Vitis riparia* grape genome. *Sci. Data* 6:127.
- Gong, H. J., Blackmore, D. H., Clingeleffer, P. R., Sykes, S. R., and Walker, R. R. (2014). Variation for potassium and sodium accumulation in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri. *Vitis* 53, 65–72.
- Gonzalez-Camacho, J. M., Crossa, J., Perez-Rodriguez, P., Ornella, L., and Gianola, D. (2016). Genome-enabled prediction using probabilistic neural network classifiers. *BMC Genomics* 17:208. doi: 10.1186/s12864-016-2553-1
- Grzeskowiak, L., Costantini, L., Lorenzi, S., and Grando, M. S. (2013). Candidate loci for phenology and fruitfulness contributing to the phenotypic variability observed in grapevine. *Theor. Appl. Genet.* 126, 2763–2776. doi: 10.1007/s00122-013-2170-1
- Guillaumie, S., Ilg, A., Rety, S., Brette, M., Trossat-Magnin, C., Decroocq, S., et al. (2013). Genetic analysis of the biosynthesis of 2-methoxy-3-isobutylpyrazine, a major grape-derived aroma compound impacting wine quality. *Plant Physiol.* 162, 604–615. doi: 10.1104/pp.113.218313
- Guo, D. L., Zhao, H. L., Li, Q., Zhang, G. H., Jiang, J. F., Liu, C. H., et al. (2019). Genome-wide association study of berry-related traits in grape *Vitis vinifera* L. based on genotyping-by-sequencing markers. *Hort. Res.* 6:11.
- Harris, Z. N., Kovacs, L. G., and Londo, J. P. (2017). RNA-seq-based genome annotation and identification of long-noncoding RNAs in the grapevine cultivar 'Riesling'. *BMC Genomics* 18:937. doi: 10.1186/s12864-017-4346-6
- Henikoff, S., Till, B. J., and Comai, L. (2004). TILLING. Traditional mutagenesis meets functional genomics. *Plant Physiol.* 135, 630–636. doi: 10.1104/pp.104.041061
- Hopper, D. W., Ghan, R., Schlauch, K. A., and Cramer, G. R. (2016). Transcriptomic network analyses of leaf dehydration responses identify highly connected ABA and ethylene signaling hubs in three grapevine species differing in drought tolerance. *BMC Plant Biol.* 16:118. doi: 10.1186/s12870-016-0804-6
- Houel, C., Chatbanyong, R., Doligez, A., Rienth, M., Folia, S., Luchaire, N., et al. (2015). Identification of stable QTLs for vegetative and reproductive traits in the microvine (*Vitis vinifera* L.) using the 18 K Infinium chip. *BMC Plant Biol.* 15:205. doi: 10.1186/s12870-015-0588-0
- Huang, Y. F., Doligez, A., Fournier-Level, A., Le Cunff, L., Bertrand, Y., Canaguier, A., et al. (2012). Dissecting genetic architecture of grape proanthocyanidin composition through quantitative trait locus mapping. *BMC Plant Biol.* 12:30. doi: 10.1186/1471-2229-12-30
- Huang, Y. F., Viallet, S., Guiraud, J. L., Torregrosa, L., Bertrand, Y., Cheynier, V., et al. (2014). A negative MYB regulator of proanthocyanidin accumulation, identified through expression quantitative locus mapping in the grape berry. *New Phytol.* 201, 795–809. doi: 10.1111/nph.12557
- Ilc, T., Halter, D., Miesch, L., Lauvoisard, F., Kriegshauser, L., Ilg, A., et al. (2017). A grapevine cytochrome P450 generates the precursor of wine lactone, a key odorant in wine. *New Phytol.* 213, 264–274. doi: 10.1111/nph.14139
- IPCC (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Summary for Policy Makers*. Cambridge: Cambridge University Press.
- Jaillon, O., Aury, J. M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., et al. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449, 463–467. doi: 10.1038/nature06148
- Jia, H., Zhang, Z., Zhang, S., Fu, W., Su, L., Fang, J., et al. (2020). Effect of the methylation level on the grape fruit development process. *J. Agric. Food Chem.* 68, 2099–2115.
- Jiang, J., Liu, X., Liu, C., Liu, G., Li, S., and Wang, L. (2017). Integrating omics and alternative splicing reveals insights into grape response to high temperature. *Plant Physiol.* 173, 1502–1518. doi: 10.1104/pp.16.01305
- Jin, W., Dong, J., Hu, Y., Zhongping, L., Xu, X., and Han, Z. (2008). Improved cold-tolerance performance in transgenic grape (*Vitis vinifera* L.) overexpressing cold-inducible transcription factor AtDREB1b. *HortScience* 44, 35–39. doi: 10.21273/hortsci.44.1.35
- Jones, G. V., White, M. A., Cooper, O. R., and Storchmann, K. (2005). Climate change and global wine quality. *Clim. Change* 73, 319–343. doi: 10.1007/s10584-005-4704-2
- Jones, H. D. (2015). Regulatory uncertainty over genome editing. *Nat. Plants* 1:14011.
- Karlbach, G., Hansen, P., Veiga, D. F., Steinhaus, R., Danis, D., Li, S., et al. (2020). HBA-DEALS: accurate and simultaneous identification of differential expression and splicing using hierarchical Bayesian analysis. *Genome Biol.* 21:171.
- Kiselev, K. V., Tyunin, A. P., and Zhuravlev, Y. N. (2013). Involvement of DNA methylation in the regulation of STS10 gene expression in *Vitis amurensis*. *Planta* 237, 933–941. doi: 10.1007/s00425-012-1806-8
- Kliwer, W. M., Howarth, L., and Omori, M. (1967). Concentrations of tartaric acid and malic acids and their salts in *Vitis vinifera* grapes. *Am. J. Enol. Vitic.* 18, 42–54.
- Kliwer, W. M., and Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. *Am. J. Enol. Vitic.* 23, 71–77. doi: 10.17660/actahortic.1995.378.9
- Kobayashi, S., Goto-Yamamoto, N., and Hirochika, H. (2004). Retrotransposon-induced mutations in grape skin color. *Science* 304:982. doi: 10.1126/science.1095011
- Kodur, S., Tisdall, J. M., Clingeleffer, P. R., and Walker, R. R. (2013). Regulation of berry quality parameters in 'Shiraz' grapevines through rootstocks (*Vitis*). *Vitis* 52, 125–128.
- Konate, M., Wilkinson, M. J., Taylor, J., Scott, E. S., Berger, B., and Rodriguez Lopez, C. M. (2020). Greenhouse spatial effects detected in the barley (*Hordeum vulgare* L.) epigenome underlie stochasticity of DNA methylation. *Front. Plant Sci.* 11:553907. doi: 10.3389/fpls.2020.553907
- Krol, A., and Weidner, S. (2017). Changes in the proteome of grapevine leaves (*Vitis vinifera* L.) during long-term drought stress. *J. Plant Physiol.* 211, 114–126. doi: 10.1016/j.jplph.2016.11.016
- Kwasniewski, M. T., Vanden Heuvel, J. E., Pan, B. S., and Sacks, G. L. (2010). Timing of cluster light environment manipulation during grape development affects C13 norisoprenoid and carotenoid concentrations in Riesling. *J. Agric. Food Chem.* 58, 6841–6849. doi: 10.1021/jf904555p
- Lacombe, T., Boursiquot, J. M., Laucou, V., Di Vecchi-Staraz, M., Peros, J. P., and This, P. (2013). Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vinifera* L.). *Theor. Appl. Genet.* 126, 401–414. doi: 10.1007/s00122-012-1988-2
- Laloum, T., Martin, G., and Duque, P. (2018). Alternative splicing control of abiotic stress responses. *Trends Plant Sci.* 23, 140–150. doi: 10.1016/j.tplants.2017.09.019
- Lan, P., Li, W., and Schmidt, W. (2012). Complementary proteome and transcriptome profiling in phosphate-deficient *Arabidopsis* roots reveals multiple levels of gene regulation. *Mol. Cell. Proteomics* 11, 1156–1166. doi: 10.1074/mcp.m112.020461
- Latrasse, D., Rodriguez-Granados, N. Y., Veluchamy, A., Mariappan, K. G., Bevilacqua, C., Crapart, N., et al. (2017). The quest for epigenetic regulation underlying unisexual flower development in *Cucumis melo*. *Epigenet. Chrom.* 10:22.
- Laucou, V., Launay, A., Bacilieri, R., Lacombe, T., Adam-Blondon, A. F., Berard, A., et al. (2018). Extended diversity analysis of cultivated grapevine *Vitis vinifera*

- with 10K genome-wide SNPs. *PLoS One* 13:e0192540. doi: 10.1371/journal.pone.0192540
- Lauresergues, D., Couzigou, J. M., Clemente, H. S., Martinez, Y., Dunand, C., Becard, G., et al. (2015). Primary transcripts of microRNAs encode regulatory peptides. *Nature* 520, 90–93. doi: 10.1038/nature14346
- Lecourieux, D., Kappel, C., Claverol, S., Pieri, P., Feil, R., Lunn, J. E., et al. (2020). Proteomic and metabolomic profiling underlines the stage- and time-dependent effects of high temperature on grape berry metabolism. *J. Integr. Plant Biol.* 62, 1132–1158. doi: 10.1111/jipb.12894
- Lecourieux, F., Kappel, C., Pieri, P., Charon, J., Pillet, J., Hilbert, G., et al. (2017). Dissecting the biochemical and transcriptomic effects of a locally applied heat treatment on developing cabernet sauvignon grape berries. *Front. Plant Sci.* 8:53. doi: 10.3389/fpls.2017.00053
- Leng, X., Wang, P., Zhu, X., Li, X., Zheng, T., Shangquan, L., et al. (2017). Ectopic expression of CSD1 and CSD2 targeting genes of miR398 in grapevine is associated with oxidative stress tolerance. *Funct. Integr. Genomics* 17, 697–710. doi: 10.1007/s10142-017-0565-9
- Leolini, L., Costafreda-Aumedes, S. A., Santos, J., Menz, C., Fraga, H., Molitor, D., et al. (2020). Phenological model intercomparison for estimating grapevine budbreak date (*Vitis vinifera* L.). *Europe. Appl. Sci. Basel* 10:3800. doi: 10.3390/app10113800
- Liang, Z. C., Duan, S. C., Sheng, J., Zhu, S. S., Ni, X. M., Shao, J. H., et al. (2019). Whole-genome resequencing of 472 *Vitis* accessions for grapevine diversity and demographic history analyses. *Nat. Commun.* 10:1190.
- Ling, Y., Serrano, N., Gao, G., Atia, M., Mokhtar, M., Woo, Y. H., et al. (2018). Thermopriming triggers splicing memory in *Arabidopsis*. *J. Exp. Bot.* 69, 2659–2675. doi: 10.1093/jxb/ery062
- Liu, G. T., Jiang, J. F., Liu, X. N., Jiang, J. Z., Sun, L., Duan, W., et al. (2019). New insights into the heat responses of grape leaves via combined phosphoproteomic and acetylproteomic analyses. *Hort. Res.* 6:100.
- Liu, G. T., Ma, L., Duan, W., Wang, B. C., Li, J. H., Xu, H. G., et al. (2014). Differential proteomic analysis of grapevine leaves by iTRAQ reveals responses to heat stress and subsequent recovery. *BMC Plant Biol.* 14:110. doi: 10.1186/1471-2229-14-110
- Liu, H.-F., Wu, B.-H., Fan, P.-G., Li, S.-H., and Li, L.-S. (2006). Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *J. Sci. Food Agric.* 86, 1526–1536. doi: 10.1002/jsfa.2541
- Liu, T., Wu, P., Wang, Q., Wang, W., Zhang, C., Sun, F., et al. (2018). Comparative transcriptome discovery and elucidation of the mechanism of long noncoding RNAs during vernalization in *Brassica rapa*. *Plant Growth Regul.* 85, 27–39. doi: 10.1007/s10725-018-0371-y
- Ma, X., Zhang, Q., Zhu, Q., Liu, W., Chen, Y., Qiu, R., et al. (2015). A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Mol. Plant* 8, 1274–1284. doi: 10.1016/j.molp.2015.04.007
- Malnoy, M., Viola, R., Hung, M.-H., Koo, O.-J., Kim, S., Velasco, R., et al. (2016). DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. *Front. Plant Sci.* 7:1904. doi: 10.3389/fpls.2016.01904
- Marais, J., Van Wyk, C. J., and Rapp, A. (1992). Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin blanc grapes and Weisser Riesling Wines. *S. Afr. J. Enol. Vitic.* 13, 23–32.
- Marfil, C., Ibanez, V., Alonso, R., Varela, A., Bottini, R., Masuelli, R., et al. (2019). Changes in grapevine DNA methylation and polyphenols content induced by solar ultraviolet-B radiation, water deficit and abscisic acid spray treatments. *Plant Physiol. Biochem.* 135, 287–294. doi: 10.1016/j.plaphy.2018.12.021
- Marguerit, E., Brendel, O., Lebon, E., Van Leeuwen, C., and Ollat, N. (2012). Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. *New Phytol.* 194, 416–429. doi: 10.1111/j.1469-8137.2012.04059.x
- Martinelli, L., and Mandolino, G. (1994). Genetic transformation and regeneration of transgenic plants in grapevine (*Vitis rupestris* S.). *Theor. Appl. Genet.* 88, 621–628. doi: 10.1007/bf01253963
- Martinez, de Toda, F., Sancha, J. C., Zheng, W., and Balda, P. (2014). Leaf area reduction by trimming, a growing technique to restore the anthocyanins: sugars ratio decoupled by the warming climate. *Vitis* 53, 189–192.
- Martinez-Luscher, J., Morales, F., Delrot, S., Sanchez-Diaz, M., Gomes, E., Aguirreola, J., et al. (2015a). Characterization of the adaptive response of grapevine (cv. Tempranillo) to UV-B radiation under water deficit conditions. *Plant Sci.* 232, 13–22. doi: 10.1016/j.plantsci.2014.12.013
- Martinez-Luscher, J., Morales, F., Sanchez-Diaz, M., Delrot, S., Aguirreola, J., Gomes, E., et al. (2015b). Climate change conditions (elevated CO₂ and temperature) and UV-B radiation affect grapevine (*Vitis vinifera* cv. Tempranillo) leaf carbon assimilation, altering fruit ripening rates. *Plant Sci.* 236, 168–176. doi: 10.1016/j.plantsci.2015.04.001
- Martinez-Lüscher, J., Sanchez-Diaz, M., Delrot, S., Aguirreola, J., Pascual, I., and Gomès, E. (2014). Ultraviolet-B radiation and water deficit interact to alter flavonol and anthocyanin profiles in grapevine berries through transcriptomic regulation. *Plant Cell Physiol.* 55, 1925–1936. doi: 10.1093/pcp/pcu121
- Martinez-Lüscher, J., Sanchez-Diaz, M., Delrot, S., Aguirreola, J., Pascual, I., and Gomès, E. (2016). Ultraviolet-B alleviates the uncoupling effect of elevated CO₂ and increased temperature on grape berry (*Vitis vinifera* cv. Tempranillo) anthocyanin and sugar accumulation. *Aust. J. Grape Wine Res.* 22, 87–95. doi: 10.1111/ajgw.12213
- Mateo, J. J., and Jimenez, M. (2000). Monoterpenes in grape juice and wines. *J. Chromatogr. A* 881, 557–567. doi: 10.1016/S0021-9673(99)00342-4
- Matthews, M. A., and Anderson, M. M. (1989). Reproductive development in grape (*Vitis vinifera* L.): responses to seasonal water deficit. *Am. J. Enol. Vitic.* 40, 52–60.
- Matzke, M. A., Kanno, T., and Matzke, A. J. M. (2015). “RNA-Directed DNA methylation: the evolution of a complex epigenetic pathway in flowering plants,” in *Annual Review of Plant Biology*, Vol. 66, ed. S. S. Merchant (Santa Clara, CA: Palo Alto), 243–267. doi: 10.1146/annurev-arplant-043014-114633
- Mauch-Mani, B., Baccelli, I., Luna, E., and Flors, V. (2017). Defense priming: an adaptive part of induced resistance. *Annu. Rev. Plant Biol.* 68, 485–512. doi: 10.1146/annurev-arplant-042916-041132
- Merdinoglu, D., Schneider, C., Prado, E., Wiedemann-Merdinoglu, S., and Mestre, P. (2018). Breeding for durable resistance to downy and powdery mildew in grapevine. *OENO One* 52, 203–209. doi: 10.20870/oeno-one.2018.52.3.2116
- Meuwissen, T. H. E., Hayes, B. J., and Goddard, M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157:1819.
- Migicovsky, Z., Sawler, J., Gardner, K. M., Aradhya, M. K., Prins, B. H., Schwaninger, H. R., et al. (2017). Patterns of genomic and phenomic diversity in wine and table grapes. *Hort. Res.* 4:17035.
- Minio, A., Massonnet, M., Figueroa-Balderas, R., Vondras, A. M., Blanco-Ulate, B., and Cantu, D. (2019). Iso-seq allows genome-independent transcriptome profiling of grape berry development. *G3 Genes Genomes Genet.* 9, 755–767.
- Molitor, D., and Junk, J. (2019). Climate change is implicating a two-fold impact on air temperature increase in the ripening period under the conditions of the Luxembourgish grapegrowing region. *OENO One* 53, 409–422.
- Morales-Castilla, I., Garcia, De Cortazar-Atauri, I., Cook, B. I., Lacombe, T., Parker, A., et al. (2020). Diversity buffers winegrowing regions from climate change losses. *Proc. Natl. Acad. Sci. U.S.A.* 117, 2864–2869. doi: 10.1073/pnas.1906731117
- Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1935–1945. doi: 10.1093/jxb/erm055
- Moriondo, M., Bindi, M., Fagarazzi, C., Ferrise, R., and Trombi, G. (2010). Framework for high-resolution climate change impact assessment on grapevines at a regional scale. *Reg. Envir. Chang.* 11, 553–567. doi: 10.1007/s10113-010-0171-z
- Mozsár, J., Viczián, O., and Süle, S. (1998). *Agrobacterium*-mediated genetic transformation of an interspecific grapevine. *Vitis* 37, 127–130.
- Nibau, C., Gallem, M., Dadarou, D., Doonan, J. H., and Cavallari, N. (2019). Thermo-sensitive alternative splicing of FLOWERING LOCUS M is modulated by cyclin dependent kinase G2. *Front. Plant Sci.* 10:1680. doi: 10.3389/fpls.2019.01680
- Nicolas, S. D., Peros, J. P., Lacombe, T., Launay, A., Le Paslier, M. C., Berard, A., et al. (2016). Genetic diversity, linkage disequilibrium and power of a large grapevine (*Vitis vinifera* L.) diversity panel newly designed for association studies. *BMC Plant Biol.* 16:74. doi: 10.1186/s12870-016-0754-z
- Ocana, J., Walter, B., and Schellenbaum, P. (2013). Stable MSAP markers for the distinction of *Vitis vinifera* cv Pinot noir clones. *Mol. Biotechnol.* 55, 236–248. doi: 10.1007/s12033-013-9675-3

- Pagliarani, C., and Gambino, G. (2019). Small RNA mobility: spread of rna silencing effectors and its effect on developmental processes and stress adaptation in plants. *Int. J. Mol. Sci.* 20:4306. doi: 10.3390/ijms20174306
- Pagliarani, C., Vitali, M., Ferrero, M., Vitulo, N., Incarbone, M., Lovisolio, C., et al. (2017). The accumulation of miRNAs differentially modulated by drought stress is affected by grafting in grapevine. *Plant Physiol.* 173, 2180–2195. doi: 10.1104/pp.16.01119
- Pajaro, A., Severing, E., Angenent, G. C., and Immink, R. G. H. (2017). Histone H3 lysine 36 methylation affects temperature-induced alternative splicing and flowering in plants. *Genome Biol.* 18:102.
- Palumbo, M., Zenoni, S., Fasoli, M., Massonet, M., Farina, L., Castiglione, F., et al. (2014). Integrated network analysis identifies fight-club nodes as a class of hubs encompassing key putative switch genes that induce major transcriptome reprogramming during grapevine development. *Plant Cell* 26, 4617–4635. doi: 10.1105/tpc.114.133710
- Palusa, S. G., Ali, G. S., and Reddy, A. S. (2007). Alternative splicing of pre-mRNAs of *Arabidopsis* serine/arginine-rich proteins: regulation by hormones and stresses. *Plant J.* 49, 1091–1107. doi: 10.1111/j.1365-313x.2006.03020.x
- Pan, Z., Zeng, Y., An, J., Ye, J., Xu, Q., and Deng, X. (2012). An integrative analysis of transcriptome and proteome provides new insights into carotenoid biosynthesis and regulation in sweet orange fruits. *J. Proteomics* 75, 2670–2684. doi: 10.1016/j.jprot.2012.03.016
- Pantaleo, V., Vitali, M., Boccacci, P., Miozzi, L., Cuozzo, D., Chitarra, W., et al. (2016). Novel functional microRNAs from virus-free and infected *Vitis vinifera* plants under water stress. *Sci. Rep.* 6:20167.
- Park, Y. J., Lee, J. H., Kim, J. Y., and Park, C. M. (2019). Alternative RNA splicing expands the developmental plasticity of flowering transition. *Front. Plant Sci.* 10:606. doi: 10.3389/fpls.2019.00606
- Parker, A., De Cortázar-Atauri, I. G., Chuine, I., Barbeau, G., Bois, B., Boursiquot, J.-M., et al. (2013). Classification of varieties for their timing of flowering and veraison using a modelling approach: a case study for the grapevine species *Vitis vinifera* L. *Agr. Forest Meteorol.* 180, 249–264. doi: 10.1016/j.agrformet.2013.06.005
- Pastore, C., Dal Santo, S., Zenoni, S., Movahed, N., Allegro, G., Valentini, G., et al. (2017). Whole plant temperature manipulation affects flavonoid metabolism and the transcriptome of grapevine berries. *Front. Plant Sci.* 8:929. doi: 10.3389/fpls.2017.00929
- Pastore, C., Filippetti, I., Zenoni, S., Fasoli, M., Ferrarini, A., Pezzotti, M., et al. (2014). Differential expression of genes in berries of cv. "Sangiovese" (*Vitis vinifera* L.) during ripening following cluster thinning at veraison. *Acta Hort.* 1046, 441–448. doi: 10.17660/actahortic.2014.1046.60
- Pelsy, F., Hocquigny, S., Moncada, X., Barbeau, G., Forget, D., Hinrichsen, P., et al. (2010). An extensive study of the genetic diversity within seven French wine grape variety collections. *Theor. Appl. Genet.* 120, 1219–1231. doi: 10.1007/s00122-009-1250-8
- Perrone, I., Gambino, G., Chitarra, W., Vitali, M., Pagliarani, C., Riccomagno, N., et al. (2012). The grapevine root-specific aquaporin *VvPIP2;4N* controls hydraulic conductance and leaf gas exchange under well-watered conditions but not under water stress. *Plant Physiol.* 160, 965–977. doi: 10.1104/pp.112.203455
- Petrie, P. R., and Clingeleffer, P. R. (2005). Effects of temperature and light (before and after budburst) on inflorescence morphology and flower number of Chardonnay grapevines (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 11, 59–65. doi: 10.1111/j.1755-0238.2005.tb00279.x
- Pons, A., Allamy, L., Schüttler, A., Rauhut, D., Thibon, C., and Darriet, P. (2017). What is the expected impact of climate change on wine aroma compounds and their precursors in grape? *OENO One* 51, 141–146. doi: 10.20870/oeno-one.2016.0.0.1868
- Prudent, M., Lecomte, A., Bouchet, J. P., Bertin, N., Causse, M., and Genard, M. (2011). Combining ecophysiological modelling and quantitative trait locus analysis to identify key elementary processes underlying tomato fruit sugar concentration. *J. Exp. Bot.* 62, 907–919. doi: 10.1093/jxb/erq318
- Quilot-Turion, B., Genard, M., Valsesia, P., and Memmah, M. M. (2016). Optimization of allelic combinations controlling parameters of a peach quality model. *Front. Plant Sci.* 7:1873. doi: 10.3389/fpls.2016.01873
- Rahhal, R., and Seto, E. (2019). Emerging roles of histone modifications and HDACs in RNA splicing. *Nucleic Acids Res.* 47, 4911–4926. doi: 10.1093/nar/gkz292
- Reddy, A. S., Marquez, Y., Kalyna, M., and Barta, A. (2013). Complexity of the alternative splicing landscape in plants. *Plant Cell* 25, 3657–3683. doi: 10.1105/tpc.113.117523
- Ren, C., Liu, X., Zhang, Z., Wang, Y., Duan, W., Li, S., et al. (2016). CRISPR/Cas9-mediated efficient targeted mutagenesis in Chardonnay (*Vitis vinifera* L.). *Sci. Rep.* 6:32289.
- Reymond, M., Muller, B., Leonardi, A., Charcosset, A., and Tardieu, F. (2003). Combining quantitative trait Loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiol.* 131, 664–675. doi: 10.1104/pp.013839
- Rienth, M., Torregrosa, L., Luchaire, N., Chatbanyong, R., Lecourieux, D., Kelly, M. T., et al. (2014). Day and night heat stress trigger different transcriptomic responses in green and ripening grapevine (*Vitis vinifera*) fruit. *BMC Plant Biol.* 14:108. doi: 10.1186/1471-2229-14-108
- Rienth, M., Torregrosa, L., Sarah, G., Ardisson, M., Brillouet, J.-M., and Romieu, C. (2016). Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome. *BMC Plant Biol.* 16:164. doi: 10.1186/s12870-016-0850-0
- Rinaldo, A., Cavallini, E., Yong, J., Moss, S., McDavid, D., Hooper, L., et al. (2015). A grapevine anthocyanin acyltransferase, transcriptionally regulated by VvMYBA, can produce most acylated anthocyanins present in grape skins. *Plant Physiol.* 169, 1897–1916.
- Roderick, M. L., Hobbins, M. T., and Farquhar, G. D. (2009). Pan evaporation trends and the terrestrial water balance. II. Energy balance and interpretation. *Geogr. Compass* 3, 761–780. doi: 10.1111/j.1749-8198.2008.00214.x
- Rosen, A., Hasan, Y., Briggs, W., and Uptmoor, R. (2018). Genome-based prediction of time to curd induction in Cauliflower. *Front. Plant Sci.* 9:78. doi: 10.3389/fpls.2018.00078
- Rossmann, S., Richter, R., Sun, H., Schneeberger, K., Topfer, R., Zyprian, E., et al. (2020). Mutations in the miR396 binding site of the growth-regulating factor gene *VvGRF4* modulate inflorescence architecture in grapevine. *Plant J.* 101, 1234–1248. doi: 10.1111/tpj.14588
- Salmon, J.-M., Ojeda, H., and Escudier, J.-L. (2018). Disease resistant grapevine varieties and quality: the case of Bouquet varieties. *OENO One* 52, 225–230. doi: 10.20870/oeno-one.2018.52.3.2139
- Schmidt, S. M., Belisle, M., and Frommer, W. B. (2020). The evolving landscape around genome editing in agriculture: many countries have exempted or move to exempt forms of genome editing from GMO regulation of crop plants. *Embo Rep.* 21:e50680.
- Schultz, H. R. (2000). Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. *Aust. J. Grape Wine Res.* 6, 2–12. doi: 10.1111/j.1755-0238.2000.tb00156.x
- Schultz, H. R. (2017). Issues to be considered for strategic adaptation to climate evolution – is atmospheric evaporative demand changing? *OENO One* 51, 107–114. doi: 10.20870/oeno-one.2016.0.0.1619
- Scorza, R., Cordts, J., Ramming, D., and Emershad, R. (1995). Transformation of grape (*Vitis vinifera* L.) zygotic-derived somatic embryos and regeneration of transgenic plants. *Plant Cell Rep.* 14, 589–592.
- Serra, I., Strever, A., Myburgh, P. A., and Deloire, A. (2014). Review: the interaction between rootstocks and cultivars (*Vitis vinifera* L.) to enhance drought tolerance in grapevine. *Aust. J. Grape Wine Res.* 20, 1–14. doi: 10.1111/ajgw.12054
- Seymour, D. K., and Becker, C. (2017). The causes and consequences of DNA methylome variation in plants. *Curr. Opin. Plant Biol.* 36, 56–63. doi: 10.1016/j.pbi.2017.01.005
- Sgubin, G., Swingedouw, D., Dayon, G., García, De Cortázar-Atauri, I., Ollat, N., et al. (2018). The risk of tardive frost damage in French vineyards in a changing climate. *Agric. Forest Meteorol.* 250–251, 226–242. doi: 10.1016/j.agrformet.2017.12.253
- Shangguan, L., Fang, X., Jia, H., Chen, M., Zhang, K., and Fang, J. (2020). Characterization of DNA methylation variations during fruit development and ripening of *Vitis vinifera* (cv. 'Fujiminori'). *Physiol. Mol. Biol. Plants* 26, 617–637. doi: 10.1007/s12298-020-00759-5
- Sharma, A., Badola, P. K., Bhatia, C., Sharma, D., and Trivedi, P. K. (2020). Primary transcript of miR858 encodes regulatory peptide and controls flavonoid biosynthesis and development in *Arabidopsis*. *Nat. Plants* 6, 1262–1274. doi: 10.1038/s41477-020-00769-x

- Shiraishi, M. (1995). Proposed descriptors for organic acids to evaluate grape germplasm. *Euphytica* 81, 13–20. doi: 10.1007/bf00022454
- Siebert, T. E., Wood, C., Elsey, G. M., and Pollnitz, A. P. (2008). Determination of rotundone, the pepper aroma impact compound, in grapes and wine. *J. Agric. Food Chem.* 56, 3745–3748. doi: 10.1021/jf800184t
- Simonneau, T., Lebon, E., Coupel-Ledru, A., Marguerit, E., Rossdeutsch, L., and Ollat, N. (2017). Adapting plant material to face water stress in vineyards: which physiological targets for an optimal control of plant water status? *OENO One* 51, 167–179. doi: 10.20870/oeno-one.2017.51.2.1870
- Sun, X., Fan, G., Su, L., Wang, W., Liang, Z., Li, S., et al. (2015). Identification of cold-inducible microRNAs in grapevine. *Front. Plant Sci.* 6:595. doi: 10.3389/fpls.2015.00595
- Syed, N. H., Kalyna, M., Marquez, Y., Barta, A., and Brown, J. W. (2012). Alternative splicing in plants—coming of age. *Trends Plant. Sci.* 17, 616–623. doi: 10.1016/j.tplants.2012.06.001
- Takase, H., Sasaki, K., Shinmori, H., Shinohara, A., Mochizuki, C., Kobayashi, H., et al. (2016). Cytochrome P450 CYP71BE5 in grapevine (*Vitis vinifera*) catalyzes the formation of the spicy aroma compound (-)-rotundone. *J. Exp. Bot.* 67, 787–798. doi: 10.1093/jxb/erv496
- Tamiru, M., Hardcastle, T. J., and Lewsey, M. G. (2018). Regulation of genome-wide DNA methylation by mobile small RNAs. *New Phytol.* 217, 540–546. doi: 10.1111/nph.14874
- Tandonnet, J. P., Marguerit, E., Cookson, S. J., and Ollat, N. (2018). Genetic architecture of aerial and root traits in field-grown grafted grapevines is largely independent. *Theor. Appl. Genet.* 131, 903–915. doi: 10.1007/s00122-017-3046-6
- Tello, J., Roux, C., Chouiki, H., Laucou, V., Sarah, G., Weber, A., et al. (2019). A novel high-density grapevine (*Vitis vinifera* L.) integrated linkage map using GBS in a half-diallel population. *Theor. Appl. Genet.* 132, 2237–2252. doi: 10.1007/s00122-019-03351-y
- Tempere, S., Pérès, S., Espinoza, A. F., Darriet, P., Giraud-Héraud, E., and Pons, A. (2019). Consumer preferences for different red wine styles and repeated exposure effects. *Food. Qual. Prefer.* 73, 110–116. doi: 10.1016/j.foodqual.2018.12.009
- Terrier, N., Ageorges, A., Abbal, P., and Romieu, C. (2011). Generation of ESTs from grape berry at various developmental stages. *J. Plant Physiol.* 148, 1575–1583. doi: 10.1078/0176-1617-00566
- Terrier, N., Glissant, D., Grimplet, J., Barrieu, F., Abbal, P., Couture, C., et al. (2006). Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. *Planta* 222, 832–847. doi: 10.1007/s00425-005-0017-y
- Tillett, R. L., Wheatley, M. D., Tattersall, E. A., Schlauch, K. A., Cramer, G. R., and Cushman, J. C. (2012). The *Vitis vinifera* C-repeat binding protein 4 (*VvCBF4*) transcriptional factor enhances freezing tolerance in wine grape. *Plant Biotechnol. J.* 10, 105–124. doi: 10.1111/j.1467-7652.2011.00648.x
- Tirumalai, V., Swetha, C., Nair, A., Pandit, A., and Shivaprasad, P. V. (2019). miR828 and miR858 regulate VvMYB114 to promote anthocyanin and flavonol accumulation in grapes. *J. Exp. Bot.* 70, 4775–4792. doi: 10.1093/jxb/erz264
- Tomás, M., Medrano, H., Escalona, J. M., Martorell, S., Pou, A., Ribas-Carbó, M., et al. (2014). Variability of water use efficiency in grapevines. *Environ. Exp. Bot.* 103, 148–157. doi: 10.1016/j.envexpbot.2013.09.003
- Tonietto, J., and Carbonneau, A. (1998). Facteurs mésoclimatiques de la typicité du raisin de table de l'A.O.C. Muscat du Ventoux dans le département de Vaucluse, France. *Progr. Agric. Vitic.* 115, 271–279.
- Tonietto, J., and Carbonneau, A. (2004). A multicriteria climatic classification system for grape-growing regions worldwide. *Agric. Forest Meteorol.* 124, 81–97. doi: 10.1016/j.agrformet.2003.06.001
- Torregrosa, L., Bigard, A., Doligez, A., Lecourieux, D., Rienth, M., Luchaire, N., et al. (2017). Developmental, molecular and genetic studies on grapevine response to temperature open breeding strategies for adaptation to warming. *OENO One* 51:11.
- Tricker, P. J., Lopez, C. M., Gibbings, G., Hadley, P., and Wilkinson, M. J. (2013). Transgenerational, dynamic methylation of stomata genes in response to low relative humidity. *Int. J. Mol. Sci.* 14, 6674–6689. doi: 10.3390/ijms14046674
- van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchene, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy* 9:514. doi: 10.3390/agronomy9090514
- Varela, A., Ibanez, V. N., Alonso, R., Zavallo, D., Asurmendi, S., Gomez Talquenca, S., et al. (2020). Vineyard environments influence Malbec grapevine phenotypic traits and DNA methylation patterns in a clone-dependent way. *Plant Cell Rep.* 40, 111–125. doi: 10.1007/s00299-020-02617-w
- Viana, A. P., Riaz, S., and Walker, M. A. (2013). Genetic dissection of agronomic traits within a segregating population of breeding table grapes. *Genet. Mol. Res.* 12, 951–964. doi: 10.4238/2013.april.2.11
- Villano, C., Carputo, D., Frusciante, L., Santoro, X., and Aversano, R. (2014). Use of SSR and retrotransposon-based markers to interpret the population structure of native grapevines from southern Italy. *Mol. Biotechnol.* 56, 1011–1020. doi: 10.1007/s12033-014-9780-y
- Vincent, D., Ergul, A., Bohlman, M. C., Tattersall, E. A., Tillett, R. L., Wheatley, M. D., et al. (2007). Proteomic analysis reveals differences between *Vitis vinifera* L. cv. Chardonnay and cv. Cabernet Sauvignon and their responses to water deficit and salinity. *J. Exp. Bot.* 58, 1873–1892. doi: 10.1093/jxb/erm012
- Visentin, I., Pagliarani, C., Deva, E., Caracci, A., Tureckova, V., Novak, O., et al. (2020). A novel strigolactone-miR156 module controls stomatal behaviour during drought recovery. *Plant Cell Environ.* 43, 1613–1624. doi: 10.1111/pce.13758
- Vitulo, N., Forcato, C., Carpinelli, E. C., Telatin, A., Campagna, D., D'angelo, M., et al. (2014). A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and genotype. *BMC Plant Biol.* 14:99. doi: 10.1186/1471-2229-14-99
- Walker, A. R., Lee, E., Bogs, J., McDavid, D. A., Thomas, M. R., and Robinson, S. P. (2007). White grapes arose through the mutation of two similar and adjacent regulatory genes. *Plant J.* 49, 772–785. doi: 10.1111/j.1365-3113.2006.02997.x
- Waltz, E. (2012). Tiptoeing around transgenic. *Nat. biotechnol.* 30, 215–217. doi: 10.1038/nbt.2143
- Wan, D. Y., Guo, Y., Cheng, Y., Hu, Y., Xiao, S., Wang, Y., et al. (2020). CRISPR/Cas9-mediated mutagenesis of *VvMLO3* results in enhanced resistance to powdery mildew in grapevine (*Vitis vinifera*). *Hort. Res.* 7:116.
- Wang, M., Sun, X., Wang, C., Cui, L., Chen, L., Zhang, C., et al. (2017). Characterization of miR061 and its target genes in grapevine responding to exogenous gibberellic acid. *Funct. Integr. Genomics* 17, 537–549. doi: 10.1007/s10142-017-0554-z
- Wang, P., Dai, L., Ai, J., Wang, Y., and Ren, F. (2019). Identification and functional prediction of cold-related long non-coding RNA (lncRNA) in grapevine. *Sci. Rep.* 9:6638.
- Wang, X., Yang, M., Ren, D., Terzaghi, W., Deng, X. W., and He, G. (2019). Cis-regulated alternative splicing divergence and its potential contribution to environmental responses in *Arabidopsis*. *Plant J.* 97, 555–570.
- Wang, S., Tian, L., Liu, H., Li, X., Zhang, J., Chen, X., et al. (2020). Large-scale discovery of non-conventional peptides in maize and arabidopsis through an integrated peptidogenomic pipeline. *Mol. Plant* 13, 1078–1093. doi: 10.1016/j.molp.2020.05.012
- Wang, Y., Liu, X., Ren, C., Zhong, G. Y., Yang, L., Li, S., et al. (2016). Identification of genomic sites for CRISPR/Cas9-based genome editing in the *Vitis vinifera* genome. *BMC Plant Biol.* 16:96. doi: 10.1186/s12870-016-0787-3
- Wang, Z., Ji, H., Yuan, B., Wang, S., Su, C., Yao, B., et al. (2015). ABA signalling is fine-tuned by antagonistic HABI variants. *Nat. Commun.* 6:8138.
- Wiggans, G. R., Cole, J. B., Hubbard, S. M., and Sonstegard, T. S. (2017). Genomic selection in dairy cattle: the USDA experience. *Annu. Rev. Anim. Biosci.* 5, 309–327. doi: 10.1146/annurev-animal-021815-111422
- Wohlfahrt, Y., Smith, J., Tittmann, S., Honermeier, B., and Stoll, M. (2018). Primary productivity and physiological responses of *Vitis vinifera* L. cvs. under Free Air Carbon dioxide Enrichment (FACE). *Eur. J. Agron.* 101, 149–162. doi: 10.1016/j.eja.2018.09.005
- Wu, J., Xu, Z., Zhang, Y., Chai, L., Yi, H., and Deng, X. (2014). An integrative analysis of the transcriptome and proteome of the pulp of a spontaneous late-ripening sweet orange mutant and its wild type improves our understanding of fruit ripening in citrus. *J. Exp. Bot.* 65, 1651–1671. doi: 10.1093/jxb/eru044
- Xie, H., Konate, M., Sai, N., Tesfamichael, K. G., Cavnagaro, T., Gilliam, M., et al. (2017). Global DNA methylation patterns can play a role in defining terroir in grapevine (*Vitis vinifera* cv. Shiraz). *Front. Plant Sci.* 8:1860. doi: 10.3389/fpls.2017.01860
- Xu, J., Hou, Q. M., Khare, T., Verma, S. K., and Kumar, V. (2019). Exploring miRNAs for developing climate-resilient crops: a perspective review. *Sci. Total Environ.* 653, 91–104. doi: 10.1016/j.scitotenv.2018.10.340

- Yamamoto, T., Iketani, H., Ieki, H., Nishizawa, Y., Notsuka, K., Hibi, T., et al. (2000). Transgenic grapevine plants expressing a rice chitinase with enhanced resistance to fungal pathogens. *Plant Cell Rep.* 19, 639–646. doi: 10.1007/s002999900174
- Yang, S., Fresnedo-Ramirez, J., Sun, Q., Manns, D. C., Sacks, G. L., Mansfield, A. K., et al. (2016). Next generation mapping of enological traits in an F2 interspecific grapevine hybrid family. *PLoS One* 11:e0149560. doi: 10.1371/journal.pone.0149560
- Yang, X., Zhang, H., and Li, L. (2012). Alternative mRNA processing increases the complexity of microRNA-based gene regulation in *Arabidopsis*. *Plant J.* 70, 421–431. doi: 10.1111/j.1365-313x.2011.04882.x
- Zarrouk, O., Brunetti, C., Egipto, R., Pinheiro, C., Genebra, T., Gori, A., et al. (2016). Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy. *Front. Plant Sci.* 7:1640. doi: 10.3389/fpls.2016.01640
- Zenoni, S., Ferrantini, A., Giacomelli, E., Xumerle, L., Fasoli, M., Malerba, G., et al. (2010). Characterization of transcriptional complexity during berry development in *Vitis vinifera* using RNA-seq. *Plant Physiol.* 152, 1787–1795. doi: 10.1104/pp.109.149716
- Zhang, Z., and Xiao, B. (2018). Comparative alternative splicing analysis of two contrasting rice cultivars under drought stress and association of differential splicing genes with drought response QTLs. *Euphytica* 214:73.
- Zyprian, E., Ochssner, I., Schwander, F., Simon, S., Hausmann, L., Bonow-Rex, M., et al. (2016). Quantitative trait loci affecting pathogen resistance and ripening of grapevines. *Mol. Genet. Genomics* 291, 1573–1594. doi: 10.1007/s00438-016-1200-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Gomès, Maillot and Duchêne. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Projected Wine Grape Cultivar Shifts Due to Climate Change in New Zealand

Anne-Gaelle E. Ausseil^{1*}, Richard M. Law², Amber K. Parker³, Edmar I. Teixeira⁴ and Abha Sood⁵

¹ Manaaki Whenua — Landcare Research, Wellington, New Zealand, ² Manaaki Whenua — Landcare Research, Palmerston North, New Zealand, ³ Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, New Zealand, ⁴ Plant and Food Research, Lincoln, New Zealand, ⁵ National Institute of Water and Atmospheric Research, Wellington, New Zealand

OPEN ACCESS

Edited by:

Chris Winefield,
Lincoln University, New Zealand

Reviewed by:

Oswaldo Failla,
University of Milan, Italy
Steven Schultze,
University of South Alabama,
United States

*Correspondence:

Anne-Gaelle E. Ausseil
ausseila@landcareresearch.co.nz

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 16 October 2020

Accepted: 26 March 2021

Published: 21 April 2021

Citation:

Ausseil A-GE, Law RM, Parker AK,
Teixeira EI and Sood A (2021)
Projected Wine Grape Cultivar
Shifts Due to Climate Change in
New Zealand.
Front. Plant Sci. 12:618039.
doi: 10.3389/fpls.2021.618039

Climate change has already been affecting the regional suitability of grapevines with significant advances in phenology being observed globally in the last few decades. This has significant implications for New Zealand, where the wine industry represents a major share of the horticultural industry revenue. We modeled key crop phenological stages to better understand temporal and spatial shifts in three important regions of New Zealand (Marlborough, Hawke's Bay, Central Otago) for three dominant cultivars (Merlot, Pinot noir, and Sauvignon blanc) and one potential new and later ripening cultivar (Grenache). Simulations show an overall advance in flowering, véraison, and sugar ripeness by mid-century with more pronounced advance by the end of the century. Results show the magnitude of changes depends on the combination of greenhouse gas emission pathway, grape cultivar, and region. By mid-century, in the Marlborough region for instance, the four cultivars would flower 3 to 7 days earlier and reach sugar ripeness 7 to 15 days earlier depending on the greenhouse gas emission pathway. For growers to maintain the same timing of key phenological stages would require shifting planting of cultivars to more Southern parts of the country or implement adaptation strategies. Results also show the compression of time between flowering and véraison for all three dominant cultivars is due to a proportionally greater advance in véraison, particularly for Merlot in the Hawke's Bay and Pinot noir in Central Otago. Cross-regional analysis also raises the likelihood of the different regional cultivars ripening within a smaller window of time, complicating harvesting schedules across the country. However, considering New Zealand primarily accommodates cool climate viticulture cultivars, our results suggest that late ripening cultivars or extended ripening window in cooler regions may be advantageous in the face of climate change. These insights can inform New Zealand winegrowers with climate change adaptation options for their cultivar choices.

Keywords: adaptation, cumulative thermal time, climate change, cultivar, wine grape, phenology

1. INTRODUCTION

Climate change poses major challenges to the wine industry. Research on past observations have shown that in recent decades, climate change has led to advances in phenology, higher sugar concentrations at harvest and compressed or earlier harvests, as well as changes in yield and risk profile (Chuine et al., 2004; Duchêne and Schneider, 2005; Webb and Barlow, 2007;

García de Cortázar-Atauri et al., 2010; Molitor et al., 2014a; van Leeuwen and Darriet, 2016; Wolkovich and Morales-Castilla, 2019). For instance, if a grape cultivar ripens too early, véraison to harvest may coincide with the hottest period of the season which potentially leads to negative effects on flavor, aroma, and alcohol content of grapes (van Leeuwen and Seguin, 2006; Duchêne et al., 2010). One of the most critical climatic drivers accelerating phenology is warmer temperature over the full cycle of development (Jones et al., 2005a,b; Jones, 2012; Cook and Wolkovich, 2016; van Leeuwen and Darriet, 2016; Schultze and Sabbatini, 2019).

This issue is particularly relevant for New Zealand where there is little cultivar diversification and significant regional concentration of production. In 2019, the value of New Zealand wine exports was nearly \$1.8 billion (New Zealand Winegrowers, 2020) making wine the second-most significant horticultural export in New Zealand, contributing to 30% of horticultural produce exports in 2019 by value (Ministry for Primary Industries, 2020). Eighty percentage of the total grape production area in New Zealand is a combination of three major cultivars: Sauvignon blanc, Pinot noir, and Merlot (New Zealand Winegrowers, 2020). The growth in area has been considerable in recent years going from 23 to 31 thousand hectares for the period 2010–2019. The Marlborough wine region alone represents 70% of the national production area (New Zealand Winegrowers, 2020), of which 85% of this region's production area is Sauvignon blanc, as well as the region being the largest production area of Pinot noir (Wine Marlborough, 2019).

Phenological modeling can deliver valuable insights into the potential strategies for adaptation to climate change. Research into modeling phenological changes now enables simulations of the time to flowering and véraison (GFV; Grapevine Flowering Véraison model; Parker et al., 2011, 2013) and the time to target sugar ripeness (GSR; Grapevine Sugar Ripeness model; Parker et al., 2020a) for a wide range of cultivars. These models are driven by temperature which allows cultivar suitability to be investigated under the warmer conditions of future climate scenarios across a given country or winegrowing region. The use of models such as GFV and GSR provides a more dynamic way to quantify developmental progression rather than using fixed harvest dates which can be influenced by multiple factors such as end use (e.g., sparkling wine harvest at lower sugar concentrations), logistics of harvest or disease pressure (García de Cortázar-Atauri et al., 2010). Although Morales-Castilla et al. (2020) characterized increased cultivar diversity for NZ under future climate scenarios, this was not at the scale of individual wine regions. Therefore, using the GFV and GSR models to simulate the timing of key phenological stages at regional scale is of interest to understand cultivar choices as an adaptation strategy to climate change.

In this study, we simulated flowering, véraison and target sugar ripeness (represented as the time to reach a 200 g/l target sugar concentration) using the GFV and GSR models at national scale for a range of cultivars. We considered six global circulation models to project future climate, applied on a 0.05° latitude/longitude grid (approximately 4–5 km at New Zealand latitudes) (Tait et al., 2006). The analysis focused on

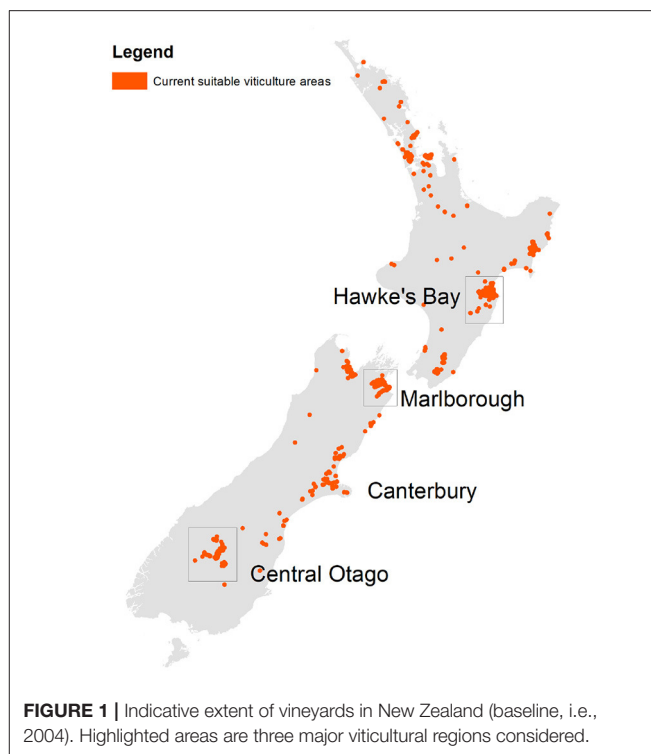


FIGURE 1 | Indicative extent of vineyards in New Zealand (baseline, i.e., 2004). Highlighted areas are three major viticultural regions considered.

two time periods for mid-century (2046–2065) and late century (2081–2100). Cultivar suitability and implications for the wine industry were explored by analyzing spatial and temporal shifts in phenology. Results focus on three key regional cultivars of importance to New Zealand's wine industry (Sauvignon blanc, Pinot noir, and Merlot) and one potential future later-ripening cultivar (Grenache) to explore the intra- and cross-regional impacts under different climate change scenarios.

2. MATERIALS AND METHODS

2.1. Vineyard Suitable Area

Land suitable for viticulture was identified with a combination of a 5 km expansion of the 2004 distribution of viticulture in New Zealand (Manaaki Whenua – Landcare Research, 2004), constrained by a land use capability (LUC) classification (Manaaki Whenua – Landcare Research, 2018) to ensure that land is not affected by fundamental characteristics that would preclude viticulture (e.g., a high water table, or a hazardous area). The dataset was resampled to match the 0.05° climate dataset used to perform the climate change scenario analysis (see **Supplementary Material** for more details). **Figure 1** demonstrates the extent of general suitability for viticulture in New Zealand given this definition.

Three key wine producing regions were considered: Hawke's Bay, Marlborough, and Central Otago (**Figure 1**). Marlborough is the largest producing region, with Sauvignon blanc representing 76% of national production (New Zealand Winegrowers, 2020). Central Otago is the southernmost growing region in NZ and has an important production area of Pinot noir (80% of Central

TABLE 1 | Summary of current temperature (from <https://niwa.co.nz/our-science/climate/publications/regional-climatologies>) and projections range (for RCP 2.6–8.5) in the study regions (Ministry for the Environment, 2018).

Region	Current average daily temperature (°C)		Mid-century (compared to 1995)	End of century (compared to 1995)	Mean regional number of days where maximum temperature is over 32°C (end of century, RCP 8.5)	Mean regional number of days where maximum temperature is over 35°C (end of century, RCP 8.5)
	Winter	Summer				
Marlborough	2–3	21–23	+0.7–1°C	+0.7–3°C	2.4 days/year	0.27 days/year
Hawke's Bay	3–5	21–24	+0.7–1.1°C	+0.7–3.1°C	5.6 days/year	0.4 days/year
Otago	–4–+1	19–22	+0.6–0.9°C	+0.6–2.8°C	17.8 days/year	1.87 days/year

Otago viticultural areas), the second most planted grape cultivar in NZ. Hawke's Bay represents the second biggest production area in NZ of which Merlot is an important red cultivar (New Zealand Winegrowers, 2020). Internationally the three cultivars selected in this study are within the top 10 planted cultivars worldwide (Anderson and Aryal, 2013).

2.2. Climate Change Projections

Climate outcomes are based on a set of Representative Concentration Pathways (RCP) representing scenarios for approximate total radiative forcing at 2100, relative to 1750. The fifth IPCC assessment (Intergovernmental Panel on Climate Change, 2014) selected four scenarios referenced as RCP 2.6, 4.5, 6.0, and 8.5 (for respective radiative forcing of 2.6, 4.5, 6.0, and 8.5 W.m^{-2}). Six Global Circulation Models (GCM) from the Coupled Model Inter-comparison Project (CMIP5), were previously used to downscale simulations of the RCP for New Zealand (Tait et al., 2016; Ministry for the Environment, 2018). The GCMs considered in the analysis were BCC-CSM1.1, CESM1-CAM5, GFDL-CM3, GISSER-R, HadGEM2-ES, and NorESM1-M. The output variables from these models included daily precipitation, maximum and minimum daily air temperature, daily average relative humidity, daily average solar radiation, and daily average wind speed at 10 m. Daily mean temperatures for the period 1979–2120, considering three mean temperatures were calculated as the arithmetic mean of the minimum and maximum daily temperatures from datasets downscaled by the New Zealand National Institute of Water and Atmospheric Research (NIWA).

The three periods of interest were determined as current (1971–2005), mid-century (2046–2065), and end of century (2081–2100).

A summary of current and future temperature change in the three regions of interest is shown in **Table 1**. The range of shifts in temperature is around 0.5 to 3 °C warmer depending on the RCP. Optimal temperature for grapes are around 30 °C, with signs of heat stress above 35 °C (Kliewer, 1977; Hunter and Bonnardot, 2011; Hochberg et al., 2015). For the projections considered in this study, this threshold of maximum temperature was only crossed up to 1.9 days/year under RCP 8.5 by the end of the century in the Otago region.

2.3. Phenology Projection

We used the GFV model developed by Parker et al. (2011) to simulate the time of key phenological stages of flowering and véraison defined as the time at which 50% capfall had occurred and when 50% of berries softened or changed from

TABLE 2 | F^* values for four cultivars, Pinot noir, Merlot, Sauvignon blanc, and Grenache as determined by the GFV model (Parker et al., 2011, 2013) for flowering and véraison, and GSR model (Parker et al., 2020a) for the time to 200 g/l sugar concentration.

	Flowering	Véraison	Sugar concentration 200 g/l
Pinot noir	1 219	2 511	2 838
Merlot	1 269	2 636	2 856
Grenache	1 277	2 761	2 967
Sauvignon blanc	1 282	2 528	2 820

green to translucent for white cultivars or changed color from green to red for red cultivars (Parker et al., 2011). This model is a linear growing degrees days model that relates cumulative daily temperature to the day of the year (DOY) when flowering or véraison occurs. The thermal summation (F^*) is specific to each cultivar. In the case of the GFV model, the cumulative summation starts on the 60th DOY in the Northern Hemisphere (t_0) corresponding to the 242nd DOY in the Southern Hemisphere and uses a base temperature (T_b) of 0 °C. The Grapevine Sugar Ripeness (GSR) model (Parker et al., 2020a) is also a linear model used to simulate the time to reach a target sugar concentration in the berries. The GSR model has $T_b = 0$ and $t_0 = 273$ (Southern Hemisphere). A target sugar concentration of 200 g/l was selected because it represents a mid-target sugar concentration provided within the range presented in Parker et al. (2020a), and it is close to that of an accepted target for Sauvignon blanc (21.5°Brix) as determined by winegrowers in Trought and Bramley (2011).

The corresponding DOY values of flowering, véraison (using GFV) and target sugar ripeness (using GSR) per year were calculated for selected cultivars and F^* values, derived from (Parker et al., 2013, 2020a) (**Table 2**). These raster grids were recorded in a static database for all RCPs and GCMs, with an output resolution of 243x260 pixels (same as the input climate data), covering all of mainland NZ. To summarize the change in phenology for future periods, we recorded the simulated flowering, véraison and target sugar ripeness for 2046–2065 to represent mid-century, and 2081–2100 to represent end of century. For each region of interest, we then summarized the median DOY across the region, for each GCM and each year within the three periods (current, mid-century, and end of century). Areas not capable or not suitable for wine production were masked out for all periods.

The GFV and GSR were selected for the study because they represent some of the most extensively calibrated and validated phenological models to date for the grapevine (Parker et al., 2011, 2020a). The GFV database (which was divided into calibration and validation data) consisted of observations from 1960 to 2007, from 123 locations including 12 of the principal viticulture regions of France, Changins in Switzerland, Veneto and Tuscany in Italy, and the Peloponnese region in Greece, and 81 cultivars. This equated to 2 278 flowering observations and 2 088 véraison observations (Parker et al., 2011). The GSR database covered six different target sugar concentrations, spanning the period 1963–2014, nine of the principal viticulture regions of France, as well as Germany, Greece, Italy, Luxembourg, Portugal, Spain, and Switzerland, and 65 cultivars. Depending on the target sugar concentration there was up to 1 223 observations (quantity used for calibration and validation for 170 g/l sugar concentration) (Parker et al., 2020a). To date, these models represent the most spatially and temporally robust temperature-based models currently in application for phenological predictions that can be used in new sites, new climates, or new cultivars for the grapevine. The GFV model and the GSR model have also been validated in the New Zealand context for Marlborough Sauvignon blanc, the cultivar for which there is extensive data available (Parker et al., 2014, 2015, 2020b). In the most recent validation (Parker et al., 2020b), the model was tested for the period 2004–2020 and it was found the goodness-of-fit (root mean squared error) was 6.67, 4.67, and 9.67 days for flowering, véraison and time to 200 g/l sugar concentrations respectively which was within prediction ranges of the original calibrations and validations of cultivars by the models (Parker et al., 2011, 2013, 2020a). In addition, the temperature relationships for the GSR and GFV models have been successfully validated beyond the original model development datasets (Verdugo-Vásquez et al., 2019; Parker et al., 2020b; Ramos and de Toda, 2020; Wang et al., 2020) including other cool climate areas such as Champagne (Parker et al., 2020a) and areas in China (Wang et al., 2020). They have also been applied in combination with future climate change projections (van Leeuwen et al., 2019; de Rességuier et al., 2020; Parker et al., 2020b; Ramos and de Toda, 2020). Together, these tests and applications of the GSR and GFV models illustrate the models' broad applicability to new sites and different climates.

For cross-regional analysis, the combined overall median range for the time of flowering, véraison, and target sugar ripeness for the current period was determined for Sauvignon blanc (Marlborough), Hawke's Bay (Merlot), and Pinot noir (Otago) as well as the combined overall projected median range for all RCPs. The cultivar-by-region projections were then compared among the regions and the different RCPs.

3. RESULTS

3.1. Current and Projected Phenology at Regional Scale

Few differences among GCMs were observed for any given RCPs for flowering, véraison, and time to 200 g/l sugar

concentration of the three cultivar-region combinations of interest (**Supplementary Material**). As the GCM variability tended to be similar across temporal scale (mid- to end of the century), we used the mean GCM to summarize our findings. Since conclusions can be drawn in the three regions of interest, we presented results for Marlborough, with results for Hawke's Bay and Central Otago available in **Supplementary Material**.

For the average GCM data, the magnitude of difference between future and current dates in timing of phenological stages progressively increased with increased radiative forcing (**Figures 2, 3**). The differences between RCPs also increased for later phenological stages. For example, the shift in flowering was similar across RCP scenarios for flowering for Sauvignon blanc (4 to 7 days difference from current period) by the mid-century. However, differences between RCPs were larger for véraison and for target sugar ripeness occurring 14 and 16 days earlier for RCP 8.5 compared with RCP 2.6. Results clearly show a compression of time between flowering and target sugar ripeness corresponding with higher greenhouse gas emissions pathways.

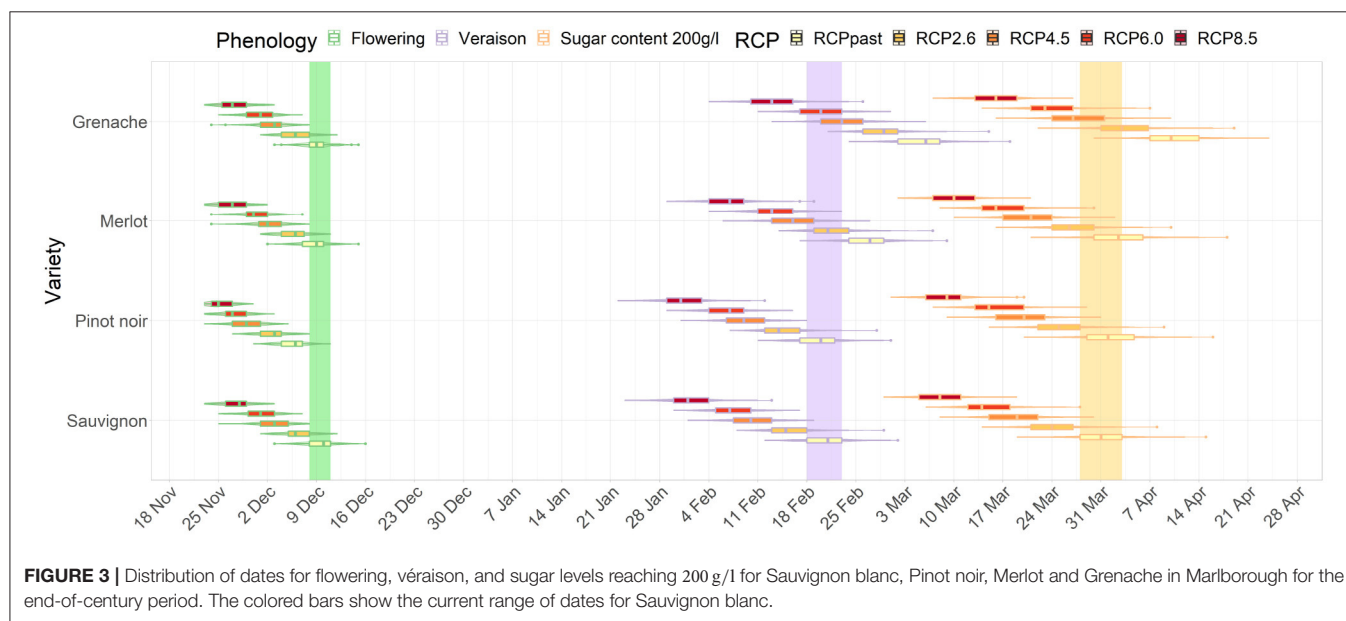
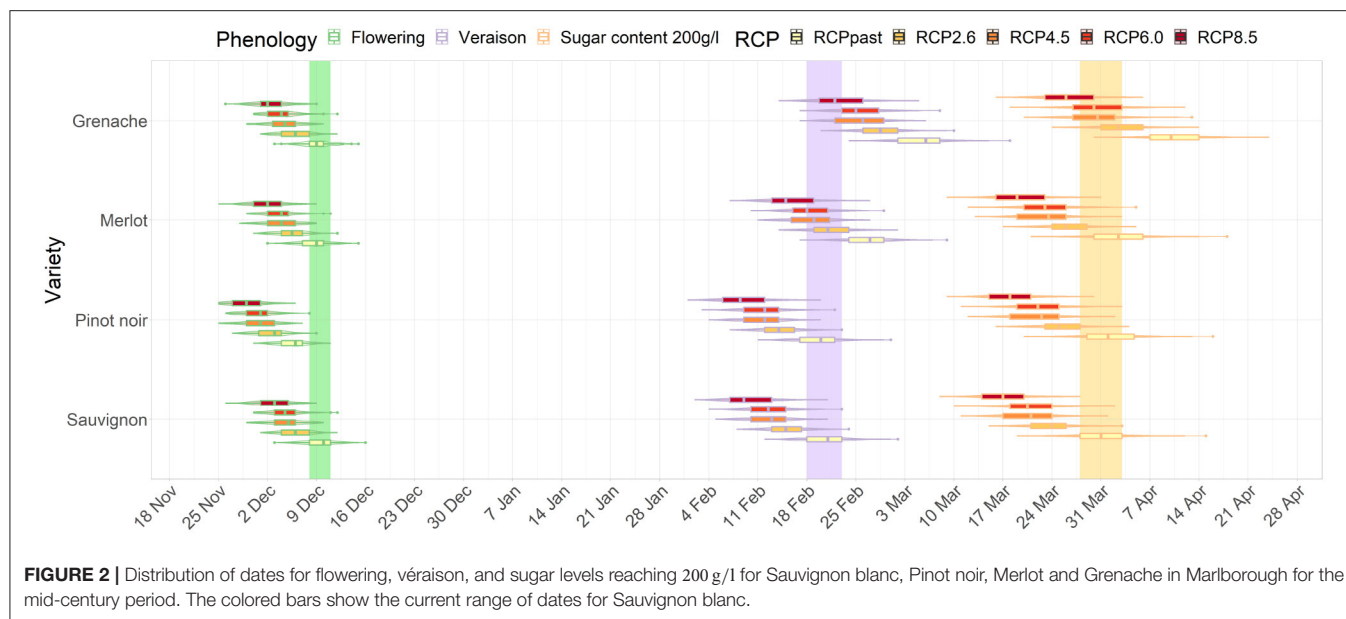
In Marlborough, flowering dates for all cultivars and RCPs advanced earlier than the current flowering window for Sauvignon blanc (green bar on **Figure 2**). Pinot noir, which is also extensively planted in the Marlborough region, in a high emission (RCP 8.5) scenario, flowering advanced 7 days. The timing of véraison advanced for all RCPs for Pinot Noir and Sauvignon blanc in a similar range (purple bar on **Figure 2**). Merlot, which usually has a later véraison date than Sauvignon blanc, reached a similar véraison period as a current Sauvignon blanc under RCP 2.6 and 4.5. However, for Grenache, the timing of véraison overlapped the current Sauvignon blanc véraison period only in the case of RCP 8.5 (**Figure 2**).

For the mid-century projections target sugar ripeness, Pinot noir and Merlot showed similar magnitudes of advancements to Sauvignon blanc. All RCPs' projections for Grenache overlapped with the current period defined for Sauvignon blanc (orange bar on **Figure 2**), except Grenache dates were earlier than current Sauvignon blanc dates under RCP 8.5 (**Figure 2**).

By the end of the century, the projected differences between RCP 2.6 and 8.5 became more dramatic than for the mid-century projections of all three stages of development. Under RCP 2.6, Grenache cultivar reached a target sugar ripeness at a similar time to the current period for Sauvignon blanc. However, under RCP 8.5, all cultivars including Grenache have projected véraison dates and sugar ripeness dates earlier than the current period for Sauvignon blanc. Grenache projections indicated that its target sugar ripeness would be attained 2 to 3 weeks earlier the current period for Sauvignon blanc (**Figure 3**).

3.2. Current and Projected Flowering Dates at Regional Scale

The climate change impacts on phenology for different wine grape cultivars in New Zealand demonstrated advances in dates for flowering, véraison, and the target sugar ripeness for the three key regions: Marlborough, Hawke's Bay, and Central Otago. For a given cultivar and RCP scenario, this shift occurs homogeneously across the studied regions. However, each developmental stage



would be reached at a similar time in more southern parts of New Zealand. We examined the date at which flowering occurred currently in Marlborough for Sauvignon blanc (approximately 8th December), and mapped the relative difference between this date and the projected flowering dates that could occur in suitable wine areas of New Zealand for RCP 8.5, using the GFV model. The results indicated that a Sauvignon blanc would reach the 8th of December flowering date in Canterbury and Central Otago by the middle of the century and South Canterbury by the end of the century (Figure 4).

Similarly, the current flowering date of Merlot for Hawke's Bay was determined to be around the 4th of December. Because this date is close to the flowering date of a Sauvignon blanc in Marlborough, we also found that a Merlot would flower around

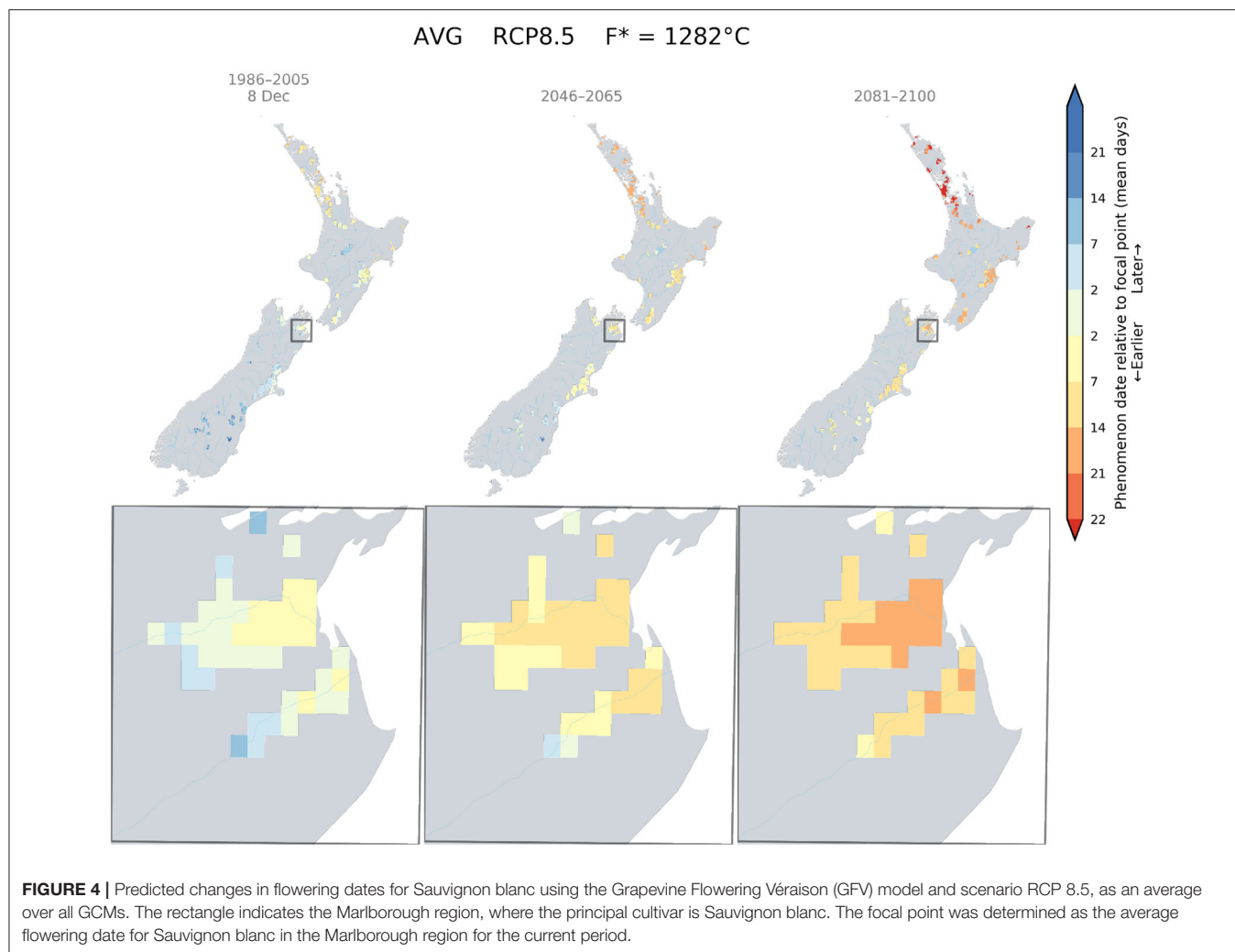
the 4th of December in Canterbury by mid-century, and in Central Otago and South Canterbury by the end of the century (Figure 5).

Currently Pinot noir in Central Otago was found to flower around the 16th of December. This flowering date was only attainable in South Canterbury by mid-century and a small area of this region by the end of the century (Figure 6).

Similar shifts and spatial patterns were observed for véraison and target sugar ripeness dates (Supplementary Material).

3.3. Cross-Regional Analysis

The development stages for three cultivar-region combinations occur across a wide range of dates in the current period (light bands, Figures 7, 8). For example, the median véraison date



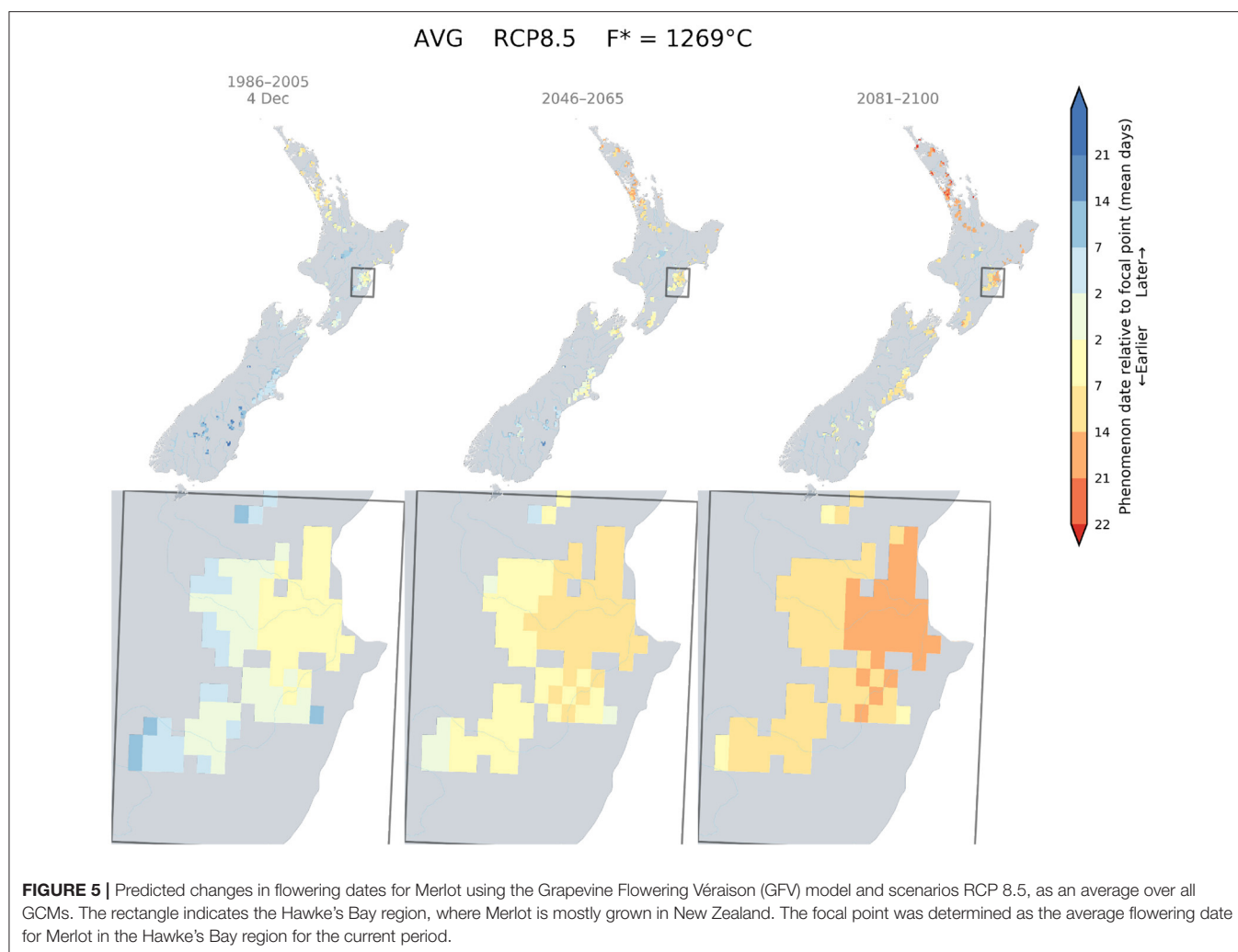
occurs around the 17th of February, 24th of February, and 8th of March for a Sauvignon blanc cultivar in Marlborough, a Merlot in Hawke's Bay, and a Pinot noir in Central Otago respectively, creating a window of véraison dates of 19 days. That range of dates compressed for future periods (mid- and end of century, dark bands **Figures 7, 8**) under RCP 8.5, as véraison would happen for all three cultivars across a shorter period (between the 10th and the 18th of February). The range of dates for target sugar ripeness across the three cultivar-regional combinations compressed from 26 to 12 days by mid-century under RCP 8.5 (**Figure 7**), down to a 7 day period by the end of the century (**Figure 8**).

For all cultivar-region combinations, the differences among the RCPs in the projected time of target sugar ripeness were greater than differences among RCP projections for flowering and véraison. When comparing between regions, the advances in timing of the three stages irrespective of RCP were similar for Hawke's Bay Merlot and Sauvignon blanc Marlborough, but Central Otago Pinot was comparatively less advanced and showed greater variation in projections. By the end of the century, the projected time of target sugar ripeness for Merlot

in Hawke's Bay and Sauvignon blanc in Marlborough overlapped (boxplot in **Figure 8**) with the time range of véraison for the three cultivars under RCP 2.6 and 4.5. There was also an overlap for the future timing of target sugar ripeness in Central Otago under RCP 8.5 with its current timing of véraison (light purple band, **Figure 8**).

4. DISCUSSION

The application of the GFV and GSR empirical models on selected New Zealand wine grape cultivars for a range of different RCPs and GCMs allowed us to explore the impacts of climate change on timing of three important crop phenological stages. Our analysis showed that dates for flowering, véraison and target sugar ripeness advanced as a function of warming in the RCP scenarios considered. The differences between high- and low-emissions pathways were more prominent by the end of century. We found greater differences between RCPs for véraison and target sugar ripeness dates compared to flowering dates. This shows that tracking toward a lower RCP would likely minimize the impact on these two key phenological stages. Results also

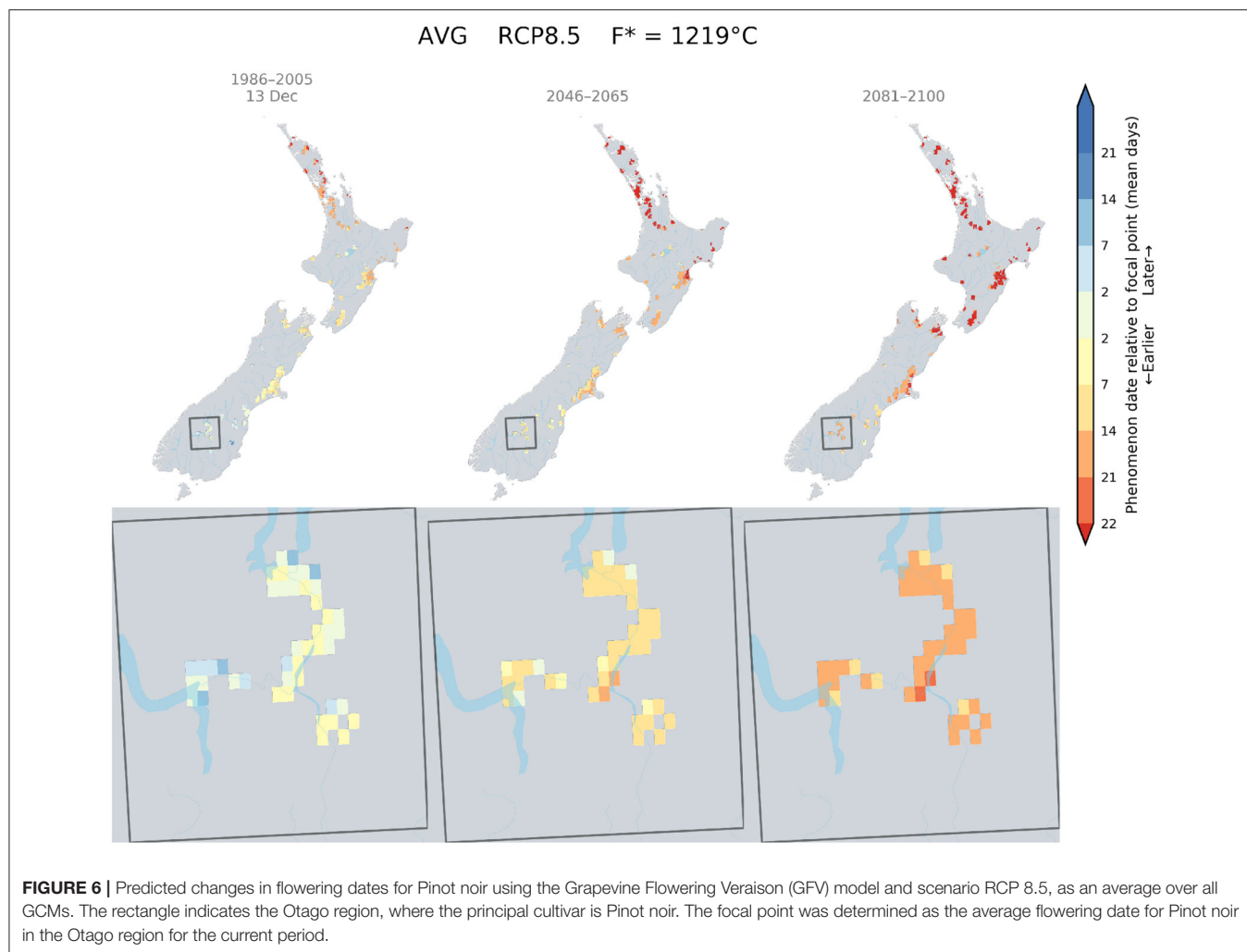


suggest that cultivar shift as a potential adaptation strategy is possible if winegrowers aim to maintain the same window of time for each development stage in the current regions of study. For instance, depending on the emissions pathway, it was shown that a later flowering and ripening cultivar such as Grenache may flower, go through véraison or reach a 200 g/l target sugar ripeness at a similar time to that of currently planted Sauvignon blanc. Similarly, if growers aim to maintain the main cultivars currently grown in New Zealand (Sauvignon blanc, Pinot noir, and Merlot) with similar calendar of phenological stages, a spatial shift of producing areas to more Southern regions would be required in the future, or application of field management to maintain the timing of phenology.

We looked at whether the phenological shifts were occurring at the same rate across the various climate change scenarios for the three main cultivars of interest in each respective region of their growth (Sauvignon blanc in Marlborough, Merlot in Hawke's Bay, and Pinot noir in Central Otago). We showed the rate of change in phenological stages is different between cultivars, a similar conclusion to observations made in Australia (Petrie and Sadras, 2008), resulting in a compression in the range

of maturity dates, particularly at higher emission scenarios. For instance, RCP 2.6 shows an even shift in dates for the three cultivars however under RCP 4.5 to 8.5, the timing for reaching phenological stages becomes uneven across the three cultivars. Pinot noir in particular shows a much faster rate of change, resulting in all three cultivars reaching the same stage within up to half its current range of dates by the end of the century. This compression in time may be a concern for the wine industry, as grapes will likely mature at a similar time, thus putting pressure on scheduling of harvesting and transport of harvested grape to facilities.

From a varietal change perspective, a cultivar with later ripening than the currently used ones may become more suitable if the target is to keep the same calendar of phenological events. The workflow developed with the GFV and GSR models can be further extended to explore such adaptive strategies as all the required datasets are now pre-computed, which enables the testing of potential phenological changes across grape cultivars and New Zealand regions for the four RCP scenarios. We also showed that it was possible to use the GFV model to test and select substitutable cultivars, i.e., to target flowering in a region



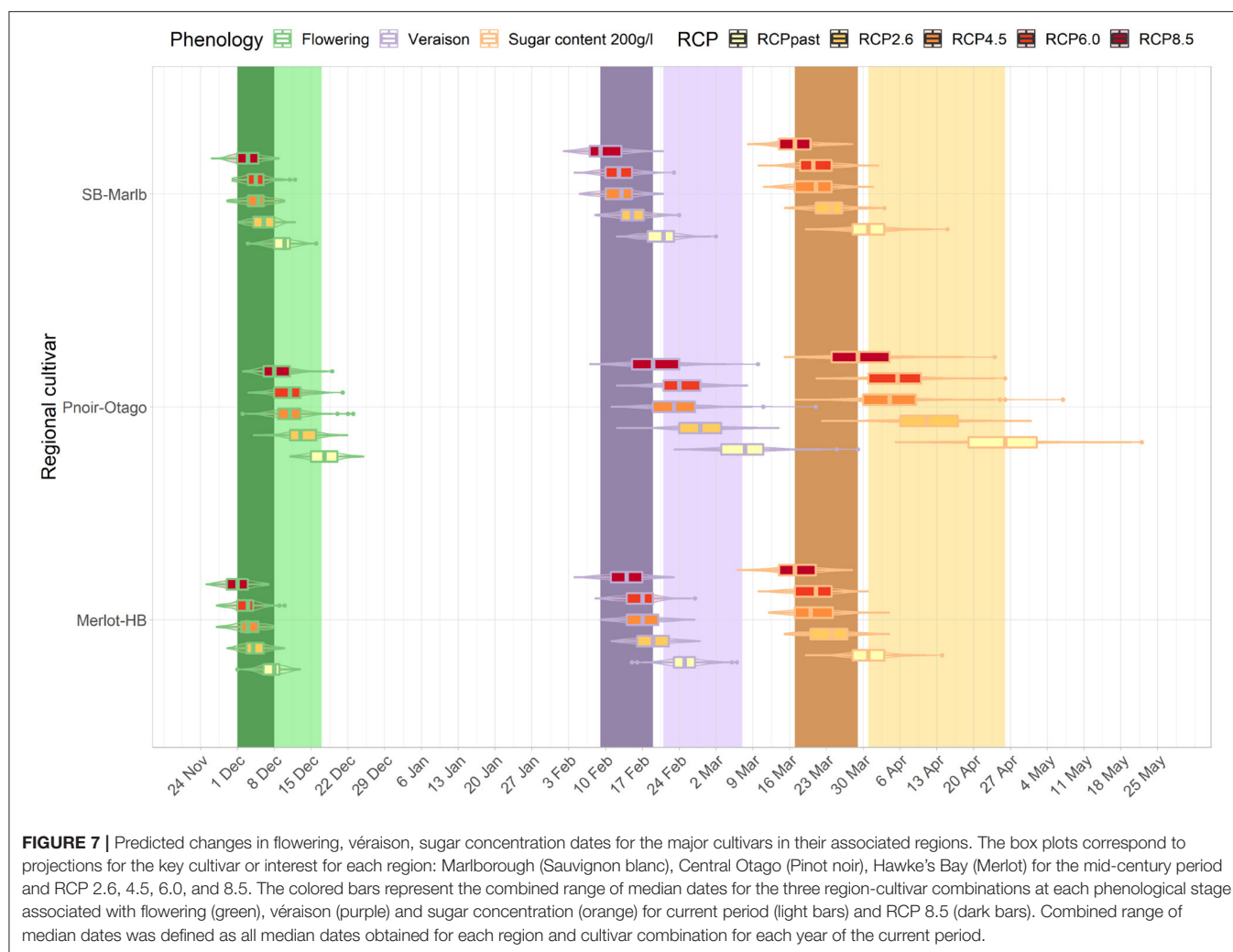
at a specific date to keep existing phenological calendars similar as in historical climate. This could allow, for example, to explore benefits of minimizing drastic shifts in temporal patterns of phenological events.

Our results on spatial phenology stage shifts focused on areas that are currently suitable for viticulture in New Zealand. However, new areas could become suitable under climate change, opening new opportunities for key cultivars in regions of New Zealand that are not traditionally known as wine-growing regions. Under climate change, southern parts of New Zealand that are presently too cool (i.e., a flowering date beyond mid-December) could eventually exhibit earlier flowering dates. Concurrently in these regions, the risks to frost may also become less of a constraint under warmer temperature, although this would need to be combined with earlier timing for budbreak (Mosedale et al., 2015; Sgubin et al., 2018). For a comprehensive assessment of potential gains of production across New Zealand viticulture regions in the future (Morales-Castilla et al., 2020), other aspects influenced by edapho-climatic factors would need to be considered, including water supply, risk of biotic stresses and cultivar responses to photoperiod (Parker et al., 2013).

4.1. Scope, Implications, and Limitations of This Study

Although the land use capability (LUC; Manaaki Whenua – Landcare Research, 2018) delineated potential growing regions for this study, it is acknowledged that there may be opportunities to consider expansion to new production areas in the future. Our projections are available across the country at 0.05° resolution, so it is possible to change the extent of potential areas for viticulture. Another important consideration is that the projections are based on temperature only. While temperature is the key driver of phenology (Cook and Wolkovich, 2016), other climatic drivers such as projected rainfall changes may also impact on suitable regions in the future. To further establish the impact of rainfall for determining suitable regions, broader assessment of suitability thresholds for viticulture would need to be considered in future research.

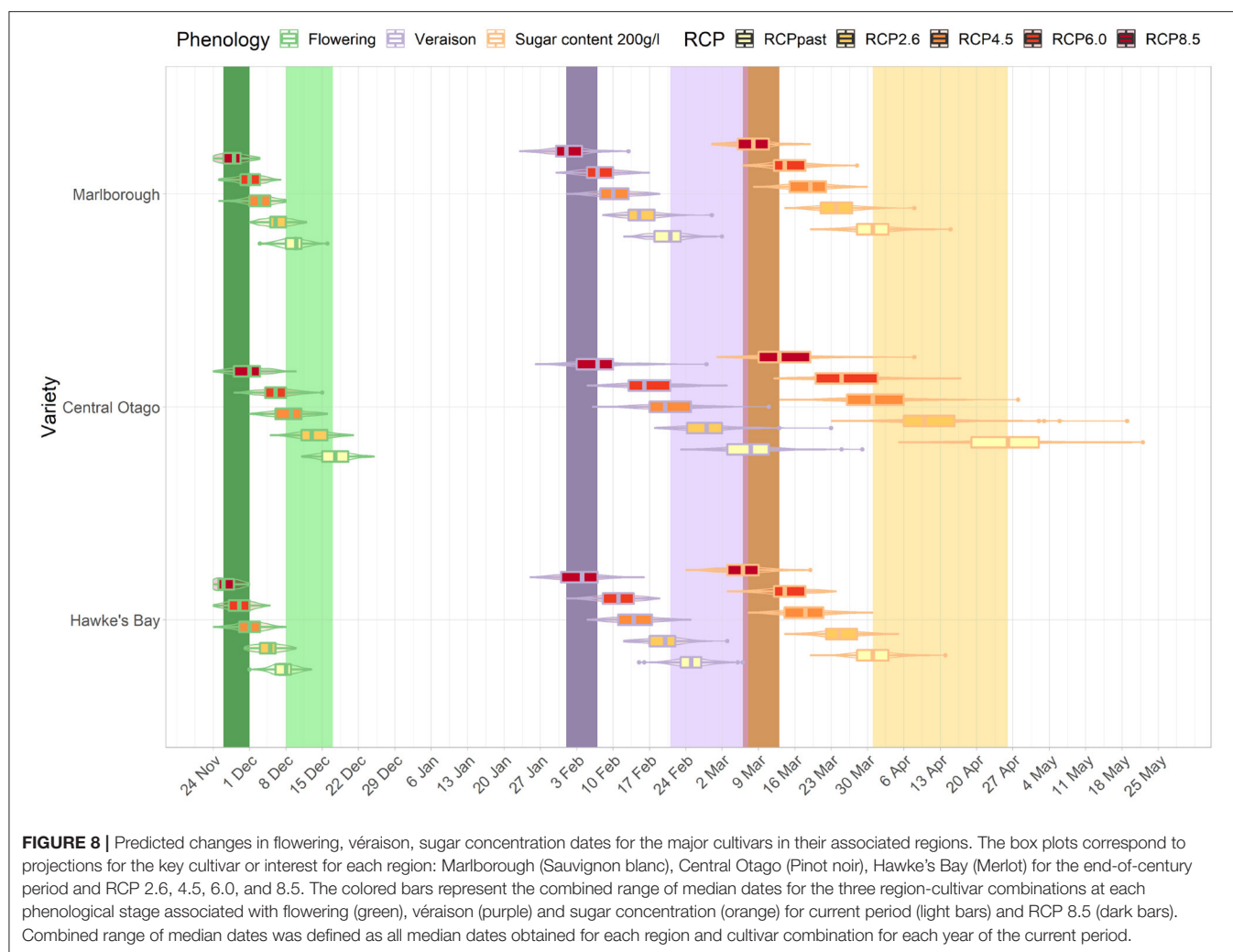
The spatial resolution (0.05° grid) used to perform the climate change scenario analysis enabled us to produce information at the regional level, but it does not account for finer aspects of topography and complex climate interactions that can occur at a lower resolution, particularly in the context of the NZ



maritime temperate climate (Parker et al., 2015; Sturman et al., 2017). Given the complex terrain of NZ, the extension of our analysis to finer spatial resolutions would likely provide additional insights on phenological responses. Such research has been carried out in St. Emilion, Bordeaux, and the results indicate that within region variability also plays an important role in future projections (Le Roux et al., 2016; de Rességuier et al., 2020). Future research will benefit from the availability of downscaled input datasets at finer resolutions and analytical methods to aggregate simulations across scales, an active area of research in the field (Ewert et al., 2015).

