

Mycorrhizal symbiosis improve fruit quality in Tempranillo grapevine sensitive to low-moderate warming

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ABSTRACT

An adequate clonal selection could help the adaptation of *Vitis vinifera* cv. Tempranillo to warming. Moreover, the resilience of Tempranillo to elevated air temperatures increases when associated with arbuscular mycorrhizal fungi (AMF). Our objective was to assess if mycorrhizal association can counteract the deleterious effect of elevated temperatures on plant performance and fruit quality in clones (CL) highly sensitive to warming. Fruit bearing cuttings of Tempranillo CL-843 were cultivated under greenhouse conditions. Assay included plants inoculated (+M) or not (-M) with AMF and grown at either 24/14°C or 28/18°C day/night air temperatures. Elevated temperatures shortened the period between fruit set and veraison in both -M and +M plants and also the period between veraison and maturity in +M plants. Photosynthetic rates were higher under warm temperatures irrespective of mycorrhizal inoculation, but sugars and proteins in leaves decreased in -M plants under these environmental conditions. Warming induced the accumulation of Ca, P, Cu and Mn in leaves of all plants and those of Mg and Zn in a greater extent in +M plants. Only in +M plants mature berries maintained the balance of sugars to organic acids and increased the Arg-to Pro-ratio under elevated temperatures. The association of Tempranillo with AMF may result in a more adequate source of N for yeasts during the must fermentation process and could mitigate the increased pH and ethanol levels found in the wines elaborated with grapes developed under low-moderate warming. However, extrapolating these findings to fields in the Mediterranean region or areas subjected to intense warming and frequent heatwaves deserves further study.

1. Introduction

Human activity, especially during the last 50 years, has clearly contributed to the increase in the concentration of greenhouse gases, responsible for global warming (Webb et al., 2013). Climate change is affecting southern Europe and the most recent data support the upward trend in temperatures over the last 30 years (Spinoni et al., 2015). According to the Intergovernmental Panel on Climate Change (IPCC, 2014), the global average temperature may increase by 4°C in the next 100 years, which will mean that the future climate scenario of the southern Mediterranean basin will not be favourable for viticulture

(Lionello et al., 2014). Forecasts for the Mediterranean area have not improved according to recent IPCC (2022). Experts affirm that air temperature and heat waves will increase throughout the 21st century above the global average, precipitation will decrease in most Mediterranean regions by between 4 and 22%, depending on the emission scenario and episodes of torrential rains will increase in the northern part and drought will be more prevalent in most areas. It is predicted that the continuous warming will be associated with more frequent, longer and more intense heatwaves, but even low to moderate warming may exert strong and early effect on groundwater storage and evapotranspiration (Condon et al., 2020).

Abbreviations: AMF, arbuscular mycorrhizal fungi.

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Respiration, transpiration and, in a larger extent, photosynthesis are metabolic processes strongly affected by temperature fluctuations (Venios et al., 2020). Therefore, both primary and secondary metabolisms are very influenced by elevated temperatures, which, ultimately, is reflected in the composition and nutritional quality of leaves and fruits in commercial (Torres et al., 2015, 2017, 2018a) and local (Goicoechea et al., 2021) varieties of grapevines. The imbalance between the accumulation of sugars and the phenolic maturity of the grape (Martínez-Lüscher et al., 2016; Arrizabalaga et al., 2018, 2020) contributes to the fact that not always the appropriate phenological and aromatic maturity is reached for the subsequent production of wine.

In Spain, the improvement of vine varieties for winemaking has been promoted through clonal selection, due to the existing Protected Designations of Origin for wine (Ibáñez et al., 2015). Thus, the selection of clones within any of these authorized varieties gives rise to plant material that is immediately accepted by the protection body. Clonal selection tries to exploit the intravarietal biodiversity originating from somatic mutations, selecting those plants with useful characteristics for viticulturists (Franco et al., 2020). Tempranillo grapevine, also known as Tinta Roriz, Aragonez or Valdepeñas and widely cultivated in several countries over the world for its wine of high quality, is an example of a commercial grapevine subjected to the selection of clones. Several studies (Arrizabalaga et al., 2018, 2020, 2021a;2021b; Torres et al., 2016, 2017, 2018b, 2018c) have demonstrated that the clonal biodiversity of Tempranillo translated into different abilities to respond to environmental constraints related to the climate change, so that an adequate clonal selection could help the adaptation of the vines to the warming conditions and increases in the atmospheric CO₂ levels predicted for the European Mediterranean area. Moreover, the association of Tempranillo with arbuscular mycorrhizal fungi (AMF) can enhance its resilience against environmental stresses, such as elevated air temperatures, water deficit or their interaction, in terms of improved antioxidant metabolism (Torres et al., 2015) and grape quality (Torres et al., 2018b, 2018c). Specifically, Tempranillo clone (CL) CL-843 increased its mycorrhizal dependency when subjected to low warming and/or drought conditions (Torres et al., 2018b), may be because of its high sensitivity to environmental stresses, reflected in the clear imbalance between sugars and anthocyanins found in mature berries when plants were not associated with AMF (Torres et al., 2017).

Considering all these precedent results, the general objective of this study was to assess if mycorrhizal symbiosis can counteract the deleterious effect of increased temperatures on fruit quality in Tempranillo clones highly sensitive to warming. Special attention was paid to the effect of mycorrhization on the phenology and physiology of the vine during the fruit development under different day/night temperature regimens. Research was carried out using CL-843 of Tempranillo due to its high sensitivity to moderate environmental constraints and its mycorrhizal dependence under abiotic stresses.

2. Material and methods

2.1. Biological material

Three-node segments of *V. vinifera* (L.) cv. Tempranillo CL-843, native of Oyón (Álava, Spain), were collected in the winter from an experimental vineyard of the Institute of Sciences of Vine and Wine (Logroño, Spain). Fruit bearing cuttings were produced as initially described in Mullins (1966) and then modified by Ollat et al. (1998) and Antolín et al. (2010). Rooting was made in a heat-bed (27°C) kept in a cool room (4°C) for 30 days. Fruit-bearing cuttings stand out as a useful model to study grapevine physiology under controlled environments (Morales et al., 2016) because their phenological stages and physiological responses to environmental factors are similar to those observed for conventional vines (Carbonell-Berejano et al., 2013).

Once rooting was successful, the cuttings were planted in 0.8 L plastic pots containing a mixture of vermiculite-sand-light peat (2.5/

