Impact of 2100-Projected Air Temperature, Carbon Dioxide and Water Scarcity on Grape Primary and Secondary Metabolites of Different *Vitis vinifera* cv. Tempranillo Clones

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ABSTRACT

The exploration of the grapevine (*Vitis vinifera* L.) intra-varietal diversity can be an interesting approach for the adaptation of viticulture to climate change. We evaluated the response of four Tempranillo clones to simulated year-2100-expected air temperature, CO_2, and relative humidity (RH) conditions: climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1} CO_2, and 35 %/53 % RH) *vs* current situation conditions (CS; 24 °C/14 °C, 400 µmol mol^{-1} CO_2 and 45 %/63 % RH), under two irrigation regimes, "well-watered" (WW) *vs* "water deficit" (WD). The treatments were applied to fruit-bearing cuttings grown under research-oriented greenhouse controlled conditions. CC increased sugar accumulation and hastened grape phenology, an effect that was mitigated by water deficit. Both CC and water deficit modified amino acid concentrations and accumulation profiles with different intensities, depending on the clone. Combined CC and water deficit decreased anthocyanins and the anthocyanin to total soluble solids (TSS) ratio. The results suggest differences in the response of the clones to the 2100-projected conditions, which are not always solely explained by differences observed in the ripening dynamics. Among the clones studied, RJ43 and CL306 were the most affected by CC/WD conditions, meanwhile 1084 was globally less affected than the other clones.

Keywords: amino acids; anthocyanins; climate change; grapevine (*Vitis vinifera* L.); organic acids; sugars; Tempranillo; water deficit

INTRODUCTION

Nowadays, one of the most worrying environmental issues for viticulture is the modification of air composition and the consequences that this swift change is expected to provoke in the near future. The Intergovernmental Panel on Climate Change (IPCC) considers different scenarios for the end of the century (Representative Concentration Pathways, RCTs). In the worst cases (scenarios RCP 6.0 and RCP 8.5), the IPCC estimates a rise in the global mean temperature between 2.2 ± 0.5 °C and 3.7 ± 0.7 °C and an atmospheric CO_2 concentration between 669.7 and 935.9 ppm for 2100 [1]. In addition, a swift in the precipitation regime around the globe is expected. In some areas, such as the Mediterranean region, medium or long drought periods are forecasted to be 3 to 8 times more frequent than nowadays [2]. In addition, near-surface land relative humidity (RH) is estimated to be reduced in the coming years as a result of climate change [3]. These environmental changes are expected to severely affect fruit crops performance due to its great sensitivity on abiotic factors.

Fruit crops yield and quality are affected by different environmental factors including air temperature, atmospheric CO_2, and water availability. In the case of grapevine, the research done so far points towards an earlier harvest, mainly associated with the increase in air temperature [4–7]. In addition, changes in grape composition have been reported in response to warm temperatures, with reductions in total must acidity and malic acid concentration [8–10] concomitant with higher sugar accumulation rates [11]. High temperatures are also known to reduce anthocyanin accumulation [8,12,13], as well as to produce an imbalance between anthocyanins and sugars in ripe berries [14]. However, the impact of elevated CO_2 on grape composition has been less thoroughly studied. Some authors reported a decrease in must acidity and an increase in total soluble solids (TSS) [10]. Other studies concluded, however, that the expected rise in CO_2 may not cause negative repercussions on the quality of the grapes [15,16, 17]. In some cases, elevated CO_2 led to a decrease in some grape constituents, such as malic acid and amino acid contents, without affecting sugar and anthocyanin levels [18].

Research on water scarcity effects has determined that drought might provoke a reduction of berry size [19] and organic acid concentration [20], an increase in sugars [21], and the promotion of the anthocyanin biosynthetic pathway [22]. Furthermore, Deluc et al. reported an impact of water stress on the amino acid content of a red grape cultivar (Cabernet Sauvignon) but not on a white one (Chardonnay) [23].

Although the literature about the combined effects of these environmental factors is not so extensive, authors suggest that CO_2 does not seem to modify the impact of elevated temperature on malic acid, sugars, and anthocyanins [6], while we have recently reported their cumulative effects on malic acid breakdown and sugar accumulation [18]. Additionally, elevated temperature, high CO_2 and drought applied simultaneously reduced berry malic acid content and total polyphenol index, increasing color density in grapes of Tempranillo, without affecting the total anthocyanin concentration [24]. In the case of amino acids, some authors have reported that both drought [25] and its combination with high temperatures [11] increased the amino acid concentration. However, studies in the recent literature are too scarce to draw a clear picture of the interactive effects of high temperature, elevated CO_2, and water deficit on the accumulation of grape quality related metabolites.

Different strategies have been suggested to mitigate the potential negative impact of the projected future environmental conditions on grape composition. Among them, selecting a better adapted plant material has been proposed as one of the most powerful tools to face climate change [26]. Clonal diversity within grapevine cultivars has been studied for a broad range of characteristics, including phenological development, probable alcohol and titratable acidity, color for the red varieties or aroma for the white ones, as well as disease resistance [27]. This diversity may allow the selection of clones with traits that are expected to reduce the potential negative impact of climate change. Thus, the use of late-ripening clones has been proposed as an adaptive approach to be explored in order to compensate the shortening of the ripening period lead by warmer conditions [28].

The present work is focused on Tempranillo, a variety well settled in Spain, more specifically in La Rioja, País Vasco and Navarra. This cultivar has a large number of clones characterized and commercialized [27], even

though a few studies have actually explored their performance under the projected environmental conditions, including changes in the most important climate change-related factors such as temperature, air CO_2, or water availability. We have previously highlighted that Tempranillo clones presented differences in their response to elevated temperature regarding sugar and anthocyanin accumulation [29]. We have also reported that Tempranillo clones showed different phenological developments in response to elevated CO_2 and increased temperature, particularly for vegetative production and carbon partitioning into organs [30] as in terms of grape composition [18]. Moreover, the differences among clones observed in terms of phenological development seemed to condition the impact of the environmental conditions assayed on the vegetative growth, especially that of water deficit [31]. Because of this, we hypothesized that clones with a longer reproductive cycle may be less affected than the early ripening clones in terms of berry composition, since the former ripening would take place under colder conditions. Then, differences in the length of the ripening period may modulate the impact of climate change related factors on grape composition. The objective of this work was to evaluate the response of four Tempranillo clones with different reproductive cycle lengths to a combination of high temperature, elevated air CO_2 concentration and low relative humidity, under different water availabilities, focusing on plant phenology, the evolution over the ripening period of must characteristics (organic acids, sugars and amino acids) and skin anthocyanin levels.

MATERIAL AND METHODS

Plant Material: Origin and Development

Four clones of grapevine (*V. vinifera*) cv. Tempranillo were used in the experiment: RJ43, CL306, VN31, and 1084. The plant material was obtained from the germplasm bank of: Estación de Viticultura y Enología de Navarra (EVENA, Navarra, Spain), RJ43 and CL306 (the most widely used Tempranillo clones in Spain); Vitis Navarra (Navarra, Spain), VN31; and Instituto de Ciencias de la Vid y del Vino (ICVV, La Rioja, Spain), 1084 (non-commercialized). These clones were selected on the basis of differences in their reproductive cycle length, previously characterized by the providers as intermediate-reproductive cycle (RJ43) [32], short-reproductive cycle (CL306) [33], and long-reproductive cycle (both VN31 and 1084) [29,34].

The selection, activation, and growth of 400–500 mm-long dormant cuttings were carried out according to an adapted protocol from Mullins and Rajasekaran [35] described in detail by Arrizabalaga-Arriazu et al. [29] Only a single inflorescence per plant was allowed to grow and develop into a cluster, and the irrigation throughout all the experiment was done with the nutritive solution described by Ollat et al. [36]

Experimental Design

A detailed description of the experimental design was previously reported [31]. Briefly, at fruit set, plants of the four clones with similar phenological stage and bunch size characteristics were divided homogeneously and placed in growth chamber-greenhouses (GCGs) settled at different temperature, CO_2 concentration, and relative humidity (T/CO_2/RH) regimes: 24 °C/14 °C (day/night), 400 µmol mol^{-1} CO_2, and RH of 45%/63% (day/night) (current situation conditions, CS) *vs* 28 °C/18 °C (day/night), 700 µmol mol^{-1} CO_2, and RH of 35%/53% (day/night) (climate change conditions, CC). In order to maintain a photoperiod of 14h during plant growth, natural light was supplemented with a system of high-pressure sodium lamps (HQT-TS 400W/D Osram, Augsburg, Germany), which was triggered when photosynthetically active radiation (PAR) dropped below a photon flux density of 1000 µmol m^{-2} s^{-1}. Within each greenhouse, plants were subjected to two irrigation regimes: well-watered (WW) *vs* water deficit (WD, receiving 60% the water applied to the WW plants). IPCC predictions were considered for setting the temperature and CO_2 conditions of the CC treatment, [1] while the RH conditions were set according to ENSEMBLES models developed based on the MPI-ECHAM5 Max Planck Institute model and IPCC data. According to those models, in 2100, the RH during summer for the area of Navarra and La Rioja will be 12% lower than current situation [37]. The WD treatment was determined according to the expected conditions predicted by the model of the Max Planck Institute for the North of Spain at the end of the present century, referring a summer precipitation 40% lower [37]. Soil water content was monitored with soil moisture sensors (EC-5 Soil Moisture Sensors, Decagon Devices Inc., Pullman, WA, USA). Plants under WD treatment were watered when the correspondent sensor marked a soil humidity lower than 10% (m^3 H_2O × 100 m^{-3} substrate) [24]. Then, they received 60% of the equivalent volume of solution used for watering WW plants during the corresponding drought period. In the case of WW plants,

moisture levels were kept at ca. 80-90% of the substrate field capacity (sensor value between 30 and 40 %, m^3 H_2O × 100 m^{-3} substrate). Pre-dawn leaf water potential was measured at mid-veraison and 2 weeks after mid-veraison using a pressure chamber SKYE SKPM 1400 (Skye Instruments Ltd, Llandrindod, Wales, UK), according to Arrizabalaga- Arriazu et al.[31]. Average values are included in Table S1. Plants of all the treatments received the same amount of nutrients. For that, WW plants were irrigated alternating nutrient solution with plain water, whereas WD plants were always irrigated with nutrient solution. Each clone was represented with between 7 and 8 plants per treatment.