It is important that model complexity and fit within temperature boundaries are considered when selecting models to address specific research questions in different environments, as more complex model structures with additional parameters could be considered for future improvement. Over-optimal temperatures have a negative impact on plant development, causing a decreased rate of development. Non-linearity and threshold responses of plant development to temperature have therefore been calibrated in other grapevine phenophase models

(García de Cortázar-Atauri et al., 2010; Cuccia et al., 2014; Molitor et al., 2014b, 2020; Morales-Castilla et al., 2020; Prats-Llinàs et al., 2020) and these could be incorporated in the future to enhance our methodological approach. So far, test comparisons between the Wang and Engel (1998) model, which has a temperature threshold, and the GFV model have shown few differences for Pinot noir (Burgundy) (Cuccia et al., 2014) in cool-climate regions that are comparable to New Zealand at degrees of warming of up to 5 °C. Only when considering the warmer region of Seville were minor differences detected between the two models (Cuccia et al., 2014). Besides, curvilinear temperature relationships were tested for both the GSR and GFV models, but the model accuracy did not improve within the mean minimum and maximum temperature of calibration datasets (ranging from 15–32 °C for the GFV, 14–34 °C for the GSR) (Parker et al., 2011, 2020a). As the temperature ranges for the future climate scenarios did not exceed those of the temperatures used for the GFV and GSR model calibration (Table 1), these models were considered fit to assess the different cultivars responses to future temperature in our study. However, the



structure and parameterization of phenological models suitable for future climate change studies warrants continual review for any crop, grapevine included, as more information on patterns of response to extreme temperatures is made available to be integrated in models (e.g., Rosenzweig et al., 2013).

The GFV is an empirical model that has been calibrated on phenology date and underlying understanding that temperature is the main driver of change. By projecting the changes in temperature and inferring phenology shifts, some key uncertainties need to be noted. First, the climate change projections showed more variability between GCMs by the end of the century, leading to more uncertainties on the range of shifts in dates. Second, the model itself assumes that the empirical relationship between temperature and phenological development will stay consistent in time. While temperature-based phenological models are used for many plant species for climate change studies, we do not know to what extent other variables might influence future projections. For instance, some studies suggest that CO₂ concentrations might change the optimum temperature for photosynthesis, and influence

biomass production, sugar concentrations, acidity levels, and water use efficiency (Bindi et al., 2001; Schultz and Stoll, 2010). Determining regional suitability for particular cultivars still requires careful consideration of additional variables. For instance, while the period from dormancy to flowering is mainly determined by temperature (and thus adequately captured by the GFV model), the period from flowering to véraison can also be influenced by water deficit (Martínez-Lüscher et al., 2016). Management aspects such as leaf area to fruit weight ratio manipulations (Parker et al., 2014) and the interaction of factors (CO₂, water stress, and temperature) also need to be considered to fully represent vine physiological responses in the context of climate change (Martínez-Lüscher et al., 2016). Furthermore, climate change may have downstream effects on the suitability of pests and pathogens, changing defence mechanisms of the plant but also the life cycle of some insects (Santos et al., 2020). The increased asynchrony between plant and pest phenology may have both positive and negative impacts (Reineke and Thiéry, 2016). Third, it is valuable to test the variability of these relationships across different soil and climate environments

as new datasets are made available. For instance, while our analysis is appropriate to draw conclusions at the regional scale, the influence of soil and micro-climate may require further analysis and downscaled information to understand impacts at a local scale.

The projected shifts in wine grape phenology in New Zealand may have some important implications for biophysical dimensions of production systems. For instance, warmer temperatures as noted in the 2017–2018 heatwave in NZ, resulted in earlier and compressed flowering, improved fruit set and improved bunch initiation, both of which lead to increased yields for the 2017–18 and 2018–19 season (Salinger et al., 2019, 2020). Therefore, in cool climate viticulture production there could be potential production benefits if temperatures do not exceed optimum physiological thresholds. Excessive heat may have adverse consequences for production especially from flowering to véraison stages when berry grapes are most sensitive (Belliveau et al., 2006). The ripening period might shift toward the hotter part of the season, leading to changes in temperatures during the ripening period (Trought et al., 2015), which could not only change grape sugar concentrations, but also flavor and aroma profiles. However, recent research has indicated that in current seasons where phenology has advanced due to heatwaves, a reciprocal increase in temperature during the ripening period did not occur (Salinger et al., 2020). Therefore, understanding the biophysical changes at specific times of the growing season at local and national levels will be important for assessing the implications of climate change on grape and wine production. The projected changes could also have equally important implication for the human dimension of the production system. If temperatures increase, it is not only the impact on grapevines that need to be considered but also the increasing risk to workers' exposure to summer heat at labor-intensive stages (Ioannou et al., 2017). Our projections also show that phenology shifts are uneven across the country, leading to different degrees of compression in time of reaching target sugar concentrations for the three regions of interest. This has implications in preparedness for upgrading infrastructure and creates a shorter harvesting time span causing competition for seasonal laborers (Petrie and Sadras, 2008; Cradock-Henry and Fountain, 2019). Therefore, it is essential to consider grape and wine production in terms of linked social-ecological or human-environmental systems, addressing changes in biophysical processes as well as human and management activities within a complex adaptive system (Berkes and Folke, 1994; Cradock-Henry and Fountain, 2019).

In response to shifts in phenology, winegrowers have several adaptation measures that could be considered. Adaptation strategies are often distinguished short-term, seasonal or interannual responses or tactical adaptation; and longer-term, more strategic actions. In the shorter term, tactical adaptation could be adopted by shifting viticultural techniques to delay ripeness (van Leeuwen et al., 2019). Often these techniques such as leaf area manipulations or delayed pruning only delay ripeness by 1–3 weeks (Friend and Trought, 2007; Parker et al., 2014; Petrie et al., 2017; Moran et al., 2019). Depending on the RCP scenario considered, this may be insufficient for

successful adaptation and more strategic, even transformational changes may be needed (Fleming et al., 2015). In the longer term, a key adaptation strategy to climate change for wine growers remains to change cultivars to buffer winegrowing regions' losses under future climate conditions (Morales-Castilla et al., 2020). Flexibility in cultivar choice allows winegrowers to maintain harvest dates close to optimal windows and to enhance resilience to climate change impacts (van Leeuwen et al., 2019). By managing harvest timing, growers can influence environmental conditions prevalent during ripening. The choice on new cultivars may also be reviewed in combination with other desired adaptation management decisions (such as rootstock height, trunk height or leaf area to fruit ratio or delayed pruning) that would increase resilience to other climate-change related stressors such as droughts, storms, or heat stress. Increasing genetic diversity in a crop may also improve resilience to other risks such as prevalence to pests and diseases (van Leeuwen et al., 2019).

It is important to note that other broader considerations are relevant for the selection of most appropriate local adaptive measures. The shift in sensory profiles of wines may change the concept of "terroir" and the wine typicity that some regions are well-known for (van Leeuwen and Seguin, 2006; Santos et al., 2020). Other considerations include for example socio-economic aspects, market preferences, transportation and logistics, and risks of other natural hazards that also affect the vulnerability of the NZ wine industry (Mosedale et al., 2016; Cradock-Henry and Fountain, 2019).

5. CONCLUSION

Our study has implemented two empirical modeling approaches (GFV and GSR models) to assess climate change impacts to flowering, véraison and sugar ripeness of grapevines in New Zealand, with uncertainty represented by six GCMs and four RCPs. Results indicate that warmer temperatures due to climate change are likely to advance the phenological stages of grape vines in New Zealand. Specifically, grapes will reach flowering and véraison at earlier dates which influences timing and subsequent berry ripening as demonstrated by advances in sugar ripeness. Projected changes in grapevine development indicates that cultivar shifts represent a possible option to adapt to climate change. Nevertheless, compression of key phenological stages may still occur.

Further understanding phenological characteristics of a wide range of cultivars is a key aspect that should be considered for increasing resilience of the wine industry to climate change. Future research is also needed to encompass a wider range of risk factors, identifying vulnerability, sensitivity, and adaptive capacity for a well-informed industry.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

A-GA and AP: substantial contributions to the conception of the work and drafting the work. A-GA and RL: substantial contributions to the analysis of the work. AS provided critical climate change datasets. ET provided review, writing, and editing input. All authors critically revised the manuscript for important intellectual content. All authors agreed to be accountable for the content of the work.

FUNDING

This work was co-funded by Manaaki Whenua — Landcare Research Strategic Science Investment Funding for Crown Research Institutes and the New Zealand Ministry for Business, Innovation and Employment's Our Land and Water National

REFERENCES

- Anderson, K., and Aryal, N. R. (2013). *Which Winegrape Varieties Are Grown Where?: A Global Empirical Picture*. Adelaide: University of Adelaide Press.
- Belliveau, S., Smit, B., and Bradshaw, B. (2006). Multiple exposures and dynamic vulnerability: evidence from the grape industry in the Okanagan Valley, Canada. *Glob. Environ. Change* 16, 364–378. doi: 10.1016/j.gloenvcha.2006.03.003
- Berkes, F., and Folke, C. (1994). "Linking social and ecological systems for resilience and sustainability," in *Linking Social and Ecological Systems*, eds F. Berkes and C. Folke (Cambridge: Cambridge University Press), 1–25.
- Bindi, M., Fibbi, L., and Miglietta, F. (2001). Free air CO₂ enrichment (FACE) of grapevine (*Vitis vinifera* L.): II. Growth and quality of grape and wine in response to elevated CO₂ concentrations. *Eur. J. Agron.* 14, 145–155. doi: 10.1016/S1161-0301(00)00093-9
- Chuine, I., Yiou, P., Viovy, N., Seguin, B., Daux, V., and Ladurie, E. L. R. (2004). Grape ripening as a past climate indicator. *Nature* 432, 289–290. doi: 10.1038/432289a
- Cook, B. I., and Wolkovich, E. M. (2016). Climate change decouples drought from early wine grape harvests in France. *Nat. Clim. Change* 6, 715–719. doi: 10.1038/nclimate2960
- Cradock-Henry, N. A., and Fountain, J. (2019). Characterising resilience in the wine industry: insights and evidence from Marlborough, New Zealand. *Environ. Sci. Policy* 94, 182–190. doi: 10.1016/j.envsci.2019.01.015
- Cuccia, C., Bois, B., Richard, Y., Parker, A. K., García de Cortázar-Atauri, I., van Leeuwen, C., et al. (2014). Phenological model performance to warmer conditions: application to Pinot noir in Burgundy. *OENO One* 48, 169–178. doi: 10.20870/oeno-one.2014.48.3.1572
- de Rességuier, L., Mary, S., Le Roux, R., Petitjean, T., Quénel, H., and van Leeuwen, C. (2020). Temperature variability at local scale in the Bordeaux area. Relations with environmental factors and impact on vine phenology. *Front. Plant Sci.* 11:515. doi: 10.3389/fpls.2020.00515
- Duchêne, E., Huard, F., Vincent, D., Schneider, C., and Merdinoglu, D. (2010). The challenge of adapting grapevine varieties to climate change. *Clim. Res.* 41, 193–204. doi: 10.3354/cr00850
- Duchêne, E., and Schneider, C. (2005). Grapevine and climatic changes: a glance at the situation in Alsace. *Agron. Sustain. Dev.* 25, 93–99. doi: 10.1051/agro:2004057
- Ewert, F., van Bussel, L. G. J., Zhao, G., Hoffmann, H., Gaiser, T., Specka, X., et al. (2015). "Uncertainties in scaling-up crop models for large-area climate change impact assessments," in *Handbook of Climate Change and Agroecosystems: The Agricultural Model Intercomparison and Improvement Project (AgMIP)*, eds C. Rosenzweig and D. Hillel (London: World Scientific Publishing—Imperial College Press), 261–277.
- Fleming, A., Park, S. E., and Marshall, N. A. (2015). Enhancing adaptation outcomes for transformation: climate change in the Australian wine industry. *J. Wine Res.* 26, 99–114. doi: 10.1080/09571264.2015.1031883
- Friend, A. P., and Trought, M. C. (2007). Delayed winter spur-pruning in New Zealand can alter yield components of Merlot grapevines. *Aust. J. Grape Wine Res.* 13, 157–164. doi: 10.1111/j.1755-0238.2007.tb00246.x
- García de Cortázar-Atauri, I., Daux, V., Garnier, E., Yiou, P., Viovy, N., Seguin, B., et al. (2010). Climate reconstructions from grape harvest dates: methodology and uncertainties. *Holocene* 20, 599–608. doi: 10.1177/0959683609356585
- Hochberg, U., Batushansky, A., Degu, A., Rachmilevitch, S., and Fait, A. (2015). Metabolic and physiological responses of Shiraz and Cabernet Sauvignon (*Vitis vinifera* L.) to near optimal temperatures of 25 and 35 C. *Int. J. Mol. Sci.* 16, 24276–24294. doi: 10.3390/ijms161024276
- Hunter, J., and Bonnardot, V. (2011). Suitability of some climatic parameters for grapevine cultivation in South Africa, with focus on key physiological processes. *South Afr. J. Enol. Viticult.* 32, 137–154. doi: 10.21548/32-1-1374
- Intergovernmental Panel on Climate Change (2014). *Climate Change 2013 - The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press.
- Ioannou, L. G., Tsoutsoubi, L., Samoutis, G., Bogataj, L. K., Kenny, G. P., Nybo, L., et al. (2017). Time-motion analysis as a novel approach for evaluating the impact of environmental heat exposure on labor loss in agriculture workers. *Temperature* 4, 330–340. doi: 10.1080/23328940.2017.1338210
- Jones, G., Duchene, E., Tomasi, D., Yuste, J., Braslavskaya, O., Schultz, H., et al. (2005a). "Changes in European winegrape phenology and relationships with climate," in *XIV International GESCO Viticulture Congress* [Geisenheim: Groupe d'Etude des Systemes de Conduite de la Vigne (GESCO)], 54–61.
- Jones, G. V., White, M. A., Cooper, O. R., and Storchmann, K. (2005b). Climate change and global wine quality. *Clim. Change* 73, 319–343. doi: 10.1007/s10584-005-4704-2
- Jones, N. K. (2012). The influence of recent climate change on wine regions in Québec, Canada. *J. Wine Res.* 23, 103–113. doi: 10.1080/09571264.2012.678933
- Kliewer, W. (1977). Effect of high temperatures during the bloom-set period on fruit-set, ovule fertility, and berry growth of several grape cultivars. *Am. J. Enol. Viticult.* 28, 215–222.
- Le Roux, R., Taturji, M., Zavar-Reza, P., de Resseguier, L., Sturman, A., van Leeuwen, C., et al. (2016). "A fine scale approach to map bioclimatic indices using and comparing dynamical and geostatistical methods," in *11th International Terroir Congress* (Willamette Valley).
- Manaaki Whenua – Landcare Research (2004). *New Zealand Landcover Database (LCDB) (version 2)*. Available online at: <https://koordinates.com/layer/1072-land-cover-database-version-2-lcdb2>

Science Challenge (Toitu te Whenua, Toiora te Wai) and Deep South Challenge, contract C10X1507, as part of the Incorporating Climate change impacts in Land-use suitability programme.

ACKNOWLEDGMENTS

We would like to acknowledge Sharn Hainsworth, Nicholas Cradock-Henry, and two reviewers for their advice and useful comments that have improved the quality of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.618039/full#supplementary-material>

- Manaaki Whenua – Landcare Research (2018). *New Zealand Land Resource Inventory (NZLRI) Land Use Capability (revision 5)*. Available online at: <https://lris.scinfo.org.nz/layer/48076-nzlri-land-use-capability>
- Martínez-Lüscher, J., Kizildeniz, T., Vučetić, V., Dai, Z., Luedeling, E., van Leeuwen, C., et al. (2016). Sensitivity of grapevine phenology to water availability, temperature and CO₂ concentration. *Front. Environ. Sci.* 4:48. doi: 10.3389/fenvs.2016.00048
- Ministry for Primary Industries (2020). *Situation and Outlook for Primary Industries*. Technical report, Ministry for Primary Industries (Economic Intelligence Unit), Wellington, New Zealand.
- Ministry for the Environment (2018). *Climate Change Projections for New Zealand: Atmosphere Projections Based on Simulations From the IPCC Fifth Assessment*. Technical report, Ministry for the Environment, Wellington, New Zealand.
- Molitor, D., Caffarra, A., Sinigoi, P., Pertot, I., Hoffmann, L., and Junk, J. (2014a). Late frost damage risk for viticulture under future climate conditions: a case study for the Luxembourgish winegrowing region. *Aust. J. Grape Wine Res.* 20, 160–168. doi: 10.1111/ajgw.12059
- Molitor, D., Fraga, H., and Junk, J. (2020). UniPhen—a unified high resolution model approach to simulate the phenological development of a broad range of grape cultivars as well as a potential new bioclimatic indicator. *Agric. For. Meteorol.* 291:108024. doi: 10.1016/j.agrformet.2020.108024
- Molitor, D., Junk, J., Evers, D., Hoffmann, L., and Beyer, M. (2014b). A high-resolution cumulative degree day-based model to simulate phenological development of grapevine. *Am. J. Enol. Viticult.* 65, 72–80. doi: 10.5344/ajev.2013.13066
- Morales-Castilla, I., García de Cortázar-Atauri, I., Cook, B. I., Lacombe, T., Parker, A., van Leeuwen, C., et al. (2020). Diversity buffers winegrowing regions from climate change losses. *Proc. Natl. Acad. Sci. U.S.A.* 117, 2864–2869. doi: 10.1073/pnas.1906731117
- Moran, M., Petrie, P., and Sadras, V. (2019). Effects of late pruning and elevated temperature on phenology, yield components, and berry traits in Shiraz. *Am. J. Enol. Viticult.* 70, 9–18. doi: 10.5344/ajev.2018.18031
- Mosedale, J. R., Abernethy, K. E., Smart, R. E., Wilson, R. J., and Maclean, I. M. (2016). Climate change impacts and adaptive strategies: lessons from the grapevine. *Glob. Change Biol.* 22, 3814–3828. doi: 10.1111/gcb.13406
- Mosedale, J. R., Wilson, R. J., and Maclean, I. M. (2015). Climate change and crop exposure to adverse weather: changes to frost risk and grapevine flowering conditions. *PLOS ONE* 10:e0141218. doi: 10.1371/journal.pone.0141218
- New Zealand Winegrowers (2020). *Vineyard Register Report 2019–2022*. Technical report, New Zealand Winegrowers.
- Parker, A., García de Cortázar-Atauri, I., Chuine, I., Barbeau, G., Bois, B., Boursiquot, J.-M., et al. (2013). Classification of varieties for their timing of flowering and véraison using a modelling approach: a case study for the grapevine species *Vitis vinifera* L. *Agric. For. Meteorol.* 180, 249–264. doi: 10.1016/j.agrformet.2013.06.005
- Parker, A., Schulmann, T., Sturman, A., Agnew, R., Zavar-Reza, P., Katurji, M., et al. (2014). “Grapevine phenology of the Marlborough region, New Zealand,” in *10th International Terroir Congress* (Tokaj), 105–109.
- Parker, A., Schulmann, T., Sturman, A., Agnew, R., Zavar-Reza, P., Katurji, M., et al. (2015). “Understanding flowering of Sauvignon blanc in the Marlborough region, New Zealand, using high-resolution weather forecasting and the grapevine flowering véraison model,” in *19th International Symposium of the Group of International Experts of Vitivinicultural Systems for Co-Operation (GIESCO)* (Gruissan), 271–276.
- Parker, A. K., García de Cortázar-Atauri, I., Génys, L., Spring, J.-L., Destrac, A., Schultz, H., et al. (2020a). Temperature-based grapevine sugar ripeness modelling for a wide range of *Vitis vinifera* L. cultivars. *Agric. For. Meteorol.* 285:107902. doi: 10.1016/j.agrformet.2020.107902
- Parker, A. K., García de Cortázar-Atauri, I., Trought, M., Destrac, A., Agnew, R., Sturman, A., et al. (2020b). Adaptation to climate change by determining grapevine cultivar differences using temperature-based phenology models. *OENO One* 34, 955–974. doi: 10.20870/oeno-one.2020.54.4.3861
- Parker, A. K., García de Cortázar-Atauri, I., van Leeuwen, C., and Chuine, I. (2011). General phenological model to characterise the timing of flowering and véraison of *Vitis vinifera* L. *Aust. J. Grape Wine Res.* 17, 206–216. doi: 10.1111/j.1755-0238.2011.00140.x
- Petrie, P. R., Brooke, S., Moran, M. A., and Sadras, V. O. (2017). Pruning after budburst to delay and spread grape maturity. *Aust. J. Grape Wine Res.* 23, 378–389. doi: 10.1111/ajgw.12303
- Petrie, P. R., and Sadras, V. O. (2008). Advancement of grapevine maturity in Australia between 1993 and 2006: putative causes, magnitude of trends and viticultural consequences. *Aust. J. Grape Wine Res.* 14, 33–45. doi: 10.1111/j.1755-0238.2008.00005.x
- Prats-Llinàs, M. T., Nieto, H., DeJong, T. M., Girona, J., and Marsal, J. (2020). Using forced regrowth to manipulate Chardonnay grapevine (*Vitis vinifera* L.) development to evaluate phenological stage responses to temperature. *Sci. Hortic.* 262:109065. doi: 10.1016/j.scienta.2019.109065
- Ramos, M. C., and de Toda, F. M. (2020). Variability in the potential effects of climate change on phenology and on grape composition of Tempranillo in three zones of the Rioja DOCA (Spain). *Eur. J. Agron.* 115:126014. doi: 10.1016/j.eja.2020.126014
- Reineke, A., and Thiéry, D. (2016). Grapevine insect pests and their natural enemies in the age of global warming. *J. Pest Sci.* 89, 313–328. doi: 10.1007/s10340-016-0761-8
- Rosenzweig, C., Jones, J. W., Hatfield, J. L., Ruane, A. C., Boote, K. J., Thorburn, P., et al. (2013). The agricultural model intercomparison and improvement project (AgMIP): protocols and pilot studies. *Agric. For. Meteorol.* 170, 166–182. doi: 10.1016/j.agrformet.2012.09.011
- Salinger, M. J., Diamond, H. J., Behrens, E., Fernandez, D., Fitzharris, B. B., Herold, N., et al. (2020). Unparalleled coupled ocean-atmosphere summer heatwaves in the New Zealand region: drivers, mechanisms and impacts. *Clim. Change* 162, 485–506. doi: 10.1007/s10584-020-02730-5
- Salinger, M. J., Renwick, J., Behrens, E., Mullan, A. B., Diamond, H. J., Sirguy, P., et al. (2019). The unprecedented coupled ocean-atmosphere summer heatwave in the New Zealand region 2017/18: drivers, mechanisms and impacts. *Environ. Res. Lett.* 14:044023. doi: 10.1088/1748-9326/ab012a
- Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L. T., Correia, C., et al. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Appl. Sci.* 10, 1–28. doi: 10.3390/app10093092
- Schultz, H. R., and Stoll, M. (2010). Some critical issues in environmental physiology of grapevines: future challenges and current limitations. *Aust. J. Grape Wine Res.* 16, 4–24. doi: 10.1111/j.1755-0238.2009.00074.x
- Schultze, S. R., and Sabbatini, P. (2019). Implications of a climate-changed atmosphere on cool-climate viticulture. *J. Appl. Meteorol. Climatol.* 58, 1141–1153. doi: 10.1175/JAMC-D-18-0183.1
- Sgubin, G., Swingedouw, D., Dayon, G., García de Cortázar-Atauri, I., Ollat, N., Pagé, C., et al. (2018). The risk of tardive frost damage in french vineyards in a changing climate. *Agric. For. Meteorol.* 250, 226–242. doi: 10.1016/j.agrformet.2017.12.253
- Sturman, A., Zavar-Reza, P., Soltanzadeh, I., Katurji, M., Bonnardot, V., Parker, A. K., et al. (2017). The application of high-resolution atmospheric modelling to weather and climate variability in vineyard regions. *OENO One* 51, 99–105. doi: 10.20870/oeno-one.2017.51.2.1538
- Tait, A., Henderson, R., Turner, R., and Zheng, X. (2006). Thin plate smoothing spline interpolation of daily rainfall for New Zealand using a climatological rainfall surface. *Int. J. Climatol. A J. R. Meteorol. Soc.* 26, 2097–2115. doi: 10.1002/joc.1350
- Tait, A., Sood, A., Mullan, B., Stuart, S., Bodeker, G., Kremser, S., and Lewis, J. (2016). *Updated Climate Change Projections for New Zealand for Use in Impact Studies. Synthesis Report RA1. Climate Changes, Impacts and Implications (ccii) for New Zealand to 2100*. Technical report, National Institute of Water and Atmospheric Research (NIWA). MBIE contract C01X1225.
- Trought, M. C., and Bramley, R. G. (2011). Vineyard variability in Marlborough, New Zealand: characterising spatial and temporal changes in fruit composition and juice quality in the vineyard. *Aust. J. Grape Wine Res.* 17, 79–89. doi: 10.1111/j.1755-0238.2010.00120.x
- Trought, M. C. T., Parker, A. K., and van Leeuwen, C. (2015). “Can a change in vineyard practice mitigate warming due to climate change?,” in *ISHS Acta Horticulturae 1082: XI International Conference on Grapevine Breeding and Genetics 1082* (Yanqing), 397–402.
- van Leeuwen, C., and Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *J. Wine Econ.* 11:150. doi: 10.1017/jwe.2015.21

- van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy* 9:514. doi: 10.3390/agronomy9090514
- van Leeuwen, C., and Seguin, G. (2006). The concept of terroir in viticulture. *J. Wine Res.* 17, 1–10. doi: 10.1080/09571260600633135
- Verdugo-Vásquez, N., Acevedo-Opazo, C., Valdés-Gómez, H., Ingram, B., García de Cortázar-Atauri, I., and Tisseyre, B. (2019). Towards an empirical model to estimate the spatial variability of grapevine phenology at the within field scale. *Precis. Agric.* 21, 107–130. doi: 10.1007/s11119-019-09657-7
- Wang, E., and Engel, T. (1998). Simulation of phenological development of wheat crops. *Agric. Syst.* 1, 1–24.
- Wang, X., Li, H., and García de Cortázar-Atauri, I. (2020). Assessing grapevine phenological models under Chinese climatic conditions. *OENO One* 54, 189–197. doi: 10.20870/oeno-one.2020.54.3.3195
- Webb, L. B., Whetton, P. H. and Barlow, E. W. R. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. *Aust. J. Grape Wine Res.* 3, 165–175. doi: 10.1111/j.1755-0238.2007.tb00247.x
- Wine Marlborough (2019). *Wine Marlborough Website*. Technical report, Wine Marlborough, New Zealand.
- Wolkovich, E., and Morales-Castilla, I. (2019). Why varietal diversity is critical to winegrowing's warmer future. *Wine Viticult. J.* 1, 48–53.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a shared affiliation with one of the authors AP at the time of the review.

Copyright © 2021 Ausseil, Law, Parker, Teixeira and Sood. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Cold Hardiness Dynamics and Spring Phenology: Climate-Driven Changes and New Molecular Insights Into Grapevine Adaptive Potential

Valeria De Rosa, Giannina Vizzotto* and Rachele Falchi*

Department of Agricultural, Food, Environmental, and Animal Sciences, University of Udine, Udine, Italy

OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Artur Conde,
University of Minho, Portugal
Jason P. Londo,
United States Department of
Agriculture, United States

*Correspondence:

Giannina Vizzotto
giannina.vizzotto@uniud.it
Rachele Falchi
rachele.falchi@uniud.it

Specialty section:

This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

Received: 21 December 2020

Accepted: 17 March 2021

Published: 29 April 2021

Citation:

De Rosa V, Vizzotto G and
Falchi R (2021) Cold Hardiness
Dynamics and Spring Phenology:
Climate-Driven Changes and New
Molecular Insights Into Grapevine
Adaptive Potential.
Front. Plant Sci. 12:644528.
doi: 10.3389/fpls.2021.644528

Climate change has become a topic of increasing significance in viticulture, severely challenged by this issue. Average global temperatures are increasing, but frost events, with a large variability depending on geographical locations, have been predicted to be a potential risk for grapevine cultivation. Grape cold hardiness encompasses both midwinter and spring frost hardiness, whereas the avoidance of spring frost damage due to late budbreak is crucial in cold resilience. Cold hardiness kinetics and budbreak phenology are closely related and affected by bud's dormancy state. On the other hand, budbreak progress is also affected by temperatures during both winter and spring. Genetic control of bud phenology in grapevine is still largely undiscovered, but several studies have recently aimed at identifying the molecular drivers of cold hardiness loss and the mechanisms that control deacclimation and budbreak. A review of these related traits and their variability in different genotypes is proposed, possibly contributing to develop the sustainability of grapevine production as climate-related challenges rise.

Keywords: *Vitis vinifera*, chilling requirement, deacclimation, budburst, spring frost, gene expression, demethylation

INTRODUCTION

Climate change is a proven reality whose consequences on human activities and natural systems have reached an undeniable magnitude all around the world (IPCC, 2014). Global mean surface temperatures are predicted to increase by 0.3–4.8°C by the end of the 21st century, depending on the trend of anthropogenic greenhouse gas emissions, compared to the reference time-frame 1986–2005 (IPCC, 2014). Many plant species are expected to be unable to shift their geographical range quickly enough to keep up with these changes, and production will be negatively impacted if no adaptation occurs. Rainfall changes are likely to differ depending on the region, whereas radiation and extreme weather events are expected to increase (IPCC, 2019). Agriculture and viticulture, in particular, greatly depend on thermal regimen, soil composition, and water availability, in terms of fruit yield and metabolite composition (van Leeuwen and Darriet, 2016). Grapevine holds great economic value as it can be used fresh (table grape) or dry (raisin) and for winemaking (Delrot et al., 2020). Climate variations in wine-producing regions induce the so-called “vintage effect,” the year-to-year variations in yield, quality, and typicity (van Leeuwen and Darriet, 2016). Grape berry composition also

depends on “terroir,” defined as the complete natural environment in which a wine is produced, in which climate plays a major role, with the interplay of human activity (Delrot et al., 2020; Santos et al., 2020). Grapevine phenology and fruit ripening are greatly affected by temperature conditions. Berry composition is key in determining the subsequent quality of wines. The increase in temperature has been shown to cause a rise of berry sugar concentration (Coombe, 1987), whereas some secondary metabolites, such as malic acid or anthocyanins (Kliewer and Torres, 1972), are negatively affected. Higher temperatures produce an advance of phenology, causing earlier harvest dates (van Leeuwen and Darriet, 2016) and decoupling sugar and phenolic compound accumulation at maturity, thus leading to unbalanced wines (Sadras and Moran, 2012; Bonada et al., 2015). High temperatures during the final stages of berry growth, together with high precipitations, can also be the cause of cracks and rots (Molitor et al., 2016). Although rainfall tendencies are difficult to predict, the increase in evapotranspiration caused by temperature increase will cause plants to experience water stress even when rainfall does not directly decrease (van Leeuwen and Darriet, 2016).

The new climate change scenario will lead to increasing difficulty in the production of traditional wines in their areas of origin if no adaptation occurs. Therefore, adaptation measures are necessary as wine quality greatly depends on ripening conditions (Bonada et al., 2015), which in turn are a direct consequence of the timing of several phenological phases starting with budbreak.

Although the impacts of climate change are expected to be diverse in different wine-making regions (Santillán et al., 2019) and among cultivars with different phenological rhythms (McIntyre et al., 1982), several adaptation practices may be able to cope with the short-term effects of climate change and maintain wine typicity, and new training systems could be developed for the middle term (Duchêne, 2016). Remarkably, several variations in training systems and cultural practices have been adopted and tested in recent times with the aim to lower the risk of freezing damage in spring. Trimming, hedging, or pruning has been evaluated in order to mitigate the short-term impacts of climate change (Herrera et al., 2015; Frioni et al., 2016; Palliotti et al., 2017; Abad et al., 2019). In the past, late winter pruning was shown to be effective in delaying bud burst in cool climate areas (Trought et al., 1999), although it could not be applied for grapevine grown in different environments, in which both yield increase (Friend and Trought, 2007) and loss (Frioni et al., 2016) were observed. Recently, a double-pruning approach has shown a potential budburst delay of up to 4 weeks, depending on the timing of the second pruning (Palliotti et al., 2017). As regards the direct avoidance of spring frost damage, several methods, encompassing active and passive types, have been used in the past (Liu and Sherif, 2019). Active approaches include the use of wind machines and helicopters to force the warmer air toward the ground, or heaters and irrigation, to exploit the fusion heat of water. Efficacy of such methods depends greatly on external factors and cannot guarantee a complete avoidance of damage. Moreover, these approaches are costly and environmentally unsustainable

and require coordinated action by growers to avoid the rise of production costs and to ensure the effectiveness in the short term (Unterberger et al., 2018). Additionally, the application of chemicals (e.g., Amigo oil, FrostShield, and ProTone) and plant growth regulators (i.e., ethephon) has been shown to delay budbreak, although these results remain inconsistent (Qrunfleh and Read, 2013; Centinari et al., 2018; Kovaleski and Londo, 2019; Liu and Sherif, 2019; Wang and Dami, 2020).

In this context, the genetic improvement of grapevine has been taken into consideration to cope with the effects of climate change in the long run. Cultivated grapevines all around the world are usually grafted, and this adds a layer of complication to the understanding of plant–environment interactions. Moreover, the communication between scion and rootstock is often unclear or unexplored as the connection that is immediately established at grafting may evolve as the plant ages (Delrot et al., 2020). Therefore, despite the numerous aspects to consider, the investigation of unexploited varieties in germplasm collections, for both rootstock and scion, could be an interesting opportunity, strengthened by the continuous evolution of sequencing technologies and gene-mapping approaches. Efficient phenotyping methods also need to be developed to assess the effectiveness of varietal selection and the plasticity of the phenotype in different scion–rootstocks combinations (Warschefsky et al., 2016). As an example, recent studies have shown that different clone–rootstock combinations can influence and level cold hardiness differences among cultivars (Hébert-Haché et al., 2020). However, the possibility that the variability within clones of the *Vitis vinifera* species might be insufficient to compensate the phenological shifts caused by climate change must be contemplated; the need to introduce new varieties with the abandonment of the traditional ones will eventually arise if no measure is taken (Duchêne, 2016). Moreover, in addition to the already existing varieties, new ones could be generated through traditional breeding approaches or even genetic engineering. In any case, the comparison and analysis of different *Vitis* species could, first, help in clarifying the molecular regulators and drivers of cold hardiness, deacclimation, and budbreak and, second, allow the identification of targets to optimize clone selection and breeding efforts.

In this review, spring frost frequency and trends for different geographical regions are reported, together with the recent findings about the potential pathways involved in cold deacclimation and budbreak. We aim to provide an update on current status of research regarding the effects of climate change on grapevine phenology, with a focus on cold hardiness dynamics, budbreak, and the key molecular players involved in these processes. This will hopefully help in developing new ways to face current and future climate-related contingencies to allow berry ripening and harvest to be achieved in favorable conditions.

EFFECTS OF CLIMATE CHANGE ON GRAPEVINE PHENOLOGY

Several studies have assessed the impact of climate change on grapevine phenology and viticulture in the past and in the

present (Biasi et al., 2019), and numerous models have been tested to predict future consequences (Caffarra and Eccel, 2011; Bonfante et al., 2017; Alikadic et al., 2019; Costa et al., 2019; Ramos and de Toda, 2020). Agroclimatic indices are considered more reliable than individual climatic variables to describe climate change effects (Santos et al., 2020); these tools allow to closely follow and simulate plant development in different scenarios and can be used to evaluate the potential of different areas for viticulture (Molitor et al., 2014; Blanco-Ward et al., 2019). Redistribution of wine production within continents is a likely perspective, and the change in viticultural suitability for different geographic regions has been calculated, showing agreement among 17 global climate models. Wine-producing regions will possibly decrease by 2050 (mainly in Mediterranean climate area), whereas expanding suitability has been predicted an increase for New Zealand, western North America, and Northern Europe (Hannah et al., 2013).

However, commonly bioclimatic indices used in viticulture (e.g., Huglin Index, Winkler Index, Dryness Index, Cool Night Index) are arguably replaced by dynamic crop models (e.g., STICS, BRIN), which combine several indices and integrate phenotype, soil, weather data, and management practices into a more comprehensive picture (Cortázar-atauri et al., 2009; Moriondo et al., 2013; Fraga et al., 2016). Heat requirements, determined in terms of growing-degree days (GDD), represent the climatic constraint that allows grape to successfully complete its annual cycle when met. Distinct phenological phases need different climatic conditions to take place (e.g., release from ecodormancy; Ruml et al., 2016). Higher temperatures lead to an acceleration of plant development, being a potential cause of premature loss of bud cold hardiness (Pagter and Arora, 2013; Londo and Kowaleski, 2017; Kowaleski et al., 2018). In fact, early events such as budbreak and flowering have been shown to be the most sensitive to temperature-driven variations as compared to later phases (Jones et al., 2005). This increases the chances of vulnerable green tissues to be exposed to late spring frost events, which have been known to be the cause of great yield losses in the past (Gu et al., 2008). The timing of budbreak is strictly linked to the end of dormancy, a genetically programmed state of self-arrest in which the bud stops its development to avoid breaking at unfavorable times (Lang et al., 1987; Horvath et al., 2003). Whether the risk of damage due to spring frosts is globally increasing is up to debate, although recent reports suggest the relevance of this phenomenon in several locations (Augspurger, 2013; Ma et al., 2018; Sgubin et al., 2018). Effects are expected to vary, depending on the geographical position, and changes in water availability need to be taken into account together with temperature variations. Great attention has been always given to budbreak timing as early dormancy release in cold winter regions can cause significant crop losses, and frost-protecting measures represent a notable cost for producers. To the contrary, warmer regions can be affected by low rates of budburst and lower productivity due to insufficient chilling during winter, making the use of artificial dormancy-breakers a necessity. Vineyards located in southern Europe

(e.g., Italy, Spain, Portugal) are expected to experience increased water stress conditions especially during summer, leading, together with warming, to yield and quality reduction (Fraga et al., 2017; Santillán et al., 2019). Severe dryness is, in fact, the main reason impairing viticulture suitability in these areas. On the other hand, increasing average temperature has been predicted to have positive outcomes on winemaking regions in central and Western Europe and to allow the extension of viticultural areas in the north and east (Gaal et al., 2012; Cardell et al., 2019). This will favor the introduction of new currently inaccessible varieties in colder areas, as frost is expected to decrease and optimal ripening temperatures to be reached (e.g., Northern Europe, North America; Santillán et al., 2019); moreover, wine-producing suitable areas are expected to develop up to the 55°N by 2070 (Fraga et al., 2016).

Cold Hardiness Variations

Dormancy encompasses endodormancy, determined by internal factors, which allows buds to cold acclimate and reach a state of hardiness to survive freezing temperatures during winter. Cold acclimation is a process in which physiological, biochemical, and epigenetic changes driven by cold temperatures confer freezing tolerance (Wisniewski et al., 2018). Exposure to chilling temperatures, with difference depending on cultivar (Anzanello et al., 2018), is required to resume bud responsiveness to environmental signals and avoid growth start if mild temperatures occur during winter (Rohde and Bhalerao, 2007). Internal signals also prevent growth resumption in late summer or early autumn, which would cause the death of the bud in unfavorable environmental conditions (Lang et al., 1987; Horvath et al., 2003).

The productivity of grapevine and temperate plants is related to the capability of buds, both reproductive and vegetative, to tolerate freezing temperatures. Cold hardiness correlation with winter temperatures has been measured (Kowaleski et al., 2018). In general, sudden or recurring warm spells in winter can endanger the survival of woody perennials to freezing temperatures because the deacclimation process, during which cold tolerance is lost, is relatively fast (Pagter and Arora, 2013). Although deacclimation and acclimation cycles seem possible and efficient in several herbaceous plants (Vyse et al., 2019), it appears diverse for woody perennials with cold acclimation being restored only in part (Shin et al., 2015). Various grapevine species have been shown to be differently responsive to temperature variations during dormancy, likely related to the dissimilar chilling requirements that allow the transition from endodormancy to ecodormancy, at distinct timings. In addition, maximal cold hardiness is not reached automatically, and a cold sustained winter is needed (Londo and Kowaleski, 2017). Depending on the species, grapevine buds' cold hardiness can reach temperatures below -30°C (Londo and Kowaleski, 2017). However, once buds begin to swell and dehardening during the deacclimation process, their freezing tolerance quickly reduces, and the observed advancements in phenological timings may possibly increase the exposure of vulnerable plant structures to late frost events.

Spring Frost Risk

Late spring frosts have often resulted in great damage to cultivated fruit trees and in important economic losses (Gu et al., 2008; Marino et al., 2011; Ault et al., 2013; Vitasse and Rebetez, 2018). In the bigger picture, these phenomena can alter the ecosystem and evolution of entire populations because of competition among species and parasite opportunism (Inouye, 2000; Reineke and Thiéry, 2016). As previously stated, the vulnerability of plant structures to freezing temperatures differs, depending on their level of cold hardiness, which varies seasonally, and on their intrinsic ability to sustain lower temperatures. Green tissues, flowers, and fruit are, in fact, significantly more susceptible to lower temperatures than wooden tissues as their hydration levels are considerably higher, and their supercooling capabilities lower (Fennell, 2004). Budburst and leafout have been delineated as the most critical, as several trees have been shown to be the most vulnerable at that specific time (Vitasse et al., 2014; Lenz et al., 2016). Moreover, a lower temperature stability is expected during winter in the future, which will require the use of cultivars with a lower response to so-called “false springs” (Londo and Kowaleski, 2019). A “false spring” can be empirically defined as a period of warm temperatures with premature rapid vegetative growth, followed by a freeze (Gu et al., 2008; Ault et al., 2013); several mathematical approaches to evaluate these phenomena have been attempted (Marino et al., 2011). Freezing temperatures following a “false spring” can culminate in more serious damage, which affects photosynthetic tissue and reproductive tissue alike with consequences spread on multiple years of development (Carmona et al., 2008). In general, the influence of climate change on late frost events frequency and distribution remains unclear, and whether risk is increasing for temperate trees remains up for debate. The analysis of remote-sensing data showed that frost day in which the temperature drops below 0°C during the growing season have increased in the Northern Hemisphere (Liu et al., 2018). Concerning Europe, phenological and climate records were used to analyze the evolution of spring frost risk as regards several tree species, between 1950 and 2013, with a focus on determining variations in the frequency of the phenomenon (Ma et al., 2018). These results showed that species whose phenology is more responsive to temperature increases tend to experience a higher risk of being subjected to frost occurrences and damage. Maritime areas in Europe were also more exposed to frost compared to continental ones (Ma et al., 2018). Besides, high-altitude areas could experience decreased risk as the rate of warming seems to be amplified with elevation (Pepin et al., 2015). The effects of late frosts on the distribution of grapevine in Europe were analyzed (Leolini et al., 2018). The results, simulated under future scenarios, described in the AR5 IPCC (2014) report, show that budbreak and flowering advancement are more pronounced in Northeastern Europe compared to the Southwest. The simulations showed that changes in the phenology stages of grapevine might expose it to higher frequency of extreme events, with the effects being strictly linked to the phenological cycle of the considered variety (Leolini et al., 2018). An increased risk of spring frost damage is also predicted in several regions of France, supported by

two budburst day simulation models (Sgubin et al., 2018). Similarly, a high probability of spring frost damage for several woody species in Illinois (United States) was reported, by integrating field observations of temperature, phenology, and frost damage over long timeframes (Augsburger, 2013). “False spring” occurrences were reviewed across the United States over the 1920–2013 interval by taking into consideration the trends of vegetation start dates, spring freezes, and a sensitivity analysis, which indicated a decrease in spring frost exposure (Peterson and Abatzoglou, 2014), pointing out distinct tendencies for different geographical locations.

LONG-TERM RESILIENCE TO CLIMATE CHANGE

Breeding Approaches

Passive spring frost damage avoidance approaches are used preemptively and are suited to work on the long-term and include breeding and selection of new fitter varieties (Liu and Sherif, 2019). Traditional breeding approaches have been successfully used in the past to select new cultivars with characteristics of economic interest and in a perennial crop such as grapevine the entire traditional breeding procedure and evaluation process can take many years to be completed (Eibach and Töpfer, 2015). As cultivated grapevines are propagated clonally to fix and maintain specific production parameters, somatic variations that can accumulate during clonal propagation are almost the only source of genetic diversity (Carbonell-Bejerano et al., 2017; van Houten et al., 2020), greatly lower than intervarietal diversity (Roach et al., 2018). Clone collections exist and are available worldwide and represent a source that should be accessed to search for interesting genotypes (Duchêne, 2016). A possible adaptation for the current grape-growing areas should consist in the selection of varieties with a later ripening period; such varieties can be obtained from germplasm collections or through breeding processes (Duchêne et al., 2012).

Fruit trees must fulfill a chilling requirement to transition from endodormancy to ecodormancy, a phase of dormancy in which buds are responsive to growth-promoting conditions. The amount of chilling hours required to do so depends on the genotype, and genotypes that require less chilling have been shown to deacclimate earlier. In any case, the models describing winter chill accumulation are purely empirical or based on experiments in controlled conditions, and the physiological processes occurring in plants during winter are still poorly understood (Luedeling and Brown, 2011). The most popular chilling-hours accumulation models estimate effective chilling temperatures to be included in the 0–7.2°C interval (Dokoozlian, 1999), although different models attribute varying effectiveness to specific temperatures or even negative impacts of higher temperatures on previously accumulated chill (Darbyshire et al., 2011). The widely applied and possibly most accurate Dynamic Model also suggests that the same temperatures might have inconsistent effectiveness, depending on which time of the season they are registered, making it difficult to transfer available information from one location

to another (Luedeling, 2012). Cultivated grapevines are generally considered low-chilling-requiring species compared to other woody perennials; however, chilling requirements can differ significantly in high- and low-chill varieties and fast- or slow-burst phenotypes (Londo and Johnson, 2014). Production located at higher latitudes could benefit from the use of grapevines characterized by higher chilling requirements and slower budburst rates, which would allow lowering the risk of spring frost damage (Londo and Johnson, 2014). Wild grapevines presented a continuous range of chilling requirements and budburst rates, making them an interesting source of variability. In detail, *Vitis amurensis*, *Vitis labrusca*, and *Vitis riparia* were classified as low-chill and fast-burst species, whereas *Vitis rupestris*, *Vitis aestivalis*, and *Vitis vulpina* showed higher chilling requirements (>1,000 h) and longer budburst timings (>14 days). Different latitudes were also proposed as seemingly having an adaptive effect. In fact, North-distributed genotypes (*V. riparia*, *V. labrusca*, and *V. amurensis*) were all classified as low-chill, fast-bursting species. On the contrary, southern varieties (*V. aestivalis*, *V. cinerea*, *V. rupestris*, and *V. vulpina*) were all characterized by higher chilling requirements and slower budburst timings (Londo and Johnson, 2014).

Hybrid crosses were shown to allow lowering the deepest level of cold hardiness, although this could also introduce enhanced midwinter responsiveness in areas where climate warming produces mild winter temperatures (Londo and Kowaleski, 2019). Deacclimation rates were also observed to be much faster in wild varieties *V. riparia* and *V. amurensis*, commonly used by breeders to increase freezing tolerance in cultivated varieties. This could contribute to increased risks of deacclimation during warmer winters and of spring frost damage (Kowaleski et al., 2018). These phenomena could be explained by the evolutionary necessity of these varieties to develop rapidly during short growing seasons typical of their area of origin (Ferguson et al., 2014). Paradoxically, this would make the varieties with the deepest levels of cold hardiness also the most vulnerable to spring frost damage (Ferguson et al., 2014), and considering the observed advancement of spring phenology, winter-hardy varieties could display unwanted phenotypes. For these reasons, focusing breeding efforts on the production of delayed growth-start cultivars could be an alternative favorable approach. A prerequisite for this strategy is the gaining of a comprehensive understanding of the biochemical and molecular mechanisms responsible for dormancy establishment and release in grapevine buds.

Rootstocks are traditionally used to protect scions from soil-borne pests and to improve tolerance to various abiotic stresses; however, their effects on the entirety of the plant often remain obscure (Ollat et al., 2016). The breeding of new rootstocks needs to be considered as a long-term strategy to cope with the consequences of climate change as the substitution of traditional scions with new ones is not going to be accepted as easily. The genetic background of commonly used rootstocks can be difficult to understand as their heritage is often mixed (Poczei et al., 2013), but efforts to improve breeding by enhancing the knowledge of genetic markers have been attempted in recent years (Migliaro et al., 2019; Riaz et al., 2019).

This information is important and needs to be exploited to improve marker-assisted selection (MAS) of new rootstocks, as their influence on scion signaling molecules, response to several stresses, and even berry quality has been observed (Tramontini et al., 2013; Pagliarani et al., 2017; Martin et al., 2020; Zombardo et al., 2020). Moreover, rootstocks can alter scion development rate possibly because of their different abilities to take up nutrients and water from the soil (Zhang et al., 2016). Additionally, messenger RNA molecules and hormones have been reported to pass through the graft site in a possibly environment- and genotype-dependent manner (Nikolaou et al., 2000; Yang et al., 2015). Putative rootstock effects on grapevine phenology and, in particular, on its heat requirements have also been described (Miele, 2019).

A great boost in breeding effort can be attributed to the identification of molecular markers, the introduction of genetic mapping, and genotype–phenotype associations, considerably facilitated by the release of the complete sequence of the *V. vinifera* genome (Jaillon et al., 2007; Velasco et al., 2007). MAS can help the identification of sequences with different genetic backgrounds, aiding the potential exploitation of wild *Vitis* species carrying traits of interest (Daldoul et al., 2020).

Molecular Mechanisms Involved in Deacclimation and Budbreak

Monitoring dormancy status of the bud in real time appears really challenging, because of the absence of visual changes during the bud dormancy cycle (Or, 2009), and the use of GDD as a proxy for spring phenology is not always reliable. Therefore, a better knowledge base of the physiological mechanisms underpinning dormancy induction and release can be an important part of predicting the potential effects of global warming on grapevine. A strict correlation between budbreak and loss of winter cold hardiness (deacclimation) has been recently hypothesized, pointing out that a temperature-controlled interplay underpins these phenological changes (Kowaleski and Londo, 2019).

In this context, recent advances in the understanding of cold hardiness and spring budburst mechanisms may contribute to enhance the sustainability of viticulture, especially when acute cold weather events are expected to increase (Kowaleski and Londo, 2019). On the other hand, traditional breeding is also empirical and requires a deep knowledge of the physiological characteristics of the selected cultivars in past and present cultivated areas. Recently introduced molecular approaches allowed new methods of “molecular breeding” to be applied, allowing speedier and refined crosses (Delrot et al., 2020).

Unfortunately, phenological traits, such as budburst, are often regulated by many quantitative trait loci (QTLs), which are highly responsive to environmental factors. For this reason, the mapping and cloning of genes related to phenological traits are really challenging, and the reproducibility of these QTLs remains low (Delrot et al., 2020).

Recently, several works have identified QTLs associated with budbreak. For example, two independent QTLs on chromosomes

4 and 19 were identified using a genetic map build with microsatellites markers on varieties Riesling and Gewürztraminer (Duchêne et al., 2012). The WRKY transcription factor VvWRKY3 was found within the confidence interval on chromosome 19; a similar transcription factor, AtWRKY2 from *Arabidopsis*, was shown to mediate ABA (abscisic acid) control on seed germination (Jiang and Yu, 2009). Moreover, several genes encoding glutathione S-transferases (GSTs) were also identified on both chromosomes 4 and 19. Increased levels of expression of these genes were registered after both HC (hydrogen cyanamide) application (Or, 2009), a dormancy-breaking agent, and after the natural fulfillment of chilling requirements (Pacey-Miller et al., 2003). Similarly, simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) were used to map another QTL related to budburst on chromosome 15, overlapping on QTLs related to veraison (Grzeskowiak et al., 2013). Genes on chromosome 15 included several transcription factors involved in bud and fruit development (Grzeskowiak et al., 2013).

With regard to cold hardiness control, the progeny resulting from the cross between cold-vulnerable cv. Cabernet Sauvignon and the cold-tolerant hybrid Zuoyouhong was used for the construction of a high-density genetic linkage map on which cold hardiness-related QTLs were mapped (Su et al., 2020). Six QTLs located on chromosomes 2, 3, and 15 were identified, and four cold-responsive candidate genes were proposed. In detail, a dehydration-responsive protein containing a *cis*-DRE (dehydration responsive) element was identified. CRT (C-repeat)/DRE elements, containing a core CCGAC sequence designated as C-repeat, are present in single or multiple copies in the promoter regions of plant COR (cold-responsive) genes, which are induced by low-temperature exposure (Stockinger et al., 1997). The COP9 signalosome (CSN) subunit 1 was also individuated; CSN was shown to be required for the expression of COR genes in *Arabidopsis* (Schwechheimer et al., 2002). Additionally, an RRM (RNA recognition motif)-containing protein was found to be putatively involved in cold hardiness as well. RRM modules were found in cold-responsive RNA-binding proteins from cyanobacteria (Maruyama et al., 1999). Lastly, a MYB-related gene's expression was also reported to be enhanced by cold exposure. Its overexpression in *Arabidopsis* was previously shown to confer increased tolerance to cold (Sun et al., 2018).

Transcriptomic tools have led to new insights into the gene expression processes that take place in dormant tissues. Dormancy release is regulated by a multitude of independent genes whose mechanisms of action are still unclear, together with their conservation among species (Table 1). Growth resumption happens simultaneously with cold deacclimation, although most hardiness is already lost when new tissue is visible (Kovaleski and Londo, 2019). Growth start is also subordinate to the fulfillment of the chilling requirement and the transition from endodormancy to ecodormancy, in which the bud becomes sensitive to favorable environmental conditions. CBFs/DREBs (C-repeat binding factors/dehydration responsive element binding) are important cold-response regulators stimulated by low temperatures. These transcription

TABLE 1 | Genes with putative involvement in cold deacclimation and budbreak regulation.

Gene	Physiological role	Reference
CBFs/DREBs <i>bHLH</i>	Low-temperature response	Xiao et al., 2006 Tillett et al., 2012 Xu et al., 2014 Rubio et al., 2019a Su et al., 2020
<i>VpERF2</i> <i>VpERF3</i> <i>VvA8H</i> <i>VvWRKY3</i>	ABA regulation	Zhu et al., 2013 Gibbs et al., 2014 Duchêne et al., 2012 Zheng et al., 2015 Zheng et al., 2018a
<i>WICS2</i> <i>VvNPR1</i> <i>VvWRKY70</i> <i>VaCPK20</i> <i>CNGCs</i> <i>FAD5</i> <i>GSTs</i>	Defense mechanisms	Zheng et al., 2018b Orrantia-Araujo et al., 2020
<i>ERF-ViIs</i> <i>RBOHF</i> <i>EBB1</i> <i>DMLs</i>	Ca ²⁺ transport	Dubrovina et al., 2013 Kovaleski and Londo, 2019
	Membrane fluidity	Kovaleski and Londo, 2019
	Hypoxia response and oxidative stress	Duchêne et al., 2012 Grzeskowiak et al., 2013 Meitha et al., 2018 Kovaleski and Londo, 2019
	Growth resumption	Busov et al., 2016
	Chilling-responsive demethylation	Conde et al., 2017 Shangguan et al., 2020

factors act as a part of a signaling cascade in which they are induced by ICEs (inducers of CBF expression) and activate COR genes by binding to the CRT/DRE *cis*-elements in their promoter regions and thus conferring freezing tolerance to the plant (Chinnusamy et al., 2010; Thomashow, 2010). Another cold-responsive transcription factor, bHLH, was characterized in both *V. vinifera* cv. Cabernet Sauvignon and wild *V. amurensis* with a proposed putative regulatory role in cold stress response in a CBF-dependent way (Xu et al., 2014). Changes in expression levels and timing of *VvbHLH* and *VabHLH* were observed, possibly caused by differences in the *cis*-regulatory elements in their sequence (Xu et al., 2014). CBFs/DREBs have been identified in several woody species as well as *Arabidopsis*, and their functions are highly conserved (Wisniewski et al., 2014). Several CBFs/DREBs are known in grapevine (Xiao et al., 2006; Tillett et al., 2012; Rubio et al., 2019a) and show increased mRNA expression following exposure to freezing temperatures (Xiao et al., 2006, 2008). The most well-known targets of CBFs/DREBs are DHNs (dehydrins), part of the LEA (late embryogenesis abundant) proteins. DHNs accumulate during dormancy induction and cold acclimation and protect cells from dehydration damage (Wisniewski et al., 2014). Four grape DHNs have been identified (Yang et al., 2012). DHNs were reported to be differently expressed in wild *V. riparia* and in cultivated variety Chardonnay following cold exposure (Xiao and Nassuth, 2006). Increased freezing tolerance is also observed in case of *VvCBFs* overexpression (Tillett et al., 2012). Moreover, the synergistic effect of low temperatures and ABA application in stimulating the expression of CBFs/DREBs in grapevine dormant buds has been recently assessed (Rubio et al., 2019a). ABA has a key role in plant dormancy regulation as ABA variations

have been correlated to different degrees of seed dormancy (Nambara et al., 2010). ABA's role in bud dormancy in woody perennials has been hypothesized, although the regulation mechanism is complex and is still obscure. Recently, several studies showed that the highest levels of ABA were reached at the maximum depth of dormancy and started decreasing at the end of endodormancy in grapevine buds (Kovaleski and Londo, 2019; Rubio et al., 2019b). ABA was also observed to promote starch synthesis in dormant buds, thus promoting their sink capacity and regulating dormancy depth this way (Rubio et al., 2019b). Changing ABA balance in the buds is also the mechanism by which dormancy-breaking agents, such as HC, seem to accomplish their effect (Zheng et al., 2015; Rubio et al., 2019b). In detail, the budbreaking effect of HC in grapevine was reported to be exerted by the stimulation of the ABA-degrading enzyme ABA 8'-hydrolase (A8H), encoded by the *VvA8H-CYP707A4* gene (Zheng et al., 2015). A8H and ABA catabolite increase was also observed during natural dormancy release (Zheng et al., 2015). Moreover, the reversible ability of ABA to prevent loss of cold hardiness and deacclimation after several days of prolonged application on grapevine buds was observed (Kovaleski and Londo, 2019). Together, these results suggest an important role of ABA in endodormancy maintenance and dormancy release, but not in its induction. More recent studies showed that transgenic vines overexpressing *VvA8H-CYP707A4* show both a higher catabolism of ABA and an enhancement of budbreak. Hypoxia and ethylene, which are both considered dormancy release stimulants, enhance the expression of *VvA8H-CYP707A4* (Zheng et al., 2018a). Multiple studies have shed light on the role of other hormones in dormancy release and budbreak; for example, a recent work focused on the expression of several genes involved in the gibberellin (GA) biosynthetic pathway and the interaction of GAs with cytokinins (CKs) in grapevine buds (Zheng et al., 2018b). Although further studies are required, the authors propose an inhibitory effect of GA on budbreak that would give account of the low levels of this hormone registered during dormancy. Authors also hypothesize that this inhibition results from the antagonistic effect of GAs on CK responses, which are required for bud meristem reactivation; only following meristem activation higher levels of GA could be required to sustain growth and budbreak (Zheng et al., 2018b). In addition to this, the effects of cold temperatures on the concentration of salicylic acid (SA) and the expression of genes in its biosynthetic pathway in dormant grapevine buds were also explored (Orrantia-Araujo et al., 2020). Buds exposed to longer periods of chilling hours showed a higher content of endogenous SA once transferred in forcing conditions. The expression of genes *ICS2* (isochorismate synthase 2), *NPR1* (nonexpressor of PR genes 1) and *WRKY70* showed variations in buds subjected to cold treatment compared to control ones. *ICS2* takes part in the biosynthesis pathway of SA, *NPR1* is a master regulator of SA-mediated defense signaling, and *WRKY70* participates in both positive and negative regulation of SA signaling. These results indicate that cold accumulation could stimulate the synthesis of SA in grapevine buds and introduce the possibility

of a role of SA-mediated defense signaling in bud dormancy release (Orrantia-Araujo et al., 2020).

The discovery and characterization of the *EBB1* gene with a role in shoot growth resumption after winter have been carried out both in *Populus* (Yordanov et al., 2014) and in peach, where RNA-seq analysis confirmed that *EBB1* is involved in budbreak by taking part into the regulation of several pathways that act synergistically and involve hormones, cell division, and cell wall modifications (Zhao et al., 2020). The conservation of this AP2/ERF family transcription factor was evidenced by the identification of several homologs in various woody perennial species, among which also is *V. vinifera* (Busov et al., 2016). Consistently with the *EBB1* expression in *Poplar*, *VvEBB1* resulted greatly downregulated during dormancy and upregulated before budbreak.

It is well-known that genomic DNA methylation is a mechanism that influences gene expression. In plants, a subgroup of DNA glycosylase-lyases, known as DEMETER-LIKE DNA demethylases (DMLs), can actively demethylate DNA and have been shown to be involved in abiotic stress responses in *Arabidopsis* (Le et al., 2014), developmental transitions in tomato (Liu et al., 2015), and nodule development in *Medicago truncatula* (Satgé et al., 2016). A *Populus trichocarpa* DML, *PtaDML10*, was proposed to be responsible for DML-mediated demethylation at the shoot apical meristem in budbreak regulation (Conde et al., 2017). A loss-of-function analysis confirmed the chilling-responsive demethylation performed by *DML10* in proximity to dormancy release. RNA-seq combined with methylome data analysis revealed that the *DML10* gene targets are genetically associated with budbreak (Conde et al., 2017). Moreover, no overlap was found between the targets of *DML10*-mediated demethylation and *EBB1* targets in poplar. This seemingly confirms that these genes act on separate pathways (Conde et al., 2017). No evidence on the role of DML genes on grapevine dormancy release currently exists, although several DML demethylases have been identified (Shangguan et al., 2020).

Additionally, regulated hypoxia has been found to be a development signal in several stages of plant life (Gibbs et al., 2014; Abbas et al., 2015), and many responses to hypoxia are regulated by group VII of ethylene responsive transcription factors (ERF-VIIs) (Gibbs et al., 2014). For these reasons, the role of oxygen-dependent signaling in transcriptional and metabolic reactivation during budburst in grapevine was investigated (Meitha et al., 2018). The data support that oxygen-dependent signaling through grape ERFs is involved in the transition from dormancy to budburst. Moreover, approximately 20% of grapevine genes presenting a HRPE (hypoxia-responsive promoter element) motif in their promoter were differently expressed in the first 24 h of budburst (Meitha et al., 2018). These results strongly suggest an important developmental function of oxygen-dependent signaling through *VvERF-VIIs* in determining timing and coordination of budburst in grapevines. Further support of the role of oxidative stress response pathways in grapevine budbreak regulation is provided by Kovaleski and Londo (2019), proposing the expression of *RBOHF* (respiratory burst oxidase homolog protein F) as a marker for budbreak. *RBOHF* is involved in ABA and ethylene signaling through H_2O_2 production (Kwak et al., 2003). In addition

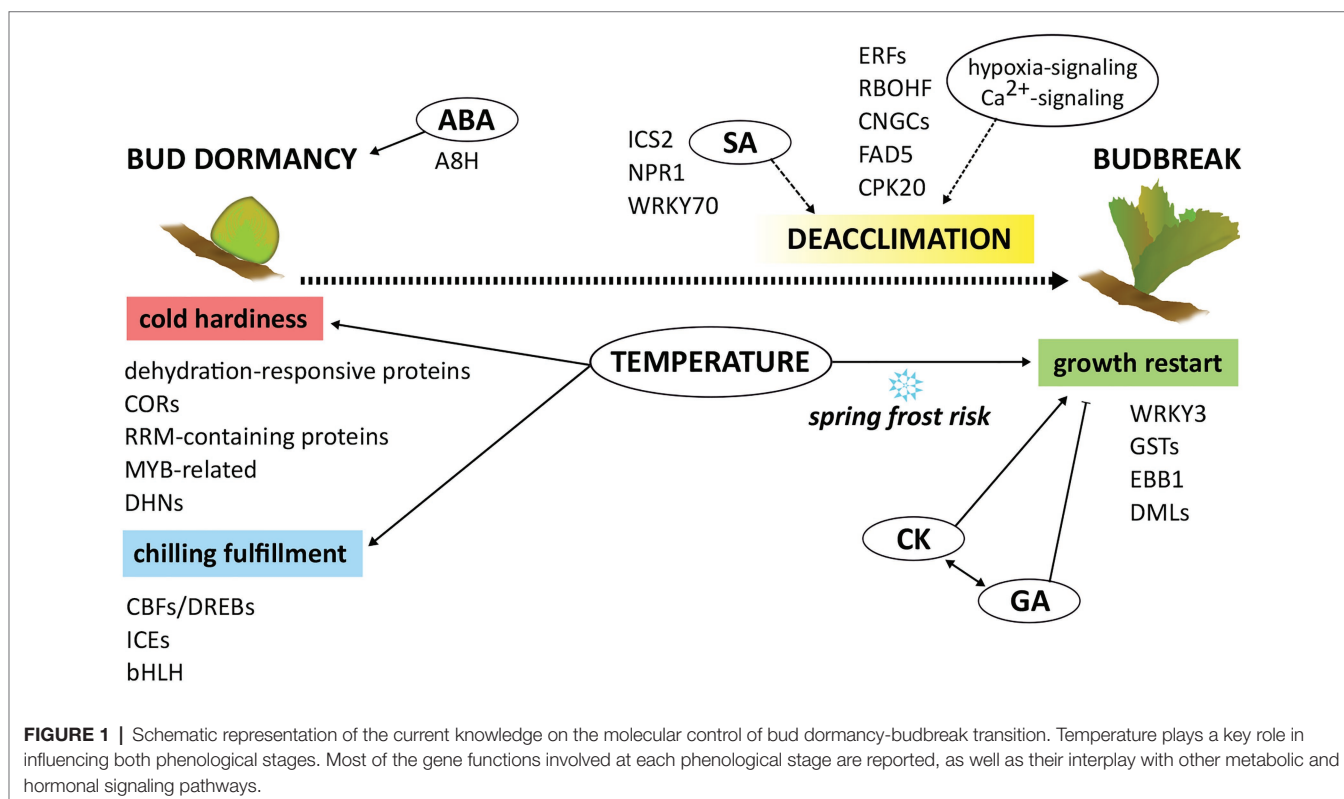
to this, two ERF genes from Chinese wild *Vitis pseudoreticulata*, *VpERF2* and *VpERF3*, were reported to be involved in abiotic stress response pathways including cold exposure (Zhu et al., 2013). Overexpression studies also pointed out a role of these transcription factors in pathogenesis-related proteins accumulation. Moreover, ABA-dependent expression of *VpERF2* and SA-dependent expression of *VpERF3* were shown through exogenous hormone application on leaves (Zhu et al., 2013).

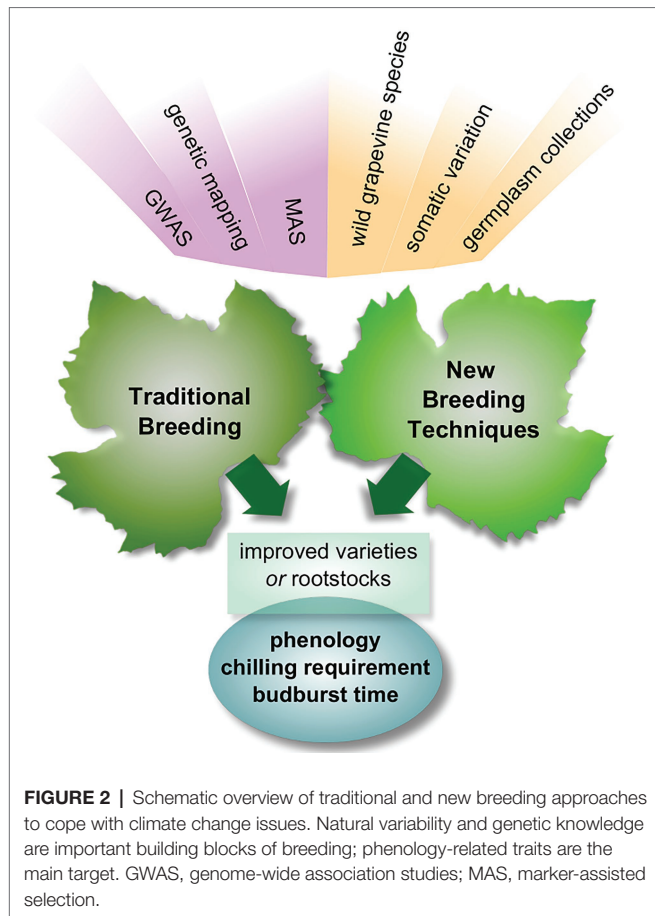
Recently, dormant buds of several *Vitis* genotypes, belonging to different species, were observed to sense the stimulus for dormancy release and deacclimation simultaneously when put into the same forcing conditions (Kovaleski and Londo, 2019). The observed differences in budbreak timings would then be attributed to the ability of the specific genotypes to restart growth. In fact, temperature sensing is believed to be the first step toward bud growth. Among the first sensors, membrane CNGCs (cyclic nongated ion channels) are very responsive to temperature changes. These nonselective Ca^{2+} channels are placed as very first components of the thermosensing pathways in *Arabidopsis* and *Physcomitrella* (Finka et al., 2012) and possibly have the ability to sense membrane fluidity changes caused by temperature shifts (Finka and Goloubinoff, 2014). Synchronous downregulation of nuclear-localized CNGC15 and FAD5 (fatty acid desaturase 5) was reported, suggesting a role of nuclear Ca^{2+} signaling during dormancy in grapevine buds (Kovaleski and Londo, 2019). A role in cold and water stress response of Ca^{2+} flux sensor *VaCPK20* (calcium-dependent protein kinase) from wild *V. amurensis* vines was also suggested (Dubrovina et al., 2013).

CONCLUDING REMARKS

Spring frost damage risk cannot be overlooked in the future in several areas of the world, making the identification of effective adaptive measures an issue of the present. Understanding the molecular mechanisms underlying cold hardiness loss/deacclimation and budbreak is essential for improving crop sustainability and adaptation in the future changing climate. The observations gathered so far on cold deacclimation and dormancy release regulation in grapevine outline a very complex scenario in which many pathways are involved (Figure 1). As chilling requirement, deacclimation dynamics, and budbreak timing appear tightly connected, a major regulatory role can be ascribed to temperature-sensing related genes, common among different genotypes. Hormonal interplay, at times synergistic as well as antagonistic or seemingly independent, should also draw great attention as not only ABA's expected involvement seems ascertained, but also growth reactivation-related, defense-related, and oxidative stress-related hormones putatively perform actively in the regulation of these phenomena. A third valuable and worthy of notice opportunity concerns epigenetics and epigenetic regulators, which add an extra layer of complexity. Defining the extent of the role and significance of each component of this intricate net of regulators requires further studies.

Breeding efforts need to focus on the potential of wild *Vitis* varieties to bear favorable traits, starting from changing chilling requirements and budburst rates. In this regard, the accuracy of all most popularly used chilling-hours accumulation





REFERENCES

- Abad, F. J., Marín, D., Loidi, M., Miranda, C., Royo, J. B., Urrestarazu, J., et al. (2019). Evaluation of the incidence of severe trimming on grapevine (*Vitis vinifera* L.) water consumption. *Agric. Water Manag.* 213, 646–653. doi: 10.1016/j.agwat.2018.10.015
- Abbas, M., Berckhan, S., Rooney, D. J., Gibbs, D. J., Vicente Conde, J., Sousa Correia, C., et al. (2015). Oxygen sensing coordinates photomorphogenesis to facilitate seedling survival. *Curr. Biol.* 25, 1483–1488. doi: 10.1016/j.cub.2015.03.060
- Alikadic, A., Pertot, I., Eccel, E., Dolci, C., Zarbo, C., Caffarra, A., et al. (2019). The impact of climate change on grapevine phenology and the influence of altitude: a regional study. *Agric. For. Meteorol.* 271, 73–82. doi: 10.1016/j.agrformet.2019.02.030
- Anzanello, R., Fialho, F. B., and dos Santos, H. P. (2018). Chilling requirements and dormancy evolution in grapevine buds. *Cienci. Agrotec.* 42, 364–371. doi: 10.1590/1413-70542018424014618
- Augsburger, C. K. (2013). Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage risk is increasing. *Ecology* 94, 41–50. doi: 10.1890/12-0200.1
- Ault, T. R., Henebry, G. M., de Beurs, K. M., Schwartz, M. D., Betancourt, J. L., and Moore, D. (2013). The false spring of 2012, earliest in north American record. *Eos* 94, 181–182. doi: 10.1002/2013EO200001
- Biasi, R., Brunori, E., Ferrara, C., and Salvati, L. (2019). Assessing impacts of climate change on phenology and quality traits of *Vitis vinifera* L.: the contribution of local knowledge. *Plants* 8:121. doi: 10.3390/plants8050121
- Blanco-Ward, D., Ribeiro, A., Barreales, D., Castro, J., Verdial, J., Feliciano, M., et al. (2019). Climate change potential effects on bioclimatic indices: a casa study for the Portuguese demarcated Douro region (Portugal). *BIO Web Conf.* 12:01013. doi: 10.1051/bioconf/20191201013

models needs to be standardized in order to select varieties suitable to changing conditions is specific areas. An intense application of genetic mapping approaches is required to locate and isolate the genetic *loci* that are responsible for the phenotypic expression of these characteristics so that traditional or new plant breeding techniques can be carried out more swiftly and purposefully (Figure 2). Despite the complexity of the full picture and the uncertainties about the connections among the players, the variety of elements involved allows tackling the problem through a multitude of approaches and should be considered encouraging.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

VDR activity was supported by a grant of the Italian Ministry of Education, Research and University (MIUR).

ACKNOWLEDGMENTS

We would like to thank Department of Agricultural, Food, Environmental, and Animal Sciences, University of Udine for funding newly hired researchers.

- Bonada, M., Jeffery, D. W., Petrie, P. R., Moran, M. A., and Sadras, V. O. (2015). Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Aust. J. Grape Wine Res.* 21, 240–253. doi: 10.1111/ajgw.12142
- Bonfante, A., Alfieri, S. M., Albrizio, R., Basile, A., De Mascellis, R., Gambuti, A., et al. (2017). Evaluation of the effects of future climate change on grape quality through a physically based model application: a case study for the Aglianico grapevine in Campania region, Italy. *Agric. Syst.* 152, 100–109. doi: 10.1016/j.agry.2016.12.009
- Busov, V., Carneros, E., and Yakovlev, I. (2016). EARLY BUD-BREAK1 (EBB1) defines a conserved mechanism for control of BUD-BREAK in woody perennials. *Plant Signal. Behav.* 11:e1073873. doi: 10.1080/15592324.2015.1073873
- Caffarra, A., and Eccel, E. (2011). Projecting the impacts of climate change on the phenology of grapevine in a mountain area. *Aust. J. Grape Wine Res.* 17, 52–61. doi: 10.1111/j.1755-0238.2010.00118.x
- Carbonell-Bejerano, P., Royo, C., Torres-Pérez, R., Grimplet, J., Fernandez, L., Franco-Zorrilla, J. M., et al. (2017). Catastrophic unbalanced genome rearrangements cause somatic loss of berry color in grapevine. *Plant Physiol.* 175, 786–801. doi: 10.1104/pp.17.00715
- Cardell, M. F., Amengual, A., and Romero, R. (2019). Future effects of climate change on the suitability of wine grape production across Europe. *Reg. Environ. Chang.* 19, 2299–2310. doi: 10.1007/s10113-019-01502-x
- Carmona, M. J., Chaïb, J., Martínez-Zapater, J. M., and Thomas, M. R. (2008). A molecular genetic perspective of reproductive development in grapevine. *J. Exp. Bot.* 59, 2579–2596. doi: 10.1093/jxb/ern160
- Centinari, M., Gardner, D. M., Smith, D. E., and Smith, M. S. (2018). Impact of amigo oil and KDL on grapevine postbudburst freeze damage, yield components, and fruit and wine composition. *Am. J. Enol. Vitic.* 69, 77–88. doi: 10.5344/ajev.2017.17030

- Chinnusamy, V., Zhu, J. K., and Sunkar, R. (2010). Gene regulation during cold stress acclimation in plants. *Methods Mol. Biol.* 639, 39–55. doi: 10.1007/978-1-60761-702-0_3
- Conde, D., Le Gac, A. L., Perales, M., Dervinis, C., Kirst, M., Maury, S., et al. (2017). Chilling-responsive DEMETER-LIKE DNA demethylase mediates in poplar bud break. *Plant Cell Environ.* 40, 2236–2249. doi: 10.1111/pce.13019
- Coombe, B. G. (1987). Influence of temperature on composition and quality of grapes. *Acta Hort.* 206, 23–36. doi: 10.17660/actahortic.1987.206.1
- Cortázar-atauri, I. G. D., Brisson, N., and Gaudillere, J. P. (2009). Performance of several models for predicting budburst date of grapevine (*Vitis vinifera* L.). *Int. J. Biometeorol.* 53, 317–326. doi: 10.1007/s00484-009-0217-4
- Costa, R., Fraga, H., Fonseca, A., de Cortazar-Atauri, I. G., Val, M. C., Carlos, C., et al. (2019). Grapevine phenology of cv. Touriga Franca and Touriga Nacional in the Douro wine region: Modelling and climate change projections. *Agronomy* 9:210. doi: 10.3390/agronomy9040210
- Daldoul, S., Boubakri, H., Gargouri, M., and Mliki, A. (2020). Recent advances in biotechnological studies on wild grapevines as valuable resistance sources for smart viticulture. *Mol. Biol. Rep.* 47, 3141–3153. doi: 10.1007/s11033-020-05363-0
- Darbyshire, R., Webb, L., Goodwin, I., and Barlow, S. (2011). Winter chilling trends for deciduous fruit trees in Australia. *Agric. For. Meteorol.* 151, 1074–1085. doi: 10.1016/j.agrformet.2011.03.010
- Delrot, S., Grimplet, J., Carbonell-Bejerano, P., Schwandner, A., Bert, P.-F., Bavaresco, L., et al. (2020). “Genetic and genomic approaches for adaptation of grapevine to climate change” in *Genomic Designing of Climate-Smart Fruit Crops*. ed. C. Kole (Cham: Springer), 157–270.
- Dokoozlian, N. K. (1999). Chilling temperature and duration interact on the budbreak of “Perlette” grapevine cuttings. *HortScience* 34, 1054–1056. doi: 10.21273/hortsci.34.6.1
- Dubrovina, A. S., Kiselev, K. V., and Khristenko, V. S. (2013). Expression of calcium-dependent protein kinase (CDPK) genes under abiotic stress conditions in wild-growing grapevine *Vitis amurensis*. *J. Plant Physiol.* 170, 1491–1500. doi: 10.1016/j.jplph.2013.06.014
- Duchêne, É. (2016). How can grapevine genetics contribute to the adaptation to climate change? *Oeno One* 50, 113–124. doi: 10.20870/oeno-one.2016.50.3.98
- Duchêne, E., Butterlin, G., Dumas, V., and Merdinoglu, D. (2012). Towards the adaptation of grapevine varieties to climate change: QTL and candidate genes for developmental stages. *Theor. Appl. Genet.* 124, 623–635. doi: 10.1007/s00122-011-1734-1
- Eibach, R., and Töpfer, R. (2015). “Traditional grapevine breeding techniques” in *Grapevine Breeding Programs for the Wine Industry*. ed. A. Reynolds (Woodhead Publishing), 3–22.
- Fennell, A. (2004). Freezing tolerance and injury in grapevines. *J. Crop Improv.* 10, 201–235. doi: 10.1300/J411v10n01_09
- Ferguson, J. C., Moyer, M. M., Mills, L. J., Hoogenboom, G., and Keller, M. (2014). Modeling dormant bud cold hardiness and Budbreak in twenty-three *Vitis* genotypes reveals variation by region of origin. *Am. J. Enol. Vitic.* 65, 59–71. doi: 10.5344/ajev.2013.13098
- Finka, A., Cuendet, A. F. H., Maathuis, F. J. M., Saidi, Y., and Goloubinoff, P. (2012). Plasma membrane cyclic nucleotide gated calcium channels control land plant thermal sensing and acquired thermotolerance. *Plant Cell* 24, 3333–3348. doi: 10.1105/tpc.112.095844
- Finka, A., and Goloubinoff, P. (2014). The CNGC_b and CNGC_d genes from *Physcomitrella patens* moss encode for thermosensory calcium channels responding to fluidity changes in the plasma membrane. *Cell Stress Chaperones* 19, 83–90. doi: 10.1007/s12192-013-0436-9
- Fraga, H., de Cortázar Atauri, G. I., Malheiro, A. C., Moutinho-Pereira, J., and Santos, J. A. (2017). Viticulture in Portugal: a review of recent trends and climate change projections. *Oeno One* 51, 61–69. doi: 10.20870/oeno-one.2017.51.2.1621
- Fraga, H., de Cortázar Atauri, I. G., Malheiro, A. C., and Santos, J. A. (2016). Modelling climate change impacts on viticultural yield, phenology and stress conditions in Europe. *Glob. Chang. Biol.* 22, 3774–3788. doi: 10.1111/gcb.13382
- Friend, A. P., and Trought, M. C. T. (2007). Delayed winter spur-pruning in New Zealand can alter yield components of merlot grapevines. *Aust. J. Grape Wine Res.* 13, 157–164. doi: 10.1111/j.1755-0238.2007.tb00246.x
- Frioni, T., Tombesi, S., Silvestroni, O., Lanari, V., Bellincontro, A., Sabbatini, P., et al. (2016). Spur-pruning applied after bud burst limits yield and delayed fruit sugar accumulation in cv. Sangiovese in Central Italy. *Am. J. Enol. Vitic.* 67, 419–425. doi: 10.5344/ajev.2016.15120
- Gaal, M., Moriondo, M., and Bindi, M. (2012). Modelling the impact of climate change on the Hungarian wine regions using random forest. *Appl. Ecol. Environ. Res.* 10, 121–140. doi: 10.15666/aer/1002_121140
- Gibbs, D. J., MdIsa, N., Movahedi, M., Lozano-Juste, J., Mendiondo, G. M., Berckhan, S., et al. (2014). Nitric oxide sensing in plants is mediated by Proteolytic control of group VII ERF transcription factors. *Mol. Cell* 53, 369–379. doi: 10.1016/j.molcel.2013.12.020
- Grzeskowiak, L., Costantini, L., Lorenzi, S., and Grando, M. S. (2013). Candidate loci for phenology and fruitfulness contributing to the phenotypic variability observed in grapevine. *Theor. Appl. Genet.* 126, 2763–2776. doi: 10.1007/s00122-013-2170-1
- Gu, L., Hanson, P. J., Post, W. M., Kaiser, D. P., Yang, B., Nemani, R., et al. (2008). The 2007 eastern US spring freeze: increased cold damage in a warming world? *Bioscience* 28, 253–262. doi: 10.1641/B580311
- Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., et al. (2013). Climate change, wine, and conservation. *PNAS* 110, 6907–6912. doi: 10.1073/pnas.1210127110
- Hébert-Haché, A., Inglis, D., Kemp, B., and Willwerth, J. J. (2020). Clone and rootstock interactions influence the cold hardiness of *Vitis vinifera* cvs. Riesling and sauvignon blanc. *Am. J. Enol. Vitic.* 72:ajev.2020.20025. doi: 10.5344/ajev.2020.20025
- Herrera, J. C., Bucchetti, B., Sabbatini, P., Comuzzo, P., Zulini, L., Vecchione, A., et al. (2015). Effect of water deficit and severe shoot trimming on the composition of *Vitis vinifera* L. merlot grapes and wines. *Aust. J. Grape Wine Res.* 21, 254–265. doi: 10.1111/ajgw.12143
- Horvath, D. P., Anderson, J. V., Chao, W. S., and Foley, M. E. (2003). Knowing when to grow: signals regulating bud dormancy. *Trends Plant Sci.* 8, 534–540. doi: 10.1016/j.tplants.2003.09.013
- Inouye, D. W. (2000). The ecological and evolutionary significance of frost in the context of climate change. *Ecol. Lett.* 3, 457–463. doi: 10.1046/j.1461-0248.2000.00165.x
- IPCC (2014). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. eds. Core Writing Team, R. K. Pachauri and L. A. Meyer (Geneva: IPCC), 151.
- IPCC (2019). *Climate Change and Land: An IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse Gas Fluxes in Terrestrial Ecosystems*. eds. P. R. Shukla, J. Skea, E. C. Buendia, V. Masson-Delmotte, H. O. Pörtner and D. C. Roberts et al. (In press).
- Jaillon, O., Aury, J. M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., et al. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449, 463–467. doi: 10.1038/nature06148
- Jiang, W., and Yu, D. (2009). Arabidopsis WRKY2 transcription factor mediates seed germination and postgermination arrest of development by abscisic acid. *BMC Plant Biol.* 9:96. doi: 10.1186/1471-2229-9-96
- Jones, G. V., Duchene, E., Tomasi, D., Yuste, J., Braslavka, O., Schultz, H., et al. (2005). “Change in European Winegrape phenology and relationship with climate” in *Proceedings of XIV GESCO Symposium*. Vol. 1. 55–61.
- Kliever, W. M., and Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. *Am. J. Enol. Vitic.* 23, 71–77.
- Kovaleski, A. P., and Londo, J. P. (2019). Tempo of gene regulation in wild and cultivated *Vitis* species shows coordination between cold deacclimation and budbreak. *Plant Sci.* 287:110178. doi: 10.1016/j.plantsci.2019.110178
- Kovaleski, A. P., Reisch, B. I., and Londo, J. P. (2018). Deacclimation kinetics as a quantitative phenotype for delineating the dormancy transition and thermal efficiency for budbreak in *Vitis* species. *AoB Plants* 10:ply066. doi: 10.1093/aobpla/ply066
- Kwak, J. M., Mori, I. C., Pei, Z. M., Leonhard, N., Angel Torres, M., Dangl, J. L., et al. (2003). NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in arabidopsis. *EMBO J.* 22, 2623–2633. doi: 10.1093/emboj/cdg277
- Lang, G. A., Early, J. D., Martin, G. C., and Darnell, R. L. (1987). Endo-, Para-, and ecodormancy: physiological terminology and classification for dormancy research. *Hortic. Sci.* 22, 371–377.