2.5/1, v/v/v). Peat (N: 70–150 mg L⁻¹; P₂O₅: 80–180 mg L⁻¹; K₂O: 140–220 mg L⁻¹; pH: 5.2–6.0) (Floragard, Vilassar de Mar, Barcelona, Spain) was previously sterilized at 100°C for 1 h on three consecutive days. At transplanting, half of the plants (+M) were inoculated with the mycorrhizal inoculum Bioradis Gel (Bioera SLU, Tarragona, Spain). The inoculum was a mixture of five AMF (*Septoglomus deserticola*, *Funneliformis mosseae*, *Rhizogloium intraradices*, *Rhizogloium clarum* and *Glomus aggregatum*), and contained 100 spores per g of inoculum and a mixture of plant growth promoting rhizobacteria (PGPRs) belonging to the genera *Bacillus* and *Paenibacillus* (2×10^6 CFU g⁻¹). Mycorrhizal inoculum was produced by using trap plants for each type of mycorrhizal fungus, and then all AMF were mixed according the commercial formulation. The microbial preparation was diluted in distilled water (1:20) to ensure that each plant could receive 1 g of product. The inoculation was performed by submerging roots of fruit-bearing cuttings in the Bioradis Gel for 15 min. In order to restore rhizobacteria and other soil free-living microorganisms accompanying AMF, uninoculated plants (-M) were submerged for 15 min in a filtrate of the above-mentioned mycorrhizal inoculum. The filtrate was obtained by passing mycorrhizal inoculum through a layer of 15–20 µm filter paper with particle retention of 2.5 µm (Whatman 42; GE Healthcare, Little Chalfont, UK). Microorganisms accompanying AMF play an important role in the uptake of soil resources as well as on the infectivity and efficiency of AMF isolates (Agnolucci et al., 2015) and some PGPRs, such as *Bacillus* spp. isolated from vineyards, are known to benefit the basal immunity of grapevines against some pathogens (Trotel-Aziz et al., 2019). By restoring the bacterial component of the mycorrhizal inoculum in the rhizosphere of the -M plants, differences between -M and +M plants are expected to be mainly due to the presence of AMF associated with +M plants.

Then plants were placed in 6.5 L plastic pots (1 plant per pot) and transferred to two Growth Chamber Greenhouses (GCGs) adapted to simulate climate change conditions (Morales et al., 2014). Initial growth conditions were 25/15°C and 50/90% relative humidity (day/night) regime and natural daylight (photosynthetic photon flux density, PPFD, was on average 850 µmol m⁻² s⁻¹ at midday) supplemented with high-pressure sodium lamps (SON-T Agro Phillips, Eindhoven, Netherlands) to extend the photoperiod up to 15 h and ensure a minimum PPFD of 350 µmol m⁻² s⁻¹. Humidity and temperature were controlled by using M22W2HT4X transmitters (Rotronic Instrument Corp., Hauppauge, USA). PPFD was monitored with a LI-190SZ quantum sensor (LI-COR, Lincoln, USA). Plants were watered twice per day (140 mL day⁻¹) with the nutrient solution detailed by Ollat et al. (1998). The electric conductivity of the nutrient solution adjusted to pH 5.5 was 1.46 ± 0.15 mS cm⁻¹ as determined with a conductivity metre 524 Crison (Crison Instruments S.A., Alella, Spain) and the phosphorus (P) level was 9.78 mg L⁻¹.

2.2. Experimental design

After fruit set (Eichhorn and Lorenz (E-L) fruit stage 27) (Coombe, 1995) that took place about 50 days after bud-break, 12 -M and 12 + M plants were exposed to two temperature regimes (24/14°C or 28/18°C day/night), so that we had 6 + M plants and 6 -M plants at 24/14°C and 6 + M plants and 6 -M plants at 28/18°C. Temperature regimes were chosen according to the average temperature registered in La Rioja (Spain) during the growing season (1981–2010) (AEMET, Spain) and the projected rise in the global average temperatures of 4°C for 2081–2100 (IPCC, 2014). The excessive soil warming, which can negatively affect AMF infection, was strongly reduced by wrapping the pots with a reflecting material (Passioura, 2006; Poorter et al., 2012). Soil temperature was measured at 5 cm soil depth using probes PT100 (Coreterm, Valencia, Spain) and reached 23 ± 0.5°C and 28 ± 0.5°C for 24/14°C and 28/18°C temperatures, respectively. Plants remained in the GCGs until the berries reached commercial maturity (E-L 38 stage) (for 60 days, approximately) and were harvested separately based on the sugar

level (21–23°Brix) measured in berry subsamples (2–3 berries) weekly harvested. Leaves were harvested coinciding with the commercially ripe berries (E-L38 stage) and immediately frozen at -80°C for further analysis of organic solutes and minerals.

2.3. Mycorrhizal dependency of Tempranillo CL-843

The mycorrhizal dependency of grapevines was tested by calculating two parameters according to [Bagyaraj \(1992\)](#): The Relative Mycorrhizal Dependency (RMD) and the Mycorrhizal Inoculation Effect (MIE). The RMD gives information on the extent of growth or yield increase attributed to the mycorrhizal condition. In our study, RMD was calculated by using yield parameters as follows: dry weight of bunches produced by mycorrhizal grapevines/ dry weight of bunches produced by non-mycorrhizal grapevines. The MIE index allows the assessment of the extent to which inoculated fungi cause growth response. In our study MIE was calculated as follows: (dry weight of bunches produced by inoculated grapevines - dry weight of bunches produced by non-inoculated grapevines)/dry weight of bunches in inoculated grapevines. Data on RMD and MIE were expressed as percentages.

2.4. Determination of CO₂ exchange rate (CER), leaf conductance to water vapour (g_w) and transpiration rate (T)

CO₂ exchange rates (CER), leaf conductance to water vapour (g_w) and transpiration rates (T) were measured with a portable photosynthesis system (ADC-LCi, BioScientific Ltd., Hoddesdon, UK) in fully developed young leaves from 10.00 to 12.00 h under the above-mentioned greenhouse conditions with a photosynthetically active photon flux density (PPFD) of 1200 μmol m⁻² s⁻¹. Measurements were made coinciding with four stages of berry development: 1) when berries began to soften (E-L 34 stage, green berries); 2) when berries began to colour and enlarge (E-L 35 stage, veraison); 3) seven days after veraison (E-L 36 stage); and 4) fourteen days after veraison (E-L 37 stage).

2.5. Minerals in leaves at fruit harvest (E-L38)

Leaf samples (0.5 g dry weight, DW) were dry-ashed and dissolved in HCl according to [Duque \(1971\)](#). Phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu) were determined using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, USA). The operating parameters of the ICP-OES were: radio frequency power, 1300 W; nebulizer flow, 0.85 L min⁻¹; nebulizer pressure, 30 psi; auxiliary gas flow, 0.2 L min⁻¹; sample introduction, 1 mL min⁻¹ and three replicates per sample. Total nitrogen (N) and carbon (C) were quantified after combustion (950°C) of leaf DW with pure oxygen by an elemental analyser provided with a thermal conductivity detector (TruSpec CN, Leco, USA).

2.6. Total soluble proteins (TSP), proline (Pro), total soluble sugars (TSS) and starch in leaves at fruit harvest (E-L38)

Determination of TSP, Pro, TSS and starch was performed on 0.5 g of fresh leaves which were ground in an ice-cold mortar and pestle containing potassium phosphate buffer (50 mM, pH 7.0). The homogenates were filtered through four layers of cheese cloth and centrifuged at 28,710 × g at 4°C for 15 min. The supernatant was collected and stored at 4°C for TSP, Pro-and TSS determinations. The pellet was used to determine starch after iodine reaction ([Jarvis and Walker, 1993](#)). TSP were analysed with the protein dye-binding method ([Bradford, 1976](#)) and TSS with the anthrone reagent ([Yemm and Willis, 1954](#)) using, respectively, bovine serum albumin (BSA) and glucose as standards. Proline was analysed as described by [Rienth et al. \(2014\)](#). 500 mg of fresh leaves were powdered in liquid nitrogen, diluted 5 fold with deionized water and centrifuged at 3,000 × g for 10 min at 4°C. 750 μL

of the supernatant were mixed with the same volume of formic acid in a vortex for two min. Then, 750 μL of 3% ninhydrin in dimethylsulfoxide (daily prepared) were added and the mixture was heated at 100°C for 15 min. The absorbance was read at 520 nm.