Phenological Development

The dates of fruit set, mid-veraison (half of the berries in the bunch had started to change color) and maturity (total soluble solid content, TSS, of ca. 22 °Brix) were annotated for each plant individually, making it possible to calculate the elapsed time between fruit set and mid-veraison and between mid-veraison and maturity. The determination of both fruit set and mid-veraison was accomplished visually (berry diameter around 2 mm and half of the berries in the bunch turned red color, respectively). Maturity was determined by measuring periodically the levels of TSS of two berries per bunch during the last weeks of development (every 2 or 3 days) until they reached a TSS level of ca. 22 °Brix.

Sample Collection

Berries were harvested at mid-veraison, 1 week after mid-veraison, 2 weeks after mid-veraison and maturity (defined previously), frozen and stored at -80 °C. The number of berries per bunch sampled was either 3 or 4, except at maturity, when 10 berries per bunch were taken. Berry volume was estimated by measuring the diameters of 3 berries per bunch (10 berries at maturity) and applying the formula of the volume of a spheroid ($volume = \frac{4}{3}\pi \times r_1^2 \times r_2$; being r_1 the equatorial radius and r_2 the polar radius).

For carrying out the analyses, pools of berries taken from two or three different plants (3– 4 berries per plant) were prepared and berries were handled according to a protocol adapted from and Torres et al. [11] Berries were weighed, and the skin, pulp, and seeds were separated. The skin and seeds were weighed, and

the relative skin mass determined using the quotient between skin fresh weight (FW) and berry FW, expressed as a percentage. Frozen pulp was ground using an MM200 ball grinder (Retsch, Haan, Germany). The skin was ground with a MM200 ball grinder (Retsch, Haan, Germany) after freeze-drying (Alph1-4, CHRIST, Osterode, Germany) to carry out the anthocyanin analyses. The fresh bunch weights determined at maturity were previously reported [31]. The data are resumed in Figure S1.

Total Soluble Solids, Sugars, Organic Acids and Amino Acids Profiles

Total soluble solids (TSS) content in must was measured as described by Arrizabalaga- Arriazu et al.[18] Primary metabolites in berries were extracted according to an adapted protocol from Torres et al. [11] Briefly, 250 mg of frozen powdered pulp was extracted with decreasing concentrations of ethanol (80%, 50% and 0% ethanol (v/v)), dried using a Speed-Vac (SAVANT SC 110A, Thermo Fisher Scientific, Waltham, MA, USA), and re-suspended in ultrapure water. The obtained extracts were used for the analyses of sugars, organic acids, and amino acids.

Sugar analysis was carried out according to the manufacturer, by measuring enzymatically both the glucose and fructose concentration with an automated absorbance microplate reader (Elx800UV, Biotek Instruments Inc., Winooski, VT, USA) using the Glucose/Fructose kit from BioSenTec (Toulouse, France). The results are presented as the sum of glucose and fructose and referred to as total sugars.

Malic and tartaric acids were analyzed with automated colorimetric methods using a Bran and Luebbe TRAACS 800 autoanalyzer (Bran & Luebbe, Plaisir, France) as previously described by Arrizabalaga-Arriazu et al. [18] Malic acid determination was based on the detection of NADH at 340 nm, formed by the reduction of NAD^+ during the enzymatic conversion of L-malate to oxaloacetate by L-malate dehydrogenase (L-MDH). Tartaric acid determination was based on a colorimetric method with ammonium vanadate reactions. The results are presented as the concentration of malic acid and the sum of malic and tartaric acid (referred to as total acidity according to Iland et al.[38]).

For the free amino acid determination at maturity, samples extracts were derived with 6-aminoquinolyl-N-hydroxy-succinimidyl-carbamate (AccQ-Tag derivatization reagent, Waters, Milford, MA, USA) according to

Arrizabalaga-Arriazu et al. [18] and the references within, 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). Free amino acid separation was achieved by using an 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). Chromatographic analyses were carried out using an excitation wavelength of 250 nm and an emission wavelength of 395 nm. The identification and quantification of 19 amino acids (excluding tryptophan) were done as previously described [18]. In order to keep a stable baseline and a consistent retention time over the analysis, a control analysis was carried out as described by Torres et al. [11]

Total Anthocyanins

Anthocyanin concentration in berry skins was determined according to Arrizabalaga-Arriazu et al. [18] Briefly, ground dried skins were extracted with methanol containing 0.1 % HCl (v/v) and the obtained solution was filtered using a polypropylene syringe filter of 0.45 µm (Pall Gelman Corp., Ann Arbor, USA). The separation of the different compounds was carried out with a Syncronis C18, 2.1 × 100 mm, 1.7 µm column (Thermo Fisher Scientific, Waltham, MA, USA) in an UltiMate 3000 UHPLC system (Thermo Electron SAS, Waltham, MA, USA) equipped with a DAD-3000 diode array detector (Thermo Electron SAS, Waltham, MA, USA). The chromatographic analysis was done with a detection wavelength of 520 nm and malvidin-3-O-glucoside as the external standard sample (Extrasynthese, Genay, France). The obtained chromatograms were analyzed with the Chromeleon software (version 7.1) (Thermo Electron SAS, Waltham, MA, USA) in order to calculate concentration from the peak area of each individual anthocyanin. The concentration of total anthocyanins was calculated as the sum of the concentration of the individual anthocyanins determined.

Statistical Analysis

The statistical analysis carried out for each parameter consisted in a three-way ANOVA (clone, T/CO_2/RH regime and irrigation regime) and a Fisher's least significant difference (LSD) as a post-hoc test when

statistically significant differences were found ($P < 0.05$). The analyses were done using the software R (3.5.3).

RESULTS

Phenological Development

The length of the period between fruit set and mid-veraison and, especially, between mid-veraison and maturity was significantly different among clones, the 1084 accession having the longest ripening period (mid-veraison to maturity) (Figure 1A). Considering the clones altogether, CC significantly reduced the number of days to reach mid-veraison compared with CS plants, but it did not affect the length between mid-veraison and maturity (Figure 1B). Also, WD slowed down, in general, the phenological development. However, a significant interaction between the clone and irrigation regime was observed for the elapsed time between mid-veraison and maturity. In this way, the 1084 accession was much less affected by water deficit than the other clones (Figure 1A).

Berry Volume and Relative Skin Mass

The volume of the grapes differed among clones from mid-veraison onward, the 1084 and RJ43 accessions showing the biggest berries and VN31 the smallest ones at maturity (Table 1). CC significantly reduced berry volume at mid-veraison, but this effect disappeared during the rest of the ripening period, and bunch weight at maturity was similar under CS and CC conditions (Figure S1). Water deficit significantly reduced berry volume whatever the developmental stage considered and globally reduced bunch weight at maturity. A significant interaction among the clone, T/CO_2/RH regime, and irrigation was observed at maturity for berry volume. Notably, the RJ43 accession was very affected by water deficit, especially under CC conditions, while the impact of WD on CL306 and VN31 was similar under CC and CS conditions. However, the bunch weight of CL306 was drastically reduced at maturity by water deficit under CC conditions. Also, a berry volume of 1084 was strongly affected by water deficit under CS but not under CC conditions (Table 1).

In general, WD significantly increased the grape relative skin mass at mid-veraison, 1 week after mid-veraison, and maturity, and CC conditions had a similar effect 2 weeks after mid-veraison and at maturity (Table 2). However, at maturity, a significant interaction between the clone and T/CO_2/RH regime as well as between the clone and irrigation regime was observed, which was reflected in the significant reduction in the relative skin mass of the 1084 grapes when CC and WD were combined, contrary to what happened in the rest of the clones. At maturity, the 1084 accession exhibited the lowest relative skin mass values; meanwhile, RJ43 and CL306 presented the highest values, and this effect being more evident under CC and WD conditions.