- Le, T. N., Schumann, U., Smith, N. A., Tiwari, S., Khang Au, P. C., Zhu, Q. H., et al. (2014). DNA demethylases target promoter transposable elements to positively regulate stress responsive genes in Arabidopsis. *Genome Biol.* 15:458. doi: 10.1186/s13059-014-0458-3
- Lenz, A., Hoch, G., Körner, C., and Vitas, Y. (2016). Convergence of leaf-out towards minimum risk of freezing damage in temperate trees. *Funct. Ecol.* 30, 1480–1490. doi: 10.1111/1365-2435.12623
- Leolini, L., Moriondo, M., Fila, G., Costafreda-Aumedes, S., Ferrise, R., and Bindi, M. (2018). Late spring frost impacts on future grapevine distribution in Europe. *Field Crop Res.* 222, 197–208. doi: 10.1016/j.fcr.2017.11.018
- Liu, R., How-Kit, A., Stammiti, L., Teyssier, E., Rolin, D., Mortain-Bertrand, A., et al. (2015). A DEMETER-like DNA demethylase governs tomato fruit ripening. *Proc. Natl. Acad. Sci. U. S. A.* 112, 10804–10809. doi: 10.1073/pnas.1503362112
- Liu, Q., Piao, S., Janssens, I. A., Fu, Y., Peng, S., Lian, X., et al. (2018). Extension of the growing season increases vegetation exposure to frost. *Nat. Commun.* 9:426. doi: 10.1038/s41467-017-02690-y
- Liu, J., and Sherif, S. M. (2019). Combating spring frost with ethylene. *Front. Plant Sci.* 10:1408. doi: 10.3389/fpls.2019.01408
- Londo, J. P., and Johnson, L. M. (2014). Variation in the chilling requirement and budburst rate of wild *Vitis* species. *Environ. Exp. Bot.* 106, 138–147. doi: 10.1016/j.envexpbot.2013.12.012
- Londo, J. P., and Kowaleski, A. P. (2017). Characterization of wild north American grapevine cold hardiness using differential thermal analysis. *Am. J. Enol. Vitic.* 68, 203–212. doi: 10.5344/ajev.2016.16090
- Londo, J. P., and Kowaleski, A. P. (2019). Deconstructing cold hardiness: variation in supercooling ability and chilling requirements in the wild grapevine *Vitis riparia*. *Aust. J. Grape Wine R.* 25, 276–285. doi: 10.1111/ajgw.12389
- Luedeling, E. (2012). Climate change impacts on winter chill for temperate fruit and nut production: a review. *Sci. Hortic.* 144, 218–229. doi: 10.1016/j.scienta.2012.07.011
- Luedeling, E., and Brown, P. H. (2011). A global analysis of the comparability of winter chill models for fruit and nut trees. *Int. J. Biometeorol.* 55, 411–421. doi: 10.1007/s00484-010-0352-y
- Ma, Q., Huang, J. G., Hänninen, H., and Berninger, F. (2018). Divergent trends in the risk of spring frost damage to trees in Europe with recent warming. *Glob. Chang. Biol.* 25, 351–360. doi: 10.1111/gcb.14479
- Marino, G. P., Kaiser, D. P., Gu, L., and Ricciuto, D. M. (2011). Reconstruction of false spring occurrences over the southeastern United States 1901–2007: an increasing risk of spring freeze damage? *Environ. Res. Lett.* 6:024015. doi: 10.1088/1748-9326/6/2/024015
- Martin, L., Vila, H., Bottini, R., and Berli, F. (2020). Rootstocks increase grapevine tolerance to NaCl through ion compartmentalization and exclusion. *Acta Physiol. Plant.* 42:145. doi: 10.1007/s11738-020-03136-7
- Maruyama, K., Sato, N., and Ohta, N. (1999). Conservation of structure and cold-regulation of RNA-binding proteins in cyanobacteria: probable convergent evolution with eukaryotic glycine-rich RNA-binding proteins. *Nucleic Acids Res.* 27, 2029–2036. doi: 10.1093/nar/27.9.2029
- McIntyre, G. N., Lider, L. A., and Ferrari, N. L. (1982). The chronological classification of grapevine phenology. *Am. J. Enol. Vitic.* 33, 80–85.
- Meitha, K., Agudelo-Romero, P., Signorelli, S., Gibbs, D. J., Considine, J. A., Foyer, C. H., et al. (2018). Developmental control of hypoxia during bud burst in grapevine. *Plant Cell Environ.* 41, 1154–1170. doi: 10.1111/pce.13141
- Miele, A. (2019). Rootstock-scion interaction: 6. Phenology, chilling and heat requirements of cabernet sauvignon grapevine. *Rev. Bras. Frutic.* 41:e-446. doi: 10.1590/0100-29452019446
- Migliaro, D., De Lorenzo, G., Di Lorenzo, G. S., Nardi, B. De, Gardiman, M., Failla, O., et al. (2019). Grapevine non-vinifera genetic diversity assessed by simple sequence repeat markers as a starting point for new rootstock breeding programs. *Am. J. Enol. Vitic.* 70, 390–397. doi: 10.5344/ajev.2019.18054
- Molitor, D., Baus, O., Hoffmann, L., and Beyer, M. (2016). Meteorological conditions determine the thermal-temporal position of the annual botrytis bunch rot epidemic on *Vitis vinifera* L. cv. Riesling grapes. *Oeno One* 50, 231–244. doi: 10.20870/oeno-one.2016.50.4.36
- Molitor, D., Junk, J., Evers, D., Hoffmann, L., and Beyer, M. (2014). A high-resolution cumulative degree day-based model to simulate phenological development of grapevine. *Am. J. Enol. Vitic.* 65, 72–80. doi: 10.5344/ajev.2013.13066
- Moriondo, M., Jones, G. V., Bois, B., Dibari, C., Ferrise, R., Trombi, G., et al. (2013). Projected shifts of wine regions in response to climate change. *Clim. Chang.* 119, 825–839. doi: 10.1007/s10584-013-0739-y
- Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M., and Kamiya, Y. (2010). Abscissic acid and the control of seed dormancy and germination. *Seed Sci. Res.* 20, 55–67. doi: 10.1017/S0960258510000012
- Nikolaou, N., Koukourikou, A. M., and Karagiannidis, N. (2000). Effects of various rootstocks on xylem exudates cytokinin content, nutrient uptake and growth patterns of grapevine *Vitis vinifera* L. cv. Thompson seedless. *Agronomie* 20, 363–373. doi: 10.1051/agro:2000133
- Ollat, N., Bordenave, L., Tandonnet, J. P., Boursiquot, J. M., and Marguerit, E. (2016). Grapevine rootstocks: origins and perspectives. *Acta Hortic.* 1136, 11–22. doi: 10.17660/ActaHortic.2016.1136.2
- Or, E. (2009). “Grape Bud Dormancy Release - The Molecular Aspect” in *Grapevine Molecular Physiology and Biotechnology. 2nd Edn.* ed. K. A. Roubelakis-Angelakis (Netherlands: Springer), 1–29.
- Orrantia-Araujo, M. A., Martínez-Téllez, M. Á., Rivera-Domínguez, M., Hernández-Oñate, M. Á., and Vargas-Arispuro, I. (2020). Changes in the endogenous content and gene expression of salicylic acid correlate with grapevine bud dormancy release. *J. Plant Growth Regul.* 40, 254–262. doi: 10.1007/s00344-020-10100-9
- Pacey-Miller, T., Scott, K., Ablett, E., Tingey, S., Ching, A., and Henry, R. (2003). Genes associated with the end of dormancy in grapes. *Funct. Integr. Genomics* 3, 144–152. doi: 10.1007/s10142-003-0094-6
- Pagliarini, C., Vitali, M., Ferrero, M., Vitolo, N., Incarbone, M., Lovisolo, C., et al. (2017). The accumulation of miRNAs differentially modulated by drought stress is affected by grafting in grapevine. *Plant Physiol.* 173, 2180–2195. doi: 10.1104/pp.16.01119
- Pagter, M., and Arora, R. (2013). Winter survival and deacclimation of perennials under warming climate: physiological perspectives. *Physiol. Plant.* 147, 75–87. doi: 10.1111/j.1399-3054.2012.01650.x
- Pallioti, A., Frioni, T., Tombesi, S., Sabbatini, P., Cruz-Castillo, J. G., Lanari, V., et al. (2017). Double-pruning grapevines as a management tool to delay berry ripening and control yield. *Am. J. Enol. Vitic.* 68, 412–421. doi: 10.5344/ajev.2017.17011
- Pepin, N., Bradley, R. S., Diaz, H. F., Baraer, M., Caceres, E. B., Forsythe, N., et al. (2015). Elevation-dependent warming in mountain regions of the world. *Nature Clim. Change* 5, 424–430. doi: 10.1038/nclimate2563
- Peterson, A. G., and Abatzoglou, J. T. (2014). Observed changes in false springs over the contiguous United States. *Geophys. Res. Lett.* 41, 2156–2162. doi: 10.1002/2014GL059266
- Poccai, P., Hyvönen, J., Tallér, J., Jahnke, G., and Kocsis, L. (2013). Phylogenetic analyses of Teleki grapevine rootstocks using three chloroplast DNA markers. *Plant Mol. Biol. Report.* 31, 371–386. doi: 10.1007/s11105-012-0512-9
- Qrunfleh, I. M., and Read, P. E. (2013). Delaying bud break in ‘Edelweiss’ grapevines to avoid spring frost injury by NAA and vegetable oil applications. *Adv. Hortic. Sci.* 27, 18–24. doi: 10.1400/220118
- Ramos, M. C., and de Toda, F. M. (2020). Variability in the potential effects of climate change on phenology and on grape composition of Tempranillo in three zones of the Rjoja DOCa (Spain). *Eur. J. Agron.* 111:126014. doi: 10.1016/j.eja.2020.126014
- Reineke, A., and Thiéry, D. (2016). Grapevine insect pests and their natural enemies in the age of global warming. *J. Pest. Sci.* 89, 313–328. doi: 10.1007/s10340-016-0761-8
- Riaz, S., Pap, D., Uretsky, J., Laucou, V., Boursiquot, J. M., Kocsis, L., et al. (2019). Genetic diversity and parentage analysis of grape rootstocks. *Theor. Appl. Genet.* 132, 1847–1860. doi: 10.1007/s00122-019-03320-5
- Roach, M. J., Johnson, D. L., Bohlmann, J., van Vuuren, H. J. J., Jones, S. J. M., Pretorius, I. S., et al. (2018). Population sequencing reveals clonal diversity and ancestral inbreeding in the grapevine cultivar chardonnay. *PLoS Genet.* 14:e1007807. doi: 10.1371/journal.pgen.1007807
- Rohde, A., and Bhalerao, R. P. (2007). Plant dormancy in the perennial context. *Trends Plant Sci.* 12, 217–223. doi: 10.1016/j.tplants.2007.03.012
- Rubio, S., Noriega, X., and Pérez, F. J. (2019a). Abscissic acid (ABA) and low temperatures synergistically increase the expression of CBF/DREB1 transcription factors and cold-hardiness in grapevine dormant buds. *Ann. Bot.* 123, 681–689. doi: 10.1093/aob/mcy201
- Rubio, S., Noriega, X., and Pérez, F. J. (2019b). ABA promotes starch synthesis and storage metabolism in dormant grapevine buds. *J. Plant Physiol.* 234–235, 1–8. doi: 10.1016/j.jplph.2019.01.004

- Ruml, M., Korać, N., Ćirković, D., Vujadinović, M., and Vuković, A. (2016). Heat requirements for red grapevine cultivars in the wine-producing region of Sremski Karlovci, Serbia. *Acta Hort.* 1139, 409–412. doi: 10.17660/ActaHortic.2016.1139.71
- Sadras, V. O., and Moran, M. A. (2012). Elevated temperature decouples anthocyanins and sugars in berries of shiraz and cabernet franc. *Aust. J. Grape Wine Res.* 18, 115–122. doi: 10.1111/j.1755-0238.2012.00180.x
- Santillán, D., Iglesias, A., La Jeunesse, I., Garrote, L., and Sotes, V. (2019). Vineyards in transition: a global assessment of the adaptation needs of grape producing regions under climate change. *Sci. Total Environ.* 657, 839–852. doi: 10.1016/j.scitotenv.2018.12.079
- Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L.-T., Correia, C., et al. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Appl. Sci.* 10:3092. doi: 10.3390/app10093092
- Satgé, C., Moreau, S., Sallet, E., Lefort, G., Auriac, M. C., Remblière, C., et al. (2016). Reprogramming of DNA methylation is critical for nodule development in *Medicago truncatula*. *Nat. Plants.* 2:16166. doi: 10.1038/nplants.2016.166
- Schwechheimer, C., Serino, G., and Deng, X. W. (2002). Multiple ubiquitin ligase-mediated processes require COP9 signalosome and AXR1 function. *Plant Cell* 14, 2553–2563. doi: 10.1105/tpc.003434
- Sgubin, G., Swingedouw, D., Dayon, G., García de Cortázar-Atauri, I., Ollat, N., Pagé, C., et al. (2018). The risk of tardive frost damage in French vineyards in a changing climate. *Agric. For. Meteorol.* 250–251, 226–242. doi: 10.1016/j.agrformet.2017.12.253
- Shangguan, L., Fang, X., Jia, H., Chen, M., Zhang, K., and Fang, J. (2020). Characterization of DNA methylation variations during fruit development and ripening of *Vitis vinifera* (cv. 'Fujiminori'). *Physiol. Mol. Biol. Plants* 26, 617–637. doi: 10.1007/s12298-020-00759-5
- Shin, H., Oh, Y., and Kim, D. (2015). Differences in cold hardiness, carbohydrates, dehydrins and related gene expressions under an experimental deacclimation and reacclimation in *Prunus persica*. *Physiol. Plant.* 154, 485–499. doi: 10.1111/ppl.12293
- Stockinger, E. J., Gilmour, S. J., and Thomashow, M. F. (1997). *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc. Natl. Acad. Sci. U. S. A.* 94, 1035–1040. doi: 10.1073/pnas.94.3.1035
- Su, K., Xing, H., Guo, Y., Zhao, F., Liu, Z., Li, K., et al. (2020). High-density genetic linkage map construction and cane cold hardiness QTL mapping for Vitis based on restriction site-associated DNA sequencing. *BMC Genomics* 21:419. doi: 10.1186/s12864-020-06836-z
- Sun, X., Matus, J. T., Wong, D. C. J., Wang, Z., Chai, F., Zhang, L., et al. (2018). The GARP/MYB-related grape transcription factor AQUILLO improves cold tolerance and promotes the accumulation of raffinose family oligosaccharides. *J. Exp. Bot.* 69, 1749–1764. doi: 10.1093/jxb/ery020
- Thomashow, M. F. (2010). Molecular basis of plant cold acclimation: insights gained from studying the CBF cold response pathway. *Plant Physiol.* 154, 571–577. doi: 10.1104/pp.110.161794
- Tillett, R. L., Wheatley, M. D., Tattersall, E. A. R., Schlauch, K. A., Cramer, G. R., and Cushman, J. C. (2012). The *Vitis vinifera* C-repeat binding protein 4 (VvCBF4) transcriptional factor enhances freezing tolerance in wine grape. *Plant Biotechnol. J.* 10, 105–124. doi: 10.1111/j.1467-7652.2011.00648.x
- Tramontini, S., Vitali, M., Centioni, L., Schubert, A., and Lovisolo, C. (2013). Rootstock control of scion response to water stress in grapevine. *Environ. Exp. Bot.* 93, 20–26. doi: 10.1016/j.envexpbot.2013.04.001
- Trought, M. C. T., Howell, G. S., and Cherry, N. (1999). Practical Considerations for Reducing Frost Damage in Vineyards. *Report to New Zealand Winegrowers*.
- Unterberger, C., Brunner, L., Nabernegg, S., Steininger, K. W., Steiner, A. K., Stabentheiner, E., et al. (2018). Spring frost risk for regional apple production under a warmer climate. *PLoS One* 13:e0200201. doi: 10.1371/journal.pone.0200201
- van Houten, S., Muñoz, C., Bree, L., Bergamín, D., Sola, C., and Lijavetzky, D. (2020). Natural genetic variation for grapevine phenology as a tool for climate change adaptation. *Appl. Sci.* 10:5573. doi: 10.3390/app10165573
- van Leeuwen, C., and Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *J. Wine Econ.* 11, 150–167. doi: 10.1017/jwe.2015.21
- Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D. A., Cestaro, A., Pruss, D., et al. (2007). A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS One* 2:e1326. doi: 10.1371/journal.pone.0001326
- Vitasse, Y., Lenz, A., and Körner, C. (2014). The interaction between freezing tolerance and phenology in temperate deciduous trees. *Front. Plant Sci.* 5:541. doi: 10.3389/fpls.2014.00541
- Vitasse, Y., and Rebetez, M. (2018). Unprecedented risk of spring frost damage in Switzerland and Germany in 2017. *Clim. Chang.* 149, 233–246. doi: 10.1007/s10584-018-2234-y
- Vyse, K., Pagter, M., Zuther, E., and Hinch, D. K. (2019). Deacclimation after cold acclimation - a crucial, but widely neglected part of plant winter survival. *J. Exp. Bot.* 70, 4595–4604. doi: 10.1093/jxb/erz229
- Wang, H., and Dami, I. E. (2020). Evaluation of Budbreak-delaying products to avoid spring frost injury in grapevines. *Am. J. Enol. Vitic.* 71, 181–190. doi: 10.5344/ajev.2020.19074
- Warschefsky, E. J., Klein, L. L., Frank, M. H., Chitwood, D. H., Londo, J. P., von Wettberg, E. J. B., et al. (2016). Rootstocks: diversity, domestication, and impacts on shoot phenotypes. *Trends Plant Sci.* 21, 418–437. doi: 10.1016/j.tplants.2015.11.008
- Wisniewski, M., Nassuth, A., and Arora, R. (2018). Cold hardiness in trees: a mini-review. *Front. Plant Sci.* 9:1394. doi: 10.3389/fpls.2018.01394
- Wisniewski, M., Nassuth, A., Teulières, C., Marque, C., Rowland, J., Cao, P. B., et al. (2014). Genomics of cold hardiness in Woody plants. *CRC. Crit. Rev. Plant Sci.* 33, 92–124. doi: 10.1080/07352689.2014.870408
- Xiao, H., and Nassuth, A. (2006). Stress- and development-induced expression of spliced and unspliced transcripts from two highly similar dehydrin 1 genes in *V. riparia* and *V. vinifera*. *Plant Cell Rep.* 25, 968–977. doi: 10.1007/s00299-006-0151-4
- Xiao, H., Siddiqua, M., Braybrook, S., and Nassuth, A. (2006). Three grape CBF/DREB1 genes respond to low temperature, drought and abscisic acid. *Plant Cell Environ.* 29, 1410–1421. doi: 10.1111/j.1365-3040.2006.01524.x
- Xiao, H., Tattersall, E. A. R., Siddiqua, M. K., Cramer, G. R., and Nassuth, A. (2008). CBF4 is a unique member of the CBF transcription factor family of *Vitis vinifera* and *Vitis riparia*. *Plant Cell Environ.* 31, 1–10. doi: 10.1111/j.1365-3040.2007.01741.x
- Xu, W., Zhang, N., Jiao, Y., Li, R., Xiao, D., and Wang, Z. (2014). The grapevine basic helix-loop-helix (bHLH) transcription factor positively modulates CBF-pathway and confers tolerance to cold-stress in Arabidopsis. *Mol. Biol. Rep.* 41, 5329–5342. doi: 10.1007/s11033-014-3404-2
- Yang, Y., He, M., Zhu, Z., Li, S., Xu, Y., Zhang, C., et al. (2012). Identification of the dehydrin gene family from grapevine species and analysis of their responsiveness to various forms of abiotic and biotic stress. *BMC Plant Biol.* 12:140. doi: 10.1186/1471-2229-12-140
- Yang, Y., Mao, L., Jittayasothon, Y., Kang, Y., Jiao, C., Fei, Z., et al. (2015). Messenger RNA exchange between scions and rootstocks in grafted grapevines. *BMC Plant Biol.* 15:251. doi: 10.1186/s12870-015-0626-y
- Yordanov, Y. S., Ma, C., Strauss, S. H., and Busov, V. B. (2014). EARLY BUD-BREAK 1 (EBB1) is a regulator of release from seasonal dormancy in poplar trees. *Proc. Natl. Acad. Sci. U. S. A.* 111, 10001–10006. doi: 10.1073/pnas.1405621111
- Zhang, L., Marguerit, E., Rossdeutsch, L., Ollat, N., and Gambetta, G. A. (2016). The influence of grapevine rootstocks on scion growth and drought resistance. *Theor. Exp. Plant Physiol.* 28, 143–157. doi: 10.1007/s40626-016-0070-x
- Zhao, X., Han, X., Wang, Q., Wang, X., Chen, X., Li, L., et al. (2020). EARLY BUD BREAK 1 triggers bud break in peach trees by regulating hormone metabolism, the cell cycle, and cell wall modifications. *J. Exp. Bot.* 71, 3512–3523. doi: 10.1093/jxb/eraa119
- Zheng, C., Acheampong, A. K., Shi, Z., Mugzech, A., Halaly-Basha, T., Shaya, F., et al. (2018a). Abscisic acid catabolism enhances dormancy release of grapevine buds. *Plant Cell Environ.* 41, 2490–2503. doi: 10.1111/pce.13371
- Zheng, C., Halaly, T., Acheampong, A. K., Takebayashi, Y., Jikumaru, Y., Kamiya, Y., et al. (2015). Abscisic acid (ABA) regulates grape bud dormancy, and dormancy release stimuli may act through modification of ABA metabolism. *J. Exp. Bot.* 66, 1527–1542. doi: 10.1093/jxb/eru519

- Zheng, C., Kwame Acheampong, A., Shi, Z., Halaly, T., Kamiya, Y., Ophir, R., et al. (2018b). Distinct gibberellin functions during and after grapevine bud dormancy release. *J. Exp. Bot.* 69, 1635–1648. doi: 10.1093/jxb/ery022
- Zhu, Z., Shi, J., Xu, W., Li, H., He, M., Xu, Y., et al. (2013). Three ERF transcription factors from Chinese wild grapevine *Vitis pseudoreticulata* participate in different biotic and abiotic stress-responsive pathways. *J. Plant Physiol.* 170, 923–933. doi: 10.1016/j.jplph.2013.01.017
- Zombardo, A., Crosatti, C., Bagnaresi, P., Bassolino, L., Reshef, N., Puccioni, S., et al. (2020). Transcriptomic and biochemical investigations support the role of rootstock-scion interaction in grapevine berry quality. *BMC Genomics* 21:468. doi: 10.1186/s12864-020-06795-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 De Rosa, Vizzotto and Falchi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Accuracy of Interpolated Versus In-Vineyard Sensor Climate Data for Heat Accumulation Modelling of Phenology

Paula Pipan^{1,2*}, Andrew Hall³, Suzy Y. Rogiers² and Bruno P. Holzapfel²

¹ School of Agriculture and Wine Science, Charles Sturt University, Wagga Wagga, NSW, Australia, ² National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, NSW, Australia, ³ Institute for Land, Water and Society, Charles Sturt University, Albury, NSW, Australia

OPEN ACCESS

Edited by:

Tommaso Frioni,
Catholic University of the Sacred
Heart, Italy

Reviewed by:

Gabriele Cola,
University of Milan, Italy
Steven Schultze,
University of South Alabama,
United States

*Correspondence:

Paula Pipan
ppipan@gotafe.vic.edu.au

Specialty section:

This article was submitted to
Plant Science Crop and Product
Physiology,
a section of the journal
Frontiers in Plant Science

Received: 30 November 2020

Accepted: 16 June 2021

Published: 13 July 2021

Citation:

Pipan P, Hall A, Rogiers SY and
Holzapfel BP (2021) Accuracy
of Interpolated Versus In-Vineyard
Sensor Climate Data for Heat
Accumulation Modelling
of Phenology.
Front. Plant Sci. 12:635299.
doi: 10.3389/fpls.2021.635299

Background and Aims: In response to global heating, accurate climate data are required to calculate climatic indices for long-term decisions about vineyard management, vineyard site selection, varieties planted and to predict phenological development. The availability of spatially interpolated climate data has the potential to make viticultural climate analyses possible at specific sites without the expense and uncertainty of collecting climate data within vineyards. The aim of this study was to compare the accuracy and precision of climatic indices calculated using an on-site climate sensor and an interpolated climate dataset to assess whether the effect of spatial variability in climate at this fine spatial scale significantly affects phenological modelling outcomes.

Methods and Results: Four sites comprising two topographically homogenous vineyards and two topographically diverse vineyards in three wine regions in Victoria (Australia) were studied across four growing seasons. A freely available database of interpolated Australian climate data based on government climate station records (Scientific Information for Land Owners, SILO) provided temperature data for grid cells containing the sites (resolution 0.05° latitude by 0.05° longitude, approximately 5 km × 5 km). In-vineyard data loggers collected temperature data for the same time period. The results indicated that the only significant difference between the two climate data sources was the minimum temperatures in the topographically varied vineyards where night-time thermal layering is likely to occur.

Conclusion: The interpolated climate data closely matched the in-vineyard recorded maximum temperatures in all cases and minimum temperatures for the topographically homogeneous vineyards. However, minimum temperatures were not as accurately predicted by the interpolated data for the topographically complex sites. Therefore, this specific interpolated dataset was a reasonable substitute for in-vineyard collected data only for vineyard sites that are unlikely to experience night-time thermal layering.

Significance of the Study: Access to accurate climate data from a free interpolation service, such as SILO provides a valuable tool to manage blocks or sections within

vineyards more precisely for vineyards that do not have a weather station on site. Care, nevertheless, is required to account for minimum temperature discrepancies in topographically varied vineyards, due to the potential for cool air pooling at night, that may not be reflected in interpolated climate data.

Keywords: viticulture, climate change, phenology, climatic indices, climate data

INTRODUCTION

The concept of “terroir” has long been applied to wine regions. It is a French word, which can be defined as “an elusive combination of the effects of sun, soil, weather and history” Deloire (2008). In Australia, there is a rapidly growing movement and consumer demand for regionally authentic and recognisable wines. Quantifying the components that contribute to the distinct, recognizable flavour profile of a wine from a specific region or indeed from a specific vineyard is important. This quantification will aid in understanding how, if possible, to mitigate the effects of global heating in order to sustain that distinct, recognisable and marketable flavour profile. Of all the aspects of terroir contributing to wine flavour, climate has been found to have the greatest effect (Webb et al., 2008; Bonada et al., 2015; Pons et al., 2017; Geffroy et al., 2019), because the stages of grapevine growth (phenology) are driven by climate, or more specifically by temperature (Gladstones, 1992, 2011; Jones and Davis, 2000).

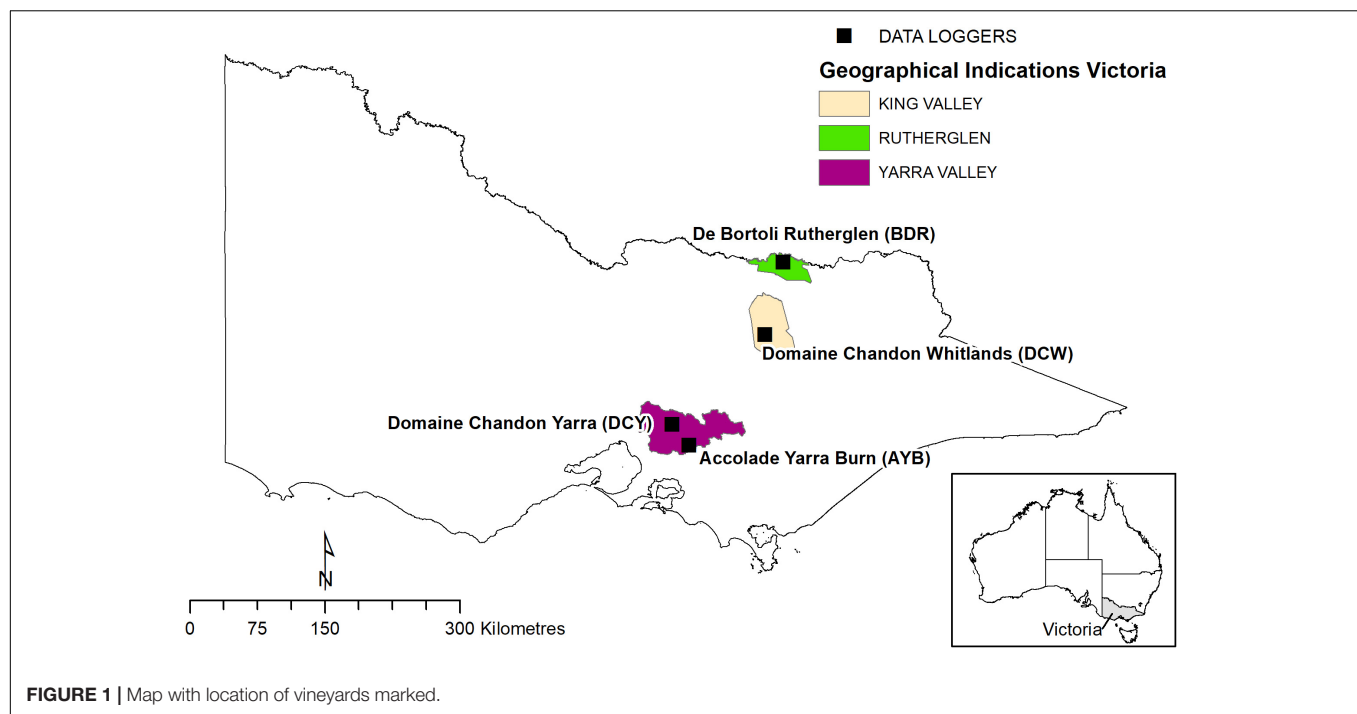
Climate is driven by the amount of solar radiation (insolation) received by a surface (Oke, 2002), hence the latitude, altitude, slope, and aspect of a vineyard site will influence the insolation and therefore the climate it experiences (Jacquet and Morlat, 1997; Gladstones, 2011; Neethling et al., 2019). The amount of insolation that reaches a surface depends on the angle (slope) of that surface (Jones, 2007). Slope and aspect are interconnected. The aspect of a slope will determine how much insolation it receives so the aspect that most directly faces the sun, receives the most insolation (Oke, 2002; Jones, 2007). Water availability, insolation, and temperature are the main drivers of photosynthesis in the grapevine which controls the production of carbohydrates and the phenological stages after budbreak (Medrano et al., 2003; Holzapfel and Smith, 2012). They also influence soil temperature, which mediates post-harvest carbohydrate accumulation (Holzapfel and Smith, 2012; Hall et al., 2016) and enhanced vegetative and reproductive growth (Field et al., 2009; Rogiers et al., 2011, 2014; Clarke et al., 2015). Heat accumulation over time determines phenological stages. In vineyards worldwide, the advancement of phenological stages has been observed due to climate change (Caffarra and Eccel, 2011; Bonnefoy et al., 2013; Malheiro et al., 2013; Cola et al., 2017; Jarvis et al., 2017; Alikadic et al., 2019).

The categorisation of the climate of a vineyard site, referred to as a mesoclimate (Coombe and Dry, 1988) or a wine region, referred to as a macroclimate (Coombe and Dry, 1988) uses a number of climatic indices. Climatic indices combine daily temperature data to produce a single index figure, which can then be categorised. These categories have been developed for grape growers to determine the suitability of a site for the

growth habits and phenological development of a particular grape variety. The mapping at a macroclimate scale of climatic indices of wine growing regions has been undertaken in numerous studies around the world (Tonietto and Carbonneau, 2004; Jones et al., 2009; Hall and Jones, 2010; Irimi et al., 2014; Remenyi et al., 2019). The categorisation of viticultural regions enables the identification of climate analogues, i.e., identification of locations whose historical climate is similar to the anticipated future climate at a reference location (Grenier et al., 2013). Climate analogues have been identified by Australian grape growers as being useful when making long-term vineyard management decisions (6 to 10 years) (Dunn et al., 2015).

Temporal variation in climate or climate variability is often used to denote deviations of climatic statistics over a given period of time (e.g., a month, season or year) when compared to long-term statistics for the same calendar period. The World Meteorological Organisation (2019) defines it as variations in the mean state and other statistics of the climate on all temporal and spatial scales, beyond individual weather events. Care is required when comparing seasonal climate data to climatic index categories, as season to season climate variability can be quite extensive (Hall and Blackman, 2019).

However, spatial variation within a vineyard has been identified as being directly related to the flavour profile of wines (Marais et al., 2001; Bramley et al., 2011; Scarlett et al., 2014). Vineyards on steep sites experience thermal layering at night, as by day the earth's surface is heated by the sun so there is thermal mixing by convection as an upward transfer of heat from the warmed surface to the cooler atmosphere occurs. By night, when the earth's surface cools rapidly, heat is transferred downward which suppresses mixing and the formation of cold layers near the surface is observed (Oke, 2002). Therefore, climate data at a macroclimate scale are unlikely to provide accurate climatic indices at the fine spatial scale of a specific vineyard site, particularly if it is topographically varied. Hence, for long-term decisions about vineyard management, varieties to be planted, change of training system, row orientation, vineyard sites and to predict phenological development, accurate climate data at a mesoclimate scale are required to calculate climatic indices. Interpolated climate databases are available worldwide that provided climate data at the mesoclimate scale (Hijmans et al., 2005; Spittlehouse, 2006; Mbogga et al., 2010; Moreno and Hasenauer, 2016; Wang et al., 2016; Fick and Hijmans, 2017). This study investigated two sources of climate data in Australia and compared their capacity to categorise climatic indices in vineyards with both homogenous topography (open, flat plain) and diverse topography (at elevation with multiple angles of slopes and aspects). Vineyards, or other agricultural enterprises in other parts of the world



could verify results in their location with local interpolated climate databases.

MATERIALS AND METHODS

Locations and Vineyards

Four vineyard sites were selected for this study in three Victorian (Australia) wine regions, varying in topographic complexity (Figures 1, 2).

Two vineyards were classified as topographically diverse (TD):

1. Accolade Yarra Burn (AYB) Beenak Road Vineyard, Hoddles Creek (Yarra Valley GI), which is a steep hilly site with a top elevation of 446 m. Coordinates -37.887S, 145.601E
2. Domaine Chandon (DCW), Mansfield-Whitfield Road, Whitlands Vineyard (King Valley GI), which is an undulating site with a top elevation of 790 m. Coordinates -36.781S, 146.360E

Another two vineyards were classified as topographically homogenous (TH):

3. Domaine Chandon (DCY), Maroondah Hwy Vineyard, Coldstream (Yarra Valley GI) which is a relatively even, flat valley site at an elevation of 150 m. Coordinates -37.677S, 145.431E
4. De Bortoli (DBR) (formerly Rutherglen Estates), Great Northern Road vineyard, Rutherglen (Rutherglen GI) which is a relatively even, flat wide valley site at an elevation of 150 m. Coordinates -36.055S, 146.540E

The cultivars grown in the case study vineyards are those most suited to the climate of that vineyard. Hence, the data logger placement was in Chardonnay blocks for three of the vineyards: AYB, DCW, and DCY. The DBR vineyard is a much warmer site, where no Chardonnay is grown, so the data logger was in a Shiraz block. Since the study is mainly concerned with within vineyard differences caused by varying data sources, it is unlikely the different variety at DBR will significantly impact the overall results or conclusions around spatial variability in climate that can be drawn from the study.

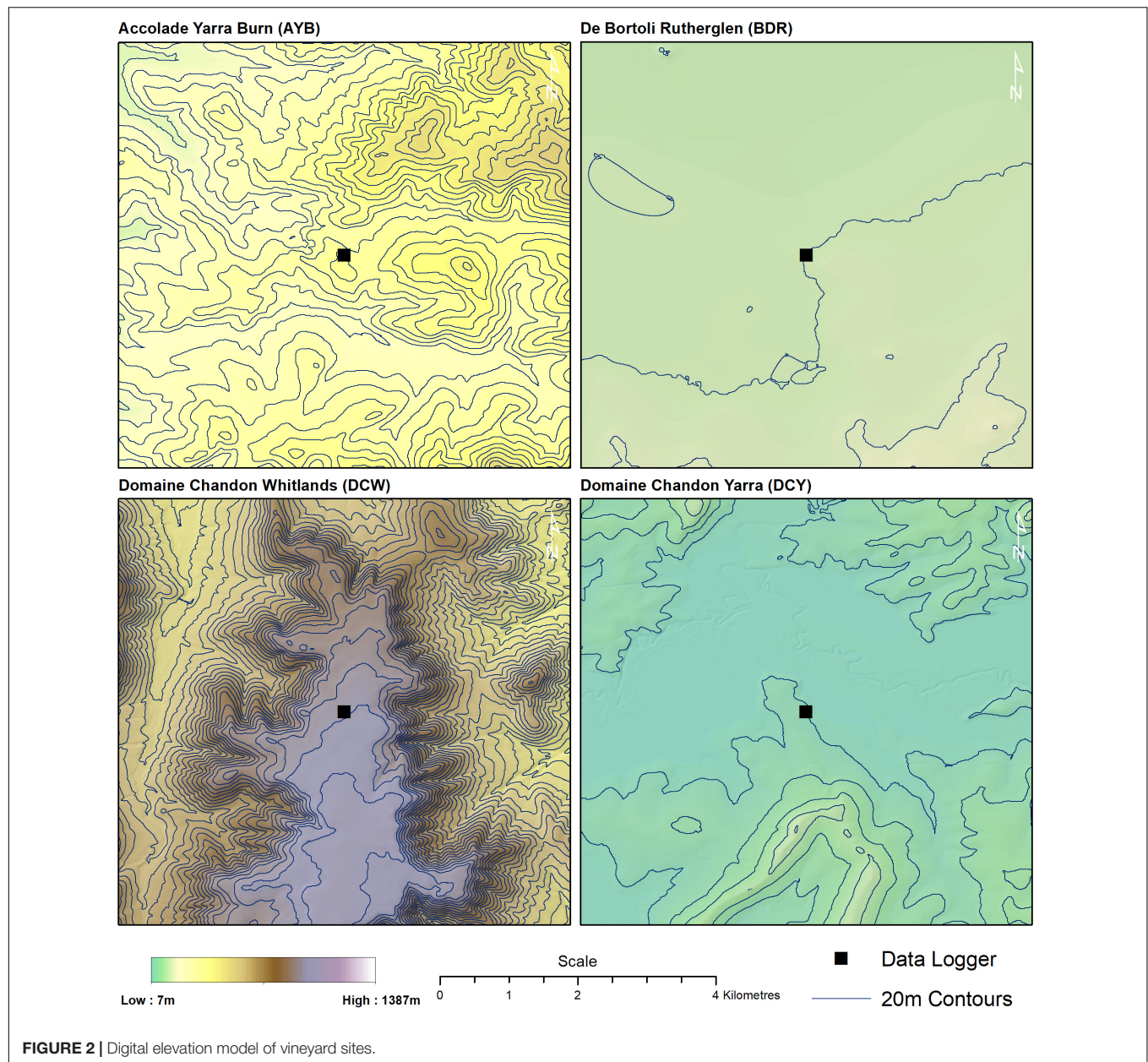
All sites are irrigated, so it was assumed that vine water status was optimally maintained, minimising soil effects on vine water status and vine growth.

Data Loggers

A Tinytag TGP-4500 data logger (TT) from Gemini Data Loggers Ltd (Chichester, West Sussex, United Kingdom) (calibrated by manufacturer) housed in a Stevenson screen was attached to the tops of trellising posts in the middle of each of the four vineyards recording temperature, humidity and dewpoint every 30 min for four consecutive growing seasons of 2015-16, 2016-17, 2017-18, and 2018-19.

Interpolated Climate Data Source

Scientific Information for Landowners (SILO) climatic data corresponding to the above four vineyard sites for the four growing seasons of the study was downloaded. Scientific Information for Landowners uses temperature data from 4600 Australian Bureau of Meteorology (BOM) weather stations and applies smoothing splines to generate interpolated surfaces on a regular 0.05° grid (approximately $5 \text{ km} \times 5 \text{ km}$) of Australia (Jeffrey et al., 2001) with latitude, longitude and elevation as



independent variables. SILO data is freely available and easily accessible to grape growers from <https://www.longpaddock.qld.gov.au/silo/>. It is acknowledged that the $5 \text{ km} \times 5 \text{ km}$ pixel may present issues in adequately accounting for the spatial variation within the pixel, in particular at the higher altitude and topographically varied vineyard sites at AYB and DCW. Scientific Information for Landowners data contains measurements for many climatic indicators, but for this project, maximum and minimum air temperature SILO data were used.

Climate Summaries

The BOM climate summaries archive for the state of Victoria gave the following descriptions of the four growing seasons monitored in this study (**Table 1**):

Climate Data Analysis

The research produced 16 sets of minimum and maximum temperature data (four vineyards, four growing seasons) from two sources: SILO and in-vineyard Tinytag (TT) data logger. These data were analysed, and climatic indices were calculated using Excel 2016 and RStudio1.3 software as described below.

1. The growing season average minimum (GSminTave) and maximum (GSmaxTave) temperatures for the seven months from October to April from each study year at each site were calculated from the in-vineyard TTdata logger and from SILO data.
2. The growing season average minimum and maximum temperatures recorded by the in-vineyard TT data

logger and interpolated by SILO from each study year at each site was analysed with Student's *t*-test to determine if there was a statistically significant difference ($P \leq 0.05$) between them.

3. Daily minimum and maximum temperatures for each site for each season from both in-vineyard TT data and SILO data were then used to calculate the climatic indices as listed below.
 - a. Average growing season temperatures (GSTavg): the mean air temperature of all days between October 1 and April 30 (Jones, 2006), which were categorised according to Jones (2006).
 - b. Growing degree days (GDD): the summation of daily average air temperature above 10°C during the 7 month growing season from October to April (Amerine and Winkler, 1944) were calculated using the following formula:

$$\Sigma \text{GDD}_{10} = \max[(T_{\max} + T_{\min})/2 - 10, 0]$$

These results were categorised into the Winkler Index for the classification of wine growing regions.

- c. Heliothermal index of Huglin (HI). The summation of daily average air temperature above 10°C during the six months of the growing season from October 1 to March 31 in the southern hemisphere, incorporating a length of day coefficient with the addition of a latitude correction factor, *K* (Huglin, 1978).

$$\text{HI} = \sum_{d=1}^n \max((T_{\text{mean}} - 10 + T_{\text{max}} - 10) / 2, 0) K$$

The in-vineyard derived and SILO derived HI were compared and classified according to Huglin (1978).

- d. Mean January Temperature (MJT) is the mean temperature of the warmest month (January in the southern hemisphere), classified according to Smart and Dry (1980). Mean January Temperature is well correlated with GDD. The in-vineyard TT derived and SILO derived MJT were compared and classified according to Smart and Dry (1980).

Phenology

Two grapevine phenological stages were recorded for all four vineyards in each of the four growing seasons of the study.

1. Budbreak: was defined as the date when 50% of vines reached stage four of the modified EL system (Coombe, 1995), when green leaf tips are visible on buds. Daily vineyard observations by vineyard staff determined this stage. It is acknowledged that these observations by different staff at the four sites may vary and as such are a potential source of error. The budbreak dates were compared to regional average phenological dates determined by Hall et al. (2016). The regional predicted dates are based on three scenarios: a 1975–2004 base period using climate records, and two projected climate scenarios described in terms of the mean temperature anomalies (MTA) from the base period, i.e., +1.26 and +2.61°C. Mean temperature anomalies are the spatially average temperature increase across Australia for specific future scenarios from a selected global climate model (Hall et al., 2016).
2. Maturity: this is usually defined as modified EL stage 38 (Coombe, 1995). For this study, harvest dates were used to determine maturity. It is acknowledged that the use of harvest dates to determine maturity is a potential source of error. The harvest dates were compared to regional average phenological dates determined by Hall et al. (2016). The regional predicted dates are based on the three scenarios as described for budbreak (above).

The calculated climatic indices provided information on heat accumulation which drives the phenological stages of budburst and maturity. Comparison of the climatic indices determined whether those calculated from the SILO interpolated data matched those from the in-vineyard TT data logger. The phenological stages of budburst and maturity were compared to regional average phenological dates to determine whether they were within the base period ranges or within either of the two projected climate change scenarios. In the currently warmest wine grape-producing regions, the warming trend will likely lead to the ripening period taking place earlier, in a warmer

TABLE 1 | Victorian climate descriptions for the four seasons of the study from the Bureau of Meteorology.

	Spring (Sept–Nov)	Summer (Dec–Feb)	Autumn (Mar–May)
2015–16	Rainfall 47% below average of 181 mm Mean temp 2.05 °C above long-term average	Rainfall near average of 120 mm Mean temp +1.73 °C above long-term average	Rainfall near average of 156.8 mm Mean temp 1.88 °C above long-term average, highest on record
2016–17	Rainfall 42% above average of 181 mm Mean temp 0.10 °C below long-term average	Rainfall 7% below average of 120 mm Mean temp 0.87 °C above long-term average	Rainfall near average of 156.8 mm Mean temp 1.08 °C above long-term average, 4th warmest autumn on record
2017–18	Rainfall slightly below average of 181 mm Mean temp 1.64 °C above long-term average	Rainfall 6 % above average of 120 mm Mean temp above average in top 10% of all summers on record.	Rainfall 39.2% below average of 156.8 mm Mean temp 1.17 °C above long-term average
2018–19	Rainfall 42.7% below average of 181 mm Mean temp 0.86 °C above long-term average	Rainfall 12% below average of 120 mm Mean temp 2.54 °C above average, highest on record	Rainfall 21% below average of 156.8 mm Mean temp 1.04 °C above long-term average

part of summer, which, in addition to the general pattern of warming, can greatly accelerate ripening leading to a potential loss of fruit quality and wine value. Jones (2015) refers to the balance of the four ripeness clocks of sugar accumulation, acid respiration, phenolic ripeness, and fruit character being disrupted by this warming pattern. This has been seen in other studies (Boss et al., 2014; Gaiotti et al., 2018; Geffroy et al., 2019).

RESULTS

Seasonal Temperatures

During the four growing seasons of the study in the two topographically diverse (TD) vineyards, the average growing season minimum temperature (GSminTave) at Accolade Yarra Burn (AYB) were 0.7 to 0.9°C lower for the in-vineyard TT data logger results than for SILO results but were 0.7 to 2.3°C higher at Domaine Chandon Whitlands (DCW) (**Figure 3**). The GSminTave in the both of the topographically homogenous vineyards (TH) at Domaine Chandon Yarra (DCY) and De Bortoli Rutherglen (DBR) showed no consistent trend between the in-vineyard TT data logger results and the SILO results (**Figure 3**).

For the four seasons of the study, the maximum (GSmaxTave) temperatures for the in-vineyard TT data logger results were 0 to 1.8°C higher than the SILO results for both the TH vineyards at DCY and DBR and the TD vineyard at AYB. However, they were 0 to 1.0°C lower at the TD vineyard at DCW (**Figure 3**).

GSminTave in the TD vineyards of AYB and DCW showed significant differences ($P \leq 0.05$) using Student's *t*-test between the in-vineyard measured and SILO interpolated data (**Table 2**). For the TH vineyards, there were no statistically significant differences in minimum temperatures except at DCY in the hot 2017–18 growing season.

The Student's *t*-test results for the average growing season maximum temperatures only showed significant differences ($P \leq 0.05$) between the in-vineyard data loggers and SILO data in the TH DBR vineyard in the two warmer seasons of 2015–16 and 2017–18, when in-vineyard temperatures showed consistently higher values (**Table 2**).

Climatic Indices

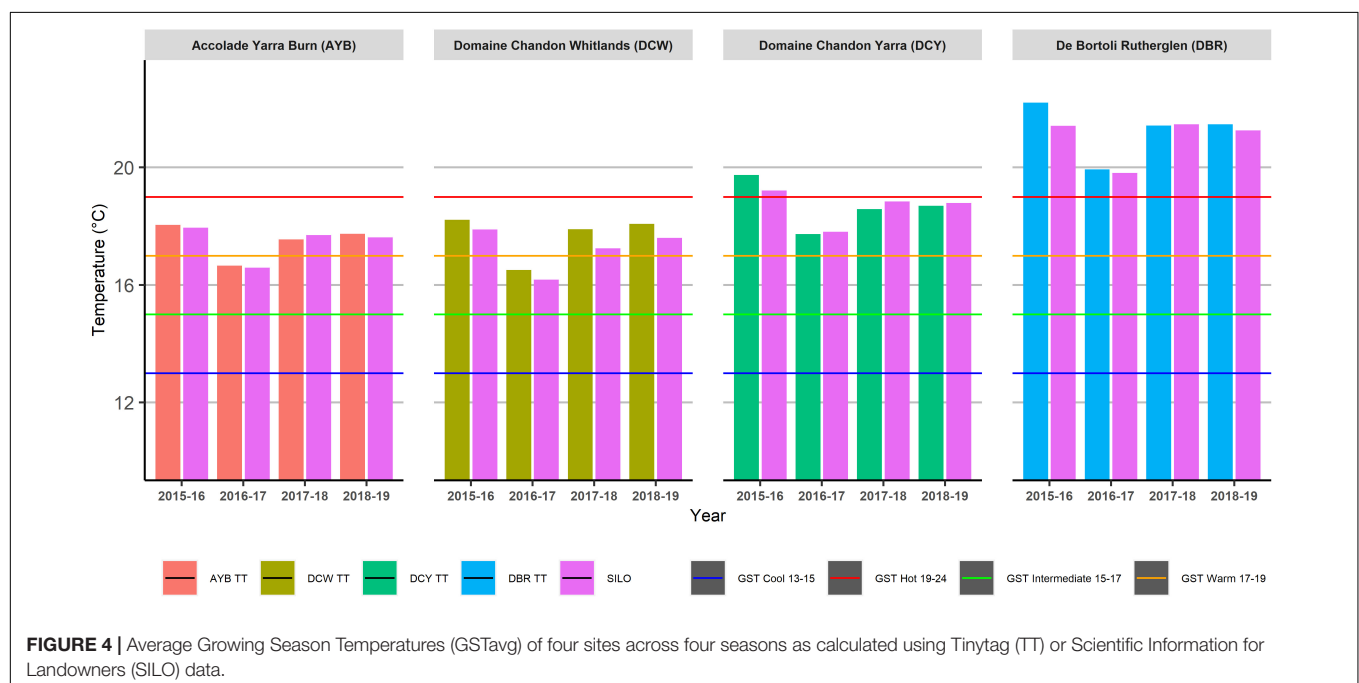
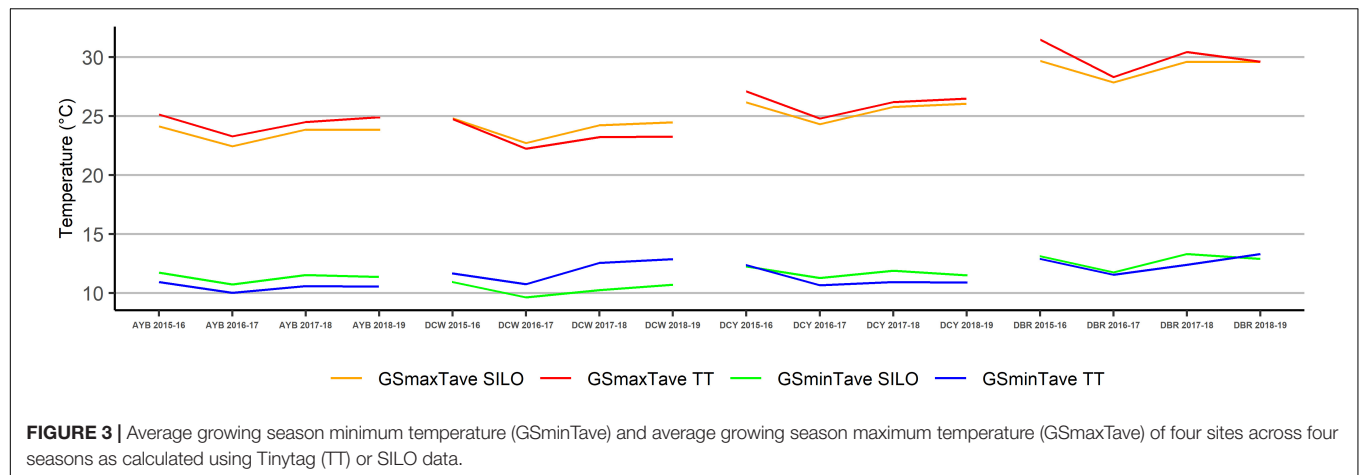
All sites for the four seasons of the study were categorised in the same viticultural classification for average growing season temperature (GSTavg) (**Figure 4**) for both the in-vineyard TT data and the SILO data. Despite the topographically diverse vineyards having significant differences in average minimum temperatures, this did not influence the GSTavg classifications.

However, the classifications showed temporal variations. In the cool growing season (2016–17), in both of the TD vineyards at AYB and DCW the GSTavg classification was “Intermediate” but was “Warm” in the other three growing seasons. For the TH vineyard at DCY, GSTavg classification in the hot growing season (2015–16) was “Hot” but was “Warm” in the other three

TABLE 2 | Comparing average growing season minimum and maximum temperature data sets (Π , SILO) for four sites and four growing seasons.

	AYB	AYB	AYB	AYB	DCW	DCW	DCW	DCW	DCY	DCY	DCY	DCY	DCY	DBR	DBR	DBR	DBR
	2015-16	2016-17	2017-18	2018-19	2015-16	2016-17	2017-18	2018-19	2015-16	2016-17	2017-18	2018-19	2015-16	2016-17	2017-18	2018-19	
°C diff min temp SILO vs. TT	0.80	0.71	0.93	0.85	-0.79	-1.14	-2.32	-2.16	-0.10	0.63	0.95	0.58	0.22	0.20	-0.09	-0.33	
Student's Statistic	-2.35	-1.94	-2.58	-2.04	1.97	2.55	5.47	4.42	0.26	-1.61	-2.37	-1.31	-0.48	-0.41	0.17	0.54	
P(T = t) two-tail mean min T	0.02	0.05	0.01	0.04	0.05	0.01	0.00	0.00	0.80	0.11	0.02	0.19	0.63	0.68	0.86	0.59	
°C diff max temp SILO vs. TT	-0.99	-0.85	-0.63	-1.07	0.05	0.48	0.99	1.17	-0.95	-0.47	-0.43	-0.42	-1.81	-0.45	-1.07	-0.28	
Student's Statistic	1.81	1.44	1.15	1.52	-0.10	-0.81	-1.97	-1.91	1.64	0.79	0.80	0.70	3.66	0.70	2.01	0.46	
P(T = t) two-tail mean max T	0.07	0.15	0.25	0.13	0.92	0.42	0.05	0.06	0.10	0.43	0.42	0.49	0.00	0.48	0.04	0.65	

Yellow indicates statistically significant difference; green indicates no statistically significant difference.



growing seasons. De Bortoli Rutherglen (DBR) remained in the “Hot” classification across all four growing seasons.

In the TD vineyard at AYB, the GDD classification based on Winkler (**Figure 5**) remained the same within growing seasons for both in-vineyard TT and SILO data. However, it was a Region III classification in the hot growing season (2015–16) and Region II in the other three seasons of the study.

The TD DCW vineyard had a higher GDD classification (Region II) in the cool season (2016–17) for in-vineyard TT data compared to SILO data (Region Ib) and also for the 2017–18 and 2018–19 seasons where in-vineyard TT data gave a classification of Region III compared to Region II with SILO data. The hot growing season (2015–16) showed classification of Region III for both data sets.

The TH DCY vineyard showed consistent GDD classifications between in-vineyard TT data logger and SILO data within

the same season, but changed from Region IV in the hot 2015–16 season, to Region II in the cool season (2016–17) and to Region III in the intermediate seasons (2017–18 and 2018–19).

The TH DBR vineyard classifications were consistent between in-vineyard TT and SILO data within the same season; however, the classification changed from Region IV in the cooler season (2016–17) to Region V in the other three seasons.

The TH vineyards at DCY and DBR showed consistent classifications within the same season for Heliothermal index of Huglin (HI) for both data collection methods, except for the hotter 2015–16 season at DBR where TT data gave a “Very Warm” classification and SILO gave a “Warm” classification (**Figure 6**). Huglin classification remained consistent in both TH vineyards for all seasons, except the hotter growing season (2015–16).



FIGURE 5 | Growing Degree Days (GDD) for four sites across four growing seasons as calculated using Tinytag (TT) or Scientific Information for Landowners (SILO) data.



FIGURE 6 | Heliothermal index of Huglin (HI) across four sites and four seasons as calculated using Tinytag (TT) or Scientific Information for Landowners (SILO) data.

The HI classifications in the TD vineyards at AYB and DCY remained the same for both sets of data in the cooler season (2016–17) and the intermediate growing season (2017–18). However, in the other seasons (2015–16 and 2018–19), the SILO data gave a cooler HI classification than the TT data at AYB, and a warmer HI classification in 2018–19 at DCY.

Mean January Temperature (MJT) results were consistent between in-vineyard TT data and SILO data within the

same season for all sites across all four growing seasons, although all sites recorded their highest MJT in the 2018–19 season (Figure 7).

Phenology

Topographically diverse (TD) AYB vineyard had consistent, early budbreak dates in the second week of September, across the four growing seasons (Table 3), with a range of five days between the earliest and latest budbreak dates. The highest elevation

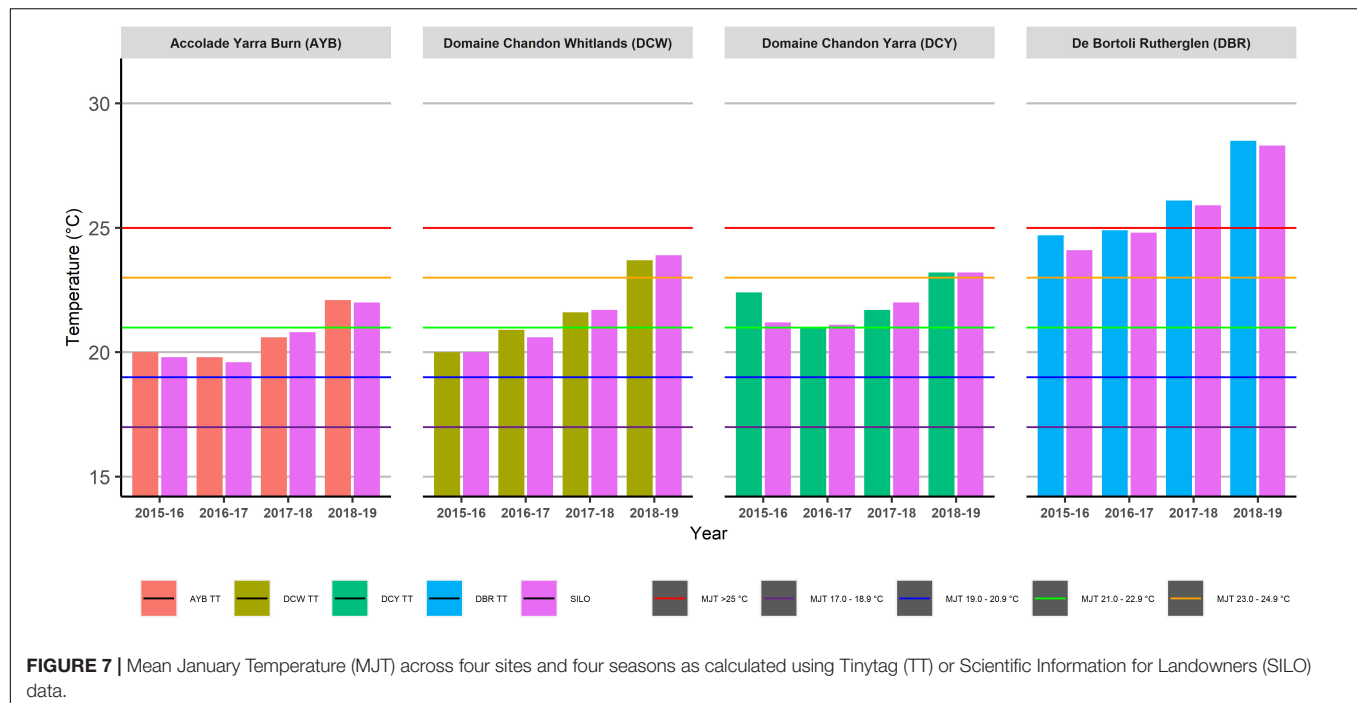


TABLE 3 | Budbreak and maturity (harvest) dates in number of days from July 1 and number of days between both stages. Ch = Chardonnay, Sh = Shiraz.

Vineyard	Budbreak (No. of days from July 1)	Harvest date (No. of days from July 1)	No. of days between budbreak and harvest
AYB 2015–16 (Ch)	71	240	169
AYB 2016–17 (Ch)	71	251	180
AYB 2017–18 (Ch)	76	257	181
AYB 2018–19 (Ch)	76	240	164
DCW 2015–16 (Ch)	101	245	143
DCW 2016–17 (Ch)	113	258	144
DCW 2017–18 (Ch)	106	249	142
DCW 2018–19 (Ch)	98	245	147
DCY 2015–16 (Ch)	79	227	148
DCY 2016–17 (Ch)	68	242	174
DCY 2017–18 (Ch)	81	228	147
DCY 2018–19 (Ch)	72	237	165
DBR 2015–16 (Sh)	83	230	147
DBR 2016–17 (Sh)	74	247	173
DBR 2017–18 (Sh)	98	246	148
DBR 2018–19 (Sh)	91	241	150

vineyard at DCW (790 m) recorded late budbreak dates in early to mid-October each year, with a fair degree of variation between the earliest and latest budbreak dates (15-day range over the four seasons). The TH vineyards at DCY and DBR showed a large variation in budbreak dates from early September to early October between growing seasons (13 days and 24 days, respectively), with the earliest budbreak at both vineyards occurring in the cooler (as calculated by heat accumulation) 2016–17 season.

Accolade Yarra Burn (AYB) had a range of 17 days between the earliest and latest harvest dates over the four seasons; DCW had a range of 14 days; DCY 15 days, and DBR 17 days (Table 3).

The number of days between budbreak and maturity/harvest date (Table 3) for the two TH vineyards at DCY and DBR showed a marked increase in the cooler 2016–17 season, but no clear pattern was observed in the TD vineyards at AYB and DCW. The highest elevation and cooler vineyard at DCW had a consistent length of vintage, with only a five-day difference in number of days between budbreak and harvest across the four seasons. Topographically diverse vineyard AYB had a 16-day difference between its longest and shortest vintage, and the TH vineyards at DCY and DBR had large differences in vintage length at 27 and 26 days, respectively.

Budbreak dates were compared to the regional predicted dates after Hall et al. (2016) (Table 4). Both the TH and TD vineyards in the Yarra Valley (DCY and AYB, respectively) had budbreak dates between the minimum and maximum values of the MTA 1.26 scenario. The TD and highest elevation vineyard at DCW was within the minimum and maximum modelled dates without an MTA scenario applied. The TH DBR vineyard had the greatest range of budburst, coinciding with the MTA 1.26 scenario in the 2018–19 season, and the MTA 2.61 scenario in the 2016–17 season.

Harvest dates were compared to the regional predicted dates after Hall et al. (2016) (Table 5). The TD vineyard in the Yarra Valley, AYB had harvest dates coincident with the MTA 1.26 scenario. Harvest dates in the TH Yarra vineyard, DCY coincided with both the MTA 1.26 and the MTA 2.61 scenarios. The TD and highest elevation vineyard at DCW was within the maturity dates determined for the 1974–2005 base period.

TABLE 4 | Modelled budbreak dates (number of days from July 1) using a 1975–2004 base period and projected mean temperature anomalies (MTA) of 1.26 and 2.61°C (after Hall et al., 2016).

Region	Min	Q1	Median	Q3	Max
Yarra	75	82	86	89	93
Yarra MTA1.26	66	73	76	79	82
King Valley	93	94	98	105	113
Rutherglen	90	93	96	98	114
Rutherglen MTA1.26	80	83	85	88	100
Rutherglen MTA2.61	71	73	75	77	88

TABLE 5 | Modelled maturity dates (number of days from July 1) for base period 1975–2004 and projected mean temperature anomalies (MTA) of 1.26 and 2.61°C (after Hall et al., 2016).

Region	Min	Q1	Median	Q3	Max
Yarra	259	277	284	297	329
Yarra MTA1.26	237	249	252	258	265
Yarra MTA2.61	220	230	232	236	240
King Valley	242	246	254	272	305
Rutherglen	233	237	242	247	281
Rutherglen 1.26	219	223	227	230	250
Rutherglen 2.61	205	209	212	216	231

The TH DBR vineyard had harvest dates coincident with the MTA 1.26 scenario.

DISCUSSION

Scientific Information for Landowners (SILO) interpolation generates climate maps for Australia by applying smoothing splines to data at weather station locations on a 0.05° grid (Jeffrey et al., 2001). Interpolations at this scale are unable to represent fine spatial variability in climate for topographically complex sites at the within vineyard management unit scale.

Temperatures

The main significant differences that were found when comparing the data from the two sources were in the minimum temperatures in the topographically diverse vineyards at AYB and DCW. This would be consistent with the thermal layering that would occur at night in these vineyards (Oke, 2002) which would make interpolations of minimum temperatures more difficult on the hilly sites where there would be numerous layers at different temperatures. Due to daytime thermal mixing, maximum daily temperatures would be vertically homogenous and therefore more accurately interpolated by SILO in both topographically homogenous and topographically diverse areas. This was found to be the case (Table 2) with maximum daily temperatures not differing significantly from each other. Of note was the direction of the difference in minimum temperatures between TT and SILO data. Scientific Information for Landowners overestimated the minimum temperatures at AYB and underestimated the minimum temperatures at DCW. Modelling at this scale uses a broad environmental lapse rate (a rate of temperature change

with respect to elevation) and cool air pooling cannot be represented at the within-vineyard scale. Accolade Yarra Burn was topographically the most complex site, as can be seen in Figure 2 compared to the high plateau of DCW. Cool air pooling and multiple thermal layers were more likely at AYB, resulting in observed minimum temperatures being lower than those interpolated by SILO. At the undulating high plateau at DBW, the potential exists for warmer than interpolated minimum temperatures due to flatter terrain retaining greater heat than sloping sites (Oke, 2002; Jones, 2007).

No correlation was found between the coolness of the day and the magnitude of the difference between the minimum temperatures recorded by the data logger and that interpolated by SILO (data not shown). The lack of correlation is probably due to weather factors that influence cool air pooling, such as clear atmospheric conditions when the ground becomes relatively cooler than the air temperature on that day (Oke, 2002, p. 180). This can happen at any level of minimum temperature, not just on cool days. Another weather factor that can affect cool air pooling is wind speed, with more geostrophic wind resulting in a mixed boundary layer, preventing cool air pooling at the surface. Day to day weather, therefore, is more likely to be a factor that determines the level of cool air pooling than simply an assessment of minimum temperatures.

Climatic Indices

Daily temperature data from both data sources were used to calculate heat accumulation climatic indices commonly employed in viticulture to make long-term vineyard management decisions. The two topographically diverse vineyards at AYB in the Yarra Valley and DCW in the King Valley were also the cooler sites in this study, based on their GSTavg (Figure 4), GDD (Figure 5), HI (Figure 6), and MJT (Figure 7). It had been expected that climatic indices calculated from SILO climate data would be more likely to closely match climatic indices calculated from data collected in-vineyard for TH vineyards (DCY and DBR) than TD vineyards at higher elevations (AYB and DCW). Furthermore, it was expected that the calculation of the climatic indices in the TD vineyards may have resulted in different classifications from the two sources of data (Figures 4–7) within the same season. In fact, the classifications did differ within the same season for GDD in the highest elevation and TD vineyard at DCW for three of the seasons studied and with the HI for both of the TD vineyards for two of the seasons. As expected, the climatic indices calculated from both sources at the topographically homogenous vineyards at DCY and DBR had consistent classifications of climatic indices within the same season, with the exception of HI at DBR in the highest heat accumulation season in 2015–16.

Temporal Variation

Temporal variation between growing seasons was greater than any spatial scale differences observed in the vineyards. As evidenced in other studies (Holzapfel and Smith, 2012; Hall and Blackman, 2019; Priori et al., 2019), the dominant effect of climate variability due to seasonal changes in weather patterns is not unusual. The greatest heat accumulation occurred in the 2015–16

season when all four sites recorded their highest GSTavg, GDD, and HI. The lowest heat accumulation occurred in the 2016–17 season while the other two seasons tracked slightly cooler than 2015–16. The MJT was warmest at all four sites in 2019. This is consistent with the BOM climate summaries (**Table 1**) where the warmest summer on record was in 2019. These temporal variations were also noted when the results were compared to average indices calculated regionally, over 30 years (Hall and Jones, 2010; Jarvis et al., 2017) (data not shown). Climatic indices GSTavg, GDD, and HI at the TH sites at DCY and DBR were higher than the regionally calculated indices for all seasons except the cooler 2016–17. In contrast the TD vineyards at ABY and DCW were within the Hall and Jones (2010) ranges compared to the single averages given by Jarvis et al. (2017). This would indicate that as noted by Jones (2006), warmer vineyard regions can expect the effects of global warming to be more significant than in cooler regions.

Phenology and Climate Change

Budbreak

It has been found that the actual bud temperature, rather than air temperature drives the timing of budbreak (Keller and Tarara, 2010). This would be related to the amount of insolation received by the plant, which is dependent on the latitude, altitude, slope and aspect of the vineyard site (Jacquet and Morlat, 1997; Gladstones, 2011; Neethling et al., 2019). There is the added complexity of differing budbreak heat sums for clones of the same variety (Ladányi et al., 2010), for different rootstocks (Jogaiah et al., 2013) and for viticultural practices such as late pruning (Silvestroni et al., 2018) which all influence the required heat accumulation for budbreak. It needs to be acknowledged that in an ideal situation all vineyard management practises including pruning dates for the four study vineyards would have been the same but considering these were commercial vineyards this was not possible. Aside from temperature, these inconsistent practises will likely have impacted to some extent on the results. For example, increasingly later pruning into early spring had been adopted at the TH vineyard at DBR to delay budbreak. The 2015–16 season was pruned in mid-August, the following three seasons were each pruned a week later than the year before (M. Partridge, vineyard manager, personal communication, February 18, 2020). However, delaying pruning had no consistent effects on date of budburst. This highlights the dominant effect of climate variability from season to season (Hall and Blackman, 2019) on heat accumulation, therefore affecting phenological development.

Maturity

In this study, the actual harvest dates were used in the comparison with predicted maturity dates of Hall et al. (2016). This is a potential source of error, as harvest date is determined by wine style, and not determined by EL stage 38 (Coombe, 1995). The three Chardonnay vineyards at ABY, DCW and DCY were harvested quite early for sparkling wine, which requires lower sugar ripeness (around 10.5 Baumé) and higher acid levels (Rankine, 2007) than traditional table wine. A regionally acquired prediction model based on table wine maturity may not be able to

fully account for the early picking for sparkling wine. Similarly, at DBR, the winemaker explained that although the sugar ripeness in the Shiraz may well have been reached earlier, the flavour and tannin ripeness may not have been achieved. He stated that they usually harvested at 14.2 to 14.5 Baumé in order to avoid “green” flavour and tannins (M. Scalzo, personal communication February 18, 2020). This is corroborated by Hall and Jones (2009) who note that the period from veraison to maturity is particularly important for the production of desirable wine grapes. A long period and optimum temperature enables the fruit to develop flavours that add value to a finished wine. Night temperatures in particular have been found to be an important determinant of wine composition (Mori et al., 2005; Cohen et al., 2008; Gaiotti et al., 2018). In some cooler regions worldwide, the shortening of the period between budbreak and maturity due to global warming has actually led to an improvement in grape quality (Van Leeuwen and Darriet, 2016; Koch and Oehl, 2018).

The seasonal effects on phenology in this study were considerable. Maturity dates were up to 28 days apart from year to year and the number of days between budbreak and maturity being 30 days longer in both TH vineyards at DCY and DBR in the cool season of 2016–17 compared to the warmer seasons in 2015–16 and 2017–18 (**Table 3**). This shortening of time between phenological stages in warmer seasons is consistent with other studies (Jones et al., 2005; Malheiro et al., 2013). This is also consistent with studies into the effect of increased soil temperature on post-harvest carbohydrate accumulation (Holzapfel and Smith, 2012; Hall et al., 2016) and enhanced vegetative and reproductive growth (Field et al., 2009; Rogiers et al., 2011, 2014; Clarke et al., 2015).

Over the four years of the study, all budbreak and harvest dates were already in the projected MTA 1.26 or 2.61 ranges of Hall et al. (2016) at all sites except the highest (790 m) vineyard at DCW. This is in contrast with the findings of Alikadic et al. (2019) that with climate change, advances in phenology were more pronounced at higher elevation. There is some evidence that vines have different phenological behaviour at higher elevation sites (Caffarra and Eccel, 2010) where lower average temperatures could lead to phenotypical adaptation of growth rates. However, comparing the timing of phenological events at a particular vineyard with regionally calculated dates requires some caution. It has been useful to make a brief comparison to projected future phenology for the regions, however, no firm conclusions about long-term trends in phenological stages can be drawn as four years of data does not provide enough statistical power to allow definite determinations.

CONCLUSION

The aim of this study was to compare climatic indices calculated from in-vineyard collected climate data (TT) and an interpolated climate dataset (specifically, SILO) for two spatially homogenous vineyards and two topographically diverse vineyards in three wine regions in Victoria over four growing seasons. The data retrieved from SILO for maximum temperatures generally correlated well with the data collected from the data loggers at all

sites. There was also good correlation for minimum temperatures in the spatially homogenous vineyards but not in the spatially diverse vineyards where night-time thermal layering is likely to occur. Night temperatures are a significant determinant of grape composition. Hence, from a practical point of view, the use of the SILO data for the calculation of climatic indices in spatially homogenous vineyards in order to plan vineyard management for future climate scenarios or to investigate climate analogues or to predict phenological phases, can be considered to have similar accuracies to within-vineyard collected climate data. However, caution would need to be exercised by spatially diverse vineyards where cool air pooling occurs at night.

Even at topographically complex sites, knowledge of local conditions would allow interpretation of SILO derived indices in order to gain climate information unique to the site terroir, which would be more useful than published regionally derived indices. Due to the readily accessible, downloadable nature of the SILO data, this will allow any vineyard, anywhere in Australia to calculate their own 30-year average climatic indices and track these annually, providing them with an excellent tool for long-term vineyard management decisions, albeit with some interpretation required at hilly sites. Similar interpolated climate

databases exist worldwide. Vineyards, or other agricultural enterprises, in other parts of the world could verify results in their location with the local interpolated climate database.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

PP and AH conceived and designed the analysis with support from BH and SR. PP collected the data, performed the analysis with support from AH, and wrote the manuscript with input from BH, SR, and AH. AH contributed analysis tools. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by the Charles Sturt University.

REFERENCES

- Alikadic, A., Pertot, I., Eccel, E., Dolci, C., Zarbo, C., Caffarra, A., et al. (2019). The impact of climate change on grapevine phenology and the influence of altitude: a regional study. *Agric. Forest Meteorol.* 271, 73–82. doi: 10.1016/j.agrformet.2019.02.030
- Amerine, M., and Winkler, A. (1944). Composition and quality of musts and wines of California grapes. *Hilgardia* 15, 493–675. doi: 10.3733/hilg.v15n06p493
- Bonada, M., Jeffery, D. W., Petrie, P. R., Moran, M. A., and Sadras, V. O. (2015). Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Austr. J. Grape Wine Res.* 21, 240–253. doi: 10.1111/ajgw.12142
- Bonnefoy, C., Quenol, H., Bonnardot, V., Barbeau, G., Madelin, M., Planchon, O., et al. (2013). Temporal and spatial analyses of temperature in a French wine-producing area: the Loire Valley. *Int. J. Climatol.* 33, 1849–1862. doi: 10.1002/joc.3552
- Boss, P. K., Bottcher, C., and Davies, C. (2014). Various influences of harvest date and fruit sugar content on different wine flavor and aroma compounds. *Am. J. Enol. Viticu.* 65, 341–353. doi: 10.5344/ajev.2014.13137
- Bramley, R. G. V., Ouzman, J., and Boss, P. K. (2011). Variation in vine vigour, grape yield and vineyard soils and topography as indicators of variation in the chemical composition of grapes, wine and wine sensory attributes. *Austr. J. Grape Wine Res.* 17, 217–229. doi: 10.1111/j.1755-0238.2011.00136.x
- Caffarra, A., and Eccel, E. (2010). Increasing the robustness of phenological models for *Vitis vinifera* cv. Chardonnay. *Int. J. Biometeorol.* 54, 255–267. doi: 10.1007/s00484-009-0277-5
- Caffarra, A., and Eccel, E. (2011). Projecting the impacts of climate change on the phenology of grapevine in a mountain area. *Austr. J. Grape Wine Res.* 17, 52–61. doi: 10.1111/j.1755-0238.2010.00118.x
- Clarke, S. J., Lamont, K., Pan, H., Barry, L., Hall, A., and Rogiers, S. Y. (2015). Spring root-zone temperature regulates root growth, nutrient uptake and shoot growth dynamics in grapevines. *Austr. J. Grape Wine Res.* 21, 479–489. doi: 10.1111/ajgw.12160
- Cohen, S. D., Tarara, J. M., and Kennedy, J. A. (2008). Assessing the impact of temperature on grape phenolic metabolism. *Anal. Chim. Acta* 621, 57–67. doi: 10.1016/j.aca.2007.11.029
- Cola, G., Failla, O., Maghradze, D., Megrelidze, L., and Mariani, L. (2017). Grapevine phenology and climate change in Georgia. *Int. J. Biometeorol.* 61, 761–773. doi: 10.1007/s00484-016-1241-9
- Coombe, B. (1995). Adoption of a system for identifying grapevine growth stages. *Austr. J. Grape Wine Res.* 1, 104–110. doi: 10.1111/j.1755-0238.1995.tb00086.x
- Coombe, B. G., and Dry, P. R. (1988). *Viticulture*. Australia: Australian Industrial Publishers.
- Deloire, A. (2008). Unravelling the terroir mystique—an agro-socio-economic perspective. *CAB Rev. Perspect. Agric. Veterinary Sci. Nutrit. Nat. Resour.* 3:18. doi: 10.1079/pavsnr.20083032
- Dunn, M. R., Lindesay, J. A., and Howden, M. (2015). Spatial and temporal scales of future climate information for climate change adaptation in viticulture: a case study of User needs in the Australian winegrape sector. *Austr. J. Grape Wine Res.* 21, 226–239. doi: 10.1111/ajgw.12138
- Fick, S. E., and Hijmans, R. J. (2017). WorldClim 2: new 1–km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* 37, 4302–4315. doi: 10.1002/joc.5086
- Field, S. K., Smith, J. P., Holzapfel, B. P., Hardie, W. J., and Emery, R. N. (2009). Grapevine response to soil temperature: xylem cytokinins and carbohydrate reserve mobilization from budbreak to anthesis. *Am. J. Enol. Viticult.* 60, 164–172.
- Gaiotti, F., Pastore, C., Filippetti, I., Lovat, L., Belfiore, N., and Tomasi, D. (2018). Low night temperature at veraison enhances the accumulation of anthocyanins in *Corvina* grapes (*Vitis vinifera* L.). *Sci. Rep.* 8:8719.
- Geffroy, O., Descôtes, J., Levasseur-Garcia, C., Debord, C., Denux, J.-P., and Dufourcq, T. (2019). A 2-year multisite study of viticultural and environmental factors affecting rotundone concentration in Duras red wine. *OENO One* 53, 589–592.
- Gladstones, J. (1992). *Viticulture and Environment*. Australia: Winetitles.
- Gladstones, J. (2011). *Wine, Terroir and Climate Change*. Australia: Wakefield Press.
- Grenier, P., Parent, A.-C., Huard, D., Anctil, F., and Chaumont, D. (2013). An assessment of six dissimilarity metrics for climate analogs. *J. Appl. Meteorol. Climatol.* 52, 733–752. doi: 10.1175/jamc-d-12-0170.1
- Hall, A., and Blackman, J. (2019). Modelling within-region spatiotemporal variability in grapevine phenology with high resolution temperature data. *OENO One* 53, 147–159.
- Hall, A., and Jones, G. V. (2009). Effect of potential atmospheric warming on temperature-based indices describing Australian winegrape growing conditions. *Austr. J. Grape Wine Res.* 15, 97–119. doi: 10.1111/j.1755-0238.2008.00035.x

- Hall, A., and Jones, G. V. (2010). Spatial analysis of climate in winegrape-growing regions in Australia. *Austr. J. Grape Wine Res.* 16, 389–404. doi: 10.1111/j.1755-0238.2010.00100.x
- Hall, A., Mathews, A. J., and Holzapfel, B. P. (2016). Potential effect of atmospheric warming on grapevine phenology and post-harvest heat accumulation across a range of climates. *Int. J. Biometeorol.* 60, 1405–1422. doi: 10.1007/s00484-016-1133-z
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., and Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol. J. R. Meteorol. Soc.* 25, 1965–1978. doi: 10.1002/joc.1276
- Holzapfel, B. P., and Smith, J. P. (2012). Developmental stage and climatic factors impact more on carbohydrate reserve dynamics of Shiraz than cultural practice. *Am. J. Enol. Viticult.* 63, 333–342. doi: 10.5344/ajev.2012.11071
- Huglin, P. (1978). Nouveau mode d'évaluation des possibilités héliothermiques d'un milieu viticole. *Comptes Rendus de l'Académie d'Agricu. de France* 64, 1117–1126.
- Irimi, L., Patriche, C. V., Quéno, H., Planchon, O., and Sfăcă, L. (2014). Characteristics of the baseline climate of the Cotnari (Romania) wine growing region. *Cercetari Agronom. Moldova* 47, 99–111. doi: 10.1515/cerce-2015-0008
- Jacquet, A., and Morlat, R. (1997). Characterization of the climatic variability in the loire valley vineyard. influence of landscape and physical characteristics of the environment. *Agronomie* 9, 465–480.
- Jarvis, C., Barlow, E., Darbyshire, R., Eckard, R., and Goodwin, I. (2017). Relationship between viticultural climatic indices and grape maturity in Australia. *Int. J. Biometeorol.* 61, 1849–1862. doi: 10.1007/s00484-017-1370-9
- Jeffrey, S. J., Carter, J. O., Moodie, K. B., and Beswick, A. R. (2001). Using spatial interpolation to construct a comprehensive archive of Australian climate data. *Environ. Mod. Softw.* 16, 309–330. doi: 10.1016/s1364-8152(01)00008-1
- Jogaiah, S., Oulkar, D., Banerjee, K., Sharma, J., Patil, A., Maske, S., et al. (2013). Biochemically induced variations during some phenological stages in thompson seedless grapevines grafted on different rootstocks. *South Afr. J. Enol. Viticult.* 34, 36–45.
- Jones, G. (2015). *Climate Grapes and Wine: Terroir and the Importance of Climate to Winegrape Production*. Petaluma, CA: GuildSomm.
- Jones, G., Duchene, E., Tomasi, D., Yuste, J., Braslavskaya, O., Schultz, H., et al. (2005). "Changes in European winegrape phenology and relationships with climate," in *Paper Presented at the XIV International GESCO Viticulture Congress*, (Geisenheim), 23–27.
- Jones, G., Moriondo, M., Bois, B., Hall, A., and Duff, A. (2009). Analysis of the spatial climate structure in viticulture regions worldwide. *Bull. de l'OIV* 82:507.
- Jones, G. V. (2006). Climate and terroir: impacts of climate variability and change on wine. *Fine Wine Terroir Geosci. Perspect.* 9, 1–14.
- Jones, G. V. (2007). Climate change: observations, projections, and general implications for viticulture and wine production. *Econom. Department Working Paper* 7:14.
- Jones, G. V., and Davis, R. E. (2000). Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. *Am. J. Enol. Viticult.* 51, 249–261.
- Keller, M., and Tarara, J. M. (2010). Warm spring temperatures induce persistent season-long changes in shoot development in grapevines. *Ann. Bot.* 106, 131–141. doi: 10.1093/aob/mcq091
- Koch, B., and Oehl, F. (2018). *Climate Change Favors Grapevine Production in Temperate Zones*. Germany: Scientific Research Publishing Irvine USA.
- Ladányi, M., Hlaszny, E., Pernes, G., and Bisztray, G. (2010). "Climate change impact study based on grapevine phenology modelling," in *Paper Presented at the VIIIth International Terroir Congress*.
- Malheiro, A. C., Campos, R., Fraga, H., Eiras-Dias, J., Silvestre, J., and Santos, J. A. (2013). Winegrape phenology and temperature relationships in the lisbon wine region, portugal. *OENO One* 47, 287–299. doi: 10.20870/oeno-one.2013.47.4.1558
- Marais, J., Calitz, F., and Haasbroek, P. (2001). Relationship between microclimatic data, aroma component concentrations and wine quality parameters in the prediction of Sauvignon Blanc wine quality. *South Afr. J. Enol. Viticult.* 22, 22–26.
- Mbogga, M., Hansen, C., Wang, W., and Hamann, A. (2010). *A Comprehensive Set of Interpolated Climate Data for Alberta*. Publication No. Ref. T/235. ISBN: 978-0-7785-9183-2.
- Medrano, H., Escalona, J. M., Cifre, J., Bota, J., and Flexas, J. (2003). A ten-year study on the physiology of two spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Functional Plant Biol.* 30, 607–619. doi: 10.1071/fp02110
- Moreno, A., and Hasenauer, H. (2016). Spatial downscaling of European climate data. *Int. J. Climatol.* 36, 1444–1458. doi: 10.1002/joc.4436
- Mori, K., Sugaya, S., and Gemma, H. (2005). Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Sci. Horticul.* 105, 319–330. doi: 10.1016/j.scienta.2005.01.032
- Neethling, E., Barbeau, G., Coulon-Leroy, C., and Quéno, H. (2019). Spatial complexity and temporal dynamics in viticulture: a review of climate-driven scales. *Agric. Forest Meteorol.* 276:107618. doi: 10.1016/j.agrformet.2019.107618
- Oke, T. R. (2002). *Boundary Layer Climates*. Milton Park: Routledge.
- Pons, A., Allamy, L., Schüttler, A., Rauhut, D., Thibon, C., and Darriet, P. (2017). What is the expected impact of climate change on wine aroma compounds and their precursors in grape? *OENO One* 51, 141–146. doi: 10.20870/oeno-one.2016.0.0.1868
- Priori, S., Pellegrini, S., Perria, R., Puccioni, S., Storch, P., Valboa, G., et al. (2019). Scale effect of terroir under three contrasting vintages in the Chianti Classico area (Tuscany, Italy). *Geoderma* 334, 99–112. doi: 10.1016/j.geoderma.2018.07.048
- Rankine, B. (2007). *Making good wine*. Sydney, NSW: Macmillan Publishers Australia.
- Remenyi, T., Rollins, D., Love, P., Earl, N., Bindoff, N., and Harris, R. (2019). *Australia's Wine Future A Climate Atlas*, University of Tasmania, Hobart, Tasmania. ISBN: 978-1-922352-06-4.
- Rogiers, S. Y., Clarke, S. J., and Schmidtke, L. M. (2014). Elevated root-zone temperature hastens vegetative and reproductive development in S hiraz grapevines. *Austr. J. Grape Wine Res.* 20, 123–133. doi: 10.1111/ajgw.12053
- Rogiers, S. Y., Smith, J. P., Holzapfel, B. P., and Hardie, W. J. (2011). Soil temperature moderates grapevine carbohydrate reserves after bud break and conditions fruit set responses to photoassimilatory stress. *Functional Plant Biol.* 38, 899–909. doi: 10.1071/fp10240
- Scarlett, N. J., Bramley, R. G. V., and Siebert, T. E. (2014). Within-vineyard variation in the 'pepper' compound terundone is spatially structured and related to variation in the land underlying the vineyard. *Austr. J. Grape Wine Res.* 20, 214–222. doi: 10.1111/ajgw.12075
- Silvestroni, O., Lanari, V., Lattanzi, T., and Palliotti, A. (2018). Delaying winter pruning, after pre-pruning, alters budburst, leaf area, photosynthesis, yield and berry composition in Sangiovese (*Vitis vinifera* L.). *Austr. J. Grape Wine Res.* 24, 478–486. doi: 10.1111/ajgw.12361
- Smart, R., and Dry, P. (1980). *A Climatic Classification for Australian Viticultural Regions*. Adelaide: Annual Technical Issue.
- Spittlehouse, D. (2006). Climate BC: your access to interpolated climate data for BC. *Streamline Water. Manage. Bull.* 9, 16–21.
- Tonietto, J., and Carbonneau, A. (2004). A multicriteria climatic classification system for grape-growing regions worldwide. *Agric. Forest Meteorol.* 124, 81–97. doi: 10.1016/j.agrformet.2003.06.001
- Van Leeuwen, C., and Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *J. Wine Econ.* 11:150. doi: 10.1017/jwe.2015.21
- Wang, T., Hamann, A., Spittlehouse, D., and Carroll, C. (2016). Locally downscaled and spatially customizable climate data for historical and future periods for North America. *PLoS One* 11:e0156720. doi: 10.1371/journal.pone.0156720
- Webb, L., Whetton, P., and Barlow, E. (2008). Modelling the relationship between climate, winegrape price and winegrape quality in Australia. *Climate Res.* 36, 89–98. doi: 10.3354/cr00739
- World Meteorological Organisation. (2019). *What is Climate Variability?* Available online at: <https://www.wmo.int/pages/prog/wcp/ccl/faqs.php> (accessed May 13, 2021).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Pipán, Hall, Rogiers and Holzapfel. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Under-Vine Vegetation Mitigates the Impacts of Excessive Precipitation in Vineyards

Justine Vanden Heuvel¹ and Michela Centinari^{2*}

¹School of Integrative Plant Science, Cornell University, Ithaca, NY, United States, ²Department of Plant Science, The Pennsylvania State University, University Park, PA, United States

OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Stefania Savoi,
Montpellier SupAgro, France
Michaela Griesser,
University of Natural Resources and
Life Sciences Vienna, Austria

*Correspondence:

Michela Centinari
mzc22@psu.edu

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 21 May 2021

Accepted: 01 July 2021

Published: 26 July 2021

Citation:

Vanden Heuvel J and
Centinari M (2021) Under-Vine
Vegetation Mitigates the Impacts of
Excessive Precipitation in Vineyards.
Front. Plant Sci. 12:713135.
doi: 10.3389/fpls.2021.713135

Excessive precipitation events have greatly increased in several grape growing regions due to human-caused climate change. These heavy downpours result in a myriad of problems in the vineyard including soil aggregate breakdown, soil runoff, nutrient leaching, excessive vine vegetative growth, and diseased fruit. The negative impacts of excessive precipitation events on vineyards are exacerbated by the maintenance of bare soil under the vines. Exposure of bare soil results in soil erosion and runoff which pollutes nearby watersheds; raindrops weaken and break apart soil aggregates, leading to increased soil erosivity and contributing to the formation of surface crusts. In addition to excessive precipitation events, some grape growing regions can be characterized by fertile soils. The availability of ample water and nutrients can lead to highly vigorous vines with shoot growth continuing through harvest. Long shoots and large leaves result in shaded fruit, a humid vine microclimate, and excessive cluster rot. In this review, we examined how either natural (i.e., resident) or seeded under-vine vegetation (UVV) can help mitigate many of the problems associated with excessive precipitation. Through providing vegetative coverage to reduce the force of raindrops, increasing soil organic matter and enhancing soil microbial diversity, UVV can reduce the soil degradation and off-site impacts caused by excessive precipitation events. Through competition for soil resources, UVV can reduce excessive vegetative growth of vines and decrease cluster rot incidence and severity, although grapevine response to UVV can be highly variable. We discussed recent advances in understanding below and aboveground vine response and acclimation to UVV and presented current evidence of factors influencing the impact of UVV on vine growth and productivity to assist practitioners in making informed decisions and maximize the ecosystem services provided by UVV.

Keywords: climate change, competition, cover crop, soil health, vigor, *Vitis*

INTRODUCTION

There has been increased interest in understanding grapevine response and acclimation to changes in water availability induced by climate changes in order to adapt management strategies. Although increased drought is a major agricultural challenge and considerable emphasis has been placed to ameliorate the impact of lower water availability on wine grape production

worldwide, some grape growing regions are facing challenges relating to more erratic rainfall patterns and excess water availability. In general, heavy precipitation events (measured as observed change in total annual precipitation falling in the heaviest 1% of events) are becoming more intense and more frequent across most of the United States (U.S. Global Change Research Program, 2014). This increasing trend is particularly strong in the northeastern United States, where excessive precipitation events increased by 71% between 1958 and 2012. In this region, rainfall events greater than 150 mm over 24 h increased in frequency from six events between 1979 and 1996 to 25 events from 1997 to 2014, a 317% increase (Howarth et al., 2019). In central Europe, heavy precipitation, defined as the 95th percentile of daily precipitation, increased from 1 to 3 mm/day per decade in the last 100 years, while during the last 60 years extreme winter precipitation intensified by 6–8% per decade in western Europe (Zolina, 2012). Both observations and climate model projections indicate strong increases in extreme precipitation in northern Europe as well. Climate model projections also show increases in extreme precipitation and flood discharge in the 21st century throughout Europe (reviewed by Madsen et al., 2014). Hosseinzadehtalaei et al. (2020) suggest that the frequency of extreme precipitation events in Europe will be tripled under the representative concentration pathway (RCP8.5) high emissions global scenario.

These heavy precipitation events can lead to a multitude of detrimental impacts on plants and soil in vineyards, particularly those with a lack of soil cover, as well as their neighboring ecosystems. Exposure of bare soil results in greater impact from raindrops, which weaken and break soil aggregates apart, increasing the erosivity of soils and contributing to the formation of crusting on the soil surface (Epstein and Grant, 1973). In vineyards in central Spain, runoff from bare soil was more than three times higher than from soil with vegetation cover, while nitrates lost in the runoff were almost six times greater from bare soil than from covered soil (García-Díaz et al., 2017). Even in regions where vegetation cover between rows is a common practice, nitrogen (N) and dissolved organic carbon can leach at a greater rate from under-vine bare soil compared to vegetation-covered soil (Karl et al., 2016a). Runoff and leaching cause decreases in soil fertility and eutrophication in downstream bodies of water.

Grapevines themselves can also be detrimentally impacted by increased and/or excessive precipitation. An increase in plant available water – and hence nutrient availability – can lead to greater vegetative growth (Giese et al., 2015) and berry size (Karl et al., 2016b) as well as extended growth of shoot tips (Centinari et al., 2016). Increased vegetative growth can cause cluster rots through decreased air flow in the canopy, while increased cluster compactness (due to increased berry size) also contributes to fungal pressure on the cluster (Valdes-Gomez et al., 2008; Guilpart et al., 2017). Therefore, highly vegetative vines might require more extensive and costly management practices to remediate these potential issues.

In grape growing regions with high precipitation, where inter-row vegetation is already maintained, researchers have been experimenting with under-vine vegetation (UVV) to

alleviate some of the detrimental effects to soil and plants caused by ample precipitation and provide further benefits to the vineyard ecosystem. Both annual and perennial cover crop species have been intentionally planted in the area beneath the vines, but adoption of natural vegetation (i.e., managed weed growth) has also been explored. In addition to the eastern United States, UVV has been trialed in a range of climates including wine regions in France (Delpuech and Metay, 2018), Spain (Abad et al., 2020), Australia (Penfold and Howie, 2019), and New Zealand (Merfield, 2019), where excessive precipitation might not be a concern.

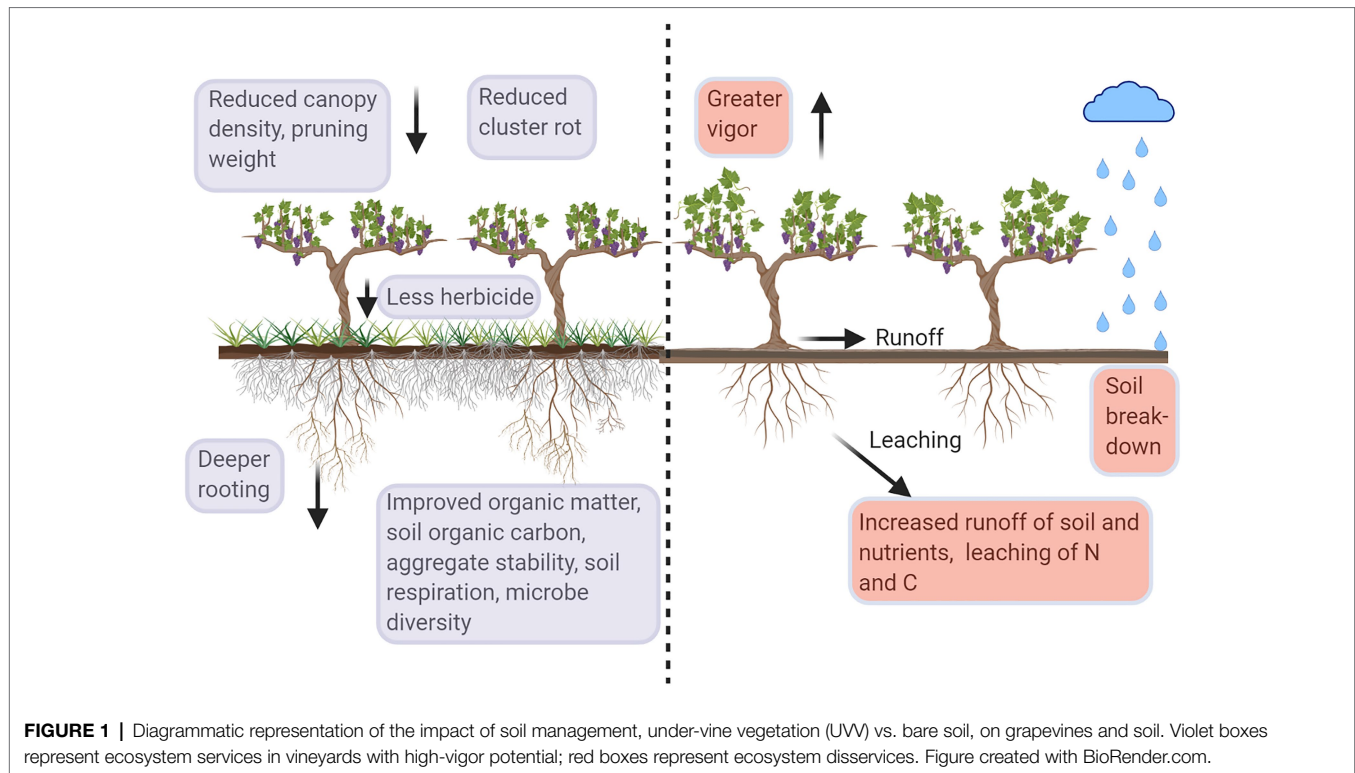
This review details how UVV can help ameliorate climate challenges related to increased heavy precipitation; we focus on key soil and plant traits that could be impacted by implementing UVV, describe the current understanding of the complex UVV-vine interactions, and identify knowledge gaps in the published literature. Our discussions are intended to provide a framework that can guide future research and increase UVV adoption. Due to the common use of inter-row cover crops in many wine regions with high precipitation, we focus on the additional impact UVV provides in vineyards where inter-row vegetation is already maintained.

SOIL HEALTH AND ECOSYSTEM SERVICES

Protecting soil from degradation is important for the long-term sustainability of a vineyard. Introducing cover crops into what was traditionally considered a perennial monoculture system can help achieve this goal (Garcia et al., 2018). Cover cropping between rows has been extensively studied in vineyards around the world, but information on complete vineyard floor cover crops (between row plus under-row) is limited. Below we summarize how introducing UVV can positively influence important parameters of soil health. These results, however, should be maintained in the context of short-term effects, within 2 or 3 years from UVV establishment as scientific investigations are often limited to a few years of field data collection.

Soil Organic Matter and Soil Carbon

Soil organic matter (SOM), soil organic carbon (SOC, a component of SOM), and total carbon (C) can markedly increase or decrease as a function of soil management, although the response to management can take many years to become detectable. Repeated herbicide applications and cultivation result in bare soil, negatively impacting SOM and SOC (Figure 1). Plants can contribute biomass as well as rhizodeposits, directly increasing soil C. Indirectly, plants play a role in modifying soil C pools through microbial stimulation and reductions in soil erosion. Contributions of UVV to SOM and soil C are likely dependent on whether the vegetation is incorporated into the soil (e.g., annual species) or whether it remains in place over multiple years (e.g., perennial species). Working with annual species as UVV for 3 years, Karl et al. (2016a)



reported an increase in SOM of only 0.6% compared to under-vine plots managed with herbicide. In a cold climate vineyard in Iowa, United States, changes in SOM were only apparent in shallow soil after 6 years of UVV treatments (DeVetter et al., 2015). Changes in SOM in vineyards with inter-row cover crops are also reported to be slow (Garcia et al., 2018).

An additional benefit of using cover crops in vineyards is the possibility of sequestering atmospheric C and N by increasing their concentration across the soil profile. A recent study analyzed changes in soil C and N across the under-vine soil profile (0–100 cm) 3 years after UVV (red fescue, *Festuca rubra*) was planted in a young vineyard (Fleishman et al., 2021). Planting an under-vine grass with a dense root system rather than maintaining bare soil significantly increased soil C by 56 and 44% at 1–20 cm and 21–40 cm soil depth, respectively. Total N in the UVV plots was 37 and 19% at 1–20 and 21–40 cm soil depth, respectively, higher than in the bare soil plots only after 3 years of vine-UVV coexistence. The increases in total C and N in the shallow soil layers were explained by UVV root biomass which colonized most of the shallow soil. It is unknown if over a longer time UVV will contribute to increases in deep soil C (ex. below 40 cm) in vineyards.

Soil Physical Characteristics

Exposure of bare soil results in greater impact from raindrops, which weakens and breaks soil aggregates apart, increasing the potential of erosion and contributing to the formation of surface crusts (Epstein and Grant, 1973; Figure 1). Karl et al. (2016a) reported that using white clover (*Trifolium repens*) as

an UVV for 4 years resulted in 36% greater aggregate stability than under-vine soils maintained with the herbicide glyphosate and 23% greater than those maintained with cultivation. Soils from the under-vine white clover plots maintained almost 75% of aggregate mass after a simulated rain event (Karl et al., 2016a). In a nearby vineyard, Chou and Vanden Heuvel (2019) reported increases of 82% in soil aggregate stability between glyphosate-maintained soil and natural vegetation (i.e., managed weed growth) after 3 years of UVV.