2.7. Sugars, organic acids and amino acids profiles

Samples of 5–10 berries per plant (three plants for each treatment) were separated into skin and flesh. Berry flesh was crushed and filtered through gauze, and then extracts were centrifuged at 4100 × g at 4°C for 10 min. The supernatant was used for the determination of must total soluble solids by using a temperature-compensating refractometer (Zuzi model 315; Auxilab, Beriáin, Spain), and titratable acidity measured by titration with NaOH ([OIV, 2018](#)). The maturity index was calculated as the ratio of total soluble solids and titratable acidity.

Skins were powdered separately in an MM200 ball grinder (Retsch, Haan, Germany) and then, freeze dried in a Vir Tis Bench Top K lyophilizer (SP Scientific, Warminster, Philadelphia, PA, USA). Skins from each plant were used to analyse metabolites. Metabolites were extracted according to [Bobeica et al. \(2015\)](#) with minor modification. Subsamples of 50 mg fine powder of skins were extracted with 80% ethanol (v/v) at 80°C for 15 min followed by two extractions with 50% ethanol (v/v) and ultrapure water, respectively, dried in Speed-Vac, and re-dissolved in ultrapure water. The resultant extracts were used for determinations of sugars, organic acids and amino acids.

Sugars were measured enzymatically with an automated microplate reader (Elx800UV, Biotek Instruments Inc., Winooski, VT, USA) using the Glucose/Fructose kit from BioSenTec (Toulouse, France). Malic acid was determined using an enzyme-coupled spectrophotometric method that measures the change in absorbance at 340 nm from the reduction of NAD⁺ to NADH. Tartaric acid was assessed by using the colorimetric method based on ammonium vanadate reactions ([Pereira et al., 2006](#)). Both compounds were quantified with a Bran and Luebbe TRAACS 800 autoanalyzer (Bran & Luebbe, Plaisir, France).

After derivation with 6-aminoquinolyl-N-hydroxy-succinimidyl-carbamate (AccQ-Tag derivatization reagent, Waters, Milford, MA, USA) according to [Hilbert et al. \(2003\)](#), free amino acids were measured according to [Habran et al. \(2016\)](#). Briefly, amino acids were analysed using an UltiMate 3000 UHPLC system (Thermo Electron SAS, Waltham, MA USA) equipped with an FLD-3000 Fluorescence Detector (Thermo Electron SAS, Waltham, MA USA). Separation was performed on a AccQ-Tag Ultra column, 2.1 × 100 mm, 1.7 μm (Waters, Milford, MA, USA) at 37°C with elution at 0.5 mL min⁻¹ (eluent A, sodium acetate buffer, 140 mM at pH 5.7; eluent B, acetonitrile; eluent C, water) according to the gradient described by [Habran et al. \(2016\)](#). To maintain consistent retention time and a stable baseline, a control was performed before each run of 18 samples. Chromeleon software, version 7.1 (Thermo Electron SAS, Waltham, MA USA) was used to calculate peak area. A standard of 20 amino acids (Alanine, Arginine, Aspartic acid, Asparagine, Cysteine, GABA, Glycine, Glutamic acid, Glutamine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine, Valine) purchased from Sigma (St Louis, Missouri, USA) was used after the control and in the middle of each run to calibrate amino acid quantification. Seventeen amino acids were identified and quantified in skin extracts as described by [Pereira et al. \(2006\)](#). Results were expressed in μmol g⁻¹ dry matter (DM) of skin.

2.8. Anthocyanin and flavonol profiles in berry skins

Another subsample of powdered berry skins was used in order to analyse individual anthocyanins and flavonols. Samples were extracted according to [Acevedo de la Cruz et al. \(2012\)](#) then analysed as described in [Martínez-Lüscher et al. \(2014\)](#) with some modifications. Extracts were analysed using an UltiMate 3000 UHPLC system (Thermo Electron SAS, Waltham, MA USA) equipped with DAD-3000 diode array detector operating at 520 nm and at 360 nm (Thermo Electron SAS, Waltham,

MA USA). Separation was performed on a Syncronis C18, 2.1 × 100 mm, 1.7 µm Column (Thermo Fisher Scientific, Waltham, MA USA) at 25°C with elution at 0.368 mL min⁻¹ according to the following gradient (v/v): 0 min 92.2% A 7.8% B, 9.6 min 73% A 27% B, 14.1 min 70% A 30% B, 14.8 min 92.2% A 7.8% B (eluent A, water and formic acid, 90/10 v/v; eluent B, acetonitrile). Identification and peak assignment of phenolic compounds were based on comparison of their retention times and UV-vis spectrometric data with that of pure standards. Formal identification of flavonoids was performed by liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance spectrometry as in previous studies (Acevedo de la Cruz et al., 2012; Hilbert et al., 2015). Chromeleon software, version 7.1 (Thermo Electron SAS, Waltham, MA USA) was used to calculate peak area. The concentration of individual flavonoids was calculated in milligrams per gram (mg g⁻¹) of dry skin weight (DM) using malvidin-3-O-glucoside was used as external standard for all the quantified anthocyanins (at 520 nm), and quercetin-3-O-glucoside was used for all the quantified flavonols (at 360 nm) (Extrasynthese, Genay, France)

2.9. Statistical analysis

Statistical analyses were carried out using statistical software the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA) version 21.0 for Windows. After establishing the normal distribution of the residuals with the Shapiro-Wilk normality test due to the small sample size ($n = 3-5$) and the homogeneity of variance with the Levene test, data within each clone were subjected to a two-way analysis of variance (ANOVA) with or without Welch correction, taking into account if the requirement of the homogeneity of variances was fulfilled or not. The test allowed assessing the main effect of the factors temperature (T) (24/14 °C, 24 and 28/18 °C, 28), and AMF inoculation (M, +M and -M) and the interaction between them. Means ± standard errors (SE) were calculated and when the F ratio was significant ($P \leq 0.05$), a Duncan test was applied. Two-way ANOVA was performed to determine significant differences in measured parameters. Pearson's analyses were performed to determine the correlations between different parameters. Significant levels were always set at $p \leq 0.05$.

3. Results

Table 1 summarizes and compares the agronomic characteristics of the Tempranillo CL-843 cultivated in field or under greenhouse conditions, inoculated (+M) or not (-M) with AMF and grown at different day/night temperatures. The number of days since fruit set to veraison was very similar in the grapevines grown at field and in the +M grapevines cultivated in greenhouse at 24/14 °C. The increase of day/night temperatures by 4 °C caused a significant advancement of the veraison and, in +M plants, also shortened the period between veraison and maturity of fruits. Warming also caused the decrease in the bunch mass, being this effect especially evident in -M grapevines.

Under 24/14 °C, the Relative Mycorrhizal Dependency (RMD) of

Tempranillo CL-843 was below 100%, which indicates that under these environmental conditions mycorrhizal symbiosis did not have a beneficial effect on yield (Fig. 1). In contrast, mycorrhizal association favoured yield when plants were cultivated at 28/18 °C (RMD achieved values of 140%). Values of Mycorrhizal Inoculation Effect (MIE) corroborated that the application of AMF increased yield (by 20%) only when grapevines were subjected to elevated temperatures.