Malic Acid and Total Acidity

Malic acid concentration and total acidity (the sum of malic and tartaric acids) in the must decreased throughout the ripening process and differed among clones (Figure 2A,B). The 1084 accession had significantly lower malic acid levels compared with the rest of the clones at mid-veraison and maturity, regardless of the T/CO_2/RH and irrigation regimes (Figure 2A), whereas VN31 had a lower total acidity than the rest of the clones at mid-veraison, 2 weeks after mid-veraison, and maturity (Figure 2B). When analyzed altogether, grapes from all clones grown under CC conditions showed a higher malic acid concentration and total acidity at mid-veraison, compared with CS. However, in later stages, these levels dropped faster in the CC treatment, reaching lower values than in CS plants both 2 weeks after mid-veraison and at maturity (Figure 2A,B). WD did not affect significantly the concentration of malic acid in grapes. However, total acidity at maturity was generally lower in WD plants compared with WW plants (Figure 2B,C) due to a significant reduction in tartaric acid from 4.42 ± 0.14 mg g^{-1} pulp FW to 3.97 ± 0.13 mg g^{-1} pulp FW ($P < 0.001$, data not shown). At maturity, the total acidity of grapes ripened under CC/WD was significantly lower than in grapes ripened at CS/WW (Figure 2C).

Sugars and Total Soluble Solids

The concentration of total sugars (the sum of glucose and fructose) was lower in the berries of the 1084 accession, from 1 week after mid-veraison onward, compared with the other clones (Figure 3A). Considering

the clones altogether, compared to CS, CC significantly increased the sugar concentration 1 and 2 weeks after mid-veraison (in mg g^{-1} pulp FW, from 72.73 ± 2.96 to 89.03 ± 3.70 and from 142.20 ± 4.28 to 162.89 ± 4.22, respectively). WD reduced the sugar levels during the whole ripening process compared with WW plants. One week after mid-veraison, there was a significant interaction between the T/CO_2/RH and irrigation regimes, the plants grown under combined CC and WW conditions showing the highest sugar contents at this stage (Figure 3A). The total sugar concentration 2 weeks after mid-veraison was similar in plants grown at CS/WW and CC/WD (Figure 3B). Similar trends were observed for total soluble solids compared to sugars, regarding 1084 accession's behavior or CC and WD impact, individually or combined (Table S2).

Amino Acids

The concentration of total amino acids at maturity was significantly different among clones, the 1084 one having the lowest values (Figure 4A). As the significant interaction among factors indicates, the effect of CC depended on the water availability in a different manner depending on the clone, while WD increased the amino acid concentration differently among clones. Thus, CC tended to reduce the amino acid levels relative to CS conditions in the RJ43, VN31 and 1084 accessions regardless of water availability, and especially in VN31 plants grown at WD conditions. In the case of CL306 and RJ43, the grape total amino acid concentration significantly increased in plants grown under CC/WD conditions when compared to CS/WW (Figure 4A). Finally, the 1084 accession was the less affected by the T/CO_2/RH regime and water availability.

The amino acid profile at maturity also varied among clones and treatments (Figure 4B and Table S2). Amino acids derived from α-ketoglutarate were the most abundant group in every case, but its proportion in 1084 was significantly lower than in the other clones, mainly because of the relatively low glutamine content in that clone. Aspartate derivatives were the second most abundant group in all the clones, wherein threonine and asparagine had the highest relative abundance. The 1084 accession exhibited a significantly higher relative abundance of aspartate derivatives compared with other accessions. Pyruvate, phosphoglycerate and shikimate derivatives were the least abundant amino acid groups and their proportions were similar among all the clones studied. The relative abundance of aspartate derivatives significantly increased with

WD, especially in the 1084 accession (Figure 4B and Table S2). In contrast, WD reduced the relative abundance of shikimate and phosphoglycerate derivatives (except for the 1084 accession under CC conditions), this effect being more obvious in the CL306 accession. A significant interaction between the clone and irrigation regime was observed for the relative abundance of α-ketoglutarate derivatives, as it was reduced by WD in 1084, in opposition to VN31, where WD tended to increase it (Figure 4B and Table S2). CC conditions increased the proportion of valine, leucine, isoleucine, tyrosine, and serine and reduced the relative abundance of asparagine and histidine, the latter under WW conditions in RJ43 and CL306 and under both WW and WD in VN31 and 1084 (Table S2).

Total Anthocyanins and Anthocyanin to TSS Ratio

Clones showed differences in their anthocyanin levels only at maturity, the 1084 accession having the lowest concentration (Figure 5A,B). CC conditions increased the levels of anthocyanins at mid-veraison and 1 week after mid-veraison. Grapes ripened under WD conditions had lower levels of anthocyanins 2 weeks after mid-veraison (Figure 5A). At maturity, there was an interaction between the CO_2/T/RH and irrigation regime as, considering the clones altogether, CC significantly reduced the anthocyanin levels when combined with WD conditions but not with WW (Figure 5C).

Regarding the relationship between anthocyanins and TSS at maturity, RJ43 and VN31 had the highest ratios and 1084 the lowest ones (Figure 6A). Considering the clones altogether, the CC/WD treatment significantly reduced the anthocyanin to TSS ratio with respect to the CS/WW treatment (Figure 6B). When studied independently for each clone, this reduction was more obvious in RJ43 and less marked in other clones such as VN31 or 1084 (Figure 6C).

DISCUSSIONS

In the future, changes in grape composition are expected in the Mediterranean area, as a result of one or more abiotic factors related to climate change. In the present study, the response of four Tempranillo clones

to the foreseen T/CO_2/RH conditions by the end of the present century, combined or not with water deficit, was studied, focusing on the evolution of grape components throughout the ripening period.

Tartaric and malic acid are the principal organic acids of grape berry and represent the most significant influences on the acidity and pH of the juice[38]. Organic acids (especially malic acid) are degraded along the ripening period, thus decreasing their concentration up to maturity. In the present study, the degradation of malic acid was enhanced by CC conditions; consequently, the levels of malic acid and total acidity were lower in CC compared to CS at maturity. The results agree with previous studies in red Tempranillo that report a lower concentration of malic acid in berries ripened under combined high temperature and elevated CO_2[6,18,24]. A higher malate export rate from the vacuole to the cytoplasm and altered expression and activity of enzymes involved in malate catabolism have been described as responsible for the enhancement of malate degradation under high temperatures [9,39]. Among the clones studied, 1084 was the accession that showed the lowest levels of malic acid at maturity despite its similar bunch weights to RJ43 and CL306, agreeing with previous results we have reported[31]. This result suggests that the low concentration of malic acid in 1084 was not related to the bunch size. Rather than a higher sensitivity of this clone to the projected environmental conditions assayed, this result might be a consequence of the longer ripening period of this accession. Phenology is the first source of genetic variation of grape acidity at harvest[40].

Decrease in malic acid in response to water supply limitation has been described on various grapevine cultivars[41]. Some authors justify this decrease by a higher respiration rate due to an increase in cluster temperature or caused by a reduced vegetative growth under drought conditions[42]. In our study, however, we did not observe a significant effect of water deficit on malic acid concentration, as also reported by Berdeja et al.[43] At maturity, the organic acid content of CL306 was not more affected by WD under CC conditions, despite its stronger reduction in bunch weight compared to the other clones. This results confirmed that the composition of the grape was not related to the bunch weight in our experimental conditions. The tightly controlled environmental conditions within the greenhouse, which minimized differences in cluster temperature between WW and WD plants grown under the same T/CO_2/RH regime, may explain the lack of differences in malic acid between irrigation regimes. In contrast, total acidity

measured at maturity was lower in the plants subjected to WD, due to a significant reduction in tartaric acid. Although tartaric acid is less sensitive to climatic conditions during ripening[40], some authors have reported a reduction in tartaric acid levels in grapes of cv. Tempranillo ripened under water deficit conditions and ambient temperature [24].

The phenology of grape development has been described as a process highly dependent on environmental factors, especially on temperature [5,44]. Our results suggest a higher impact of the CC treatment on the period before veraison, shortening the elapsed time between fruit set and mid-veraison. In contrast, WD had a stronger effect after mid-veraison, slowing down the ripening rate. Therefore, the results indicate that WD compensated the impact of CC on grape sugar accumulation and phenological development. Although mild water deficit has proven to enhance ripening through several processes, such as altering plant abscisic acid signaling, reduction in berry size, or increase in berry sugar concentration [22,23,44,45], severe water deficit can induce stomatal closure, thus limiting carbon fixation and delaying berry ripening. [5,46] In our case, the lower sugar concentration observed from 1 week after mid-veraison onward in the grapes subjected to WD suggests a lower sugar accumulation rate, probably associated with a limitation in photosynthetic activity under these conditions. Clones exhibited differences in their phenological development; however, differences between mid-veraison and maturity were greater than between fruit set and mid-veraison, in a similar trend reported in previous experiments considering several Tempranillo clones [29].

The effect of CC/WD treatment on berry volume varied among clones, 1084 and VN31 being the least affected, when plants grown at CS/WW were compared with those grown at CC/WD. However, it shall be mentioned that big berries are not desirable for viticulturists, the small size being the trait of interest for breeders [47]. CC increased the relative skin mass proportion, meaning that berry skins were thicker in the CC than in CS treatment, even though 1084 relative skin mass was reduced by CC.