Based on the positive impact inter-row cover crops have had on improving water infiltration in vineyards (Celette et al., 2009), it is possible that UVV holds potential for improving soil infiltration through maintaining favorable soil structure and porosity, thereby reducing the opportunity for runoff along the soil surface following excessive precipitation. DeVetter et al. (2015) reported a massive but statistically insignificant increase in infiltration in a UVV treatment (creeping red fescue) compared to herbicide (0.6 min and 14.7 min for 444 ml of water to infiltrate the soil covered by UVV and maintained with herbicide, respectively) on a fine loam soil (DeVetter et al., 2015). However, Karl et al. (2016b) reported no differences in saturated soil infiltration rate among under-vine treatments of vegetation and bare soil over 4 years on a silt loam soil.

Soil Microbial Activity

Microbial activity responds quickly to changes in soil management practices, often indicating changes in the flux of labile C before differences in SOM are apparent. Soil respiration is often used as a proxy for microbial activity of a soil, particularly when

quantified in the absence of roots. Soil from UVV treatments had the greatest soil respiration rates in a number of studies (**Figure 1**). In the Finger Lakes region of New York, United States, soil respiration in the UVV natural vegetation treatment was 43% greater than in under-vine plots maintained with glyphosate and 45% greater than in plots maintained with cultivation (Karl et al., 2016a). In a nearby vineyard, soil respiration was 49% greater in under-vine plots with natural vegetation compared to those maintained with glyphosate, while plots with planted UVV, such as tall fescue (*Festuca arundinacea*), chicory (*Cichorium intybus*), tillage radish (*Raphanus sativus*), and alfalfa (*Medicago sativa*), had soil respiration rates up to 75% greater than plots maintained with glyphosate (Chou and Vanden Heuvel, 2019). The trend of greater soil respiration with UVV compared to herbicide or cultivation indicates that lack of vegetation decreases the input of biodegradable substrates to the soil, diminishing microbial activity and potentially lowering the rate of N mineralization into plant available forms (Rustad et al., 2001).

A more diverse community of soil microbes tends to be associated with decreased incidence of plant diseases as well as improved plant productivity (Vukicevich et al., 2016). The impact of floor management practices on vineyard microbiome has been overlooked until recently but is of significant interest as soil may be considered the vineyard microbial pool (Zarraonaindia et al., 2015). As UVV expands the diversity of plants in the vineyard, microbes associated with those herbaceous plants can broaden overall soil microbial diversity. In California, vineyard floor management impacted the composition of soil bacteria, but a potential association between soil, rhizosphere, and fruit microbiome was not investigated (Burns et al., 2016). In the cool, wet climate of upstate New York, Chou et al. (2018) studied the impact of three under-vine practices (herbicide application, soil cultivation, and natural vegetation as UVV). The authors reported that soil bacterial and fungal composition in the UVV treatment differed from the plots maintained with glyphosate (Chou et al., 2018). Although several studies have proved that cover crops planted either in the inter-row or under-vine area can affect the soil microbiome pool, we are still far from understanding the subsequent impact on vine functioning and productivity.

Additional Ecosystem Services/ Disservices

Other off-site impacts of concern in regions where vineyards are predominantly located on slopes – particularly in close proximity to bodies of water – are runoff and leaching of nutrients and agrochemicals (**Figure 1**). Lack of soil cover can increase the severity of soil runoff (Battany and Grismer, 2000). While the additional contribution of UVV to inter-row cover crops on soil runoff has not been directly quantified, UVV presumably provides a physical barrier that further reduces runoff when rows are planted perpendicular to hillsides. Greater dissolved SOC leaching from under-vine soils in comparison with those with UVV was reported by Karl et al. (2016a), indicating C loss from the agroecosystem. Total N leaching was great in the glyphosate-maintained plots as well as the

legume white clover plots. Other vineyard groundcover studies found greater N leaching in herbicide-treated inter-rows, although this result is not simply a function of bare soil as cultivated plots had lower leaching of N (Steenwerth and Belina, 2010). A greater presence of soil C, microbial biomass, and plant residues has been linked with reduced N leaching in vineyard systems (Steenwerth and Belina, 2008). Greater leaching of nitrate can lead to increased emissions of the greenhouse gas nitrous oxide from soils (Steenwerth and Belina, 2010).

In a recent review, Garcia et al. (2018) summarized the important role cover crops play in weed control, pest and disease status, water availability, field trafficability, soil biodiversity, and C sequestration. These impacts are defined as ecosystem services, which are the conditions and processes through which ecosystems sustain human life. In Mediterranean-climate regions, competition for soil resources, chiefly water and nutrients, between the cover crops and grapevine is typically viewed as an ecosystem disservice because it can negatively suppress vegetative growth, reduce yield potential, and fruit composition (Garcia et al., 2018). However, the balance between service and disservice is dynamic and the ability of cover crops to provide ecosystem services or disservices varies depending on climate and soil conditions, the species of cover crop, as well as the coverage of the soil among other factors. For example, competition for soil resources from complete vineyard floor cover might provide a beneficial regulation of vegetative growth in a region and/or season with high precipitation and be considered an ecosystem service rather than a disservice. In other instances, less competitive cover crops (for example, annual herbaceous species or leguminous species) can be used as UVV to provide ecosystem services while limiting potential effects on vine growth and production (Jordan et al., 2016; Abad et al., 2020).

Another example of an important ecosystem service provided by cover crops is the biological control of pests. Beneficial insect presence in an ecosystem is usually positively correlated with vegetation abundance and diversity (Letourneau et al., 2011). Between-row cover crops can enhance populations of natural enemies of pests, reducing spider mite and some leafhopper populations on grapes (Costello and Daane, 2003; English-Loeb et al., 2003). The impact of UVV on vineyard pests has not been directly investigated, although Wolf and Giese (2020) warn of potential vole and cutworm damage in UVV plots. Research on UVV impacts on diseases has been preliminary; a reduction in gray mold (*Botrytis cinerea*) was recorded when grapes were harvested from plots managed with UVV rather than bare soil (Coniberti et al., 2018b).

GRAPEVINE-UVV INTERACTION

Aboveground Growth and Yield Responses

In addition to ecological benefits, UVV can be planted in vineyards to limit root uptake capacity and decrease vine growth. However, vegetative growth and yield reductions are not easily predicted. Grapevine-UVV interaction can produce a wide range of aboveground effects from no influence (Jordan et al., 2016) to significant reductions in vegetative growth

(Hatch et al., 2011; Giese et al., 2014; Karl et al., 2016b; Coniberti et al., 2018a; Fleishman et al., 2019). While lower vegetative growth is often considered an ecosystem service in regions with ample precipitation (Giese et al., 2014; DeVetter et al., 2015; Hickey et al., 2016), it might potentially become a disservice if growth reductions are considered excessive (Karl et al., 2016b). Many factors affect responses of grapevines to UVV competition, including soil resource availability and demand from both plant species. Even within the same site, vegetative growth and yield reductions can fluctuate with annual shifts in resource availability and vine acclimation strategies (Giese et al., 2014; Hickey et al., 2016). Grapevine demand for water and nutrients is influenced by environmental conditions but also endogenous factors, such as vine age and rootstock (or root system genotype), which in turn affect root system volume and uptake capacity.

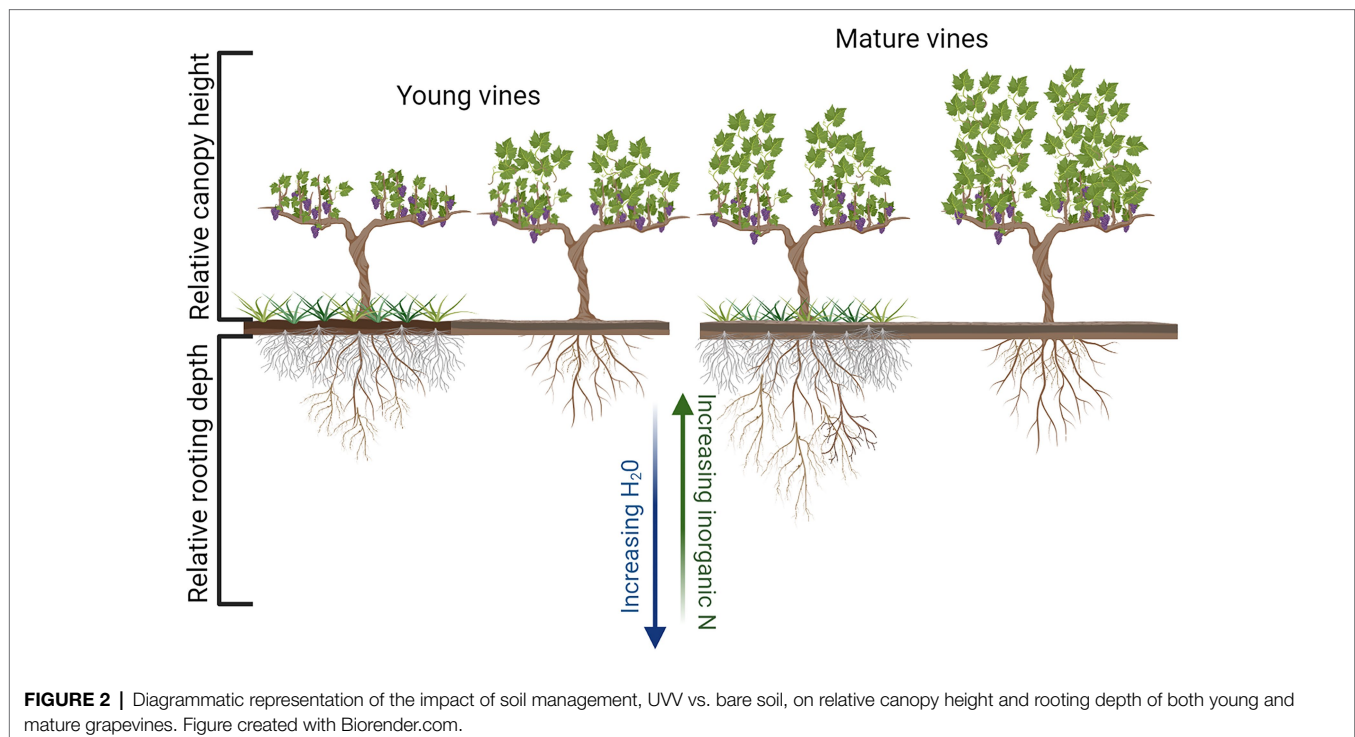
The influence of UVV on vegetative growth and yield varied across studies, vineyard sites, and years within the same site though some commonalities can be identified. For example, reductions in vegetative and reproductive growth induced by UVV are typically greater in younger vines than in older and more established vines (**Figure 2**), at least in regions with ample soil resources. Red fescue planted as an UVV in the fall of the second year of vineyard establishment induced yield and pruning weight reductions up to 39 and 46%, respectively, in the following growing season as compared to vines grown with herbicide-treated under-vine (Hatch et al., 2011). When white clover and natural vegetation were used as UVV in a young vineyard, pruning weight was reduced by approximately 50% by the fifth year, while yield was reduced by 16% in the natural vegetation treatment compared to plots maintained with

under-vine herbicide (Karl et al., 2016b). Results from these studies and other work (Hatch et al., 2011; Fleishman et al., 2019) suggest that UVV can be used in the early years of a vineyard to favorably limit vine size at sites with high growth potential, although such significant vegetative growth and yield reductions may be considered an ecosystem disservice depending on the production goals of the grower. When UVV is implemented in older vineyards (10 years of age or more) the impact on vine size and yield has been considerably less (Giese et al., 2014; DeVetter et al., 2015; Jordan et al., 2016), likely due to more developed root systems which are able to access enough water and nutrients to maintain growth (**Figure 2**).

Another trend observed across studies is the more pronounced reduction in vegetative rather than reproductive growth when UVV is used (Giese et al., 2014; DeVetter et al., 2015; Hickey et al., 2016; Karl et al., 2016b; Chou and Vanden Heuvel, 2019), resulting in an increased Ravaz index (calculated as yield-to-pruning weight ratio). In one study, yield of vines growing with UVV was actually significantly higher than that of vines in the bare soil under-vine plots in a drier than average season (Chou and Vanden Heuvel, 2019). It is plausible that water content was higher in soil covered by UVV than in bare soil exposed to cultivations as reported in a previous work (DeVetter et al., 2015); if UVV is not actively growing it might form a green mulch and reduce loss of water *via* evapotranspiration.

Grapevine-UVV Spatial Interaction

Belowground investigations can help better understand how grapevine and UVV interact to predict aboveground effects. Although studying root dynamics and functions present logistic



and labor challenges, understanding how grapevine root systems respond and acclimate to UVV may help explain the variability, but also commonalities, of aboveground effects reported in the previous work.

The adoption of UVV creates a greater and more direct interaction between the root systems of the perennial (grapevines) and herbaceous (cover crop) species compared to using inter-row cover crops only, which could result in a greater resource uptake limitation and aboveground effects. When cover crops or natural vegetation grow in the inter-row only, the grapevine root system mainly colonizes the under-row area while the cover crops the inter-row, suggesting a compartmentalization of space and resources (Celette et al., 2005). In drier climates, this spatial separation might limit the access of grapevine roots to water and nutrients to a point that decreases vine vegetative growth (Celette et al., 2008, 2009). However, in climates with high precipitation and fertile soils, resources in the under-row area are often sufficient to support ample vine vegetative growth; therefore, the more direct spatial interaction imposed by UVV might be necessary to limit excessive grapevine vegetative growth. In a vineyard with complete cover crop floor coverage (inter- and under-row) soil volume available for grapevine root growth could be very limited; however, grapevines are considered plastic plants with one of the deepest rooting patterns (Smart et al., 2006) and in deep soils they could shift root distribution below the soil layers colonized by UVV.

A common belowground response to UVV, which was also reported in other fruit crop systems (Yao et al., 2009; Atucha et al., 2013), is a reduction of root production in shallow soil layers (20 cm), defined as a zone of high nutrient competition (Centinari et al., 2016; Klodd et al., 2016; Fleishman et al., 2019, 2021; **Figure 2**). Lower root growth in the top 20–30 cm of soil could reduce vine access to nutrients, as shallow soil strata have typically greater nutrient availability compared to deeper soil, which instead have higher soil water content (Klodd et al., 2016). This root distribution response can be described as inter-plant avoidance (Maina et al., 2002) and it was observed in both young and mature vines exposed to UVV from the year following vineyard planting (Klodd et al., 2016; Fleishman et al., 2019). The spatial segregation response reported in several studies is not surprising; some of the UVV species used, such as grasses, typically have a much denser root system compared to grapevines; and grass root length density (RLD; cm root/cm³ soil) can be up 10 times that of young grapevines (Fleishman et al., 2019). However, vines exposed to UVV later on, when in full production, might (Centinari et al., 2016) or might not (Giese et al., 2016) show an avoidance response.

A smaller and/or deeper root system (both as a proportion of the whole root system and as absolute RLD) could decrease vine access to specific soil resources (e.g., N vs. water) which are not homogeneously distributed across the soil profile (**Figure 2**). The entity of these growth reductions could vary greatly depending on the soil environment and volume explored by the root system; thus, it is not surprising that the aboveground growth reductions were observed in some studies but not in others, as previously discussed. A deeper root system might also be a useful trait in regions with ample precipitation that occasionally experience

extended drought periods. Vineyards in regions with high precipitation events are often unirrigated and if vines can rely on deep water they might better withstand reduced water availability than those with a more shallow root system. Benefits of UVV under variable seasonal water availability are still speculative, but future studies could explore UVV potential to stabilize grapevine growth responses to variable soil moisture availability.

Grapevine-UVV Temporal Interaction

The seasonal dynamics of root growth can affect the temporal interaction between the UVV and grapevines. Although belowground grapevine phenology is less predictable and not strictly coupled to aboveground phenological phases (Radville et al., 2016), vine root production typically exhibits a unimodal trend, with a major flush of root growth between bloom and veraison (Comas et al., 2010). A moderate water deficit is typically desired after bloom to reduce excessive vine vegetative growth without affecting C assimilation. If UVV roots grow before grapevines reach their seasonal peak of root production, they might limit grapevine root growth in the under-vine area. New roots are mainly absorptive, responsible for resource uptake; therefore, decreased root production might restrict vine water and nutrient uptake resulting in beneficial reduction of vine growth. The extent of these aboveground growth reductions, however, is less predictable than results obtained through deficit irrigation strategies in dry climates. Regardless, UVV still offers an opportunity to alleviate detrimental effects of excessive precipitation.

Cover crops with different growth cycles (e.g., perennial species vs. summer annual species) might introduce competition at different times of the vine growth cycle. Cool-season grasses exhibit a growth pattern that parallels the fast spring vegetative growth of vines and can be more effective in suppressing primary shoot growth compared to summer annual cover crop species which are planted later in the season. Competition for resources during early stages of berry development may, however, decrease berry size and thus yield potential. If summer annual UVV competes later in the season with the grapevines they could decrease the duration of lateral shoot growth (Centinari et al., 2016) or, in other instances, have no impact on vine size (Jordan et al., 2016). In addition to different competition timing, annual cover crops exhibit a shorter growth cycle and have smaller root systems relative to perennial cover crops, thus they tend to be less competitive for soil resources, at least in the first couple of years of establishment (Centinari et al., 2016; Jordan et al., 2016). Previous work indicated that perennial UVV species, mainly cool-season grasses, tend to be more competitive and effective in reducing vegetative growth than annual species (Giese et al., 2015; Hickey et al., 2016). However, repeated establishment of annual UVV over the years might still deplete shallow soil of water and nutrients and induce belowground vine response (Centinari et al., 2016).

Grapevine-UVV Interaction Can Alter Root Traits

In some instances, but not always, shifts in root distribution induced by UVV were coupled with changes in other root traits,

which suggest a plastic belowground vine response. For example, absorptive roots of vines growing with annual UVV species, such as annual ryegrass (*Lolium multiflorum*) and buckwheat (*Fagopyrum esculentum*), had longer median life span than those in the under-vine bare soil plots (Centinari et al., 2016). Shifts toward deeper root distribution induced by UVV could explain these differences, as roots produced in deeper soils tend to live longer than those growing in shallow soils (Anderson et al., 2003; Centinari et al., 2016). Results differ when grapevine roots growing in the UVV plots and in the zone of major interaction (0–40 cm) were sorted depending on their proximity to a UVV root. Grapevine roots growing without neighboring UVV roots lived much longer (over 300 days) compared to those growing nearby UVV roots (106 and 72 days in neighborhoods of annual ryegrass or buckwheat, respectively). This suggests that vines growing with UVV may maintain roots longer in soil patches with lower competition pressure, while shedding those in high competition areas (near UVV roots) to optimize resource uptake strategy (Centinari et al., 2016).

Other studies explored UVV-induced responses of absorptive root traits which are typically associated with increased efficiency of nutrient uptake, such as production of absorptive roots with smaller diameter, greater root length to mass ratio (specific root length, SRL, cm/g), and greater branching intensity (Klodd et al., 2016; Fleishman et al., 2019). Effects of UVV on these root traits, however, were not consistent between sites. For example, when young Noiret (*Vitis* hybrid sp.) vines grafted either on 101-14 Mgt (*V. riparia* × *V. rupestris*) or Riparia (*Riparia gloire*) were exposed to UVV (red fescue) for 1 year, they were able to compensate for reduction of absorptive RLD in the shallow soil (0–20 cm) with greater root length in deeper soil (21–40 cm), which was described as a zone of lower competition compared to the 0–20 cm depth increment (Fleishman et al., 2019). The same vines grown with UVV also had higher SRL and lower absorptive root diameter. However, despite these observed belowground changes, UVV vines still had lower macronutrients (particularly N) concentration and content in vegetative tissues and fruit compared to vines maintained with under-vine herbicide. Reduction in nutrient uptake was likely the main cause of the lower pruning weight induced by UVV reported in this study. In contrast, mature Cabernet Sauvignon (*Vitis vinifera*) vines grafted on the same rootstock (101-14 Mgt) of Fleishman et al. (2019) and with the same UVV species for 7 years exhibited only modest reductions in vegetative growth and no apparent changes in root morphological traits (e.g., root diameter, SRL, and branching intensity) compared to vines in plots with herbicide-treated under-vine (Klodd et al., 2016). More studies are needed to confirm if these contrasting results are related to the age of the vines and/or length (years) of UVV-vine interaction.

Grapevine-UVV Competition for Water and Nutrients

Limited water and nutrient uptake affect many metabolic processes. Growth processes (i.e., shoot growth and early season

berry growth) are the most sensitive and first affected by water deficits and nutrient deficiencies. Reduction in nutrients in vineyards with UVV could be direct, through lower soil availability, or indirect, *via* reduced water availability which can decrease nutrient movement toward the roots by mass flow or diffusion (Tinker and Nye, 2000) and N mineralization (Celette et al., 2009).

Most work from regions with ample precipitation noted no or minimal competition for water in vineyards with UVV (Jordan et al., 2016; Klodd et al., 2016; Karl et al., 2016a; Fleishman et al., 2019). Only a few studies reported a positive correlation between decreased vine growth/yield and decreased soil moisture and vine water status (Hatch et al., 2011; Centinari et al., 2016). When examined across soil depths, UVV tended to decrease soil moisture below the zone colonized by perennial grasses (e.g., between 40 and 60 cm; Hickey et al., 2016; Klodd et al., 2016; Fleishman et al., 2019), but these differences were considered modest and did not affect the overall soil water storage. Soil moisture at shallow depths (0–20 cm) was reduced by one UVV species (white clover) planted annually in a vineyard in upstate NY but not by another UVV species (natural vegetation; Karl et al., 2016a). In both seasons of measurement, soil moisture differed among treatments until mid-summer as the vegetation established (Figure 3), but by veraison, there were no differences among treatments. These differences in shallow soil moisture (Figure 3) were not linked to differences in pruning weight (Karl et al., 2016b).

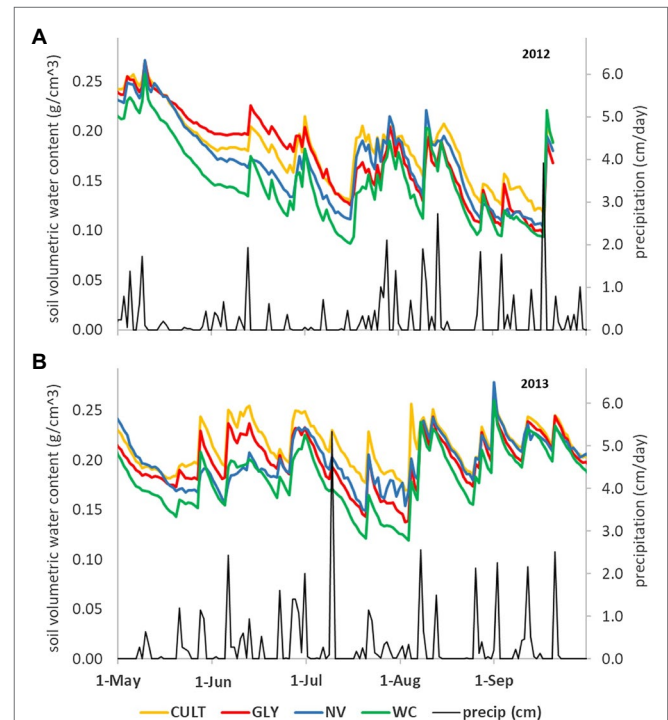


FIGURE 3 | Soil water content (g/cm³) measured at midday under four UVV treatments in Lansing, NY, United States in (A) 2012 and (B) 2013 measured at 20 cm soil depth. CULT, cultivation; GLY, glyphosate; NV, natural vegetation; and WC, white clover. Data from Karl (2015), partially reported in Karl et al. (2016a).

Although UVV might affect soil moisture, there is no evidence that vines growing with UVV can adsorb water at deeper depths than those without UVV under wet weather conditions (Klodd et al., 2016; Fleishman et al., 2019). In general, it is hard to correlate soil moisture patterns with depth of water uptake in regions that do not experience prolonged dry-down periods because of the frequent and erratic rainfall events. The role of deep roots in water uptake might be more relevant in drier regions or seasons.

In regions where water availability is not typically a limiting factor, competition for nutrients might be more relevant. Compared to vines maintained with under-vine herbicide, UVV decreased plant N status (Giese et al., 2014; Fleishman et al., 2019) and extractable soil nitrates at shallow depths (<20 cm; Klodd et al., 2016) when perennial grasses were used. Competition for nutrients was less apparent when UVV species were planted on an annual basis (Karl et al., 2016b; Chou and Vanden Heuvel, 2019). However, interpretation of nutrient results among studies could be difficult due to different tissues (leaf petiole vs. blade) and methodologies used for collecting the tissue (e.g., time of the season and position on the canopy). Future studies should focus on a minimum of quantifying leaf blade nutrients to examine nutrient limitations since they are the most accurate tissue for measuring N and phosphorus (P) status of grapevines than leaf petioles (Schreiner et al., 2013).

It still unclear if decreased soil resource uptake induced by UVV competition has a meaningful impact the capability of a vine to assimilate C and on the allocation ratios among C sinks (e.g., shoot growth vs. fruit growth and ripening vs. storage in perennial tissues). Centinari et al. (2016) reported lower leaf C assimilation rate with the implementation of annual ryegrass under the vine in two of the 3 years of the study, but these observations were not always associated to lower leaf transpiration rate. Reduced C availability was listed as one of the potential causes of decreased grapevine fine root production for vines growing with annual ryegrass UVV. Hatch et al. (2011) reported lower C assimilation for vines in UVV plots compared to those managed with herbicide in only out of four dates, while leaf transpiration, stomatal conductance, and intercellular CO₂ concentration of vines were unaffected by treatment. More research is needed in this area.

ABOVE AND BELOWGROUND ACCLIMATION STRATEGIES

Acclimation Strategies Could Depend on Vine Age

Interaction between a woody and herbaceous species is a dynamic process and acclimation strategies, although still understudied, might evolve over the years. Long-term studies (>5 years) of UVV in vineyards are needed to assess the sustainability of this practice across the lifetime of the vineyard. To date, we are aware of only two studies conducted in the humid eastern United States that assess aboveground vine response to UVV over 7 years (Giese et al., 2014; Hickey et al., 2016). In the first study (Giese et al., 2014), several cool-season perennial

grasses were established in a vineyard already in full production (6 years after vineyard planting), while in the second trial (Hickey et al., 2016), a cool-season perennial grass was established under young vines (2 years after vineyard planting). At both sites, UVV favorably reduced vegetative growth compared to vines maintained with under-vine herbicide, but when UVV interacted with mature vines the reduction in pruning weight did not diminish or increase over time and appeared to be mainly driven by seasonal weather conditions. Additionally, there was no indication of root redistribution (Giese et al., 2016). In contrast, when vines were exposed to UVV starting at a young age, an acclimation to UVV competition was observed over the years. Differences in dormant pruning weight between vines in UVV and herbicide-treated plots diminished over 6 years and were mainly attributed to larger relative increases in size of UVV vines compared to vines maintained with under-vine herbicide over time (Hickey et al., 2016). Yield differences between under-vine management treatments also disappeared over time. It is plausible that in regions with ample precipitation and soil depth, young vines are able to acclimate to UVV competition over the years to a point that they can maintain or have limited reduction in aboveground growth despite having a much smaller absorptive root system than vines in bare soil under-vine plots (Klodd et al., 2016).

In addition to investigating shifts in root growth and morphological traits in response to UVV, as described earlier, several studies explored grapevine root association with beneficial microbes as a vine acclimation strategy to UVV. To date, investigations were mainly focused on arbuscular mycorrhizal fungi (AMF); however, other root-associated microbes that impact vine functioning might be affected by UVV as well. The guiding hypothesis was that vines growing with UVV would have greater AMF colonization than those growing with bare soil under-vine to improve efficiency of nutrient absorption especially in deeper soil layers not colonized by UVV roots. This could provide the vines with enough resources to maintain growth in competitive soil environments. However, to date, there is no indication that vines increase AMF colonization in response to UVV across the soil profile (0–100 cm) of young and mature vineyards (Klodd et al., 2016; Fleishman et al., 2019).

Root System Genotype Might Affect Vine Response and Acclimation

Grapevine interaction with UVV over time can also be influenced by the root system genotype, but strong evidence is still lacking. Rootstocks are usually classified from low- to high-vigor based on their influence on the scion vegetative growth. If high-vigor rootstocks have greater RLD and higher soil water and nutrient depletion than low-vigor rootstocks they could more readily acclimate to belowground UVV competition. They might also be able to explore deep soil layers faster (both as a proportion of the whole root system and as absolute RLD) and therefore use more water in deeper soil too. These root traits could lead to a different aboveground response to UVV, such as less relative growth reduction for a grapevine grafted on a

high-vigor compared to low-vigor rootstock. We could also speculate that, while differences between rootstocks might be exacerbated by competition with UVV, they would also diminish over time if the vines are able to acclimate to competition.

Two studies conducted at the same site examined below and aboveground responses to UVV competition of young grapevines (Noiret) grafted on two rootstocks that are considered to impart low (*Riparia*; *Riparia Gloire*) or moderate (101-14 Mgt) vigor, 1 year after UVV establishment and 2 years later (Fleishman et al., 2019, 2021). In general, the young low-vigor and medium-vigor rootstocks had a similar root redistribution response 1 year after UVV establishment. In response to UVV, both rootstocks had lower RLD in the shallow, high nutrient competition soil depth (0–20 cm) and greater RLD in the deeper, lower competition zone (21–40 cm). In contrast, 2 years later the medium-vigor rootstock displayed a more plastic belowground response to UVV competition than the low-vigor rootstock (Fleishman et al., 2021). While both rootstocks markedly and similarly decreased total root mass density (mg/cm³ of soil) between 0 and 20 cm, at deeper depths only the medium-vigor root system was influenced by the presence of UVV. These results suggest that root system genotypes might differ in their response to UVV competition, but that it might take a few years to observe significant differences. However, it is still unclear if differences in belowground rootstock-UVV interaction will lead to significant changes in aboveground growth.

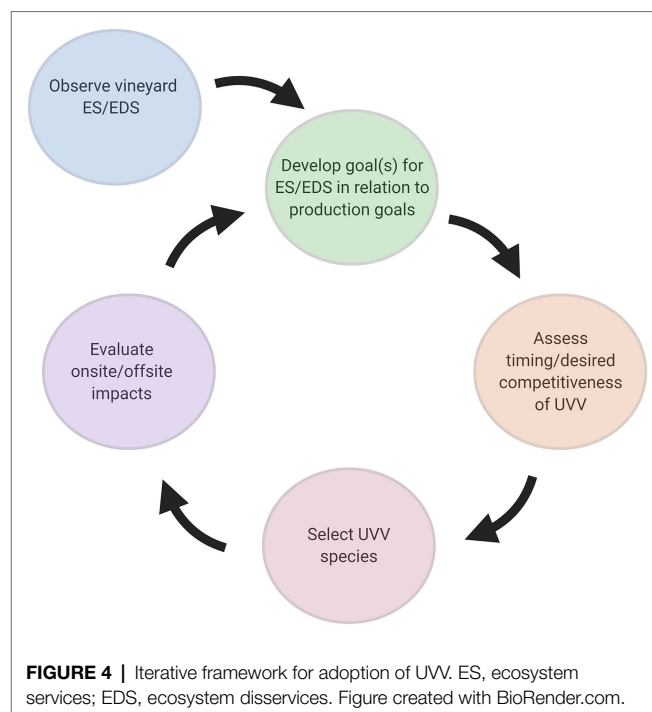
PRACTICAL CONSIDERATIONS FOR FARM ADOPTION

Adoption of UVV in vineyards will require a flexible management plan due to both inter- and intra-annual variation in weather conditions (i.e., temperature and precipitation) and the practitioner's production goals. The balance between ecosystem services and disservices provided by UVV is dynamic and there are a large number of factors that will influence vine response to UVV. Practitioners should carefully consider these factors if deciding to adopt UVV (Figure 4). While factors influencing vine response acclimation to UVV were examined above, they are discussed here in the specific context of their influence on adoption in commercial vineyards.

Species of UVV

Studies on implementation of UVV in environments with high precipitation have investigated both perennial and annual species. In colder climates, annual species were selected since soil is generally hilled above the graft union for winter protection of scion buds on the trunk. Annual species are then replanted after the hill is pulled down in the spring. As a function of the hilling/unhilling, UVV in these vineyards will potentially compete with the vine for a shorter period of time compared to perennial UVV.

Choice of species will determine the competitiveness of the UVV within the context of the site and season. Species range



not only in their competitiveness for water and nutrients, but also in the timing of when they are most competitive. For example, natural vegetation (i.e., managed weed growth) will generally provide almost season-long competition as a function of the diversity in species in the stand (Karl, 2015) although the species that comprise the stand will change by site and year (Jordan, 2014). In contrast, buckwheat establishes early in the growing season (prior to grapevine bloom in the northeastern United States) by easily out-competing weeds and then provides little competition for water and nutrients (Centinari et al., 2016) while chicory can provide intense competition that significantly reduces vine growth through the spring and summer (Jordan, 2014; Chou and Vanden Heuvel, 2019). Perennial grasses can provide enough competition to reduce growth rate throughout the season (Giese et al., 2015) without affecting wine water status in a region with high precipitation (Giese et al., 2014).

Legumes can release N depending on management, which can be an ecosystem service or disservice depending on production goals (Wise and Walter-Peterson, 2018). Release of N from a leguminous cover crop into nearby waterways (Karl et al., 2016a) is clearly an ecosystem disservice; however, the risk of N leaching from various legumes planted as UVV is unknown. Timing and amount of N release from a legume UVV were unpredictable both throughout the growing season and among seasons (Karl et al., 2016a). As the pruning weight of vines with white clover UVV was 30–57% lower than vines maintained with the under-vine herbicide, it is unlikely that a considerable amount N release from the white clover UVV was uptaken by the vines to support their growth (Karl et al., 2016b). The criteria for choosing species of UVV should primarily be based on timing and amount of desired competition

with the vine, ability to establish and grow in the climate, desired height, and ease of management.

UVV Planting and Management

Planting of UVV can be accomplished by hand or mechanically (Wise and Walter-Peterson, 2018; Wolf and Giese, 2020). Mowing is generally required unless dwarf species are well established and can be completed with a dedicated under-vine mower or a combination of row middle mowing and weed whacking (Wise and Walter-Peterson, 2018; Wolf and Giese, 2020). Tall or climbing weeds that reach the grapevine canopy can block sunlight from fruit, potentially reducing ripening and interfering with harvest. Location of the fruiting zone is impacted by the training system and will dictate the need to mow UVV and/or weeds.

As UVV can compete with vines, water and nutrient status must be carefully monitored through the season to ensure vines have the required resources (Wise and Walter-Peterson, 2018). The impact of UVV on pest pressure has not been studied, although Wolf and Giese (2020) warn of potential vole and cutworm damage to vines if vegetation is thick around grapevine trunks.

Factors Affecting UVV Competitiveness and Vine Acclimation

Both vineyard and environmental factors will impact the competitiveness of the UVV with the vine as well as the ability of the vine to adapt to the competitive environment. These factors include vine vigor, vine age, soil properties, and soil nutrient and water availability.

Vigorous vines can withstand greater competition from UVV as vegetative growth of the vine tends to be reduced prior to reproductive growth (Chou and Vanden Heuvel, 2019; Fleishman et al., 2019). Presumably a function of rooting depth and volume as well as carbohydrate and nutrient storage capacity of permanent vine structures (cordons, trunk, and roots), the vegetative growth and reproductive growth of young vineyards are more impacted by UVV; as the vines mature the impacts of UVV are lessened (Hickey et al., 2016).

The physical, chemical, and biological characteristics of the soil will mediate competition for water and nutrients between the vine and the UVV. Soils lower in SOM will provide fewer nutrients and water holding capacity will be reduced compared to high SOM soils, potentially resulting in greater competition between the vine and UVV. Soil depth will impact the ability of the vine root systems to explore a greater soil volume in response to UVV competition, impacting vine nutrient and water status (Kallas, 2017). In vineyards with high SOM and deep soils, competition for resources by UVV is often an ecosystem service.

Adjusting Management Practices

Both water and nutrient management plans may need to be adjusted with the adoption of UVV although interventions

and timings of those interventions will be dependent on soil and environmental conditions. Pre-bloom irrigation may be necessary with the adoption of UVV in drier climates (Coniberti et al., 2018c). In climates with higher precipitation, nutrient additions may be needed, particularly during the critical phase of bloom through veraison (D'Attilio, 2014). Alternate forms of nutrient additions that are less dependent on water uptake – such as foliar applications – should be considered to offset nutrient deficiencies (D'Attilio, 2014). Use of a leguminous UVV cannot reliably increase N concentrations in vine vegetative tissue (Karl et al., 2016a) as sometimes only a small proportion of N from decomposing legumes is taken up by the vine (Brunetto et al., 2011). Wise and Walter-Petersen (2018) characterize the release of N from a clover used as a UVV as unpredictable.

Impacts on Fruit Composition and Wine Sensory Perception

The impact of UVV on fruit composition and wine sensory perception would likely be indirect, with flavor and aroma compounds potentially impacted by plant adaptation to UVV through changes in berry size, leaf area to fruit ratio, fruiting zone microclimate (light exposure and temperature), and soil resource availability. The ability to study the impact of UVV on wine sensory characteristics has been hampered by the use of laboratory-style winemaking practices (i.e., chaptalization to standard sugar levels and lack of oak) as opposed to commercial fermentations. Nonetheless, a handful of studies have investigated the impact of UVV on consumers' ability to differentiate resulting wines but the results are inconsistent among years and studies (Jordan et al., 2016; Karl et al., 2016b; Coniberti et al., 2018a).

Cost of Adoption

Adopting and maintaining UVV includes the following potential costs: site preparation, seed, planting, mowing, and additional irrigation and fertilization. However, savings for producers may be realized through elimination of herbicide application and/or cultivation as well as reduced need for canopy management (e.g., hedging). Labor costs of establishment and maintenance of under-vine bare soil compared to UVV are difficult to gauge as it depends on the cover crop species (i.e., cost of seeds and rate of seeding) used and its management needs, such as number of herbicide applications, cultivations, or mowing practices. Specific information on the cost of adoption and maintenance of UVV is sparse. Karl et al. (2016b) estimated that the cost of adoption and maintenance of UVV was around \$84 and \$169 per hectare for natural vegetation and white clover, respectively, compared to herbicide (glyphosate) and cultivation which was \$548 and \$1,036 per hectare, respectively.

Reduction in vegetative growth induced by UVV might require less labor-intensive canopy management practices, such as leaf removal and hedging. Labor savings can be hard to quantify, but a study conducted on Cabernet Sauvignon

in a humid climate indicated that vines growing with a perennial fescue as UVV had smaller canopies, which were hedged in about half of the time compared to vines maintained with under-vine herbicide which were more vigorous (Hill, 2017). Similarly, time needed for leaf removal was reduced by 28% in UVV plots compared to herbicide plots. Use of UVV, however, can have a negative impact on economic returns if yield is significantly reduced. When differing under-vine management practices were implemented in the third year after planting on Cabernet Franc vines in the Finger Lakes region of New York State, yield was diminished from 11.5 t/ha in vines maintained with an under-vine herbicide to 8.4 t/ha in vines growing with white clover as UVV (Karl et al., 2016b).

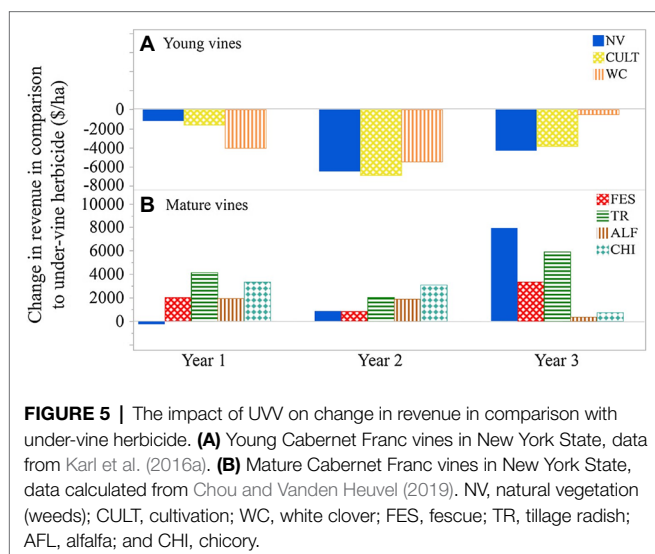
A partial budget analysis could be used to estimate the financial implications of using UVV over more traditional under-vine management practices (Figure 5; Karl et al., 2016b). For example, when crop value per hectare was considered with the cost of planting and maintaining UVV, plots with under-vine herbicide had the highest economic return in a young vineyard (Figure 5A) when yield was reduced by early UVV implementation. However, when 15-year-old Cabernet Franc vines were subjected to three under-vine treatments over a three-year period in the same region, yield was either unaffected or increased significantly through the use of UVV, resulting in a positive impact on revenue per hectare (Figure 5B). While partial budgeting suggests that use of UVV has the potential to increase economic returns, additional benefits may arise from the ability for producers to market their wines with sustainability characteristics for quality differentiation (Schaufele and Hamm, 2017). Recent research suggests that consumers' willingness to pay for wines may increase if a certification that takes into account vineyard biodiversity is on the bottle (Mazzocchi et al., 2019); wine consumers might also not be dissuaded by a modest price increase (\$1 per bottle) if the wine was made with environmentally friendly farm practices, such as

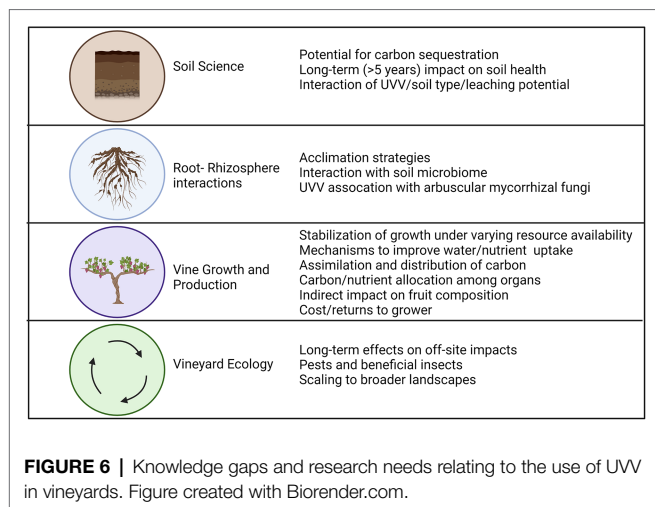
UVV (Kelley et al., 2017). Further analyses that consider economic costs and returns to the producer are needed to encourage adoption of UVV in appropriate regions.

CONCLUSION

Adapting management strategies in the face of climate change are critical for maintaining and improving wine grape production worldwide. Several studies have demonstrated the potential of UVV to preserve soil health in grape growing regions with fertile soil and increasingly excessive precipitation, while also reducing herbicide input and excessive vine growth. This review discussed progress in several research areas which could help explain effects on vine growth and production and vineyard ecosystems (Figure 6). Many UVV species trialed to date can improve several parameters of soil health although long-term (>5 years) effects are still unknown. UVV effects on vine growth and productivity remain less predictable, but some similarities in vine responses to UVV competition have been identified across studies, such as a stronger reduction in vine vegetative rather than reproductive growth and greater UVV effects on vine nutrient rather than water status in regions with ample precipitation. There is also growing evidence of an age-dependent response of vines to UVV competition and that vines are able to redistribute their fine roots to areas of lower competition that are not highly colonized by UVV roots.

To promote widespread adoption of UVV in this changing climate, practitioners need guidelines on under-vine management options that best serve their production goals while maximizing the number of ecosystem services provided. Numerous knowledge gaps still exist which might prevent practitioners from more clearly predicting vine response and acclimation to UVV over the years. Figure 6 summarizes important research needs that were identified throughout the review. Although they were classified by discipline or research area, the research needs are cross-disciplinary with required approaches spanning from soil science through plant ecophysiology to crop production to address these knowledge gaps. A transdisciplinary approach is critical for linking shifts of root distribution in response to UVV to changes in soil environment (e.g., resources and microbiome), vine functioning, and fruit composition. For instance, an integrated approach will help clarify if deeper root systems of vines growing with UVV, which has been reported in several studies, can stabilize vine productivity under more erratic rainfall patterns (i.e., more intensive rainfall alternated by dry periods) associated with climate change. Furthermore, there is little evidence that vines growing with UVV can improve efficiency of resource uptake by modifying morphological root traits. Work in this area is still limited with contrasting results likely due to differing vine age, root system genotype (i.e., rootstock), and the time of vine UVV coexistence, among other reasons. Future work should explore if grapevines exhibit mechanisms which will improve nutrient or water uptake capacity in a highly competitive soil environment, and if these mechanisms evolve over the years, allowing vines





to acclimate to the presence of UVV and maintain above ground growth and/or production despite a smaller root system.

AUTHOR CONTRIBUTIONS

JV and MC developed the concept and wrote the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

- Abad, J., Marin, D., Santesteban, L. G., Cibrián, J. F., and Sagüés, A. (2020). Under-vine cover crops: impact on weed development, yield and grape composition. *OENO One* 54, 975–983. doi: 10.20870/oeno-one.2020.54.4.4149
- Anderson, L. J., Comas, L. H., Lakso, A. N., and Eissenstat, D. M. (2003). Multiple risk factors in root survivorship: a 4-year study in Concord grape. *New Phytol.* 158, 489–501. doi: 10.1046/j.1469-8137.2003.00757.x
- Atucha, A., Merwin, I. A., Brown, M. G., Gardiazabal, F., Mena, F., Adriazola, C., et al. (2013). Root distribution and demography in an avocado (*Persea americana*) orchard under groundcover management systems. *Funct. Plant Biol.* 40, 507–515. doi: 10.1071/FP12317
- Battany, M. C., and Grismer, M. E. (2000). Rainfall runoff and erosion in Napa Valley vineyards: effects of slope, cover and surface roughness. *Hydrol. Process.* 14, 1289–1304. doi: 10.1002/(SICI)1099-1085(200005)14:7<1289::AID-HYP43>3.0.CO;2-R
- Brunetto, G., Ventura, M., Scandellari, F., Ceratta, C. A., Kaminski, J., Wellington de Melo, G., et al. (2011). Nutrient release during the decomposition of mowed perennial ryegrass and white clover and its contribution to nitrogen nutrition of grapevine. *Nutr. Cycl. Agroecosyst.* 90, 299–308. doi: 10.1007/s10705-011-9430-8
- Burns, K. N., Kluepfel, D. A., Strauss, S. L., Bokulich, N. A., Cantu, D., and Steenwerth, K. L. (2016). Vineyard soil bacterial diversity and composition revealed by 16s rRNA genes: differentiation by geographic features. *Soil Biol. Biochem.* 91, 232–247. doi: 10.1016/j.soilbio.2015.09.002
- Celette, F., Findeling, A., and Gary, C. (2009). Competition for nitrogen in an unfertilized intercropping system: the case of an association of grapevine and grass cover in a Mediterranean climate. *Eur. J. Agron.* 30, 41–51. doi: 10.1016/j.eja.2008.07.003
- Celette, F., Gaudin, R., and Gary, C. (2008). Spatial and temporal changes to the water regime of a Mediterranean vineyard due to the adoption of cover cropping. *Eur. J. Agron.* 29, 153–162. doi: 10.1016/j.eja.2008.04.007
- Celette, F., Wery, J., Chantelot, E., Celette, J., and Gary, C. (2005). Belowground interactions in a vine (*Vitis vinifera* L.)-tall fescue (*Festuca arundinacea* Shreb.) intercropping system: water relations and growth. *Plant Soil* 276, 205–217. doi: 10.1007/s11104-005-4415-5
- Centinari, M., Vanden Heuvel, J. E., Goebel, M., and Bauerle, T. L. (2016). Root-zone management practices impact above and below-ground growth in cabernet franc grapevines. *Aust. J. Grape Wine Res.* 22, 137–148. doi: 10.1111/ajgw.12162
- Chou, M., and Vanden Heuvel, J. E. (2019). Under-vine cover crops reduce vine vigor without reducing yield in cabernet franc. *Am. J. Enol. Vitic.* 70, 98–108. doi: 10.5344/ajev.2018.18037
- Chou, M., Vanden Heuvel, J. E., Bell, T. H., Panke-Buisse, K., and Kao-Kniffen, J. (2018). Vineyard under-vine floor management alters soil microbial composition, while the fruit microbiome shows no corresponding shifts. *Sci. Rep.* 8:11039. doi: 10.1038/s41598-018-29346-1
- Comas, L. H., Bauerle, T. L., and Eissenstat, D. M. (2010). Biological and environmental factors controlling root dynamics and function: effects of root ageing and soil moisture. *Aust. J. Grape Wine Res.* 16, 131–137. doi: 10.1111/j.1755-0238.2009.00078.x
- Coniberti, A., Ferrari, V., Disegna, E., Dellacassa, E., and Lakso, A. N. (2018a). Under-trellis cover crop and deficit irrigation to regulate water availability and enhance Tannat wine sensory attributes in a humid climate. *Sci. Hort.* 235, 244–252. doi: 10.1016/j.scienta.2018.03.018
- Coniberti, A., Ferrari, V., Disegna, E., Garcia Petillo, M., and Lakso, A. N. (2018b). Complete vineyard floor cover crop to reduce grapevine susceptibility to bunch rot. *Eur. J. Agron.* 99, 167–176. doi: 10.1016/j.eja.2018.07.006
- Coniberti, A., Ferrari, V., Disegna, E., Garcia Petillo, M., and Lakso, A. N. (2018c). Under-trellis cover crop and planting density to achieve vine balance in a humid climate. *Sci. Hort.* 227, 65–74. doi: 10.1016/j.scienta.2017.09.012
- Costello, M. J., and Daane, K. M. (2003). Spider and leafhopper (*Erythroneura* spp.) response to vineyard ground cover. *Environ. Entomol.* 32, 1085–1098. doi: 10.1603/0046-225X-32.5.1085
- Delpuech, X., and Metay, A. (2018). Adapting cover crop soil coverage to soil depth to limit competition for water in a Mediterranean vineyard. *Eur. J. Agron.* 97, 60–69. doi: 10.1016/j.eja.2018.04.013

FUNDING

This manuscript is partially based on a series of studies that were supported by the Towards Sustainability Foundation, Lacroute, Kaplan, and Saltonstall endowments at Cornell AgriTech, National Institute of Food and Agriculture (NIFA), US Department of Agriculture (USDA) under agreement no. 2010-51181-21599, USDA NIFA Foundational program (Accession number 1014758) and the USDA NIFA Federal Appropriation under project PEN0 4628 and Accession number 1014131, as well as through the Northeast Sustainable Agriculture Research and Education program under subaward numbers GNE16-119, GNE13-062, and LNE17-360, New York State Department of Agriculture and Markets Specialty Crop Block Grant Program, the Pennsylvania Wine Marketing and Research Program, and The Pennsylvania State University Undergraduate Research Grant Program.

ACKNOWLEDGMENTS

The authors thank Steve Lerch, Michael Brown, Mariam Berdeja, and Don Smith for technical assistance, former and present graduate students (Ming-Yi Chou, Suzanne Fleishman, Rebecca Herveux, Lindsay Jordan, Raquel Kallas, Adam Karl, Anne Klodd, and Taylor Mattus), and their collaborators Drs. Tony Wolf, Ian Merwin, Taryn Bauerle, Thomas Bjorkman, and David Eissenstat.

- DeVetter, L. W., Dilley, C. A., and Nonnecke, G. R. (2015). Mulches reduce weeds, maintain yield, and promote soil quality in a continental-climate vineyard. *Am. J. Enol. Vitic.* 66, 54–64. doi: 10.5344/ajev.2014.14064
- D'Attilio, D. (2014). Optimizing nitrogen fertilization practices under intensive vineyard cover cropping floor management systems. dissertation/master's thesis. Blacksburg, VA: Virginia Polytechnic Institute and State University.
- English-Loeb, G., Rhainds, M., Martinson, T., and Ugine, T. (2003). Influence of flowering cover crops on *Anagrus* parasitoids (hymenoptera: Mymaridae) and *Erythroneura* leafhoppers (Homoptera: Cicadellidae) in New York vineyards. *Agr. For. Ent.* 5, 173–181. doi: 10.1046/j.1461-9563.2003.00179.x
- Epstein, E., and Grant, W. J. (1973). "Soil crust formation as affected by raindrop impact" in *Physical Aspects of Soil Water and Salts in Ecosystems*. eds. A. Hadas, D. Swartzendruber, P. E. Rijtema, M. Fuchs and B. Yaron (Springer-Verlag, Berlin, Germany), 195–201.
- Fleishman, S. M., Bock, H. W., Eissenstat, D. M., and Centinari, M. (2021). Undervine groundcover substantially increases shallow but not deep soil carbon in a temperate vineyard. *Agric. Ecosyst. Environ.* 313:107362. doi: 10.1016/j.agee.2021.107362
- Fleishman, S. M., Eissenstat, D. M., and Centinari, M. (2019). Rootstock vigor shifts aboveground response to groundcover competition in young grapevines. *Plant Soil* 440, 151–165. doi: 10.1007/s11104-019-04059-0
- García, L., Celette, F., Gary, C., Ripoche, A., Valdés-Gómez, H., and Metay, A. (2018). Management of service crops for the provision of ecosystem services in vineyards: a review. *Agric. Ecosyst. Environ.* 251, 158–170. doi: 10.1016/j.agee.2017.09.030
- García-Díaz, A., Bienes, R., Sastre, B., Novara, A., Gristina, L., and Cerdà, A. (2017). Nitrogen losses in vineyards under different types of soil groundcover. A field runoff simulator approach in Central Spain. *Agric. Ecosyst. Environ.* 236, 256–267. doi: 10.1016/j.agee.2016.12.013
- Giese, G., Velasco-Cruz, C., Roberts, L., Heitman, J., and Wolf, T. K. (2014). Complete vineyard floor cover crops favorably limit grapevine vegetative growth. *Sci. Hort.* 170, 256–266. doi: 10.1016/j.scienta.2014.03.011
- Giese, G., Wolf, T. K., Velasco-Cruz, C., and Roberts, L. (2016). Cover crop and root pruning effects on the rooting pattern of SO4 rootstock grafted to Cabernet sauvignon. *Am. J. Enol. Vitic.* 67, 105–115. doi: 10.5344/ajev.2015.15066
- Giese, G., Wolf, T. K., Velasco-Cruz, C., Roberts, L., and Heitman, J. (2015). Cover crop and root pruning impacts on vegetative growth, crop yield components, and grape composition of cabernet sauvignon. *Am. J. Enol. Vitic.* 66, 212–226. doi: 10.5344/ajev.2014.14100
- Guilpart, N., Roux, S., Gary, C., and Metay, A. (2017). The trade-off between grape yield and grapevine susceptibility to powdery mildew and grey mould depends on inter-annual variations in water stress. *Agric. For. Meteorol.* 234, 203–211. doi: 10.1016/j.agrformet.2016.12.023
- Hatch, T. A., Hickey, C. C., and Wolf, T. K. (2011). Cover crop, rootstock, and root restriction regulate vegetative growth of cabernet sauvignon in a humid environment. *Am. J. Enol. Vitic.* 62, 298–311. doi: 10.5344/ajev.2011.11001
- Hickey, C. C., Hatch, T. A., Stallings, J., and Wolf, T. K. (2016). Under-trellis cover crop and rootstock affect growth, yield components, and fruit composition of cabernet sauvignon. *Am. J. Enol. Vitic.* 67, 281–295. doi: 10.5344/ajev.2016.15079
- Hill, B. T. (2017). Root restriction, under-trellis cover cropping, and rootstock modify vine size and berry composition of Cabernet Sauvignon. dissertation/master's thesis. Blacksburg, VA: Virginia Tech University.
- Hosseinzadehtalaei, P., Tabari, H., and Willems, P. (2020). Climate change impact on short-duration extreme precipitation and intensity-duration-frequency curves over Europe. *J. Hydrol.* 590:125249. doi: 10.1016/j.jhydrol.2020.125249
- Howarth, M. E., Thornicroft, C. D., and Bosart, L. H. (2019). Changes in extreme precipitation in the Northeast United States: 1979–2014. *J. Hydrometeorol.* 20, 673–689. doi: 10.1175/JHM-D-18-0155.1
- Jordan, L. M. (2014). Evaluating the effects of using annually established under-vine cover crops in Northeastern Riesling vineyards. dissertation/master's thesis. Ithaca, NY: Cornell University.
- Jordan, L. M., Bjorkman, T. J., and Vanden Heuvel, J. E. (2016). Using under-vine cover crops did not impact vine growth or fruit composition of mature cool climate 'Riesling' grapevines. *HortTechnology* 26, 36–45. doi: 10.21273/HORTTECH.26.1.36
- Kallas, R. F. (2017). Evaluating the effects of under-vine cover crops on Noiret vines in a commercial vineyard in New York State. dissertation/master's thesis. Ithaca, NY: Cornell University.
- Karl, A. D. (2015). Impact of under-vine management in a Finger Lakes Cabernet Franc vineyard. dissertation/master's thesis. Ithaca, NY: Cornell University.
- Karl, A. D., Merwin, I. M., Brown, M. G., Hervieux, R., and Vanden Heuvel, J. E. (2016a). Under-vine management impacts soil properties and leachate composition in a New York state vineyard. *HortScience* 51, 941–949. doi: 10.21273/HORTSCI.51.7.941
- Karl, A. D., Merwin, I. M., Brown, M. G., Hervieux, R., and Vanden Heuvel, J. E. (2016b). Impact of under-vine management on vine growth, yield, fruit composition, and wine sensory analyses of Cabernet franc. *Am. J. Enol. Vitic.* 67, 269–280. doi: 10.5344/ajev.2016.15061
- Kelley, K. M., Zelinskie, J., Centinari, M., Gardner, D. M., Govindasamy, R., Hyde, J., et al. (2017). Consumer preferences for sustainable wine attributes: a conjoint analysis. *J. Wine Econ.* 12, 416–425. doi: 10.1017/jwe.2017.40
- Klodt, A. E., Eissenstat, D. M., Wolf, T. K., and Centinari, M. (2016). Coping with cover crop competition in mature grapevines. *Plant Soil* 400, 391–402. doi: 10.1007/s11104-015-2748-2
- Letourneau, D. K., Armbrrecht, I., Salguero Rivera, B., Montoya Lerma, J., Jimenez Carmona, E., Constanza Daza, M., et al. (2011). Does plant diversity benefit agroecosystems? A synthetic review. *Ecol. Appl.* 21, 9–21. doi: 10.1890/09-2026.1
- Madsen, H., Lawrence, D., Lang, M., Martinkova, M., and Kjeldsen, T. R. (2014). Review of trend analysis and climate change projections of extreme precipitation and floods in Europe. *J. Hydrol.* 519, 3634–3650. doi: 10.1016/j.jhydrol.2014.11.003
- Maina, G. G., Brown, J. S., and Gersani, M. (2002). Intra-plant versus inter-plant root competition in beans: avoidance, resource matching or tragedy of the commons. *Plant Ecol.* 160, 235–247. doi: 10.1023/A:1015822003011
- Mazzocchi, C., Ruggeri, G., and Corsi, S. (2019). Consumers' preferences for biodiversity in vineyards: a choice experiment on wine. *Wine Econ. Policy* 8, 155–164. doi: 10.1016/j.wep.2019.09.002
- Merfield, C. N. (2019). Vineyard floor management: a sustainability nexus with a focus on undervine weeding. The BHU Future Farming Centre Report 04–19. Available at: <https://documentcloud.adobe.com/link/track?uri=urn:aa:scds:US:fb048a15-9973-47a3-b2d8-84b3a4ab66a8#pageNum=1> (Accessed May 5, 2021).
- Penfold, C., and Howie, J. (2019). Under-vine cover cropping. *Wine Australia Factsheet*. Available at: https://www.wineaustralia.com/getmedia/384c2ac3-b0b1-4c7b-9c7c-a8904090a69b/CORD_Factsheets_CoverCropsUndervine_V2.pdf (Accessed May 5, 2021).
- Radville, L., Bauerle, T. L., Comas, L. H., Marchetto, K. A., Lakso, A. N., Smart, D. R., et al. (2016). Limited linkages of aboveground and belowground phenology: a study in grape. *Am. J. Bot.* 103, 1897–1911. doi: 10.3732/ajb.1600212
- Rustad, L. E., Campbell, J. L., Marion, G. M., Norby, R. J., Mitchell, M. J., Hartley, A. E., et al. (2001). A metaanalysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126, 543–562. doi: 10.1007/s004420000544
- Schaufele, I., and Hamm, U. (2017). Consumers' perceptions, preferences and willingness-to-pay for wine with sustainability characteristics: a review. *J. Clean. Prod.* 147, 379–394. doi: 10.1016/j.jclepro.2017.01.118
- Schreiner, R. P., Lee, J., and Skinkis, P. A. (2013). N, P, and K supply to pinot noir grapevines: impact on vine nutrient status, growth, physiology, and yield. *Am. J. Enol. Vitic.* 64, 26–38. doi: 10.5344/ajev.2012.12064
- Smart, D. R., Schwass, E., Lakso, A., and Morano, L. (2006). Grapevine rooting patterns: a comprehensive analysis and a review. *Am. J. Enol. Vitic.* 57, 89–104.
- Steenwerth, K., and Belina, K. M. (2008). Cover crops enhance soil organic matter, carbon dynamics and microbiological function in a vineyard agroecosystem. *Appl. Soil Ecol.* 40, 359–369. doi: 10.1016/j.apsoil.2008.06.006
- Steenwerth, K., and Belina, K. M. (2010). Vineyard weed management practices influence nitrate leaching and nitrous oxide emissions. *Agric. Ecosyst. Environ.* 138, 127–131. doi: 10.1016/j.agee.2010.03.016

- Tinker, P. B., and Nye, P. H. (2000). *Solute Movement in the Rhizosphere*. New York: Oxford University Press, 370.
- U.S. Global Change Research Program (2014). Available at: <https://www.c2es.org/content/extreme-precipitation-and-climate-change/> (Accessed May 5, 2021).
- Valdes-Gomez, H., Fermaud, M., Roudet, J., Colonnec, A., and Gary, C. (2008). Grey mould incidence is reduced on grapevine with lower vegetative and reproductive growth. *Crop Prot.* 27, 1174–1186. doi: 10.1016/j.cropro.2008.02.003
- Vukicevich, E., Lowery, T., Bowen, P., Urbez-Torres, J. R., and Hart, M. (2016). Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. *Agron. Sustain. Dev.* 36:48. doi: 10.1007/s13593-016-0385-7
- Wise, A., and Walter-Peterson, H. (2018). Expanding the use of under-vine cover crops in New York vineyards. Appellation Cornell, 2018-2. Available at: <https://grapesandwine.cals.cornell.edu/sites/grapesandwine.cals.cornell.edu/files/shared/Research%20Focus%202018-2%20May.pdf> (Accessed May 5, 2021).
- Wolf, T., and Giese, G. (2020). Floor management strategies for Virginia vineyards. Virginia Cooperative Extension, Publication SPES-209. Available at: https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/spes/spes-209/SPES-209.pdf (Accessed May 5, 2021).
- Yao, S., Merwin, I. A., and Brown, M. G. (2009). Apple root growth, turnover, and distribution under different orchard groundcover management systems. *Hortic. Sci.* 44, 168–175. doi: 10.21273/HORTSCI.44.1.168
- Zarraonaindia, I., Owens, S., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., et al. (2015). The soil microbiome influences grapevine-associated microbiota. *mBio* 6:e02527. doi: 10.1128/mBio.02527-14
- Zolina, O. (2012). “Changes in intense precipitation in Europe,” in *Changes in Flood Risk in Europe, IAHS Special Publication 10*. ed. Z. W. Kundzewicz (International Association of Hydrological Sciences and CRC Press/Balkema), 97–120.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Vanden Heuvel and Centinari. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Melatonin Relieves Ozone Stress in Grape Leaves by Inhibiting Ethylene Biosynthesis

Chuang Liut, Hui Kang†, Yafang Wang, Yuxin Yao, Zhen Gao* and Yuanpeng Du*

State Key Laboratory of Crop Biology, Collaborative Innovation Center of Fruit & Vegetable Quality and Efficient Production in Shandong, College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an, China

OPEN ACCESS

Edited by:

Chris Winefield,
Lincoln University, New Zealand

Reviewed by:

Tse-Min Lee,
National Sun Yat-sen University,
Taiwan
Weiqiang Li,
RIKEN, Japan

*Correspondence:

Yuanpeng Du
duyuanpeng001@163.com
Zhen Gao
gaoz89@sda.edu.cn

†These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 30 April 2021

Accepted: 30 June 2021

Published: 28 July 2021

Citation:

Liu C, Kang H, Wang Y, Yao Y,
Gao Z and Du Y (2021) Melatonin
Relieves Ozone Stress in Grape
Leaves by Inhibiting Ethylene
Biosynthesis.
Front. Plant Sci. 12:702874.
doi: 10.3389/fpls.2021.702874

Ozone (O₃) stress severely affects the normal growth of grape (*Vitis vinifera* L.) leaves. Melatonin (MT) plays a significant role in plant response to various abiotic stresses, but its role in O₃ stress and related mechanisms are poorly understood. In order to understand the mechanism of MT in alleviate O₃ stress in grape leaves, we perform a transcriptome analyses of grapes leaves under O₃ stress with or without MT treatment. Transcriptome analysis showed that the processes of ethylene biosynthesis and signaling were clearly changed in “Cabernet Sauvignon” grapes under O₃ and MT treatment. O₃ stress induced the expression of genes related to ethylene biosynthesis and signal transduction, while MT treatment significantly inhibited the ethylene response mediated by O₃ stress. Further experiments showed that both MT and aminoethoxyvinylglycine (AVG, an inhibitor of ethylene biosynthesis) enhanced the photosynthetic and antioxidant capacities of grape leaves under O₃ stress, while ethephon inhibited those capacities. The combined treatment effect of MT and ethylene inhibitor was similar to that of MT alone. Exogenous MT reduced ethylene production in grape leaves under O₃ stress, while ethephon and ethylene inhibitors had little effect on the MT content of grape leaves after O₃ stress. However, overexpression of *VvACO2* (1-aminocyclopropane-1-carboxylate oxidase2) in grape leaves endogenously induced ethylene accumulation and aggravated O₃ stress. Overexpression of the MT synthesis gene *VvASMT1* (acetylserotonin methyltransferase1) in tobacco (*Nicotiana tabacum* L.) alleviated O₃ stress and reduced ethylene biosynthesis after O₃ stress. In summary, MT can alleviate O₃ stress in grape leaves by inhibiting ethylene biosynthesis.

Keywords: grape leaves, melatonin, ozone stress, ethylene, antioxidant capacity

INTRODUCTION

Ozone (O₃) in the troposphere is a highly oxidizing atmospheric pollutant. Elevated O₃ concentration severely affects the growth and development of plants (Serengil et al., 2011), as well as human health (Karnosky et al., 2007; Borowiak, 2013). At present, the near-surface O₃ concentration is increasing at an annual rate of 0.5–2.0% (Vingarzan, 2004) and is projected to increase by 40–60% at the end of the 21st century, when the tropospheric O₃ concentration will reach 80 nL L⁻¹ (Fiscus et al., 2005). O₃ stress induces the release of large amounts of ethylene from leaf stomata, the damage of plant leaves caused by O₃ is correlated with the release of

ethylene (Tingey et al., 1976; Mehlhorn and Wellburn, 1987). As an important signal molecule, ethylene plays an important role in plant response to abiotic stress (Zhang M. et al., 2016). Ethylene biosynthesis begins with the formation of S-adenosyl-L-methionine (SAM) from methionine by SAM synthetase. Then, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) catalyzes SAM to produce ACC, and ACC oxidase (ACO) oxidizes ACC to ethylene (Najeeb et al., 2018).

Grapes (*Vitis vinifera* L.) are an important fruit crop grown worldwide. Previous investigation suggested that O₃ stress adversely affects the photosynthetic system of grape (cultivar Cabernet Sauvignon) leaves, and exogenous MT treatment can alleviate O₃ stress (Geng et al., 2016; Chen et al., 2020). Grapes and other plants have evolved various strategies to withstand abiotic stresses, such as regulating interactive hormone networks, including MT and ethylene (Arnao and Hernández-Ruiz, 2015; Ryu and Cho, 2015; Nguyen et al., 2016).

Melatonin is an indole derivative of tryptophan that is ubiquitous in plants and animals and has high efficiency, conservation, and strong antioxidant effects (Galano et al., 2011; Yang et al., 2018). MT synthesis in plants requires the participation of many enzymes, and the last step of the reaction is catalyzed by acetylserotonin o-methyltransferase (ASMT) (Kang et al., 2011). MT is an essential plant growth regulator, and both external application and endogenous induction can improve plant tolerance to drought, salinity, and other abiotic stresses (Zuo et al., 2014; Arnao and Hernández-Ruiz, 2015). For example, exogenous MT treatment can enhance the antioxidant capacity of cabbage (*Brassica oleracea* L.) (Zhang N. et al., 2016) and tea tree (*Camellia sinensis* L.) (Li et al., 2021) by increasing their anthocyanin content. In apple (*Malus domestica*), MT can regulate reactive oxygen species (ROS) signaling and activate the CBL1-CIPK23 (calcineurin B-like 1-interacting protein kinases23) pathway to regulate the expression of potassium channel protein genes, thereby improving salt tolerance (Li et al., 2016). Furthermore, exogenous MT can reduce the ion poisoning of mushrooms (*Agaricus campestris*) (Gao et al., 2020) and wheat (*Triticum aestivum* L.) (Al-Huqail et al., 2020). MT can also interact with other plant hormones [abscisic acid (ABA), jasmonic acid, salicylic acid, ethylene, etc.] to form a significant component of the plant immune system (Arnao and Hernández-Ruiz, 2018). For example, MT can increase GA (gibberellin) and reduce ABA content by regulating the expression of GA and ABA synthesis-related genes in cucumber seedlings and alleviating the inhibitory effect of a high salt environment on seedlings (Zhang et al., 2014). In *Arabidopsis thaliana*, MT reduces root meristem size by inhibiting auxin synthesis and polar transport (Wang Q. et al., 2016) and interacts with ethylene signaling pathways to improve disease resistance (Lee et al., 2014).

So far, the relationship between MT and other signaling molecules under abiotic stress is obscure, especially under O₃ stress. Thus, this experiment has explored the key metabolic changes caused by increasing the MT content in grape leaves under O₃ stress and its possible action mechanism. This research will promote the application of MT in improving the O₃ tolerance of grapes and reveal the potential molecular mechanism of MT in regulating other signal molecules under O₃ stress.

MATERIALS AND METHODS

O₃ Fumigation System

Two O₃ fumigation systems were set up in the school vineyard (36°11'N, 117°06'E), which were divided into four parts, including open-top air chambers (OTCs), gas supply systems, O₃ generation system, and O₃ concentration monitoring system (Geng et al., 2017). The mainframe of the OTC is composed of galvanized steel pipes with a 3 cm diameter and is divided into two parts: the lower part is a regular octagonal prism with length and height of 1.1 and 2.2 m. The upper part is a regular octagonal pedestal; the area of the upper base of the pedestal is one in third of the area of the lower base, and the angle between the side and the vertical is 45°. The top is open to the atmosphere, and the sides are covered with particular polyethylene plastic film for the greenhouse; the outside is covered with a sunshade net. The installation height of the LED light source (LED cold light source plant light, SP501-N, 405 W, Shanghai Sanhao Electromechanical Co., Ltd.) is 1.5 m. To ensure the stability of the gas concentration in the OTC, the gap between the exhaust ports is gradually reduced from the center of the OTC to the four sides. The O₃ generating system (WJ-HY5, Jinan Sankang) is a high-frequency O₃ generator. The oxygen intake of the O₃ generator can be modulated by adjusting the rotor flowmeter to control the O₃ concentration. The O₃ concentration monitor (DR70C-O₃ type) in the OTC was used to measure the O₃ concentration in real-time and transmits the data to the computer for observation and storage.

Plant Materials, Growth Conditions, and Experimental Treatments

Two-year-old potted seedlings of grapevine cultivar “Cabernet Sauvignon” were used to explore the effects of exogenous MT and ethylene on grape leaves under O₃ stress. Cuttings were planted in cylindrical pots with a diameter of 25 cm and a height of 35 cm (substrate:sand:soil = 2:1:1). The potted seedlings were cultivated in a greenhouse. When the new shoot leaves grew to 10–12 pieces, the plants with the same growth potential were selected and treated with water, 50 μM MT (Xu et al., 2019), 250 mg L⁻¹ ethephon (Ma et al., 2021), or 2 μM aminoethoxyvinylglycine (AVG) (Xu et al., 2019) every 2 days at 6 p.m. (three times in total), and each treatment (1.5 L) was replicated in five plants. After that, the plants were exposed to 110 nL L⁻¹ O₃ for 3 h at 800 μmol m⁻² s⁻¹ light intensity at 8 a.m. (Geng et al., 2016, 2017).

All treatments were as follows: The roots were irrigated with clean water and the leaves sprayed with clean water without O₃ treatment (control); the roots were irrigated with clean water, the leaves sprayed with clean water, and then plants were exposed to O₃ (O₃); The roots were irrigated with 50 μM MT, the leaves sprayed with water, and then plants were exposed to O₃ (MT + O₃); the leaves were sprayed with 250 mg L⁻¹ ethephon, the roots irrigated with clear water, and then plants were exposed to O₃ (Ethephon + O₃); The leaves were sprayed with 2 μM AVG, the roots irrigated with clean water, and then plants were exposed to O₃ (AVG + O₃); the roots were irrigated with 50 μM

MT, the leaves sprayed with 2 μM AVG, and then plants were exposed to O_3 (MT + AVG + O_3). After the treatment, leaves with similar nodes and sizes were selected for RNA-Seq analysis and determination of physiological indexes.

“Cabernet Sauvignon” tissue culture seedlings were used to evaluate the effect of increasing endogenous ethylene content on grape leaves under O_3 stress. Healthy apical growth tips of “Cabernet Sauvignon” vines were removed in early summer to establish grapevine *in vitro* shoot cultures. The plant materials were sterilized (75% alcohol for 2 min, 4% sodium hypochlorite for 15 min) and cultured on MS medium supplemented with 30 g L^{-1} sucrose, 7.5 g L^{-1} agar powder, and 0.2 mg L^{-1} indole-3-butyric acid (IBA). The plants were kept in a growth chamber maintained at 25/20°C, with a photoperiod of 16 h light/8 h dark, and branches with at least one bud and leaf were used for subculture every month. Healthy 2-month-old seedlings with consistent growth were selected for infection treatment. The tissue culture bottle caps were opened one week before treatment adapt the seedlings to the external environment gradually.

Chlorophyll Fluorescence Imaging and Determination of Related Enzyme Activities and Physiological Indexes

Rapid chlorophyll fluorescence imaging of grape leaves was performed using a fluorescence imaging system (PSI, Czechia). Hydrogen peroxide (H_2O_2) contents in leaves were estimated using the trichloroacetic acid (TCA) method at 390 nm (Velikova et al., 2000). Superoxide radical ($\text{O}_2^{\cdot-}$) was measured as described by Elstner and Heupel (1976) by monitoring the nitrite formation from hydroxylamine in the presence of $\text{O}_2^{\cdot-}$. The tissue staining methods of H_2O_2 and $\text{O}_2^{\cdot-}$ were according to Thordal-Christensen et al. (1997) and Orozco-Cardenas and Ryan (1999), respectively. Reduced ascorbic acid (AsA) content was measured by bipyridine colorimetry, while reduced glutathione (GSH) was determined using 5,5'-Dithio-bis (2-nitrobenzoic acid) (DTNB) (Zhou et al., 2005). The total glutathione content was determined by the method of previous described (Kosugi and Kikugawa, 1985). Oxidized glutathione (GSSG) content was calculated by the difference between total glutathione content and GSH content, and then GSH/GSSG value was obtained. Determination of superoxide dismutase (SOD) was by photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries, 1977). The SOD activity unit U was 50% inhibition of NBT photochemical reduction. Peroxidase (POD) activity was determined by monitoring the increase in absorbance at 470 nm, caused by guaiacol oxidation (Scebba et al., 2001). One unit of POD activity was defined as the change of A470 by 0.01 per min. The activity of catalase (CAT) was determined according to the method of Cakmak and Marschner (1992). CAT can decompose H_2O_2 , and H_2O_2 has a strong absorption peak at 240 nm wavelength, reducing A₂₄₀ by 0.1 per minute to a unit (U) of CAT enzyme activity. The chlorophyll content was measured by UV (ultraviolet) spectrophotometry (Yang et al., 2009), while the activity of ascorbate peroxidase (APX) was measured as previously reported (Nakano and Asada, 1981). One unit of activity for APX was defined as the amount of enzyme that

degraded 1 μmol of AsA per min. The activities of glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) were measured with a kit (Keming Biotechnology Co., Ltd., Suzhou, China). One unit of activity for GR was defined as catalyzing the oxidation of 1 nmol NADPH (nicotinamide adenine dinucleotide phosphate) per gram of sample per min. One unit of MDHAR activity was defined as 1 nmol NADH (nicotinamide adenine dinucleotide) oxidized per min per gram of sample. One unit of DHAR activity was defined as 1 nmol AsA produced per min per gram of sample.

Determination of MT, Ethylene, and ACC Contents

The MT content was determined in reference to the previously reported method (Qianqian et al., 2015), with some modifications. The sample weight was 3.0 g, and the MT was extracted with analytical grade methanol; the final extract was purified by the C₁₈ solid-phase extraction cartridge (ProElutTM, DIKMA, China) with the help of a vacuum pump, and then the volume was adjusted to 1 mL. The ethylene production rate was measured by gas chromatography (Shimadzu GC-16A, Japan) and repeated three times (Farmer et al., 1986). In order to avoid the wounding effect on ethylene production, the wound was wrapped in cotton with water after sampling and then sealed with a sealing film. The leaves were immediately put into a container and sealed, maintained in a light incubator at 25°C for 24 h, and then the gas was extracted into a 1 mL syringe for determination. The extraction and determination of ACC were according to Tucker et al. (2010). After O_3 treatment, the samples were stored in liquid nitrogen.

RNA-Seq and Quantitative Real-Time PCR

Transcriptome sequencing was conducted by OE Biotech Co., Ltd. (Shanghai, China). Total RNAs were extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, United States), and the mRNA was enriched using magnetic beads containing Oligo (dT). The quality of the constructed gene library was checked by the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, United States). After passing the quality test, the HiSeq X Ten sequencer of Illumina Company was used for sequencing, and the double-terminal data of 150 bp was produced. Raw data (raw reads) were processed using Trimmomatic (Bolger et al., 2014). The reads containing ploy-N and the low quality reads were removed to obtain the clean reads. Then the clean reads were mapped to reference genome¹ using hisat2 (Kim et al., 2015). The reads were reassembled using StringTie (Pertea et al., 2015). The protein-coding gene expression was calculated in FPKM (Fragments Per kb Per Million Reads). The default screening difference condition was $P < 0.05$ and \log_2 (fold change) > 1 . FDR (false discovery rate) error control method was used for P -value multiple hypothesis testing and correction. GO (Gene Ontology) enrichment and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis of differentially

¹<http://genomes.cribi.unipd.it/grape/>

expressed genes (DEGs) were respectively performed using R based on the hypergeometric distribution. The quantitative real-time PCR (qRT-PCR) analysis was carried out with the UltraSYBR Mixture kit (CWBI, Beijing, China) on a Bio-Rad iQ5 (Hercules, CA, United States) instrument. The reaction mixture was 20 μ L: double distilled water (ddH₂O) 7.0 μ L, forward primer (10 μ mol L⁻¹) 1.0 μ L, reverse primer (10 μ mol L⁻¹) 1.0 μ L, 2 \times UltraSYBR Mixture 10.0 μ L, cDNA 1.0 μ L. The *Vvactin* gene was used as an internal reference. The relative quantitative gene expression values were calculated using the $2^{-\Delta\Delta CT}$ method from three replicates. The primer sequences used are shown in **Supplementary Table 1**.

Genetic Transformation of ACO2 and ASMT1 in Grape and Tobacco

The open reading frames (ORFs) of *ACO2* and *ASMT1* from “Cabernet Sauvignon” leaves were cloned and then respectively ligated to the pRI101-AN expression vector driven by the 35S promoter. Then, the plasmid was transformed into *Agrobacterium tumefaciens* strain GV3101 by the heat shock method. The “Cabernet Sauvignon” tissue culture seedlings were immersed in an *Agrobacterium* suspension adjusted to OD₆₀₀ = 0.6, placed in a closed container, and then the bacteria solution was completely immersed in grape leaves (with obvious water stains) by vacuum extraction. The bacterial suspension on the leaf surfaces was dried, and the seedlings were cultured in bottles with medium. After 2 days, a qRT-PCR analysis was done to detect the expression level, and the overexpression strain was used for the O₃ treatment experiment. The plants infected with an empty carrier were used as a control, and each line was set with three replicates. The tobacco was infected by the leaf disc method (Wang F. et al., 2016), and the T0 tobacco plants overexpressing *VvASMT1* were obtained after screening in selection medium. The transgenic lines were further identified by PCR and confirmed by qRT-PCR, after which T2 transgenic lines were obtained for experimental treatment.

Statistical Analysis

All statistical analyses were performed by SPSS 24.0 software. A one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was employed, standard deviation (SD) was calculated from three replicates. The differences between individual means were deemed to be significant at $P < 0.05$.

RESULTS

Exogenous MT Inhibits the Ethylene Biosynthesis and Signaling Caused by O₃ Stress

To explore the mechanism by which MT alleviates O₃ stress in grape leaves, RNA-Seq analysis was performed on “Cabernet Sauvignon” grape leaves treated with control, O₃, and MT + O₃. DEGs were represented by a Venn diagram (**Figure 1A**). Compared with the control, O₃ significantly (P -value < 0.05) up-regulated and down-regulated 5121 and 2935 genes in grape

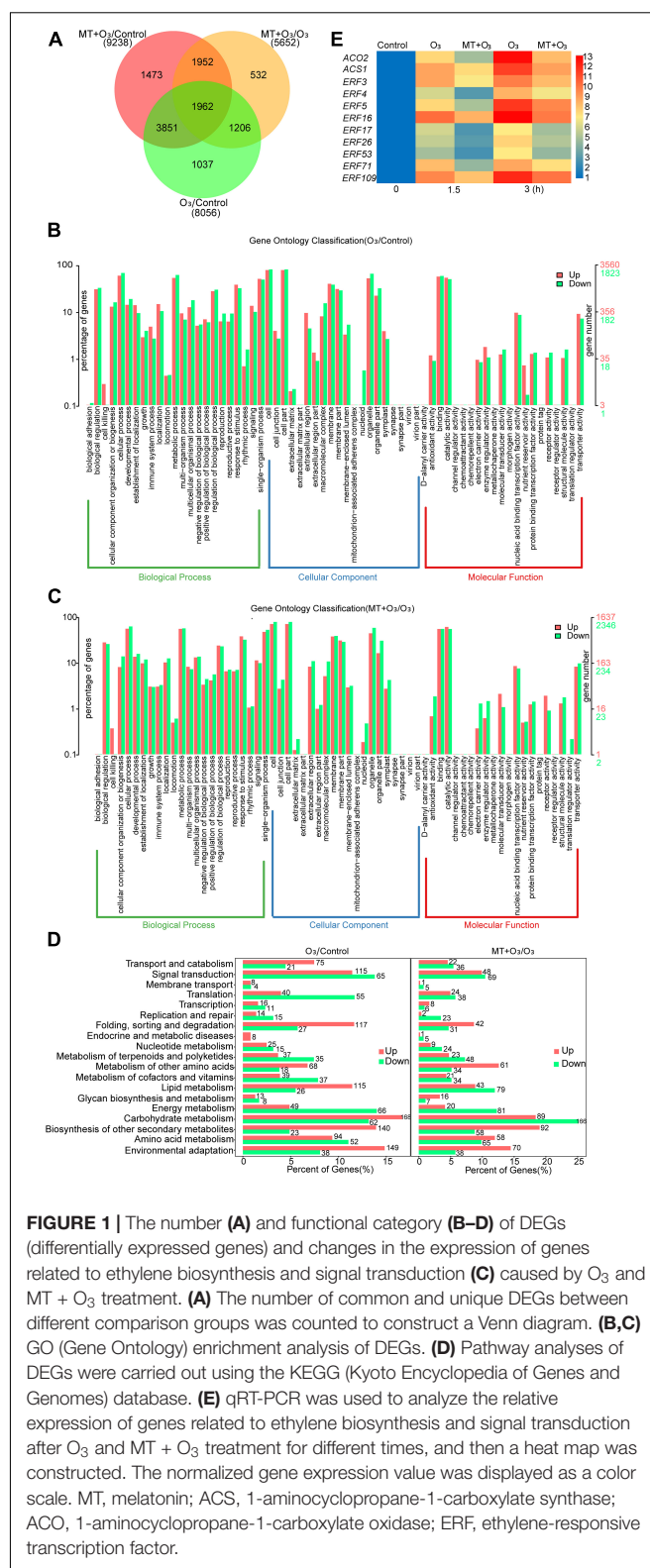


FIGURE 1 | The number (A) and functional category (B–D) of DEGs (differentially expressed genes) and changes in the expression of genes related to ethylene biosynthesis and signal transduction (C) caused by O₃ and MT + O₃ treatment. (A) The number of common and unique DEGs between different comparison groups was counted to construct a Venn diagram. (B,C) GO (Gene Ontology) enrichment analysis of DEGs. (D) Pathway analyses of DEGs were carried out using the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. (E) qRT-PCR was used to analyze the relative expression of genes related to ethylene biosynthesis and signal transduction after O₃ and MT + O₃ treatment for different times, and then a heat map was constructed. The normalized gene expression value was displayed as a color scale. MT, melatonin; ACS, 1-aminocyclopropane-1-carboxylate synthase; ACO, 1-aminocyclopropane-1-carboxylate oxidase; ERF, ethylene-responsive transcription factor.

leaves, respectively (**Supplementary Table 3**). Compared with O₃ treatment, MT + O₃ up-regulated and down-regulated 2342 and 3310 genes, respectively (**Supplementary Table 4**).

GO enrichment analysis showed that the DEGs were primarily associated with biological regulation, cellular process, metabolic process, signaling, cell, cell part, membrane, membrane part, binding, catalytic activity, nucleic acid binding transcription factor activity, and transporter activity (Figures 1B,C). KEGG enrichment analysis indicated that all of the annotated DEGs were primarily related to signal transduction, amino acid metabolism, biosynthesis of secondary metabolites, carbohydrate metabolism, and environmental adaptation (Figure 1D). In the classification of signal transduction pathways, the most apparent change in the number of DEGs occurred in the ethylene signal pathway (Supplementary Tables 5, 6). Compared with the control, O₃ resulted in significant changes in the expression levels of 71 genes related to ethylene biosynthesis and signaling pathways. Except for the obvious down-regulation of three genes, all the others were up-regulated; the expression of ACO2 was up-regulated by 5.2-fold (Supplementary Table 5). Compared with O₃ treatment, MT + O₃ caused significant changes in the expression of 38 ethylene biosynthesis and signaling pathway-related genes. Among these 38 genes, 22 were significantly down-regulated, and ACO2 was down-regulated by 1.03-folds (Supplementary Table 6). When the expression levels of these 22 genes in O₃ treatment and control were compared, it was found that 19 had higher expression levels than the O₃ treatment (Supplementary Table 7). qRT-PCR analysis was done to further determine changes in the expression of ethylene-related genes under O₃ and MT + O₃ treatments. The results showed that the expression levels of 11 genes increased significantly with the extension of O₃ treatment time, among which the expressions of ACO2 and ERF16 (*ethylene-responsive transcription factor16*) were up-regulated by 12.7 and 12.9-folds, respectively (Figure 1E). During stress, the relative expression levels of all detected genes under the MT + O₃ treatment were significantly lower than that under the O₃ treatment (Figure 1E), which was similar to the transcriptome analysis results.

MT Relieves O₃ Stress by Regulating the Ethylene Pathway

To determine the effects of MT and ethylene on grape leaves under O₃ stress, “Cabernet Sauvignon” grapes were treated differently. O₃ stress caused more yellowing spots on grape leaves, and ethephon aggravated leaf damage symptoms under O₃ stress, causing obvious chlorosis. In addition, AVG, MT, and a combination of the two treatments significantly reduced the leaf injury symptoms after O₃ stress, and the yellowing area was smaller (Figure 2A). To further explore the mutual influence of MT and ethylene under O₃ stress, the contents of MT and ethylene under different treatments were determined (Figures 2B–D). The results showed that compared with the control, O₃ stress increased the ethylene release rate and the ACC content of “Cabernet Sauvignon” leaves by 100.8 and 82.19%, respectively. Meanwhile, the ethylene production after MT + O₃ treatment was significantly less than in the O₃ treatment (Figures 2B,C). Compared with the control, the MT content after O₃ stress was significantly reduced by 59.25% (Figure 2D). After watering, the MT content in grape leaves increased by 97.64%

relative to the control (Figure 2D). Compared with O₃ treatment, the MT content after MT + O₃ and MT + AVG + O₃ treatment increased by 41.48 and 35.7%, respectively; however, there was no significant difference between ethephon + O₃, AVG + O₃, and the O₃ treatment (Figure 2D).

Effects of MT and Ethylene on F_v/F_m and Reactive Oxygen Species in Grape Leaves After O₃ Stress

Compared with the control, the F_v/F_m of grape leaves after O₃ stress decreased by 28.01% (Figure 3A). Compared with O₃ treatment, the F_v/F_m of grape leaves treated with ethephon + O₃ decreased by 23.34%, while the F_v/F_m increased by 28.35, 10.25, and 28.17% after MT + O₃, AVG + O₃, and MT + AVG + O₃ treatment, respectively (Figure 3A). After O₃ stress, the H₂O₂ content and O₂^{•−} production rate increased significantly by 43.55 and 163.30%, respectively, relative to the control. Compared with the O₃ treatment, the H₂O₂ content and O₂^{•−} production rate of grape leaves increased by 23.37 and 22.30% after treatment with ethephon + O₃, while MT + O₃, AVG + O₃, and AVG + MT + O₃ decreased by 24.18 and 42.51, 15.31, and 25.78, 19.29, and 46.70%, respectively (Figures 3B,C).

Effects of MT and Ethylene on Antioxidant System in Grape Leaves After O₃ Stress

Ascorbic acid and GSH are antioxidants involved in scavenging of active oxygen free radicals under stress conditions. Compared with the control, O₃ stress significantly reduced GSH, AsA contents, and GSH/GSSG in grape leaves, while increased GSSG content (Figures 4A–D). Compared with O₃ treatment, the GSH, AsA content, and GSH/GSSG were increased after MT + O₃, AVG + O₃, and AVG + MT + O₃ treatments. However, ethephon + O₃ treatment reduced GSH, AsA content, and GSH/GSSG, but increased the content of GSSG (Figures 4A–D).

Compared with the control, the GR, CAT, SOD, and POD activities in grape leaves increased significantly after O₃ stress. In contrast, the activities of MDHAR, DHAR, and APX were significantly inhibited (Figures 4E–K). Compared with the O₃ treatment, MT + O₃, AVG + O₃, and AVG + MT + O₃ treatments increased the GR, MDHAR, DHAR, APX, CAT, SOD, and POD activities of grape leaves, but the difference between AVG + MT + O₃ and MT + O₃ treatment was not significant (Figures 4E–K). Compared with the O₃ treatment, the SOD and POD activities of grape leaves after treatment with ethephon + O₃ were significantly increased, the activities of MDHAR and APX were inhibited. In contrast, the activities of GR, CAT, and DHAR did not change (Figures 4E–K). The above results indicate that both MT and AVG can alleviate the damage caused by O₃ stress on grape leaves by regulating the antioxidant system.

Overexpression of VvACO2 Intensifies O₃ Stress in Grape Leaves

To further verify that ethylene can exacerbate the stress effect of O₃ on grape leaves, the VvACO2 gene was transiently overexpressed in grape leaves to promote ethylene biosynthesis.