In general terms, photosynthetic rates were higher at 28/18 °C than at 24/14 °C during the whole process of fruit ripening in both -M and +M plants (Fig. 2a). This pattern of the photosynthetic rate was not parallel to that of the leaf conductance (Fig. 2b). Leaf conductance was similar in all treatments, irrespective of air temperature and mycorrhizal inoculation with the exception of the peak measured in leaves of -M plants at E-L36 stage. The behaviour of transpiration (Fig. 2c) was quite similar to that of leaf conductance, being transpiration values slightly higher in plants cultivated at 28/18 °C.

The concentrations of mineral nutrients and organic solutes in leaves are shown in Table 2. The two main factors included in the experimental assay, mycorrhization and temperature, influenced on the concentrations of several macronutrients. Mycorrhizal inoculation favoured the accumulation of Mg, but decreased the levels of K. Elevated temperatures induced the accumulation of Ca, P and Mg in leaves. Moreover, temperature was the factor that most affected the micronutrients concentrations in leaves. The levels of Cu and Mn clearly increased in leaves of grapevines grown at 28/18 °C. In contrast, the levels of starch, TSS and soluble proteins declined in leaves of grapevines subjected to warm temperatures, although the application of mycorrhizal inoculum allowed maintaining the sugars and proteins in levels similar to those measured at 24/14 °C.

The effect of mycorrhizal inoculation, air temperature and their interaction on the concentrations of soluble sugars and organic acids in grapes are shown in Table 3. Warm temperatures produced significant increases in the levels of soluble solids in both must (mainly, sugars) and skin (glucose and fructose), but only in grapes collected from -M plants. Moreover, must titratable acidity and skin tartaric acid sharply decreased in grapes from -M plants grown at 28/18 °C, but not in those from +M plants.

A total of 17 amino acids were detected in the skins of mature grapes (Table 4), being the most abundant arginine and proline. Air temperature affected the concentrations of asparagine, threonine, histidine and arginine irrespective grapevines had been or not inoculated with AMF. Except for asparagine, elevated temperatures induced the accumulation of these amino acids. Mycorrhizal inoculation was the most influencing factor on the levels of methionine since this amino acid was less abundant in the skin of grapes collected from +M plants. The amount of glutamic acid and valine significantly increased in -M grapevines when cultivated under elevated day/night temperatures and a similar behaviour was observed for glycine in grapes from +M grapevines. In contrast, elevated temperatures decreased the concentration of isoleucine in the skin of fruits from +M plants.

The profiles of anthocyanin derivatives in the skin of grapes (Table 5)

Table 1

Summary of the agronomic characteristics of the Tempranillo clon 843 (CL 843) according to the data obtained from greenhouse-grown fruit-bearing cuttings inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown either at 24/14°C or 28/18°C (day/night) temperature regimes, and the data collected from plants grown in field over the 2009–2012 period and provided by the Institute of Sciences of Vine and Wine (Logroño, Spain).

		Greenhouse-grown fruit-bearing cuttings				ANOVA			Field-grown plants
		Treatments				M	T	M × T	
		24/14°C		28/18°C					
		-M	+M	-M	+M				
Reproductive cycle	Fruit set-veraison (days)	53	60	39 b	40 a	ns	***	ns	61
	Veraison-maturity (days)	38 ab	50 a	44 ab	36 b	ns	ns	*	56
Yield components	Bunch mass (g bunch ⁻¹)	225 a	144 b	122 b	156 b	ns	**	**	199

Values from greenhouse-grown fruit-bearing cuttings represent means ($n = 3$) separated by Duncan test ($P \leq 0.05$) and different letters within rows indicate significant differences as affected by the main factors 'mycorrhizal inoculation, M', 'temperature, T', and their interaction (M × T). ns, *, ** and *** indicate non-significance or significance at 5%, 1% and 0.1% probability levels, respectively.

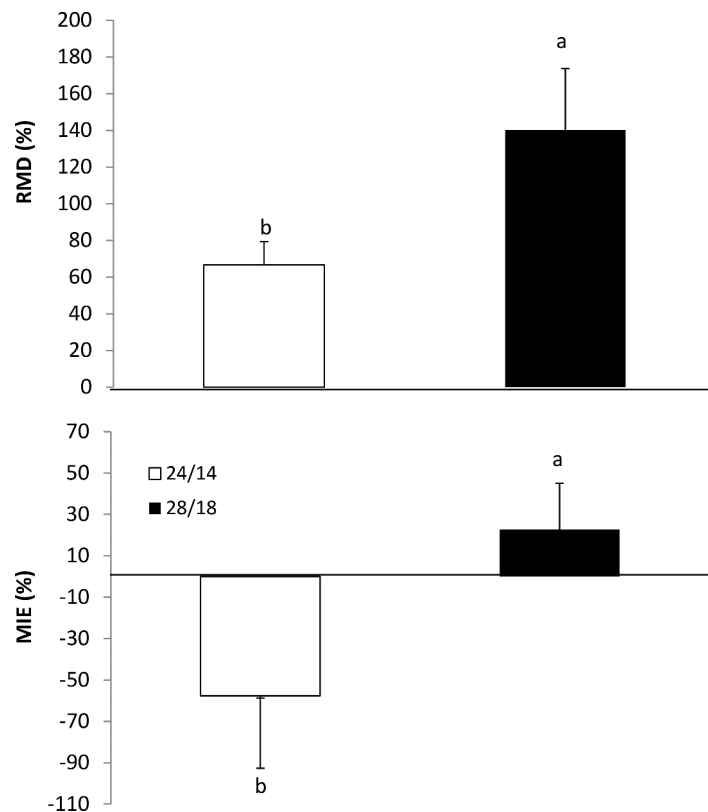


Fig. 1. Relative Mycorrhizal Dependency (RMD) and Mycorrhizal Inoculation Effect (MIE) in Tempranillo CL-843 grown at 24/14 °C (24/14) or 28/18 °C (28/18) day/night temperatures during berry growth and ripening. Values represent means \pm S.E. ($n = 4-6$). Within each graph, different letters indicate significant differences ($p \leq 0.05$) between temperature regimes according to Duncan test.

showed that the 3-monoglucosides represented more than 90% of the total in fruits collected from grapevines cultivated at 24/14 °C. This percentage lowered and reached values of 76–81% in grapes from plants grown at 28/18 °C. In contrast, warm temperatures increased 3-Acetylglucosides and 3-*p*-Coumaroyl-glucosides fractions. Air temperature was the factor that most influenced the levels of anthocyanin derivatives in grape skins; in general terms, the amount of these compounds decreased under elevated temperature. However, mycorrhizal association stopped the decrease of malvidin in the 3-Acetyl-glucosides and 3-*p*-Coumaroyl-glucosides fractions under warm day/night temperatures.

Six flavonols were detected in the skin of grapes at harvest, being myricetin-3-O-glucoside the most abundant irrespective of air temperature during fruit ripening or the presence of mycorrhizal fungi colonizing grapevine roots (Table 6). Elevated temperatures favoured the accumulation of quercetin-3-O-galactoside and laricitrin-3-O-glucoside, especially in fruits from -M plants. Under 24/14 °C, only grapes from +M plants accumulated kaempferol-3-O-glucoside; however, these levels significantly decreased under elevated temperatures.