In grapes, nitrogen is present as inorganic (ammonium and ammonium salts) and organic (proteins and amino acids) forms. Together with ammonia, free amino acids (except proline and hydroxyproline) are components of the yeast assimilable nitrogen (YAN), thus having important implication for must

fermentation[48]. Thus, the lower levels of total amino acids in the 1084 accession may imply a lower N availability during the fermentation process. Regarding the impact of environmental factors, the concentration of total free amino acids decreased under CC, considering the clones altogether. This result agrees with our previous results and with Martínez-Lüscher et al., who reported a reduction in α-amino nitrogen in grapes of Tempranillo developed under high temperatures combined with elevated CO_2 [7,18]. Studies of Torres et al. and Sweetman et al. on the effect of high temperature applied as a single factor showed an increase in the amino acid content in grapes, [9,11] while Wohlfahrt et al. reported no impact of elevated CO_2 on these compounds' level [17]. However, Torres et al. [11] performed the amino acid analysis on the skin, and, in the study of Sweetman et al. [9], the temperatures assayed were much more extreme than in the present work (35 °C/28 °C day/night as high temperature treatment compared with 28 °C/18 °C of the present study). Conversely, in the study of Wohlfahrt et al. [17], the CO_2 level was less elevated than in the present work (480 µmol mol^{-1} as elevated CO_2 treatment compared with 700 µmol mol^{-1} of the present study) and, moreover, the studies took place in a vineyard instead of growth chamber-greenhouses. These differences in the methodologies may explain the contradictory results.

It should be noted that the response of total amino acids to CC conditions depended on the clone studied as well as on the irrigation regime, as highlighted by the significant interaction among the three factors. In particular, the combination of CC and WD conditions increased the total amino acid concentration in RJ43 and CL306, two of the most widely distributed Tempranillo clones, but did not modified total amino acid content in VN31 and 1084 compared to CS/WW conditions. Disparity of responses to drought have been already seen among different grapevine cultivars [23,49], but the present results also suggest variability in the response of amino acids to changes in the T/CO_2/RH regime and water availability among clones within the same cultivar.

Qualitative differences in the grape amino acid profiles were noticeable at maturity among clones, with the 1084 accession having a higher relative abundance of aspartate derivatives at the expense of α-ketoglutarate derivatives as already seen in previous experiments[18]. Even though the relative abundance of some individual amino acids was affected by CC conditions, the relative proportions of amino acid families

according to their precursor were not modified. These results may indicate that the T/CO_2/RH regime did not impact the relative accumulation of precursors but it affected later stages of their biosynthetic pathways. WD had a higher impact on the amino acid profile than CC conditions. Despite the fact that relative abundance of proline was reduced under WD conditions, its concentration increased, supporting its osmoprotector role and agreeing with other authors' reports [23,50]. Moreover, WD effect was in some cases, dependent on the clone. For example, the reduction in the relative abundance of shikimate and phosphoglycerate derivatives was more evident in CL306 and less marked in 1084. The amplitude of the response of Tempranillo genotypes to the projected environmental conditions will most probably have implications in the organoleptic properties of the wine produced from these grapes since the shikimate route is responsible for the biosynthesis of aromatic amino acids [51], including the precursor of the phenylpropanoid pathway (phenylalanine)[52].

Together with sugars and acids, phenolic compounds are the most abundant constituents present in grapes. Among them, anthocyanins play an essential role in the grape and wine color in red berry varieties[44]. The lower values in anthocyanin concentration, as well as in the anthocyanin to TSS ratio of berries of clone 1084, reveal the existence of intra-varietal diversity among Tempranillo clones for this trait in agreement with previous studies [29]. The decrease in the accumulation of anthocyanins in grapes due to high temperatures has been widely described, and it has been associated to a reduction in anthocyanin biosynthesis, through the inhibition of mRNA transcription of the biosynthetic genes, as well as to an enhancement of anthocyanin degradation[40]. Also, elevated temperature has been shown to uncouple berry organoleptic traits such as the accumulation of anthocyanins and sugars, thus decreasing the anthocyanins to sugars ratio [14]. Conversely, high CO_2 applied as a single environmental factor did not affect total anthocyanins in cv. Touriga Franca [15] or cv. Cabernet Sauvignon [53] and we reported previously similar results in Tempranillo[18]. In our present study, the combination of elevated temperature and elevated CO_2 did not have a great impact either on anthocyanin concentration or on the anthocyanins to TSS ratio when applied under WW conditions. Decreases of these two parameters occurred when the CO_2/T/RH conditions foreseen for 2100 were combined with WD. The results suggest that the effect of CC conditions on anthocyanin levels

as well as on the anthocyanins to TSS ratio may be more intense in a future climate under low water availability. Similarly, Zarrouk et al., in one of the 2 years of a field experiment, observed no differences in the anthocyanin content of east- and west–exposed berries (temperature 5 °C - 9 °C higher in the west-exposed berries) in the treatment with low water deficit[54]. However, under most severe water stresses, anthocyanin levels decreased in the west-exposed berries. In the same line, Kizildeniz et al. reported no consistent decreases in anthocyanins or anthocyanins to sugars ratio in response to elevated temperature, but observed consistent decreases in both parameters in response to water stress [10]. In general, a moderate water stress is reported to increase anthocyanins, through the up-regulation of genes involved in their biosynthetic pathway [22,23]. However, when a certain threshold of water stress is exceeded, anthocyanin concentration can be negatively affected[10,24,54]. Such negative impact of water stress on anthocyanins can result from the repression of biosynthesis at the onset of ripening and from degradation at later stages[54]. Regarding the performance of the clones, RJ43 and CL306, some of the most widely cultivated Tempranillo clones, were the most affected by CC/WD conditions. In contrast, the VN31 clone sustained a relatively high anthocyanin concentration with low effects in anthocyanin to TSS ratio under the same conditions.

To sum up, considering the environmental conditions projected for 2100, the results indicate a modification of grape berry composition that might affect wine quality. Climate change conditions (elevated temperature, elevated CO_2, and reduce RH) hastened grape sugar accumulation and consequently advanced ripeness, whereas water deficit partially compensated such effects slowing down the ripening period and sugar accumulation. The impact of climate change on grape amino acids depended on the irrigation level and on the clone, the 1084 accession being less affected than the other clones. Water deficit was the factor that impacted the most amino acid families, decreasing shikimate and phosphoglycerate derivatives at the expense of aspartate derivatives, with different intensities depending on the clone studied. The combination of climate change and water deficit reduced the anthocyanin concentration as well as the anthocyanins to TSS ratio. The results suggest that differences in the response of the Tempranillo clones studied to the environmental conditions projected for 2100 are not always necessarily associated with differences in the length of their reproductive period. However, this is a 1 year study conducted under controlled conditions

with young potted plants. Consequently, we cannot directly extrapolate these results to natural conditions, where grapevines are adult field grown plants that have probably different source-sink relationships, higher transport capacity, and photosynthetic behavior than fruit-bearing cuttings because of their constraintless root system. Despite these limitations, this is the first step to explore the impact of multi-stresses on grapevine physiology before a future validation under field conditions, including the study of the effect of high light stress, which is also expected to be due to climate change. This study also brings in information that can be useful in order to design adaptive strategies in the vineyard to cope with climate change-induced challenge for viticulture. In particular, exploiting the grapevine genotypic diversity can be a suitable tool to optimize the adaptation of traditional varieties to the foreseen climate scenarios using genotype-environment interactions.

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SUPPLEMENTARY INFORMATION DESCRIPTION

Table S1. Pre-dawn leaf water potential at veraison and two weeks after mid-veraison.

Table S2. Total soluble solids in berries during ripening

Table S3. Total free amino acid content in berries and relative abundance of individual amino acids at maturity.

Figure S1. Bunch weight at maturity.

This material is available free of charge via the Internet at http://pubs.acs.org.