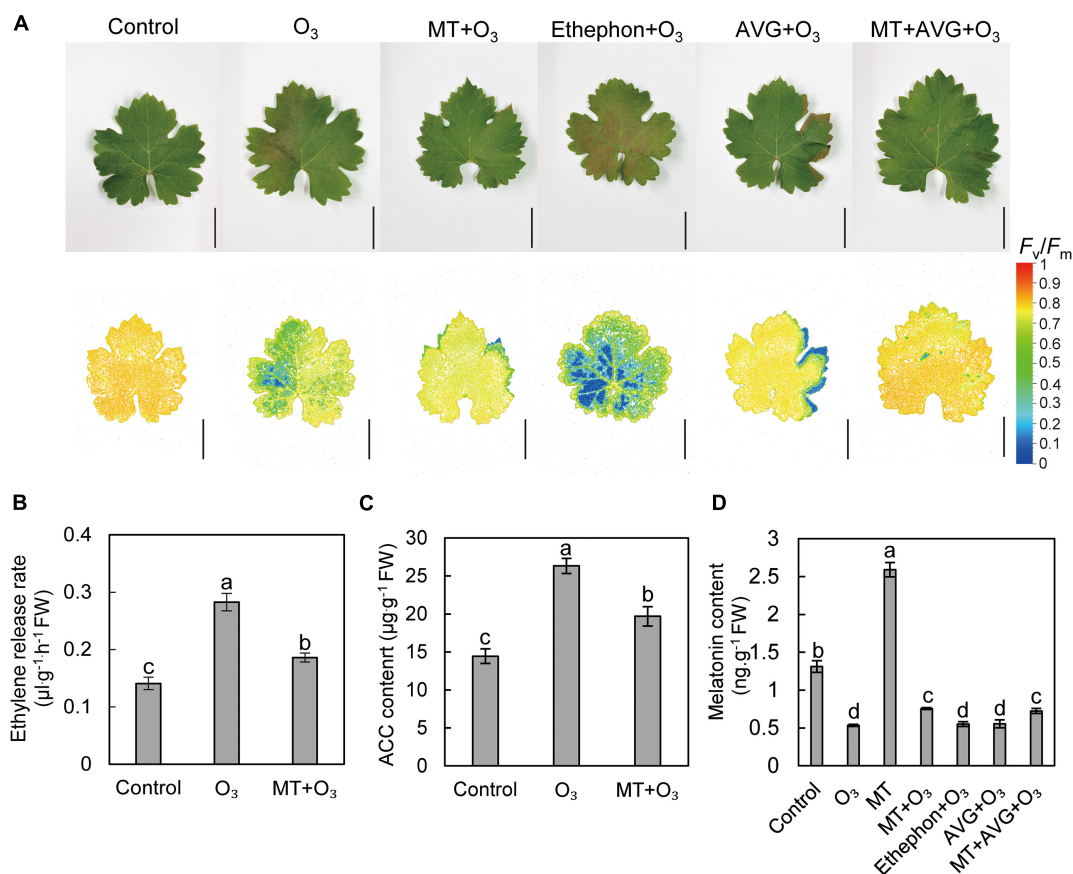


FIGURE 2 | Effects of MT and ethylene on the apparent symptoms and chlorophyll fluorescence (A), ethylene release rate (B), ACC (C), and MT (D) contents in grape leaves after O_3 stress. AVG, aminoethoxyvinylglycine; ACC, 1-aminocyclopropane-1-carboxylic acid. Values represent the mean of three replicates \pm SD. The difference was not significant at a 5% significance level among values labeled with the same lowercase letter. Bars, 5 cm.

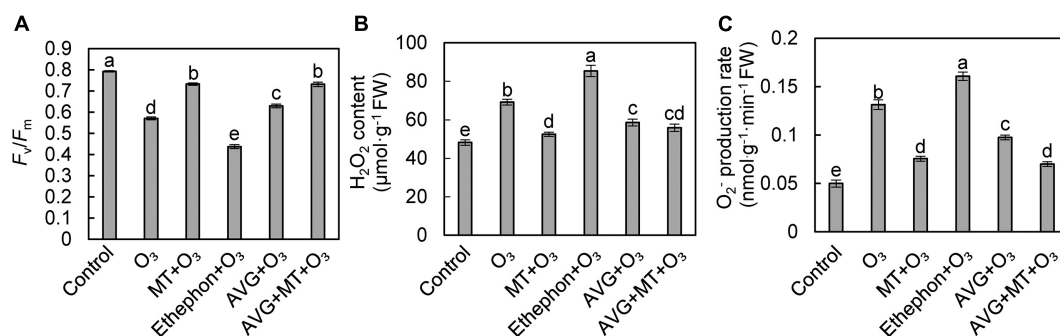


FIGURE 3 | Effects of O_3 , MT + O_3 , Ethephon + O_3 , AVG + O_3 , and AVG + MT + O_3 treatments on F_v/F_m (A) and ROS (B,C) of "Cabernet Sauvignon" grape leaves. Values represent the mean of three replicates \pm SD. Different lowercase letters above the bars indicate significant difference ($P < 0.05$).

The expression levels of the two plants infected with 35S:VvACO2 carrier were 7.98 and 10.83-folds that of plants infected with the empty carrier, respectively (Figure 5A). These results confirm the successful expression of VvACO2 in grape leaves. The ethylene release rate and ACC content were also significantly higher in the leaves of plants overexpressing VvACO2 than those of the control group (Figures 5B,C). Under normal

conditions, the growth of the control and overexpression plants was the same. After 110 nL L^{-1} O_3 treatment for 3 h, the yellowing degree (Figure 5D), H_2O_2 content (Figure 5E), and O_2^- production rate (Figure 5F) in the leaves of overexpressed plants were significantly higher than those of the control group. Meanwhile, the chlorophyll content (Figure 5G) of overexpressed plant leaves was significantly lower than that of

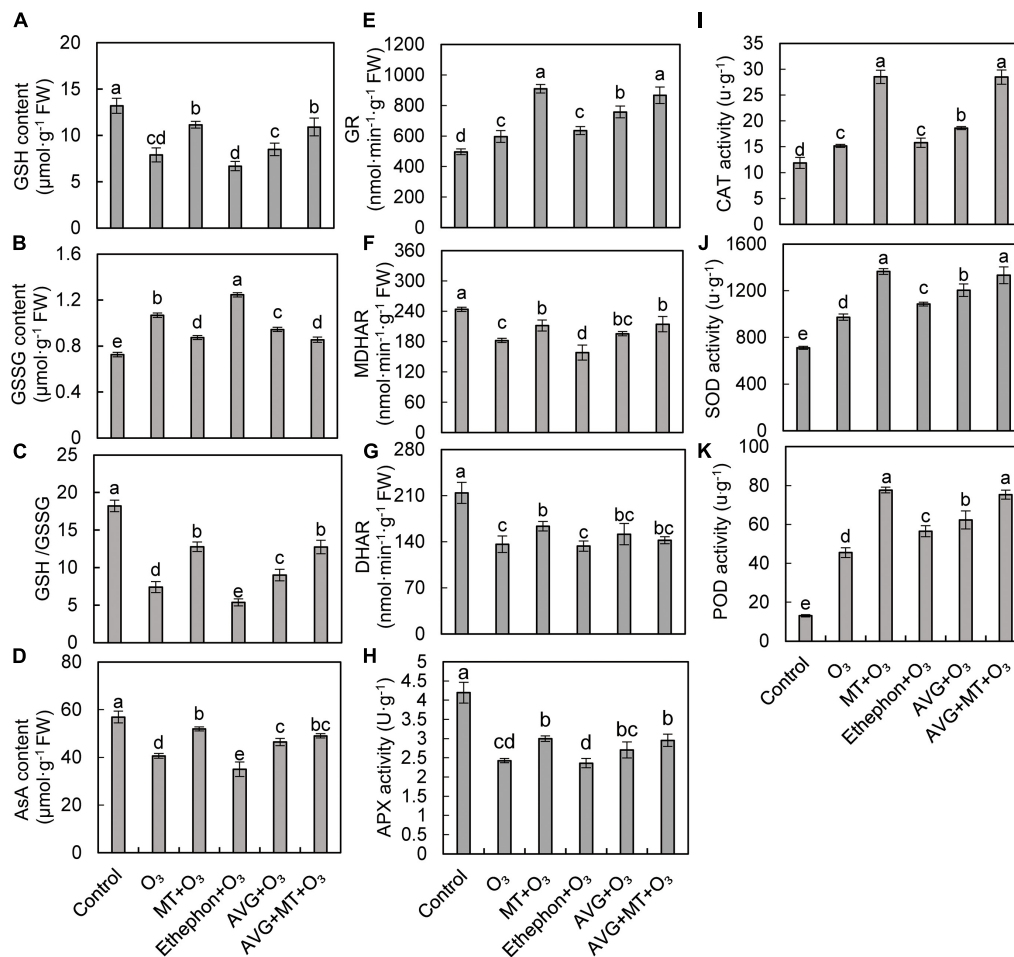


FIGURE 4 | Effects of O₃, MT + O₃, Ethephon + O₃, AVG + O₃, and AVG + MT + O₃ treatments on reduced glutathione (GSH, **A**) and oxidized glutathione (GSSG, **B**) contents, GSH/GSSG (**C**), reduced ascorbic acid (AsA, **D**) content, glutathione reductase (GR, **E**), monodehydroascorbate reductase (MDHAR, **F**), dehydroascorbate reductase (DHAR, **G**), ascorbate peroxidase (APX, **H**), catalase (CAT, **I**), superoxide dismutase (SOD, **J**), and peroxidase (POD, **K**) activities in “Cabernet Sauvignon” grape leaves. Values represent the mean of three replicates ± SD. For the values labeled with the same lowercase letter, the difference was not significant, according to Duncan's multiple range test at a 5% significance level.

the control group. These results show that the endogenous induction of ethylene biosynthesis in grape leaves aggravated O₃ damage to the leaves.

Overexpression of *VvASMT1* Enhances Tobacco Tolerance to O₃

To analyze the effect of endogenous MT on plant O₃ tolerance, the overexpression vector 35S: *VvASMT1* was transformed into tobacco to increase the endogenous MT content and test its O₃ resistance. The upstream primer of the 35S promoter and the downstream primer for amplifying the *ASMT* gene were used for PCR amplification. The results showed no specific band in the control group, while specific bands appeared in the five tobacco lines. The band sizes were the sum of the 35S promoter fragment length and the *ASMT* ORF sequence length (Figure 6A). The results indicated that *VvASMT1* was successfully transferred into tobacco. Two lines with

moderate expression and high expression, line 1 and line 3, respectively, were selected for functional analysis (Figure 6B). The results showed that the MT content in the leaves of the wild-type and the two transgenic tobacco lines were 0.2299, 0.4957, and 0.5676 ng g⁻¹, respectively (Figure 6C). After treatment with 110 nL L⁻¹ O₃ for 3 h, the tobacco showed yellowing and wilting symptoms, and the stress degree of transgenic tobacco was significantly lower than that of wild-type tobacco (Figure 6D). The leaf color in the transgenic lines after H₂O₂ and O₂⁻ staining was significantly lighter than that of the wild-type (Figures 6E,F), indicating that the ROS content was significantly lower than that of the wild-type. Compared with the wild-type, overexpression of *VvASMT1* significantly increased GSH, AsA content, and related antioxidant enzymes (GR, SOD, POD, CAT, MDHAR, DHAR, and APX) activities after stress (Supplementary Figure 1). After O₃ treatment, the ethylene release rate and ACC content of transgenic tobacco leaves were significantly lower than the

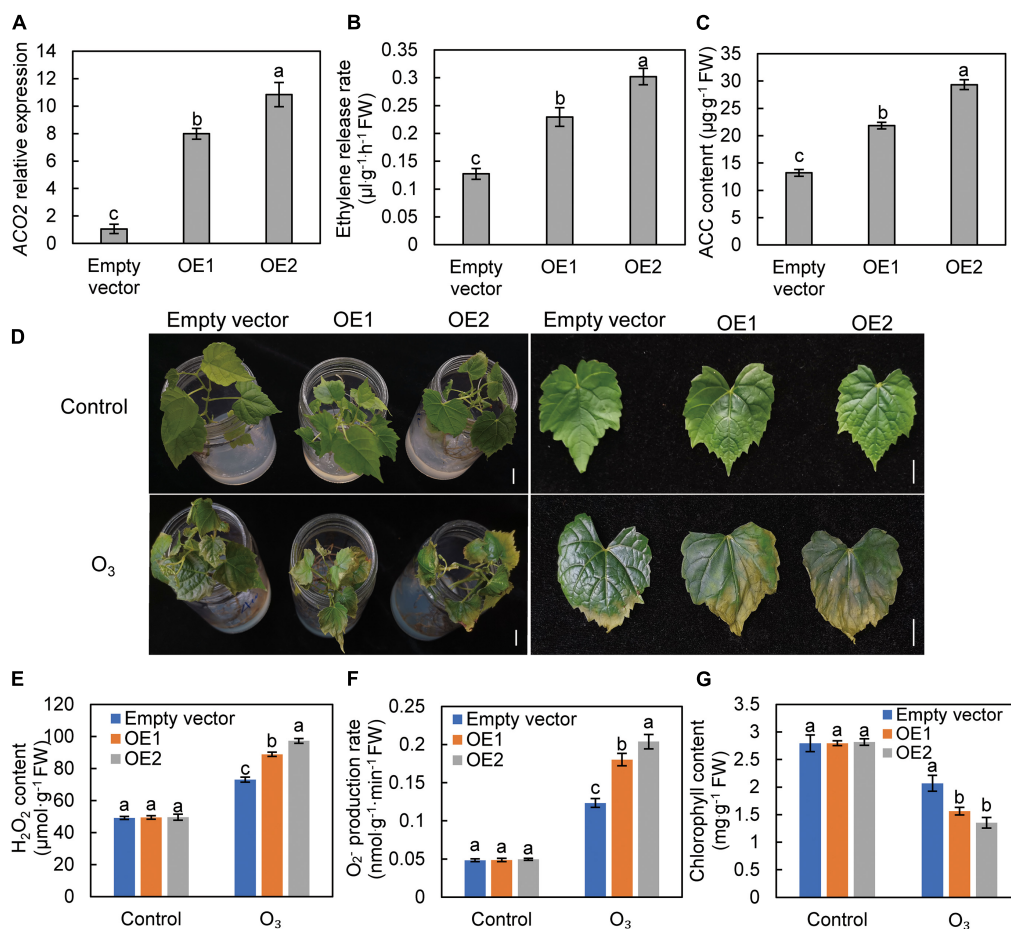


FIGURE 5 | The effect of *VvACO2* overexpression on the response of grape leaves to O_3 stress. The *ACO2* relative expression (A), ethylene release rate (B), and ACC content (C) in over-expressing (OE) and control (empty vector). The leaf appearance (D), ROS (E,F), and chlorophyll content (G) of the tissue culture seedlings of “Cabernet Sauvignon” grape after O_3 stress. Letters marked by the same lowercase letters are not significant at $P < 0.05$ (Duncan's multiple range test). Bars, 1 cm.

wild-type (Figures 6G,H). The above results indicate that overexpression of the *VvASMT1* gene in tobacco increased the MT content, alleviated the O_3 stress, and reduced the ethylene content after stress.

DISCUSSION

At present, only a few studies have examined the molecular mechanism of O_3 stress on fruit trees, especially grapes (Valletta et al., 2016). O_3 enters plant leaves mainly through the stomata and increases ROS production (Langebartels et al., 2002). Thus, ROS level can be an essential indicator to determine the degree of cellular oxidative stress (Collén et al., 2003). When large amounts of ROS are produced under stress, its clearance system gets damaged, resulting in membrane lipid peroxidation, massive chlorophyll degradation, hindrance of photosynthetic electron transfer, inactivation of antioxidant enzymes, and inhibiting plant growth development (Degl'Innocenti et al., 2007; Iriti and Faoro, 2008; Bose et al., 2014). PSII is the most sensitive component

of the photosynthetic electron transport chain under O_3 stress (Tran et al., 2013). Similarly, our research showed that O_3 stress could damage grape PSII and significantly reduce the chlorophyll content in grape leaves. Stressed leaves also produced large amounts of ROS and changed the activity of antioxidant enzymes, resulting in yellowing and wilting of grape leaves. Further, O_3 stress significantly induced the upregulation of genes related to ethylene biosynthesis and some ethylene responsive transcription factors, as well as the increase of ethylene release rate and ACC content. In our study, increase in exogenous and/or endogenous ethylene content aggravated O_3 stress. Therefore, it was speculated that O_3 destroys the photosynthetic and antioxidant grapes systems by increasing the ethylene content.

Many studies have shown that MT can alleviate various abiotic stresses; plants with higher MT content are more tolerant to O_3 (Dubbels et al., 1995). The present study also proved that MT could relieve O_3 stress in grape leaves and improve various physiological indexes. Exogenous MT application significantly increased the F_v/F_m of “Cabernet Sauvignon” grape leaves under O_3 stress and alleviated leaf chlorosis. Similarly, MT can reduce

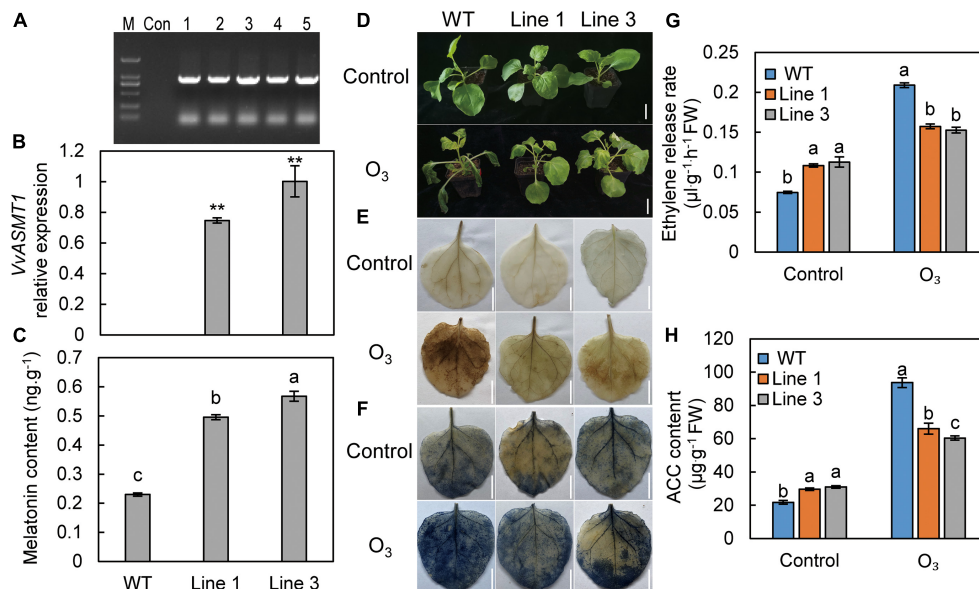


FIGURE 6 | Tobacco plants overexpressing *VvASMT1* showed increased tolerance to O_3 . **(A)** PCR detection of *VvASMT1* overexpression in tobacco. **(B)** The expression level of *VvASMT1* in transgenic and wild-type tobacco. **(C)** The content of MT in transgenic and wild-type tobacco. Phenotypes **(D)**, H_2O_2 staining **(E)**, O_2^- staining **(F)**, ethylene release rate **(G)**, and ACC content **(H)** of wild-type and transgenic lines after O_3 treatment. The numbers 1–5 represent five different tobacco lines; M, DNA marker; Con, control; ASMT, acetylserotonin methyltransferase; WT, wild-type. Values represent the means \pm SD of three replicates. **Highly significant difference, $P < 0.01$. Values indicated by the same lowercase letters are not significant at $P < 0.05$. Bars, 3 cm in **(D)**, 2 cm in **(E,F)**.

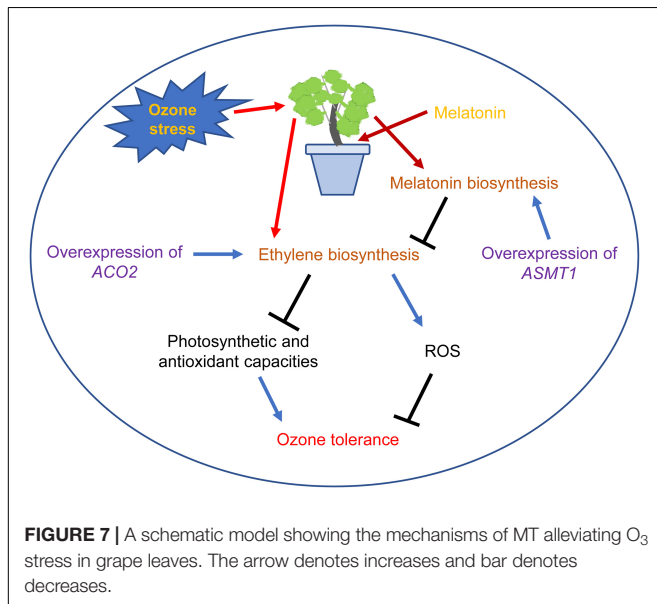


FIGURE 7 | A schematic model showing the mechanisms of MT alleviating O_3 stress in grape leaves. The arrow denotes increases and bar denotes decreases.

the damage of barley leaf photosystem II (PSII) under stress and maintain the chlorophyll content (Arnao and Hernández-Ruiz, 2009). These results may be related to the protection of chloroplast structure by MT (Zhao et al., 2016). Additionally, this experiment found that watering the roots of “Cabernet Sauvignon” with MT could increase the leaf MT content. Similarly, treating grapes with MT at the rhizosphere increased the MT levels of the roots, but also in the leaves, thus enhancing

salt tolerance in “Crimson seedless” grapevines (Xu et al., 2019). This result suggests that the MT provided through external sources is absorbed in plants (Tan et al., 2007) and can accumulate in distant organs through long-distance transportation to tolerate abiotic stress. In addition, our experiment for the first time verified that overexpression of *VvASMT* in tobacco could alleviate O_3 stress by increasing the MT content. This result is similar to that obtained following overexpression of the key enzyme caffeic acid-O-methyltransferase (*COMT*) for MT synthesis, which increased the endogenous MT content of tomatoes and enhanced salt resistance (Sun et al., 2019).

Plants can resist ROS damage through a defense system composed of enzymatic and non-enzymatic ROS scavenging systems (Apel and Hirt, 2004). Antioxidant enzyme scavenging systems mainly include SOD, POD, CAT, etc., while non-enzymatic systems include the AsA-GSH cycle. GSH can catalyze the degradation of excess H_2O_2 and activate various defense mechanisms by participating in redox signal transduction (Szalai et al., 2009). The increase in GR activity reduces the cellular glutathione pool, providing sufficient GSH for DHAR to reduce dehydroascorbate (DHA) to AsA (Noctor and Foyer, 1998). MT can enhance the scavenging ability of the ROS system to improve stress tolerance. For example, the application of $0.5 \mu\text{mol L}^{-1}$ MT can improve salt tolerance in tomatoes by increasing the antioxidant enzyme activity and the accumulation of AsA and GSH (Liu et al., 2015). Moreover, the leaves of O_3 -tolerant soybean varieties maintain higher AsA levels than susceptible varieties (Chutteang et al., 2015). Thus, varieties with high antioxidant contents in the leaves are more resistant to the

O₃ damage. This experiment also found that MT could protect antioxidant enzymes and the AsA-GSH signaling system under O₃ stress, while ethylene had the opposite effect. MT may act as an antioxidant to antagonize ethylene and remove excessive ROS, thereby sharing the pressure of other antioxidants.

Melatonin has opposite regulatory effects on ethylene in different crop species. For example, MT can promote the ripening of grape berries by increasing the ethylene content (Xu et al., 2018) and enhancing the salt tolerance of grapes by promoting *VviMYB108A*-mediated ethylene biosynthesis (Xu et al., 2019). On the contrary, MT treatment reduces ethylene release and improves fruit quality by inhibiting the expression of genes related to ethylene biosynthesis during apple storage (Onik et al., 2020). In our experiment, MT treatment of O₃ stressed plants down-regulated the expression of ethylene biosynthesis and signal transduction genes in grape leaves. MT treatment also reduced the ethylene release rate and ACC content in O₃ stressed leaves, thus alleviating O₃ stress. The increase of endogenous MT in tobacco also reduced ethylene biosynthesis after stress and alleviated O₃ stress. However, MT treatment of 'Crimson seedless' grapevines roots increased the ethylene release rate of leaves (Xu et al., 2019). These contrasting effects of MT may be due to the multi-pathway characteristics of MT synthesis, making it functionally specific in the developmental stage, tissues, and organs. In addition, the physiological effects of MT are pleiotropic (Byeon and Back, 2014; Zhang et al., 2014), and its regulation of ethylene could be indirect. The different inducing effects could also change the mutual regulation with other hormones; thus, the specific mechanism needs to be further explored. Although both MT and ethylene inhibitor significantly alleviated O₃ stress in grapes, the effect of MT treatment was better than treatment with ethylene inhibitor treatment. In addition, the combined treatment effect of MT and ethylene inhibitor was similar to that of MT alone, and the treatment of ethylene and ethylene inhibitor did not affect the MT content under O₃ stress. It can be seen that MT and ethylene may play upstream and downstream roles, respectively, in the signal pathway under O₃ stress.

Finally, as depicted in **Figure 7**, O₃ stress increased ROS content and decreased the photosynthetic and antioxidant capacities of grape leaves by inducing ethylene biosynthesis. Melatonin pretreatment or overexpression of *ASMT1* can enhance the *in vivo* melatonin level, reduce ethylene production

in grape leaves under O₃ stress and increase plant O₃ tolerance. In addition, overexpression of *ACO2* in grape leaves decreased O₃ tolerance by increasing endogenous ethylene content. Taken together, MT can alleviate O₃ damage to grape leaves by inhibiting ethylene biosynthesis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: NCBI repository, accession number: PRJNA733572.

AUTHOR CONTRIBUTIONS

CL performed most parts of the experiment, analyzed the data, and wrote the manuscript. HK and YW participated in performing the experiments. ZG participated in the manuscript writing and revision. YD and YY designed the research. All authors have read and approved the final manuscript.

FUNDING

This work was supported by the National Key Research and Development Program of China (2019YFD1000101), National Natural Sciences Foundation of China in 2016 (31572084), and China Agriculture Research System of MOF and MARA.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.702874/full#supplementary-material>

Supplementary Figure 1 | Effects of ozone stress on GSH (A), AsA (B), and related antioxidant enzyme activities (C–I) of wild-type and transgenic tobacco.

Supplementary Table 1 | Primers used in this study.

Supplementary Table 2 | RNA-Seq profiles of grape leaves after different treatments, including control, O₃, and MT + O₃. The default condition for screening differences is *P* < 0.05 and fold change > 2.

REFERENCES

- Al-Huqail, A. A., Khan, M. N., Ali, H. M., Siddiqui, M. H., and Al-Humaid, L. A. (2020). Exogenous melatonin mitigates boron toxicity in wheat. *Ecotoxicol. Environ. Saf.* 201:110822. doi: 10.1016/j.ecoenv.2020.110822
- Apel, K., and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55, 373–399. doi: 10.1146/annurev.arplant.55.031903.141701
- Arnao, M. B., and Hernández-Ruiz, J. (2018). Melatonin and its relationship to plant hormones. *Ann. Bot.* 121, 195–207. doi: 10.1093/aob/mcx114
- Arnao, M. B., and Hernández-Ruiz, J. (2009). Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *J. Pineal Res.* 46, 58–63. doi: 10.1111/j.1600-079X.2008.00625.x
- Arnao, M. B., and Hernández-Ruiz, J. (2015). Functions of melatonin in plants: a review. *J. Pineal Res.* 59, 133–150. doi: 10.1111/jpi.12253
- Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170
- Borowiak, K. (2013). Morphological changes in two tobacco and petunia cultivars under exposure to tropospheric ozone. *Acta Biol. Cracov. Ser. Bot.* 55, 58–66. doi: 10.2478/abcsb-2013-0002
- Bose, J., Rodrigo-Moreno, A., and Shabala, S. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. *J. Exp. Bot.* 65, 1241–1257. doi: 10.1093/jxb/ert430
- Byeon, Y., and Back, K. (2014). Melatonin synthesis in rice seedlings *in vivo* is enhanced at high temperatures and under dark conditions due to increased

- serotonin N-acetyltransferase and N-acetylserotonin methyltransferase activities. *J. Pineal Res.* 56, 189–195. doi: 10.1111/jpi.12111
- Cakmak, I., and Marschner, H. (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. *Plant Physiol.* 98, 1222–1227. doi: 10.1104/pp.98.4.1222
- Chen, Z., Gao, Z., Sun, Y., Wang, Y., Yao, Y., Zhai, H., et al. (2020). Analyzing the grape leaf proteome and photosynthetic process provides insights into the injury mechanisms of ozone stress. *Plant Growth Regul.* 91, 143–155. doi: 10.1007/s10725-020-00593-5
- Chutteang, C., Booker, F. L., Na-Ngern, P., Burton, A., Aoki, M., and Burkey, K. O. (2015). Biochemical and physiological processes associated with the differential ozone response in ozone-tolerant and sensitive soybean genotypes. *Plant Biol.* 2015:12347. doi: 10.1111/plb.12347
- Collén, J., Pinto, E., Pedersen, M., and Colepiccolo, P. (2003). Induction of oxidative stress in the red macroalga *Gracilaria tenuistipitata* by pollutant metals. *Arch. Environ. Contam. Toxicol.* 45, 337–342. doi: 10.1007/s00244-003-0196-0
- Degl'Innocenti, E., Guidi, L., and Soldatini, G. (2007). Effects of elevated ozone on chlorophyll a fluorescence in symptomatic and asymptomatic leaves of two tomato genotypes. *Biol. Plant.* 51, 313–321. doi: 10.1007/s10535-007-0061-5
- Dubbels, R., Reiter, R., Klenke, E., Goebel, A., Schnakenberg, E., Ehlers, C., et al. (1995). Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography–mass spectrometry. *J. Pineal Res.* 18, 28–31. doi: 10.1111/j.1600-079X.1995.tb00136.x
- Elstner, E. F., and Heupel, A. (1976). Inhibition of nitrite formation from hydroxylammonium chloride: a simple assay for superoxide dismutase. *Anal. Biochem.* 70, 616–620. doi: 10.1016/0003-2697(76)90488-7
- Farmer, P., Bailey, E., Gorf, S., Törnqvist, M., Osterman-Golkar, S., Kautiainen, A., et al. (1986). Monitoring human exposure to ethylene oxide by the determination of haemoglobin adducts using gas chromatography–mass spectrometry. *Carcinogenesis* 7, 637–640. doi: 10.1093/carcin/7.4.637
- Fiscus, E. L., Booker, F. L., and Burkey, K. O. (2005). Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. *Plant Cell Environ.* 28, 997–1011. doi: 10.1111/j.1365-3040.2005.01349.x
- Galano, A., Tan, D. X., and Reiter, R. J. (2011). Melatonin as a natural ally against oxidative stress: a physicochemical examination. *J. Pineal Res.* 51, 1–16. doi: 10.1111/j.1600-079X.2011.00916.x
- Gao, Y., Wang, Y., Qian, J., Si, W., Tan, Q., Xu, J., et al. (2020). Melatonin enhances the cadmium tolerance of mushrooms through antioxidant-related metabolites and enzymes. *Food Chem.* 2020:127263. doi: 10.1016/j.foodchem.2020.127263
- Geng, Q. W., Xing, H., Sun, Y. J., Hao, G. M., Zhai, H., and Du, Y. P. (2017). Analysis of the interaction effects of light and O₃ on fluorescence properties of 'Cabernet Sauvignon' grapes based on response surface methodology. *Sci. Hortic.* 225, 599–606. doi: 10.1016/j.scienta.2017.07.030
- Geng, Q. W., Xing, H., Hao, G. M., Sun, Y. J., Zhai, H., and Du, Y. P. (2016). Effect of exogenous melatonin on photosynthesis of 'cabernet sauvignon' grape leaves under ozone stress. *Acta Hortic. Sin.* 43, 1463–1472. doi: 10.16420/j.issn.0513-353x.2016-0123
- Giannopolitis, C. N., and Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* 59, 309–314. doi: 10.1104/pp.59.2.309
- Iriti, M., and Faoro, F. (2008). Oxidative stress, the paradigm of ozone toxicity in plants and animals. *Water Air Soil Pollut.* 187, 285–301. doi: 10.1007/s11270-007-9517-7
- Kang, K., Kong, K., Park, S., Natsagdorj, U., Kim, Y. S., and Back, K. (2011). Molecular cloning of a plant N-acetylserotonin methyltransferase and its expression characteristics in rice. *J. Pineal Res.* 50, 304–309. doi: 10.1111/j.1600-079X.2010.00841.x
- Karnosky, D. F., Skelly, J. M., Percy, K. E., and Chappelka, A. H. (2007). Perspectives regarding 50 years of research on effects of tropospheric ozone air pollution on US forests. *Environ. Pollut.* 147, 489–506. doi: 10.1016/j.envpol.2006.08.043
- Kim, D., Langmead, B., and Salzberg, S. L. (2015). HISAT: a fast spliced aligner with low memory requirements. *Nat. Methods* 12, 357–360. doi: 10.1038/nmeth.3317
- Kosugi, H., and Kikugawa, K. (1985). Thiobarbituric acid reaction of aldehydes and oxidized lipids in glacial acetic acid. *Lipids* 20, 915–921. doi: 10.1007/BF02534777
- Langebartels, C., Wohlgenuth, H., Kschieschan, S., Grün, S., and Sandermann, H. (2002). Oxidative burst and cell death in ozone-exposed plants. *Plant Physiol. Biochem.* 40, 567–575. doi: 10.1016/S0981-9428(02)01416-X
- Lee, H. Y., Byeon, Y., and Back, K. (2014). Melatonin as a signal molecule triggering defense responses against pathogen attack in *Arabidopsis* and tobacco. *J. Pineal Res.* 57, 262–268. doi: 10.1111/jpi.12165
- Li, C., Liang, B., Chang, C., Wei, Z., Zhou, S., and Ma, F. (2016). Exogenous melatonin improved potassium content in *Malus* under different stress conditions. *J. Pineal Res.* 61, 218–229. doi: 10.1111/jpi.12342
- Li, X., Ahammed, G. J., Zhang, X. N., Zhang, L., Yan, P., Zhang, L. P., et al. (2021). Melatonin-mediated regulation of anthocyanin biosynthesis and antioxidant defense confer tolerance to arsenic stress in *Camellia sinensis* L. *J. Hazard. Mater.* 403:123922. doi: 10.1016/j.jhazmat.2020.123922
- Liu, N., Gong, B., Jin, Z., Wang, X., Wei, M., Yang, F., et al. (2015). Sodic alkaline stress mitigation by exogenous melatonin in tomato needs nitric oxide as a downstream signal. *J. Plant Physiol.* 2015, 68–77. doi: 10.1016/j.jplph.2015.07.012
- Ma, W., Xu, L., Gao, S., Lyu, X., Cao, X., and Yao, Y. (2021). Melatonin alters the secondary metabolite profile of grape berry skin by promoting VvMYB14-mediated ethylene biosynthesis. *Hortic. Res.* 8, 1–15. doi: 10.1038/s41438-021-00478-2
- Mehlhorn, H., and Wellburn, A. R. (1987). Stress ethylene formation determines plant sensitivity to ozone. *Nature* 327, 417–418. doi: 10.1038/327417a0
- Najeeb, U., Tan, D. K., Bange, M. P., and Atwell, B. J. (2018). Protecting cotton crops under elevated CO₂ from waterlogging by managing ethylene. *Funct. Plant Biol.* 45, 340–349. doi: 10.1071/FP17184
- Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22, 867–880. doi: 10.1093/oxfordjournals.pcp.a076232
- Nguyen, D., Rieu, I., Mariani, C., and van Dam, N. M. (2016). How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. *Plant Mol. Biol.* 91, 727–740. doi: 10.1007/s11103-016-0481-8
- Noctor, G., and Foyer, C. H. (1998). Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 249–279. doi: 10.1146/annurev.arplant.49.1.249
- Onik, J. C., Wai, S. C., Li, A., Lin, Q., Sun, Q., Wang, Z., et al. (2020). Melatonin treatment reduces ethylene production and maintains fruit quality in apple during postharvest storage. *Food Chem.* 337:127753. doi: 10.1016/j.foodchem.2020.127753
- Orozco-Cardenas, M., and Ryan, C. A. (1999). Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. *Proc. Natl. Acad. Sci.* 96, 6553–6557. doi: 10.1073/pnas.96.11.6553
- Pertea, M., Pertea, G. M., Antonescu, C. M., Chang, T. C., Mendell, J. T., and Salzberg, S. L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat. Biotechnol.* 33, 290–295. doi: 10.1038/nbt.3122
- Qianqian, S., Na, Z., Jinfang, W., Haijun, Z., Dianbo, L., Jin, S., et al. (2015). Melatonin promotes ripening and improves quality of tomato fruit during postharvest life. *J. Exp. Bot.* 66, 657–668. doi: 10.1093/jxb/eru332
- Ryu, H., and Cho, Y. G. (2015). Plant hormones in salt stress tolerance. *J. Plant Biol.* 58, 147–155. doi: 10.1007/s12374-015-0103-z
- Scebbia, F., Sebastiani, L., and Vitagliano, C. (2001). Activities of antioxidant enzymes during senescence of *Prunus armeniaca* leaves. *Biol. Plant.* 44, 41–46. doi: 10.1023/A:1017962102950
- Serengil, Y., Augustaitis, A., Bytnerowicz, A., Grulke, N., Kozovitz, A., Matyssek, R., et al. (2011). Adaptation of forest ecosystems to air pollution and climate change: a global assessment on research priorities. *iForest* 4, 44–48. doi: 10.3832/for0566-004
- Sun, S., Wen, D., Yang, W., Meng, Q., Shi, Q., and Gong, B. (2019). Overexpression of caffeic acid o-methyltransferase 1 (*COMT1*) increases melatonin level and salt stress tolerance in tomato plant. *J. Plant Growth Regul.* 2019, 1–15. doi: 10.1007/s00344-019-10058-3
- Szalai, G., Kells, T., Galiba, G., and Kocsy, G. (2009). Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. *J. Plant Growth Regul.* 28, 66–80. doi: 10.1007/s00344-008-9075-2
- Tan, D. X., Manchester, L. C., Di Mascio, P., Martinez, G. R., Prado, F. M., and Reiter, R. J. (2007). Novel rhythms of

- N1-acetyl-N2-formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phytoremediation. *Faseb J.* 21, 1724–1729. doi: 10.1096/fj.06-7745com
- Thordal-Christensen, H., Zhang, Z., Wei, Y., and Collinge, D. B. (1997). Subcellular localization of H₂O₂ in plants. H₂O₂ accumulation in papillae and hypersensitive response during the barley—powdery mildew interaction. *Plant J.* 11, 1187–1194. doi: 10.1046/j.1365-3113.1997.11061187.x
- Tingey, D. T., Standley, C., and Field, R. W. (1976). Stress ethylene evolution: A measure of ozone effects on plants. *Atmos. Environ.* 10, 969–974. doi: 10.1016/0004-6981(76)90204-3
- Tran, T. A., Vassileva, V., Petrov, P., and Popova, L. P. (2013). Cadmium-induced structural disturbances in *Pisum sativum* leaves are alleviated by nitric oxide. *Turk. J. Bot.* 37, 698–707. doi: 10.3906/bot-1209-8
- Tucker, M. L., Xue, P., and Yang, R. (2010). 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration and ACC synthase expression in soybean roots, root tips, and soybean cyst nematode (*Heterodera glycines*)-infected roots. *J. Exp. Bot.* 61, 463–472. doi: 10.1093/jxb/erp317
- Valletta, A., Salvatori, E., Rita Santamaria, A., Nicoletti, M., Toniolo, C., Caboni, E., et al. (2016). Ecophysiological and phytochemical response to ozone of wine grape cultivars of *Vitis vinifera* L. *Nat. Prod. Res.* 30, 2514–2522. doi: 10.1080/14786419.2015.1118631
- Velikova, V., Yordanov, I., and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sci.* 151, 59–66. doi: 10.1016/S0168-9452(99)00197-1
- Vingarzan, R. (2004). A review of surface ozone background levels and trends. *Atmos. Environ.* 38, 3431–3442. doi: 10.1016/j.atmosenv.2004.03.030
- Wang, F., Wang, C., Yan, Y., Jia, H., and Guo, X. (2016). Overexpression of cotton *GhMPK11* decreases disease resistance through the gibberellin signaling pathway in transgenic *Nicotiana benthamiana*. *Front. Plant Sci.* 7:689. doi: 10.3389/fpls.2016.00689
- Wang, Q., An, B., Wei, Y., Reiter, R. J., Shi, H., Luo, H., et al. (2016). Melatonin regulates root meristem by repressing auxin synthesis and polar auxin transport in *Arabidopsis*. *Front. Plant Sci.* 7:1882. doi: 10.3389/fpls.2016.01882
- Xu, L., Xiang, G., Sun, Q., Ni, Y., Jin, Z., Gao, S., et al. (2019). Melatonin enhances salt tolerance by promoting MYB108A-mediated ethylene biosynthesis in grapevines. *Hortic. Res.* 6, 1–14. doi: 10.1038/s41438-019-0197-4
- Xu, L., Yue, Q., Xiang, G., Bian, F., and Yao, Y. (2018). Melatonin promotes ripening of grape berry via increasing the levels of ABA, H₂O₂, and particularly ethylene. *Hortic. Res.* 5:41. doi: 10.1038/s41438-018-0045-y
- Yang, Q., Chen, Z. Z., Zhou, X. F., Yin, H. B., Li, X., Xin, X. F., et al. (2009). Overexpression of SOS (Salt Overly Sensitive) genes increases salt tolerance in transgenic *Arabidopsis*. *Mol. Plant* 2, 22–31. doi: 10.1093/mp/ssn058
- Yang, Y., Yan, L., Yana, S., Tao, L., Yanchun, C., Dake, Z., et al. (2018). The role of phyto-melatonin and related metabolites in response to stress. *Molecules* 23:1887. doi: 10.3390/molecules23081887
- Zhang, H. J., Zhang, N., Yang, R. C., Wang, L., Sun, Q. Q., Li, D. B., et al. (2014). Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA4 interaction in cucumber (*Cucumis sativus* L.). *J. Pineal Res.* 57, 269–279. doi: 10.1111/jpi.12167
- Zhang, M., Smith, J. A. C., Harberd, N. P., and Jiang, C. (2016). The regulatory roles of ethylene and reactive oxygen species (ROS) in plant salt stress responses. *Plant Mol. Biol.* 91, 651–659. doi: 10.1007/s11103-016-0488-1
- Zhang, N., Sun, Q., Li, H., Li, X., Cao, Y., Zhang, H., et al. (2016). Melatonin improved anthocyanin accumulation by regulating gene expressions and resulted in high reactive oxygen species scavenging capacity in cabbage. *Front. Plant Sci.* 7:197. doi: 10.3389/fpls.2016.00197
- Zhao, H., Ye, L., Wang, Y., Zhou, X., Yang, J., Wang, J., et al. (2016). Melatonin increases the chilling tolerance of chloroplast in cucumber seedlings by regulating photosynthetic electron flux and the ascorbate-glutathione cycle. *Front. Plant Sci.* 7:1814. doi: 10.3389/fpls.2016.01814
- Zhou, B., Guo, Z., and Liu, Z. (2005). Effects of abscisic acid on antioxidant systems of *Stylosanthes guianensis* (Aublet) Sw. under chilling stress. *Crop Sci.* 45, 599–605. doi: 10.2135/cropsci2005.0599
- Zuo, B., Zheng, X., He, P., Wang, L., Lei, Q., Feng, C., et al. (2014). Overexpression of *MzASMT* improves melatonin production and enhances drought tolerance in transgenic *Arabidopsis thaliana* plants. *J. Pineal Res.* 57, 408–417. doi: 10.1111/jpi.12180

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Liu, Kang, Wang, Yao, Gao and Du. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



1-Aminocyclopropane-1-Carboxylate Deaminase-Producing Plant Growth-Promoting Rhizobacteria Improve Drought Stress Tolerance in Grapevine (*Vitis vinifera* L.)

Bingbing Duan¹, Lin Li¹, Guoqiao Chen¹, Chenxing Su-Zhou¹, Yashan Li^{1,2}, Hasmik Merkeryan¹, Wei Liu³ and Xu Liu^{1,4*}

¹College of Enology, Northwest A&F University, Yangling, China, ²School of Chemistry and Life Sciences, Chuxiong Normal University, Chuxiong, China, ³Horticulture Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu, China, ⁴Ningxia Eastern Foot of Helan Mountain Wine Station, Northwest A&F University, Yinchuan, China

OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Youry Pili,
Free University of Bozen-Bolzano,
Italy
Anabela Fernandes-Silva,
Centre for the Research and
Technology of Agro-Environmental
and Biological Sciences (CITAB),
Portugal

*Correspondence:

Xu Liu
liuxu@nwfau.edu.cn

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 08 May 2021

Accepted: 06 August 2021

Published: 03 September 2021

Citation:

Duan B, Li L, Chen G, Su-Zhou C,
Li Y, Merkeryan H, Liu W and
Liu X (2021) 1-Aminocyclopropane-
1-Carboxylate Deaminase-Producing
Plant Growth-Promoting
Rhizobacteria Improve Drought
Stress Tolerance in Grapevine
(*Vitis vinifera* L.).
Front. Plant Sci. 12:706990.
doi: 10.3389/fpls.2021.706990

Plant growth-promoting rhizobacteria (PGPRs) that produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase are capable of reducing limits to plant growth due to water-deficient conditions. Here, seven PGPR strains that can produce ACC deaminase were successfully obtained from the rhizosphere soil of grapevine (*Vitis vinifera* L.) in arid regions of China. The strains belonged to three different genera: *Pseudomonas*, *Enterobacter*, and *Achromobacter*, according to their 16S rDNA sequencing analysis. A drought tolerance experiment revealed two PGPR strains (DR3 and DR6) with exceptionally high phosphate solubilization, nitrogen fixation, indoleacetic acid (IAA), and exopolysaccharides secretion potential. Both strains were selected for use in a pot experiment to evaluate their growth-promoting effects on grapevines under drought conditions. Each of these two PGPRs and their mixed inoculation into grapevines were expected to alleviate the comprehensive growth inhibition of grapevines caused by drought stress. The mixed inoculation was hypothesized to elicit the best growth-promoting effects. Inoculation with the PGPRs not only enhanced the root-adhering soil/root tissue ratios and soil aggregate stability, but it also increased the nitrogen and phosphorus levels in the soil and plant leaves. Further, inoculation with PGPRs significantly altered the plant height, biomass of shoot and root organs, relative water contents, and net photosynthetic rate of leaves, enabling grapevines to better cope with drought. Moreover, the contents of IAA, abscisic acid, and malondialdehyde in these grapevines under drought stress were significantly changed by PGPRs. They indirectly affected biochemical and physiological properties of grapevines to alleviate their drought stress. Taken together, these results demonstrate that the DR3 and DR6 PGPRs might be useful for effectively weakening the growth inhibition caused by drought in grapevines. The strains might also be applied as effective bioinoculants to maintain the quality of wine grapes.

Keywords: 1-aminocyclopropane-1-carboxylate deaminase, drought stress, *Enterobacter* sp., plant growth-promoting rhizobacteria, *Pseudomonas* sp., *Vitis vinifera* L.

INTRODUCTION

With their continuous deterioration under global climate change, ecological systems are becoming seriously damaged. Drought, an environmental stress, is prevalent in arid regions, where it limits plant growth and threatens agricultural production (Vurukonda et al., 2016). As a root-borne stress, drought would normally lead to osmotic and oxidative stress, resulting in changed physiological, biochemical, and molecular properties of plants that jointly cause losses in crop production. In order to improve the drought tolerance of plants, various approaches have been explored, such as breeding drought-tolerant varieties and implementing water-saving irrigation (Evans and Sadler, 2008; Luo et al., 2019; Zhang et al., 2020). Yet, due to their advanced technological requirements and high labor and costs, certain approaches are not easy to apply in practice. Therefore, enhancing the drought tolerance of plants *via* targeted physical and biological methods is becoming a hot research topic in agricultural science.

Plant growth-promoting rhizobacteria (PGPRs) are bacterial groups obtained from rhizosphere soil. They can promote plant growth by means of biological control against soil-borne pathogens, biological nitrogen fixation, and root growth promotion (Pii et al., 2015). Therefore, they can cope with various environmental stresses and alleviate limitations to plant growth, effectively mitigating the loss of crop productivity. The growth-promoting effects of PGPRs on plants have been widely reported for many species (Mir et al., 2020; Zhang et al., 2020; Alemneh et al., 2021; Kalozoumis et al., 2021). Nevertheless, there are different genera of PGPRs as well as differences in the mechanisms by which they promote the growth of plants (Broadbent et al., 1977; Kaushal and Wani, 2016). PGPRs could directly and indirectly promote plant growth *via* nitrogen fixation, exopolysaccharides (EPS), and phytohormones (Vurukonda et al., 2016; Niu et al., 2018). Notably, 1-aminocyclopropane-1-carboxylate (ACC) deaminase-producing PGPRs, which are capable of hydrolyzing ACC to α -ketobutyrate and ammonia, could lower ethylene levels in plants and alleviate growth inhibition caused by excessive ethylene under environmental stressful conditions, drought in particular (Singh et al., 2015; Tiwari et al., 2018). Moreover, indoleacetic acid (IAA) is able to promote cell division and cell elongation, and thereby regulate root development and architecture (Spaepen et al., 2008; Naveed et al., 2015). The IAA content is reportedly increased after inoculation with PGPRs under environmental stress, and this alleviated exogenous stress incurred by the plants. In addition, EPS that attach to the surface of roots to form biofilms could protect these organs from drying out (Janczarek and Rachwał, 2013). Therefore, the mechanisms underpinning PGPRs' alleviation of environmental stress and promotion of plant growth may be complicated, entailing a combination of many pathways (Cohen et al., 2015; Ortiz et al., 2015).

The wine grape (*Vitis vinifera* L.) plant is a particularly important perennial fruit vine growing in arid regions of China, where its production is a major economic activity. It is well known that the quality of wine depends on the grape

berry's chemical composition, which is the outcome of interactions between fruiting vines and their biotic and abiotic factors in their environment, such as local climate and soil physicochemical properties (Novello et al., 2017; Mazzei et al., 2019). Drought disturbances in arid regions have seriously impacted the quality of wine grapes and restricted the development of the grape industry (You et al., 2020). The rhizobacteria associated with grapevines in arid regions are prone to water shortages, and so they may have adapted to drought stress conditions; if so, they could help their host plants also adapt to drought stress. Some researchers did report on PGPRs isolated from grapevine rhizosphere soil that improved the quality of wine grapes (Aballay et al., 2011). Therefore, to cope with drought stress, it is imperative we try to isolate PGPRs for use in wine grape cultivation in these regions, to enhance both the quality of grapes produced and increase the incomes of local wine growers.

Accordingly, this study had three objectives: (1) to isolate PGPRs with ACC deaminase activity from the rhizosphere soil of wine grapevines; (2) to evaluate the effects of inoculations of selected PGPRs upon grapevines' growth under imposed drought stress; and (3) to reveal the potential mechanisms of PGPRs' alleviation of drought stress for grapevines by taking a comprehensive perspective. To the best of our knowledge, this is the first report to determine the effect and mechanism of ACC deaminase-producing PGPRs on grapevines under drought stress. We hoped to find effective PGPR strains for consideration as biological fertilizers for ameliorating drought stress incurred by grapevines in arid regions, to enable the sustainable development of the grape industry.

MATERIALS AND METHODS

Isolation of Rhizobacteria With ACC Deaminase Activity

The rhizosphere soil used for the bacterial isolation was collected from 8-year-old vines of *V. vinifera* cv. Cabernet Sauvignon growing in the Xige winery on Helan Mountain, in Ningxia, China (38°3'44"N, 105°56'11"E), in 2018. Located in a BWk climate according to the Koppen-Geiger classification, this winery has a mean annual temperature of 10.8°C, maximum and minimum temperatures of 39°C and 21°C, respectively. The mean annual rainfall was 147 mm, which mainly distributed from June to September. The annual reference evapotranspiration was 1208.5 mm. The soil is loamy sand soil, classified as a Calcic Cambisol (FAO/UNESCO/ISRIC, 1988). The rhizosphere soils were obtained by gently shaking the plant roots; to isolate from them the bacteria with ACC deaminase activity, we followed Penrose and Glick's (2003) method. Briefly, 1 g rhizosphere soil was inoculated in a 50-ml sterile DF medium, which contained 3 mm ACC as the sole nitrogen (N) source (Dworkin and Foster, 1958). Then, these media were incubated in a shaking incubator at 200 rpm and 28°C for 24 h. The ensuing isolates were kept for further use in Luria-Bertani slants (at 4°C).

Bacterial Identification Using a 16S rDNA Sequencing Analysis

For each of the seven ACC deaminase-producing strains obtained, the 16S ribosomal DNA gene was amplified by PCR using the primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTACGACTT-3') at the genus level (Niu et al., 2018). The 16S rRNA sequences were determined with an ABI3730-XL DNA sequencer (Sangon Biotechnology Ltd., Shanghai, China). The detailed steps are described by Zhang et al. (2020). The obtained sequences were aligned and analyzed by the BLAST algorithm for comparison with published sequences in the National Center for Biotechnology Information nucleotide database,¹ and submitted to GenBank. The phylogenetic tree based on 16S rRNA sequencing analysis was constructed in MEGA v7.0, by using the neighbor-joining method applied to distance matrices.

Analysis of Drought Tolerance and Plant Growth-Promoting Traits of Isolated Strains

The drought tolerance experiment of the isolated strains was fully described by Zhang et al. (2020). To evaluate the drought tolerance of isolates, different concentrations of polyethylene glycol 6000 were added to nutrient broth medium to create water potentials of different gradients (0, -0.05, -0.30, and -0.49 MPa; Michel and Kaufmann, 1973). These media were inoculated with 1% bacterial strains isolated by ACC as the sole N sources and then incubated. Next, the growth-promoting traits of these strains were measured using the spectrophotometric method at 600 nm. Phosphate solubilization was measured using method of the Mo-blue (Watanabe and Olsen, 1965; Chen et al., 2006). Döbereiner's nitrogen-free medium was used to grow the bacterial strains to determine their putative N fixation ability (Day and Döbereiner, 1976; Cattelan et al., 1999). The IAA was detected using Salkowski's reagent (Hassan et al., 2014). The EPS was measured using the phenol-sulfuric acid method (Khan et al., 2017). The ACC deaminase activity was detected by the method of Penrose and Glick (2003), whereby the amount of α -ketobutyrate degraded from ACC by isolated strains is monitored. According to the Bradford (1976) method, the protein content of toluenized cells was estimated.

Pot Experiment Design

The pot experiment had a completely randomized design and was performed in a greenhouse from April to September in 2019 on the campus of Northwest A&F University, China (34°17'23"N, 108°4'14"E). Environmental conditions in the greenhouse during the drought stress were as follows: air temperature of 26–32°C, solar radiation of 300–600 W/m², reference evapotranspiration of 3–5 mm/d, and vapor pressure deficit of 3.5–4.5 Kpa. Based on the above results, *Pseudomonas corrugata* (DR3) and *Enterobacter soli* (DR6) were selected for this pot experiment. Separate inoculums of *P. corrugata* (DR3) and *E. soli* (DR6) were prepared by diluting the cultures to

a final concentration of 10⁸ CFU/ml. The soil for the pot experiment was also collected in the vineyard of Xige winery and classified as sandy loam, and heat-sterilized at 121°C for 3 h (Barnawal et al., 2016b). Soil sterilization was applied to avoid interference of the native microbial communities and thus ameliorate colonizing efficiency of the inocula. Soil chemical properties for the pot experiment were as follows: pH of 8.03, organic matter (OM) content of 11.38 g/kg, total nitrogen (TN) content of 0.51 g/kg, total phosphorus (TP) content of 0.39 g/kg, and an available P (AP) content of 4.35 mg/kg.

For the bacterial inoculations, four treatments were implemented as follows: (1) *P. corrugata* alone (T1); (2) *E. soli* alone (T2); (3) a 1:1 volume mixture of *P. corrugata* and *E. soli* (T3); and (4) control without any bacteria (CK). For the drought treatment, plants were grown at two levels: at 75% field capacity [control, no drought stress (ND)] or 35% field capacity (drought stress, DS). Therefore, in this 2 × 4 factorial experiment, there was a total of 8 treatment combinations (4 bacterial inoculations × 2 drought conditions), with 15 replicates of each treatment. To apply ACC deaminase-producing bacteria *P. corrugata*, *E. soli*, and their mixture, a syringe was used, through which 150 ml of a given bacterial suspension was inoculated into the middle of a grapevine's root system. The control grapevines received 150 ml of ddH₂O with no bacteria.

The Cabernet Sauvignon (*V. vinifera*) grapevines were obtained from the commercial vineyard of Xixia King Industry Co. Ltd. in Ningxia. These grapevines were cultivated with the hardwood cuttings using a conventional cutting propagation method in the last year. A 1-year-old own-rooted Cabernet Sauvignon grapevine was planted in each polyethylene pot (25 cm diameter × 30 cm height) as one replicate in early April. The plants were subjected to drought stress in early August. Daily weighing of the pots was used to regulate the water content, and any lost water was replenished at 18:00 every day. The imposed drought conditions lasted for 25 days, after which the soil and plant samples were collected for determination of the growth, physiological and biochemical characteristics of plants, and soil physicochemical properties to evaluate the plant growth-promoting effects of strains.

Sample Collection

After grapevine growth for 25 days under drought stress, plant photosynthetic characteristics were measured in early September. Then, soil and grapevines were all sampled for determination of various indices of soil and plants. Roots and shoots were separately collected by clipping grapevines at the soil surface and directly measured the plant height.

All roots were rinsed with distilled water for determination of root morphology. All the leaves were collected separately and divided into three subsamples: One sample was preserved in liquid nitrogen for antioxidant indicators, one sample was oven-dried at 75°C for leaf nutrients, and the third one that leaf at fifth or sixth nodes of shoots was for leaf relative water content (RWC). Soil samples were divided into two subsamples: One fresh soil sample was used to determine the potential activity of urease and alkaline phosphatase, and the other one

¹<http://www.ncbi.nlm.nih.gov/>

sample was air-dried to measure the aggregate stability and nutrient contents.

Dependent Variables Measured

The root-adhering soil/root tissue (RAS/RT) ratio was determined, as described by Sandhya et al. (2009). Distilled water was used to separate the RAS, after which the roots were dried at 105°C. Soil aggregate stability was then determined according to the amounts of water-stable aggregates (Chaudhary and Kar, 1972; Sandhya et al., 2009). Aggregate stability was assessed according to the amounts of water-stable aggregates. Soil available nitrogen (AN) was quantified using a continuous flow injection analyzer (Seal Auto Analyzer 3-AA3; Zhang et al., 2016). Soil AP was determined by the molybdenum antimony colorimetric method, following the methodology used by Page et al. (1982). The potential activity of urease and alkaline phosphatase was, respectively, assayed using the phenol sodium standard colorimetric and disodium phenyl phosphate standard colorimetric method (Saiya-Cork et al., 2002).

Plant growth characteristics were analyzed by determining the height and stem diameters, biomass of shoot and root parts, root parameters, leaf nutrients, and RWC. Plant height and diameter were measured on a metric scale. To obtain the shoot and root biomass, they were weighed after being dried in a forced hot-air oven at 70°C for 2 days (Ryle et al., 1981). Root morphology was examined by scanning the roots on a flatbed scanner (EPSON, Perfection V-750), for which total root length, root surface area, and volume were obtained for each plant by analyzing the root images in WinRHIZO Arabidopsis 2017a software (WinRHIZO, RRID:SCR_017120; Oddiraju et al., 1996; Himmelbauer et al., 2004).

For leaf nutrients, nitrogen and phosphorus contents were quantified using the method described by Guo et al. (2005) and Chandra et al. (2019). Leaf sample (0.20 g) was weighed and extracted with H₂SO₄-HClO₄. The content of TN was determined at 645 nm by indophenol blue colorimetry, and the content of TP was measured by molybdenum antimony colorimetry. RWC of leaves was determined using the methodology of Meher et al. (2018) and calculated according to Sharp et al. (1990). Briefly, a total of 10 leaves per treatment were weighed and immersed in distilled water at 4°C for 12 h. The leaves were then dried the water on the surface and re-weighed as saturated fresh weight. The immersed leaves were placed in an aluminum box and baked at 105°C for 15 min, and then dried at 75°C to constant weight. When cool to room temperature, the leaves were weighed again to obtain dry weight.

The chlorophyll in leaves was extracted in 10 ml of 80% (v/v) acetone and then measured at the wavelengths of 663 nm and 645 nm by spectrometry, respectively (Calvo-Polanco et al., 2016). Plant photosynthetic characteristics of 10 leaves at sixth or seventh nodes of shoots for each treatment were also evaluated at 9:00–11:00 am on the day after drought stress was finished. Their photosynthetic rates (Pn) and stomatal conductance (Gs) were quantified with a portable photosynthesis system (LI-COR-6400, Lincoln, NE, United States). The flow rate of air was

750 µmol/min. The light source was red and blue LEDs with 1800 µmol/(m²s) of light intensity. The water use efficiency (WUE) was calculated according to the photosynthetic parameters.

The phydetek-IAA and phydetek-abscisic acid (ABA) immunoassay kits (Agdia, Elkhart, IN, United States) were, respectively, used to determine the IAA and ABA contents of leaves. The contents of these two phytohormones were determined based on the manufacturer's protocol.

The grapevines were also used for an assessment of key antioxidant indicators, namely, their malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) levels. MDA content was measured following Heath and Packer (1968). Briefly, leaves tissue (0.50 g) was added into 2 ml of 0.1% trichloroacetic acid (v/v) and ground into homogenate, and then was extracted with 5 ml of 0.5% thiobarbituric acid (v/v) solution. The extract solution was shaken, boiled at boiling water bath for 10 min. After cooling to room temperature, the resulting extraction was centrifuged, and then, the suspension was determined at the 532 nm and 600 nm on a spectrophotometer, respectively.

The SOD, POD, and CAT enzymes were extracted using the method described by Li et al. (2017). Briefly, leaves tissue (0.50 g) was homogenized with precooled 1.5 ml of Tris-HCl buffer (pH 7.5), containing 0.1% mercaptoethanol (v/v), and 5% sucrose (w/v). The homogenate was then centrifuged, and the supernatants of crude enzymes were used to determine the activity of SOD, POD, and CAT. SOD activity was determined by Beyer and Fridovich (1987). One unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction rate of nitroblue tetrazolium by 50% at 25°C. POD activity was measured according to the previous method (Zhou and Leul, 1998). According to Beer and Sizer (1952), CAT activity was determined by monitoring the disappearance of H₂O₂ at 240 nm.

Statistical Analysis

One-way ANOVA was used to analyze the differences of the data with SPSS 19.0. Duncan's multiple range tests compared the means of these variables in a pairwise manner at a significant level of $P < 0.05$. The graphs were drawn in OriginPro 9.0.

RESULTS

Isolation and Identification of Bacterial Strains With ACC Deaminase Activity

Seven ACC deaminase-producing bacterial strains were obtained from the rhizosphere soil of grapevine. These isolated bacterial strains differed in their ability to produce ACC deaminase (Table 1). The ACC deaminase activity of these two stains, isolate DR3 and DR6, was higher than the left five stains. Notably, the ACC deaminase activity of isolate DR3 was 60.11 µmol α-KB/(mg Pr h), which was about 50% higher than the 41.18 µmol α-KB/(mg Pr h) produced by isolate DR6. Next, these seven ACC deaminase-producing

TABLE 1 | 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of the isolated bacteria associated with grapevine rhizosphere soil.

Isolate no.	Genera	Nearest-type strain	Accession no.	Similarity (%) of the 16S rRNA gene	ACC deaminase activity [$\mu\text{mol } \alpha\text{-KB}/(\text{mg Prh})$]
DR1	<i>Pseudomonas fluorescens</i>	GU358073	MK774791	99.72%	15.69 \pm 0.31 g
DR2	<i>Pseudomonas corrugata</i>	MF077202	MK346043	99.86%	22.87 \pm 0.60e
DR3	<i>P. corrugata</i>	MK240442	MK411217	99.93%	60.11 \pm 0.94a
DR4	<i>Pseudomonas frederiksbergensis</i>	MK849865	MK611658	99.72%	26.21 \pm 0.66d
DR5	<i>Pseudomonas frederiksbergensis</i>	KU958696	MK611660	99.86%	34.25 \pm 0.64c
DR6	<i>Enterobacter soli</i>	JQ682636	MK611659	99.51%	41.18 \pm 0.93b
DR7	<i>Achromobacter xylosoxidans</i>	JQ659946	MK611665	99.72%	19.39 \pm 0.45f

Values are the mean \pm SE ($n=3$). Different lowercase letters indicate significant differences according to Duncan's multiple range test at the $P<0.05$ level.

bacterial strains were identified by the 16S rDNA sequencing analysis; their similarity with the closest known type strains was 99–100% (Table 1). The phylogenetic analysis revealed these strains belonged to three different genera: *Pseudomonas* (five isolates), *Enterobacter* (one isolate), and *Achromobacter* (one isolate; Figure 1).

Drought Tolerance, Plant Growth-Promoting Properties, and EPS Production of Isolates

Although all seven isolates had nitrogen fixing, phosphate solubilization, and IAA secretion abilities, the three isolates DR1, DR3, and DR6 were able to survive under drought conditions of -0.30 MPa water potential (Table 2). Furthermore, all strains except DR7 had the ability to secrete EPS, while DR3 and DR6 were distinguished by higher phosphate solubilization, nitrogen fixation, and EPS secretion potential (Table 2). For strain DR3, its phosphate dissolving capacity and EPS production were, respectively, $64.07 \mu\text{g}/\text{ml}$ and $9.43 \text{ mg}/\text{mg}$ protein, with slight lower corresponding values for strain DR6, at $58.97 \mu\text{g}/\text{ml}$ and $7.38 \text{ mg}/\text{mg}$ protein. Further, DR3 and DR6 harbored the greatest IAA production ability (at 10.29 and $11.14 \mu\text{g}/\text{ml}$, respectively). Hence, the DR3 and DR6 strains were used in the pot experiment.

RAS/RT Ratio, Soil Aggregate Stability, Soil Nutrient Contents, and Related Enzyme Activity in Pot Experiment

Drought stress significantly increased both the RAS/RT ratio and soil aggregate stability (Table 3). Moreover, PGPRs also positively influenced the RAS/RT ratio and soil aggregate stability. Under drought, the RAS/RT ratios of treatments with inoculations of T1, T2, and T3 were, respectively, increased by 25.52, 29.35, and 56.47% over the control group; their corresponding soil aggregate stabilities were increased by 19.65, 22.66, and 39.68%. The RAS/RT ratios and soil aggregate stability were significantly higher in the mixed inoculation treatment (T3) than with the inoculation of either bacterial strain alone ($p<0.05$).

The soil available N and available P contents were decreased significantly by drought stress, but this was alleviated after inoculation with PGPRs (Table 3). Compared with the control (CK) under drought stress conditions, the AN and AP

contents under inoculation with PGPRs were increased considerably, by 27.80–93.50% and 20.38–81.84%, respectively. The soil AN content ($39.61 \text{ mg}/\text{kg}$) and AP content ($4.72 \text{ mg}/\text{kg}$) under the T3 inoculation exceeded those in the other treatments ($p<0.05$), under both non-drought and drought stress conditions. Similar patterns characterized the urease and alkaline phosphatase responses (Table 3). Under drought, the urease activity of the T1, T2, and T3 inoculation treatment increased by 53.41, 59.09, and 123.86%, respectively; the corresponding increases for alkaline phosphatase activity were 54.24, 84.67, and 164.57%. Thus, inoculating the rhizosphere with PGPRs can increase the local availability of soil nutrients and reduce the impact of a drought condition on grapevines.

Growth and Photosynthetic Characteristics Responses of Grapevines

Plant height, shoot, root, and leaf growth parameters were all decreased by drought stress (Table 4). However, these negative effects of drought stress were ameliorated after inoculation with different strains, especially the mixed inoculation (T3). Plant height at mixed inoculation (T3) under no drought stress and drought stress were 73.84 cm and 69.46 cm , respectively. Compared with the control, the inoculation of the PGPR strains under drought stress increased plant height by 12.30–32.68%; moreover, the dry weight of shoot and root components was, respectively, increased by 19.84–36.64%, and 28.03–64.74%. In addition, the strains significantly improved the diameter of grapevines' shoot, as well as the length, surface area, volume, and activity of their roots. In particular, the T3 treatment increased the root activity by 115.12%, directly affecting plant growth and nutrient contents.

The growth and photosynthetic characteristics of grapevines were markedly decreased while under drought stress (Tables 4 and 5), whereas inoculation of the strains was able to effectively counter this impact. Leaf total nitrogen, total phosphorus, RWC, chlorophyll contents, Pn, Gs, and WUE were all increased at treatments with inoculations of the strains by 50.77–110.99%, 68.99–220.25%, 15.17–31.07%, 31.63–61.22%, 30.04–61.31%, 52.89–198.10%, and 41.67–103.13%, respectively, when compared with the control under drought stress. Among the three treatments, the mixed inoculation (T3) best improved the growth and photosynthetic characteristics of grapevines.

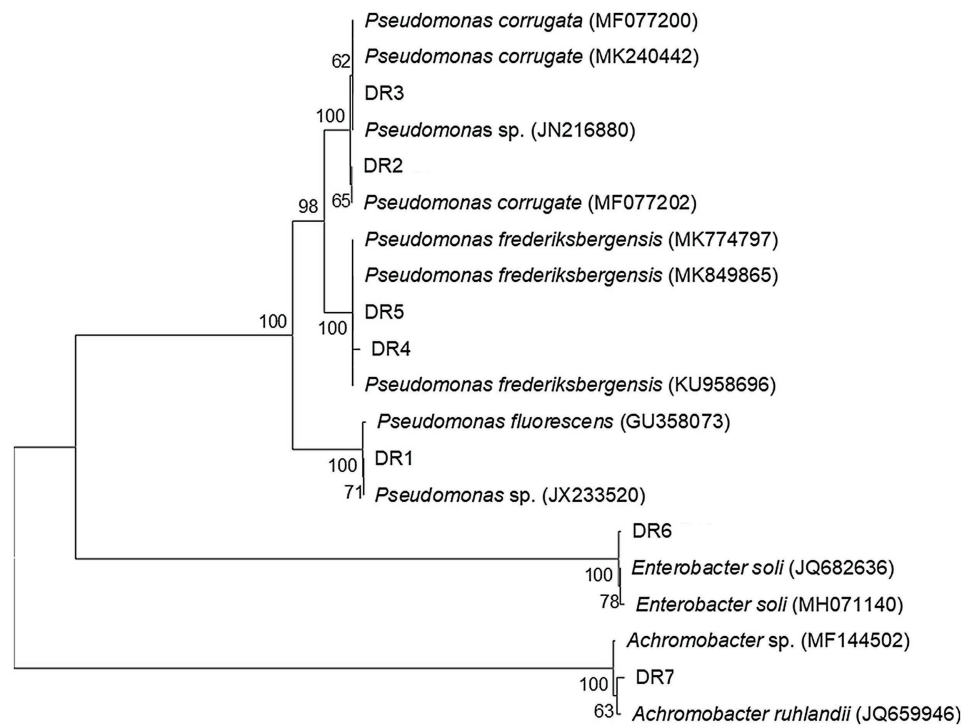


FIGURE 1 | Phylogenetic tree of seven ACC deaminase-producing strains isolated from the rhizosphere of grapevines. Distance and clustering analyses were performed, using the neighbor-joining method, in MEGA v7.0. Bootstrap values ($n = 1,000$) are given as percentages at the branching points.

TABLE 2 | Plant growth-promoting characteristics of the isolates from grapevine rhizosphere soil.

Strain	Drought tolerance (OD ₆₀₀ at −0.30 MPa)	Indoleacetic acid (IAA) production (μg/ml)	Nitrogen fixation	Phosphorus-solubilizing circle (mm)	Phosphate dissolving (μg/ml)	Exopolysaccharides production (mg/mg protein)
DR1	0.25	4.46 ± 0.27f	+	6.51 ± 0.29d	49.37 ± 0.39e	4.10
DR2	–	8.33 ± 0.44cd	+	7.62 ± 0.30d	52.25 ± 0.64d	4.44
DR3	1.05	10.29 ± 0.21b	+	14.17 ± 0.08a	64.07 ± 0.79a	9.43
DR4	–	9.08 ± 0.20c	+	9.91 ± 0.24c	57.06 ± 0.48c	4.16
DR5	–	8.07 ± 0.13d	+	7.36 ± 0.13d	51.89 ± 0.33d	3.52
DR6	0.84	11.14 ± 0.11a	+	11.65 ± 0.91b	58.97 ± 0.17b	7.38
DR7	–	5.44 ± 0.41e	+	7.60 ± 0.11d	51.84 ± 0.28d	–

Values are the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences according to Duncan's multiple range test at the $P < 0.05$ level.

IAA and ABA Contents

Drought stress and the bacterial strains' inoculation significantly influenced the phytohormones, IAA, and ABA of grapevines (Figure 2). Their IAA content was significantly decreased by drought stress, but it was significantly 18.9–78.8% and 55.2–131.4% higher in the PGPR inoculation treatments than the controls without and with drought stress, respectively. Among the inoculation treatments under both non-drought and drought stress conditions, the IAA content of grapevines inoculated with T3—the mixed inoculation of *P. corrugata* and *E. soli*—was always the highest, while their ABA contents were the lowest. However, the ABA content of grapevines

was significantly increased by drought stress. Compared with the control under drought stress, ABA contents of inoculation treatments T1 (*P. corrugata*), T2 (*E. soli*), and T3 (*P. corrugata* and *E. soli*) were reduced by 9.94, 11.54, and 35.42%, respectively.

MDA Content and Antioxidant Enzymes Activity

Drought stress caused a significant increment in MDA content and augmentation in the activity of antioxidant enzymes (SOD, POD, and CAT; Figure 3). Compared with the non-inoculated grapevines, the MDA content decreased by 12.7–25.9%, and

TABLE 3 | Effects of inoculation with ACC deaminase-producing plant growth-promoting rhizobacteria (PGPRs) on root-adhering soil/root tissue (RAS/RT) ratio, soil aggregate stability, available N, available P and enzyme activities under non-drought (ND) and drought stress (DS) conditions.

Drought stress	Treatment	RAS/RT	Aggregate stability (%)	Available N (mg/kg)	Available P (mg/kg)	Urease (mg/g)	Alkaline phosphatase (mg/kg)
ND	CK	23.52±0.70d	34.75±0.84d	30.96±0.84c	4.23±0.11d	1.34±0.07c	14.75±0.84d
	T1	30.27±1.13c	42.56±2.04c	38.71±1.61b	5.47±0.28b	1.82±0.18b	22.56±1.61c
	T2	31.17±2.59c	42.95±0.87c	39.04±0.87b	5.67±0.08b	1.91±0.04b	25.95±0.87b
	T3	32.02±1.26c	49.66±2.64b	47.90±0.91a	6.52±0.13a	2.67±0.04a	37.66±0.91a
DS	CK	33.15±0.97c	43.20±1.17c	20.47±0.66e	2.60±0.06f	0.88±0.08d	9.20±0.66e
	T1	41.61±1.87b	51.69±1.90b	26.16±0.94d	3.13±0.05e	1.35±0.19c	14.19±0.94d
	T2	42.88±1.70b	52.99±2.19b	30.97±1.04c	3.31±0.06e	1.40±0.04c	16.99±1.04d
	T3	51.87±2.73a	60.34±1.45a	39.61±0.88b	4.72±0.08c	1.97±0.12b	24.34±0.88bc

Values are the mean ± SE (n=3). Different lowercase letters indicate significant differences according to Duncan's multiple range test at the $P < 0.05$ level.

the SOD, POD, and CAT activities improved by 11.8–74.1%, 44.2–118.4%, and 28.5–103.6%, respectively. Under drought, the MDA content was significantly decreased by the PGPR inoculations, reaching its lowest value, 23.98 nmol/g, under T3. Conversely, antioxidant enzymes (SOD, POD, and CAT) activities were significantly increased after the inoculation with PGPRs, attaining their highest values under T3. These results showed that PGPR inoculations could reduce the accumulation of MDA in leaves of grapevines under drought conditions, thereby lessening the severity of incurred membrane damage and improving their drought resistance.

Soil and Plant Growth Properties Associated With the Phytohormones and MDA

The phytohormones IAA and ABA, and MDA, each had significantly positive correlations with the grapevines' root and shoot properties and photosynthetic characteristics, and the soil properties (Table 6). Yet, only SOD, POD, and CAT were positively correlated with soil properties. Specifically, IAA was positively correlated with the dry weight of root as well as the dry weight of shoot and the R/S ratio (Figure 4). In addition, IAA content was also positively correlated with the soil AN and AP contents. By contrast, ABA was negatively correlated with the dry weight of root and shoot, the R/S ratio as well as Gs, and also AN and AP. Lastly, MDA was negatively correlated with RWC.

DISCUSSION

The PGPRs with ACC deaminase activity could reduce ethylene's inhibition of plant growth and induce plant stress resistance via phytohormone signaling pathways, thus positively impacting plant growth and alleviating abiotic stresses, like drought. Unsurprisingly, such PGPRs are sought/isolated and widely studied for various crops to help them cope with adverse growing conditions. In this study, a total of seven ACC deaminase-possessing strains from grapevine rhizosphere soil in the arid region were isolated. Their ACC deaminase activities

varied almost 4-fold, from 15.69 to 60.11 $\mu\text{mol } \alpha\text{-KB}/(\text{mg Prh})$. The levels of ACC deaminase activity measured in our study are generally higher than those of strains isolated from rhizosphere soil of other crops, such as 1.89–39.40 $\mu\text{mol } \alpha\text{-KB}/(\text{mg Prh})$ in foxtail millet (Niu et al., 2018) and 1.82–41.58 $\mu\text{mol } \alpha\text{-KB}/(\text{mg Prh})$ in wheat (Ansari et al., 2021). These differences may be due to the different species studied, as well as the degree of stress incurred by the host plants (Niu et al., 2018), given that an environmental stress likely induces the strain to develop tolerance to that stress (Hoffmann and Hercus, 2000). More than 20 genera of rhizosphere bacteria are now known to harbor potential plant disease prevention and growth promotion benefits, such as *Pseudomonas*, *Bacillus* (Broadbent et al., 1977), *Agrobacterium*, and *Eriwinia*. Among these genera, *Pseudomonas* is the dominant genus, accounting for 60–93% of the PGPRs identified to date. Further, the genera of isolated strains mainly depend on the species identity of their host plants (Gontia-Mishra et al., 2017; Niu et al., 2018); for example, *Pseudomonas* and *Arthrobacter* were isolated from rhizosphere soil of millet, and likewise *Acinetobacter* in addition to *Bacillus* from that of wheat (Timmusk et al., 2014; Gontia-Mishra et al., 2017). Among the seven isolated strains in our study, at a frequency of 74.1%, *Pseudomonas* clearly was the dominant class in the rhizosphere soil of grapevine. Further, of the seven strains, two strains (*P. corrugata* DR3 and *E. soli* DR6) not only could survive under the -0.3 MPa condition but also featured the greatest ACC deaminase activity (DR3: 60.11 $\mu\text{mol } \alpha\text{-KB}/(\text{mg Prh})$; DR6: 41.18 $\mu\text{mol } \alpha\text{-KB}/(\text{mg Prh})$). This key trait could, to a considerable extent, relieve the ethylene stress to plants induced by drought, along with positively affecting their growth under drought conditions. Therefore, these two strains were used in the pot experiment to test the effects of PGPR on grapevines and discern their mechanisms.

In this study, drought decreased the plant height, the dry matter and diameter of shoots, the root length and volume, and leaf nutrient and photosynthetic characteristics, thus impairing the growth of grapevines. These results are supported by other findings that drought can alter physiological, biochemical, and molecular processes in plants, leading to productivity losses (Kaushal and Wani, 2016). Some studies reported that a PGPR inoculation could modulate key

TABLE 4 | Effects of inoculation with ACC deaminase-producing PGPRs on the growth parameters and nutrient uptake of grapevines under non-drought (ND) and drought stress (DS) conditions.

Drought stress	Treatments	Plant height (cm)		Shoot		Root			Leaf		
		DW ^a (g)	Diameter (mm)	DW (g)	Length (cm)	Surface area (cm ²)	Volume (cm ³)	Activity (μg/g/h)	TN ^b (g/kg)	TP ^c (g/kg)	RWC ^d (%)
ND	CK	5.79±0.04c	4.45±0.21c	4.99±0.13cd	3,192±83c	706±9c	10.42±0.43d	63.81±1.32d	13.69±0.07f	2.43±0.15d	80.29±0.76c
	T1	6.84±0.50b	5.43±0.14b	5.84±0.17b	3,302±92bc	767±21b	15.62±0.72b	75.96±4.74c	19.71±0.18d	3.54±0.16c	87.10±0.65b
	T2	7.02±0.38b	5.56±0.16b	5.99±0.29b	3,465±57b	789±8b	17.25±1.73b	87.21±1.03b	22.04±0.04c	4.93±0.46b	87.78±0.80b
	T3	8.04±0.21a	6.18±0.23a	7.70±0.22a	3,843±54a	863±14a	20.98±0.69a	103.18±1.52a	29.92±0.04a	6.40±0.34a	92.39±0.75a
DS	CK	4.94±0.09d	3.67±0.12d	3.46±0.07f	2,277±85e	508±5f	6.76±0.47e	29.97±0.62f	11.01±0.08g	1.58±0.26e	62.48±0.81e
	T1	6.68±0.05b	4.52±0.18c	4.43±0.52d	2,588±56d	566±10e	10.38±0.44d	51.03±3.85e	16.60±0.19e	2.67±0.28d	71.96±0.56d
	T2	5.92±0.15c	4.68±0.17c	4.79±0.24d	2,715±51d	636±14d	10.57±0.33d	57.27±3.91de	19.65±0.04d	3.96±0.12c	74.50±1.04d
	T3	6.75±0.08b	5.39±0.12b	5.70±0.17bc	3,199±104c	705±8c	13.07±0.33c	64.47±1.22d	23.23±0.12b	5.06±0.30b	81.89±1.24c

^aDW: dry weight.^bTN: total nitrogen.^cTP: total phosphorus.^dRWC: relative water content.

Values are the mean ±SE (n=3). Different lowercase letters indicate significant differences according to Duncan's multiple range test at the P<0.05 level.

morphological and biochemical processes to mitigate the drought stress incurred by crop plants or herbs, such as maize (Jochum et al., 2019), wheat (Yaseen et al., 2019), and *Chlorophytum* (Barnawal et al., 2016a). Accordingly, in the present study, the growth traits and physiological properties of grapevines were measured under non-drought and drought stress conditions. This demonstrated that single-PGPR inoculations of *P. corrugata* or *E. soli* promoted the plant growth. Importantly, applying the mixture inoculation of both PGPRs had stronger promotion effects than applying either alone. Specifically, plant height, shoot dry matter and diameter, root length and volume, and leaf nutrients were all significantly higher for the mixture of PGPRs than either single-strain inoculation or none at all (control) under drought condition. These results are consistent with other findings (Jochum et al., 2019; Zhang et al., 2020). For example, inoculation with PGPRs improved the growth of spring wheat plants (Baris et al., 2014), whose dry matter content and growth were higher when treated with the mixed than single inoculations (Şahin et al., 2004; Baris et al., 2014). Therefore, two PGPRs, *P. corrugata* and *E. soli*, are capable of effectively enhancing the drought tolerance of grapevine and ameliorating the negative effects to it caused by drought.

Although both PGPRs *P. corrugata* and *E. soli* were isolated, selected, and inoculated to improve drought tolerance and growth of grapevine plants, their mechanisms may nonetheless be complicated and likely involve a complex combination of many pathways (Cohen et al., 2015; Ortiz et al., 2015). For example, PGPRs are known to benefit plant growth under drought conditions in multiple ways (Figure 5), because of their role in various processes: absorption of nutrients and water, root proliferation, aggregate stability, EPS production, and regulating phytohormone secretion (Sandhya et al., 2009; Khan et al., 2017). Nitrogen (N) participates in chlorophyll synthesis, so higher leaf concentrations of N could improve the photosynthetic rate of plants (Chen et al., 2005). Phosphorus (P) could participate in energy synthesis and promote plant growth, which are both determined by the relative supply of N and P in soil (Longstreth and Nobel, 1980; Mattos et al., 2003). Here, adding PGPRs augmented the available N and P contents, along with N, P-related enzyme activities and urease and alkaline phosphatase activities, to promote the 1-year-old own-rooted grapevine growth; however, the mechanisms responsible are different. We know that PGPRs capable of nitrogen fixation and P-solubilization can promote plants' absorption of specific nutrients and increase their utilization rate of nutrients (Islam et al., 2014; Wang et al., 2020). In this way, PGPRs that promote nitrogen fixation will enhance the uptake of N by plants, thereby increasing the N content and photosynthetic rate of leaves. These positive effects on plants would feedback belowground, leading to more plant root exudates being formed and released into the rhizosphere, including ACC. Meanwhile, those PGPRs with ACC deaminase will decompose ACC as an N resource. This could drive a concentration gradient of ACC between the internal and external plant roots, and continuously promote ACC exudation from roots, which would also mitigate the ethylene stress induced by drought.

TABLE 5 | Effects of inoculation with ACC deaminase-producing PGPRs on the chlorophyll contents, Pn, Gs, and water use efficiency (WUE) of grapevine leaves under non-drought (ND) and drought stress (DS) conditions.

Drought stress	Treatments	Chlorophyll contents (mg/g)	Pn ^a (μmol/m ² s)	Gs ^b (mmol/m ² s)	WUE ^c (μmol/mmol)
ND	CK	2.62 ± 0.05c	8.01 ± 0.55cd	80.73 ± 3.58d	1.40 ± 0.12d
	T1	3.05 ± 0.03b	10.11 ± 0.47b	93.46 ± 3.64c	2.52 ± 0.16b
	T2	3.09 ± 0.05b	10.27 ± 0.40b	112.10 ± 4.34b	2.57 ± 0.11b
	T3	3.43 ± 0.08a	11.88 ± 0.38a	144.78 ± 5.68a	3.04 ± 0.16a
DS	CK	1.96 ± 0.01d	5.66 ± 0.49e	26.85 ± 2.45g	0.96 ± 0.08e
	T1	2.58 ± 0.07c	7.36 ± 0.49d	41.05 ± 2.93f	1.46 ± 0.11d
	T2	2.71 ± 0.10c	7.02 ± 0.31d	66.51 ± 3.78e	1.36 ± 0.11d
	T3	3.16 ± 0.15b	9.13 ± 0.52bc	80.04 ± 3.61d	1.95 ± 0.12c

^aPn: photosynthetic rates.^bGs: stomatal conductance.^cWUE: water use efficiency.

Values are the mean ± SE (n=3). Different lowercase letters indicate significant differences according to Duncan's multiple range test at the P<0.05 level.

Because P typically has only one source—mainly from the weathering of bedrock material—the relatively low P content of soil would restrict plant growth (Del Campillo et al., 1999; Ezawa et al., 2002; Vassilev and Vassileva, 2003). Regarding the increased available P contents in soil in this study, it is likely driven by the P-solubilization of PGPRs, this increasing the production of P-related enzymes by PGPRs, which stimulates the availability of P in soil, as suggested by our **Table 5** results. These findings are supported by several studies (Vacheron et al., 2013; Bargaz et al., 2018; Enebe and Babalola, 2018). Moreover, some work has shown that *Pseudomonas* and *Escherichia coli* used in present study function well as phosphate solubilizers (Park et al., 2008). Compared with the single strain, the inoculation of mixed strains had a more pronounced effect on soil fertility. This further proved that applying PGPRs could improve the nutrient contents of soil and promote the absorption and utilization of soil nutrients by plants. Therefore, inoculation with PGPRs could be used as good biofertilizers to regulate the soil nutrient elements and improve drought resistance of grapevines under drought conditions.

Drought is a root-borne stress, because the corresponding root metabolism that occurs under drought mainly will influence the photosynthesis process. Therefore, the alternations to root system architecture under drought conditions are a response best understood as a stress defensive mechanism. The EPS, a high molecular weight type of carbohydrate, could help bacteria attach themselves to the plants root surface for the formation of biofilm which protect the roots from drying (Janczarek and Rachwał, 2013) and improves their drought tolerance. In addition, research has shown that EPS-producing PGPR could improve water uptake and soil nutrients absorption by improving the RAS/RT ratio and macro-aggregate stability under drought conditions (Janczarek et al., 2015). Our results showed that single and mixed inoculations of EPS-producing DR3 and DR6 into the rhizosphere did not only significantly increase the RAS/RT, RWC, and soil aggregation stability of grapevines under drought stress, but they also led to the development of an extensive root system and greater total dry weight. Thus, we may conclude that the main role of EPS production under

drought conditions is to augment the levels of rhizosphere soil nutrients and regulate water.

Drought stress can induce the excessive production of reactive oxygen species (ROS), which could impair or degrade normal cell metabolism functioning *via* oxidative damage of DNA, membrane proteins, and lipids, ultimately limiting the growth of plants (Song et al., 2014; Yang et al., 2015). Notably, MDA, the final decomposition product of membrane lipid peroxidation, could be used to gauge the degree to which a plant has been damaged in the face of adversity (Peng et al., 2020). This view is supported by our study's results, in that imposing drought significantly increased the MDA in grapevine. To overcome the negative effects caused by ROS, the antioxidant defense systems of plants, which include SOD, POD, and CAT enzymes, are activated under drought stress (Jesus et al., 2015) to eliminate excessive ROS. In our study, the SOD, POD, and CAT activities of the grapevines inoculated under drought stress were significantly higher than those without inoculation and accompanied by a decreased MDA. Therefore, our results support the view that the PGPR inoculations are very effective at reducing oxidative damage under different stress conditions (Barnawal et al., 2016a; Zhang et al., 2020). While facing drought stress conditions, this study's experimental injections of *P. corrugata*, *E. soli*, and their mixed inoculation into the rhizosphere were able to significantly reduce the MDA content of the grapevines. Accordingly, we anticipate the three inoculums could reduce the MDA content by increasing the activity of SOD and POD under drought conditions, thereby improving the antioxidant defense activity and drought resistance of grapevines in field settings.

Further, the hormonal balance of a plant can be modulated by phytohormones produced by PGPRs. This could regulate morphological and physiological characteristics of plants indirectly, thus promoting plant growth under stressful conditions. Our results revealed that drought stress significantly decreased IAA, whereas the two strains have stronger capacity to promote the synthesis of IAA (*P. corrugata*: 10.29 μg/ml; *E. soli*: 11.14 μg/ml). Inoculation with PGPRs increased the endogenous IAA production compared with the control under both normal

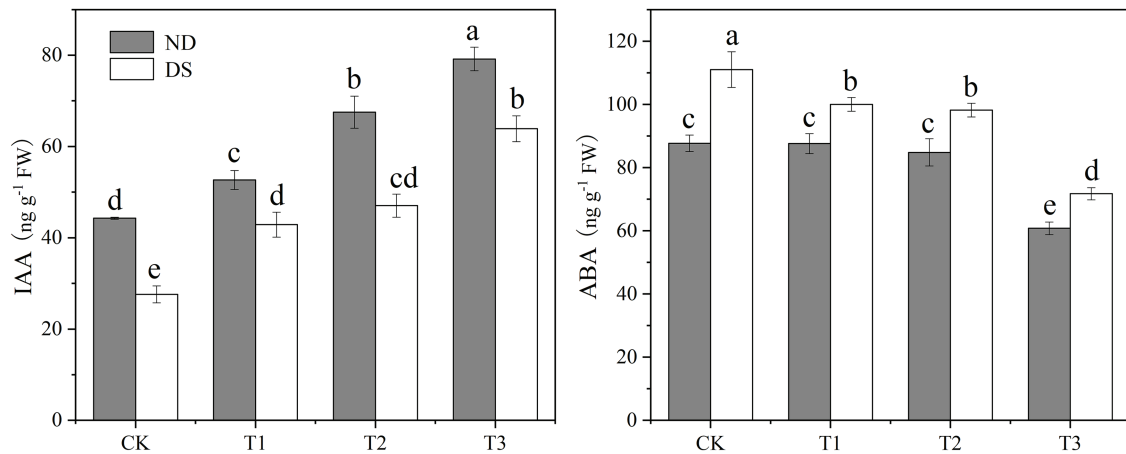


FIGURE 2 | Effects of inoculation with ACC deaminase-producing PGPRs on IAA and ABA contents of grapevine leaves under non-drought (ND) and drought stress (DS) conditions. Bars represent the mean ± SE (n = 3).

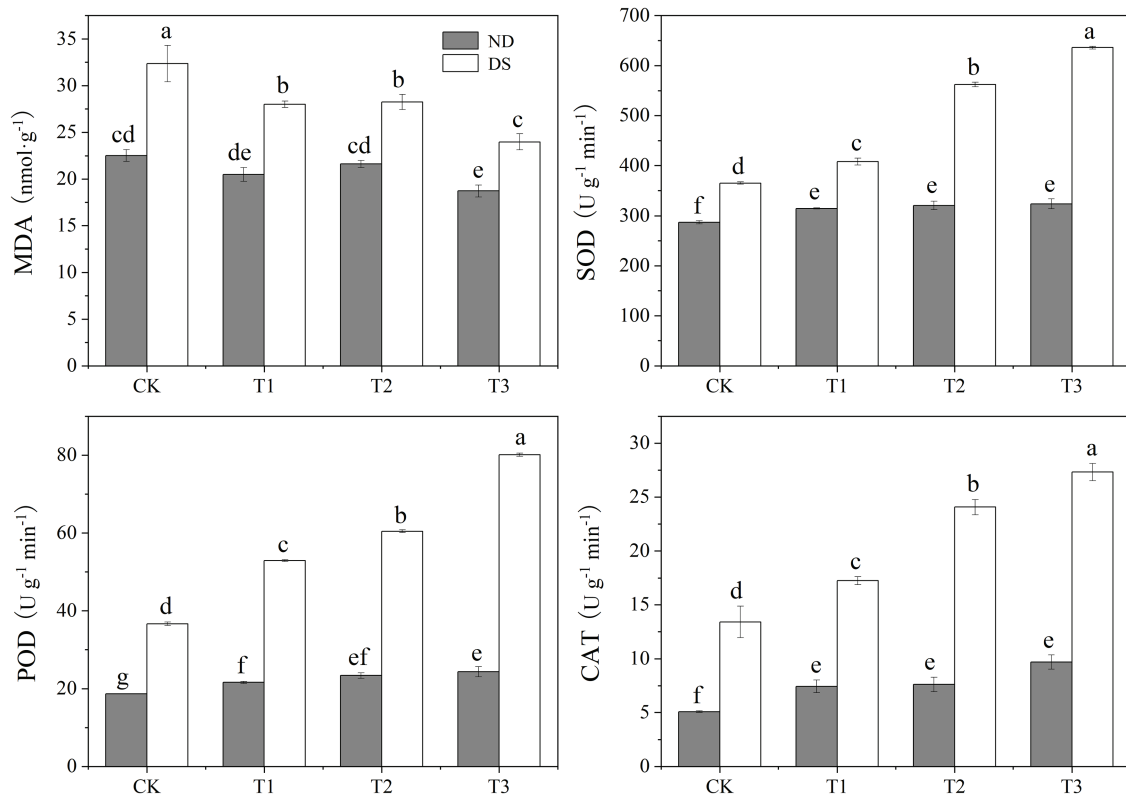


FIGURE 3 | Effects of inoculation with ACC deaminase-producing PGPRs on the malondialdehyde (MDA) content and antioxidant enzymes activity of grapevine leaves under non-drought (ND) and drought stress (DS) conditions. Bars represent the mean ± SE (n = 3).