4. Discussion

The effect of elevated temperatures on the stomatal conductance and thus on transpiration rates have been studied in several white and red varieties of grapevines. However, results of assays that examined the direct dependence of stomatal conductance on air temperature have been controversial: while stomatal conductance increased with increasing temperatures in grapevines grown in vineyards, it remained unchanged or even decreased in grapevines subjected to warming under controlled conditions (Galat Giorgi et al., 2019). According to Venios et al. (2020), apart from different sensitivities amongst varieties, the most influential factor on gas exchange parameters is the interaction between temperature and vapour pressure deficit (VPD). In our study,

warm day/night temperatures affected in a higher extent photosynthetic and transpiration rates (Figs. 2a, 2c) than leaf conductance (Fig. 2b) in Tempranillo CL-843. The increased photosynthesis in plants cultivated at 28/18 °C may be related to the fact that the carboxylation capacity of Rubisco can be enhanced in warm-grown plants thus compensating decreased Rubisco content (Cavanagh et al., 2022). In general terms, leaf conductance achieved similar values in grapevines cultivated under warm temperatures than in those grown at ambient temperature, which reveals that stomata did not close under high air temperatures. This fact leads us to conclude that Tempranillo CL-843 behaved as an anisohydric cultivar. This anisohydric behaviour is also supported by the higher transpiration rates found in grapevines subjected to high temperatures compared with those found in plants under ambient temperature (Fig. 2c), which may have contributed to both heat dissipation in order to reduce the damaging effects linked to elevated temperatures (Sade et al., 2012; Galat Giorgi et al., 2019) and enhanced mineral nutrient translocation from the roots to the leaves. In fact, the foliar concentrations of Ca, P, Mg, Cu and Mn were higher in grapevines subjected to elevated temperatures (Table 2) regardless they were or not inoculated with AMF. However, inoculation of AMF favoured the accumulation of Mg (Table 2; $M, p \leq 0.01$), independently of air temperature, and also that of Zn under elevated air temperatures (Table 2; $M \times T, p \leq 0.05$). In contrast with these results Torres et al. (2018c) did not observe a clear relationship between the transpiration rate at final fruit harvest (E-L38) and the levels of minerals in leaves of other clones of Tempranillo. The present study demonstrates that the accumulation of minerals in the vegetative organs of Tempranillo is dependant on the transpiration rates during the whole process of the fruit ripening, which was longer in -M than in +M plants (Table 1). The shorter period of time from the veraison to the maturity in +M grapevines together with the limited soil volume in pots may have restricted the effectiveness of AMF for improving the uptake of mineral nutrients in the present study, but the

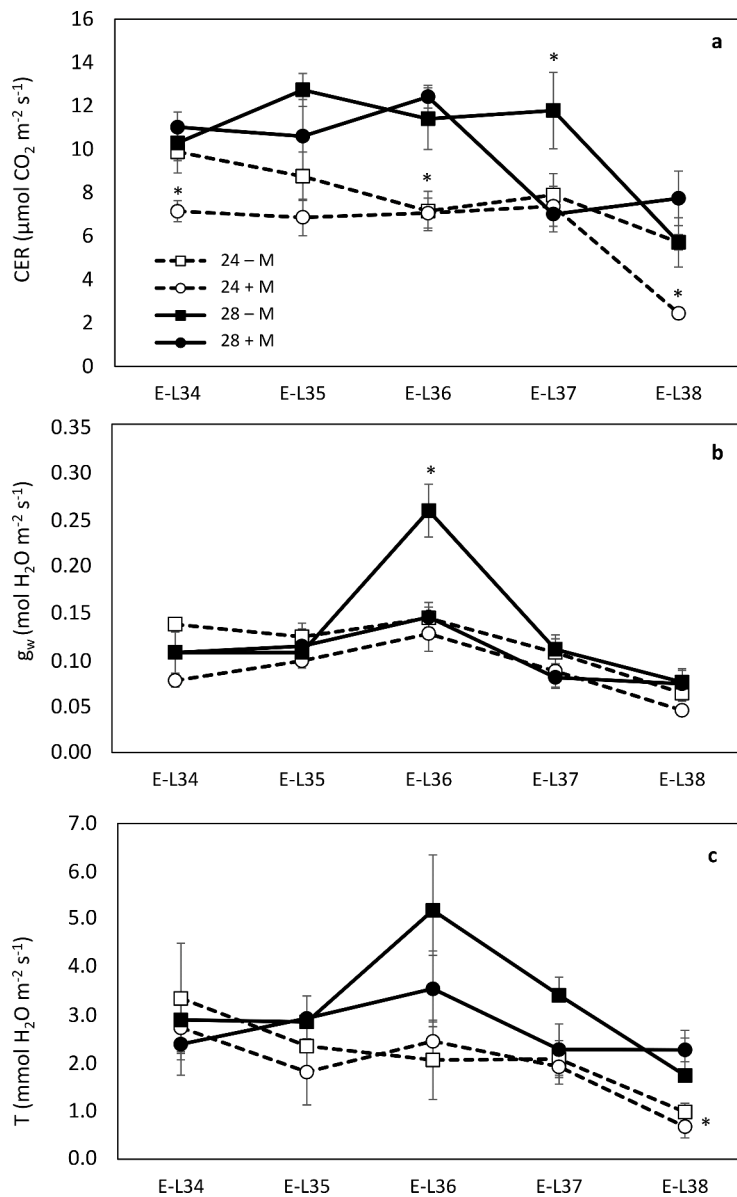


Fig. 2. CO_2 exchange rate (CER) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (a), leaf conductance to water vapour (g_w) ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (b) and transpiration rate (T) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (c) in leaves of Tempranillo CL-843 inoculated (+M) or not (-M) with arbuscular mycorrhizal fungi and grown at 24/14°C (24) or 28/18°C (28) day/night temperatures during berry growth and ripening. Values represent means \pm S.E. ($n = 4-6$). Asterisks indicate that value significantly differed ($p \leq 0.05$) from the rest of treatments according to Duncan test.

positive effect of AMF on the absorption of Mg and Zn under elevated temperatures might have enhanced the resilience of +M grapevines to heat stress (Khalil et al., 2018) since Mg helps to maintain the chloroplast structures and Zn favours the turgidity of the membranes. Similarly, the increased concentration of Ca in leaf tissues of -M and +M grapevines under elevated temperatures might have reduced the oxidative stress and benefited photosynthesis (Fig. 2a) (Khalil et al., 2018). Moreover, in +M grapevines, increased concentration of Ca in leaves may have mitigated the reduction in the levels of total soluble proteins (Table 2) under day/night warm temperatures (Goswami et al., 2015).