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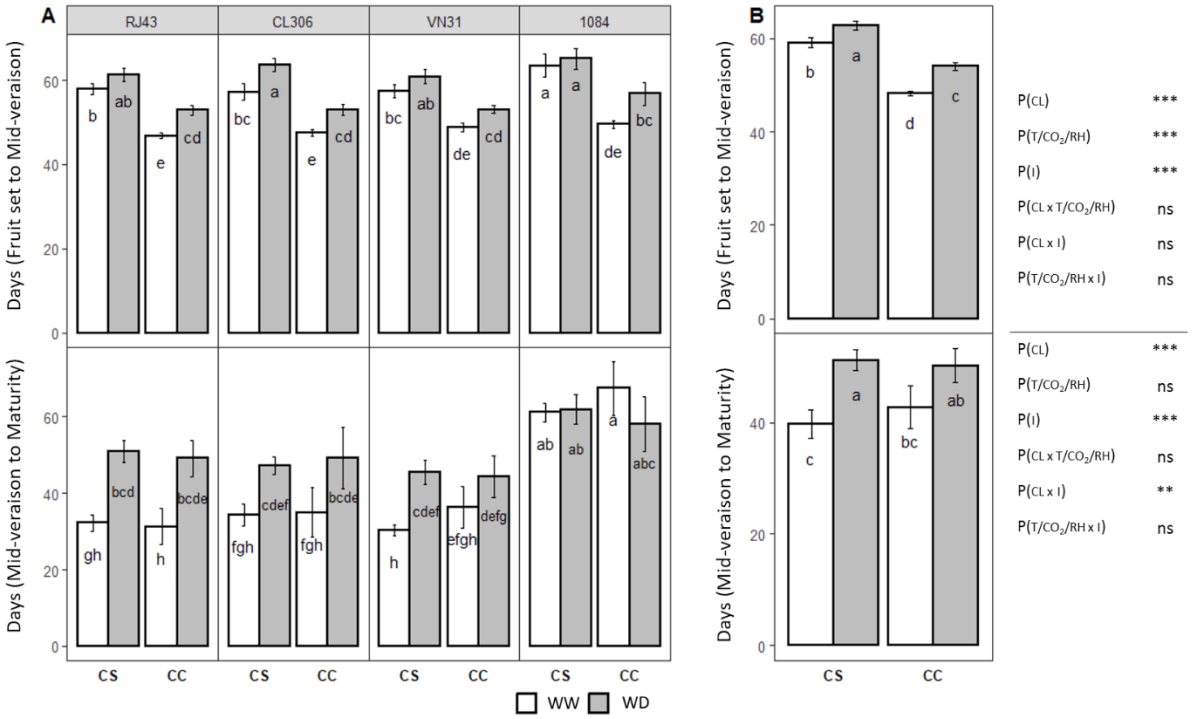


Figure 1. Elapsed time between fruit set and mid-veraison and between mid-veraison and maturity of Tempranillo clones grown under two T/CO_2/RH conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1} and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1} and 33 %/53 % RH) combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Data (values are means ± SE) are represented according to the T/CO_2/RH and irrigation regimes (A) considering each clone individually (n = 6-8) and (B) considering all the clones as a whole (n = 28-31). Means with letters in common within the same chart (A or B) and parameter are not significantly different according to LSD test (P > 0.05). Probability values (P) for the main effects of clone, P(CL); T/CO_2/RH regime, P(T/CO_2/RH); irrigation regime, P(I); and their interactions, P(CL x T/CO_2/RH), P(CL x I) and P(T/CO_2/RH x I). ***, P < 0.001; **, P < 0.01; ns, not significant. Interaction of all factors P(CL x T/CO_2/RH x I) was statistically not significant (P > 0.05).

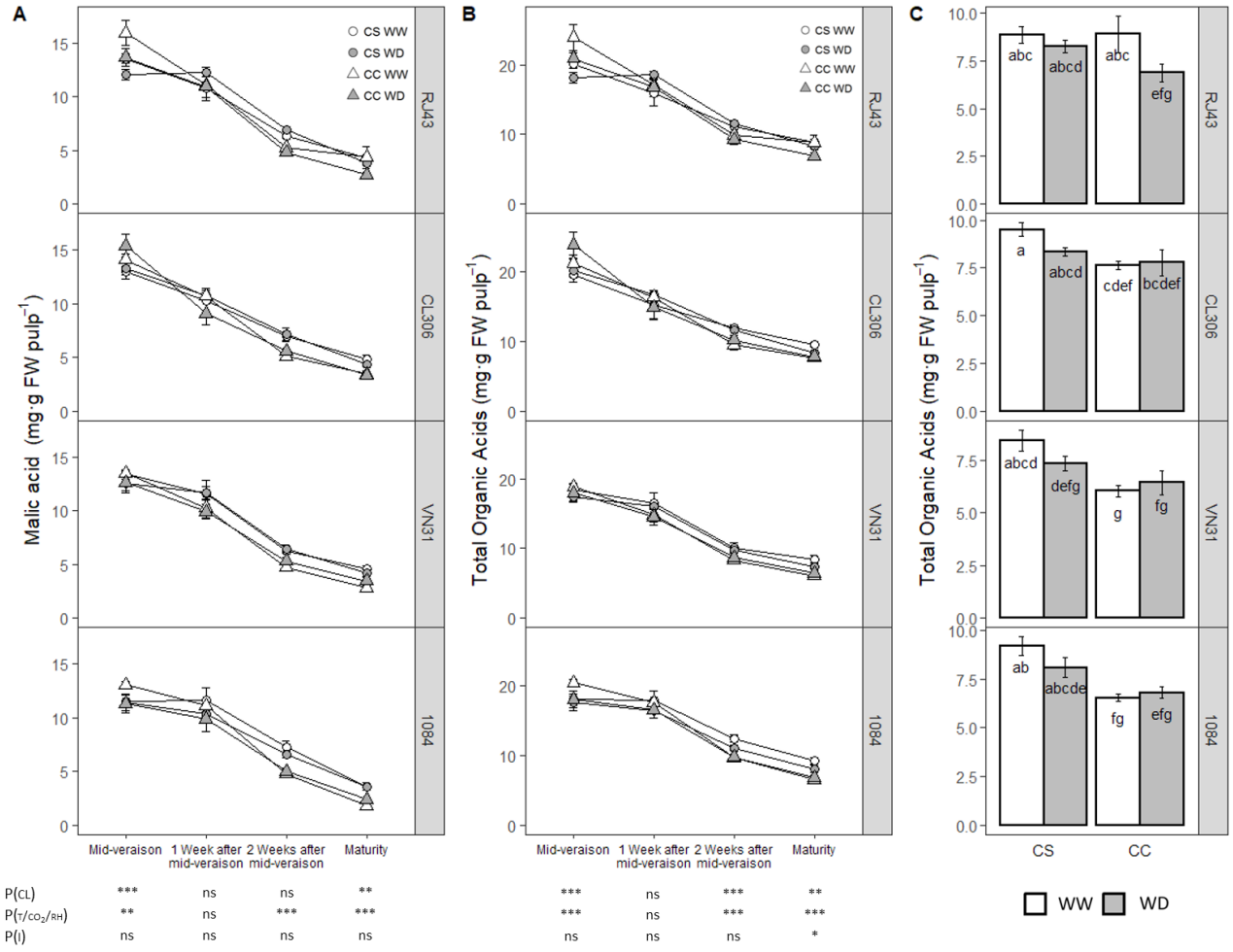


Figure 2. Evolution of the concentration of malic acid (A) and total acidity (B), and detail of total acidity at maturity (C) of Tempranillo clones grown under two T/CO_2/RH conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1} and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1} and 33 %/53 % RH) combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are represented according to the T/CO_2/RH and irrigation regimes and considering clones independently (n = 3-4). 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); T/CO_2/RH regime, P(T/CO_2/RH); irrigation regime, P(I). All probability values for the interactions of factors (P(CL x T/CO_2/RH), P(CL x I), P(T/CO_2/RH x I) and P(CL x T/CO_2/RH x I)) were statistically not significant (P > 0.05).