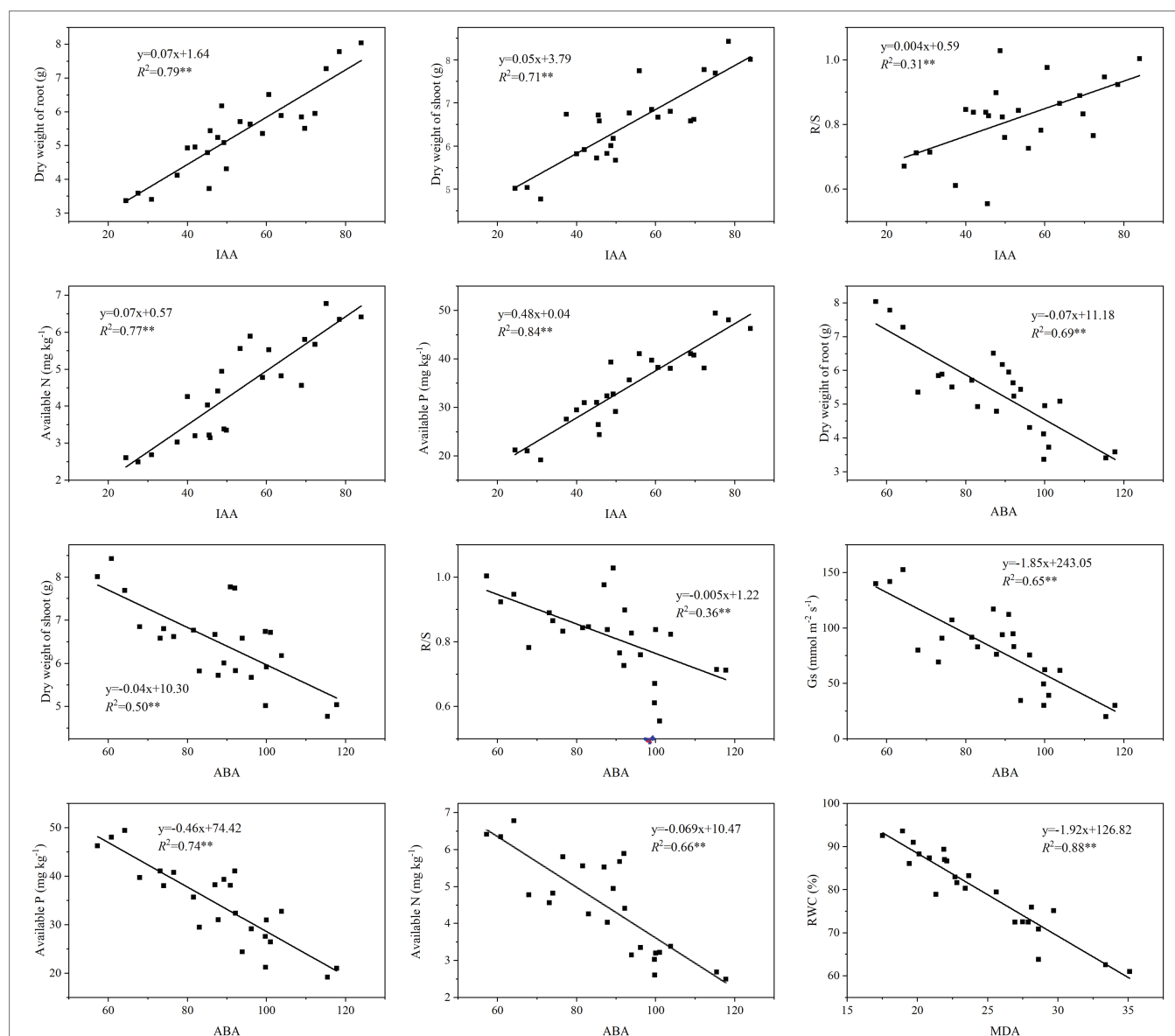
and drought conditions. In this way, the root biomass and surface area would be increased, which then promoted the uptake of water and nutrients, thus ensuring plant growth and survival when incurring drought stress. This is supported by other findings that IAA is one of the vital factors which can alleviate exogenous stress upon plants, by stimulating plant

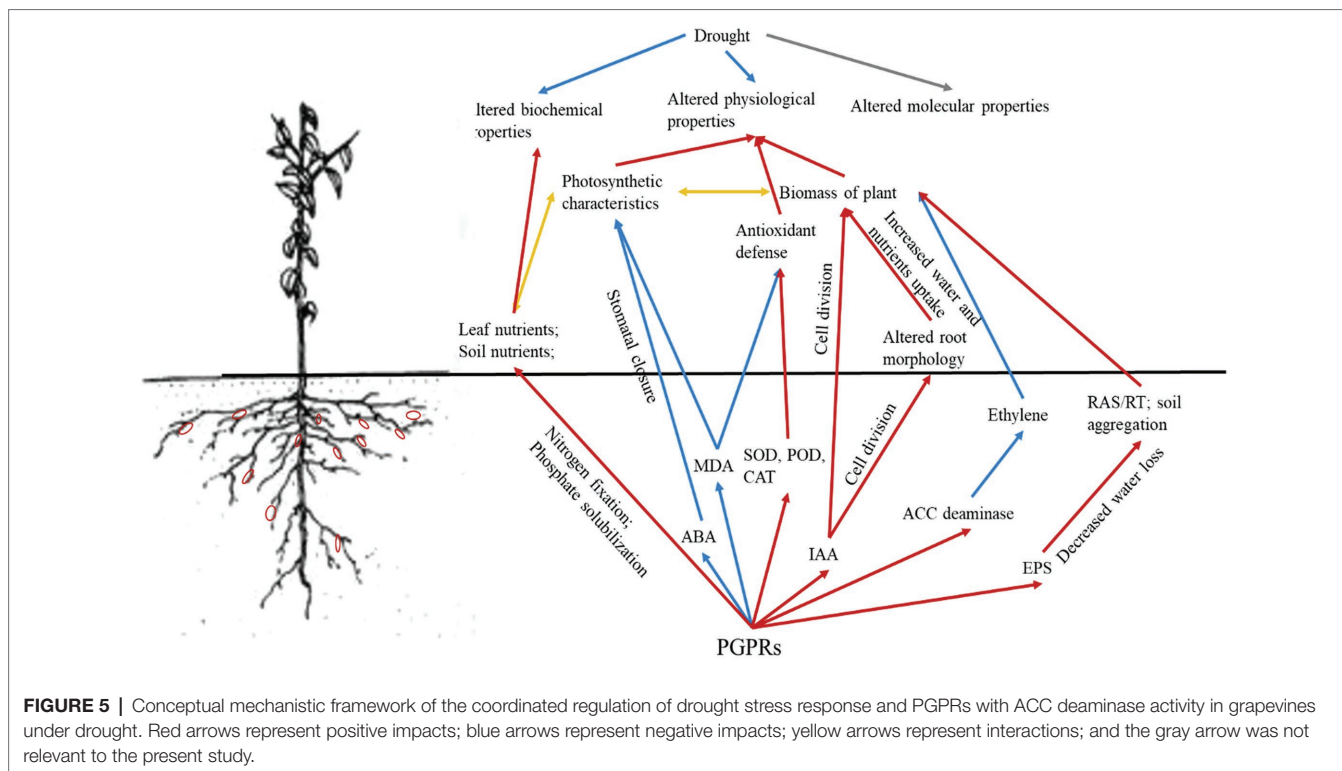
development, significantly increasing dry matter content, shoot or root lengths, and affecting the absorption of nutrients by plants (Baris et al., 2014; Pal et al., 2019; Zerrouk et al., 2019). Previous studies have also shown that IAA could promote the growth of cotton (Kapgate et al., 1989), soybeans (Sarkar et al., 2002), and *Brassica juncea* (Mir et al., 2020). In addition, some

TABLE 6 | Mantel test results for the correlations between the grapevines' phytohormones, MDA, and antioxidant enzymes and their root, shoot, photosynthesis, and soil properties.

Parameters	Root properties		Shoot properties		Photosynthetic properties		Soil properties	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
IAA	0.66	0.00	0.80	0.00	0.62	0.00	0.57	0.00
ABA	0.69	0.00	0.68	0.00	0.55	0.00	0.53	0.00
MDA	0.89	0.00	0.75	0.00	0.76	0.00	0.50	0.00
SOD	−0.05	0.65	−0.08	0.77	−0.08	0.79	0.34	0.00
POD	0.09	0.15	0.01	0.39	0.03	0.31	0.46	0.00
CAT	0.04	0.28	−0.03	0.60	−0.02	0.52	0.41	0.00

Bold numbers indicate significant correlations ($P < 0.05$).

**FIGURE 4 |** Linear regressions showing effects on the physiological and biochemical properties of grapevines from the phytohormones and MDA that were altered by inoculation with ACC deaminase-producing PGPRs.



studies demonstrated IAA does not only regulate growth of the root system to increase the absorption of water and nutrients, but also enhance stomatal conductance, photosynthetic rate, and the activity of antioxidant enzymes by removing excess ROS (Shi et al., 2014; Li et al., 2019).

Another important stress hormone, ABA, has been well-studied for how it enhances drought tolerance in plant through regulating stress-induced genes as well as signaling the stomatal closure under drought conditions (During, 1984; Jochum et al., 2019). Diminished stomatal conductance is beneficial for lessening transpiration, thus reducing water losses, but it may lead to insufficient CO₂ and a lowering of the plant photosynthesis rate. Our results showed that drought significantly increased ABA and indirectly decreased Gs to reduce transpiration in grapevines, thereby increasing their WUE. Inoculation with PGPRs significantly decreased the ABA concentration in grapevines, compared with non-inoculated grapevines under drought stress. Similar results were reported for maize (Porcel et al., 2014) and tomato (Belimov et al., 2015). Therefore, we speculated that PGPRs with ACC deaminase activity might alleviate the diminished photosynthetic characteristics due to drought stress by reducing the content of ABA. Our experiment confirmed this hypothesis, in that the chlorophyll content of leaves, Pn, Gs, and WUE were all significantly increased by inoculation strains under drought conditions. Those results are supported by Liu et al. (2019), who determined the photosynthetic characteristics of *Sambucus williamsii* Hance seedling leaves in response to inoculation with *Acinetobacter calcoaceticus* X128. Therefore, PGPRs can promote plant growth and increase dry matter accumulation by increasing the photosynthetic rate (Liu et al., 2013).

CONCLUSION

This study shows that several ACC deaminase-producing rhizobacteria exhibit high tolerance to drought stress. Hence, roots of wine grapevines can be selected as a resource to isolate PGPRs that might be used to protect plants from drought stress impacts. Our study suggests that inoculations with *Pseudomonas corrugate* DR3 and *E. soli* DR6 isolated from rhizosphere soil of wine grape could effectively alleviate drought stress damage and promote the growth of 1-year-old own-rooted grapevines, with their mixed inoculation showing the best promotion effects. The mechanisms by which PGPRs alleviate environmental stress and promote plant growth rely on complex combination of numerous pathways. These two strains improved soil nutrients and then promoted plant growth by contributing to enhanced nitrogen fixation and phosphate solubilization. Additionally, the PGPRs' ability to partly regulate phytohormones and induce the ROS defense system indirectly affects biochemical and physiological properties of grapevines, ameliorating the drought stress incurred by plants. Therefore, *P. corrugata* DR3 and *E. soli* DR6 both offer great potential as biological fertilizers in arid regions for the sustainable development of the grape industry.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession numbers can be found at: <https://www.ncbi.nlm.nih.gov/>,

MK774791; MK346043; MK411217; MK611658; MK611660; MK611659; and MK611665.

AUTHOR CONTRIBUTIONS

XL designed the experiments and was responsible for the manuscript revision. GC performed the experiment and laboratory analysis. BD and LL co-wrote the paper. CS-Z, YL, HM, and WL analyzed the data and edited the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

- Aballay, E., Mårtensson, A., and Persson, P. (2011). Screening of rhizosphere bacteria from grapevine for their suppressive effect on *Xiphinema* index Thorne & Allen on *in vitro* grape plants. *Plant Soil* 1, 313–325. doi: 10.1007/s11104-011-0851-6
- Alemneh, A. A., Zhou, Y., Ryder, M. H., and Denton, M. D. (2021). Large-scale screening of rhizobacteria to enhance the chickpea-Mesorhizobium symbiosis using a plant-based strategy. *Rhizosphere* 18:100361. doi: 10.1016/j.rhishp.2021.100361
- Ansari, F. A., Jabeen, M., and Ahmad, I. (2021). *Pseudomonas azotoformans* FAP5, a novel biofilm-forming PGPR strain, alleviates drought stress in wheat plant. *Int. J. Environ. Sci. Technol.* 1–16. doi: 10.1007/s13762-020-03045-9
- Bargaz, A., Iyamlouli, K., Chtouki, M., Zeroual, Y., and Dhiba, D. (2018). Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. *Front. Microbiol.* 9:1606. doi: 10.3389/fmicb.2018.01606
- Baris, O., Sahin, F., Turan, M., Orhan, F., and Gulluce, M. (2014). Use of plant-growth-promoting rhizobacteria (PGPR) seed inoculation as alternative fertilizer inputs in wheat and barley production. *Commun. Soil Sci. Plant Anal.* 45, 2457–2467. doi: 10.1080/00103624.2014.912296
- Barnawal, D., Bharti, N., Tripathi, A., Pandey, S. S., Chantotiya, C. S., and Kalra, A. (2016a). ACC-deaminase-producing endophyte *Brachybacterium paraconglomeratum* strain SMR20 ameliorates *Chlorophytum* salinity stress via altering phytohormone generation. *J. Plant Growth Regul.* 35, 553–564. doi: 10.1007/s00344-015-9560-3
- Barnawal, D., Pandey, S. S., Bharti, N., Pandey, A., Ray, T., Singh, S., et al. (2016b). ACC deaminase-containing plant growth-promoting rhizobacteria protect *Papaver somniferum* from downy mildew. *J. Appl. Microbiol.* 122, 1286–1298. doi: 10.1111/jam.13417
- Beer, R. F. J., and Sizer, I. W. A. (1952). Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* 195, 133–140. doi: 10.1016/S0021-9258(19)50881-X
- Belimov, A. A., Dodd, I. C., Safronova, V. I., Shaposhnikov, A. I., Azarova, T. S., Makarova, N. M., et al. (2015). Rhizobacteria that produce auxins and contain 1-aminocyclopropane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*). *Ann. Appl. Biol.* 167, 11–25. doi: 10.1111/aab.12203
- Beyer, W. F. Jr., and Fridovich, I. (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.* 161, 559–566. doi: 10.1016/0003-2697(87)90489-1
- Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. doi: 10.1016/0003-2697(76)90527-3
- Broadbent, P., Baker, K. F., Franks, N., and Holland, J. (1977). Effect of *Bacillus* spp. on increased growth of seedling in steamd and in nontreated soil. *Phytopathology* 67, 1027–1034. doi: 10.1094/phyto-67-1027
- Calvo-Polanco, M., Sanchez-Romera, B., Aroca, R., Asins, M. J., Declerck, S., Dodd, I. C., et al. (2016). Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato. *Environ. Exp. Bot.* 131, 47–57. doi: 10.1016/j.envexpbot.2016.06.015

FUNDING

All sources of funding received for the research being submitted here. The National Key Research and Development of China, Key Research and Development of Shaanxi province, and Key Research and Development of Sichuan Province supported the experiments conducted in field and greenhouse. Besides, the Fundamental Research Funds for the Central Universities gave support for the publication fees and sampling analysis.

- Cattelan, A. J., Hartel, P. G., and Fuhrmann, J. J. (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil Sci. Soc. Am. J.* 63, 1670–1680. doi: 10.2136/sssaj1999.6361670x
- Chandra, R., Amit, , and Ghosh, U. K. (2019). Effects of various abiotic factors on biomass growth and lipid yield of *Chlorella minutissima* for sustainable biodiesel production. *Environ. Sci. Pollut. Res. Int.* 26, 3848–3861. doi: 10.1007/s11356-018-3696-1
- Chaudhary, T. N., and Kar, S. (1972). “Aggregate and clod size distribution,” in *Theory and Practice in Agrophysics Measurements*. eds. R. P. Gupta and B. P. Ghildyal (New Delhi: Allied publishers Ltd), 61–70.
- Chen, S. P., Bai, Y. F., Zhang, L. X., and Han, X. G. (2005). Comparing physiological responses of two dominant grass species to nitrogen addition in Xilin River Basin of China. *Environ. Exp. Bot.* 53, 65–75. doi: 10.1016/j.envexpbot.2004.03.002
- Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A., and Young, C. C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* 34, 33–41. doi: 10.1016/j.apsoil.2005.12.002
- Cohen, A. C., Bottini, R., Pontin, M., Berli, F. J., Moreno, D., Boccanandro, H., et al. (2015). Azospirillum brasilense ameliorates the response of Arabidopsis thaliana to drought mainly via enhancement of ABA levels. *Physiol. Plant.* 153, 79–90. doi: 10.1111/ppl.12221
- Day, J. M., and Döbereiner, J. (1976). Physiological aspects of N₂-fixation by a Spirillum from Digitaria roots. *Soil Biol. Biochem.* 8, 45–50. doi: 10.1016/0038-0717(76)90020-1
- Del Campillo, M. C., Van der Zee, S., and Torrent, J. (1999). Modelling long-term phosphorus leaching and changes in phosphorus fertility in excessively fertilized acid sandy soils. *Eur. J. Soil Sci.* 50, 391–399. doi: 10.1046/j.1365-2389.1999.00244.x
- During, B. (1984). Diurnal changes in water relations and abscisic acid in field-grown *Vitis vinifera* cultivars: II. Absciscic acid changes under semi-arid conditions. *New Phytol.* 97, 37–47. doi: 10.2307/2434193
- Dworkin, M., and Foster, J. (1958). Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol.* 75, 592–601. doi: 10.1128/jb.75.5.592-603.1958
- Enebe, M. C., and Babalola, O. O. (2018). The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. *Appl. Microbiol. Biotechnol.* 102, 7821–7835. doi: 10.1007/s00253-018-9214-z
- Evans, R. G., and Sadler, E. J. (2008). Methods and technologies to improve efficiency of water use. *Water Resour. Res.* 44, 767–768. doi: 10.1029/2007WR006200
- Ezawa, T., Smith, S. E., and Smith, F. A. (2002). P metabolism and transport in AM fungi. *Plant Soil* 244, 221–230. doi: 10.1023/A:1020258325010
- Gontia-Mishra, I., Sapre, S., Kachare, S., and Tiwari, S. (2017). Molecular diversity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing PGPR from wheat (*Triticum aestivum* L.) rhizosphere. *Plant Soil* 414, 213–227. doi: 10.1007/s11104-016-3119-3
- Guo, J. F., Yang, Y. S., Chen, G. S., and Lin, P. (2005). Dissolved organic carbon and nitrogen in precipitation, throughfall and stemflow from *Schima superba* and *Cunninghamia lanceolata* plantations in subtropical China. *J. Forestry Res.* 16, 19–22. doi: 10.1007/BF02856847
- Hassan, W., Bano, R., Bashir, F., and David, J. (2014). Comparative effectiveness of ACC-deaminase and/or nitrogen-fixing rhizobacteria in promotion of maize (*Zea mays* L.) growth under lead pollution. *Environ. Sci. Pollut. Res. Int.* 21, 10983–10996. doi: 10.1007/s11356-014-3083-5

- Heath, R. L., and Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 189–198. doi: 10.1016/0003-9861(68)90654-1
- Himmelbauer, M. L., Loiskandl, W., and Kastanek, F. (2004). Estimating length, average diameter and surface area of roots using two different image analyses systems. *Plant Soil* 260, 111–120. doi: 10.1023/B:PLSO.0000030171.28821.55
- Hoffmann, A. A., and Hercus, M. J. (2000). Environmental stress as an evolutionary force. *Bioscience* 50, 217–226. doi: 10.1641/0006-3568(2000)050[0217:ESAAEF] 2.3.CO;2
- Islam, F., Yasmeen, T., Ali, Q., Ali, S., Arif, M. S., Hussain, S., et al. (2014). Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress. *Ecotoxicol. Environ. Saf.* 104, 285–293. doi: 10.1016/j.ecoenv.2014.03.008
- Janczarek, M., and Rachwał, K. (2013). Mutation in the pssA gene involved in exopolysaccharide synthesis leads to several physiological and symbiotic defects in *Rhizobium leguminosarum* bv. *Trifolii*. *Int. J. Mol. Sci.* 14, 23711–23735. doi: 10.3390/ijms141223711
- Janczarek, M., Rachwał, K., Cieśla, J., Ginalska, G., and Bieganski, A. (2015). Production of exopolysaccharide by *Rhizobium leguminosarum* bv. *Trifolii* and its role in bacterial attachment and surface properties. *Plant Soil* 388, 211–227. doi: 10.1007/s11104-014-2320-5
- Jesus, J. M., Danko, A. S., Fiúza, A., and Borges, M. T. (2015). Phytoremediation of salt-affected soils: a review of processes, applicability, and the impact of climate change. *Environ. Sci. Pollut. Res.* 22, 6511–6525. doi: 10.1007/s11356-015-4205-4
- Jochum, M. D., McWilliams, K. L., Borrego, E. J., Kolomiets, M. V., Niu, G. H., Pierson, E. A., et al. (2019). Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. *Front. Microbiol.* 10:2106. doi: 10.3389/fmicb.2019.02106
- Kalozeumis, P., Savvas, D., Aliferis, K., Ntatsi, G., Marakis, G., Simou, E., et al. (2021). Impact of plant growth-promoting rhizobacteria inoculation and grafting on tolerance of tomato to combined water and nutrient stress assessed via metabolomics analysis. *Front. Plant Sci.* 12:670236. doi: 10.3389/fpls.2021.670236
- Kapgate, H. G., Potkile, N. N., Zode, N. G., and Dhopte, A. M. (1989). Persistence of physiological responses of upland cotton to growth regulators. *Ann. Plant Physiol.* 3, 188–195.
- Kaushal, M., and Wani, S. P. (2016). Rhizobacterial-plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. *Agric. Ecosyst. Environ.* 231, 68–78. doi: 10.1016/j.agee.2016.06.031
- Khan, N., Bano, A., and Babar, M. D. A. (2017). The root growth of wheat plants, the water conservation and fertility status of sandy soils influenced by plant growth promoting rhizobacteria. *Symbiosis* 72, 195–205. doi: 10.1007/s13199-016-0457-0
- Li, J., Guan, Y. L., Yuan, L. Y., Hou, J. F., Wang, C. G., Liu, F. F., et al. (2019). Effects of exogenous IAA in regulating photosynthetic capacity, carbohydrate metabolism and yield of *Zizania latifolia*. *Sci. Hortic.* 253, 276–285. doi: 10.1016/j.scienta.2019.04.058
- Li, H. S., Lei, P., Pang, X., Li, S., Xu, H., Xu, Z. Q., et al. (2017). Enhanced tolerance to salt stress in canola (*Brassica napus* L.) seedlings inoculated with the halotolerant *Enterobacter cloacae* HSNJ4. *Appl. Soil Ecol.* 119, 26–34. doi: 10.1016/j.apsoil.2017.05.033
- Liu, F. C., Ma, H. L., Peng, L., Du, Z. Y., Ma, B. Y., and Liu, X. H. (2019). Effect of the inoculation of plant growth-promoting rhizobacteria on the photosynthetic characteristics of *Sambucus williamsii* Hance container seedlings under drought stress. *AMB Express* 9:169. doi: 10.1186/s13568-019-0899-x
- Liu, F. C., Xing, S. J., Ma, H. L., Du, Z. Y., and Ma, B. Y. (2013). Plant growth-promoting rhizobacteria affect the growth and nutrient uptake of *Fraxinus americana* container seedlings. *Appl. Microbiol. Biotechnol.* 97, 4617–4625. doi: 10.1007/s00253-012-4255-1
- Longstreth, D. J., and Nobel, P. S. (1980). Nutrient influences on leaf photosynthesis. Effects of nitrogen, phosphorus, and potassium for *Gossypium hirsutum* L. *Plant Physiol.* 65, 541–543. doi: 10.1104/pp.65.3.541
- Luo, L. J., Xia, H., and Lu, B. R. (2019). Editorial: crop breeding for drought resistance. *Front. Plant Sci.* 10:314. doi: 10.3389/fpls.2019.00314
- Mattos, D., Graetz, D. A., and Alva, A. K. (2003). Biomass distribution and nitrogen-15 partitioning in citrus trees on a sandy entisol. *Soil Sci. Soc. Am. J.* 67, 555–563. doi: 10.2136/sssaj2003.5550
- Mazzei, P., Celano, G., Palese, A. M., Lardo, E., Drosos, M., and Piccolo, A. (2019). HRMAS-NMR metabolomics of Aglianicone grapes pulp to evaluate terroir and vintage effects, and, as assessed by the electromagnetic induction (EMI) technique, spatial variability of vineyard soils. *Food Chem.* 283, 215–223. doi: 10.1016/j.foodchem.2019.01.012
- Meher, S., Shivakrishna, P., Reddy, K. A., and Rao, D. M. (2018). Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. *Saudi J. Biol. Sci.* 25, 285–289. doi: 10.1016/j.sjbs.2017.04.008
- Michel, B. E., and Kaufmann, M. R. (1973). The osmotic potential of polyethylene glycol 6000. *Plant Physiol.* 51, 914–916. doi: 10.1104/pp.51.5.914
- Mir, A. R., Siddiqui, H., Alam, P., and Hayat, S. (2020). Foliar spray of auxin/ IAA modulates photosynthesis, elemental composition, ROS localization and antioxidant machinery to promote growth of *Brassica juncea*. *Physiol. Mol. Biol. Plants* 26, 2503–2520. doi: 10.1007/s12298-020-00914-y
- Naveed, M., Qureshi, M. A., Zahir, Z. A., Hussain, M. B., Sessitsch, A., and Mitter, B. (2015). L-tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by *Burkholderia phytofirmans* PsJN. *Ann. Microbiol.* 65, 1381–1389. doi: 10.1007/s13213-014-0976-y
- Niu, X. G., Song, L. C., Xiao, Y. N., and Ge, W. D. (2018). Drought-tolerant plant growth-promoting rhizobacteria associated with Foxtail Millet in a semi-arid agroecosystem and their potential in alleviating drought stress. *Front. Microbiol.* 8:2580. doi: 10.3389/fmicb.2017.02580
- Novello, G., Gamalero, E., Bona, E., Boatti, L., Mignone, F., Massa, N., et al. (2017). The rhizosphere bacterial microbiota of *Vitis vinifera* cv. Pinot Noir in an integrated pest management vineyard. *Front. Microbiol.* 8:1528. doi: 10.3389/fmicb.2017.01528
- Oddiraju, V. G., Beyl, C. A., and Barker, P. A. (1996). Root growth of seedlings and microcuttings of western black cherry grown in compacted soil. *HortScience* 31, 453–457. doi: 10.21273/HORTSCI.31.3.453
- Ortiz, N., Armada, E., Duque, E., Roldan, A., and Azcon, R. (2015). Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains. *J. Plant Physiol.* 174, 87–96. doi: 10.1016/j.jplph.2014.08.019
- Page, A., Miller, R., and Keeney, D. (1982). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. *Am. Soc. Agro. Soil Sci. Soc. Am* 2, 643–698.
- Pal, A. K., Mandal, S., and Sengupta, C. (2019). Exploitation of IAA Producing PGPR on mustard (*Brassica nigra* L.) seedling growth under cadmium stress condition in comparison with exogenous IAA application. *Plant Sci. Today* 6, 22–30. doi: 10.14719/pst.2019.6.1.440
- Park, K. H., Park, G. T., Kim, S. M., Lee, C. Y., and Son, H. J. (2008). Conditions for soluble phosphate production by environment-friendly biofertilizer resources, *Pseudomonas fluorescens*. *J. Environ. Sci. Int.* 17, 1033–1037. doi: 10.5322/JES.2008.17.9.1033
- Peng, Y., Li, T. L., Jiang, H. M., Gu, Y. F., Chen, Q., Yang, C. R., et al. (2020). Postharvest biochemical characteristics and ultrastructure of *Coprinus comatus*. *Peer J.* 8:e8508. doi: 10.7717/peerj.8508
- Penrose, D. M., and Glick, B. R. (2003). Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant.* 118, 10–15. doi: 10.1034/j.1399-3054.2003.00086.x
- Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., and Crecchio, C. (2015). Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol. Fert. Soils* 51, 403–415. doi: 10.1007/s00374-015-0996-1
- Porcel, R., Zamarreño, Á. M., García-Mina, J. M., and Aroca, R. (2014). Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants. *BMC Plant Biol.* 14:36. doi: 10.1186/1471-2229-14-36
- Ryle, G. J. A., Arnott, R. A., and Powell, C. E. (1981). Distribution of dry weight between root and shoot in white clover dependent on N₂ fixation or utilizing abundant nitrate nitrogen. *Plant Soil* 60, 29–39. doi: 10.1007/BF02377110
- Şahin, F., Çakmakçı, R., and Kantar, F. (2004). Sugar beet and barley yields in relation to inoculation with N₂-fixing and phosphate solubilizing bacteria. *Plant Soil* 265, 123–129. doi: 10.1007/s11104-005-0334-8
- Saiya-Cork, K. R., Sinsabaugh, R. L., and Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biol. Biochem.* 34, 1309–1315. doi: 10.1016/S0038-0717(02)00074-3

- Sandhya, V. Z. A. S. K., Grover, M., Reddy, G., and Venkateswarlu, B. (2009). Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. *Biol. Fertil. Soils* 46, 17–26. doi: 10.1007/s00374-009-0401-z
- Sarkar, P. K., Haque, M. S., and Karim, M. A. (2002). Growth analysis of soybean as influenced by GA₃ and IAA and their frequency of application. *J. Agron.* 3, 123–126. doi: 10.3923/ja.2002.123.126
- Sharp, R. E., Hsiao, T. C., and Silk, W. K. (1990). Growth of the maize primary root at low water potentials: II. Role of growth and deposition of hexose and potassium in osmotic adjustment. *Plant Physiol.* 93, 1337–1346. doi: 10.1104/pp.93.4.1337
- Shi, H., Chen, L., Ye, T., Liu, X., Ding, K., and Chan, Z. (2014). Modulation of auxin content in Arabidopsis confers improved drought stress resistance. *Plant Physiol. Biochem.* 82, 209–217. doi: 10.1016/j.plaphy.2014.06.008
- Singh, R. P., Shelke, G. M., Kumar, A., and Jha, P. N. (2015). Biochemistry and genetics of ACC deaminase: a weapon to “stress ethylene” produced in plants. *Front. Microbiol.* 6:937. doi: 10.3389/fmicb.2015.00937
- Song, Y., Miao, Y., and Song, C. (2014). Behind the scenes: the roles of reactive oxygen species in guard cells. *New Phytol.* 201, 1121–1140. doi: 10.1111/nph.12565
- Spaepen, S., Dobbelaere, S., Croonenborghs, A., and Vanderleyden, J. (2008). Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. *Plant Soil* 312, 15–23. doi: 10.1007/s11104-008-9560-1
- Timmusk, S., Abd El-Daim, I. A., Copolovicim, L., Tanilas, T., Kannaste, A., Behers, L., et al. (2014). Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. *PLoS One* 9:e96086. doi: 10.1371/journal.pone.0096086
- Tiwari, G., Duraivadivel, P., Sharma, S., and Hariprasad, P. (2018). 1-Aminocyclopropane-1-carboxylic acid deaminase producing beneficial rhizobacteria ameliorate the biomass characters of *Panicum maximum* Jacq. By mitigating drought and salt stress. *Sci. Rep.* 8:17513. doi: 10.1038/s41598-018-35565-3
- Vacheron, J., Desbrosses, G., Bouffaud, M. L., Touraine, B., Moënné-Loccoz, Y., Muller, D., et al. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Front. Plant Sci.* 4:356. doi: 10.3389/fpls.2013.00356
- Vassilev, N., and Vassileva, M. (2003). Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. *Appl. Microbiol. Biotechnol.* 61, 435–440. doi: 10.1007/s00253-003-1318-3
- Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., and SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiol. Res.* 184, 13–24. doi: 10.1016/j.micres.2015.12.003
- Wang, J. J., Li, R. V., Zhang, H., Wei, G. H., and Li, Z. F. (2020). Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. *BMC Microbiol.* 20:38. doi: 10.1186/s12866-020-1708-z
- Watanabe, F. S., and Olsen, S. R. (1965). Test of an ascorbic acid method for determining phosphorous in water and NaHCO₃ extracts from soil. *Soil Sci. Soc. Am. J.* 29, 677–678. doi: 10.2136/sssaj1965.03615995002900060025x
- Yang, L. M., Fountain, J. C., Wang, H., Ni, X. Z., Ji, P. S., Lee, R. D., et al. (2015). Stress sensitivity is associated with differential accumulation of reactive oxygen and nitrogen species in maize genotypes with contrasting levels of drought tolerance. *Int. J. Mol. Sci.* 16, 24791–24819. doi: 10.3390/ijms161024791
- Yaseen, R., Zafar-ul-Hye, M., and Hussain, M. (2019). Integrated application of ACC-deaminase containing plant growth promoting rhizobacteria and biogas slurry improves the growth and productivity of wheat under drought stress. *Int. J. Agric. Bio.* 21, 869–878. doi: 10.17957/IJAB/15.0969
- You, J. H., Gao, L., Wang, H. O., Lu, Q. Q., Zhou, L., and Li, S. (2020). Effects of drought stress on physiological indexes of nine grape rootstock varieties. *Non-wood Forest Res.* 38, 180–189. doi: 10.14067/j.cnki.1003-8981.2020.03.021
- Zerrouk, I. Z., Rahmoune, B., Khelifi, L., Mounir, K., Baluska, F., and Ludwig-Muller, J. (2019). Algerian Sahara PGPR confers maize root tolerance to salt and aluminum toxicity via ACC deaminase and IAA. *Acta Physiol. Plant.* 41:91. doi: 10.1007/s11738-019-2881-2
- Zhang, C., Liu, G., Xue, S., and Wang, G. (2016). Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau. *Soil Biol. Biochem.* 97, 40–49. doi: 10.1016/j.soilbio.2016.02.013
- Zhang, M., Yang, L., Hao, R., Bai, X., and Yu, X. (2020). Drought-tolerant plant growth-promoting rhizobacteria isolated from jujube (*Ziziphus jujuba*) and their potential to enhance drought tolerance. *Plant Soil* 452, 423–440. doi: 10.1007/s11104-020-04582-5
- Zhou, W., and Leul, M. (1998). Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. *Plant Growth Regul.* 26, 41–47. doi: 10.1023/A:1006004921265

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Duan, Li, Chen, Su-Zhou, Li, Merkeryan, Liu and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Missing Links in Predicting Berry Sunburn in Future Vineyards

Christopher Bahr*, Dominik Schmidt and Katrin Kahlen

Department of Modeling and Systems Analysis, Hochschule Geisenheim University, Geisenheim, Germany

OPEN ACCESS

Edited by:

Chiara Pastore,
University of Bologna, Italy

Reviewed by:

Andrew Hall,
Charles Sturt University, Australia
Runze Yu,
California State University, Fresno,
United States
JJ Hunter,
Agricultural Research Council of South
Africa (ARC-SA), South Africa

*Correspondence:

Christopher Bahr
christopher.bahr@hs-gm.de

Specialty section:

This article was submitted to
Plant Biophysics and Modeling,
a section of the journal
Frontiers in Plant Science

Received: 27 May 2021

Accepted: 09 September 2021

Published: 12 October 2021

Citation:

Bahr C, Schmidt D and Kahlen K
(2021) Missing Links in Predicting
Berry Sunburn in Future Vineyards.
Front. Plant Sci. 12:715906.
doi: 10.3389/fpls.2021.715906

Sunburn in grapevine berries is known as a recurring disorder causing severe yield losses and a decline in berry quality. The transition from healthy to sunburnt along a temporal trajectory is not fully understood. It is driven by light-boosted local heat impact and modulated by, e.g., past environments of the berry and its developmental state. Events of berry sunburn are often associated with heatwaves, indicating a link to climate change. In addition, the sensitivity of grapevine architecture to changing environmental condition indicates an urgent need to investigate and adapt mitigation strategies of berry sunburn in future vineyards. In this perspective, we want to identify missing links in predicting berry sunburn in vineyards and propose a modeling framework that may help us to investigate berry sunburn in future vineyards. For this, we propose to address open issues in both developing a model of berry sunburn and considering dynamic canopy growth, and canopy interaction with the environment and plant management such as shoot positioning or leaf removal. Because local environmental conditions drive sunburn, we aim at showing that identifying sunburn-reducing strategies in a vineyard under future environmental conditions can be supported by a modeling approach that integrates effects of management practices over time and takes grapevine architecture explicitly into account. We argue that functional-structural plant models may address such complex tasks. Once open issues are solved, they might be a promising tool to advance our knowledge on reducing risks of berry sunburn *in silico*.

Keywords: climate change, grapevine, heat, canopy architecture, light, functional-structural plant model

1. INTRODUCTION

Berry sunburn in grapevines is a recurring disorder that can reduce berry quality and cause severe yield loss (Keller, 2015). Recently, Gambetta et al. (2021) reviewed current knowledge on berry sunburn in grapevine. They conclude that processes resulting in sunburn are highly complex and not fully understood, but key drivers of sunburn are local light conditions and heat impact on the berry surface and a cultivar-specific susceptibility of the berry to sunburn. The latter may depend on various characteristics of the berry such as its developmental stage and its adaptation to the environment.

An increased emergence of sunburn has been observed in recent years in some vine regions in France and Germany (Gambetta et al., 2021). Given that berry sunburn is driven by extreme heat, more frequent and intense heatwaves, which can be expected in future (Masson-Delmotte et al., 2021), could indicate a link of climate change and sunburn. Thus, a more frequent occurrence of sunburn could be expected in the future (Silvestre et al., 2019; Santos et al., 2020; Gambetta et al., 2021), but only if viticulturist could not fully adapt canopy management and associated practices.

Yet, we think that climate change might have even more significant effects on sunburn patterns in a future vineyard: Climate change might further advance phenological phases (Duchêne et al., 2010; Bernardo et al., 2018) and, e.g., shift the ripening phase into periods with higher temperatures, for example, in European and Australian wine regions (Jones et al., 2005; Webb et al., 2007, 2012). In the ripening phase, berries are particularly susceptible to sunburn (Bondada and Keller, 2012); thus, climate change might aggravate sunburn risks of newly sun-exposed berries in this phase. Being less susceptible to sunburn in earlier phases (Hulands et al., 2014) does not mean that there is no potential sunburn risk. Extreme temperatures in heatwaves might counterbalance the protective trait. Thus, assuming that climate change intensifies extreme events (Perkins-Kirkpatrick and Lewis, 2020), this might add sunburn-risk periods even to the earlier growth season. Then again, elevated CO₂ (eCO₂), one driver of climate change, may change bunch architecture (i.e., longer bunches), which might affect sun exposure, and increase growth of secondary lateral shoots, but periods of high temperatures may weaken this effect (Wohlfahrt et al., 2018). Obviously, both statements neglect effects of adapted management practices (Stoll et al., 2010; Zheng et al., 2017; Gatti et al., 2018; Valentini et al., 2018, 2021; Bei et al., 2019; Lavado et al., 2019; Hunter et al., 2020; Gutiérrez-Gamboa et al., 2021; Martinez De Toda, 2021; Naulleau et al., 2021; Schäfer et al., 2021) and other limiting factors like reduced soil water availability (Lopes et al., 2018). Thus, eCO₂ might reduce sunburn risks in the later season because of shading berries by increased lateral leaf area, but high temperatures might attenuate the positive effect. On the other hand, leaf removal is a common management practice (Palliotti et al., 2013; Pastore et al., 2013; Torres et al., 2021), for example, to influence grape composition or to reduce disease pressure (Zenoni et al., 2017; Tóth, 2020; O'Brien et al., 2021; VanderWeide et al., 2021). While timing, extent, and need for leaf removal depend on local environmental factors, opening up the canopy at some point is usually recommended for promoting wine quality (Frioni et al., 2017; Hickey and Wolf, 2019; Satisha and Somkuwar, 2019; Würz et al., 2020; O'Brien et al., 2021). Even though, early leaf removal in the bunch zone can allow berries to better adapt to sunlight and, thereby, reduce their susceptibility to sunburn (Gambetta et al., 2021), leaf removal events just before or during a heatwave might dramatically increase sunburn occurrence due to newly sun-exposed berries being insufficiently adapted to the risky environment (Hayman et al., 2012; Palliotti et al., 2014). Again, if we expect more heatwaves due to climate change, this would shorten and reduce the time windows of leaf removal for protection against sunburn. In addition, strategic decisions such as row orientation, cultivar choice, and trellis system might interplay with the above-mentioned scenarios (Palliotti, 2011; Hunter et al., 2016, 2017; Zheng et al., 2017; Bernardo et al., 2018; Leeuwen et al., 2019; Chopard et al., 2021; Kurtural and Fidelibus, 2021; Sargolzaei et al., 2021). For example, in north-south oriented rows sunburn occurs often just on the west side of the rows (Spayd et al., 2002; Gambetta et al., 2021) due to an unbalanced temperature distribution with heat peaks in the afternoon (Lopes et al., 2018; Strack et al., 2021). Therefore, in order to reduce sunburn risks in such vineyards, leaf removal

is sometimes limited to the morning side (east) of the canopy. However, climate change might increase temperature to a point where the heat impact might cause sunburn on non-shaded berries on the morning side. Thus, climate change might increase the sunburn risk of hitherto low-risk berries and add new locations of possible sunburn occurrence to the grapevines.

In summary, the interplay of the discussed future climatic conditions, seasonal management practices, and strategic decisions on vineyard planning might severely affect future seasonal sunburn occurrence pattern. This underlines the importance of taking climate change explicitly into account when addressing sunburn in future vineyards. Hence, an advanced modeling tool for systematically analyzing future scenarios may then be needed to support and accelerate the development of adapted mitigation strategies. Recently, Gambetta et al. (2021) suggested predicting sunburn events based on the following modeling approach: "If the susceptibility of a given cultivar and developmental stage and the duration of adaptation were known, this information could be combined with accurate berry fruit surface temperature (FST) to predict sunburn events. In addition, modeling approaches on canopy level could provide a better insight for mitigation strategies of sunburn protection considering plant architecture and training systems in vineyards." **Figure 1** illustrates this idea.

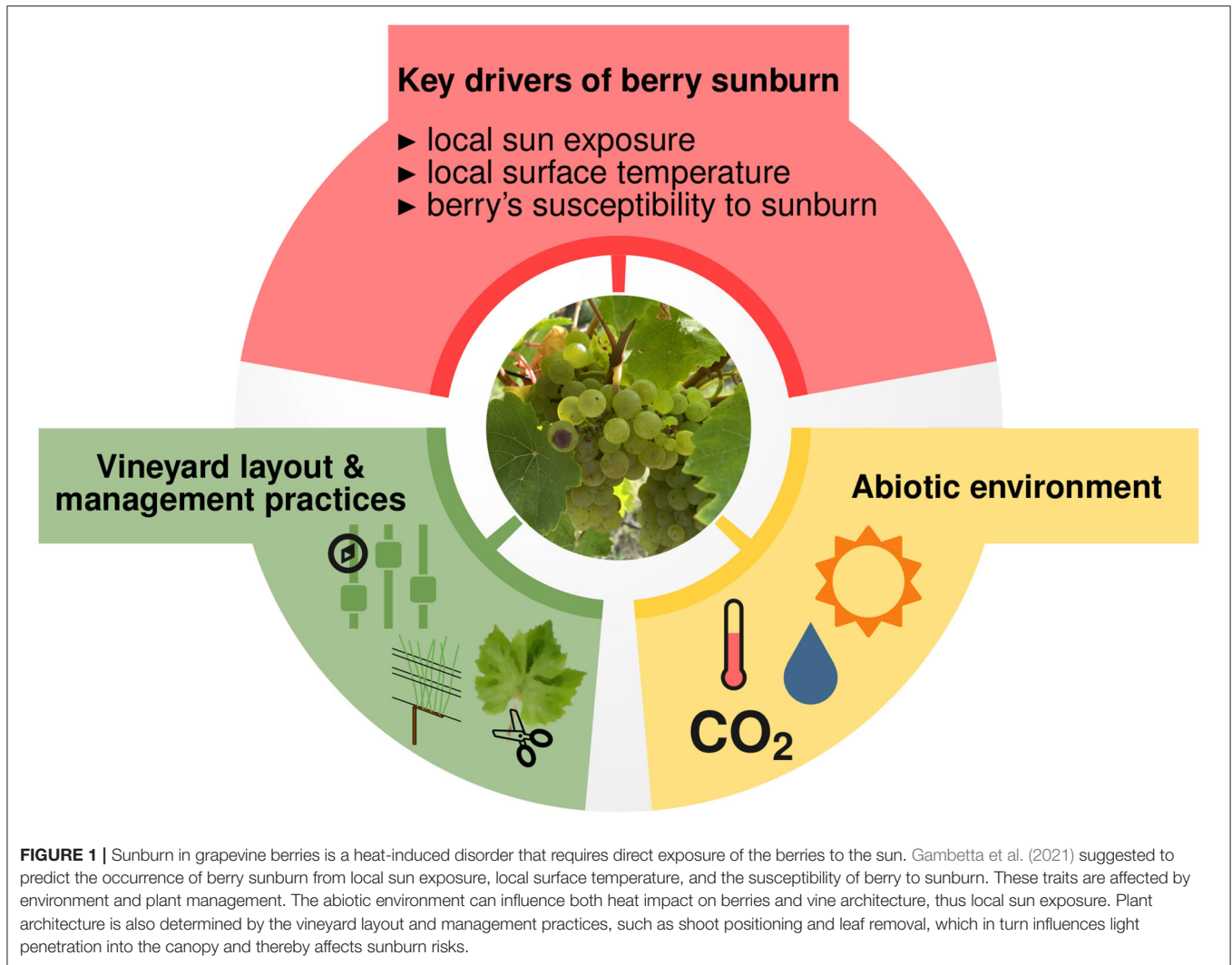
In this perspective, we want to advance this idea and propose a modeling framework that may help us to investigate the extent of berry sunburn in future vineyards, while particularly considering climate change and pointing out missing links to be resolved for such a berry sunburn prediction. Other factors effecting canopy development and hence light penetration, such as nutrition and water status (Keller, 2005; Lopes et al., 2018; Briglia et al., 2020), are assumed to be unaltered and linked to a selected reference condition, although this limits the initial scope of the modeling framework.

2. MODELING BERRY SUNBURN OF GRAPEVINES

Following Gambetta et al. (2021), a model of berry sunburn of grapevine may assume that sunburn occurrence can be predicted from the following key characteristics of the berry: susceptibility of the given cultivar to sunburn, developmental stage, and duration of adaptation and berry surface temperature.

The output of the sunburn model for a berry is its sunburn state, either healthy or sunburnt. At the onset of berry and bunch growth, all berries can be assumed healthy. This could be reflected in the model by an initial sunburn value of $SB = \text{FALSE}$ for all berries. During development, a berry either keeps this value or, if subjected to a sunburn event, its trait is set to $SB = \text{TRUE}$. For the decision of an irreversible state transition from healthy to sunburnt, the model could compare the surface temperature of the berry, FST, with a cultivar-specific threshold surface temperature T_c . If the threshold surface temperature is exceeded, sunburn occurs. This can be expressed by

$$\text{if } FST > T_c \quad \text{then the sunburn state of berry: } SB = \text{TRUE}. \quad (1)$$



The current susceptibility of a berry to sunburn could be expressed by a scaling factor f_s of the threshold temperature. This changes (Equation 1) as follows

if $FST > f_s \times T_c$ then the sunburn state of berry: $SB = TRUE$.
(2)

Based on this approach, a decreasing f_s would cause sunburn at lower FST. If the factor f_s reflects both, the developmental stage of berry and duration of adaptation, the model could echo the changing susceptibility of a berry to sunburn. Such a modeling approach seems quite appealing at the first glance, because of its simplicity and close link to observations in the field. However, we need to overcome missing links before it would be applicable. These missing links are directly related to the model components in Equation (2) but also to the fact that berry sunburn is a disorder that requires direct sun exposure of the berries and, therefore, depends on the canopy architecture of the grapevines. The reason for this is that shaded berries typically do not show sunburn symptoms at all, since the required heat impact for

sunburn is not supplied by ambient temperature alone (Gambetta et al., 2021).

Cultivar-specific threshold temperature, T_c : Since the model should consider varying susceptibility of a berry to sunburn by accounting for the developmental stage of the berry and duration of adaptation, the cultivar-specific threshold temperature, T_c , has to represent a reference condition. This reference condition could be a combination of developmental stage “véraison” (beginning of berry ripening) and lowest susceptibility of a berry to sunburn. In addition, to be useful as reference value in the model, T_c has to be a constant value. Yet, it is still an open task to show that T_c is such a robust trait to assess sunburn.

Susceptibility factor, f_s : To allow the comparison of temperatures in Equation (2), the susceptibility factor of a berry to sunburn has to be dimensionless and equal 1 for the reference condition of T_c . In order to echo observations, f_s should depend on both, developmental stage (DS) and berry skin adaptation to sun exposure (SE). The following equation mimics a simplified

modeling approach of this relation:

$$f_s = \text{function}(f_{DS}, f_{SE}) \quad (3)$$

where f_{DS} represents the dependency of sunburn susceptibility on the developmental stage of a berry, and f_{SE} includes variation in susceptibility with respect to the sustaining sun exposure of berry. Thus, f_{DS} should cause f_s to decrease with time from minimum susceptibility at the onset of berry growth to maximum susceptibility at harvest. In contrast, a minimum susceptibility should be reached at full sun adaptation reflected by a f_{SE} causing f_s to increase. However, response functions of the different aspects of berry susceptibility are unknown and it is unclear whether these aspects act independently from each other. For estimating parameters experimentally, first attempts have shown that grapes grown in different conditions can successfully be burnt applying artificial light and taking thermal images to determine a surface temperature (Müller et al., 2021).

Berry surface temperature, FST: For predicting berry sunburn in future vineyards, it would be necessary to predict berry surface temperature as well. Energy-balance models allow estimating FST of single berries grown in controlled conditions (Smart and Sinclair, 1976) and in the field (Cola et al., 2009; Ponce de León and Bailey, 2021). The model of Cola et al. (2009) is setup for red grapevine berries in a hedge-like row canopy from véraison to harvest and predicts FST from sensible heat flow, air temperature and a turbulent exchange coefficient using constant values of leaf area index and row height as input. This model estimates FST within static architectural conditions in the field sufficiently accurate for the model purpose (Cola et al., 2009). In contrast, the model of Ponce de León and Bailey (2021) successfully introduced a heat storage term for predicting rapid spatial and temporal fluctuations in berry temperature. The model is validated against experimental data and predicts average berry temperature with high accuracy (assessed by coefficients of determination above 94% and low errors). However, advancements are needed to make both models sensitive to changing canopy architecture caused by grapevine growth and interactions with the environment or plant manipulation events.

Light-exposure of the berries: Berry sunburn requires sun exposure, which, therefore, needs to be monitored as mandatory prerequisite of the above-described approach of modeling sunburn. However, the sheer number of berries in a vineyard does not permit tracking sun exposure of all berries in a vineyard simultaneously. It seems reasonable that predicting local light conditions on the berries could help to overcome these challenges. Certainly, the penetration of light into the grapevine canopy depends on many factors such as the trellis system (e.g., vertical shoot positioning), row spacing and orientation, leaf positioning within the canopy including the optical properties of canopy, but also plant management such as leaf removal (Zorer et al., 2017; Naulleau et al., 2021). A simple model of light attenuation within a canopy, such as Beer-Lambert equation, allows precise estimates in horizontally homogeneous canopies based on leaf area index and an experimentally derivable light extinction coefficient (Monsi and Saeki, 2005). However, such

an approach does not result in accurate snapshots of local light conditions within a heterogeneous grapevine row canopy. As a consequence, for modeling sunburn in vineyards, model approaches are needed that echo canopy architecture and its interplay with the incoming light in high resolution.

3. TOWARD PREDICTING BERRY SUNBURN IN FUTURE VINEYARDS

A specific class of plant models, the so-called functional-structural plant models (FSPMs), can integrate structural components of a canopy in detail and can even catch the variability of canopy (e.g., Schmidt and Kahlen, 2019; Boudon et al., 2020). They explicitly combine plant architecture and plant functioning. FSPMs can be used to deal with research questions ranging from basic research to applied sciences (Louarn and Song, 2020). Understanding plant functioning across scales and integrating multidisciplinary knowledge remain an ambitious task in FSPMs (Louarn and Song, 2020), but they have particularly proven useful for addressing complex interactions of plants and their light environment (e.g., Kahlen and Stützel, 2011).

For grapevine, there already exist several FSPM approaches. Most of them consider canopy architecture in detail but focus on static snapshots of grapevine architecture captured by digitized real plants (e.g., Louarn et al., 2005). The pioneering model *Top-vine* simulates light-sensitive differences in the variability of canopy structure of cultivar \times training system pairs for cvs. Grenache Noir and Syrah (Louarn et al., 2008). Follow-up models of *Top-vine* (Prieto et al., 2012; Prieto et al., 2019) and further grapevine-FSPMs (Zhu et al., 2018; Albasha et al., 2019) focus on linking complex physiological processes such as photosynthesis and transpiration to static architectural constraints: Prieto et al. (2012) adapted the architectural model of *Top-vine* to fit it to digitized data of a single grapevine cv. Syrah of each experimental site and used this model to examine the variability of gas exchange within the canopy, taking into account the nitrogen content of the leaves and the local adaptation to radiation in the grapevine. The latest development of this study highlights the role of N-distribution within the canopy on gas exchange of canopy architectures established by different training systems (Prieto et al., 2019). In the FSPM *GrapevineXL*, Zhu et al. (2018) linked local plant architecture to a bio-mechanical model of gas exchange and a water status model. They simulated berry quality based on carbon and water fluxes. In this study, the descriptive architecture mimicked the conditions of grapevine fruiting cuttings of cv. Cabernet Sauvignon in a greenhouse environment. The model *HydroShoot* is a FSPM that considers plant architecture for simulating transpiration and net photosynthesis rates at leaf and plant level of single grapevines (Albasha et al., 2019). To achieve this, *HydroShoot* does not take into account time-dependent changes in plant architecture.

So far, just a very few grapevine-FSPMs consider dynamic plant growth over the season (Garin et al., 2014; Schmidt et al., 2019). *Top-vine* data also served as the basis of the first dynamic grapevine-FSPM to analyze the development of

TABLE 1 | Grapevine FSPMs, their original purpose, and necessary features listed to model berry sunburn.

Grapevine FSPMs			Necessary features for berry sunburn modeling				
Model*	Purpose	Cultivar	Dynamic growth	In-season management	Berries	Berry growth	FST model
<i>Top-vine</i> ^a	canopy structure × training system, gas-exchange × nitrogen content × radiation	Grenache Noir, Syrah	no	no	no	no	no
<i>Hydroshoot</i> ^b	gas-exchange × water deficit	Syrah	no	no	no	no	no
<i>GrapevineXL</i> ^c	berry growth × water flux × carbon flux	Cabernet Sauvignon	no	no	yes	yes	no
<i>Top-vine (OpenAlea)</i> ^d	powdery mildew development	n.a	yes	no	no	no	no
<i>Virtual Riesling</i> ^e	canopy structure × training system × plant management × light interception	Riesling	yes	yes	no	no	no
<i>Helios</i> ^f	berry temperature × training system	Cabernet Sauvignon	no	no	yes	no	yes

* model specific information taken from cited publications.

^a(Louarn et al., 2005, 2008; Prieto et al., 2012; Prieto et al., 2019).

^b(Albasha et al., 2019).

^c(Zhu et al., 2018).

^d(Garin et al., 2014).

^e(Schmidt et al., 2019; Bahr et al., 2021).

^f(Ponce de León and Bailey, 2021).

powdery mildew (Garin et al., 2014). In contrast, *Virtual Riesling*, a FSPM for field-grown Riesling, was developed using repeatedly digitized vines grown in a unique vineyard facility established to catch climate change impact on grapevine (Schmidt et al., 2019). This model already allows assessing the role of changing temperatures in grapevine architecture and thereby considering management techniques such as vertical shoot positioning (Schmidt et al., 2019). Most recently, *Virtual Riesling* was coupled with a light model (Bahr et al., 2020) for analyzing the effects of leaf removal on light distribution within the canopy (Bahr et al., 2021) and it was initially calibrated for assessing the effects of elevated ambient CO₂ concentrations on grapevine growth and development (Schmidt et al., 2020). However, the current version of *Virtual Riesling* does not include generative growth and an in-depth model evaluation is still missing. Recently, Ponce de León and Bailey (2021) developed a model for simulating single berry temperature in vineyards. Their canopies representing snapshots of four different trellis systems were built using a procedural plant model generator implemented in the software environment Helios (Bailey, 2019). Hence, this approach is mainly lacking of dynamic growth features for the canopy and the berries, to be applied in the proposed context of modeling berry sunburn (cf. **Table 1**). In summary, we conclude that all the above-mentioned grapevine-FSPMs require important advancements to allow for integrating a sunburn sub model and reliably predicting sunburn in future vineyards.

4. TOWARD *IN SILICO* EXPERIMENTS FOR DEVELOPING MITIGATION STRATEGIES OF SUNBURN PROTECTION

An advanced grapevine-FSPM could be used to identify plant architectures and management strategies favorable for reducing sunburn risks under detrimental environments based on *in silico* experiments. Such *in silico* experiments are simulation studies that mimic real experiments. In other words, the advanced model would be used to simulate virtual vineyards including treatments and replications. From the *in silico* experiments, we could extract information on sunburn occurrence within virtual vineyards (location, time and probability) to identify correlations with characteristics from climatic measures (thermal course and radiation intensity), morphological measures (leaf area and bunch dimensions), phenological stages and applied management practices.

Before exploring future conditions *in silico*, an obligatory validation study comparing recent sunburn occurrence with simulated sunburn risks has to attest sufficient model accuracy. Simulations of vineyards responding to changes in environmental conditions should give us answers to the impact of climate change on sunburn. For this, a series of *in silico* experiments should be performed to estimate the effects of morphological responses to eCO₂, increased temperature, and heatwaves on sunburn occurrence. Performing simulations with various options for management practices, such as timing,

location, and intensity of leaf removal, under challenging environments would then allow us to identify optimized management practices for reducing sunburn. However, a model focusing exclusively on sunburn would not cover trade-offs between possible conflicting objectives of a viticulturist such as controlling sugar content or avoiding pests and other diseases (Santos et al., 2020). Thus, it would be of great advantage to apply newly identified strategies theoretically favorable for reducing sunburn risks in real vineyards to test their effect and also to reveal potential management conflicts (e.g., in *VineyardFACE* at Geisenheim University, Germany, e.g., Wohlfahrt et al., 2018). In addition, it could be necessary to advance the grapevine model to include further processes of interest, yet this is beyond the scope of this perspective. To summarize, we suggest to integrate a sunburn model into an advanced grapevine-FSPM, to conduct *in silico* experiments and use them to identify management strategies and plant architectures favorable for reducing sunburn risks in future vineyards, and to test them in the field.

Since almost all existing grapevine-FSPMs on vineyard level are based on data collected on a specific site, this reduces the transfer ability of any such advanced model to other sites or environmental conditions that were not considered for model development (Jones et al., 2017). Nevertheless, if the proposed approach proves to be valuable, further extensions (various varieties, scion-rootstock combinations, and cultivation methods) can follow.

5. CONCLUSION

Viticulture demands to control fruit quality and yield, while reducing pest, diseases, and disorders such as berry sunburn. Canopy management can reduce the risk of sunburn; however, climate change and particularly heatwaves might make it necessary to adapt strategies to the new environmental conditions. Sunburn events are results of the complex interplay of

environment and grapevine architecture affecting both the local heat impact on the berry surface and the susceptibility of berry to sunburn. Accordingly, a modeling approach to predict sunburn in vineyards should consider plant architecture, environment, and their interaction over time. We suggest that functional-structural plant models can be appropriate tools to integrate these sunburn aspects. However, current grapevine-FSPMs require further advancements to allow for integrating a sunburn sub-model reliably predicting sunburn in future vineyards. In this perspective, we highlighted missing links that have to be addressed. These are related to a concept and the parametrization of a berry sunburn model, the model input of berry exposure to direct sunlight and the role of dynamics in plant growth, and plant canopy management and environment. Once open issues are solved, and the proposed modeling framework should help us to better understand how climate change may affect sunburn and, thus, could provide new ideas for mitigating effects of climate change.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

KK and DS designed the overall concept. All authors wrote the manuscript and approved the final version and publication.

FUNDING

We acknowledge support by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), project numbers 449374897 and 432888308, and the Open Access Publishing Fund of Geisenheim University.

REFERENCES

- Albasha, R., Fournier, C., Pradal, C., Chelle, M., Prieto, J. A., Louarn, G., et al. (2019). Hydroshoot: a functional-structural plant model for simulating hydraulic structure, gas and energy exchange dynamics of complex plant canopies under water deficit-application to grapevine (*Vitis vinifera*). In *Silico Plants* 1:diz007. doi: 10.1093/insilicoplants/diz007
- Bahr C., Schmidt D., Friedel M., and Kahlen K. (2020). Shedding light on virtual Riesling canopies (*Vitis vinifera* L.) [abstract]. in *iCROP2020-Crop Modelling for the Future* (Montpellier).
- Bahr, C., Schmidt, D., Friedel, M., and Kahlen, K. (2021). Leaf removal effects on light absorption in virtual riesling canopies (*Vitis vinifera* L.). In *Silico Plants* 2. doi: 10.1093/insilicoplants/diab027
- Bailey, B. N. (2019). Helios: A scalable 3d plant and environmental biophysical modeling framework. *Front. Plant Sci.* 10:1185. doi: 10.3389/fpls.2019.01185
- Bei, R. D., Wang, X., Papagiannis, L., Cocco, M., O'Brien, P., Zito, M., et al. (2019). Postveraison leaf removal does not consistently delay ripening in Semillon and Shiraz in a hot Australian climate. *Am. J. Enol. Vitic.* 70, 398–410. doi: 10.5344/ajev.2019.18103
- Bernardo, S., Dinis, L.-T., Machado, N., and Moutinho-Pereira, J. (2018). Grapevine abiotic stress assessment and search for sustainable adaptation strategies in mediterranean-like climates. a review. *Agron. Sustain. Dev.* 38:66. doi: 10.1007/s13593-018-0544-0
- Bondada, B., and Keller, M. (2012). Morphoanatomical symptomatology and osmotic behavior of grape berry shrivel. *J. Am. Soc. Hortic. Sci.* 137, 20–30. doi: 10.21273/JASHS.137.1.20
- Boudon, F., Persello, S., Jestin, A., Briand, A.-S., Grechi, I., Fernique, P., et al. (2020). V-mango: a functional-structural model of mango tree growth, development and fruit production. *Ann. Bot.* 126, 745–763. doi: 10.1093/aob/mcaa089
- Briglia, N., Williams, K., Wu, D., Li, Y., Tao, S., Corke, F., et al. (2020). Image-based assessment of drought response in grapevines. *Front. Plant Sci.* 11:595. doi: 10.3389/fpls.2020.00595
- Chopard, J., Bisson, A., Lopez, G., Persello, S., Richert, C., and Fumey, D. (2021). "Development of a decision support system to evaluate crop performance under dynamic solar panels," in *Agrivoltaics2020 Conference: Launching Agrivoltaics World-Wide* (AIP Publishing).
- Cola, G., Failla, O., and Mariani, L. (2009). BerryTone—simulation model for the daily course of grape berry temperature. *Agric. Forest Meteorol.* 149, 1215–1228. doi: 10.1016/j.agrformet.2009.01.007

- Duchêne, E., Huard, F., Dumas, V., Schneider, C., and Merdinoglu, D. (2010). The challenge of adapting grapevine varieties to climate change. *Clim. Res.* 41, 193–204. doi: 10.3354/cr00850
- Frioni, T., Zhuang, S., Palliotti, A., Sivilotti, P., Falchi, R., and Sabbatini, P. (2017). Leaf removal and cluster thinning efficiencies are highly modulated by environmental conditions in cool climate viticulture. *Am. J. Enol. Vitic.* 68, 325–335. doi: 10.5344/ajev.2017.16098
- Gambetta, J. M., Holzapfel, B. P., Stoll, M., and Friedel, M. (2021). Sunburn in grapes: a review. *Front. Plant Sci.* 11:604691. doi: 10.3389/fpls.2020.604691
- Garin, G., Fournier, C., Andrieu, B., Houllès, V., Robert, C., and Pradal, C. (2014). A modelling framework to simulate foliar fungal epidemics using functional-structural plant models. *Ann. Bot.* 114, 795–812. doi: 10.1093/aob/mcu101
- Gatti, M., Garavani, A., Krajec, K., Ughini, V., Parisi, M. G., Frioni, T., et al. (2018). Mechanical mid-shoot leaf removal on ortugo (*Vitis vinifera* L.) at pre- or mid-veraison alters fruit growth and maturation. *Am. J. Enol. Vitic.* 70, 88–97. doi: 10.5344/ajev.2018.18055
- Gutiérrez-Gamboa, G., Zheng, W., and de Toda, F. M. (2021). Current viticultural techniques to mitigate the effects of global warming on grape and wine quality: a comprehensive review. *Food Res. Int.* 139:109946. doi: 10.1016/j.foodres.2020.109946
- Hayman, P., Longbottom, M., McCarthy, M., and Thomas, D. (2012). *Managing Grapevines During Heatwaves [Fact Sheet January]*. Adelaide, SA: Australian Government, Grape and Wine Research and Development Cooperation.
- Hickey, C. C., and Wolf, T. K. (2019). Intensive fruit-zone leaf thinning increases *Vitis vinifera* L. 'cabernet sauvignon' berry temperature and berry phenolics without adversely affecting berry anthocyanins in virginia. *HortScience* 54, 1181–1189. doi: 10.21273/HORTSCI13904-19
- Hulands, S., Greer, D. H., and Harper, J. (2014). The interactive effects of temperature and light intensity on *Vitis vinifera* cv. 'semillon' grapevines. ii. berry ripening and susceptibility to sunburn at harvest. *Eur. J. Hortic. Sci.* 79, 1–7.
- Hunter, J., Volschenk, C., and Booyse, M. (2017). Vineyard row orientation and grape ripeness level effects on vegetative and reproductive growth characteristics of *Vitis vinifera* L. cv. Shiraz/101-14 mgt. *Eur. J. Agronomy* 84, 47–57. doi: 10.1016/j.eja.2016.12.004
- Hunter, J., Volschenk, C., and Zorer, R. (2016). Vineyard row orientation of *Vitis vinifera* L. cv. Shiraz/101-14 mgt: climatic profiles and vine physiological status. *Agric. Forest Meteorol.* 228–229, 104–119. doi: 10.1016/j.agrformet.2016.06.013
- Hunter, J. K., Tarricone, L., Volschenk, C., Giacalone, C., Melo, M. S., and Zorer, R. (2020). Grapevine physiological response to row orientation-induced spatial radiation and microclimate changes. *OENO ONE* 54, 411–433. doi: 10.20870/oeno-one.2020.54.2.3100
- Jones, G., Duchene, E., Tomasi, D., Yuste, J., Braslavska, O., Schultz, H., et al. (2005). "Changes in european winegrape phenology and relationships with climate," in *XIV International GESCO Viticulture Congress, Geisenheim, Germany, 23–27 August, 2005* (Groupe d'Etude des Systemes de CONduite de la vigne (GESCO)), 54–61.
- Jones, J. W., Antle, J. M., Basso, B., Boote, K. J., Conant, R. T., Foster, I., et al. (2017). Brief history of agricultural systems modeling. *Agric. Syst.* 155, 240–254. doi: 10.1016/j.agsy.2016.05.014
- Kahlen, K., and Stützel, H. (2011). Modelling photo-modulated internode elongation in growing glasshouse cucumber canopies. *New Phytol.* 190, 697–708. doi: 10.1111/j.1469-8137.2010.03617.x
- Keller, M. (2005). Deficit irrigation and vine mineral nutrition. *Am. J. Enol. Vitic.* 56, 267–283.
- Keller, M. (2015). *The Science of Grapevines: Anatomy and Physiology*. London: Elsevier Science.
- Kurtural, S. K., and Fidelibus, M. W. (2021). Mechanization of pruning, canopy management, and harvest in winegrape vineyards. *Catalyst* 5, 29–44. doi: 10.5344/catalyst.2021.20011
- Lavado, N., Uriarte, D., Mancha, L. A., Moreno, D., Valdés, E., and Prieto, M. H. (2019). Effect of forcing vine regrowth on 'tempranillo' (*Vitis vinifera* L.) berry development and quality in extremadura. *Vitis* 58, 135–142. doi: 10.5073/vitis.2019.58
- Leeuwen, C. V., Pieri, P., Gowdy, M., Ollat, N., and Roby, J.-P. (2019). Reduced density is an environmental friendly and cost effective solution to increase resilience to drought in vineyards in a context of climate change. *OENO ONE* 53, 129–146. doi: 10.20870/oeno-one.2019.53.2.2420
- Lopes, C. M., Costa, J. M., Egipto, R., Zarrouk, O., and Chaves, M. M. (2018). Can mediterranean terroirs withstand climate change? case studies at the alentejo portuguese winegrowing region. *E3S Web Conf.* 50:01004. doi: 10.1051/e3sconf/20185001004
- Louarn, G., Lebon, E., and Lecoq, J. (2005). "Top-vine", a topiary approach based architectural model to simulate vine canopy structure," in *XIV International GESCO Viticulture Congress, Geisenheim, Germany, 23–27 August, 2005* (Groupe d'Etude des Systemes de CONduite de la vigne (GESCO)), 464–470.
- Louarn, G., Lecoq, J., and Lebon, E. (2008). A three-dimensional statistical reconstruction model of grapevine (*Vitis vinifera*) simulating canopy structure variability within and between cultivar/training system pairs. *Ann. Bot.* 101, 1167–1184. doi: 10.1093/aob/mcm170
- Louarn, G., and Song, Y. (2020). Two decades of functional-structural plant modelling: now addressing fundamental questions in systems biology and predictive ecology. *Ann. Bot.* 126, 501–509. doi: 10.1093/aob/mcaa143
- Martinez De Toda, F. (2021). Global warming allows two grape crops a year, with about two months apart in ripening dates and with very different grape composition - the forcing vine regrowth to obtain two crops a year. *VITIS* 60, 119–124. doi: 10.5073/vitis.2021.60.119-124
- Masson-Delmotte V., Zhai P., Pirani P., Connors S. L., Péan C., Berger S., et al. (eds.). (2021). *IPCC, 2021: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. Available online at: https://www.ipcc.ch/report/ar6/wg1/downloads/report/IPCC_AR6_WGI_Citation.pdf
- Monsi, M., and Saeki, T. (2005). On the factor light in plant communities and its importance for matter production. *Ann. Bot.* 95, 549. doi: 10.1093/aob/mci052
- Müller, K., Friedel, M., and Stoll, M. (2021). "Manipulation Von Beerenoberflächentemperaturen im Weinberg," in *60. Arbeitstagung des Forschungsrings des Deutschen Weinbaus (Online-Conference)*.
- Naulleau, A., Gary, C., Prévot, L., and Hossard, L. (2021). Evaluating strategies for adaptation to climate change in grapevine production—systematic review. *Front. Plant Sci.* 11:607859. doi: 10.3389/fpls.2020.607859
- O'Brien, P., Collins, C., and Bei, R. D. (2021). Leaf removal applied to a sprawling canopy to regulate fruit ripening in cabernet sauvignon. *Plants* 10, 1017. doi: 10.3390/plants10051017
- Palliotti, A. (2011). A new closing y-shaped training system for grapevines. *Aust. J. Grape Wine Res.* 18, 57–63. doi: 10.1111/j.1755-0238.2011.00171.x
- Palliotti, A., Panara, F., Silvestroni, O., Lanari, V., Sabbatini, P., Howell, G., et al. (2013). Influence of mechanical postveraison leaf removal apical to the cluster zone on delay of fruit ripening in sangiovese *Vitis vinifera* L. grapevines. *Aust. J. Grape Wine Res.* 19, 369–377. doi: 10.1111/ajgw.12033
- Palliotti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., and Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. *Sci. Hortic.* 178, 43–54. doi: 10.1016/j.scienta.2014.07.039
- Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, M., Tornielli, G. B., and Filippetti, I. (2013). Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. *BMC Plant Biol.* 13:30. doi: 10.1186/1471-2229-13-30
- Perkins-Kirkpatrick, S. E., and Lewis, S. C. (2020). Increasing trends in regional heatwaves. *Nat. Commun.* 11:3357. doi: 10.1038/s41467-020-16970-7
- Ponce de León, M. A., and Bailey, B. N. (2021). A 3d model for simulating spatial and temporal fluctuations in grape berry temperature. *Agric. Forest Meteorol.* 306:108431. doi: 10.1016/j.agrformet.2021.108431
- Prieto, J. A., Louarn, G., Pe na, J. P., Ojeda, H., Simonneau, T., and Lebon, E. (2019). A functional-structural plant model that simulates whole-canopy gas exchange of grapevine plants (*Vitis vinifera* L.) under different training systems. *Ann. Bot.* 126, 647–660. doi: 10.1093/aob/mcz203
- Prieto, J. A., Louarn, G., Pe na, J. P., Ojeda, H., Simonneau, T., and Lebon, E. (2012). A leaf gas exchange model that accounts for intra-canopy variability by considering leaf nitrogen content and local acclimation to radiation in grapevine (*Vitis vinifera* L.). *Plant Cell Environ.* 35, 1313–1328. doi: 10.1111/j.1365-3040.2012.02491.x
- Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L.-T., Correia, C., et al. (2020). A review of the potential climate change

- impacts and adaptation options for european viticulture. *Appl. Sci.* 10, 3092. doi: 10.3390/app10093092
- Sargolzaei, M., Rustioni, L., Cola, G., Ricciardi, V., Bianco, P. A., Maghradze, D., et al. (2021). Georgian grapevine cultivars: ancient biodiversity for future viticulture. *Front Plant Sci.* 12:630122. doi: 10.3389/fpls.2021.630122
- Satisha, J., and Somkuwar, R. (2019). Effect of leaf removal on composition of wine grape varieties grown in semiarid tropical climate of india. *J. Hortic. Sci.* 14, 115–124. doi: 10.24154/JHS.2019.v14i02.005
- Schäfer, J., Friedel, M., Molitor, D., and Stoll, M. (2021). Semi-minimal-pruned hedge (SMPH) as a climate change adaptation strategy: Impact of different yield regulation approaches on vegetative and generative development, maturity progress and grape quality in riesling. *Appl. Sci.* 11, 3304. doi: 10.3390/app11083304
- Schmidt, D., Bahr, C., Friedel, M., and Kahlen, K. (2019). Modelling approach for predicting the impact of changing temperature conditions on grapevine canopy architectures. *Agronomy* 9, 426. doi: 10.3390/agronomy9080426
- Schmidt, D., Bahr, C., Friedel, M., and Kahlen, K. (2020). “In silico analysis of grapevine architectural response to elevated CO₂,” in *FSPM2020: Towards Computable Plants*, eds K. Kahlen, T.-W. Chen, A. Fricke, and H. Stützel (Leibniz Universität Hannover and Hochschule Geisenheim University), 76–77.
- Schmidt, D., and Kahlen, K. (2019). Positional variation rather than salt stress dominates changes in three-dimensional leaf shape patterns in cucumber canopies. *in silico Plants*. 1:diz011. doi: 10.1093/insilicoplants/diz011
- Silvestre, J., Damásio, M., Egipto, R., Cunha, J., Brazão, J., Eiras-Dias, J., et al. (2019). “Tolerance to sunburn: a variable to consider in the context of climate change,” in *Proceedings of the 21st GIESCO International Meeting; June 23–28, 2019* (Thessaloniki), 681–682.
- Smart, R. E., and Sinclair, T. R. (1976). Solar heating of grape berries and other spherical fruits. *Agric. Meteorol.* 17, 241–259. doi: 10.1016/0002-1571(76)90029-7
- Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* 53, 171–182.
- Stoll, M., Lafontaine, M., and Schultz, H. R. (2010). Possibilities to reduce the velocity of berry maturation through various leaf area to fruit ratio modifications in *Vitis vinifera* L. riesling. *Progrès Agric. et Viticole* 127, 68–71.
- Strack, T., Schmidt, D., and Stoll, M. (2021). Impact of steep slope management system and row orientation on canopy microclimate. comparing terraces to downslope vineyards. *Agric. Forest Meteorol.* 307:108515. doi: 10.1016/j.agrformet.2021.108515
- Torres, N., Martínez-Lüscher, J., Porte, E., Yu, R., and Kurtural, S. K. (2021). Impacts of leaf removal and shoot thinning on cumulative daily light intensity and thermal time and their cascading effects of grapevine (*Vitis vinifera* L.) berry and wine chemistry in warm climates. *Food Chem.* 343:128447. doi: 10.1016/j.foodchem.2020.128447
- Tóth, A. M. (2020). Precision canopy management of the grapevine: early defoliation and girdling. *Acta Carolus Robertus* 2020:303969. doi: 10.33032/acr.2020.spec.107
- Valentini, G., Allegro, G., Pastore, C., Colucci, E., and Filippetti, I. (2018). Post-veraison trimming slow down sugar accumulation without modifying phenolic ripening in sangiovese vines. *J. Sci. Food Agric.* 99, 1358–1365. doi: 10.1002/jsfa.9311
- Valentini, G., Pastore, C., Allegro, G., Muzzi, E., Seghetti, L., and Filippetti, I. (2021). Application of kaolin and italian natural chabasite-rich zeolite to mitigate the effect of global warming in *tVitis vinifera* L. cv. sangiovese. *Agronomy* 11, 1035. doi: 10.3390/agronomy11061035
- VanderWeide, J., Gottschalk, C., Schultze, S. R., Nasrollahiazar, E., Poni, S., and Sabbatini, P. (2021). Impacts of pre-bloom leaf removal on wine grape production and quality parameters: a systematic review and meta-analysis. *Front Plant Sci.* 11:621585. doi: 10.3389/fpls.2020.621585
- Webb, L., Whetton, P., and Barlow, E. (2007). Modelled impact of future climate change on the phenology of winegrapes in australia. *Aust. J. Grape Wine Res.* 13, 165–175. doi: 10.1111/j.1755-0238.2007.tb00247.x
- Webb, L. B., Whetton, P. H., Bhend, J., Darbyshire, R., Briggs, P. R., and Barlow, E. W. R. (2012). Earlier wine-grape ripening driven by climatic warming and drying and management practices. *Nat. Clim. Chang* 2, 259–264. doi: 10.1038/nclimate1417
- Wohlfahrt, Y., Smith, J. P., Tittmann, S., Honermeier, B., and Stoll, M. (2018). Primary productivity and physiological responses of *Vitis vinifera* L. cvs. under Free Air Carbon dioxide Enrichment (FACE). *Eur. J. Agron.* 101, 149–162. doi: 10.1016/j.eja.2018.09.005
- Würz, D. A., Rufato, L., Bogo, A., Allebrandt, R., de Bem, B. P., Filho, J. L. M., et al. (2020). Effects of leaf removal on grape cluster architecture and control of botrytis bunch rot in sauvignon blanc grapevines in southern brazil. *Crop Prot.* 131:105079. doi: 10.1016/j.cropro.2020.105079
- Zenoni, S., Santo, S. D., Tornielli, G. B., D’Inca, E., Filippetti, I., Pastore, C., et al. (2017). Transcriptional responses to pre-flowering leaf defoliation in grapevine berry from different growing sites, years, and genotypes. *Front. Plant Sci.* 8:630. doi: 10.3389/fpls.2017.00630
- Zheng, W., García, J., Balda, P., and de Toda, F. M. (2017). Does full exposure of clusters have any negative effects on tempranillo (*Vitis vinifera* L.) grape quality in la rioja, spain? the use of severe cluster-zone leaf removal after berry set. *South Afr. J. Enol. Vitic.* 38, 228–236. doi: 10.21548/38-2-1620
- Zhu, J., Dai, Z., Vivin, P., Gambetta, G. A., Henke, M., Peccoux, A., et al. (2018). A 3-D functional-structural grapevine model that couples the dynamics of water transport with leaf gas exchange. *Ann. Bot.* 121, 833–848. doi: 10.1093/aob/mcx141
- Zorer, R., Volschenk, C., and Hunter, J. (2017). Integrating geographic information systems and hemispherical photography in the assessment of canopy light profiles in a vineyard. *Agric. Forest Meteorol.* 232, 672–681. doi: 10.1016/j.agrformet.2016.09.011

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Bahr, Schmidt and Kahlen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Potential Phenotyping Methodologies to Assess Inter- and Intravarietal Variability and to Select Grapevine Genotypes Tolerant to Abiotic Stress

Luísa C. Carvalho^{1*}, Elsa F. Gonçalves¹, Jorge Marques da Silva² and J. Miguel Costa^{1*}

¹LEAF – Linking Landscape, Environment, Agriculture and Food – Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal, ²BioISI – Biosystems and Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Lisboa, Portugal

OPEN ACCESS

Edited by:

Tommaso Frioni,
Catholic University of the Sacred
Heart, Italy

Reviewed by:

Markus Rienh,
University of Applied Sciences
and Arts of Western Switzerland,
Switzerland

Gastón Gutiérrez Gamboa,
Universidad Mayor, Chile

*Correspondence:

Luísa C. Carvalho
lcarvalho@isa.ulisboa.pt
J. Miguel Costa
miguelcosta@isa.ulisboa.pt

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 31 May 2021

Accepted: 28 September 2021

Published: 26 October 2021

Citation:

Carvalho LC, Gonçalves EF,
Marques da Silva J and
Costa JM (2021) Potential
Phenotyping Methodologies to
Assess Inter- and Intravarietal
Variability and to Select Grapevine
Genotypes Tolerant to Abiotic Stress.
Front. Plant Sci. 12:718202.
doi: 10.3389/fpls.2021.718202

Plant phenotyping is an emerging science that combines multiple methodologies and protocols to measure plant traits (e.g., growth, morphology, architecture, function, and composition) at multiple scales of organization. Manual phenotyping remains as a major bottleneck to the advance of plant and crop breeding. Such constraint fostered the development of high throughput plant phenotyping (HTPP), which is largely based on imaging approaches and automatized data retrieval and processing. Field phenotyping still poses major challenges and the progress of HTPP for field conditions can be relevant to support selection and breeding of grapevine. The aim of this review is to discuss potential and current methods to improve field phenotyping of grapevine to support characterization of inter- and intravarietal diversity. *Vitis vinifera* has a large genetic diversity that needs characterization, and the availability of methods to support selection of plant material (polyclonal or clonal) able to withstand abiotic stress is paramount. Besides being time consuming, complex and expensive, field experiments are also affected by heterogeneous and uncontrolled climate and soil conditions, mostly due to the large areas of the trials and to the high number of traits to be observed in a number of individuals ranging from hundreds to thousands. Therefore, adequate field experimental design and data gathering methodologies are crucial to obtain reliable data. Some of the major challenges posed to grapevine selection programs for tolerance to water and heat stress are described herein. Useful traits for selection and related field phenotyping methodologies are described and their adequacy for large scale screening is discussed.

Keywords: heat and water stress, imaging, phenotyping planning, planting material, selection traits, *Vitis vinifera*

INTRODUCTION

The EU is the leading global wine producer, with about 44% of the world's vine-growing area (circa 3.2 million ha) and sustaining about 57% of wine production by volume (OIV, 2020). European Mediterranean countries lead the cultivated area of grapevine for wine production worldwide (OIV, 2020) but they are also increasingly exposed to more adverse weather conditions,

with air temperatures rising from 2 to 5°C in major winemaking regions in parallel with changes in precipitation patterns or/and higher frequency of extreme weather events, such as heat waves (IPCC, 2014; Fraga, 2020; Lorenzo et al., 2021).

These changes have a serious impact on the sustainability of the wine sector in Mediterranean countries (e.g., Spain, France, Italy, Greece, and Portugal). Several agronomic strategies are already being implemented in viticulture to face climate challenges, and adapt to more severe heat and drought. The use of deficit irrigation is one of the most common (see Santesteban et al., 2019 for a review), but several others have been proposed and reviewed (see Gutiérrez-Gamboa et al., 2021 or Naulleau et al., 2021), and their economic consequences for the producers were analyzed (Merloni et al., 2018).

The use of better adapted plant material is another priority, namely in terms of late ripening varieties (Wolkovich et al., 2018), heat/drought tolerant clones (Van Leeuwen et al., 2013; Bota et al., 2016), and rootstocks adapted or modified to forthcoming climate conditions (Ollat et al., 2016; Prinsi et al., 2021). However, field phenotyping and grapevine selection are laborious and expensive, and still pose major challenges. The progress of high throughput plant phenotyping (HTPP) for field conditions can be relevant to support selection and breeding of grapevine. Therefore, the aim of this review is to identify potential strategies and methods to improve field phenotyping of grapevine to support characterization of inter- and intravarietal diversity. In fact, *Vitis vinifera* has a large genetic diversity that needs characterization to support selection of better adapted plant material (polycloonal or clonal), namely to abiotic stress.

The Impact of Heat and Water Stress on Grapevine Physiology

Stomatal behavior is a crucial functional trait and stomatal responses to the environment are determinant for plant adaptation. Stomata influence CO₂ uptake into the leaf along with water loss due to transpiration, actively regulating plant water status and leaf temperature (Jones, 1992; Matthews and Lawson, 2019). Stomata respond to chemical stimuli (biochemical control due to hormonal control) and to leaf water status (hydraulic control; Pantin et al., 2013) that mediate environmental inputs, such as light intensity and quality, air CO₂ concentration, and vapor pressure deficit (VPD; Buckley, 2019). Increasing soil water use is associated with hydraulic traits, to enable gas exchange under more negative water potentials, as observed by Dayer et al. (2020) in Semillon. Stomatal conductance to water vapor (*g_s*) on Chardonnay did not respond to air temperature below 30°C, but dropped under a combination of high air temperature and high air VPD (Greer, 2020). Different stomatal behaviors have been described for other varieties, thus the interaction between air temperature and VPD must be considered when addressing stomatal responses (Dayer et al., 2020; Greer, 2020).

Some varieties show a tight stomatal control (isohydric), whereas others show a less efficient stomatal control in response to water stress (anisohydric). Nevertheless, such classification of *Vitis* varieties as isohydric or anisohydric remains controversial

since differences in stomatal behavior among varieties are far more complex and largely depend on growing conditions (Chaves et al., 2010; Lovisolo et al., 2010; Villalobos-Gonzalez et al., 2019; Gambetta et al., 2021). In fact, it was shown that a variety can behave as both iso- and anisohydric, according to the level of water deficit, which defies the standard classification that implies a single behavior (Levin et al., 2019). Gambetta et al. (2020) suggested a more integrative definition of drought tolerance in grapevine, by resorting to four core physiological traits: maximum transpiration rate; stomatal regulation (expressed as the relation between stomatal conductance and leaf water potential); turgor loss point; and root volume. Bringing these parameters together, the authors suggested that it is possible to calculate, at any moment, for a vineyard under defined environmental conditions, the “stress distance,” i.e., the amount of time (e.g., number of days) that it withstands without watering before reaching a critical water potential.

The plasticity of leaf morphology is another factor of adaptation and evolution (Fritz et al., 2018). The role of leaf epidermis characteristics (cuticle, indumentum, pavement cells, and stomata) and mesophyll anatomy can have an impact on responses to abiotic stresses (Tomás et al., 2014; MacMillan et al., 2021). Leaf morphology and structure may affect stomatal behavior, leaf gas exchange, and mesophyll conductance (Tomás et al., 2014). Stomatal density and stomatal index can influence varietal leaf gas exchange characteristics as well as thermal regulation capacity (Gago et al., 2019). Costa et al. (2012) found no differences in stomatal density between Cabernet Sauvignon, Touriga Nacional, Syrah, Trincadeira, and Aragonese (syn. Tempranillo), but reported differences in *g_s*, leaf temperature, and leaf photosynthesis, suggesting that other factors besides the number of stomata regulate leaf gas exchange in grapevine. Gago et al. (2019) reported that Grenache Noir had significantly smaller leaf surface area than Syrah, but significantly thicker leaf blades. This calls for improved knowledge on morphological, anatomical, and physiological traits influencing the response to heat and drought of the *Vitis* germplasm.

The role of abscisic acid (ABA) in stomatal closure is well established; this hormone plays a key role particularly in isohydric or near-isohydric plants (Sampaio Filho et al., 2018; Dayer et al., 2020), by inducing faster ABA-related gene modulation (dal Santo et al., 2016). Stomatal sensitivity to ABA is variable among varieties (Rossdeutsch et al., 2016; Simonneau et al., 2017). Rossdeutsch et al. (2016) concluded that *Vitis* sp. genotypes with contrasting levels of drought adaptation differ in key steps involved in ABA metabolism and signaling, both when well-watered and drought stressed.

Grapevine's photosynthetic apparatus is defined as resilient, but extreme climate conditions will affect it negatively, through the overreduction of the photosynthetic electron carriers, production of reactive oxygen species (ROS), and photoinhibition (Mittler, 2006). In Mediterranean summer conditions, grapevine plants growing under heat and drought are usually exposed simultaneously to photoinhibitory light conditions, high air temperatures, and moderate to severe soil water deficits (Carvalho and Amâncio, 2019). If stress persists and carbon fixation is reduced, oxidative stress may

take place (Carvalho and Amâncio, 2019). When drought co-occurs with high light intensities an increase in ROS production by the photosynthetic apparatus can also arise (Mullineaux et al., 2006), leading to photoinhibition of photosynthesis.

Heat stress physiology in turn, at both leaf and berry levels, should be evaluated to better understand the impacts of drought and high soil and air temperatures on grapevine physiology and morphology of leaves, berries, and bunches (Costa et al., 2019a; Field et al., 2020). This is particularly important because berries tend to ripe earlier in warmer conditions, due to the effect of heat in anticipating phenological events (Van Leeuwen and Destrac-Irvine, 2017).

Plant phenology and growth are largely driven by air temperature and soil water availability (Parker et al., 2011). In fact, Verdugo-Vásquez et al. (2020) developed a climate-based model to estimate grapevine phenology, taking into account meteorological data and microclimate data at the plant level. Concomitantly, berry composition is affected by water availability and heat, with extreme temperatures and severe drought affecting negatively vigor, yield, and berry composition (Chaves et al., 2010), such as a lower content of anthocyanins (van Leeuwen and Darriet, 2016; Zarrouk et al., 2016). In addition, acidity, in particular related to malic acid content, decreases in high air temperature (van Leeuwen and Darriet, 2016). Consequently, the modern wine industry must find adequate varieties to maintain berry quality traits, such as acidity, under extreme and adverse climate conditions. Aspects such as berry sensitiveness to drought and sunburn were recently revised by Gambetta et al. (2021), attesting the relevance of the problem for the academy and the industry.

The Role of Plant Material to Mitigate Stress and Decrease Risks of Combined Heat Waves and Drought

Using optimal adapted plant material (rootstocks and *V. vinifera* varieties) for a specific region is a long term adaptation strategy crucial for grower's revenue and sustainability of the sector (less water, pesticides, and fertilizers required; **Figure 1**). Grapevine has a high level of phenotypic plasticity and genotypes can respond by adapting their growth morphology, leaf gas exchange, and berries' metabolic characteristics. Such plasticity was recently reported in a three season study of 30 varieties, indicating possible adaptations to climate change, such as the earlier and shorter ripening phase of white varieties to avoid the warmest period of the season (Gashu et al., 2020).

Autochthonous grapevine varieties represent a strong natural and historical mark, add great value to top quality wines, and are an essential raw-material to face future challenges. Therefore, a better characterization of existing variability between and within varieties is necessary, especially if we consider the need to adapt to scenarios of climate change. Usually, varieties original from the Mediterranean basin are perceived as drought tolerant, such as the widely-used Grenache, Cinsault, Carignan, Cabernet Sauvignon, Sangiovese, Zinfandel, and Nebbiolo (Fraga et al., 2012; Van Leeuwen et al., 2019),

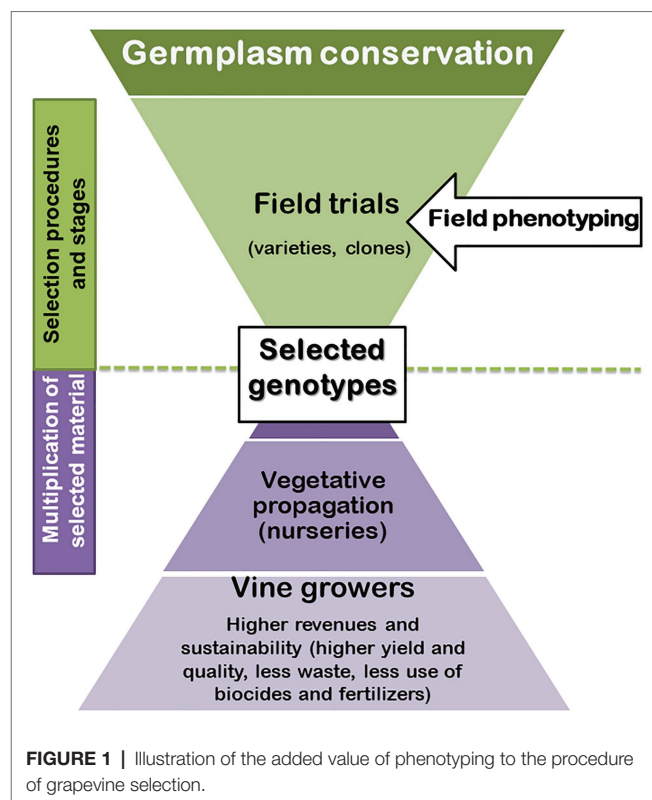


FIGURE 1 | Illustration of the added value of phenotyping to the procedure of grapevine selection.

and the less extensively spread Xinistry from Cyprus (Van Leeuwen et al., 2019). Some Portuguese varieties have also been described as well adapted to abiotic stress, such as Cerceal-Branco, Encruzado, Touriga Franca, and Viosinho (Carvalho et al., 2017). Furthermore, the existence of intravarietal variability in grapevine is the available resource for polyclonal selection (Resolution OIV-VITI 564B-2019; OIV, 2019) and clonal selection (OIV-VITI 564A-2017; OIV, 2017) aiming at climate change adaption.