The concentrations of TSS in leaves of -M grapevines at harvest time (Table 2) sharply decreased under elevated temperatures in contrast with the significant increase of glucose and fructose measured in the fruit (Table 3), which suggests an important translocation of photoassimilates from the source (leaves) to the sink (fruits) organs. This hypothesis is supported by the significant correlation ($R = -0.893$, $P \geq 0.001$) found between the concentration of TSS in the leaves and the maturity index of the must. Contrariwise, +M plants had similar levels of sugars in both leaves (Table 2) and fruits (Table 3) under ambient and high air temperatures, which may be due to the additional sink of sugars

that supposes the presence of the mycorrhizal fungus associated to the grapevine roots. Mycorrhizal status presumably caused a distribution of the photoassimilates between two strong sinks: mycorrhizal fungus colonizing roots and fruits. According to Smith and Read (2008), the carbon flow from host to AMF in roots may vary from 4 to 20% of total photoassimilates depending on the relative activity, hierarchy and developmental stage of the different sinks within the plant (Staddon et al., 2003). Moreover, the ratio between soluble sugars and titratable acidity (maturity index) in mature grapes only increased in -M plants when cultivated under warm temperatures (Table 3). Predictions for a future warmer climate include the production of wines with less organic acids, increased pH and higher ethanol levels as a consequence of enhanced accumulation of soluble sugars in fruits (Venios et al., 2020). However, all these predictions, mainly attributed to the early harvests of grapes under warm scenarios, were only evident in -M plants (Table 3). Surprisingly, in our study, the most severe shortening in the days elapsed between fruit set and ripening (Table 1) corresponded to +M plants, so those that showed the most balanced ratio between sugars and organic acids in grapes at harvest. Recently, Pascual et al. (2022) concluded that bunch transpiration is positively correlated with the accumulation of TSS in grapes of Tempranillo. Therefore, it would be interesting to study

Table 2

Concentrations of mineral nutrients and organic solutes measured at harvest in leaves of fruit-bearing cuttings of ‘Tempranillo’ CL 843 inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (–M), grown either at 24/14°C or 28/18°C (day/ night) temperature regimes.

		Treatments				Main effects				ANOVA		
		24/14°C		28/18°C		Mycorrhiza (M)		Temperature (T)		M	T	M × T
		-M	+M	-M	+M	-M	+M	24	28			
Mineral nutrients	Macronutrients					483.8	483.0	484.1	482.7			
	(mg g ⁻¹ DM)	484.6	483.7	483.1	482.1					ns	ns	ns
	C											
	N	37.6	38.3	41.9	42.2	39.8	40.0	38.0	42.0	ns	ns	ns
	Ca	13.1	14.5	19.5	18.1	16.3	16.1	13.8 b	18.9 a	ns	**	ns
	K	8.8	6.5	7.9	6.8	8.4 a	6.6 b	7.6	7.4	**	ns	ns
	P	2.0	2.1	3.3	2.8	2.6	2.4	2.0 b	3.1 a	ns	***	ns
	Mg	2.3	3.5	3.9	4.5	3.1 b	4.0 a	2.9 b	4.2 a	***	***	ns
	Micronutrients					3.8	3.8	3.1 b	4.5 a			
	(µg g ⁻¹ DM)	3.1	3.1	4.4	4.7					ns	**	ns
	Cu											
Zn	27.7 a	19.5 b	26.7 a	30.0 a	27.2	24.2	23.6	28.2	ns	*	*	
Fe	59.9	57.4	66.7	68.5	63.3	62.3	58.6	67.5	ns	ns	ns	
Mn	148.2	133.0	212.0	207.8	180.1	166.2	140.6 b	210.1 a	ns	***	ns	
Organic solutes	Starch (mg g ⁻¹ DM)	11.0	10.8	9.7	9.8	10.3	10.3	11.0 a	9.7 b	ns	**	ns
	TSS (mg g ⁻¹ DM)	25.0 a	20.0 ab	15.1 b	23.3 a	20.0	21.3	22.3	18.7	ns	ns	**
	Proteins (mg g ⁻¹ DM)	4.5 a	3.6 b	1.0 d	2.2 c	2.8	2.9	3.9	1.6	ns	***	***
	Proline (µmol g ⁻¹ DM)	2.2	1.9	1.9	1.8	2.0	1.9	2.1	1.9	ns	ns	ns

Values represent means (n=3) separated by Duncan test (P≤0.05). Different letters within rows, indicate significant differences as affected by the main factors ‘mycorrhizal inoculation, M’, ‘temperature, T’, and their interaction (M × T). ns, *, ** and *** indicate non-significance or significance at 5%, 1% and 0.1% probability levels, respectively. DM and TSS indicate, respectively dry matter and total soluble sugars.

Table 3

Concentrations of soluble sugars and organic acids measured at harvest in grapes of fruit-bearing cuttings of ‘Tempranillo’ CL 843 inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (–M), grown either at 24/14°C or 28/18°C (day/ night) temperature regimes.

		Treatments				Main effects				ANOVA		
		24/14°C		28/18°C		Mycorrhiza (M)		Temperature (T)		M	T	M × T
		-M	+M	-M	+M	-M	+M	24	28			
Must	Total soluble solids (°Brix)	20.6 c	22.1 ab	22.3 a	20.9 bc	21.4	21.5	21.4	21.6	ns	ns	**
	Titrate acidity (mg L ⁻¹)	5.9 a	5.6 ab	4.0 b	4.7 ab	4.9	5.1	5.7 a	4.3 b	ns	*	ns
	Maturity index	3.64 b	4.02 ab	5.79 a	4.45 ab	4.72	4.24	3.83 b	5.12 a	ns	*	ns
Skin	Glucose (mg g ⁻¹ DM)	125.6 b	138.7 b	191.0 a	139.4 b	158.3	139.0	132.2 b	165.2 a	ns	*	ns
	Fructose (mg g ⁻¹ DM)	133.2 b	141.9 b	192.5 a	147.0 b	162.8	144.4	137.5 b	169.7 a	ns	**	*
	Malic acid (mg g ⁻¹ DM)	33.9	38.1	29.8	31.9	31.9	35.0	36.0	30.9	ns	ns	ns
	Tartaric acid (mg g ⁻¹ DM)	40.3 a	37.7 a	24.4 b	31.8 ab	32.4	34.7	39.0 a	28.1 b	ns	*	ns

Values represent means (n=3) separated by Duncan test (P≤0.05). Different letters within rows, indicate significant differences as affected by the main factors ‘mycorrhizal inoculation, M’, ‘temperature, T’, and their interaction (M × T). ns, *, ** and *** indicate non-significance or significance at 5%, 1% and 0.1% probability levels, respectively. DM indicates dry matter.

if the association of grapevines with AMF affects the transpiration rate of bunches through changes in the amount or composition of cuticular waxes in the fruits, especially when plants are undergoing warm temperatures. Precedent findings (Goicoechea et al., 2014) have demonstrated that mycorrhization can induce the deposition of epicuticular waxes in leaves of host plants under water deficit.

The profile of aminoacids accumulated in grapes is a key factor for the wine quality and may allow differentiate wines according to the grapevine variety (Mirás-Avalos et al., 2020). Results obtained in our study (see ANOVA in Table 4) demonstrate that the aminoacidic profile of a given grapevine variety (Tempranillo in our case) can be strongly conditioned by the air temperature, mycorrhizal symbiosis or the interaction between both factors. When cultivated at ambient day/night temperatures, mycorrhizal symbiosis induced changes in the levels of some minor (tyrosine, isoleucine, glutamine or histidine, for example) aminoacids, as well as in those of the two major aminoacids (arginine, Arg, and proline, Pro) (Bell and Henschke, 2005) synthesized in grapes. Elevated day/night temperatures favoured the accumulation of Arg-and Pro-in mature fruits of –M Tempranillo (Table 4), which contrasts with the reduced levels of proteins observed in the leaves (Table 2). The significant correlation found between Arg-and leaf proteins (R = -0.847,