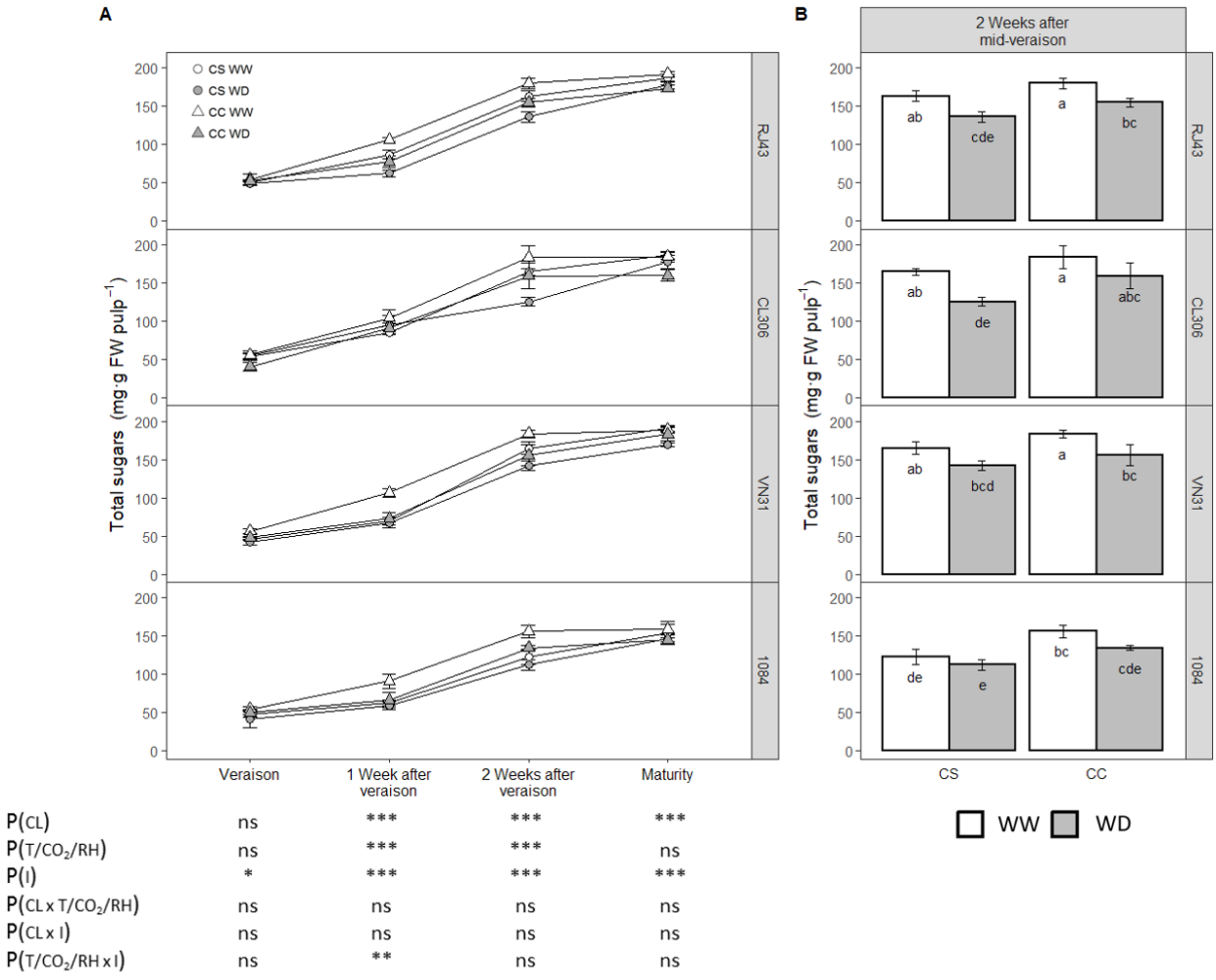


Figure 3. Evolution of the concentration of total sugars (sum of glucose and fructose) in berries (A) and detail of sugar concentration 2 weeks after mid-veraison (B) of Tempranillo clones grown under two $T/CO_2/RH$ conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1} and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1} and 33 %/53 % RH) combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are represented according to the $T/CO_2/RH$ and irrigation regimes, considering each clone individually (n = 3-4). 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); $T/CO_2/RH$ regime, P($T/CO_2/RH$); irrigation regime, P(I); and their interactions, P(CL x $T/CO_2/RH$), P(CL x I) and P($T/CO_2/RH$ x I). ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant. Interaction of all factors P(CL x $T/CO_2/RH$ x I) was statistically not significant (P > 0.05).

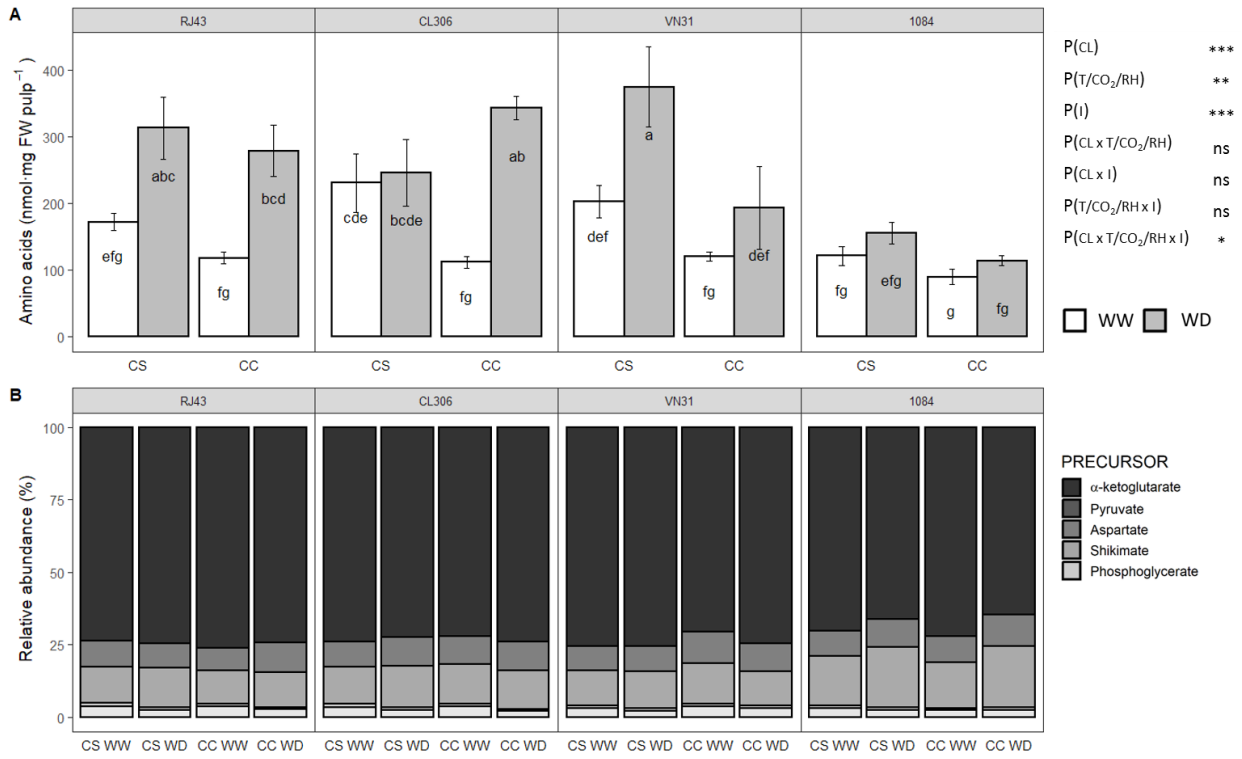
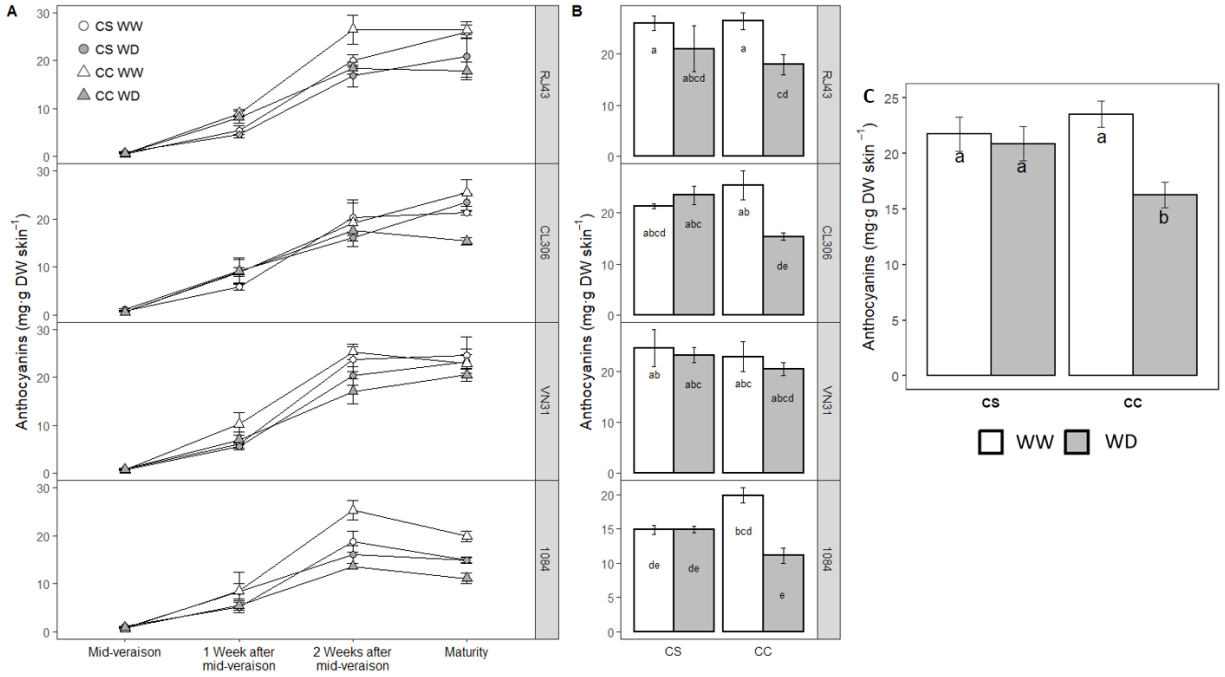


Figure 4. Total concentration of grape amino acids at maturity (A) and relative abundance of amino acids grouped according to their precursor (B) in Tempranillo clones grown under two T/CO_2/RH conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1}, and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1}, and 33 %/53 % RH) combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are represented according to the T/CO_2/RH and irrigation regimes, considering each clone individually (n = 3-4). In chart A, 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); T/CO_2/RH regime, P(T/CO_2/RH); irrigation regime, P(I); and their interactions, P(CL x T/CO_2/RH), P(CL x I), P(T/CO_2/RH x I) and P(CL x T/CO_2/RH x I). ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant.