Despite having a small land area, Portugal is extremely rich in autochthonous varieties. As a result, a coherent strategy has been developed to stop the ongoing erosion of intravarietal genetic diversity of all autochthonous varieties, to improve methods of conservation, to evaluate the intravarietal diversity for selection focused on yield, important must quality traits, and tolerance to abiotic and biotic stresses (Martins and Gonçalves, 2015; Gonçalves et al., 2016; Carvalho et al., 2020). This strategy has been implemented in the field by the National Network for Grapevine selection and by the Portuguese Association for Grapevine Diversity (PORVID).

The resources available in Portugal to perform field phenotyping to select superior clones within Portuguese grapevine varieties comprise a network of more than 185 field trials of 63 varieties, distributed along the country, and established according to efficient experimental designs to carry out selection. Rootstocks influence resistance to abiotic stress, namely to drought (Pavlousek, 2011; Harbertson and Keller, 2012). The combination of tolerant rootstocks with tolerant clones could be the most effective long term strategy to overcome adverse

climate limitations that currently affect Portugal and other Southern European countries (Santos et al., 2020). The graft-scion incompatibility remains a major issue as it can limit response to heat and drought (Tedesco et al., 2020).

Fast, robust, and accurate screening of specific traits to assess tolerance to abiotic stress of rootstocks and *V. vinifera* varieties is crucial to obtain plant material able to cope with climate change. Phenotyping technologies (for controlled and field conditions) have undergone great progress in the last decade. The latest innovations and respective application to different crops have been intensively described (Araus et al., 2018; Qiu et al., 2018; Das Choudhury et al., 2019; Pieruschka and Schurr, 2019; Roitsch et al., 2019; Jiang and Li, 2020; Li et al., 2020; Moreira et al., 2020; Yang et al., 2020; Jin et al., 2021). A multiple set of methods and technologies are now available to support the evaluation of quantitative traits, including crop yield and tolerance to abiotic stresses. In this review, the available phenotyping methodologies will be analyzed in light of their potential use to evaluate inter- and intravarietal variability and to support selection of grapevine genotypes for tolerance to abiotic stress.

PHENOTYPING IN GRAPEVINE

Definitions, Scales, and Approaches

Phenotyping is the process of systematically determining, analyzing, and predicting all or part of an organism's phenotype, and the concept was used for the first time in the 1950s. However, it was only in 2013 that Fiorani and Schurr (2013) coined the term “plant phenotyping,” defining it as “*the set of methodologies and protocols used to accurately measure plant growth, architecture, and composition at different scales.*” Phenotyping aims at providing valuable data to improve management of biodiversity resources, to foster crop/variety adaptability to the environment and resistance against pests and diseases (Costa et al., 2019b,c) as well as to identify superior traits such as yield and quality. Phenotype, as the result of the genotype (G), the environment (E), and the interaction between them (G×E) is dynamic, complex and comprises multiple quantitative traits that make it hard to study, and especially, to quantify.

Phenotyping methodologies and procedures to characterize and select individuals with particular traits and clear advantages at the level of stress resistance, yield performance, and fruit quality traits, require a systematic approach and organized data collection to facilitate further analysis. Plant phenotyping can be carried out at different levels of biological organization with similar aims but yielding different outputs. Molecular phenotyping involves transcriptomics, proteomics, metabolomics, and related areas such as lipidomics, and can be targeted to single-cell phenotyping, in which the effects of a mutation can be studied through changes in a single cell (Schiefelbein, 2015). On the other end, there are field and ecosystem level phenotyping.

Molecular phenotyping focuses on the investigation of gene function and/or biochemical pathways underpinning physiological mechanisms affecting development, productivity,

and stress responses. In grapevine, it aims at developing biotechnology programs to scan for tolerance (Ciaffi et al., 2019) or to develop improved varieties that enable the production of specific wines (DeBolt et al., 2006; Harris et al., 2013) or that are tolerant to biotic stresses (Agüero et al., 2005). At this level, phenotyping approaches are often destructive and require extensive sample manipulation and processing. Therefore, the concept of HTPP refers mainly to whole-plant phenotyping and is largely based in automated image capture and analysis [e.g., Red, Green, Blue (RGB), thermal, multispectral, and fluorescence imaging].

Modern HTPP platforms are coupled to controlled environment growth facilities, allowing large scale screening, isolation of the genetic component of the phenotype, and selection of the most promising genotypes. Large scale plant phenotyping has been extensively studied and developed under controlled conditions, especially for screening of model plants such as Arabidopsis (Merlot et al., 2002), but also for cereals (Raskin and Ladyman, 1988), canola (Knoch et al., 2019), or pepper (Toledo-Martín et al., 2016). In the last few years, good progress was made in the use of remote and proximal sensing tools to meet the phenotyping needs of annual crops, namely by using automated multi-sensor phenotyping machines.¹ Such platforms are sophisticated and costly. Therefore, low-cost or more cost-effective phenotyping options are being developed (Reynolds et al., 2019), among them, user-built cost-controlled prototypes (e.g., in the project INTERPHENO²).

However, as crops are subjected to multiple stresses, changing in duration and intensity along time, selected genotypes must be tested under conditions that are more realistic, namely in field conditions. Field phenotyping is complex, since environmental conditions cannot be controlled and it is difficult to homogenize sampling conditions. Also, field phenotyping infrastructures are not easily available due to their high costs. Some initiatives have been implemented to use field phenotyping technologies based on ground and aerial platforms. For example, the European project EMPHASIS, which is on its implementation phase, aims to create a permanent European HTPP infrastructure network, has a work package fully dedicated to field phenotyping.³

Under the plant-breeder's perspective, an efficient phenotyping must take into account two standpoints: (1) the availability of adequate tools to measure the target traits and (2) the planning of phenotyping. Concerning the first aspect, plant breeding needs simple, fast and HTPP methods well adapted to the main agronomic, physiological, and technological traits. The second aspect is related to the actions before and after phenotyping, that is, the rules that must be followed to ensure that the obtained data can be suitable for an efficient use of the acquired measurements, namely, for selection purposes and comparative experiments. This is particularly relevant to feed biodata infrastructures

¹<https://www.plant-phenotyping.org/>

²<http://interpheno.rd.ciencias.ulisboa.pt/>

³<https://emphasis.plant-phenotyping.eu/>

(ex. EU ELIXIR project or BioData.pt. which is the Portuguese distributed infrastructure for biological data).

CURRENT TECHNOLOGIES AND STRATEGIES TO SCREEN GRAPEVINE GERMPLASM

The need to identify grapevine varieties/genotypes with specific characteristics that enable them to deal with challenges posed by climate change is universally recognized. Nevertheless, we are still far from having reliable, fast, and efficient methodologies for grapevine phenotyping at reduced cost, especially in field conditions. The use of phenotyping devices in woody perennial crops with complex canopies and architecture, such as grapevine still poses difficulties. However, the principle of using indirect non-contact measurements to quantify physiological traits is suitable for grapevine field phenotyping. This approach is getting more attention in parallel with the increasing availability of proximal and remote sensing technologies, especially for “stress-tolerance” based on imaging (RGB, thermal, chlorophyll fluorescence, and hyperspectral). There is also an increasing number of available tools for image processing and analysis, and of algorithms that can support a phenotyping decision (Tsaftaris et al., 2016; Barradas et al., 2021), some developed specifically for grapevine (de Castro et al., 2018; di Gennaro et al., 2019). The potential applications of different imaging approaches for selection and stress monitoring are briefly described below and summarized in **Table 1**.

Visible RGB Imaging

Shoot growth, leaf area, and yield are important agronomical and morphological parameters used for different crops. Traditionally they are quantified by weighing or manually measuring shoot elongation and leaf area. Despite the ease of these measurements, they are very time-consuming and inadequate for large scale phenotyping. RGB imaging, currently widespread in consumer-grade digital cameras and mobile phones, but also available in a diversity of industrial devices tailored for artificial vision (Pajares et al., 2016), is an efficient method to assess leaf area and yield in grapevine (Mabrouk and Sinoquet, 1998; Nuske et al., 2011; Diago et al., 2012; Arnó et al., 2013; Dogan et al., 2018).

Pipeline image analysis performed after automatic selection of representative pixels for each category, such as “soil,” “leaves,” “wood,” or “grapes,” showed high correlation, for leaf area and fruit yield, with the values obtained by destructive methods (Diago et al., 2012). RGB images also proved to be a feasible tool to estimate yield. Berry detection was based not only on the color but also on berries geometry, specifically the radial symmetry, to distinguish them from the background even when green (Nuske et al., 2011; Abdelghafour et al., 2019; Briglia et al., 2019). More recently and using a robot as movable ground platform, Victorino et al. (2020) collected image-based indicators to support yield prediction at different phenological stages in grapevine. The authors reported that

bunch volume and bunch projected area had significantly high correlation coefficients with yield, regardless of the fruit’s occlusion.

Red, Green, Blue images have also been used to estimate the whole plant leaf area (LA) and fresh biomass in grapevine (Coupel-Ledru et al., 2014), both relevant traits to assess plant vigor. Regarding fruits, cluster compactness is an important trait to select table and wine grapes and the assessing methodology is based on the OIV descriptors, using morphological features of the clusters, and can also be estimated using image analysis, a faster and non-destructive alternative of characterization (Palacios et al., 2019). These authors developed a mobile sensing platform that automatically captures RGB images of grapevine canopies and fruiting zones at night using artificial illumination.

The distinction between the individual plants in the foreground and the vineyard in the background poses a major challenge for sensor-based phenotyping, particularly when RGB images are used, since similar color distributions occur in both. To overcome this difficulty, Klodt et al. (2015) developed a method of background subtraction based on taking two images of each plant for depth reconstruction, which were then successfully used to evaluate 3D leaf surface areas and the ratio fruit-to-leaf in new grapevine breeding lines.

Faster image data retrieval will be crucial to gain efficiency in field phenotyping. Imaging acquisition using more or less complex ground-based platforms (robots, tractors, and quads) must still be optimized for the vineyard. This poses major challenges namely related to irregular and rocky soils, different plant spacing, and orientation. Due to the frequency of image acquisition and the storage capacity, driving speed for data acquisition in field conditions has been limited to 0.5–1 km/h (Zheng et al., 2021), even though other authors refer the possibility of reaching 5 km/h for on-the-go imaging (Kicherer et al., 2015; Gutiérrez et al., 2017).

Infrared Thermography

Infrared thermography also shows potential for phenotyping in both controlled and field conditions, namely to assess drought stress (Jones et al., 2002; Costa et al., 2013; Diago et al., 2016). Stomatal conductance to water vapor correlates with the plant’s water status and regulates evaporative cooling, making plant temperature increase when stomata are closed. According to these principles there have been attempts to use thermography instead of the time-consuming leaf gas exchange measurements to assess plant water status and transpiration (Jones et al., 2002; Möller et al., 2007; Briglia et al., 2019). However, factors of environmental variability, such as wind speed, radiation, and air humidity could affect the robustness of thermal imaging data as compared to the actual plant status (Costa et al., 2013). The use of phenotyping vehicles following the concept of “mobile tunnel,” equipped with artificial broadband light sources, as is the case of the Phenoliner (Kicherer et al., 2017), may minimize those environmental disturbances. Another strategy to minimize environmental disturbances is the use of so-called thermal indexes. One of the most commonly applied, is the crop water stress index (CWSI; Clawson et al., 1989), based

TABLE 1 | List of imaging methodologies, their advantages, disadvantages, and potential application to screen grapevine plants in field conditions (genotype selection for yield, berry quality, and for abiotic stress).

Imaging methods	Phenotyping traits	Organ	Advantages	Disadvantages	Potential use for selection	
					Yield/quality	Abiotic stress
RGB visible	Morphology and Growth	Roots	Multiple solutions at low cost	Slightly (in structural/morphological analysis) to significantly (in color analysis) disturbed by light conditions Limited output on physiological data Only 2D Limited feasibility under field conditions for root analysis	No	Yes, but needs optimization for pre-selection
	Leaf color	Leaves	Fast and user friendly			
	Necrosis	Canopy	High portability and multiple platforms			
		Berries	Assessment of biotic and abiotic stress			
		Clusters	Highly adequate for field measurements			
		Shoots	Easy image analysis and processing			
		Trunk	Multiple solutions and prices			
Thermal infrared	Morphology and Growth	Leaves	High portability and multiple platforms	Impact of environment [radiation, wind speed, Tair and air vapor pressure deficit (VPD), and rain], background; Wet and dry references may induce errors Image analysis and processing (software still expensive and demanding skills)	No	Yes, but needs optimization for pre-selection
	Canopy and leaf temperature	Canopy	Assessment of biotic and abiotic stress			
	Stomatal behavior	Berries	Adequate for field measurements			
	Necrosis	Clusters				
		Trunk				
Multispectral	Leaf color	Leaves	Assessment of biotic and abiotic stress	Expensive equipment Plant architecture and light conditions may influence analysis Low capturing speed Difficult image analysis	Yes	Yes
	Chlorophyll content	Canopy	Adequate for field			
	Carotenoid content	Berries	Some information on biochemical traits			
	Secondary metabolites (anthocyanins, terpenoids)					
	Necrosis					
Hyperspectral	Leaf color	Leaves	Assessment of biotic and abiotic stress	Highly expensive equipment Growth of the plant and illumination influence analysis Lack of exhaustive info on biochemistry tissue Difficult image analysis	Yes	Yes
	Chlorophyll content	Canopy	Adequate for field			
	Carotenoid content	Berries	Some information on biochemical traits			
	Secondary metabolites (anthocyanins, terpenoids)					
	Necrosis					
Chlorophyll fluorescence	Photosynthetic efficiency	Leaves	Assessment of biotic and abiotic stress	Expensive equipment, with limited portability Complex to image full and deep canopies Strong impact of environment (light conditions)	No	Yes, but needs extensive optimization for pre-selection
	Leaf senescence degree	Berries	Adequate for field (point measurements)			
	Oxidative stress					
	Membrane integrity					
Laser, stereo (LiDAR)	Plant biomass	Leaves	3D images	Expensive Demanding skills Lack of exhaustive info about plant physiology	Yes, but needs extensive optimization	No
	Plant structure	Canopy	Assessment of biotic and abiotic stress			
	Leaf area, angle, and composition	Trunk	Adequate for field			

on the use of wet and dry reference surface temperatures. A high and stable correlation between CWSI and leaf conductance (g_L) is found when CWSI is calculated using the temperature at the center of the canopy or its sunlight fraction (Clawson et al., 1989). A high positive correlation between g_L and stem water potential (ψ_{stem}) during the season was also found (Irmak et al., 2000). Grapevine water status can be estimated through CWSI by using thermal imaging system and a RGB digital

camera (Möller et al., 2007) in which the color image is used to select pixels with specific features, such as sunlit pixels, to create masks of soil and masks of shadowed leaves to enable the analysis of the temperature in the thermal images only in sunlit leaves. In turn, Matese et al. (2018) found a high correlation between CWSI obtained from proximal and remote thermal sensing and the physiological parameters net photosynthesis (P_n) and effective quantum yield of photosystem

II (F_v'/F_m') in the varieties Vermentino, Cabernet Sauvignon, and Cagnulari. Other reports emphasize the fact that canopy size and architecture, together with leaf orientation can result in different temperature readings for identical values of stomatal conductance (Grant et al., 2007; Grant et al., 2016). Furthermore, the use of wet and dry references, required to compute CWSI or other thermal indexes (e.g., stomatal conductance index – I_c), may conflict with HTPP in field conditions, namely in air borne phenotyping. Therefore, alternative approaches, such as direct comparison between control irrigated and drought stressed plants, must be further developed.

An important and recent development in thermography, is the use of low cost equipment (e.g., thermal camera connected to a smartphone) to calculate water status indices, including CWSI and the stomatal conductance index (Petrie et al., 2019; Jouzier, 2020). Even though these instruments are less accurate, they are simpler and less expensive in monitoring plant stress responses and could also be used as pre-selection scanning to identify contrasting genotypes in terms of leaf/canopy temperature. However, when the expected temperature differences are small, such as in the case of studying intra-varietal variability, the effectiveness of this method is very limited.

Infrared sensors together with RGB sensors were also used in depth (3D) cameras. Recent technological advances that have been used for field phenotyping of grapevines have enabled the manufacture of consumer-grade depth cameras able to produce RGB information, infrared images, and 3D depth data (Milella et al., 2019). These systems might provide an alternative to the more expensive light detection and ranging (LiDAR) systems, in three-dimensional (3D) canopy reconstruction (Milella et al., 2019).

Chlorophyll Fluorescence (Conventional and Imaging)

The emission of chlorophyll fluorescence (CF) is widely used as a contactless method to assess photochemical use of energy and its non-photochemical dissipation (NPQ). The intensity of CF is variable over time depending on the photosynthetic activity and has been used to estimate plant stress, maximum potential PSII efficiency (F_v/F_m), quantum yield, and electron transport rate (ETR). Chlorophyll fluorescence has been extensively used in the assessment of biotic and abiotic stress evaluation in grapevine (Su et al., 2015; Carvalho et al., 2016; Ju et al., 2018) and it has been introduced in HTPP (Marques da Silva, 2016). At leaf level, it is measured mostly with two classes of instruments: pulse amplitude modulating fluorometers and continuous excitation fluorometers. Chlorophyll fluorescence induction (CFIN) is widely used in stress physiology research related to photosynthesis as it provides several relevant information and it is both non-destructive and cheap (since it uses continuous excitation fluorometers, much cheaper than modulated fluorometers; Humplík et al., 2015). *Vitis* species present significant interspecific and intervarietal differences in the patterns of rapid fluorescence induction (Marques da Silva et al., 2020). However, conventional fluorometry (modulated or continuous) is a point measurement, where the signal is

collected generally by an optical fiber that is in contact to or in close proximity to the leaf. This means that leaves have to be manually selected and processed, making automation impossible and thereby excluding these techniques from HTPP processes. On the contrary, chlorophyll fluorescence imaging (CFI) can collect whole-plant images and might be, therefore, included in HTPP platforms.

The use of CFI allows the study of spatial and temporal heterogeneities in fluorescence emission patterns at the level of cells, leaves, plants, or a whole field, and has potential use to identify stress tolerance and for genotype screening in breeding programs (Gorbe and Calatayud, 2012; Osório et al., 2014; Cen et al., 2017; McAusland et al., 2019; Sánchez-Moreiras et al., 2020). CFI is useful to assess stomatal patchiness and heterogeneity of photosynthetic activity (Omasa and Takayama, 2003), overcoming the problems of point measurements due to the high variability at leaf level (Ehlert and Hinch, 2008). Furthermore, imaging fluorimeters may allow the measurement of several samples (replicates) at the same time. However, assessment of fluorescence parameters that require sample dark adaptation (e.g., F_v/F_m) is not feasible in field phenotyping, but informative parameters not requiring dark adaptation (e.g., the photosynthetic ETR) can be measured, although the requirement of a low intensity modulated measuring pulse poses technical difficulties for remote measurements. Fluorescence imaging is under rapid technical development and new instruments are now available (Herritt et al., 2020). Nevertheless, the high cost and limited operational performance in field can hinder their use in large scale field phenotyping in grapevine in the near future.

Relevant information on stress conditions can also be obtained from the analysis of the spectral signature of chlorophyll fluorescence, which is collected after laser excitation in the laser induced fluorescence technique (Gameiro et al., 2016). This technique does not require sample dark adaptation or close proximity to the sample and therefore might be suitable for field HTPP (Marques da Silva, 2016; Marques da Silva and Utkin, 2018).

Multispectral and Hyperspectral Imaging

Several important photosynthesis-related parameters can be investigated through the spectral composition of the light reflected by the plant, fruits, leaves, and canopy. The principle is that reflectance differences are related to chlorophyll, carotenoids, nitrogen, or water content (Walter et al., 2015), in particular, the reflectance analyzed in the visible, near-infrared, and short wavelength infrared spectrum (SWIR). The latter is used for the estimation of plant's water status. The reflectance can be measured by spectrometers (Barradas et al., 2021), which provide point measurements (low/absent spatial resolution, very high spectral resolution), and by multispectral or hyperspectral cameras, which provide images (high spatial resolution, low/very low spectral resolution). A multitude of reflectance indexes have been published (for review, see Xue and Su, 2017), but most are suitable only for spectroscopic measurements, since they require the input of reflectance obtained at specific wavelengths, i.e., in a very narrow spectral

band. However, as discussed above, point measurements are not suitable for HTPP and, therefore, multispectral or hyperspectral imaging is necessary. These measurements, initially used for remote sensing analysis of natural ecosystems, are also suitable for plant/crop phenotyping (Humplik et al., 2015), the main limitation, as for the canopy temperature analysis, is the spatial variability to which the plants are subjected during the measurement, and also the very high costs of the hyperspectral cameras (Table 1). SWIR measurements are at the basis of indices like the normalized difference vegetation index (NDVI), an estimator of the chlorophyll (chl) content, and the proportion of chl *a* in relation to chl *b*, and the photochemical reflectance index (PRI) which allows to estimate the photosynthetic efficiency by measuring the redox status of carotenoids (Humplik et al., 2015) that is part of the non-photochemical de-excitation pathway (Demmig-Adams et al., 2012), and in turn is correlated with photosynthetic light use efficiency (Sims and Gamon, 2002). A portable apparatus for NDVI ground-based measurement was tested in grapevine to estimate plant vigor by vine leaf area index (VLAI; Drissi et al., 2009), and the authors reported that the sensor is adequate to estimate plant vigor as VLAI and canopy gap, but only before the canopy growth saturates the response.

Two indices based on reflectance measurements, R690/R600 and R740/R800, where R690 and R740 are the chlorophyll fluorescence emission peaks and R600 and R800 are bands not affected by chlorophyll fluorescence, have been used to indirectly track changes in steady-state chlorophyll fluorescence due to heat and water stress (Dobrowski et al., 2005). Both indices had a strong positive curvilinear relation with steady state fluorescence (Fs).

More recently, Gutiérrez et al. (2018) showed the feasibility of a novel approach to classify leaves from several grapevine varieties grown in field conditions. The authors used on-the-go hyperspectral imaging at considerable speed (5km/h) and different machine learning algorithms.

Near infrared (NIR) hyperspectral imaging was also used to accurately predict anthocyanin content and evolution during development of Cabernet Sauvignon grapes from veraison to ripening (Chen et al., 2015). Also, NIR hyperspectral imaging was used to predict the quantification of total phenolic anthocyanins and flavanols in grapes of two red varieties, Syrah and Aragonez (syn Tempranillo; Nogales-Bueno et al., 2015). The results identified quantifiable differences between the two varieties regarding these parameters and, interestingly the authors observed a large range of distribution of values in each variety. Another study, performed in red and white varieties of table grapes, successfully used NIR hyperspectral imaging to predict sugar, total flavonoid, and total anthocyanin contents (Gabielli et al., 2021). Sen et al. (2016) showed that the combination of visible and mid infrared (MIR, 4,000–650 cm⁻¹) ranges with methods of multivariate analysis improved the prediction of anthocyanin compounds and total phenols in wine as opposed to using NIR range alone. All these studies emphasize the importance of the robustness of the models adjusted. Furthermore, these traits are subject to high environmental variability, which can significantly change the rates of

accumulation and degradation of sugars, flavonoids, and anthocyanins (Rienth et al., 2021). Also, intracluster berry heterogeneity can also be a main bias for individual berry phenotyping (Rienth et al., 2021). However, berry composition parameters have been used in grapevine selection (Table 2) with a high degree of success, and the effects of the environment can be overcome with an appropriate experimental set up and with sampling in several seasons (Gonçalves et al., 2016). Therefore, it may be possible to apply NIR hyperspectral imaging to clonal phenotyping to obtain data on berry composition for selection.

Light Detection and Ranging

Light detection and ranging (LiDAR) is an active sensing technology that emits short-wavelength lasers, that can be visible, ultraviolet, or near-infrared light, to measure the distance from the sensor to the target according to laser speed and flight time recorded by a timer (Lin, 2015). These measurements are then translated into a 3D structure, built on the angle of the emitting laser collected by an angle encoder. LiDAR has some advantages, such as high throughput, high spatial resolution, high reproducibility, and the characteristics that make it suitable for field measurements, independency from light conditions, and the ability of the short-wavelength laser to penetrate the vegetation canopy (Jin et al., 2021). LiDAR sensors were first used in viticulture in 1998 to estimate several viticultural indices characterizing foliage distribution as well as attributes of the light microclimate in the canopy (Mabrouk and Sinoquet, 1998). The values obtained correlated well with those obtained by traditional methods and the authors were able to calculate bunch exposure and relate it with grape composition, namely sugar content, anthocyanins, and phenolics (Mabrouk and Sinoquet, 1998). This represented a major breakthrough in the estimation of key viticultural traits in an indirect, fast, reproducible, and non-destructive approach. The geometry of plant canopies can also be calculated using LiDAR, during the winter dormancy period, to calculate pruning weight, a previously laborious but extremely informative parameter to calculate plant vigor (Tagarakis et al., 2013, 2018). LAI was also successfully estimated with a laser sensor (Arnó et al., 2013), the authors obtained good correlations between LAI and canopy volume, as well as between LAI and tree area index. Nowadays, automated mobile platforms that move along rows scanning the vines are available. They are able to identify different managing systems and to calculate pruning weight, trunk, and cordon volume (Siebers et al., 2018). Water deficit can also be indirectly calculated through the measurement of plant leaf area, as it correlates well with the apparent soil electrical conductivity (EC_a), giving an indication of the plant's water needs (Tsoulas et al., 2019).

Evaluating Success of Phenotyping for Plant Breeding and Selection

A well planned phenotyping procedure is a critical task in plant breeding because it is the starting point for any efficient

TABLE 2 | Non-exhaustive list of phenotypic traits used in studies focused on agronomic, morphological, and eco-physiological characterization of grapevine genotypes.

Plant Material	Traits quantified at the following levels	
	Morphological and biophysical	Metabolic
Leaves	Individual area	Carbohydrate
	Color	ABA content
	Shape	
	Vein density	
	Thricome density	
	Cuticle thickness	
	Mesophyll thickness	
	Chlorophyll content	
	Relative water content	
	Temperature	
	Water status	
	Intrinsic water use efficiency	
Cluster	Weight	
	Color	
	Number of berries	
	Length and width	
	Compactness	
	Temperature	
	Volume	
	Projected area	
	Diameter	Carbohydrates
Trunk/shoots	Volume	
	Shoot length	
Roots*	Size	Carbohydrates
	Density	
	Inclination	
Canopy/Whole-plant	Yield	
	Biomass/vigor	
	Shoot length	
	Exposed leaf area	
	Number of leaf layers	
	3D leaf area	
	Projected leaf rea	
	Leaf area index	
	Light penetration	
		Acidity
Berries/Must		pH
		°Brix
		Anthocyanins
		Phenols
		Aroma precursors

*Only for rootstock characterization and selection; traits in **bold** are already used for selection.

selection of plants, as illustrated for grapevine in **Figure 1**. When working with quantitative traits (the most frequent and economically important ones, such as yield, tolerance, or quality), it is necessary to understand the meaning of the obtained

phenotypic value. This requires the quantification of the part of the measured trait that is due to the genotypic causes.

In classical models of quantitative genetics (i.e., balanced data with no random effects other than those associated with genotypes and error, and diagonal variance-covariance matrices), the proportion of total variance (phenotypic variance) that is genetic is called broad sense heritability (Falconer and Mackay, 1996). At the level of the mean of the genotypes, the classical concept of broad-sense heritability (H^2) is defined as

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

where σ_g^2 , σ_e^2 , and r are the genotypic variance, error variance, and number of replicates, respectively. The broad sense heritability is an important indicator of the quality of the experimental design of the trials for the evaluation of a target quantitative trait and, consequently, of the success of genetic selection. Due to its importance in the context of plant breeding of quantitative traits, several studies addressed the problem of defining the establishment of a generalized measure applicable to more complex models (Cullis et al., 2006; Oakey et al., 2006; Piepho and Möhring, 2007; Welham et al., 2010), including in the context of grapevine selection (Gonçalves et al., 2013). To summarize all these approaches, an approximate generalized measure of broad-sense heritability can be presented as

$$H^2 = 1 - \frac{\overline{PEV}}{\sigma_g^2},$$

where \overline{PEV} is the average of the predicted error variance of genotypic effects and σ_g^2 is the genotypic variance.

Another key concept is the prediction of genetic gain (R) for the several traits evaluated. In the context of ancient grapevine varieties and under the classical models, it is defined as

$$R = S \times H^2$$

where S is the differential of selection, that is, the difference between the selected group of genotypes and the mean of the population and H^2 is the broad-sense heritability (Falconer and Mackay, 1996). Similarly, the genetic gain of selection is the mean of the Empirical Best Linear Unbiased Predictors (EBLUPs) of the genotypic effects of the top-ranked selected genotypes. This last definition is also applicable for more complex models. A selection based on EBLUPs of the genotypic effects of the best model would be more efficient and lead to higher genetic gains.

To quantify and obtain high values of heritability and high predicted genetic gains, that is, to achieve precision and accuracy in the evaluation of quantitative traits, agronomic experiments demand a well-planned phenotyping, which involves the establishment of field trials with efficient experimental designs (with repetition, randomization, and efficient control of spatial variation) and correspondent appropriate models for data analysis (mixed models).

Agronomic experiments are usually large, expensive, and take many years to accomplish. Additionally, they are typically

subject to high background variability due to soil fertility and availability of water trends in the field (spatial variation) and cultural techniques, and other environmental deviations. This variability must be controlled through the type of experimental design. Typically the effective control of background variability is made by blocking or by using covariates together with sufficient replication of genotypes (Piepho and Edmondson, 2018). The experimental designs used in agriculture to reach these objectives have a long history and are routinely used in agronomic experiments, such as randomized complete block designs, latin squares, split-plot designs, and the family of incomplete block designs (Giesbrecht and Gumpertz, 2004). Nowadays, precision agriculture tools (e.g., soil water sensors, EC, and NDVI maps) can also be used to optimize the establishment of the experimental design in the field. These tools can help find homogenous patterns of soil composition and water availability that enable the definition of incomplete and complete blocks.

In grapevine, field trials for polyclonal selection comprise a representative sample of the intravarietal variability of the variety under study (Martins and Gonçalves, 2015; OIV, 2019). The experimental designs useful for screening a large number of genotypes and to provide reliable guidance to select the best genotypes are described in Gonçalves et al. (2010), and the most efficient are alpha designs and resolvable row-column designs.

The application and testing of HTPP methodologies in field trials with adequate experimental designs and the need to quantify the quality of the measurements obtained are constant concerns in the plant breeding context. For example, Tattaris et al. (2016) used spring wheat lines trials, established under an alpha-lattice design, with either two or three replications, to test HTPP monitoring of plant physiological traits (canopy temperature and a vegetation index). Tattaris et al. (2016) compared three remote sensing approaches using a low flying unmanned aerial vehicle, with that of proximal sensing, and satellite-based imagery to determine the most viable approaches for large scale crop genetic improvement. The results obtained supported the use of those techniques for HTPP for both precision and efficiency. In turn, Singh et al. (2019) demonstrated the considerable power of unmanned aerial systems or drone-based phenotyping as a HTPP alternative to visual assessments for the complex phenological trait of lodging, which significantly impacts yield and quality in many crops including wheat. They tested and validated quantitative assessment of lodging on 2,640 wheat breeding plots over the course of 2 years using differential digital elevation models. A total of 590 and 595 unique wheat entries along with the check varieties were planted in alpha-lattice field design during seasons 2016 and 2017, respectively. The broad-sense heritability of visual and digital lodging measures ranged between 0.50 and 0.59. Andrade-Sanchez et al. (2014), proved that a tractor-based phenotyping system was capable of reliably acquiring and recording data for canopy temperature, height and reflectance on experimental plots of cotton plants throughout the growing season in the field. To prove that, they evaluated field trials with 25 Pima cotton cultivars arranged as a lattice

design with four replications in a total of 200 plots. Measurements of canopy height, NDVI, and temperature all showed large differences among cultivars and expected interactions of cultivars with water regime and time of day. Broad-sense heritability ranged from 0.86 to 0.96 for canopy height, from 0.28 to 0.90 for the NDVI, and from 0.01 to 0.90 for temperature. Also in the context of high throughput phenotyping, Junker et al. (2015) highlighted some experimental procedures to optimize the quantitative evaluation of crop plant performance. In grapevine, Carvalho et al. (2020) evaluated abiotic stress tolerance, measured by the surface leaf temperature (SLT) of clones under environmental conditions of drought and extreme heat for 3 years. SLT sets the boundary condition for the latent and sensible heat transport through vegetation, soil, and atmosphere, depending on the availability of moisture at the interface soil-atmosphere (Fuchs, 1990), giving an estimate of the response of a leaf to the environmental parameters affecting it at any time (air temperature, relative humidity, solar radiation, leaf resistance, and boundary layer resistance; Udompetaikul et al., 2011). By utilizing simple measurement devices and an experimental set up that enables the separation between environmental influence and the physiological response, it is possible to study the relationship between these parameters. A plant is able to keep a SLT lower than ambient temperature by controlling stomatal aperture and thus leaf gas exchange through stomata. The capacity to control stomata opening and thus CO₂ intake for photosynthesis regardless of high air temperature gives the clones that hold it an advantage to face heat stress without loss of yield and quality of the grapes produced. The application of the methodology was done in a field trial with 255 different clones established according to a resolvable incomplete block experimental design with five complete blocks: each complete block comprised the effect of the complete block and the effect of the day; each column within each complete block, with approximately 13 plots, constituted an incomplete block, which comprised the effect of the time of day. With this type of experimental design, it was possible to prove the existence of significant genetic variability within the variety for the trait SLT and the values of generalized broad sense heritability ranged between 0.44 and 0.54, corresponding to a quantifiable genetic component difference of 3°C between the coolest and warmest of the 255 genotypes measured in three consecutive seasons.

In short, in the context of plant breeding, to perform fast, massive, or HTPP, the establishment of field trials with adequate experimental designs and the estimation of several genetic and statistical parameters, that provide information about the meaning and the quality of the data obtained, is mandatory.

Traits to Use in Phenotyping for Selection

A more sustainable viticulture must involve the use of locally adapted varieties and selected material of those varieties. Phenotyping must enable a reliable identification of genotypes with the desired traits, whether yield, specific berry composition, or tolerance to stress and should contribute to estimate their genotypic diversity.

So far, grapevine selection within ancient varieties relies on the exhaustive gathering of specific data from all the genotypes in an experimental field (with biological replicates it generally reaches more than 3,000 plants; Martins and Gonçalves, 2015). Any possibility of automation without loss of reproducibility or precise quantification should be very welcome. Moreover, the data gathered are so dependent of the effects of the environment that only an efficient experimental design allows to control those effects, and most importantly, to quantify the contribution of the genetic component, the so called broad sense heritability.

Therefore, an effective selection of grapevine genotypes takes several years, requiring much labor and costs. The need to evaluate hundreds of genotypes in several repetitions occupies between 1.0 and 2.0 ha and an efficient control of the field installation cannot allow the use of ready-made grafted plants. With all these constraints, such trials are only viable for economically prized varieties.

With respect to the data gathering itself, currently, only yield and berry composition have been exhaustively tested and quantified in selection trials (Gonçalves et al., 2016; **Table 2**). Yield is quantified by handpicking and weighing the production of each plant in the trial. However, the most time- and labor-consuming task of selection is berry composition analysis, which requires collection of individual berry samples from all grapevines and making the quantifications of pH, total acidity, and soluble solids and, in red varieties, anthocyanins, and phenols, in the lab, following standardized and well-established protocols.

The use of traits such as leaf temperature or the simpler RGB offer still limitations in assessing properly yield but mostly berry quality traits. More testing to find robust correlations between Tleaf or leaf color or canopy size and yield and quality are needed therefore, to make them used in selection for berry composition and final yield per plant.

CONCLUSION AND PROSPECTS

Screening and characterization of inter- and intravarietal variability of autochthonous grapevine varieties has a crucial importance not only for the Portuguese but also for the global wine industry. The future competitiveness and higher sustainability of the sector should be largely based on the use of well adapted plant material (rootstocks and varieties). Drought and heat stress are major driving forces for grapevine selection and breeding as means to identify the most resistant and better adapted grapevine varieties/genotypes. In fact, one of the medium/long term strategies to respond to climate change adversities and the problems of increased stress is based on the selection and use of superior genotypes.

A major future challenge for grapevine field phenotyping is to exhaustively evaluate relevant traits for selection purposes, such as tolerance to abiotic stress. Moreover, the available imaging technologies (e.g., RGB, thermal, and multispectral) need to be adapted and optimized to large field trials (**Table 1**) to provide reliable quantitative data for a robust, reproducible,

and comparable analysis at different levels (leaf, canopy, berry, and cluster). This task can be particularly challenging when dealing with intra-varietal characterization and clonal selection, attending to the potentially smaller differences between genotypes for some traits, namely those related to tolerance to abiotic stress. Also, the possibility for automation of data gathering for traits already under analysis, such as berry composition, would expedite measurements of large experiments.

Proximal and remote sensing technologies have undergone great developments in recent years and have become more accurate, cheaper and, in some cases, more user-friendly. The attention of the scientific and industry communities toward these technologies is very high due to their potential for field analysis and subsequent management of variability in field conditions. In viticulture, they are chiefly applied in the agronomic management of the vineyard as part of the so called “precision viticulture,” but some proximal and remote sensing technologies have potential for phenotyping and selection. For this purpose, it will be necessary to deepen and clarify the link between the indirect digital measurements obtained by sensors and the morphological, eco-physiological, and metabolic parameters under examination, which sometimes is still doubtful. The following step would be to develop specific and standardized protocols to apply these sensing technologies to grapevine phenotyping in field conditions, mainly focused on leaf, berry, or canopy/plant traits that are closely related to physiologically complex phenomena, such as that of tolerance to abiotic stress.

The advance in imaging technologies, robotics and computing will enable to establish and perform new assays for genotype characterization and selection that can be carried out under field conditions. This can also provide more tools to study grapevine development and behavior under climate change conditions.

AUTHOR CONTRIBUTIONS

JC: idea, writing, editing, reviewing and funding. LC and EG: writing, editing, and reviewing. JMS: writing, reviewing, and funding. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Fundação para a Ciência e Tecnologia (FCT) research unit BioISI (UID/MULTI/04046/2019) and the FCT funded R&D project INTERPHENO (PTDC/ASP-PLA/28726/2017). LC acknowledges the funding by FCT through DL57/2016/CP1382/CT0024. Linking Landscape, Environment, Agriculture, and Food (LEAF) research center has been funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the projects UID/AGR/04129/2013; UID/AGR/04129/2019, and presently UIDB/ and UIDP/04129/2020.

REFERENCES

- Abdelghafour, F., Rosu, R., Keresztes, B., Germain, C. P., and Da Costa, J. P. (2019). A Bayesian framework for joint structure and colour based pixel-wise classification of grapevine proximal images. *Comput. Electron. Agric.* 158, 345–357. doi: 10.1016/j.compag.2019.02.017
- Agüero, C. B., Uratsu, S. L., Greve, C., Powell, A. L. T., Labavitch, J. M., Meredith, C. P., et al. (2005). Evaluation of tolerance to Pierce's disease and *Botrytis* in transgenic plants of *Vitis vinifera* L. expressing the pear PGP gene. *Mol. Plant Pathol.* 6, 43–51. doi: 10.1111/J.1364-3703.2004.00262.X
- Andrade-Sanchez, P., Gore, M. A., Heun, J. T., Thorp, K. R., Carmo-Silva, A. E., French, A. N., et al. (2014). Development and evaluation of a field-based high-throughput phenotyping platform. *Funct. Plant Biol.* 41, 68–79. doi: 10.1071/FP13126
- Araus, J. L., Kefauver, S. C., Zaman-Allah, M., Olsen, M. S., and Cairns, J. E. (2018). Translating high-throughput phenotyping into genetic gain. *Trends Plant Sci.* 23, 451–466. doi: 10.1016/j.tplants.2018.02.001
- Arnó, J., Escolà, A., Vallès, J. M., Llorens, J., Sanz, R., Masip, J., et al. (2013). Leaf area index estimation in vineyards using a ground-based LiDAR scanner. *Precis. Agric.* 14, 290–306. doi: 10.1007/s11119-012-9295-0
- Barradas, A., Correia, P. M. P., Silva, S., Mariano, P., Pires, M. C., Matos, A. R., et al. (2021). Comparing machine learning methods for classifying plant drought stress from leaf reflectance spectra in *Arabidopsis thaliana*. *Appl. Sci.* 11:6392. doi: 10.3390/app11146392
- Bota, J., Tomás, M., Flexas, J., Medrano, H., and Escalona, J. M. (2016). Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. *Agric. Water Manag.* 164, 91–99. doi: 10.1016/j.agwat.2015.07.016
- Briglia, N., Montanaro, G., Petrozza, A., Summerer, S., Cellini, F., and Nuzzo, V. (2019). Drought phenotyping in *Vitis vinifera* using RGB and NIR imaging. *Sci. Hortic.* 256:108555. doi: 10.1016/j.scienta.2019.108555
- Buckley, T. N. (2019). How do stomata respond to water status? *New Phytol.* 224, 21–36. doi: 10.1111/nph.15899
- Carvalho, L. C., and Amâncio, S. (2019). Cutting the Gordian knot of abiotic stress in grapevine: From the test tube to climate change adaptation. *Physiol. Plant.* 165, 330–342. doi: 10.1111/ppl.12857
- Carvalho, L. C., Coito, J. L., Gonçalves, E. F., Chaves, M. M., and Amâncio, S. (2016). Differential physiological response of the grapevine varieties Touriga Nacional and Trincadeira to combined heat, drought and light stresses. *Plant Biol.* 18, 101–111. doi: 10.1111/plb.12410
- Carvalho, L., Gonçalves, E., Amâncio, S., and Martins, A. (2020). Selecting Aragonese genotypes able to outplay climate change-driven abiotic stress. *Front. Plant Sci.* 11:599230. doi: 10.3389/fpls.2020.599230
- Carvalho, L. C., Silva, M., Coito, J. L., Rocheta, M. P., and Amancio, S. (2017). Design of a custom RT-qPCR array for assignment of abiotic stress tolerance in traditional Portuguese grapevine varieties. *Front. Plant Sci.* 8:1835. doi: 10.3389/fpls.2017.01835
- Cen, H., Weng, H., Yao, J., He, M., Lv, J., Hua, S., et al. (2017). Chlorophyll fluorescence imaging uncovers photosynthetic fingerprint of *citrus* Huanglongbing. *Front. Plant Sci.* 8:1509. doi: 10.3389/fpls.2017.01509
- Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., et al. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.* 105, 661–676. doi: 10.1093/aob/mcq030
- Chen, S., Zhang, F., Ning, J., Liu, X., Zhang, Z., and Yang, S. (2015). Predicting the anthocyanin content of wine grapes by NIR hyperspectral imaging. *Food Chem.* 172, 788–793. doi: 10.1016/j.foodchem.2014.09.119
- Ciaffi, M., Paolacci, A. R., Paolucci, M., Alicandri, E., Bigini, V., Badiani, M., et al. (2019). Transcriptional regulation of stilbene synthases in grapevine germplasm differentially susceptible to downy mildew. *BMC Plant Biol.* 19:404. doi: 10.1186/s12870-019-2014-5
- Clawson, K. L., Jackson, R. D., and Pinter, P. J. (1989). Evaluating plant water stress with canopy temperature differences. *J. Agron.* 81, 858–863. doi: 10.2134/agronj1989.00021962008100060004x
- Costa, J. M., Egipto, R., Sánchez-Virosta, A., Lopes, C. M., and Chaves, M. M. (2019a). Canopy and soil thermal patterns to support water and heat stress management in vineyards. *Agric. Water Manag.* 216, 484–496. doi: 10.1016/j.agwat.2018.06.001
- Costa, J. M., Grant, O. M., and Chaves, M. M. (2013). Thermography to explore plant-environment interactions. *J. Exp. Bot.* 64, 3937–3949. doi: 10.1093/jxb/ert029
- Costa, J. M., Marques da Silva, J., Pinheiro, C., Barón, M., Mylona, P., Centritto, M., et al. (2019b). Opportunities and limitations of crop phenotyping in southern European countries. *Front. Plant Sci.* 10:1125. doi: 10.3389/fpls.2019.01125
- Costa, J. M., Ortuño, M. F., Lopes, C. M., and Chaves, M. M. (2012). Grapevine varieties exhibiting differences in stomatal response to water deficit. *Funct. Plant Biol.* 39, 179–189. doi: 10.1071/FP11156
- Costa, C., Schurr, U., Loreto, F., Menesatti, P., and Carpentier, S. (2019c). Plant phenotyping research trends, a science mapping approach. *Front. Plant Sci.* 9:1933. doi: 10.3389/fpls.2018.01933
- Coupel-Ledru, A., Lebon, E., Christophe, A., Doligez, A., Cabrera-Bosquet, L., Péchier, P., et al. (2014). Genetic variation in a grapevine progeny (*Vitis vinifera* L. cvs Grenache x Syrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought. *J. Exp. Bot.* 65, 6205–6218. doi: 10.1093/jxb/eru228
- Cullis, B. R., Smith, A. B., and Coombes, N. E. (2006). On the design of early generation variety trials with correlated data. *J. Agric. Biol. Environ. Stat.* 11, 381–393. doi: 10.1198/108571106X154443
- dal Santo, S., Palliotti, A., Zenoni, S., Tornielli, G. B., Fasoli, M., Paci, P., et al. (2016). Distinct transcriptome responses to water limitation in isohydric and anisohydric grapevine cultivars. *BMC Genomics* 17:815. doi: 10.1186/s12864-016-3136-x
- Das Choudhury, S., Samal, A., and Awada, T. (2019). Leveraging image analysis for high-throughput plant phenotyping. *Front. Plant Sci.* 10:508. doi: 10.3389/fpls.2019.00508
- Dayer, S., Herrera, J. C., Dai, Z., Burlett, R., Lamarque, L. J., Delzon, S., et al. (2020). The sequence and thresholds of leaf hydraulic traits underlying grapevine varietal differences in drought tolerance. *J. Exp. Bot.* 71, 4333–4344. doi: 10.1093/jxb/eraa186
- de Castro, A. I., Jiménez-Brenes, F. M., Torres-Sánchez, J., Peña, J. M., Borra-Serrano, I., and López-Granados, F. (2018). 3-D characterization of vineyards using a novel UAV imagery-based OBIA procedure for precision viticulture applications. *Remote Sens.* 10:584. doi: 10.3390/rs10040584
- DeBolt, S., Cook, D. R., and Ford, C. M. (2006). L-tartaric acid synthesis from vitamin C in higher plants. *PNAS* 103, 5608–5613. doi: 10.1073/pnas.0510864103
- Demmig-Adams, B., Cohu, C. M., Muller, O., and Adams, W. W. (2012). Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. *Photosynth. Res.* 113, 75–88. doi: 10.1007/s11220-012-9761-6
- di Gennaro, S. F., Toscano, P., Cinat, P., Berton, A., and Matese, A. (2019). A low-cost and unsupervised image recognition methodology for yield estimation in a vineyard. *Front. Plant Sci.* 10:559. doi: 10.3389/fpls.2019.00559
- Diago, M. P., Correa, C., Millán, B., Barreiro, P., Valero, C., and Tardaguila, J. (2012). Grapevine yield and leaf area estimation using supervised classification methodology on RGB images taken under field conditions. *Sensors* 12, 16988–17006. doi: 10.3390/s121216988
- Diago, M. P., Fernández-Navales, J., Fernandes, A. M., Melo-Pinto, P., and Tardaguila, J. (2016). Use of visible and short-wave near-infrared hyperspectral imaging to fingerprint anthocyanins in intact grape berries. *J. Agric. Food Chem.* 64, 7658–7666. doi: 10.1021/acs.jafc.6b01999
- Dobrowski, S. Z., Pushnik, J. C., Zarco-Tejada, P. J., and Ustin, S. L. (2005). Simple reflectance indices track heat and water stress-induced changes in steady-state chlorophyll fluorescence at the canopy scale. *Remote Sens. Environ.* 97, 403–414. doi: 10.1016/j.rse.2005.05.006
- Dogan, A., Uyak, C., Keskin, N., Akcay, A., Sensoy, R. I. G., and Ercisli, S. (2018). Grapevine leaf area measurements by using pixel values. *C. R. Acad. Bulg. Sci.* 71, 772–779. doi: 10.7546/CRABS.2018.06.07
- Drissi, R., Goutouly, J. P., Forget, D., and Gaudillere, J. P. (2009). Nondestructive measurement of grapevine leaf area by ground normalized difference vegetation index. *Agron. J.* 101, 226–231. doi: 10.2134/agronj2007.0167
- Ehlert, B., and Hinch, D. K. (2008). Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in *Arabidopsis* leaves. *Plant Methods* 4:12. doi: 10.1186/1746-4811-4-12
- Falconer, D. S., and Mackay, T.F.C. (1996). *An Introduction to Quantitative Genetics*. 4th Edn. London: Prentice Hall.
- Field, S. K., Smith, J. P., Morrison, E. N., Emery, R. J. N., and Holzapfel, B. P. (2020). Soil temperature prior to veraison alters grapevine carbon partitioning,

- xylem sap hormones, and fruit set. *Am. J. Enol. Vitic.* 71, 52–61. doi: 10.5344/ajev.2019.19038
- Fiorani, F., and Schurr, U. (2013). Future scenarios for plant phenotyping. *Annu. Rev. Plant Biol.* 64, 267–291. doi: 10.1146/annurev-arplant-050312-120137
- Fraga, H. (2020). Climate change: a new challenge for the winemaking sector. *Agronomy* 10:1465. doi: 10.3390/agronomy10101465
- Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., and Santos, J. A. (2012). An overview of climate change impacts on European viticulture. *Food Energy Secur.* 1, 94–110. doi: 10.1002/fes3.14
- Fritz, M. A., Rosa, S., and Sicard, A. (2018). Mechanisms underlying the environmentally induced plasticity of leaf morphology. *Front. Genet.* 9:478. doi: 10.3389/fgene.2018.00478
- Fuchs, M. (1990). Infrared measurement of canopy temperature and detection of plant water stress. *Theor. Appl. Climatol.* 42, 253–261. doi: 10.1007/BF00865986
- Gabrielli, M., Lançon-Verdier, V., Picouet, P., and Maury, C. (2021). Hyperspectral imaging to characterize table grapes. *Chem. Aust.* 9:71. doi: 10.3390/chemosensors9040071
- Gago, P., Conejero, G., Martínez, M. C., This, P., and Verdeil, J. L. (2019). Comparative anatomy and morphology of the leaves of grenache noir and Syrah grapevine cultivars. *SAJEV* 40, 1–9. doi: 10.21548/40-2-3031
- Gambetta, G. A., Herrera, J. C., Dayer, S., Feng, Q., Hochberg, U., and Castellari, S. D. (2020). The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. *J. Exp. Bot.* 71, 4658–4676. doi: 10.1093/jxb/era245
- Gambetta, J. M., Holzapfel, B. P., Stoll, M., and Friedel, M. (2021). Sunburn in grapes: a review. *Front. Plant Sci.* 11:604691. doi: 10.3389/fpls.2020.604691
- Gameiro, C., Utkin, A. B., Cartaxana, P., da Silva, J. M., and Matos, A. R. (2016). The use of laser induced chlorophyll fluorescence (LIF) as a fast and non-destructive method to investigate water deficit in *Arabidopsis*. *Agric. Water Manag.* 164, 127–136. doi: 10.1016/j.agwat.2015.09.008
- Gashu, K., Sikron Persi, N., Drori, E., Harcavi, E., Agam, N., Bustan, A., et al. (2020). Temperature shift between vineyards modulates berry phenology and primary metabolism in a varietal collection of wine grapevine. *Front. Plant Sci.* 11:588739. doi: 10.3389/fpls.2020.588739
- Giesbrecht, F. G., and Gumpertz, M. L. (2004). *Planning, Construction and Analysis of Comparative Experiments*. New York: Wiley.
- Gonçalves, E., Carrasquinho, I., Almeida, R., Pedrosa, V., and Martins, A. (2016). Genetic correlations in grapevine and their effects on selection. *Aust. J. Grape Wine Res.* 22, 52–63. doi: 10.1111/ajgw.12164
- Gonçalves, E., Carrasquinho, I., St. Aubyn, A., and Martins, A. (2013). Broad-sense heritability in mixed models for grapevine initial selection trials. *Euphytica* 189, 379–391. doi: 10.1007/s10681-012-0787-9
- Gonçalves, E., St. Aubyn, A., and Martins, A. (2010). Experimental designs for evaluation of genetic variability and selection of ancient grapevine varieties: A simulation study. *Heredity* 104, 552–562. doi: 10.1038/hdy.2009.153
- Gorbe, E., and Calatayud, A. (2012). Applications of chlorophyll fluorescence imaging technique in horticultural research: a review. *Sci. Hortic.* 138, 24–35. doi: 10.1016/j.scienta.2012.02.002
- Grant, O. M., Ochagavia, H., Baluja, J., Diago, M. P., and Tardaguila, J. (2016). Thermal imaging to detect spatial and temporal variation in the water status of grapevine (*Vitis vinifera* L.). *J. Hortic. Sci. Biotechnol.* 91, 43–54. doi: 10.1080/14620316.2015.1110991
- Grant, O. M., Tronina, L., Jones, H. G., and Chaves, M. M. (2007). Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes. *J. Exp. Bot.* 58, 815–825. doi: 10.1093/jxb/erl153
- Greer, D. H. (2020). Stomatal and non-stomatal limitations at different leaf temperatures to the photosynthetic process during the post-harvest period for *Vitis vinifera* cv. Chardonnay vines. *N.Z.J. Crop Hortic. Sci.* 48, 1–21. doi: 10.1080/01140671.2019.1632213
- Gutiérrez, S., Diago, M. P., Fernández-Navales, J., and Tardaguila, J. (2017). On-the-go thermal imaging for water status assessment in commercial vineyards. *Adv. Anim. Sci.* 8, 520–524. doi: 10.1017/S204047001700108
- Gutiérrez, S., Fernández-Navales, J., Diago, M. P., and Tardaguila, J. (2018). On-the-go hyperspectral imaging under field conditions and machine learning for the classification of grapevine varieties. *Front. Plant Sci.* 9:1102. doi: 10.3389/fpls.2018.01102
- Gutiérrez-Gamboa, G., Zheng, W., and Martínez de Toda, F. (2021). Current viticultural techniques to mitigate the effects of global warming on grape and wine quality: a comprehensive review. *Food Res. Int.* 139:109946. doi: 10.1016/j.foodres.2020.109946
- Harbertson, J. F., and Keller, M. (2012). Rootstock effects on deficit-irrigated winegrapes in a dry climate: grape and wine composition. *Am. J. Enol. Vitic.* 63, 40–48. doi: 10.5344/ajev.2011.11079
- Harris, N. N., Luczo, J. M., Robinson, S. P., and Walker, A. R. (2013). Transcriptional regulation of the three grapevine chalcone synthase genes and their role in flavonoid synthesis in shiraz. *Aust. J. Grape Wine Res.* 19, 221–229. doi: 10.1111/ajgw.12026
- Herritt, M. T., Pauli, D., Mockler, T. C., and Thompson, A. L. (2020). Chlorophyll fluorescence imaging captures photochemical efficiency of grain sorghum (*Sorghum bicolor*) in a field setting. *Plant Methods* 16:109. doi: 10.1186/s13007-020-00650-0
- Humplik, J. F., Lázár, D., Husíková, A., and Spíchal, L. (2015). Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses—a review. *Plant Methods* 11:29. doi: 10.1186/s13007-015-0072-8
- IPCC (2014). Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. eds. C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir et al. (Cambridge, UK and New York, USA: Cambridge University Press), 1132.
- Irmak, S., Haman, D. Z., and Bastug, R. (2000). Determination of crop water stress index for irrigation timing and yield estimation of corn. *Agron. J.* 92, 1221–1227. doi: 10.2134/agronj2000.9261221x
- Jiang, Y., and Li, C. (2020). Convolutional neural networks for image-based high-throughput plant Phenotyping: a review. *Plant Phenomics* 2020:4152816. doi: 10.34133/2020/4152816
- Jin, S., Sun, X., Wu, F., Su, Y., Li, Y., Song, S., et al. (2021). Lidar sheds new light on plant phenomics for plant breeding and management: recent advances and future prospects. *ISPRS J. Photogramm. Remote Sens.* 171, 202–223. doi: 10.1016/j.isprsjprs.2020.11.006
- Jones, H. (1992). *Plants and Microclimate. 2nd Edn.* Cambridge: Cambridge University Press
- Jones, H. G., Stoll, M., Santos, T., Sousa, C. D., Chaves, M. M., and Grant, O. M. (2002). Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. *J. Exp. Bot.* 53, 2249–2260. doi: 10.1093/jxb/erf083
- Jouzier, P. (2020). Smartphone: The winegrowers' Swiss army knife. *IVES Technical Rev.* 20. doi: 10.20870/ives-tr.2020.3622
- Ju, Y. L., Yue, X. F., Zhao, X. F., Zhao, H., and Fang, Y. L. (2018). Physiological, micro-morphological and metabolomic analysis of grapevine (*Vitis vinifera* L.) leaf of plants under water stress. *Plant Physiol. Biochem.* 130, 501–510. doi: 10.1016/j.plaphy.2018.07.036
- Junker, A., Muraya, M. M., Weigelt-Fischer, K., Arana-Ceballos, F., Klukas, C., et al. (2015). Optimizing experimental procedures for quantitative evaluation of crop plant performance in high throughput phenotyping systems. *Front. Plant Sci.* 5:770. doi: 10.3389/fpls.2014.00770
- Kicherer, A., Herzog, K., Bendel, N., Klück, H.-C., Backhaus, A., Wieland, M., et al. (2017). Phenoliner: a new field phenotyping platform for grapevine research. *Sensors* 17:1625. doi: 10.3390/s17071625
- Kicherer, A., Herzog, K., Pflanz, M., Wieland, M., Rüger, P., Kecke, S., et al. (2015). An automated field phenotyping pipeline for application in grapevine research. *Sensors* 15, 4823–4836. doi: 10.3390/s150304823
- Klodt, M., Herzog, K., Töpfer, R., and Kremers, D. (2015). Field phenotyping of grapevine growth using dense stereo reconstruction. *BMC Bioinformatics* 16:143. doi: 10.1186/s12859-015-0560-x
- Knoch, D., Abbadi, A., Grandke, F., Meyer, R. C., Samans, B., Werner, C. R., et al. (2019). Strong temporal dynamics of QTL action on plant growth progression revealed through high-throughput phenotyping in canola. *Plant Biotechnol. J.* 18, 68–82. doi: 10.1111/pbi.13171
- Levin, A. D., Williams, L. E., and Matthews, M. A. (2019). A continuum of stomatal responses to water deficits among 17 wine grape cultivars (*Vitis vinifera*). *Funct. Plant Biol.* 47, 11–25. doi: 10.1071/FP19073
- Li, Z., Guo, R., Li, M., Chen, Y., and Li, G. (2020). A review of computer vision technologies for plant phenotyping. *Comput. Electron. Agric.* 176:105672. doi: 10.1016/j.compag.2020.105672

- Lin, Y. (2015). LiDAR: An important tool for next-generation phenotyping technology of high potential for plant phenomics? *Comput. Electron. Agric.* 119, 61–73. doi: 10.1016/j.compag.2015.10.011
- Lorenzo, N., Diaz-Poso, A., and Roy, D. (2021). Heatwave intensity on the Iberian Peninsula: future climate projections. *Atmos. Res.* 258:105655. doi: 10.1016/j.atmosres.2021.105655
- Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., et al. (2010). Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non hydraulic interactions at the whole plant level: a physiological and molecular update. *Funct. Plant Biol.* 37, 98–116. doi: 10.1071/FP09191
- Mabrouk, H., and Sinoquet, H. (1998). Indices of light microclimate and canopy structure of grapevines determined by 3D digitising and image analysis, and their relationship to grape quality. *Aust. J. Grape Wine Res.* 4, 2–13. doi: 10.1111/j.1755-0238.1998.tb00129.x
- MacMillan, P., Teixeira, G., Lopes, C. M., and Monteiro, A. (2021). The role of grapevine leaf morphoanatomical traits in determining capacity for coping with abiotic stresses: a review. *Ciência Téc. Vitiv.* 36, 75–88. doi: 10.1051/ctv/ctv2021360175
- Marques da Silva, J. (2016). “Monitoring photosynthesis by *in vivo* chlorophyll fluorescence: application to high-throughput plant phenotyping,” in *Applied Photosynthesis—New Progress*. ed. M. Najafpour (Rijeka, Croatia: InTech Open), 3–22.
- Marques da Silva, J., Figueiredo, A., Cunha, J., Eiras-Dias, J. E., Silva, S., Vanneschi, L., et al. (2020). Using rapid chlorophyll fluorescence transients to classify *Vitis* genotypes. *Plan. Theory* 9:174. doi: 10.3390/plants9020174
- Marques da Silva, J., and Utkin, A. B. (2018). Application of laser-induced fluorescence in functional studies of photosynthetic biofilms. *PRO* 6:227. doi: 10.3390/pr6110227
- Martins, A., and Gonçalves, E. (2015). “Grapevine breeding programmes in Portugal,” in *Grapevine breeding programs for the wine industry*. ed. A. G. Reynolds (Amsterdam, Netherlands: Elsevier Ltd.).
- Mateo, A., Baraldi, R., Berton, A., Cesaraccio, C., Di Gennaro, S. F., Duce, P., et al. (2018). Estimation of water stress in grapevines using proximal and remote sensing methods. *Remote Sens.* 10, 1–16. doi: 10.3390/rs10010114
- Matthews, J. S. A., and Lawson, T. (2019). Climate change and stomatal physiology. *Annu. Plant Rev.* 2, 713–752. doi: 10.1002/9781119312994.apr0667
- McAusland, L., Atkinson, J. A., Lawson, T., and Murchie, E. H. (2019). High throughput procedure utilising chlorophyll fluorescence imaging to phenotype dynamic photosynthesis and photoprotection in leaves under controlled gaseous conditions. *Plant Methods* 15:109. doi: 10.1186/s13007-019-0485-x
- Merloni, E., Camanzi, L., Mulazzani, L., and Malorgio, G. (2018). Adaptive capacity to climate change in the wine industry: A Bayesian network approach. *Wine Econ. Policy* 7, 165–177. doi: 10.1016/j.wep.2018.11.002
- Merlot, S., Mustilli, A. C., Genty, B., North, H., Lefebvre, V., Sotta, B., et al. (2002). Use of infrared thermal imaging to isolate *Arabidopsis* mutants defective in stomatal regulation. *Plant J.* 30, 601–609. doi: 10.1046/j.1365-3113X.2002.01322.x
- Milella, A., Marani, R., Petitti, A., and Reina, G. (2019). In-field high throughput grapevine phenotyping with a consumer-grade depth camera. *Comput. Electron. Agric.* 156, 293–306. doi: 10.1016/j.compag.2018.11.026
- Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends Plant Sci.* 11, 15–19. doi: 10.1016/j.tplants.2005.11.002
- Möller, M., Alchanatis, V., Cohen, Y., Meron, M., Tsipris, J., Naor, A., et al. (2007). Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. *J. Exp. Bot.* 58, 827–838. doi: 10.1093/jxb/erl115
- Moreira, F. E., Oliveira, H. R., Volenec, J. J., Rainey, K. M., and Brito, L. F. (2020). Integrating high-throughput Phenotyping and statistical genomic methods to genetically improve longitudinal traits in crops. *Front. Plant Sci.* 11:681. doi: 10.3389/fpls.2020.00681
- Mullineaux, P. M., Karpinski, S., and Baker, N. R. (2006). Spatial dependence for hydrogen peroxide-directed signaling in light-stressed plants. *Plant Physiol.* 141, 346–350. doi: 10.1104/pp.106.078162
- Naulleau, A., Gary, C., Prévot, L., and Hossard, L. (2021). Evaluating strategies for adaptation to climate change in grapevine production—a systematic review. *Front. Plant Sci.* 11:607859. doi: 10.3389/fpls.2020.607859
- Nogales-Bueno, J., Baca-Bocanegra, B., Rodríguez-Pulido, F. J., Heredia, F. J., and Hernández-Hierro, J. M. (2015). Use of near infrared hyperspectral tools for the screening of extractable polyphenols in red grape skins. *Food Chem.* 172, 559–564. doi: 10.1016/j.foodchem.2014.09.112
- Nuske, S., Achar, S., Gupta, K., Narasimhan, S., and Singh, S. (2011). Visual yield estimation in vineyards: experiments with different varieties and calibration procedures. Carnegie Mellon University. 11–39.
- Oakey, H., Verbyla, A., Pitchford, W., Cullis, B., and Kuchel, H. (2006). Joint modeling of additive and non-additive genetic line effects in single field trials. *Theor. Appl. Genet.* 113, 809–819. doi: 10.1007/s00122-006-0333-z
- OIV (2017). Resolution OIV-VITI 564A-2019. International Organisation of Vine and Wine; Process for the clonal selection of vines. Available at: <http://www.oiv.int/public/medias/5382/oiv-viti-564a-2017-en.pdf> (Accessed August, 2021).
- OIV (2019). Resolution OIV-VITI 564B-2019. International Organisation of Vine and Wine; Process for the recovery and conservation of the intravarietal diversity and the polyclonal selection of the vine in grape varieties with wide genetic variability. Available at: <http://www.oiv.int/public/medias/6939/oiv-viti-564b-2019-en.pdf> (Accessed August, 2021).
- OIV (2020). State of the World Vitivinicultural Sector in 2019. International Organisation of Vine and Wine. Available at: <http://www.oiv.int/public/medias/7298/oiv-state-of-the-vitivinicultural-sector-in-2019.pdf> (Accessed August, 2021).
- Ollat, N., Peccoux, A., Papura, D., Esmenjaud, D., Marguerit, E., Tandonnet, J. P., et al. (2016). “Rootstocks as a component of adaptation to environment,” in *Grapevine in a changing environment: A molecular and Ecophysiological perspective*. 1st Edn. eds. H. Gerós, M. M. Chaves, H. Medrano and S. Delrot (West Sussex, UK: John Wiley & Sons), 68–108.
- Omasa, K., and Takayama, K. (2003). Simultaneous measurement of Stomatal conductance, non-photochemical quenching, and photochemical yield of photosystem II in intact leaves by thermal and chlorophyll fluorescence imaging. *Plant Cell Physiol.* 44, 1290–1300. doi: 10.1093/pcp/pcg165
- Osório, J., Osório, M. L., Correia, P. J., de Varennes, A., and Pestana, M. (2014). Chlorophyll fluorescence imaging as a tool to understand the impact of iron deficiency and resupply on photosynthetic performance of strawberry plants. *Sci. Hortic.* 165, 148–155. doi: 10.1016/j.scienta.2013.10.042
- Pajares, G., García-Santillán, I., Campos, Y., Montalvo, M., Guerrero, J. M., Emmi, L., et al. (2016). Machine-vision systems selection for agricultural vehicles: A guide. *J. Imaging* 2:34. doi: 10.3390/jimaging2040034
- Palacios, F., Diago, M. P., and Tardaguila, J. A. (2019). Non-invasive method based on computer vision for grapevine cluster compactness assessment using a mobile sensing platform under field conditions. *Sensors* 19:3799. doi: 10.3390/s19173799
- Pantin, F., Monnet, F., Jannaud, D., Costa, J. M., Renaud, J., Muller, B., et al. (2013). The dual effect of abscisic acid on stomata. *New Phytol.* 197, 65–72. doi: 10.1111/nph.12013
- Parker, A. K., De Cortázar-Atauri, I. G., Van Leeuwen, C., and Chuine, I. (2011). General phenological model to characterise the timing of flowering and veraison of *Vitis vinifera* L. *Aust. J. Grape Wine Res.* 17, 206–216. doi: 10.1111/j.1755-0238.2011.00140.x
- Pavlousek, P. (2011). Evaluation of drought tolerance of new grapevine rootstock hybrids. *J. Environ. Biol.* 32, 543–549.
- Petrie, P. R., Wang, Y., Liu, S., Lam, S., Whitty, M. A., and Skewes, M. A. (2019). The accuracy and utility of a low cost thermal camera and smartphone-based system to assess grapevine water status. *Biosyst. Eng.* 179, 126–139. doi: 10.1016/j.biosystemseng.2019.01.002
- Piepho, H. P., and Edmondson, R. N. (2018). A tutorial on the statistical analysis of factorial experiments with qualitative and quantitative treatment factor levels. *J. Agron. Crop Sci.* 204, 429–455. doi: 10.1111/jac.12267
- Piepho, H. P., and Möhring, J. (2007). Computing heritability and selection response from unbalanced plant breeding trials. *Genetics* 177, 1881–1888. doi: 10.1534/genetics.107.074229
- Pieruschka, R., and Schurr, U. (2019). Plant phenotyping: past, present, and future. *Plant Phenomics* 2019:7507131. doi: 10.34133/2019/7507131
- Prinsi, B., Simeoni, F., Galbiati, M., Meggio, F., Tonelli, C., Scienza, A., et al. (2021). Grapevine rootstocks differently affect physiological and molecular responses of the scion under water deficit condition. *Agronomy* 11:289. doi: 10.3390/agronomy11020289
- Qiu, R., Wei, S., Zhang, M., Li, H., Sun, H., Liu, G., et al. (2018). Sensors for measuring plant phenotyping: a review. *Int. J. Agric. Biol. Eng.* 11, 1–17. doi: 10.25165/ij.ijabe.20181102.2696

- Raskin, I., and Ladyman, J. A. R. (1988). Isolation and characterization of a barley mutant with abscisic-acid-insensitive stomata. *Planta* 173, 73–78. doi: 10.1007/BF00394490
- Reynolds, D., Baret, F., Welcker, C., Bostrom, A., Ball, J., Cellini, F., et al. (2019). What is cost-efficient phenotyping? Optimizing costs for different scenarios. *Plant Sci.* 282, 14–22. doi: 10.1016/j.plantsci.2018.06.015
- Rienth, M., Vigneron, N., Darriet, P., Sweetman, C., Burbidge, C., Bonghi, C., et al. (2021). Grape berry secondary metabolites and their modulation by abiotic factors in a climate change scenario—A review. *Front. Plant Sci.* 12:643258. doi: 10.3389/fpls.2021.643258
- Roitsch, T., Cabrera-Bosquet, L., Fournier, A., Ghamkhar, K., Jiménez-Berni, J., Pinto, F., et al. (2019). Review: new sensors and data-driven approaches—A path to next generation phenomics. *Plant Sci.* 282, 2–10. doi: 10.1016/j.plantsci.2019.01.011
- Rosdeutscher, L., Edwards, E., Cookson, S. J., Barrieu, F., Gambetta, G. A., Delrot, S., et al. (2016). ABA-mediated responses to water deficit separate grapevine genotypes by their genetic background. *BMC Plant Biol.* 16:91. doi: 10.1186/s12870-016-0778-4
- Sampaio Filho, I. D. J., Jardine, K. J., de Oliveira, R. C. A., Gimenez, B. O., Cobello, L. O., Piva, L. R. D. O., et al. (2018). Below versus above ground plant sources of abscisic acid (ABA) at the heart of tropical forest response to warming. *Int. J. Mol. Sci.* 19:2023. doi: 10.3390/ijms19072023
- Sánchez-Moreiras, A. M., Graña, E., Reigosa, M. J., and Araniti, F. (2020). Imaging of chlorophyll a fluorescence in natural compound-induced stress detection. *Front. Plant Sci.* 11:583590. doi: 10.3389/fpls.2020.583590
- Santesteban, L. G., Miranda, C., Marín, D., Sesma, B., Intrigliolo, D. S., Mirás-Avalos, J. M., et al. (2019). Discrimination ability of leaf and stem water potential at different times of the day through a meta-analysis in grapevine (*Vitis vinifera* L.). *Agric. Water Manag.* 221, 202–210. doi: 10.1016/j.agwat.2019.04.020
- Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L. T., Correia, C., et al. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Appl. Sci.* 10:3092. doi: 10.3390/app10093092
- Schieffelin, J. (2015). Molecular phenotyping of plant single cell-types enhances forward genetic analyses. *Front. Plant Sci.* 6:509. doi: 10.3389/fpls.2015.00509
- Sen, I., Ozturk, B., Tokatli, F., and Ozen, B. (2016). Combination of visible and mid-infrared spectra for the prediction of chemical parameters of wines. *Talanta* 161, 130–137. doi: 10.1016/j.talanta.2016.08.057
- Siebers, M. H., Edwards, E. J., Jimenez-Berni, J. A., Thomas, M. R., Salim, M., et al. (2018). Fast phenomics in vineyards: development of Grover, the grapevine rover, and LiDAR for assessing grapevine traits in the field. *Sensors* 18:2924. doi: 10.3390/s18092924
- Simonneau, T., Lebon, E., Coupel-Ledru, A., Marguerit, E., Rosdeutscher, L., and Ollat, N. (2017). Adapting plant material to face water stress in vineyards: which physiological targets for an optimal control of plant water status? *OENO One* 51, 167–179. doi: 10.20870/oeno-one.2017.51.2.1870
- Sims, D., and Gamon, J. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ.* 81, 337–354. doi: 10.1016/S0034-4257(02)00010-X
- Singh, D., Wang, X., Kumar, U., Gao, L., Noor, M., Imtiaz, M., et al. (2019). High-throughput phenotyping enabled genetic dissection of crop lodging in wheat. *Front. Plant Sci.* 10:394. doi: 10.3389/fpls.2019.00394
- Su, L., Dai, Z., Li, S., and Xin, H. (2015). A novel system for evaluating drought—cold tolerance of grapevines using chlorophyll fluorescence. *BMC Plant Biol.* 15:82. doi: 10.1186/s12870-015-0459-8
- Tagarakis, A. C., Koundouras, S., Fountas, S., and Gemtos, T. (2018). Evaluation of the use of LiDAR laser scanner to map pruning wood in vineyards and its potential for management zones delineation. *Precis. Agric.* 19, 334–347. doi: 10.1007/s11119-017-9519-4
- Tagarakis, A. C., Liakos, V., Chatzinikos, T., Koundouras, S., Fountas, S., and Gemtos, T. (2013). Using laser scanner to map pruning wood in vineyards. *Precis. Agric.* 13, 633–669. doi: 10.3920/978-90-8686-778-3_78
- Tattaris, M., Reynolds, M. P., and Chapman, S. C. (2016). A direct comparison of remote sensing approaches for high-throughput phenotyping in plant breeding. *Front. Plant Sci.* 7:1131. doi: 10.3389/fpls.2016.01131
- Tedesco, S., Pina, A., Fevèreiro, P., and Kragler, F. (2020). A phenotypic search on graft compatibility in grapevine. *Agronomy* 10:706. doi: 10.3390/Agron.10050706
- Toledo-Martín, E. M., García-García, M. C., Font, R., Moreno-Rojas, J. M., Gómez, P., Salinas-Navarro, M., et al. (2016). Application of visible/near-infrared reflectance spectroscopy for predicting internal and external quality in pepper: estimation of quality in pepper by NIR spectroscopy. *J. Sci. Food Agric.* 96, 3114–3125. doi: 10.1002/jsfa.7488
- Tomás, M., Medrano, H., Brugnoli, E., Escalona, J. M., Martorell, S., Pou, A., et al. (2014). Variability of mesophyll conductance in grapevine cultivars under water stress conditions in relation to leaf anatomy and water use efficiency. *Aust. J. Grape Wine Res.* 20, 272–280. doi: 10.1111/ajgw.12069
- Tsaftaris, S. A., Minervini, M., and Scharr, H. (2016). Machine learning for plant Phenotyping needs image processing. *Trends Plant Sci.* 21, 989–991. doi: 10.1016/j.tplants.2016.10.002
- Tsoulias, N., Paraforos, D. S., Fountas, S., and Zude-Sasse, M. (2019). Calculating the water deficit spatially using LiDAR laser scanner in an apple orchard. *Precis. Agric.* 19, 115–121. doi: 10.3920/978-90-8686-888-9_13
- Udompetakul, V., Upadhyaya, S. K., Slaughter, D., Lampinen, B., and Shackel, K. (2011). “Plant water stress detection using leaf temperature and microclimatic information,” in *American Society of Agricultural and Biological Engineers Annual International Meeting*; August 7–10, 2011; Louisville, Kentucky, USA, 198–208.
- van Leeuwen, C., and Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *J. Wine Econ.* 11, 150–167. doi: 10.1017/jwe.2015.21
- Van Leeuwen, C., and Destrac-Irvine, A. (2017). Modified grape composition under climate change conditions requires adaptations in the vineyard. *OENO One* 51, 147–154. doi: 10.20870/oeno-one.2016.0.0.1647
- Van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy* 9:514. doi: 10.3390/Agron.9090514
- Van Leeuwen, C., Roby, J. P., Alonso-Villaverde, V., and Gindro, K. (2013). Impact of clonal variability in *Vitis vinifera* cabernet franc on grape composition, wine quality, leaf blade stilbene content, and downy mildew resistance. *J. Agric. Food Chem.* 61, 19–24. doi: 10.1021/jf304687c
- Verdugo-Vásquez, N., Acevedo-Opazo, C., Valdés-Gómez, H., Ingram, B., García de Cortázar-Atauri, I., and Tisseyre, B. (2020). Towards an empirical model to estimate the spatial variability of grapevine phenology at the within field scale. *Precis. Agric.* 21, 107–130. doi: 10.1007/s11119-019-09657-7
- Victorino, G. F., Braga, R., Santos-Victor, J., and Lopes, C. M. (2020). Yield components detection and image-based indicators for non-invasive grapevine yield prediction at different phenological phases. *OENO One* 54, 833–848. doi: 10.20870/oeno-one.2020.54.4.3616
- Villalobos-Gonzalez, L., Muñoz-Araya, M., Franck, N., and Pastenes, C. (2019). Controversies in midday water potential regulation and stomatal behavior might result from the environment, genotype, and/or rootstock: evidence from Carménère and syrah grapevine varieties. *Front. Plant Sci.* 10:1522. doi: 10.3389/fpls.2019.01522
- Walter, A., Liebisch, F., and Hund, A. (2015). Plant phenotyping: from bean weighing to image analysis. *Plant Methods* 11:14. doi: 10.1186/s13007-015-0056-8
- Welham, S. J., Gogel, B. J., Smith, A. B., Thompson, R., and Cullis, B. R. (2010). A comparison of analysis methods for late-stage variety evaluation trials. *Aust. N. Z. J. Stat.* 52, 125–149. doi: 10.1111/j.1467-842X.2010.00570.x
- Wolkovich, E. M., García De Cortázar-Atauri, I., Morales-Castilla, I., Nicholas, K. A., and Lacombe, T. (2018). From pinot to Xinomavro in the world's future wine-growing regions. *Nat. Clim. Chang.* 8, 29–37. doi: 10.1038/s41558-017-0016-6
- Xue, J., and Su, B. (2017). Significant remote sensing vegetation indices: a review of developments and applications. *J. Sens.* 2017, 1–17. doi: 10.1155/2017/1353691
- Yang, W., Feng, H., Zhang, X., Zhang, J., Doonan, J. H., Batchelor, W. D., et al. (2020). Crop phenomics and high-throughput phenotyping: past decades, current challenges, and future perspectives. *Mol. Plant* 13, 187–214. doi: 10.1016/j.molp.2020.01.008

- Zarrouk, O., Brunetti, C., Egipto, R., Pinheiro, C., Genebra, T., Gori, A., et al. (2016). Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy. *Front. Plant Sci.* 7:1640. doi: 10.3389/fpls.2016.01640
- Zheng, X., Krause, J., Fischer, B., Gruna, R., Topfer, R., and Kicherer, A. (2021). “Phenoliner2.0: RGB and near-infrared (NIR) image acquisition for an efficient phenotyping in grapevine research,” in *Proceedings of the 5th International Conference On Optical Characterization of Materials*. eds. J. Beyerer and T. Längle; March 16, 2021 (Scientific Publishing: Karlsruhe, Germany), 57–67.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Carvalho, Gonçalves, Marques da Silva and Costa. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Physiological and Transcriptional Responses to Saline Irrigation of Young ‘Tempranillo’ Vines Grafted Onto Different Rootstocks

Ignacio Buesa^{1,2,3*}, Juan G. Pérez-Pérez¹, Fernando Visconti^{1,4}, Rebeka Strah^{5,6}, Diego S. Intrigliolo⁴, Luis Bonet¹, Kristina Gruden⁵, Maruša Pompe-Novak^{5,7} and Jose M. de Paz¹

OPEN ACCESS

Edited by:

Tommaso Frioni,
Catholic University of the Sacred
Heart, Italy

Reviewed by:

Michaela Griesser,
University of Natural Resources
and Life Sciences, Vienna, Austria
R. Andres Zurita-Silva,
Instituto de Investigaciones
Agropecuarias, Chile
Federico Berli,
Instituto de Biología Agrícola
de Mendoza (IBAM),
CONICET-UNCuyo, Argentina

*Correspondence:

Ignacio Buesa
igbuepue@gmail.com

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 30 January 2022

Accepted: 25 April 2022

Published: 06 June 2022

Citation:

Buesa I, Pérez-Pérez JG,
Visconti F, Strah R, Intrigliolo DS,
Bonet L, Gruden K, Pompe-Novak M
and de Paz JM (2022) Physiological
and Transcriptional Responses
to Saline Irrigation of Young
‘Tempranillo’ Vines Grafted Onto
Different Rootstocks.
Front. Plant Sci. 13:866053.
doi: 10.3389/fpls.2022.866053

¹ Instituto Valenciano de Investigaciones Agrarias, Centro para el Desarrollo de la Agricultura Sostenible, Unidad Asociada al CSIC “Riego en la Agricultura Mediterránea”, Valencia, Spain, ² Ecophysiologie et Génétique Fonctionnelle de la Vigne, Institut National de la Recherche Agronomique, Institut des Sciences de la Vigne et du Vin, Villenave d’Ornon, France, ³ Research Group on Plant Biology Under Mediterranean Conditions, Department of Biology, University of the Balearic Islands, Palma, Spain, ⁴ Centro de Investigaciones sobre Desertificación, Departamento de Ecología (CSIC, UV, GV), Valencia, Spain, ⁵ Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia, ⁶ Jožef Stefan International Postgraduate School Ljubljana, Ljubljana, Slovenia, ⁷ School for Viticulture and Enology, University of Nova Gorica, Vipava, Slovenia

The use of more salt stress-tolerant vine rootstocks can be a sustainable strategy for adapting traditional grapevine cultivars to future conditions. However, how the new M1 and M4 rootstocks perform against salinity compared to conventional ones, such as the 1103-Paulsen, had not been previously assessed under real field conditions. Therefore, a field trial was carried out in a young ‘Tempranillo’ (*Vitis vinifera* L.) vineyard grafted onto all three rootstocks under a semi-arid and hot-summer Mediterranean climate. The vines were irrigated with two kinds of water: a non-saline Control with EC of 0.8 dS m⁻¹ and a Saline treatment with 3.5 dS m⁻¹. Then, various physiological parameters were assessed in the scion, and, additionally, gene expression was studied by high throughput sequencing in leaf and berry tissues. Plant water relations evidenced the osmotic effect of water quality, but not that of the rootstock. Accordingly, leaf-level gas exchange rates were also reduced in all three rootstocks, with M1 inducing significantly lower net photosynthesis rates than 1103-Paulsen. Nevertheless, the expression of groups of genes involved in photosynthesis and amino acid metabolism pathways were not significantly and differentially expressed. The irrigation with saline water significantly increased leaf chloride contents in the scion onto the M-rootstocks, but not onto the 1103P. The limitation for leaf Cl⁻ and Na⁺ accumulation on the scion was conferred by rootstock. Few processes were differentially regulated in the scion in response to the saline treatment, mainly, in the groups of genes involved in the flavonoids and phenylpropanoids metabolic pathways. However, these transcriptomic effects were not fully reflected in grape phenolic ripeness, with M4 being the only one that did not cause reductions in these compounds in response to salinity, and 1103-Paulsen having the highest overall concentrations. These results suggest that all three rootstocks confer short-term salinity tolerance to the scion. The lower transcriptomic changes and the

lower accumulation of potentially phytotoxic ions in the scion grafted onto 1103-Paulsen compared to M-rootstocks point to the former being able to maintain this physiological response in the longer term. Further agronomic trials should be conducted to confirm these effects on vine physiology and transcriptomics in mature vineyards.

Keywords: osmotic adjustment, gas exchange, gene expression, water relations, *Vitis vinifera* L. (grapevine), salinity tolerance

INTRODUCTION

Changes in the Mediterranean and related semi-arid climates are expected shortly, leading to temperature increases and more frequent and longer drought periods (Döll, 2002). These will increase crop water demand, while simultaneously reducing the availability of quality water (Schultz, 2017). Since in most grapevine-growing regions, freshwater is a scarce resource (Medrano et al., 2015), the use of alternative waters, such as wastewaters often high in salts, will be more and more needed to mitigate drought stress (Mirás-Avalos and Intrigliolo, 2017). Besides, conventional waters, such as underground water, can indeed be of low quality due to excessive concentrations of soluble salts (Cl^- and/or Na^+), with an electrical conductivity over 3 dS m^{-1} (Pérez-Pérez et al., 2015). This lack of water quality poses a challenge to the sustainability of deficit irrigation in viticulture, as this irrigation strategy could aggravate the effects of salinity (van Leeuwen et al., 2019).

Excessive soil salinity can cause water loss, nutrient deficiency, oxidative stress, photoinhibition, growth inhibition, and induce many metabolic and transcriptomic changes leading to physiological damage (Walker et al., 1997; Kumari et al., 2015; Saha et al., 2015; Upadhyay et al., 2018; Zhou-Tsang et al., 2021). Previous studies have demonstrated that among plant responses to salinity, mechanisms that control ion uptake, transport, and balance, as well as hydric regulation, photosynthesis, cell division, osmotic adjustment, enzymatic activities, antioxidant production, stress signaling, and regulation of root barriers play critical roles in plant tolerance to salinity (Gong et al., 2011; Shahid et al., 2020; Zhou-Tsang et al., 2021).

The *Vitis vinifera* L. is a crop classified as moderately sensitive to salinity (Maas and Hoffman, 1977; Cramer et al., 2007), with a soil saturation extract electrical conductivity at 25°C yield threshold (EC_t) of 2.6 dS m^{-1} (Walker et al., 2002). The tolerance of grapevines to salinity depends on multiple factors and, particularly, on plant genetics, soil and climate characteristics, and the rate and length of the stress, to which vines are subjected (Maas and Hoffman, 1977; Zhang et al., 2002; Cramer et al., 2007; Chaves et al., 2009; Mirás-Avalos and Intrigliolo, 2017). Understanding the physiological and transcriptomic responses of grapevine to saline water is essential to prevent and mitigate potential negative effects on vine performance and grape composition (Ollat et al., 2016). Moreover, the contradictory effects of irrigation with saline or wastewater on vine performance and grape composition (Walker et al., 2004, 2007; Stevens et al., 2011; Mirás-Avalos and Intrigliolo, 2017) point toward the existence of important knowledge gaps regarding the effects of salinity and the salt

tolerance mechanisms in *Vitis* spp. (Zhou-Tsang et al., 2021). Microarray studies of pot-grown own-rooted vines of CVS ‘Cabernet Sauvignon,’ ‘Razegui,’ and ‘Shiraz’ revealed that salinity stress impaired photosynthesis and increased the expression of some transcription factors and genes related to ROS scavenging, abscisic acid, and osmoprotectants such as various sugars and proline (Cramer et al., 2007; Daldoul et al., 2010). High throughput sequencing studies of potted cv. ‘Thompson Seedless’ and cv. ‘Summer Black’ under greenhouse conditions implicated the activity of genes involved in cell wall modulation, various cation and ABC transporters, signal transduction genes, HSPs, and biotic stress-related genes (Guan et al., 2018; Das and Majumder, 2019).

The ‘Tempranillo’ cultivar has been specifically classified as moderately salt-sensitive as well, showing growth decreases attributable to osmotic effects rather than to ion-specific toxicities (Urdanoz and Aragüés, 2009). Nonetheless, since grapevine yield potential under saline conditions is related to the root-zone salinity, the plant portion that primarily deals with soil salinity is not the scion, but the rootstock. Among the characteristics of the different rootstock that contribute to enhancing grapevine tolerance to salinity, there is its ability to exclude and not transport salt to the shoots; besides, there is also the vigor it confers to the scion (Walker et al., 2002, 2014; Munns et al., 2020). Additionally, rootstock can have a great influence on stomatal regulation in response to water and salinity stress, even more than the scion itself (Lavoie-Lamoureux et al., 2017). For instance, rootstock can affect the osmotic adjustment response, which is one of the main physiological processes, whereby the vine responds to salinity (Keller, 2010; Haider et al., 2019). This consists of the active accumulation of solutes, thus increasing leaf relative water content and turgor (Barrios-Masias et al., 2018). Regarding this, several studies are reporting that the rootstocks with lower osmotic adjustment capacity are those with greater capacity to restrict the leaf accumulation of Na^+ and Cl^- , thus, preventing their possible phytotoxic effects (Stevens and Walker, 2002; Zhang et al., 2002), and minimizing their accumulation in the grape juice and wine in the long-term (Walker et al., 2004, 2014; Teakle and Tyerman, 2010).

American *Vitis* species, especially *V. rupestris*, *V. riparia*, and *V. berlandieri* are tolerant of saline and limestone soils (Williams et al., 1994; Ferlito et al., 2020). Some rootstocks derived from these species such as Ramsey (*V. champini*), 1103 Paulsen (1103P), 110 Richter, 140 Ruggeri, and 101–14 Mgt can exclude much salt (chiefly Na^+ and Cl^-) from root uptake and root-to-shoot transport (Walker et al., 2004, 2010; Gong et al., 2011). For instance, some of the most salinity-tolerant rootstocks, such as 140 Ruggeri and 1103 Paulsen, have an EC_t value of up

to 3.3 dS m^{-1} (Walker et al., 2002; Zhang et al., 2002; Tregeagle et al., 2006). Conversely, rootstocks, such as SO4 and 3309C, are characterized by being very sensitive to salinity with an EC_t value below 1.8 dS m^{-1} (Walker et al., 2010). Given the relatively narrow genetic pool within the commercial grapevine rootstocks and the significant genetic diversity of the genus *Vitis*, identifying salinity-tolerant grapevine rootstocks is a great opportunity to enhance viticulture sustainability (Schultz and Stoll, 2010). For instance, differential gene expression has been observed in potted *Vitis vinifera* L. ssp. *sylvestris* with different short-term salinity tolerance in greenhouse conditions (Askari et al., 2012). Therefore, a better understanding of the rootstock physiological, metabolomic, and transcriptomic mechanisms underlining salt stress tolerance is essential to improve breeding programs aimed at adapting to climate change (Ollat et al., 2016). In this sense, new information about salinity tolerance conferred by rootstocks is needed (Keller, 2010; Marín et al., 2021). Grapevine rootstock breeding programs, such as the one carried out by the University of Milan (Italy) with the M-series, are very promising for coping with water salinity (Meggio et al., 2014) and can benefit a lot from the results of field trials.

Therefore, the objective of the present research was to evaluate the physiology and transcriptomics underlying the performance against salinity of two new rootstocks, M1 and M4, compared to the well-known salinity-tolerant 1103P (Walker et al., 2010; Bianchi et al., 2020). In this work the experimental hypothesis was that the M-rootstocks may confer better salinity tolerance to the scion than the 1103P through enhanced uptake of salt-stress-contesting ions such as calcium, as well as vigor declining ability, in the case of the M1 (Porro et al., 2013; Vannozzi et al., 2017), and because of the leaf build-up of inorganic osmolytes and sodium-antagonists, such as potassium, in the case of the M4 (Meggio et al., 2014). In comparison to the M-rootstocks, the 1103P stands out for its ability to exclude Cl^- from uptake. Aiming at mimicking commercial conditions, the experiment was performed under field conditions and tried to isolate the salinity effect by fully irrigating the vines. Although the vineyard was under establishment, to our best knowledge, these grapevine rootstocks had not been previously tested against salinity under conditions so close to real practice. Besides, in contrast to previous comparative studies between these grapevine rootstocks in this work, all determinations were carried out directly in the scion. This was done considering that the scion is an integrator of rootstock-induced effects (Gambetta et al., 2012; Cookson et al., 2013). Finally, by assessing a young vineyard, i.e., one with a non-extensive root system, the physiological response to salinity could be studied ensuring that most of the roots were effectively under the intended salinity.