P ≥ 0.001) indicates that warm temperatures could induce an important translocation of N from the vegetative to the reproductive organs. However, once more, the behaviour of +M grapevines under elevated temperatures differed from that observed in –M plants (Table 4): the concentration of Arg-in grapes increased (from 36.77 mmol g⁻¹ DM at 24/14°C to 52.49 mmol g⁻¹ DM at 28/18°C), but the level of Pro-clearly decreased (from 45.26 mmol g⁻¹ DM at 24/14°C to 27.56 mmol g⁻¹ DM at 28/18°C), suggesting that at least part of Arg-was synthesized from Pro. Considering that Arg-is one of the most relevant sources of N for yeasts thanks to its easy assimilation and that Pro-is the least preferred aminoacid (Gobert et al., 2017), we can conclude that the aminoacidic profile of grapes produced by Tempranillo CL-843 under warming temperatures improved for making wines when plants were associated with AMF. Gutiérrez-Gamboa et al. (2020) affirmed that vine varieties could be classified into two separate categories depending on the ratio of assimilable (Arg) and non-assimilable (Pro) nitrogen. According the data collected by Gutiérrez-Gamboa et al. (2020), the Arg-to Pro-ratio in the juice of Tempranillo grapes is around 1.00- 1.05. In our study, the ratio Arg/Pro-in grape skin was, respectively, 1.14 and 0.86 in –M and +M Tempranillo grown at ambient temperatures while this ratio achieved values of 2.06 and 1.96 in –M and +M plants when cultivated under

Table 4

Amino acid profiles measured at harvest in grape skins of fruit-bearing cuttings of ‘Tempranillo’ CL 843 inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown either at 24/14°C or 28/18°C (day/ night) temperature regimes.

Precursor	Amino acid	Concentration (mmol g ⁻¹ DM)								ANOVA				
		Treatments				Main effects				Temperature (T)		M	T	M × T
		24/14°C		28/18°C		Mycorrhiza (M)		24	28					
		-M	+M	-M	+M	-M	+M	24	28					
3-Phosphoglycerate	Glycine	0.31 b	0.25 b	0.38 b	0.96 a	0.34	0.61	0.28	0.66	*	**	**		
	Serine	2.22	2.61	3.19	3.19	2.70	2.90	2.41	3.19	ns	ns	ns		
Phosphoenolpyruvate	Tyrosine	0.28	0.64	0.51	0.47	0.40	0.56	0.46	0.49	ns	ns	ns		
	Phenylalanine	0.46	0.58	0.46	0.22	0.46	0.40	0.52	0.34	ns	ns	ns		
Oxaloacetate	Aspartic acid	2.65	2.74	3.08	2.37	2.87	2.56	2.70	2.73	ns	ns	ns		
	Asparagine	4.19	3.47	0.86	0.69	2.53	2.08	3.83 a	0.78 b	ns	***	ns		
	Threonine	6.25	6.83	10.97	11.95	8.61	9.39	6.34b	11.46 a	ns	*	ns		
	Methionine	0.03	0.06	0.05	0.02	0.04 a	0.01 b	0.02	0.03	**	ns	ns		
	Isoleucine	0.33 b	0.99 a	0.42 b	0.31 b	0.37	0.65	0.66	0.37	**	**	***		
α-ketoglutarate	Glutamic acid	3.16 b	3.23 b	5.13 a	2.22 b	4.14	2.73	3.20	3.68	*	ns	*		
	Glutamine	8.61	5.14	5.45	3.13	7.03	4.14	6.88	4.29	ns	ns	ns		
	Histidine	0.34	0.71	1.24	1.03	0.79	0.87	0.53 b	1.14 a	ns	*	ns		
	Arginine	29.94	36.77	55.27	52.49	42.60	44.63	33.35 b	53.88 a	ns	**	ns		
	γ-aminobutyric acid	3.32	4.18	4.50	4.72	3.91	4.45	3.75	4.61	ns	ns	ns		
	Proline	27.19	45.26	27.92	27.56	27.56	36.41	36.23	27.74	ns	ns	ns		
Pyruvate	Alanine	4.46	5.23	6.60	6.15	5.53	5.69	4.85	6.37	ns	ns	ns		
	Valine	0.74 b	1.01 ab	1.26 a	0.93 ab	1.00	0.97	0.88	1.09	ns	ns	*		

Values represent means (n=3) separated by Duncan test (P<0.05). Different letters within rows, indicate significant differences as affected by the main factors ‘mycorrhizal inoculation, M), ‘temperature, T’, and their interaction (M × T). ns, *, ** and *** indicate non-significance or significance at 5%, 1% and 0.1% probability levels, respectively. DM indicates dry matter. ND: not detected.

Table 5

Anthocyanin derivatives and their distribution into different fractions measured at harvest in grape skins of fruit-bearing cuttings of ‘Tempranillo’ CL 843 inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown either at 24/14°C or 28/18°C (day/ night) temperature regimes..

Compound		Concentration (mg g ⁻¹ DM)								ANOVA				
		Treatments				Main effects				Temperature (T)		M	T	M × T
		24/14°C		28/18°C		Mycorrhiza (M)		24	28					
		-M	+M	-M	+M	-M	+M	24	28					
3-Monoglucosides	Delphinidin	8.84	7.66	2.58	2.62	5.71	5.14	8.25 a	2.60 b	ns	***	ns		
	Cyanidin	3.26	2.62	0.33	0.19	1.80	1.40	2.94 a	0.26 b	ns	***	ns		
	Petunidin	5.83	4.79	1.87	0.86	3.86	2.86	5.31 a	1.37 b	ns	***	ns		
	Peonidin	4.32	3.62	1.22	1.48	2.77	2.55	3.97 a	1.35 b	ns	**	ns		
	Malvidin	11.83	8.96	7.64	7.83	9.74	8.39	10.40 a	7.73 b	ns	*	ns		
3-Acetyl-glucosides	Delphinidin	0.27	0.23	0.20	0.17	0.23	0.20	0.25 a	0.18 b	ns	*	ns		
	Petunidin	0.18	0.15	0.28	0.25	0.23	0.21	0.16 b	0.27 a	ns	***	ns		
	Peonidin	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	ns	ns	ns		
	Malvidin	0.36 bc	0.27 c	0.48 ab	0.60 a	0.42	0.43	0.32	0.54	ns	***	*		
3 p-Coumaroyl-glucosides	Peonidin	0.35	0.27	0.26	0.23	0.30	0.25	0.31 a	0.24 b	ns	*	ns		
	Malvidin	1.59 bc	1.16 c	1.80 b	2.38 a	1.69	1.77	1.38	2.09	ns	**	*		
Fractions														
3-Monoglucosides	% of total	92.30	93.00	81.20	75.90	86.70	84.40	92.60 a	78.60 b	ns	***	ns		
3-Acetyl-glucosides	% of total	2.30	2.20	5.80	6.90	4.00	4.50	2.23 b	6.31 a	ns	***	ns		
3 p-Coumaroyl-glucosides	% of total	5.50	4.90	13.00	14.90	9.20	9.90	5.16 b	14.00 a	ns	***	ns		
Total anthocyanins		36.37 a	29.73 a	14.35 b	16.02 b	25.35	22.88	33.05 a	15.19 b	ns	***	ns		

Values represent means (n=3) separated by Duncan test (P<0.05). Different letters within rows, indicate significant differences as affected by the main factors ‘mycorrhizal inoculation, M), ‘temperature, T’, and their interaction (M × T). ns, *, ** and *** indicate non-significance or significance at 5%, 1% and 0.1% probability levels, respectively. DM indicates dry matter. ND: not detected.

elevated temperatures. Our results demonstrate that the presence or absence of AMF colonizing grapevine roots can modulate the behaviour of the same grapevine variety (in our case, Tempranillo) under warming temperatures, thus adding another factor different from others previously known (maturity of grape, genetic attributes linked to the variety, clone or rootstock, some viticultural practices such as irrigation or soil and canopy management as well as the edaphoclimatic situation of the vineyard) (Gutiérrez-Gamboa et al., 2020) for conditioning the accumulation of Arg-and Pro-in mature fruits.