P(CL)	ns	ns	ns	***
P(T/CO_2/RH)	***	*	ns	ns
P(I)	ns	ns	***	***
P(CL x T/CO_2/RH)	ns	ns	ns	ns
P(CL x I)	ns	ns	ns	ns
P(T/CO_2/RH x I)	ns	ns	ns	**
P(CL x T/CO_2/RH x I)	**	ns	ns	ns

Figure 5. Total skin anthocyanins of Tempranillo clones grown under two T/CO_2/RH conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1} and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1} and 33 %/53 % RH) combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are represented according to the T/CO_2/RH and irrigation regimes, considering each clone individually (n = 3-4), (A) during ripening evolution and (B) at maturity. Figure C shows total skin anthocyanins at maturity considering clones altogether (n = 14-15). Means with letters in common within the same chart (B or C) are not significantly different according to LSD test (P > 0.05). Probability values (P) for the main effects of clone, P(CL); T/CO_2/RH regime, P(T/CO_2/RH); irrigation regime, P(I); and their interactions, P(CL x T/CO_2/RH), P(CL x I), P(T/CO_2/RH x I) and P(CL x T/CO_2/RH x I). ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant.

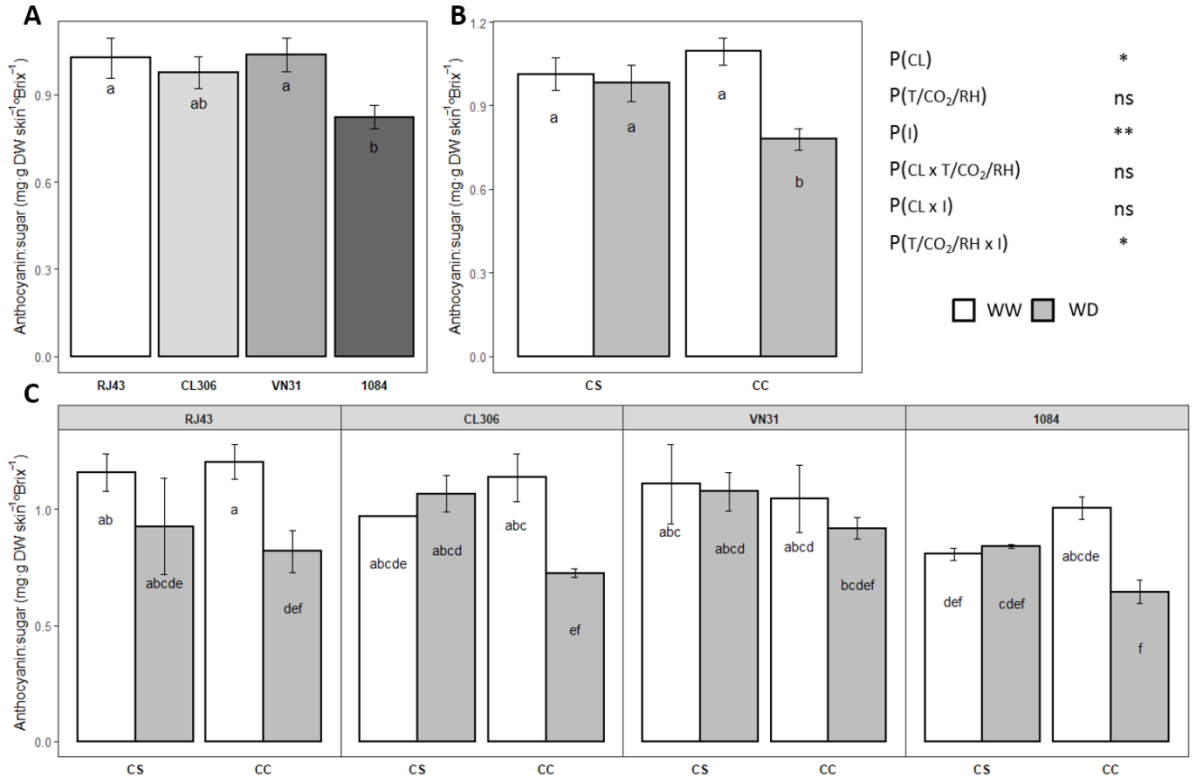


Figure 6. Anthocyanin to TSS ratio at maturity of Tempranillo clones grown under two $T/CO_2/RH$ conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1}, and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1}, and 33 %/53 % RH) combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are represented according to: (A) the clone identity (n = 12-16), (B) the $T/CO_2/RH$ and irrigation regimes (n = 14-15) and (C) the three factors together (n = 3-4). Means with letters in common within the same chart (A, B or C) are not significantly different according to LSD test ($P > 0.05$). Probability values (P) for the main effects of clone, P(CL); $T/CO_2/RH$ regime, P($T/CO_2/RH$); irrigation regime, P(I); and their interactions, P(CL x $T/CO_2/RH$), P(CL x I) and P($T/CO_2/RH$ x I). **, $P < 0.01$; *, $P < 0.05$; ns, not significant. Interaction of all factors P(CL x $T/CO_2/RH$ x I) was statistically not significant ($P > 0.05$).

Table 1. Grape berry volume of the Tempranillo clones grown under two T/CO_2/RH conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1} and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1} and 33 %/53 % RH), combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are shown according to the clone identity (n = 12-16), T/CO_2/RH regime (n = 29-30), irrigation regime (n = 29-30) and the three factors together (n = 3-4). Means with letters in common within the same stage and factor (clone, T/CO_2/RH, irrigation regime, or their interaction) are not significantly different (P > 0.05) according to LSD test. Probability values (P) for the main effects of clone, P(CL); T/CO_2/RH regime, P(T/CO_2/RH); irrigation regime, P(I); and their interactions, P(CL x T/CO_2/RH), P(CL x I), P(T/CO_2/RH x I) and P(CL x T/CO_2/RH x I).***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant.

			Berry volume (mL)			
			Mid-veraison	1 week after mid-veraison	2 weeks after mid-veraison	Maturity
RJ43			0.68 ± 0.03 b	0.80 ± 0.04 b	0.87 ± 0.03 a	0.97 ± 0.04 ab
CL306			0.60 ± 0.05 c	0.76 ± 0.04 bc	0.85 ± 0.04 ab	0.93 ± 0.04 b
VN31			0.62 ± 0.03 c	0.71 ± 0.03 c	0.78 ± 0.03 b	0.86 ± 0.03 c
1084			0.83 ± 0.04 a	0.87 ± 0.04 a	0.92 ± 0.05 a	1.00 ± 0.04 a
CS			0.75 ± 0.03 a	0.80 ± 0.03 a	0.86 ± 0.02 a	0.93 ± 0.03 a
CC			0.62 ± 0.03 b	0.77 ± 0.03 a	0.84 ± 0.03 a	0.95 ± 0.03 a
WW			0.78 ± 0.02 a	0.90 ± 0.02 a	0.95 ± 0.02 a	1.02 ± 0.02 a
WD			0.59 ± 0.02 b	0.67 ± 0.02 b	0.75 ± 0.02 b	0.86 ± 0.02 b
RJ43	CS	WW	0.81 ± 0.04 bc	0.96 ± 0.06 a	0.92 ± 0.03 abcd	1.06 ± 0.05 ab
		WD	0.71 ± 0.03 cd	0.73 ± 0.04 def	0.84 ± 0.04 cdef	0.93 ± 0.03 cdef
	CC	WW	0.72 ± 0.01 cd	0.92 ± 0.03 ab	1.01 ± 0.03 a	1.12 ± 0.04 a
		WD	0.49 ± 0.02 gh	0.59 ± 0.02 g	0.71 ± 0.04 efg	0.79 ± 0.02 gh
CL306	CS	WW	0.81 ± 0.04 bc	0.88 ± 0.04 abc	0.99 ± 0.05 abc	1.11 ± 0.09 a
		WD	0.57 ± 0.06 efg	0.68 ± 0.03 efg	0.80 ± 0.02 defg	0.89 ± 0.03 defg
	CC	WW	0.62 ± 0.01 def	0.86 ± 0.01 abcd	0.92 ± 0.06 abcd	0.95 ± 0.01 bcde
		WD	0.41 ± 0.03 h	0.61 ± 0.03 fg	0.68 ± 0.01 fg	0.76 ± 0.05 h
VN31	CS	WW	0.75 ± 0.05 bc	0.82 ± 0.04 bcd	0.87 ± 0.02 abcd	0.90 ± 0.02 defg
		WD	0.59 ± 0.02 efg	0.61 ± 0.03 fg	0.70 ± 0.02 efg	0.75 ± 0.02 h
	CC	WW	0.64 ± 0.06 de	0.80 ± 0.05 bcde	0.89 ± 0.05 abcd	0.97 ± 0.05 bcd
		WD	0.51 ± 0.03 fgh	0.62 ± 0.06 fg	0.66 ± 0.07 g	0.83 ± 0.03 efgh
1084	CS	WW	1.00 ± 0.02 a	0.99 ± 0.04 a	1.00 ± 0.02 ab	1.03 ± 0.06 abc
		WD	0.73 ± 0.10 bcd	0.73 ± 0.09 defg	0.77 ± 0.10 defg	0.81 ± 0.05 fgh
	CC	WW	0.85 ± 0.05 b	0.95 ± 0.03 a	1.01 ± 0.07 ab	1.07 ± 0.03 ab
		WD	0.71 ± 0.03 cd	0.77 ± 0.07 cde	0.85 ± 0.13 bcde	1.06 ± 0.06 ab
P(CL)			***	**	*	***
P(T/CO_2/RH)			***	ns	ns	ns
P(I)			***	***	***	***
P(CL x T/CO_2/RH)			ns	ns	ns	***
P(CL x I)			ns	ns	ns	ns
P(T/CO_2/RH x I)			ns	ns	ns	ns
P(CL)			ns	ns	ns	*

Table 2. Relative skin mass (%) of the Tempranillo clones grown under two $T/CO_2/RH$ conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1} and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1} and 33 %/53 % RH), combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are shown according to the clone identity (n = 12-16), $T/CO_2/RH$ regime (n = 29-30), irrigation regime (n = 29-30) and the three factors together (n = 3-4). Means with letters in common within the same stage and factor (clone, $T/CO_2/RH$, irrigation regime, or their interaction) are not significantly different (P > 0.05) according to LSD test. Probability values (P) for the main effects of clone, P(CL); $T/CO_2/RH$ regime, P($T/CO_2/RH$); irrigation regime, P(I); and their interactions, P(CL x $T/CO_2/RH$), P(CL x I) and P($T/CO_2/RH$ x I). ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant. Interaction of all factors P(CL x $T/CO_2/RH$ x I) was statistically not significant (P > 0.05).