MATERIALS AND METHODS

Vineyard Site and Experimental Design

The experiment was undertaken in 2019 in a ‘Tempranillo’ (*Vitis vinifera* L.) vineyard located at the IVIA’s experimental station in Moncada, Valencia, Spain ($39^\circ 35' 12'' \text{ N}$, $0^\circ 24' 1'' \text{ W}$, and 55 m.a.s.l.). In 2017, the vines were grafted onto three

rootstocks in a nursery. The rootstocks were the M1 clone 1 ($106/8 \times V. berlandieri$), the M4 clone 1 ($41\text{B} \times V. berlandieri$) and the 1103 Paulsen clone VCR119 (*V. berlandieri* cv. ‘Resseguier’ nr. 2 $\times V. rupestris$ cv. ‘Du Lot’) (Marín et al., 2021). Vines were planted in 2018 at a spacing of $0.88 \times 2.50 \text{ m}$ and guided by a vertical trellis system in a simple ‘guyot’ cordon. As it was a vineyard under establishment, it was decided to constrain the crop load to four clusters per vine to avoid overcropping. Thus, the experimental vines had an average yield of 1.75 kg , i.e., 7.9 t/ha . There were no differences in initial shoot fruitfulness or yield at harvest among treatments.

The climate in the experimental trial was hot-summer Mediterranean (Csa) according to Köppen–Geiger (Rodríguez-Ballesteros, 2016), and semi-arid according to Thornthwaite (De Paz et al., 2004), with an average annual rainfall of 392 mm and reference evapotranspiration (ET_0) of $1,137 \text{ mm}$. The soil was classified as a Petrocalcic Calcixerept according to the Soil Taxonomy (Soil Survey Staff, 2006) with the petrocalcic horizon constraining root development lying at $0.4\text{--}0.5 \text{ m}$ depth, and with loam texture (45% sand, 36% silt, and 19% clay), high calcium carbonate equivalent (40%) and, therefore, medium-to-high active calcium carbonate equivalent (6–10%), very low organic matter content (1%), and slight-to-moderate compaction ($1.56 \pm 0.13 \text{ Mg/m}^3$ of bulk density).

The vineyard was drip irrigated at 100% of crop evapotranspiration (ET_c), based on the crop coefficients reported for ‘Tempranillo’ vines by López-Urrea et al. (2012), and the ET_0 calculated with the Penman–Monteith equation (Allen et al., 1998). Weather conditions were recorded at an automated agro-meteorological station 400 m away from the plot. Importantly, no leaching fraction was adopted. Irrigation was applied through 2 L h^{-1} pressure-compensated emitters spaced at 0.88 m along a single drip line and it began 50 days after budburst, i.e., the day of the year (DOY) 133. This time was selected because then, was when midday Ψ_{stem} values reached -0.8 MPa . As a result, the vine water requirements were met by irrigation events 2-to-3 h long 3-to-5 days a week. Mineral nutrients were provided along the season by fertigation up to the cumulated rates of 30, 20, and 60 kg ha^{-1} of, respectively, N, P_2O_5 , and K_2O .

Two irrigation waters were generated by dissolving adequate amounts of reagent grade calcium and sodium chlorides in partially desalinated water. Each irrigation water featured a different electrical conductivity at 25°C (EC_{25}), but a common sodium-adsorption ratio (SAR) of $5\text{--}7 (\text{mmol L}^{-1})^{1/2}$. This way a sodification effect was avoided, which would have shown up as differences in soil structural stability and nutrient availability between the control and saline water, thus, interfering with the salinity treatment. The control water featured an EC_{25} of 0.8 dS m^{-1} with 2.7, 0.3, and 3.3 mmol L^{-1} of, respectively, Na^+ , Ca^{2+} , and Cl^- , whereas the Saline water featured an EC_{25} of 3.5 dS m^{-1} with 12.7, 6.5, and 25.7 mmol L^{-1} of, respectively, Na^+ , Ca^{2+} , and Cl^- . During the experiment, the soil on the alleyways was tilled and spontaneous weeds in the vine row were controlled by glyphosate herbicide applications.

The experiment followed a complete factorial design to assess the performance of the three rootstocks under the two water

quality levels (control and salinity). All treatments, i.e., each combination of rootstock and water quality, had three replicates, thus, resulting in 18 subplots of 10 vines each. The subplots were randomly distributed throughout the vineyard. For the determination of water relations and the measurement of gas exchange parameters, as well as for the transcriptomics, the experimental unit (biological replicate) was the 8th vine of each subplot. For the determination of the leaf nutritional status, leaf area index, and grape quality, the experimental unit consisted of the 8 vines from the 2nd to the 9th in each subplot, thus, leaving the 1st and 10th as guards.

Field Measurements and Laboratory Determinations

All field measurements and samplings were performed after more than 100 days since the treatments had begun (after 259 ± 2 mm of cumulated irrigation was applied). Specifically, the vine water relations, the gas exchange measurements, and the leaf and berry samplings were performed, on DOY 233. According to the phenological growth stages in the BBCH-scale (Lorenz et al., 1995), the vines on DOY 233 were at stage code 89, which means berries are ripe for harvesting. Total leaf area determinations and harvest were performed, respectively, on DOY 234 and 237. Each laboratory sample was analyzed in duplicate.

Vine water relations were determined in each biological replicate using a pressure chamber (Model 600, PMS Instruments Company, Albany, OR, United States) at pre-dawn ($\Psi_{\text{pre-dawn}}$) and midday. At midday, both well-exposed-to-sunlight adult leaves (Ψ_{leaf}) and bag-covered leaves (Ψ_{stem}) were measured (Santesteban et al., 2019). After the Ψ_{leaf} measurement, this leaf was frozen and stored at -20°C for determination of the leaf osmotic potential (Ψ_π). Another leaf from the same shoot was collected and re-hydrated for determination of the leaf osmotic potential at full turgor (Ψ_π^{100}). Both Ψ_π and Ψ_π^{100} were measured with a digital osmometer (Wescor, Logan, UT, United States). The leaf turgor potential (Ψ_p) was calculated as the difference between Ψ_{leaf} and Ψ_π .

The gas exchange measurements were carried out on two fully exposed and expanded young leaves of each biological replicate using an infrared open gas exchange analyzer system (Li-6400xt, Li-COR, Lincoln, NE, United States). The stomatal conductance (g_s), net photosynthesis (A_N), and intrinsic water use efficiency ($\text{WUE}_i = A_N/g_s$) were measured between 8:00 and 9:30 solar time. The CO_2 concentration inside the chamber was $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$, and an airflow of $500 \mu\text{mol min}^{-1}$ was applied. The chamber had an area of 6 cm^2 exposed to environmental light radiation, with PAR always of $1,500 \pm 2 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The relative humidity and vapor pressure deficit inside the chamber were $30 \pm 2\%$ and $2.25 \pm 0.3 \text{ kPa}$.

Leaf nutritional status was determined from samples of 20 fully expanded mature leaves per subplot. Leaves were thoroughly washed with tap water, rinsed with deionized water, and oven-dried at 65°C for 48 h. Next, they were grounded with a disk mill to pass a $200\text{-}\mu\text{m}$ mesh sieve and analyzed for the determination of various macro- and micronutrients. The concentrations of K, Ca, Mg, and Na was determined in the extracts obtained by digestion with $\text{HNO}_3:\text{HClO}_4$ (2:1)

using inductively coupled plasma atomic emission spectrometry (ICP-AES) in an iCAP series 6500 (Thermo Fisher Scientific, Franklin, MA, United States). The total N and C contents were determined by dry combustion with, final N_2 and CO_2 measurements (Horneck and Miller, 1998), respectively, using a TruSpec CHNS elemental analyzer (LECO TruSpec Micro Series, St. Joseph, MI, United States). The chloride content was determined in the aqueous extracts obtained by shaking the dried leaf material with deionized water ($\text{EC}_{25} < 1 \mu\text{S/cm}$) for two h by ion chromatography (IC) using an 850 professional IC (Metrohm, Herisau, Switzerland).

The total leaf area per vine was estimated at each biological replicate from allometric relations between shoot length (x , cm) and leaf area per shoot (y , cm^2) measured with an LI-3100 area meter (LI-COR Biosciences, Lincoln, NE, United States), separating main and lateral shoot ($y = 17.647 x$, $R^2 = 0.98^{***}$ and $y = 14.952 x$, $R^2 = 0.99^{***}$, respectively). The leaf area index (LAI) was calculated as the total leaf area per unit of ground surface area.

The berry weight and must composition were determined from 200 randomly-taken berries per subplot. The berries were crushed and hand-pressed through a metal screen filter and the must characteristics, including total soluble solids content (TSS), pH, total titratable acidity (TA), and anthocyanins and polyphenols content, were determined according to reference analysis methods (OIV, 1990).

Common Data Analyses

Two-way analysis of variance (ANOVA) was used to assess the effects of both factors, rootstock (R) and water quality (WQ), along with its interactions ($R \times WQ$), on the vine water relations, leaf gas exchange, leaf nutrient contents, vine performance, and berry composition. A significant interaction between factors in a two-way ANOVA means that the effects of the factors significantly change in magnitude or direction depending on the levels of the other factor (Snedecor and Cochran, 1989). Therefore, following the two-way ANOVAs, if significant main effects were obtained ($p < 0.05$), but significant interactions between R and WQ were not, the group means were compared using the *post hoc* Duncan test. The ANOVAs and *post hoc* tests were carried out using the Statgraphics Centurion XVI package (version 16.0.07) (Statgraphics Technologies, The Plains, VA, United States). Additionally, regressions were calculated using SigmaPlot (version 11.0) (Systat Software, San Jose, CA, United States).

RNA Extraction and Sequencing

On DOY 233, immediately after the water relations and gas exchange measurements, one sample of leaves and another one of berries were collected from each biological replicate, thus, making 18 samples in total from each plant organ. Three fully expanded young leaves per plant, from the secondary shoots, and twenty berries were cleaned with a cloth and distilled water before being cut. Leaf samples were wrapped in aluminum foil after removing the petiole. Both leaf and berry samples were immediately frozen in liquid nitrogen at the field. Afterward, samples were stored at -80°C until preparation.

Total RNA was extracted from the samples using an optimized cetyltrimethylammonium bromide (CTAB) method (adapted from Carra et al., 2007), combined with RNA purification on Zymo-Spin Columns (Direct-zol RNA MiniPrep Plus kit, Zymo Research, Irvine, CA, United States). About 50 mg of frozen and powdered plant material was further homogenized with steel beads for 10 min at maximum speed in 800 μ L CTAB buffer [Tris-HCl 100 mM, NaCl 2 M, EDTA 25 mM, CTAB 2.0% (w/v), PVP40 2.5% (w/v), and β -mercaptoethanol 2% (v/v), pH = 8] using TissueLyser (Qiagen, Hilden Germany). After the addition of an equal volume of chloroform-isoamyl alcohol 24:1, the sample was vortexed and centrifuged for 10 min at 10,000 g and 4°C. The upper aqueous phase was recovered, to which 1.5 volume of pure ethanol was added. After a 30 min precipitation at 4°C, the mixture was transferred into Zymo-Spin Columns. The RNA was further purified according to the manufacturer's instructions, with an additional washing step and a second prewashing step added to the beginning of the purification process. To elute the RNA, 30 μ L of preheated (80°C) DNase/RNase-free water was added to the column and incubated for 5 min at room temperature, before 1 min centrifugation at 14,000 g. The elution step was repeated. Isolated RNA was subjected to DNase digestion (DNase I Set, Zymo Research, Irvine, CA, United States) and cleaned up using the RNA Clean & Concentrator kit (Zymo Research, Irvine, CA, United States). RNA concentration, integrity, and purity were assessed using 2100 Bioanalyzer and RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, United States). At this point, one leaf sample from the M4 salinity treated group was excluded from further analysis due to insufficient quality. Library preparation for mRNA Illumina HiSeq 4000 sequencing, as well as preprocessing to remove adapter sequences and low-quality reads were provided by Novogene (Hong Kong).

RNA-Seq Data Analysis

The obtained 150 bp paired-end reads were trimmed to remove low-quality bases (Phred < 20), clipped to remove remaining adapter sequences, and mapped to the 12X.2 version of the PN40024 grapevine reference genome (Canaguier et al., 2017) using "CLC Genomics Workbench 12.0" (Qiagen, Hilden Germany), with the following parameters: mismatch cost 2, insertion or deletion cost 3, length fraction 1, similarity fraction 0.95, and a maximum number of hits for a read 1. The reads were annotated using the VCost.v2 annotation. Raw counts of transcripts were exported and deposited to ENA (European Nucleotide Archive) under project accession number PRJEB44658.

Normalization of the raw counts and differential expression analysis was performed in "R v3.6.3" (R Core Team, 2017), using the *limma* package v3.42.2 (Ritchie et al., 2015) with the method previously described by Dermastia et al. (2021). In short, mRNA counts with a baseline expression level of at least 50 reads mapped in at least three samples were TMM-normalized in edgeR v3.28.1 (Robinson et al., 2009) and transformed using voom (Law et al., 2014). Principal component analysis (PCA) and hierarchical clustering analysis were performed on the resulting normalized counts. PCA was performed with the *pc* package

and hierarchical clustering analysis was performed using the "pheatmap package v 1.0.12," applying 1-Pearson correlation as distance measure and Complete Linkage as the linkage method. Differential expression was obtained by contrasts. Gene Set Enrichment Analysis (GSEA) was performed as described by Subramanian et al. (2005) on normalized log-transformed expression data. Results with a false discovery rate FDR $q < 0.25$ were considered statistically significant.

Targeted Gene Expression Analysis by qPCR

Differential expression of three genes, *NCED1* (Vitvi19g01356), *MAPK2* (Vitvi16g01160), *LOX* (Vitvi06g00158), and *UBI_CF* (Vitvi19g00744) as a reference gene was confirmed by qPCR. The primers and probes used are listed in **Supplementary Table 1**. Reverse transcription was performed with the High-Capacity RNA-to-cDNA™ kit (Applied Biosystems, Waltham, MA, United States). Power SYBR™ Green PCR Master Mix was used for all assays. The following thermal cycle conditions were applied for PCR: 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min; and a climb in increments of 0.05°C from 60 to 95°C for the high-resolution melting curve. The Cq values were used for relative calculation of the initial target number from a serial dilution curve using quantGenius (Baebler et al., 2017). Then, the normalized logFC values were correlated to the values obtained from the RNA-Seq analysis by Pearson correlation coefficient.

RESULTS

Vine Physiology and Nutritional Status

The experimental season was warmer and drier than average. From DOY 1 to 233, the ET_o and rainfall were 901 and 126 mm, respectively. All rainfall events greater than 10 mm occurred

TABLE 1 | Significance of the factor effects in the two-way ANOVAs carried out for water relations and gas exchange parameters assessed in the Tempranillo cv. vines grafted onto M1, M4, and 1103-Paulsen rootstocks.

Type of parameter	Parameter	Factors		Interaction
		Rootstock	Water Quality	R × WQ
Water relations	$\Psi_{\text{pre-dawn}}$	0.33	<0.001	0.44
	Ψ_{stem}	0.06	<0.001	0.62
	Ψ_{leaf}	0.10	0.19	0.97
	Ψ_{π}	0.60	0.03	0.46
	Ψ_p	0.25	0.37	0.88
	$\Psi_{\pi 100}$	0.29	0.02	0.86
Gas exchange	A_N	<0.01	<0.001	0.17
	g_s	0.23	0.02	0.37
	WUE_i	0.25	0.07	0.67

$\Psi_{\text{pre-dawn}}$, pre-dawn leaf water potential; Ψ_{stem} , midday stem water potential; Ψ_{leaf} , midday leaf water potential; Ψ_{π} , leaf osmotic potential; Ψ_p , leaf turgor potential; $\Psi_{\pi 100}$, leaf osmotic potential at full turgor; A_N , net photosynthesis; g_s , stomatal conductance; WUE_i , intrinsic water use efficiency. Significance of effects in bold denotes statistically significant differences at $p < 0.05$.

before the start of irrigation (DOY 133). On DOY 233, when vine water relations and leaf gas exchange were measured and the berry and leaf samples were collected, the average air temperature was 23.6°C and the relative humidity was 70%. On that day an ET_o of 5 mm was recorded.

In general, the water relations of grapevine cv. ‘Tempranillo’ was significantly affected only by water quality (WQ) (Table 1), so water potential values are plotted by water quality treatment (Figure 1). According to the $\Psi_{pre-dawn}$ and Ψ_{stem} measurements, the WQ exerted a significant effect on the vine water status at both maximum hydration and maximum water demand with no differences among rootstocks (Figure 1).

Specifically, the vines from the saline treatments exhibited more negative values than the controls. These differences were -0.12 and -0.17 MPa on average for, respectively, $\Psi_{pre-dawn}$ and Ψ_{stem} . Therefore, the effects of WQ on the water status at the time of maximum hydration ($\Psi_{pre-dawn}$), were fairly maintained at the time of maximum evaporative demand (Ψ_{stem}).

According to the Ψ_{π} and Ψ_{π}^{100} measurements, neither the R nor the $R \times WQ$ had significant effects on the osmotic potential (Figure 1). Despite this, the vines from the saline treatments exhibited significantly more negative values than the controls. These differences were -0.16 MPa on average for both Ψ_{π} and

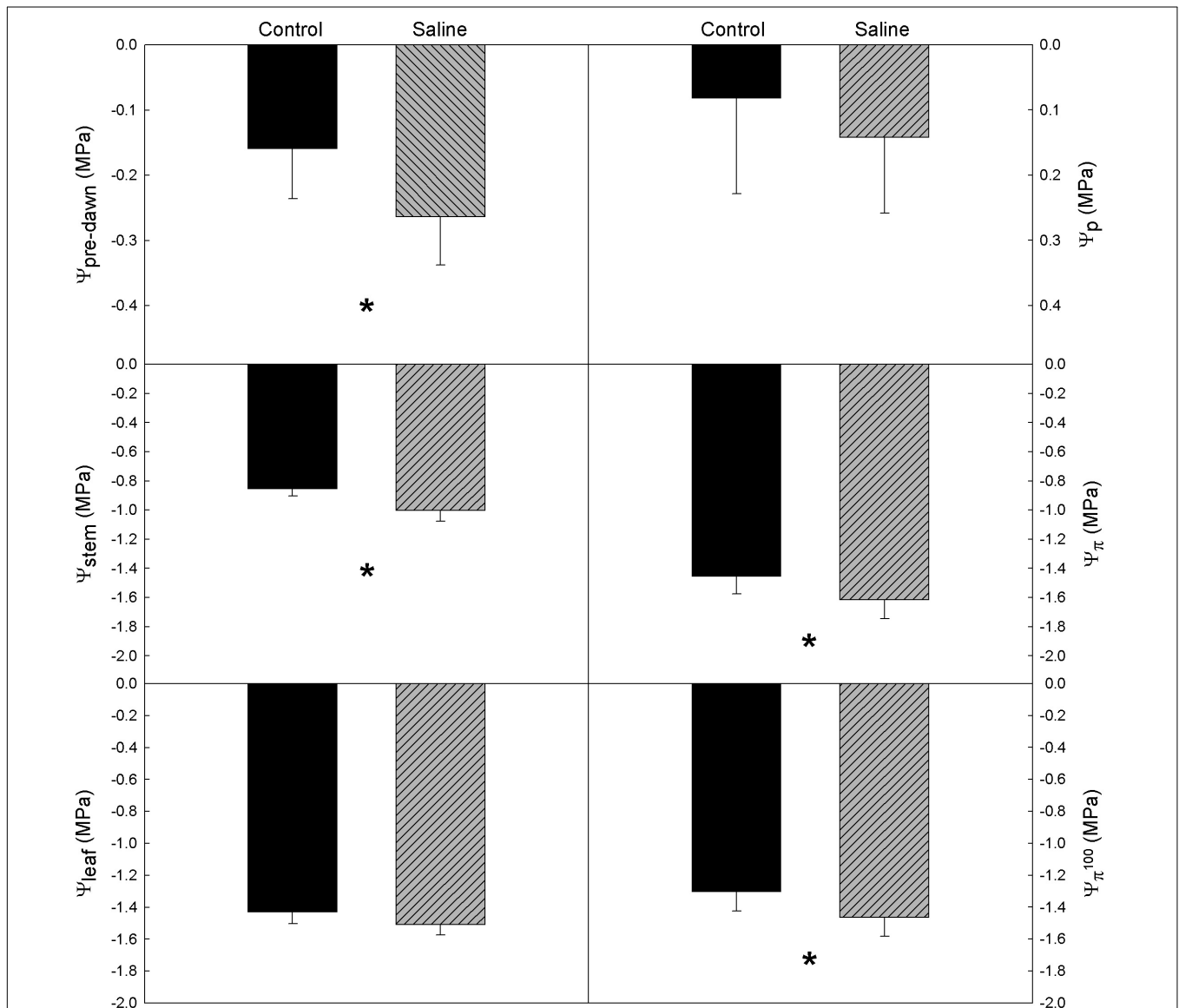


FIGURE 1 | Average values of vine water relations in a Tempranillo vineyard grafted onto M1, M4, and 1103-Paulsen (1P) rootstocks subjected to different water quality (C, control and S, saline irrigation) on DOY 233 of 2019 in Valencia, Spain. $\Psi_{pre-dawn}$, pre-dawn leaf water potential; Ψ_{stem} , midday stem water potential; Ψ_{leaf} , midday leaf water potential; Ψ_p , leaf turgor potential; Ψ_{π} , leaf osmotic potential; Ψ_{π}^{100} , leaf osmotic potential at full turgor. Data are averages and standard errors of 9 measurements per water quality. Within each parameter, an asterisk denotes significant differences between treatments at $p < 0.05$ (Duncan test).

Ψ_{π}^{100} . Both the Ψ_{leaf} and Ψ_p were unaffected by either WQ, R, or $R \times WQ$.

Regarding gas exchange parameters, both net photosynthesis rate (A_N) and leaf stomatal conductance (g_s) was significantly affected by WQ, and A_N also by R (Table 1), whereas the $R \times WQ$ interactions were non-significant. Specifically, the vines from the Saline treatments presented lower values than the controls for both parameters with an average A_N value of 14.3 and 17.2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively, and with average g_s values of 0.362 and 0.493 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$. Despite these differences in carbon

assimilation and stomatal conductance rates, no significant differences in intrinsic water use efficiency (WUE_i) in response to WQ were observed. Moreover, net photosynthetic rates of vines on 1103P were significantly higher than those on M1 (Figure 2).

The LAI was significantly affected by WQ (Table 2) due to reductions in the leaf area of lateral shoots (data not shown). Overall, the Saline treatments reduced the LAI per vine by 15% compared to the controls. This decreasing effect of WQ on the LAI was observed on the vines grafted onto the M-series rootstocks, mainly onto the M1. The concentrations

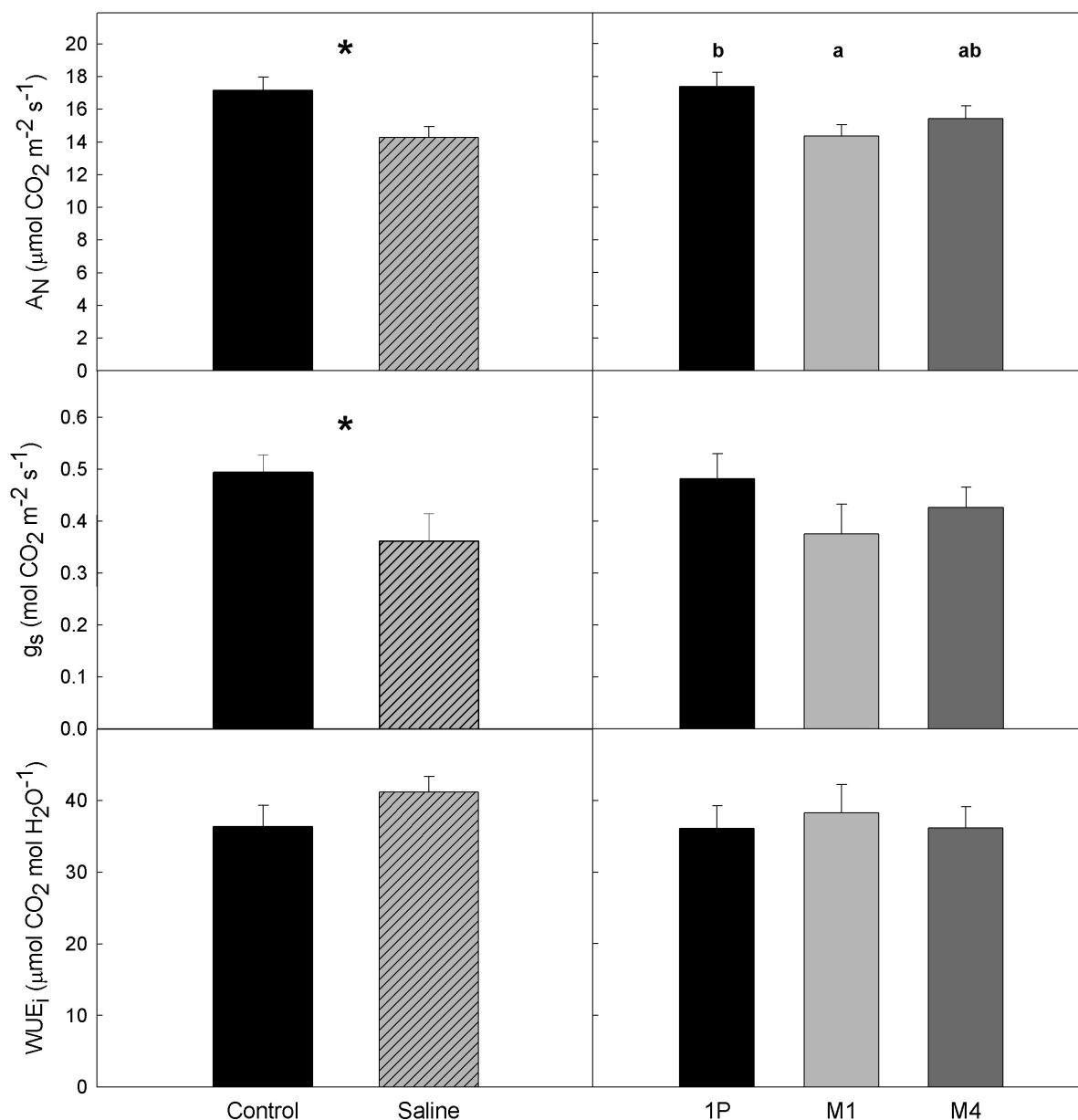


FIGURE 2 | Average values of gas exchange parameters in a Tempranillo vineyard grafted onto M1, M4, and 1103-Paulsen (1P) rootstocks subjected to different water quality (C, control and S, saline irrigation) on DOY 233 of 2019 in Valencia, Spain. A_N , net photosynthesis; g_s , stomatal conductance; WUE_i , intrinsic water use efficiency. Data are averages and standard errors of 18 and 12 measurements per water quality and rootstock, respectively. Within each parameter, asterisks or letters denote significant differences between water quality treatments or rootstocks at $p < 0.05$ (Duncan test), respectively.

TABLE 2 | Leaf area index (LAI) and leaf nutritional status in leaf blades from *Vitis vinifera* (L.) cv. Tempranillo grafted onto M1, M4 and 1103-Paulsen (1P) rootstocks subjected to different water quality (C, control and S, saline irrigation) on DOY 233 of 2019 in Valencia, Spain.

Factors	Treatment	LAI (m ² m ⁻²)	N (g 100g ⁻¹)	Cl (g 100g ⁻¹)	Ca (g 100g ⁻¹)	K (g 100g ⁻¹)	Na (g 100g ⁻¹)	Mg (g 100g ⁻¹)	K/Ca	K/Na
R	1P	1.8	2.26b	14.9a	2.01a	0.73	0.003a	0.41ab	0.37b	353.0b
	M1	1.8	2.12ab	26.9b	2.36b	0.65	0.004b	0.39a	0.28a	185.5a
	M4	1.9	2.07a	24.7b	1.94a	0.66	0.003a	0.46b	0.35ab	260.8ab
WQ	Control	2.0b	2.15	13.4a	1.93a	0.74b	0.003	0.40a	0.40	287.7a
	Saline	1.7a	2.15	30.8b	2.28b	0.61a	0.003	0.44b	0.44	245.1b
Interaction R × WQ	1P C	1.8	2.33	10.9	1.9	0.77	0.004abc	0.40	0.42	313.7
	1P S	1.8	2.19	18.9	2.1	0.68	0.002a	0.42	0.32	392.3
	M1 C	2.1	2.08	15.8	2.2	0.72	0.004bc	0.36	0.33	235.3
	M1 S	1.5	2.16	37.9	2.5	0.59	0.005c	0.43	0.24	135.6
	M4 C	2.0	2.04	13.5	1.7	0.74	0.003ab	0.44	0.44	314.2
	M4 S	1.8	2.10	35.8	2.2	0.58	0.003abc	0.48	0.27	207.3
Rootstock		0.89	0.04	<0.01	<0.001	0.25	0.02	0.05	0.04	0.05
Water Quality		0.03	0.99	<0.001	<0.001	<0.01	0.98	0.04	<0.001	0.42
R × WQ		0.08	0.33	0.09	0.49	0.72	0.05	0.61	0.49	0.27

Data are averages of 6, 9, and 3 determinations per rootstock, water quality and rootstock per water quality respectively. For each parameter, letters denote significant differences between treatments at $p < 0.05$ (Duncan test). The statistical significance effect of the rootstock (R), water quality (WQ) and their interaction are also indicated by means of the p -values from the ANOVAs. Significance of effects in bold denotes statistically significant differences at $p < 0.05$.

of the macro- and micronutrients in the vine leaves were, overall, significantly affected by both WQ and R, and even by the $R \times WQ$ interaction (Table 2), which points toward an interesting rootstock salt-stress modulating effect. On the one hand, the leaf concentrations of Cl^- , Ca^{2+} , K^+ , and Mg^{2+} depended on WQ, while N and Na^+ did not. On the other hand, the leaf concentrations of N, Cl^- , Ca^{2+} , Na^+ , and Mg^{2+} depended on R, while K^+ did not.

Nitrogen was significantly higher in the vines grafted onto the 1103P than in those grafted onto the M4 (Table 2). Specifically, the Cl^- concentration in the leaves increased 2.3-fold on average from the controls to the saline treatments. Interestingly, this increase in leaf Cl^- concentration from the controls to the saline treatments was significant in the M-series rootstocks, but not in the 1103P. The Ca^{2+} concentration in the leaves also increased significantly from the controls to the saline treatments and, similarly to Cl^- , more markedly onto the M-series than onto the 1103P (Table 2). Regarding the leaf K^+ concentrations, the effect of WQ was also significant, leading to lower K^+ concentrations from the controls to the saline treatments. Regarding leaf Na^+ , there were no significant differences in the concentrations in response to WQ, but there were depending on the rootstock and, interestingly enough, depending on the $R \times WQ$ interaction. Specifically, the M1 tended to accumulate Na^+ in the leaves in response to the Saline treatments, which is an effect not observed for 1103P or M4 (Table 2). Thus, the M1 showed the lowest K^+/Ca^{2+} ratio and the K^+/Na^+ one. Finally, there were differences in leaf Mg^{2+} concentrations in response to both WQ and R, which were statistically, but, maybe, not practically significant (Table 2).

Grape Composition

The grape composition was less affected by WQ than by R, some statistically significant interactions between both factors

were observed (Table 3). The TSS was affected by WQ and R and, in addition, the effect of WQ significantly changed in magnitude from one rootstock to the others, i.e., the interaction $R \times WQ$ was also significant. Specifically, grape TSS tended to increase from the controls to the saline treatments with a greater increment in the vines onto the M1 rootstock (Table 3). Contrary to TSS, the other grape technological composition parameters (pH, TA) were neither affected by R nor WQ nor $R \times WQ$ (Table 3).

Regarding the phenolic composition, i.e., anthocyanins and polyphenols contents, it was not significantly affected by WQ, but heavily depended on R. Besides, a significant $R \times WQ$ interaction was also revealed in the effect of WQ depending on the rootstock (Table 3). Specifically, both the polyphenols and the anthocyanins contents tended to decrease from the controls to the saline treatments onto the 1103P and on M1, with no changes onto the M4 (Table 3). Regardless of the effect of WQ on phenolic composition in grapes, the 1103P tended to have higher anthocyanins and polyphenols than the other two rootstocks.

Differential Gene Expression

High-throughput mRNA sequencing was performed on whole leaf and berry skin samples from cv. ‘Tempranillo’ was grafted onto the three different rootstocks and exposed to salinity stress. On average, 41,326,458 reads were mapped in pairs to the grapevine genome. Of the 42,413 genes annotated in grapevine, 16,790 were expressed in sufficient quantities for statistical analysis.

Although hierarchical clustering analysis and PCA of leaf and berry skin samples showed no apparent correlation in gene expression regarding either the WQ or R and no clear clustering was observed on PCA for either tissue (Supplementary Figures 1, 2), GSEA identified several processes (bins) that were

TABLE 3 | Parameters of grape composition at harvest for Tempranillo wine grapes grafted onto M1, M4, and 1103-Paulsen (1P) rootstocks subjected to different water quality (C; control and S; saline irrigation) in Valencia, Spain.

Factors	Treatment	Berry weight (g)	TSS (°)	T.A. (g L ⁻¹)	pH	Anthocyanins (mg g ⁻¹)	Polyphenols (mg g ⁻¹)
R	1P	1.6	20.1a	3.8	4.17	0.74c	4.83b
	M1	1.6	20.6b	3.3	4.17	0.53b	4.08a
	M4	1.7	20.9b	3.6	4.16	0.44a	3.73a
WQ	Control	1.7	20.2a	3.5	4.17	0.60	4.34
	Saline	1.6	20.8b	3.7	4.16	0.54	4.08
Interaction R × WQ	1P C	1.67	19.9a	3.7	4.15	0.83	5.14d
	1P S	1.61	20.2ab	3.9	4.19	0.65	4.51c
	M1 C	1.64	19.8a	3.2	4.22	0.55	4.35bc
	M1 S	1.51	21.4c	3.4	4.12	0.52	3.81a
	M4 C	1.70	20.9bc	3.5	4.15	0.42	3.54a
	M4 S	1.60	20.8bc	3.7	4.16	0.45	3.92ab
Rootstock		0.75	<0.01	0.19	0.96	<0.001	<0.001
Water Quality		0.29	<0.01	0.38	0.70	0.13	0.07
R × WQ		0.94	<0.01	0.99	0.30	0.09	0.02

TSS, total soluble solids; T.A., titratable acidity. Data are averages of 6, 9, and 3 determinations per rootstock, water quality and rootstock per water quality respectively. Within each parameter, letters denote significant differences between treatments at $p < 0.05$ (Duncan test). The statistical significance effect of the rootstock (R), water quality (WQ) and their interaction are also indicated by means of the p -values from the ANOVAs. Significance of effects in bold denotes statistically significant differences at $p < 0.05$.

statistically significantly (FDR $q < 0.25$) differentially expressed due to WQ in leaves and berries of scions grafted on the three rootstocks (Figure 3). The number of significantly differentially expressed bins was higher in leaves and berries of scions grafted on M4 and M1 rootstocks as compared to 1103P. The strongest enrichment was detected for flavonoid synthesis bins in berry skins for all three R. In them, chalcone synthases contribution prevailed (Supplementary Table 2). When examining the expression of individual genes involved in this pathway, large differences in average values were observed, with up to a fourfold difference in a uniform dominant upregulation pattern, although no statistically significant differences in gene expression were found between the control and saline treatments (Supplementary Table 3). Specifically, the differences in average values between salt-stressed and control vines were the highest in the expression of genes related to chalcone synthase (CHS) and phenylalanine ammonia-lyase (PAL) genes. This was most apparent in berry skins, where most of the PAL and CHS genes showed an upregulation pattern due to WQ (Figure 4). Moreover, the differences were highest in vines grafted onto 1103P than onto M4 and M1. However, multiple flavanone 3-hydroxylases showed a downregulation pattern in these samples. On the other hand, leaf samples showed lower differences, which were found in CHS genes in samples grafted onto M1, and some flavanone 3-hydroxylase genes in samples grafted onto M4 (Supplementary Figure 3).

Although no statistically significant differences in expression of individual genes were observed due to WQ in either leaves or the berries, some statistically significant differences due to R were observed (Supplementary Table 3). There were 15 differentially expressed genes found between the leaves of control plants grafted onto 1103P and M4. Most of them were more expressed in 1103P than in M4, but no specific pathway predominated among them.

The technical validity of RNA-Seq and the data analysis pipeline was corroborated by the targeted analysis of three genes by qPCR. The qPCR results highly correlated with RNA-Seq ($r^2 = 0.83$) (Supplementary Figure 4).

DISCUSSION

The effects of WQ and R on physiology and transcriptomics of cv. ‘Tempranillo’ vines were assessed indirectly because all determinations were carried out on the scion, not in the rootstock, which is the barrier against soil salinity. However, the scion cultivar is the genotype that ultimately bears fruit and ripens it and, therefore, confers economic value on the crop (Marguerit et al., 2012). Thus, in this approach, the scion is considered an integrator of the effects induced by the rootstock. It is important to bear this in mind when interpreting the results, especially the transcriptome analyses, because of the combination of two *Vitis* spp. Genotypes are studied by evaluating only one of them, i.e., *Vitis vinifera* L. In comparison, most of the grapevine transcriptomics responses reported in the literature have been assessed on a single genotype, i.e., directly in the own-rooted *Vitis vinifera* (Cramer et al., 2007; Guan et al., 2018; Das and Majumder, 2019; Lehr et al., 2022) or on the rootstock without grafting (Gong et al., 2011; Henderson et al., 2014; Meggio et al., 2014; Corso et al., 2015; Vannozzi et al., 2017; Fu et al., 2019; Çakır Aydemir et al., 2020), and if carried out in both the scion and the rootstock, they have been under highly controlled conditions (Upadhyay et al., 2018; Bianchi et al., 2020; Franck et al., 2020; Baggett et al., 2021), i.e., not under real field-grown conditions.

In the present trial, the water requirements of the grapevines were fully met trying to isolate the effect of WQ on the physiological and transcriptomic responses. When plant measurements and samplings were carried out, the water status

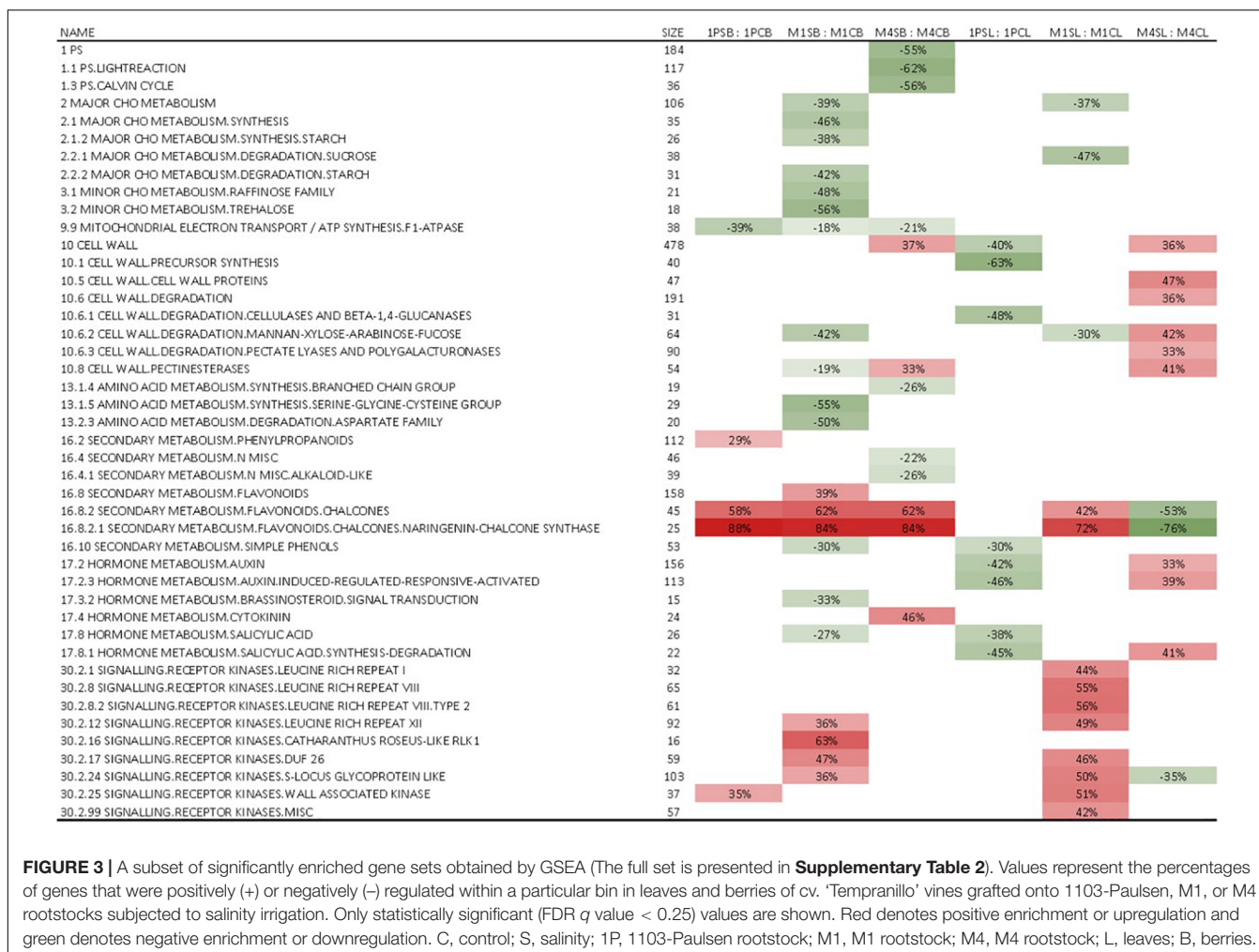


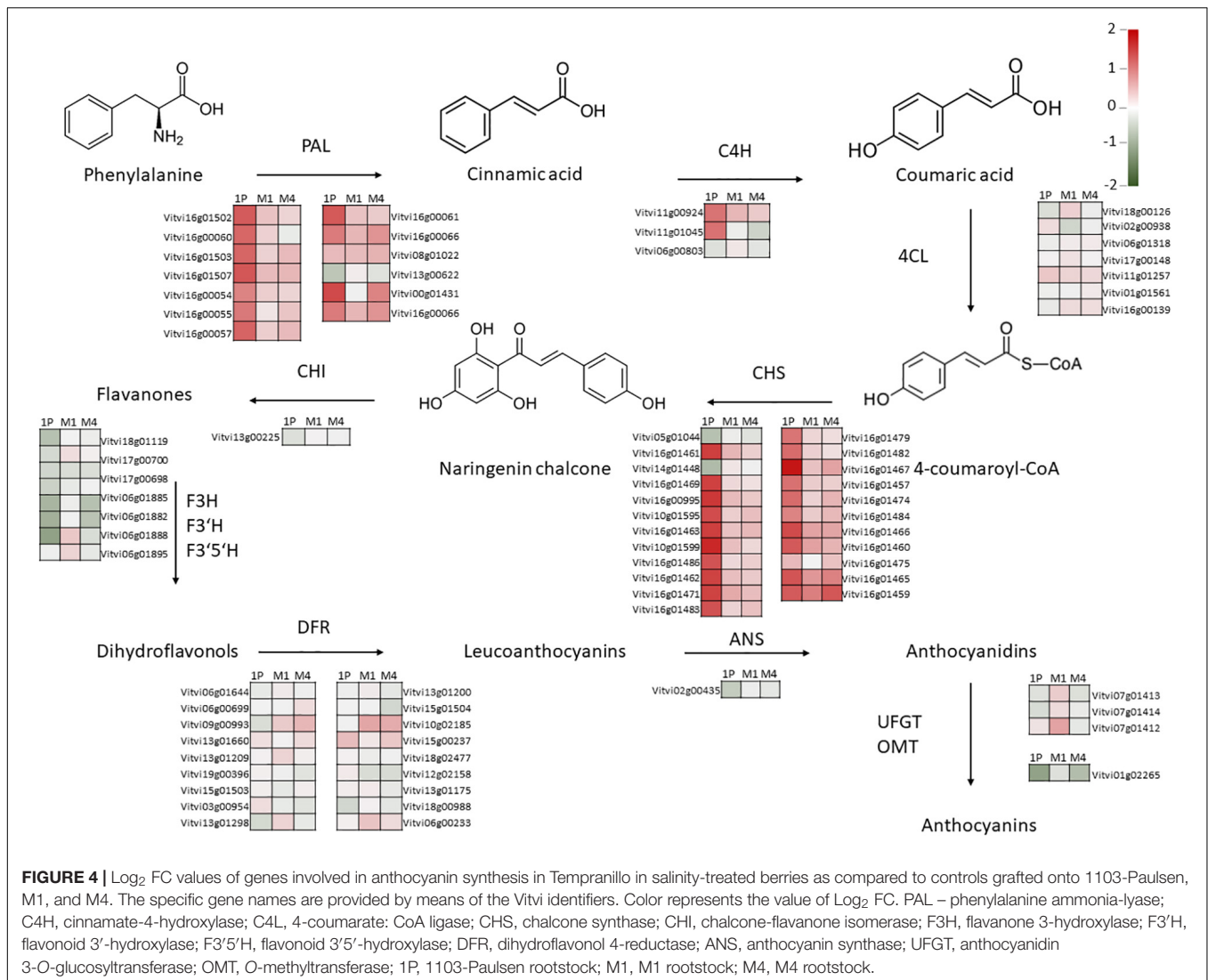
FIGURE 3 | A subset of significantly enriched gene sets obtained by GSEA (The full set is presented in **Supplementary Table 2**). Values represent the percentages of genes that were positively (+) or negatively (-) regulated within a particular bin in leaves and berries of cv. 'Tempranillo' vines grafted onto 1103-Paulsen, M1, or M4 rootstocks subjected to salinity irrigation. Only statistically significant (FDR q value < 0.25) values are shown. Red denotes positive enrichment or upregulation and green denotes negative enrichment or downregulation. C, control; S, salinity; 1P, 1103-Paulsen rootstock; M1, M1 rootstock; M4, M4 rootstock; L, leaves; B, berries.

experienced by the control vines grafted onto any of the rootstocks was indicative of very mild water stress according to Williams and Baeza (2007; **Figure 1**). This implies that irrigation largely met the evapotranspiration demand of the plants. However, it was not excessive, which would have resulted in irrigation water percolation and thus the washout of salts from the rooting depth. In fact, the ions' concentration in the soil solution of Saline treatments caused vine water stress. This was observed in the general decrease of both $\Psi_{pre-dawn}$ and Ψ_{stem} in the vines grafted onto all rootstocks under irrigation with saline water, which means a worsening of the plant water status (**Figure 1**). This physiological response is likely due to a reduction of the soil water potential by an osmotic effect (Tattersall et al., 2007), i.e., the so-called osmotic drought (Chaves et al., 2009). As expected, $\Psi_{pre-dawn}$ was in line with Ψ_{stem} (Suter et al., 2019), although plants onto M4 tended to show less negative Ψ_{stem} values than those onto 1103P, with no difference in $\Psi_{pre-dawn}$ (**Table 1**). These slight differences in Ψ_{stem} between M4 and 1103P agreed with what Frioni et al. (2020) observed in M4 under water shortage.

Plants react to salt stress and control their subsequent physiological responses using signals, which can be ionic,

osmotic, hormonal, and/or reactive oxygen species regulation (Shahid et al., 2020; Zhou-Tsang et al., 2021). Concerning the ionic, in this work the leaf ion concentrations have been observed to differ among rootstocks, notably, Cl^- , Ca^{2+} , Na^+ , and Mg^{2+} (**Table 2**). Regarding Cl^- , it usually builds up in the leaves of woody crops, and the plant's ability to avoid accumulating Cl^- in leaves is considered directly proportional to its salinity tolerance. In this work the M-series rootstocks increased the leaf Cl^- twofold in the saline treatment compared to the control. In contrast, in the 1103P the leaf Cl^- increase in the saline treatment compared to the control was not significant. These results are in agreement, on the one hand, with Meggio et al. (2014), who also reported higher leaf Cl^- in vines onto M4 in comparison to the good salt excluder 101-14 Mgt (Walker et al., 2004, 2010) and, on the other hand, with Urdanoz and Aragües (2009), who reported that the 'Tempranillo' cultivar grafted onto 1103P was able to exclude Cl^- from the leaves more efficiently than other cultivar-rootstock combinations.

The leaf Cl^- non-accumulation ability conferred by the 1103P could be due to (i) limited salt uptake, i.e., ion exclusion, and (ii) limited salt translocation from the root to the shoot. Abbaspour et al. (2013) suggested that 1103P contributes to reducing shoot



Cl⁻ concentration by root efflux and vacuolar internalization. Besides, Henderson et al. (2014) suggested that transcriptional events contributing to the Cl⁻ exclusion mechanism in grapevine are not stress-inducible, but constitutively different between contrasting genotypes. Anyway, Cl⁻ exclusion factors are yet to be identified at the transcriptomic level, and are multigenic, including transport proteins (Gong et al., 2011; Das and Majumder, 2019; Zhou-Tsang et al., 2021). This genotype-dependent, though fuzzy, transcriptomic effects agree with our GSEA results, which identified much less statistically significantly (FDR $q < 0.25$) differentially expressed bins due to WQ in 'Tempranillo' grafted onto 1103P as compared to M4 and M1 (Figure 3). Baggett et al. (2021), also similarly observed that salinity affected transcript abundance more in salt-sensitive genotypes than in salt-tolerant ones. Importantly, the leaf Cl⁻ concentrations in our trial are higher than the ones reported by Urdanoz and Aragües (2009) and Baggett et al. (2021), even though in the range of the ones found in 'Cabernet Sauvignon' onto 1103P by Dag et al. (2015) using similar WQ.

The capacity of rootstocks to restrict leaf salt buildup should not be the only parameter for rootstock selection (Zhou-Tsang et al., 2021). Regarding other criteria, several authors indicated the better M4 performance compared to other rootstocks because of an improved antioxidant capability (Meggio et al., 2014; Corso et al., 2015; Lucini et al., 2020; Prinsi et al., 2020). Furthermore, it is important to consider the likely accumulation of Cl⁻ and Na⁺ in the permanent instead of the short-lived organs of the vine (Stevens and Partington, 2013; Netzer et al., 2014), which may lead to salinity carry-over effects on the medium-to-long term. Based on our results, this would be a concern for rootstocks M1 and M4 and less for 1103P (Table 1), because of its possible detrimental effects on future bud fruitfulness (Walker et al., 2002). In fact, Dag et al. (2015) reported that irrigating the 'Cabernet-Sauvignon' scion grafted onto 1103P with water similar in salinity to the Saline treatment in this work, did not significantly affect vine performance in the first two seasons, but that Na⁺ and Cl⁻ accumulation in the wood eventually led to vine death in the third one.

Regarding Na^+ , it is less prone to build up in the leaves of grapevines than Cl^- (Henderson et al., 2018), which, given the Na^+/Cl^- ratio of the waters applied in this work, was also observed here (Table 2). However, there were differences in salt-stress modulating ability among rootstocks with the M1 more liable to leaf Na^+ accumulation as salinity increased than 1103P or M4. Regarding leaf Ca^{2+} , it increased in the Saline treatments compared to the Controls (Table 2). That leaf Ca^{2+} increased in the ‘Tempranillo’ leaves as salinity grew regardless of the rootstock suggests that all three rootstocks can maintain high $\text{Ca}^{2+}/\text{Na}^+$ ratios and thus, efficiently exclude Na^+ (Shahid et al., 2020). More interestingly, however, there were differences in leaf Ca^{2+} among the vines depending on the rootstock. Particularly, the M1 built up significantly more leaf Ca^{2+} than the 1103P and M4 (Table 2). Since Ca^{2+} can regulate plant signaling, enzyme activity, ion channel performance, and gene expression (Golldack et al., 2014), the higher leaf Ca^{2+} onto the M1 may be a positive plant adaptation as previously reported by Porro et al. (2013). Likewise, K^+ is also key in maintaining the osmotic balance and thus the ionic homeostasis in plant cells (Kumari et al., 2015; Guan et al., 2018). However, in our work, leaf K^+ decreased because of salinity, without differences among rootstocks (Table 2). Similarly, Guan et al. (2018) also found a decreasing trend in leaf K^+ in ‘Summer Black’ cv. in response to NaCl irrigation, and Munns and Tester (2008) indicated that a strong relationship between leaf K^+ and salt tolerance had not yet been reported. In our work, both the leaf $\text{K}^+/\text{Ca}^{2+}$ and K^+/Na^+ ratios were reduced by M1 compared to 1103P. This suggests that the 1103P conferred a greater salinity tolerance to the scion than the M1.

Concerning the osmolyte regulation signals, a tendency to a slight osmotic adjustment was observed in the leaves on all three rootstocks. This is because, independently of the leaf water status, i.e., Ψ_{π}^{100} , the values of the saline treatments were significantly more negative (−0.16 MPa on average) than those of the Controls (Figure 1). Through osmotic adjustment plants cope with declining soil water potential mainly because increasing osmolyte concentrations decrease the water potential within plant cells, thus increasing the leaf relative water content and turgor for a given soil water potential (Barrios-Masias et al., 2018). These osmolytes can be inorganic, which are actively and passively taken from the same soil solution, or organic, which are obtained by biosynthesis of proline, glycine-betaine, etc. However, in our work, the expression of genes involved in amino acid metabolism was not altered in leaves in response to WQ (Figure 3), whereas the concentration of Cl^- , K^+ , and Ca^{2+} did increase in the leaves (Table 2). Accordingly, the slight observed osmotic adjustment was achieved through the build-up of inorganic osmolytes, and this was controlled by the rootstock because the root is the organ that regulates the entry of the soil solution ions into the plant. The mechanisms of ion exclusion and/or upward movement along the xylem should be genetically regulated at the root level, i.e., over-expression of the cation HKT transporters genes (Deinlein et al., 2014; Fu et al., 2019; Zhou-Tsang et al., 2021), and not at the scion level. However, despite occurring at the root level, the mechanisms

may be genetically regulated in a scion-induced manner (Franck et al., 2020) and then, maybe, detected in the scion. Remarkably, among the 15 differentially expressed genes between the 1103P and M4, a lactoylglutathione lyase (Vitvi04g01424) and a Dof family transcription factor (Vitvi18g00858) were found. These genes have previously been implicated in response to abiotic stress in grapevine (Shangguan et al., 2020), as it was implicated in redox homeostasis in heat-stressed ‘Muscat Hamburg’ berries (Carbonell-Bejerano et al., 2013).

The generalized reduction found in net photosynthesis (A_N) under saline conditions, regardless of the rootstock (Figure 2), is related to stomatal and mesophyll conductance limitation, as there were no major differences in WUE_i beyond those expected, given the differences in water status (Flexas et al., 2004). Reductions are in line with those found by Flexas et al. (1999) in ‘Tempranillo’ and Baeza et al. (2007) and Baggett et al. (2021) in ‘Cabernet-Sauvignon’ cultivars. Moreover, no differences were detected in the ratio of internal to atmospheric CO_2 concentration (Ci/Ca) between treatments (0.76 and 0.75 in Control and Saline treatments, respectively; data not shown). This suggests that in this work salinity was not high enough to induce either toxic effects on the photosynthetic apparatus or cellular damage in the leaves, as confirmed using the leaf transcriptomic analysis (Figure 3), but rather that it simply increased water stress by lowering the soil water potential, which eventually showed up in g_s and, thus, A_N reduction (Figure 2). Interestingly, according to Bianchi et al. (2020), water shortcoming stress decreases stomatal conductance due to lower water potential, but the photosynthetic activity keeps high with bare differences among 1103P, M1, and M4. In contrast, in our trial, M1 performed differently from the other rootstocks by inducing an overall reduction in A_N . Moreover, Bianchi et al. (2020) did detect changes in the transcript abundances of key genes related to abscisic acid biosynthesis, but in the root, not in leaves, and studying only the wider *Vitis* spp. genotype.

The overall effects caused by salinity on decreasing leaf photosynthesis as well as LAI (Figure 2 and Table 2) should have led to reduced berry ripening (Cramer et al., 2007; Chaves et al., 2009; Liu et al., 2020; Zhou-Tsang et al., 2021). However, the opposite was observed. The Saline treatments increased TSS compared to the Control grapes. These results point toward the ability of all rootstocks to keep allocating energy resources to fruit ripening regardless of salt stress. Interestingly, Meggio et al. (2014) also highlighted the salt tolerance of these rootstocks regardless of their ability to limit specific ion accumulations in the scion, which was associated with a lower decrease in A_N and Ψ_{leaf} on M4 compared to 101–14 Mgt. This was not observed under salinity in this work, as it neither was an underwater shortage (Bianchi et al., 2020).

Effects of WQ and R on grape composition are usually not very conclusive according to studies where both factors are combined (Walker et al., 2007; Stevens et al., 2011; Hirzel et al., 2017; Mirás-Avalos and Intrigliolo, 2017). This is because there is a multitude of environmental factors that interact with rootstock response, most notably soil type (Ferlito et al., 2020). Specifically, the three rootstocks studied here perform well on soils high

in calcium carbonate like the one used in this investigation because all three come from crossings with *Vitis berlandieri*, a species that evolved on calcareous soils (Harry, 1996). In this work, there was a salt-stress modulating effect by the rootstock on grape composition, primarily TSS and, secondarily, the phenolic composition as revealed by the $R \times WQ$ interactions (Table 3). Whereas barely anything was observed on T.A., and, specifically, pH, which did not change following the decrease in leaf K^+ concentration due to salinity (Table 2) in accordance with Marín et al. (2021). Contrary to T.A., and pH, the TSS increased onto the M1 rootstock as salinity grew, whereas the other rootstocks did not respond in the same way. Moreover, the phenolic substances were also subjected to rootstock-specific modulating effects (Table 3). Despite these, the expected changes on gene expression of CHS and PAL pathways were not observed (Figure 4). This is, the significant reduction in anthocyanins content found in 1103P vines and polyphenols found in 1103P and M1 vines in response to salinity (Table 3) could not be related to the transcriptomic changes observed, nor to differences in berry size (Table 3).

Several studies have linked ultraviolet light to the induction of phenolic compound synthesis, specifically the expression of the CHS gene, a key enzyme in flavonoid biosynthesis (Merkle et al., 1994; Hernández et al., 2009; Wang et al., 2016; Reshef et al., 2018). However, these putative changes, which are related to berry exposure to sunlight in response to the saline effect on the vine leaf area (Zarrouk et al., 2016; Torres et al., 2020), would have been offset by the slight increase in the leaf area-to-production ratio (Walker et al., 2000; Bobeica et al., 2015). Moreover, flavonoid synthase is also involved in drought and osmotic stress tolerance and is controlled by rootstocks (Dal Santo et al., 2018; Bianchi et al., 2020; Zombardo et al., 2020). For instance, Zombardo et al. (2020), also in grape skin during ripening, reported some differentially expressed genes mainly involved in the synthesis and transport of phenylpropanoids (e.g., flavonoids) in response to rootstock effects. Besides, the most prominent differences in gene expression of the anthocyanin pathway usually occur during veraison, together with the differences of anthocyanin content and profile in the berry and begin to faint as the berry reaches final maturity (Castellarin et al., 2006; Castellarin and Di Gaspero, 2007). All of this highlights the complexity of relating phenotypic observations to changes in gene expression (Fu et al., 2019; Haider et al., 2019). In this regard, the next generation of omics is expected to help to identify gene function, speeding up the rootstock breeding programs for enhancing resilience to climate change in future viticulture (Marín et al., 2021).

CONCLUSION

The results of this work have shown how the grapevine M-rootstock's physiological and transcriptomic responses integrate at the scion level because of the irrigation with saline water under real field-grown conditions for the first time. The determinations carried out in the scion (i.e., cv. 'Tempranillo') permitted us to obtain some insight into the

possible mechanisms developed by the rootstocks in response to water salinity, and the differences between the three that were tested in this work. In the short period of this trial, and a vineyard under establishment, all three rootstocks similarly adjusted osmotic potential to cope with osmotic stress, and then, vine water status declined in response to irrigation with saline water compared to non-saline water. Regarding the differential response among rootstocks, based on, on the one hand, grapevine physiology and grape must composition and, on the other hand, salt accumulation in leaves and transcriptomic changes, there were differences worth highlighting. First, the M1 rootstock was the one that responded the most to salinity by reducing A_N and LAI, whereas the M4 rootstock was the one that buffered the best the effects of salinity on TSS and grape phenolic composition. Second, the 1103P rootstock was the one able to reduce the leaf Cl^- and Na^+ build-up the most and affected transcriptomic expression the least, which might have positive effects on the long-term vine performance and grape composition. Longer-term studies are needed to unravel the molecular responses occurring in mature vineyards at both the scion and rootstock levels.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ebi.ac.uk/ena>, PRJEB44658.

AUTHOR CONTRIBUTIONS

IB, JP-P, FV, DI, LB, MP-N, and JP contributed to the conception and design of the study. IB, JP-P, and RS acquired the data. IB, JP-P, FV, DI, KG, and MP-N performed the data analysis and interpretation. IB and RS prepared the first-draft. IB, JP-P, FV, RS, DI, MP-N, and JP reviewed and edited the manuscript. DI, LB, and JP supervised the work. DI, LB, MP-N, and JP acquired the funding. All authors read and approved the submitted version.

FUNDING

This work was mainly supported by European Union, Slovenian Ministry of Education, ARRS project number P4-0165, Science and Sport and Spanish Ministry of Economy and Competitiveness, co-financing Arimnet 2 project EnViRoS (grant agreement n° 618127) but also by AEI-FEDER AGL2017-83738-C3-3-R.

ACKNOWLEDGMENTS

IB and JP-P gratefully acknowledge their postdoctoral contracts from the 'Juan de la Cierva' (FJC2019-042122-I) and 'Ramón y Cajal' programs (RYC-2015-17726), respectively, supplied

by the Spanish Ministry of Economy and Competitiveness (MINECO). Thanks are also due to Mr. F. Sanz, D. Guerra, A. Yeves, M. Tasa, and P. Romero for their technical help with the fieldwork.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.866053/full#supplementary-material>

Supplementary Figure 1 | Gene expression overview in berry skin samples.

(A) Hierarchical clustering analysis of the 500 most variable genes in berry skin samples. (B) Principal component analysis of the berry skin samples. PC, principal component; 1P, 1103-Paulsen; C, control; S, salinity.

Supplementary Figure 2 | Gene expression overview in leaf samples.

(A) Hierarchical clustering analysis of the 500 most variable genes in leaf samples. (B) Principal component analysis of the berry skin samples. PC, principal component; 1P, 1103-Paulsen; C, control; S, salinity.

Supplementary Figure 3 | Log₂ FC values of genes involved in anthocyanin synthesis in Tempranillo in salinity-treatment leaves as compared to controls grafted onto 1103-Paulsen, M1, and M4. The specific gene names are provided by means of the Vitvi identifiers. Color represents the value of Log₂ FC. PAL,

phenylalanine ammonia-lyase; C4H, cinnamate-4-hydroxylase; C4L, 4-coumarate: CoA ligase; CHS, chalcone synthase; CHI, chalcone-flavanone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3'5'-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanin synthase; UFGT, anthocyanidin 3-O-glucosyltransferase; OMT, O-methyltransferase; 1P, 1103-Paulsen rootstock; M1, M1 rootstock; M4, M4 rootstock.

Supplementary Figure 4 | Correlation between RNA-Seq and qPCR differential expression represented as log₂ FC. $R^2 = 0.83$.

Supplementary Table 1 | Primers and probes used for grapevine gene expression analysis.

Supplementary Table 2 | Gene sets significantly enriched by GSEA. Values represent the percentages of genes that were positively (+) or negatively (−) regulated within a particular bin in leaves and berries of cv. Tempranillo vines grafted onto 1103-Paulsen, M1, or M4 rootstocks and subjected to salinity stress. The values are derived from comparison of control and salinity-treated plants in selected time-points. Only the values with statistical significance (FDR q value < 0.25) are listed. Red denotes positive enrichment or upregulation and green denotes negative enrichment or downregulation. The percent value represents the proportion of genes in the gene set which contributed to the enrichment. C, control; S, salinity; 1P, 1103-Paulsen rootstock; M1, M1 rootstock; M4, M4 rootstock; L, leaves; B, berries.

Supplementary Table 3 | High-throughput RNA-Seq of salinity-treated grapevine berry skin and leaf samples cv. Tempranillo grafted onto 1103-Paulsen, M1, and M4. C, control; S, salinity; 1P, 1103-Paulsen rootstock; M1, M1 rootstock; M4, M4 rootstock; L, leaves; B, berries.

REFERENCES

- Abbaspour, N., Kaiser, B., and Tyerman, S. (2013). Chloride transport and compartmentation within main and lateral roots of two grapevine rootstocks differing in salt tolerance. *Trees* 27, 1317–1325. doi: 10.1007/s00468-013-0880-2
- Allen, R. G., Pereira, L. S., Raes, D., and Smith, M. (1998). Crop evapotranspiration—Guidelines for computing crop water requirements—FAO Irrigation and drainage paper 56. *FAO Rome* 300:D05109.
- Askari, H., Daldoul, S., Ammar, A. B., Rejeb, S., Jarak, R., Rejeb, M. N., et al. (2012). Short-term response of wild grapevines (*Vitis vinifera* L. ssp. *sylvestris*) to NaCl salinity exposure: changes of some physiological and molecular characteristics. *Acta Physiol. Plant.* 34, 957–968. doi: 10.1007/s11738-011-0892-8
- Baebler, S., Svalina, M., Petek, M., Stare, K., Rotter, A., Pompe-Novak, M., et al. (2017). Quantgenius: implementation of a decision support system for qPCR-based gene quantification. *BMC Bioinform.* 18:276. doi: 10.1186/s12859-017-1688-7
- Baeza, P., Sánchez-de-Miguel, P., Centeno, A., Junquera, P., Linares, R., and Lissarrague, J. R. (2007). Water relations between leaf water potential, photosynthesis and agronomic vine response as a tool for establishing thresholds in irrigation scheduling. *Sci. Hortic.* 114, 151–158. doi: 10.1016/j.scienta.2007.06.012
- Baggett, J. P., Habibsadeh, S., Toupes, H. S., Cochetel, N., Ghan, R., Robinson, M. L., et al. (2021). Is foliar Cl[−] concentration the cause of photosynthetic decline in grapevine during mild salinity? *OENO One* 55, 33–48. doi: 10.20870/oeno-one.2021.55.4.4795
- Barrios-Masias, F. H., Knipfer, T., Walker, M. A., and McElrone, A. J. (2018). Differences in hydraulic traits of grapevine rootstocks are not conferred to a common *Vitis vinifera* scion. *Funct. Plant Biol.* 46, 228–235. doi: 10.1071/fp18110
- Bianchi, D., Caramanico, L., Grossi, D., Brancadoro, L., and Lorenzis, G. D. (2020). How do novel M-Rootstock (*Vitis* Spp.) genotypes cope with drought? *Plants* 9:1385. doi: 10.3390/plants9101385
- Bobeica, N., Poni, S., Hilbert, G., Renaud, C., Gomès, E., Delrot, S., et al. (2015). Differential responses of sugar, organic acids and anthocyanins to source-sink modulation in Cabernet Sauvignon and Sangiovese grapevines. *Front. Plant Sci.* 6:382. doi: 10.3389/fpls.2015.00382
- Çakır Aydemir, B., Yüksel Özmen, C., Kibar, U., Mutaf, F., Büyük, P. B., Bakır, M., et al. (2020). Salt stress induces endoplasmic reticulum stress-responsive genes in a grapevine rootstock. *PloS One* 15:e0236424. doi: 10.1371/journal.pone.0236424
- Canaguier, A., Grimplet, J., Di Gaspero, G., Scalabrini, S., Duchêne, E., Choise, N., et al. (2017). A new version of the grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). *Genomics Data* 14, 56–62. doi: 10.1016/j.gdata.2017.09.002
- Carbonell-Bejerano, P., Santa María, E., Torres-Pérez, R., Royo, C., Lijavetzky, D., Bravo, G., et al. (2013). Thermotolerance responses in ripening berries of *Vitis vinifera* L. cv Muscat hamburg. *Plant Cell Physiol.* 54, 1200–1216. doi: 10.1093/pcp/ptc071
- Carra, A., Gambino, G., and Schubert, A. (2007). A cetyltrimethylammonium bromide-based method to extract low-molecular-weight RNA from polysaccharide-rich plant tissues. *Anal. Biochem.* 360, 318–320. doi: 10.1016/j.ab.2006.09.022
- Castellarin, S. D., and Di Gaspero, G. (2007). Transcriptional control of anthocyanin biosynthetic genes in extreme phenotypes for berry pigmentation of naturally occurring grapevines. *BMC Plant Biol.* 7:46. doi: 10.1186/1471-2229-7-46
- Castellarin, S. D., Di Gaspero, G., Marconi, R., Nonis, A., Peterlunger, E., Paillard, S., et al. (2006). Colour variation in red grapevines (*Vitis vinifera* L.): genomic organisation, expression of flavonoid 3'-hydroxylase, flavonoid 3',5'-hydroxylase genes and related metabolite profiling of red cyanidin/blue delphinidin-based anthocyanins in berry skin. *BMC Genomics* 7:12. doi: 10.1186/1471-2164-7-12
- Chaves, M. M., Flexas, J., and Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* 103, 551–560. doi: 10.1093/aob/mcn125
- Cookson, S. J., Clemente Moreno, M. J., Hevin, C., Nyamba Mendome, L. Z., Delrot, S., Trossat-Magnin, C., et al. (2013). Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signalling, and secondary metabolism. *J. Exp. Bot.* 64, 2997–3008. doi: 10.1093/jxb/ert144
- R Core Team (2017). *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna: R Foundation.
- Corso, M., Vannozzi, A., Maza, E., Vitulo, N., Meggio, F., Pitacco, A., et al. (2015). Comprehensive transcript profiling of two grapevine rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance. *J. Exp. Bot.* 66, 5739–5752. doi: 10.1093/jxb/erv274

- Cramer, G. R., Ergul, A., Grimplet, J., Tillett, R. L., Tattersall, E. A., Bohlman, M. C., et al. (2007). Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Funct. Integr. Genomics* 7, 111–134. doi: 10.1007/s10142-006-0039-y
- Dag, A., Ben-Gal, A., Goldberger, S., Yermiyahu, U., Zipori, I., Or, E., et al. (2015). Sodium and chloride distribution in grapevines as a function of rootstock and irrigation water salinity. *Am. J. Enol. Vitic.* 66, 80–84. doi: 10.5344/ajev.2014.14019
- Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., et al. (2018). Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G×E) on the berry transcriptome. *Plant J.* 93, 1143–1159. doi: 10.1111/tpl.13834
- Daldoul, S., Guillaumie, S., Reustle, G. M., Krczal, G., Ghorbel, A., Delrot, S., et al. (2010). Isolation and expression analysis of salt induced genes from contrasting grapevine (*Vitis vinifera* L.) cultivars. *Plant Sci.* 179, 489–498. doi: 10.1016/j.plantsci.2010.07.017
- Das, P., and Majumder, A. L. (2019). Transcriptome analysis of grapevine under salinity and identification of key genes responsible for salt tolerance. *Funct. Integr. Genomics* 19, 61–73. doi: 10.1007/s10142-018-0628-6
- De Paz, J. M., Visconti, F., Zapata, R., and Sánchez, J. (2004). Integration of two simple models in a geographical information system to evaluate salinization risk in irrigated land of the Valencian Community. Spain. *Soil Use Manage.* 20, 333–342. doi: 10.1111/j.1475-2743.2004.tb00378.x
- Deinlein, U., Stephan, A. B., Horie, T., Luo, W., Xu, G., and Schroeder, J. I. (2014). Plant salt-tolerance mechanisms. *Trends Plant Sci.* 19, 371–379.
- Dermastia, M., Škrlić, B., Strah, R., Anžić, B., Tomaž, Š., Križnik, M., et al. (2021). Differential response of grapevine to infection with ‘Candidatus Phytoplasma solani’ in early and late growing season through complex regulation of mRNA and small RNA transcriptomes. *Int. J. Mol. Sci.* 22:3531. doi: 10.3390/ijms22073531
- Döll, P. (2002). Impact of climate change and variability on irrigation requirements: a global perspective. *Clim. Change* 54, 269–293. doi: 10.1098/rsta.2012.0412
- Ferlito, F., Distefano, G., Gentile, A., Allegra, M., Lakso, A. N., and Nicolosi, E. (2020). Scion–rootstock interactions influence the growth and behaviour of the grapevine root system in a heavy clay soil. *Aust. J. Grape Wine Res.* 26, 68–78. doi: 10.1111/ajgw.12415
- Flexas, J., Bota, J., Loreto, F., Cornic, G., and Sharkey, T. (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol.* 6, 269–279. doi: 10.1055/s-2004-820867
- Flexas, J., Escalona, J. M., and Medrano, H. (1999). Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. *Plant Cell Environ.* 22, 39–48. doi: 10.1046/j.1365-3040.1999.00371.x
- Franck, N., Zamorano, D., Wallberg, B., Hardy, C., Ahumada, M., Rivera, N., et al. (2020). Contrasting grapevines grafted into naturalized rootstock suggest scion-driven transcriptomic changes in response to water deficit. *Sci. Hortic.* 262:109031. doi: 10.1016/j.scienta.2019.109031
- Frióni, T., Biagioni, A., Squeri, C., Tombesi, S., Gatti, M., and Poni, S. (2020). Grafting cv. grechetto gentile vines to New M4 rootstock improves leaf gas exchange and water status as compared to commercial 1103P rootstock. *Agronomy* 10:708. doi: 10.3390/agronomy10050708
- Fu, Q.-q., Tan, Y.-z., Zhai, H., and Du, Y.-p. (2019). Evaluation of salt resistance mechanisms of grapevine hybrid rootstocks. *Sci. Hortic.* 243, 148–158. doi: 10.1016/j.scienta.2018.07.034
- Gambetta, G. A., Manuck, C. M., Drucker, S. T., Shaghasi, T., Fort, K., Matthews, M. A., et al. (2012). The relationship between root hydraulics and scion vigour across Vitis rootstocks: what role do root aquaporins play? *J. Exp. Bot.* 63, 6445–6455. doi: 10.1093/jxb/ers312
- Golldack, D., Li, C., Mohan, H., and Probst, N. (2014). Tolerance to drought and salt stress in plants: unraveling the signaling networks. *Front. Plant Sci.* 5:151. doi: 10.3389/fpls.2014.00151
- Gong, H., Blackmore, D., Clingeleffer, P., Sykes, S., Jha, D., Tester, M., et al. (2011). Contrast in chloride exclusion between two grapevine genotypes and its variation in their hybrid progeny. *J. Exp. Bot.* 62, 989–999. doi: 10.1093/jxb/erq326
- Guan, L., Haider, M. S., Khan, N., Nasim, M., Jiu, S., Fiaz, M., et al. (2018). Transcriptome sequence analysis elaborates a complex defensive mechanism of grapevine (*Vitis vinifera* L.) in response to salt stress. *Int. J. Mol. Sci.* 19:4019. doi: 10.3390/ijms19124019
- Haider, M. S., Jogaiah, S., Pervaiz, T., Yanxue, Z., Khan, N., and Fang, J. (2019). Physiological and transcriptional variations inducing complex adaptive mechanisms in grapevine by salt stress. *Environ. Exp. Bot.* 162, 455–467. doi: 10.1016/j.envexpbot.2019.03.022
- Harry, W. P. (1996). *Science, Vine, and Wine in Modern France*. Cambridge: Cambridge University Press, 9–99.
- Henderson, S. W., Baumann, U., Blackmore, D. H., Walker, A. R., Walker, R. R., and Gilliam, M. (2014). Shoot chloride exclusion and salt tolerance in grapevine is associated with differential ion transporter expression in roots. *BMC Plant Biol.* 14:273. doi: 10.1186/s12870-014-0273-8
- Henderson, S. W., Dunlevy, J. D., Wu, Y., Blackmore, D. H., Walker, R. R., Edwards, E. J., et al. (2018). Functional differences in transport properties of natural HKT1;1 variants influence shoot Na⁺ exclusion in grapevine rootstocks. *New Phytol.* 217, 1113–1127. doi: 10.1111/nph.14888
- Hernández, I., Alegre, L., Van Breusegem, F., and Munné-Bosch, S. (2009). How relevant are flavonoids as antioxidants in plants? *Trends Plant Sci.* 14, 125–132. doi: 10.1016/j.tplants.2008.12.003
- Hirzel, D. R., Steenwerth, K., Parikh, S. J., and Oberholster, A. (2017). Impact of winery wastewater irrigation on soil, grape and wine composition. *Agric. Water Manag.* 180, 178–189. doi: 10.1016/j.agwat.2016.10.019
- Horneck, D. A., and Miller, R. O. (1998). “Determination of total nitrogen in plant tissue,” in *Handbook of Reference Methods for Plant Analysis*, ed. Y. P. Kalra (Boca Raton, FL: CRC Press), 75–83.
- Keller, M. (2010). Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. *Aust. J. Grape Wine Res.* 16, 56–69. doi: 10.1111/j.1755-0238.2009.00077.x
- Kumari, A., Das, P., Parida, A. K., and Agarwal, P. K. (2015). Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophytes. *Front. Plant Sci.* 6:537. doi: 10.3389/fpls.2015.00537
- Lavoie-Lamoureux, A., Sacco, D., Risse, P.-A., and Lovisolo, C. (2017). Factors influencing stomatal conductance in response to water availability in grapevine: a meta-analysis. *Physiol. Plant.* 159, 468–482. doi: 10.1111/ppl.12530
- Law, C. W., Chen, Y., Shi, W., and Smyth, G. K. (2014). voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol.* 15:R29. doi: 10.1186/gb-2014-15-2-r29
- Lehr, P. P., Hernández-Montes, E., Ludwig-Müller, J., Keller, M., and Zörc, C. (2022). Abscisic acid and proline are not equivalent markers for heat, drought and combined stress in grapevines. *Aust. J. Grape Wine Res.* 28, 119–130. doi: 10.1111/ajgw.12523
- Liu, X., Wang, L., Wei, Y., Zhang, Z., Zhu, H., Kong, L., et al. (2020). Irrigation with magnetically treated saline water influences the growth and photosynthetic capability of *Vitis vinifera* L. seedlings. *Sci. Hortic.* 262:109056. doi: 10.1016/j.scienta.2019.109056
- López-Urrea, R., Montoro, A., Mañas, F., López-Fuster, P., and Fereres, E. (2012). Evapotranspiration and crop coefficients from lysimeter measurements of mature ‘Tempranillo’ wine grapes. *Agric. Water Manag.* 112, 13–20. doi: 10.1016/j.agwat.2012.05.009
- Lorenz, D. H., Eichhorn, K. W., Bleiholder, H., Klose, R., Meier, U., and Weber, E. (1995). Growth Stages of the grapevine: phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*)—codes and descriptions according to the extended BBCH scale. *Aust. J. Grape Wine Res.* 1, 100–103. doi: 10.1111/j.1755-0238.1995.tb00085.x
- Lucini, L., Miras-Moreno, B., Busconi, M., Marocco, A., Gatti, M., and Poni, S. (2020). Molecular basis of rootstock-related tolerance to water deficit in *Vitis vinifera* L. cv. sangiovese: a physiological and metabolomic combined approach. *Plant Sci.* 299:110600. doi: 10.1016/j.plantsci.2020.110600
- Maas, E. V., and Hoffman, G. J. (1977). Crop salt tolerance-current assessment. *J. Irrig. Drain. Div.* 103, 115–134. doi: 10.1061/jrceaa.4.0001137
- Marguerit, E., Brendel, O., Lebon, E., Van Leeuwen, C., and Ollat, N. (2012). Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. *New Phytol.* 194, 416–429. doi: 10.1111/j.1469-8137.2012.04059.x
- Marín, D., Armengol, J., Carbonell-Bejerano, P., Escalona, J., Gramaje, D., Hernández-Montes, E., et al. (2021). Challenges of viticulture adaptation to global change: tackling the issue from the roots. *Aust. J. Grape Wine Res.* 27, 8–25. doi: 10.1111/ajgw.12463
- Medrano, H., Tomás, M., Martorell, S., Escalona, J. M., Pou, A., Fuentes, S., et al. (2015). Improving water use efficiency of vineyards in semi-arid

- regions. a review. *Agron. Sustain. Dev.* 35, 499–517. doi: 10.1007/s13593-014-0280-z
- Meggio, F., Prinsi, B., Negri, A. S., Simone Di Lorenzo, G., Lucchini, G., Pitacco, A., et al. (2014). Biochemical and physiological responses of two grapevine rootstock genotypes to drought and salt treatments. *Aust. J. Grape Wine Res.* 20, 310–323. doi: 10.1111/ajgw.12071
- Merkle, T., Frohnmeyer, H., Schulze-Lefert, P., Dangl, J. L., Hahlbrock, K., and Schafer, E. (1994). Analysis of the parsley chalcone-synthase promoter in response to different light qualities. *Planta* 193, 275–282. doi: 10.1007/BF00192541
- Mirás-Avalos, J. M., and Intrigliolo, D. S. (2017). Grape composition under abiotic constraints: water stress and salinity. *Front. Plant Sci.* 8:851. doi: 10.3389/fpls.2017.00851
- Munns, R., Passioura, J. B., Colmer, T. D., and Byrt, C. S. (2020). Osmotic adjustment and energy limitations to plant growth in saline soil. *New Phytol.* 225, 1091–1096. doi: 10.1111/nph.15862
- Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681.
- Netzer, Y., Shenker, M., and Schwartz, A. (2014). Effects of irrigation using treated wastewater on table grape vineyards: dynamics of sodium accumulation in soil and plant. *Irrig. Sci.* 32, 283–294. doi: 10.1007/s00271-014-0430-8
- OIV (1990). *Recueil des Methodes Internationales d'Analyses des Vins et Des Moûts*. Paris: Office Internationale de la Vigne et du Vin.
- Ollat, N., Peccoux, A., Papura, D., Esmenjaud, D., Marguerit, E., Tandonnet, J., et al. (2016). Rootstocks as a component of adaptation to environment. *Grapevine in a Changing Environment: a Molecular and Ecophysiological Perspective* eds Gers John Wiley & Sons: Hoboken, NJ 68–108. doi: 10.1002/9781118735985.ch4
- Pérez-Pérez, J., García-Sánchez, F., Robles García, J., and Botía, P. (2015). 'Star Ruby' grapefruit and 'Clemenules' mandarin trees show different physiological and agronomic responses to irrigation with saline water. *Irrig. Sci.* 33, 191–204. doi: 10.1007/s00271-014-0459-8
- Porro, D., Pedò, S., Bertoldi, D., Bortolotti, L., Failla, O., and Zamboni, M. (2013). *Evaluation of New Rootstocks for Grapevine: Nutritional Aspects*. Leuven: International Society for Horticultural Science (ISHS), 109–115.
- Prinsi, B., Failla, O., Scienza, A., and Espen, L. (2020). Root proteomic analysis of two grapevine rootstock genotypes showing different susceptibility to salt stress. *Int. J. Mol. Sci.* 21:1076. doi: 10.3390/ijms21031076
- Reshef, N., Agam, N., and Fait, A. (2018). Grape berry acclimation to excessive solar irradiance leads to repartitioning between major flavonoid groups. *J. Agric. Food Chem.* 66, 3624–3636. doi: 10.1021/acs.jafc.7b04881
- Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., et al. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 43, e47–e47. doi: 10.1093/nar/gkv007
- Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2009). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139–140. doi: 10.1093/bioinformatics/btp616
- Rodríguez-Ballesteros, C. (2016). *Clasificación Climática de Köppen-Geiger (para España)*. *Periodo de referencia 1981–2010*. [Online]. available Online in: <https://climaenmapas.blogspot.com/p/pagina-koppen.html> (22 April 2019).
- Saha, J., Brauer, E., Upadhyay, K., Sengupta, A., Popescu, S. C., Gupta, K., et al. (2015). Polyamines as redox homeostasis regulators during salt stress in plants. *Front. Environ. Sci.* 3:21. doi: 10.3389/fenvs.2015.00021
- Santesteban, L. G., Miranda, C., Marín, D., Sesma, B., Intrigliolo, D. S., Mirás-Avalos, J. M., et al. (2019). Discrimination ability of leaf and stem water potential at different times of the day through a meta-analysis in grapevine (*Vitis vinifera* L.). *Agric. Water Manag.* 221, 202–210. doi: 10.1016/j.agwat.2019.04.020
- Schultz, H. R. (2017). Issues to be considered for strategic adaptation to climate evolution—is atmospheric evaporative demand changing? *OENO One* 51, 107–114. doi: 10.20870/oeno-one.2016.0.0.1619
- Schultz, H. R., and Stoll, M. (2010). Some critical issues in environmental physiology of grapevines: future challenges and current limitations. *Aust. J. Grape Wine Res.* 16, 4–24. doi: 10.1111/j.1755-0238.2009.00074.x
- Shahid, M., Sarkhosh, A., Khan, N., Balal, R., Ali, S., Rossi, L., et al. (2020). Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy* 10:938. doi: 10.3390/agronomy10070938
- Shangguan, L., Chen, M., Fang, X., Xie, Z., Zhang, K., Zheng, T., et al. (2020). Comparative study of DAM, Dof, and WRKY gene families in fourteen species and their expression in *Vitis vinifera*. 3. *Biotech* 10:72. doi: 10.1007/s13205-019-2039-3
- Snedecor, G. W., and Cochran, W. G. (1989). *Statistical Methods*, 8th Edn. Ames, IA: Iowa State University Press.
- Soil Survey Staff (2006). *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*, 2nd Edn. Washington DC: United States Department of Agriculture, Natural Resources Conservation Service. Agriculture Handbook Number 436.
- Stevens, R. M., Harvey, G., and Partington, D. L. (2011). Irrigation of grapevines with saline water at different growth stages: effects on leaf, wood and juice composition. *Aust. J. Grape Wine Res.* 17, 239–248. doi: 10.1111/j.1755-0238.2011.00145.x
- Stevens, R. M., and Partington, D. L. (2013). Grapevine recovery from saline irrigation was incomplete after four seasons of non-saline irrigation. *Agric. Water Manag.* 122, 39–45. doi: 10.1016/j.agwat.2013.02.003
- Stevens, R. M., and Walker, R. R. (2002). Response of grapevines to irrigation-induced saline-sodic soil conditions. *Aust. J. Exp. Agric.* 42, 323–331. doi: 10.1071/ea00143
- Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U.S.A.* 102, 15545–15550. doi: 10.1073/pnas.0506580102
- Suter, B., Triolo, R., Pernet, D., Dai, Z., and Van Leeuwen, C. (2019). Modeling stem water potential by separating the effects of soil water availability and climatic conditions on water status in grapevine (*Vitis vinifera* L.). *Front. Plant Sci.* 10:1485. doi: 10.3389/fpls.2019.01485
- Tattersall, E. A. R., Grimmett, J., DeLuc, L., Wheatley, M. D., Vincent, D., Osborne, C., et al. (2007). Transcript abundance profiles reveal larger and more complex responses of grapevine to chilling compared to osmotic and salinity stress. *Funct. Integr. Genomics* 7, 317–333. doi: 10.1007/s10142-007-0051-x
- Teakle, N. L., and Tyerman, S. D. (2010). Mechanisms of Cl⁻ transport contributing to salt tolerance. *Plant Cell Environ.* 33, 566–589. doi: 10.1111/j.1365-3040.2009.02060.x
- Torres, N., Martínez-Lüscher, J., Porte, E., and Kurtural, S. K. (2020). Optimal ranges and thresholds of grape berry solar radiation for flavonoid biosynthesis in warm climates. *Front. Plant Sci.* 11:931. doi: 10.3389/fpls.2020.00931
- Tregeagle, J. M., Tisdall, J. M., Blackmore, D. H., and Walker, R. R. (2006). A diminished capacity for chloride exclusion by grapevine rootstocks following long-term saline irrigation in an inland versus a coastal region of Australia. *Aust. J. Grape Wine Res.* 12, 178–191. doi: 10.1111/j.1755-0238.2006.tb00058.x
- Upadhyay, A., Gaonkar, T., Upadhyay, A. K., Jogaiah, S., Shinde, M. P., Kadoo, N. Y., et al. (2018). Global transcriptome analysis of grapevine (*Vitis vinifera* L.) leaves under salt stress reveals differential response at early and late stages of stress in table grape cv. thompson seedless. *Plant Physiol. Biochem.* 129, 168–179. doi: 10.1016/j.plaphy.2018.05.032
- Urdanoz, V., and Aragüés, R. (2009). Three-year field response of drip-irrigated grapevine (*Vitis vinifera* L., cv. Tempranillo) to soil salinity. *Plant Soil* 324, 219–230. doi: 10.1007/s11104-009-9948-6
- van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy* 9:514. doi: 10.3390/agronomy9090514
- Vannozzi, A., Donnini, S., Vigani, G., Corso, M., Valle, G., Vitulo, N., et al. (2017). Transcriptional characterization of a widely-used grapevine rootstock genotype under different iron-limited conditions. *Front. Plant Sci.* 7:1994. doi: 10.3389/fpls.2016.01994
- Walker, R. R., Blackmore, D. H., and Clingeleffer, P. R. (2010). Impact of rootstock on yield and ion concentrations in petioles, juice and wine of shiraz and chardonnay in different viticultural environments with different irrigation water salinity. *Aust. J. Grape Wine Res.* 16, 243–257. doi: 10.1111/j.1755-0238.2009.00081.x
- Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., and Correll, R. L. (2002). Rootstock effects on salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. Sultana): 1. yield and vigour inter-relationships. *Aust. J. Grape Wine Res.* 8, 3–14. doi: 10.1111/j.1755-0238.2002.tb00206.x
- Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., and Correll, R. L. (2004). Rootstock effects on salt tolerance of irrigated field-grown grapevines (*Vitis*

- vinifera* L. cv. Sultana) 2. Ion concentrations in leaves and juice. *Aust. J. Grape Wine Res.* 10, 90–99. doi: 10.1111/j.1755-0238.2004.tb00011.x
- Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., and Emanuelli, D. (2014). Rootstock type determines tolerance of chardonnay and shiraz to long-term saline irrigation. *Aust. J. Grape Wine Res.* 20, 496–506. doi: 10.1111/ajgw.12094
- Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., and Iacono, F. (1997). Effect of salinity and Ramsey rootstock on ion concentrations and carbon dioxide assimilation in leaves of drip-irrigated, field-grown grapevines (*Vitis vinifera* L. cv. Sultana). *Aust. J. Grape Wine Res.* 3, 66–74. doi: 10.1111/j.1755-0238.1997.tb00117.x
- Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., and Tarr, C. R. (2007). Rootstock effects on salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. Sultana). 3. fresh fruit composition and dried grape quality. *Aust. J. Grape Wine Res.* 13, 130–141. doi: 10.1111/j.1755-0238.2007.tb00243.x
- Walker, R. R., Read, P. E., and Blackmore, D. H. (2000). Rootstock and salinity effects on rates of berry maturation, ion accumulation and colour development in Shiraz grapes. *Aust. J. Grape Wine Res.* 6, 227–239. doi: 10.1111/j.1755-0238.2000.tb00183.x
- Wang, H., Wang, W., Zhan, J., Yan, A., Sun, L., Zhang, G., et al. (2016). The accumulation and localization of chalcone synthase in grapevine (*Vitis vinifera* L.). *Plant Physiol. Biochem.* 106, 165–176. doi: 10.1016/j.plaphy.2016.04.042
- Williams, L. E., and Baeza, P. (2007). Relationships among ambient temperature and vapor pressure deficit and leaf and stem water potentials of fully irrigated, field-grown grapevines. *Am. J. Enol. Vitic.* 58, 173–181.
- Williams, L. E., Dokoozlian, N. K., and Wample, R. (1994). “Grape,” in *Handbook of Environmental Physiology of Fruit Crops*, Vol. I, eds B. Schaffer and P. C. Andersen (Boca Raton, FL: CRC Press), 85–133.
- Zarrouk, O., Brunetti, C., Egipto, R., Pinheiro, C., Genebra, T., Gori, A., et al. (2016). Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy. *Front. Plant Sci.* 7:1640. doi: 10.3389/fpls.2016.01640
- Zhang, X., Walker, R. R., Stevens, R. M., and Prior, L. D. (2002). Yield-salinity relationships of different grapevine (*Vitis vinifera* L.) scion-rootstock combinations. *Aust. J. Grape Wine Res.* 8, 150–156. doi: 10.1111/j.1755-0238.2002.tb00250.x
- Zhou-Tsang, A., Wu, Y., Henderson, S. W., Walker, A. R., Borneman, A. R., Walker, R. R., et al. (2021). Grapevine salt tolerance. *Aust. J. Grape Wine Res.* 27, 149–168.
- Zombardo, A., Crosatti, C., Bagnaresi, P., Bassolino, L., Reshef, N., Puccioni, S., et al. (2020). Transcriptomic and biochemical investigations support the role of rootstock-scion interaction in grapevine berry quality. *BMC Genomics* 21:468. doi: 10.1186/s12864-020-06795-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Buesa, Pérez-Pérez, Visconti, Strah, Intrigliolo, Bonet, Gruden, Pompe-Novak and de Paz. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Advantages of publishing in Frontiers



OPEN ACCESS

Articles are free to read
for greatest visibility
and readership



FAST PUBLICATION

Around 90 days
from submission
to decision



HIGH QUALITY PEER-REVIEW

Rigorous, collaborative,
and constructive
peer-review



TRANSPARENT PEER-REVIEW

Editors and reviewers
acknowledged by name
on published articles

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org

Contact us: frontiersin.org/about/contact



REPRODUCIBILITY OF RESEARCH

Support open data
and methods to enhance
research reproducibility



DIGITAL PUBLISHING

Articles designed
for optimal readership
across devices



FOLLOW US

@frontiersin



IMPACT METRICS

Advanced article metrics
track visibility across
digital media



EXTENSIVE PROMOTION

Marketing
and promotion
of impactful research



LOOP RESEARCH NETWORK

Our network
increases your
article's readership