Flavonoids (anthocyanins and flavonols, amongst others), which are the main responsible for the colour of red grapes and wines, are very sensitive to environmental conditions. Bernardo et al. (2018) observed decreased levels of some anthocyanin derivatives (delphinidins, petunidins and peonidin-based anthocyanins) in grapes under high-temperatures while malvidin derivatives remained unchanged. We also measured reduced concentrations of the 3-Monoglucosides fraction of delphinidins, petunidins and peonidin-based anthocyanins in grapes from plants cultivated under elevated temperature (Table 5) and, in

Table 6

Individual composition of flavonols determined at harvest in grape skins of fruit-bearing cuttings of 'Tempranillo' CL 843 inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown either at 24/14°C or 28/18°C (day/ night) temperature regimes.

	Concentration (mg g ⁻¹ DM)				Main effects				ANOVA		
	Treatments		28/18°C		Mycorrhiza (M)		Temperature (T)		M	T	M × T
	-M	+M	-M	+M	-M	+M	24	28			
Myricetin-3-O-glucoside	0.61	0.59	0.60	0.47	0.61	0.53	0.60	0.54	ns	ns	ns
Quercetin-3-O-galactoside	ND	ND	0.06 a	0.04 b	0.03	0.02	0.00	0.05	ns	***	**
Quercetin-3-O-glucoside	0.13	0.11	0.15	0.08	0.14 a	0.09 b	0.12	0.11	*	ns	ns
Laricitrin-3-O-glucoside	0.05 c	0.07 c	0.17 a	0.12 b	0.11	0.10	0.06	0.15	ns	***	*
Kaempferol-3-O-glucoside	ND	0.23 a	0.10 b	0.09 b	0.05	0.16	0.12	0.09	***	ns	***
Isorhamnetin-3-O-glucoside	0.05	0.10	0.17	0.14	0.11	0.12	0.07 b	0.15 a	ns	*	ns

Values represent means (n=3) separated by Duncan test ($P \leq 0.05$). Different letters within rows, indicate significant differences as affected by the main factors 'mycorrhizal inoculation, M', 'temperature, T', and their interaction (M × T). ns, *, ** and *** indicate non-significance or significance at 5%, 1% and 0.1% probability levels, respectively. DM indicates dry matter. ND: not detected.

contrast with [Bernardo et al. \(2018\)](#), also decreased concentrations of the 3-Monoglucosides fraction of malvidins, the most abundant anthocyanin in cultivated grapes ([Durner, 2016](#)). According to [Arrizabalaga et al. \(2018\)](#), the reduced levels of anthocyanins in grapes of Tempranillo plants grown under high day/night temperatures could be explained by a relatively lower rate rather than a delayed rate of anthocyanin accumulation. Taking into account the numerous healthy properties (antioxidant and anti-inflammatory effects and beneficial role in cardiovascular and neurodegenerative diseases, diabetes and cancer) ascribed to these anthocyanins ([Salehi et al., 2020](#)) then present in red wines ([Fermo et al., 2021](#)), we can state that elevated air temperatures reduced the nutritional quality of Tempranillo grapes. In contrast, the concentrations of the 3-Acetyl-glucosides and 3-*p*-Coumaroyl-glucosides fractions of malvidins in grapes increased under warm conditions, being this increase more evident in grapes from +M plants than in those of -M plants (M × T, $P \geq 0.05$). This observation agrees with findings of [Yan et al. \(2020\)](#), who found increased proportion of acylated (both acetyl and *p*-coumaroyl) anthocyanins in berries of Merlot grapevines exposed to high temperature regimes maybe as a consequence of a regulatory mechanism at the transcriptional level. Despite the influence of elevated temperatures and mycorrhizal inoculation, the ratio of acetyl glucosides to coumaroyl glucosides in grapes of Tempranillo remained almost unchanged, ranging from 0.42 in -M plants at ambient temperature to 0.46 in +M plants under elevated temperatures, which is in line with the hypothesis that the ratio of these two fractions of anthocyanin derivatives is unique for varietal wines ([Holbach et al., 1997](#)).

The profile of flavonols in grapes of Tempranillo ([Table 6](#)) differed from that found in grapes of Merlot variety ([Yan et al., 2020](#)). In addition, these authors measured higher concentrations of flavonols in the berries from grapevines cultivated at low temperature regimes than in those collected from plants grown at intermediate or high temperature regimes, being these differences strongly related to the levels of transcripts of key flavonol genes. Contrariwise, day/night warm temperatures favoured the accumulation of some flavonols (quercetin-3-O-galactoside, laricitrin-3-O-glucoside and isorhamnetin-3-O-glucoside) in grapes of Tempranillo CL-843 ([Table 6](#)), although the presence of AMF limited the accumulation of these flavonols (M × T, $P \geq 0.01$ and $P \geq 0.05$ for quercetin-3-O-galactoside and laricitrin-3-O-glucoside, respectively). Increased levels of flavonols can provide photoprotection ([Martínez-Lüscher et al., 2014](#)) thus enhancing the resilience of Tempranillo grapevines to warm day/night temperatures. According to [Yan et al. \(2020\)](#), day temperature determines in a higher extent the accumulation of flavonoids in grapes than night temperature and [Gouot et al. \(2019\)](#) concluded that maximum bunch temperature achieved post-veraison is more critical for the levels and profiles of flavonoids than the duration of exposure to high temperatures.

5. Conclusion

The association of Tempranillo CL-843 with AMF increased the resilience of this variety to low-moderate warming. The most relevant contributions of mycorrhizal symbiosis were the maintenance of the ratio sugars to organic acids and the increased ratio Arg-to Pro-in mature grapes. Therefore, the benefit of the association of Tempranillo CL-843 with AMF could be realized in a more adequate source of N for yeasts during the fermentation process of the must and in the mitigation of the increased pH and ethanol levels found in the wines elaborated with grapes developed under warming.

However, despite these promising findings, the pessimistic perspectives for the impact of climate change in the Mediterranean region, may threaten the effectiveness of AMF for counteracting the deleterious effect of warming on grape quality. The possibility of finding low-moderate warming conditions in this area to test the beneficial effect of AMF might be limited to the vineyards established in higher altitudes. Moreover, as discussed by [Basiru and Hijri \(2022\)](#), the establishment in both soil and plant roots, persistence, effectiveness and effects on the indigenous mycorrhizal communities are difficult to predict for the mycorrhizal inoculants applied in field. Anyway, there are hopeful findings coming from Merlot grapevines in their first productive year in the vineyard since AMF inoculation benefited some berry quality traits and grapevine performance under water deficit ([Torres et al., 2021](#)).

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CRedit authorship contribution statement

Nieves Goicoechea: Conceptualization, Project administration, Resources, Supervision, Validation, Writing – review & editing, Writing – original draft. **Nazareth Torres:** Data curation, Investigation, Methodology. **Idoia Garmendia:** Data curation, Methodology. **Ghislaine Hilbert:** Methodology. **María Carmen Antolín:** Conceptualization, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they do not have any financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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