			Relative skin mass (%)			
			Mid-veraison	1 week after mid-veraison	2 weeks after mid-veraison	Maturity
RJ43			11.16 ± 0.23 a	10.14 ± 0.24 a	9.22 ± 0.26 a	11.86 ± 0.45 a
CL306			11.05 ± 0.29 a	9.72 ± 0.23 a	8.74 ± 0.18 ab	11.61 ± 0.37 ab
VN31			11.21 ± 0.23 a	10.01 ± 0.14 a	8.71 ± 0.27 ab	11.05 ± 0.21 bc
1084			10.49 ± 0.41 a	9.90 ± 0.41 a	8.35 ± 0.24 b	10.36 ± 0.33 c
CS			11.00 ± 0.21 a	9.85 ± 0.24 a	8.43 ± 0.17 b	10.86 ± 0.17 b
CC			10.96 ± 0.22 a	10.06 ± 0.13 a	9.09 ± 0.18 a	11.55 ± 0.32 a
WW			10.42 ± 0.13 b	9.64 ± 0.14 b	8.89 ± 0.18 a	10.88 ± 0.15 b
WD			11.55 ± 0.23 a	10.28 ± 0.22 a	8.63 ± 0.19 a	11.55 ± 0.34 a
RJ43	CS	WW	10.89 ± 0.31 d	9.77 ± 0.39 b	8.61 ± 0.54 cd	10.65 ± 0.26 cd
		WD	11.37 ± 0.56 abc	10.55 ± 0.63 ab	8.28 ± 0.20 d	11.69 ± 0.80 bcd
	CC	WW	10.50 ± 0.17 d	9.58 ± 0.23 ab	9.77 ± 0.39 abcd	11.04 ± 0.40 cd
		WD	11.86 ± 0.55 abcd	10.67 ± 0.49 a	10.21 ± 0.28 cd	14.07 ± 0.89 a
CL306	CS	WW	10.08 ± 0.33 cd	9.33 ± 0.25 ab	9.07 ± 0.29 abcd	10.57 ± 0.29 cd
		WD	11.25 ± 0.65 abcd	9.59 ± 0.62 ab	8.63 ± 0.53 bcd	10.76 ± 0.30 cd
	CC	WW	10.73 ± 0.14 abcd	9.29 ± 0.30 ab	8.40 ± 0.26 bcd	11.91 ± 0.72 bcd
		WD	12.16 ± 0.39 a	10.66 ± 0.12 a	8.85 ± 0.41 abcd	13.19 ± 0.10 ab
VN31	CS	WW	10.93 ± 0.18 abcd	10.20 ± 0.43 ab	8.74 ± 0.78 bcd	10.39 ± 0.33 d
		WD	11.84 ± 0.53 abcd	10.26 ± 0.22 a	8.17 ± 0.39 cd	11.10 ± 0.21 cd
	CC	WW	10.36 ± 0.33 bcd	9.84 ± 0.25 ab	9.20 ± 0.31 ab	10.76 ± 0.48 cd
		WD	11.69 ± 0.37 ab	9.74 ± 0.20 a	8.73 ± 0.69 a	11.97 ± 0.20 bc
1084	CS	WW	9.90 ± 0.19 abcd	8.75 ± 0.42 ab	8.28 ± 0.47 bcd	10.91 ± 0.44 cd
		WD	11.75 ± 1.37 ab	10.27 ± 1.93 ab	7.64 ± 0.21 cd	10.65 ± 0.81 cd
	CC	WW	9.99 ± 0.67 bcd	10.22 ± 0.34 ab	8.94 ± 0.62 abc	10.95 ± 0.33 cd
		WD	10.64 ± 0.94 abc	10.45 ± 0.39 ab	8.36 ± 0.34 bcd	8.98 ± 0.63 e
P(CL)			ns	ns	ns	***
P($T/CO_2/RH$)			ns	ns	*	**
P(I)			***	*	ns	*
P(CL x $T/CO_2/RH$)			ns	ns	ns	**
P(CL x I)			ns	ns	ns	***
P($T/CO_2/RH$ x I)			ns	ns	ns	ns

Table S1. Pre-dawn leaf water potential (MPa) at mid-veraison and two weeks after mid-veraison of the Tempranillo clones grown under two T/CO_2/RH conditions: current situation (CS: 24 °C/14 °C, 400 µmol mol^{-1}, and 45 %/65 % RH) and climate change (CC: 28 °C /18 °C, 700 µmol mol^{-1}, and 33 %/53 % RH), combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results are represented according to the T/CO_2/RH and irrigation regimes (values are means ± SE, n = 14-19). Means with letters in common are not significantly different (P > 0.05) according to LSD test. Probability values (P) for the main effects of clone, P(CL); T/CO_2/RH regime, P(T/CO_2/RH); irrigation regime, P(I); and their interactions, P(CL x T/CO_2/RH), P(CL x I) and P(T/CO_2/RH x I). ***, P < 0.001; **, P < 0.01; ns, not significant. Interaction of all factors P(CL x T/CO_2/RH x I) was statistically not significant (P > 0.05).

	Pre-dawn water potential (MPa)							
	Mid-veraison				2 weeks after mid-veraison			
CS-WW	-0.63	±	0.03	a	-0.59	±	0.03	a
CS-WD	-1.48	±	0.08	c	-1.48	±	0.09	b
CC-WW	-0.77	±	0.06	a	-0.71	±	0.04	a
CC-WD	-1.28	±	0.07	b	-1.37	±	0.07	b
P(CL)	ns				ns			
P(T/CO_2/RH)	ns				ns			
P(I)	***				***			
P(CL x T/CO_2/RH)	ns				ns			
P(CL x I)	ns				ns			
P(T/CO_2/RH x I)	**				ns			

Table S2. Total soluble solids in berries of the Tempranillo clones grown under two T/CO2/RH conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1} and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1} and 33 %/53 % RH), combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are shown according to the clone identity (n = 12-16), T/CO_2/RH regime (n = 29-30), irrigation regime (n = 29-30) and the three factors together (n = 3-4). Means with letters in common within the same stage and factor (clone, T/CO2/RH, irrigation regime, or their interaction) are not significantly different (P > 0.05) according to LSD test. Probability values (P) for the main effects of clone, P(CL); T/CO_2/RH regime, P(T/CO_2/RH); irrigation regime, P(I); and their interactions, P(CL x T/CO_2/RH), P(CL x I), P(T/CO_2/RH x I) and P(CL x T/CO_2/RH x I).***, P < 0.001; *, P < 0.05; ns, not significant.

			Total soluble solids (°Brix)			
			Mid-veraison	1 week after mid-veraison	2 weeks after mid-veraison	Maturity
RJ43			9.95 ± 0.30 a	12.51 ± 0. 47 a	16.71 ± 0.59 a	22.24 ± 0.20 a
CL306			10.26 ± 0.19 a	13.77 ± 0.44 a	16.55 ± 0.45 a	21.85 ± 0.25 a
VN31			9.99 ± 0.35 a	18.94 ± 6.36 a	17.46 ± 0.51 a	22.05 ± 0.17 a
1084			9.81 ± 0.25 a	11.75 ± 0.40 a	14.99 ± 0.60 b	18.32 ± 0.42 b
CS			9.82 ± 0.21 a	12.10 ± 0.29 a	15.74 ± 0.43 b	21.23 ± 0.38 a
CC			10.15 ± 0.19 a	16.46 ± 3.39 a	17.13 ± 0.37 a	20.99 ± 0.34 a
WW			10.60 ± 0.13 a	16.71 ± 3.37 a	17.65 ± 0.36 a	21.35 ± 0.31 a
WD			9.36 ± 0.20 b	11.83 ± 0.31 a	15.20 ± 0.34 b	20.86 ± 0.41 a
RJ43	CS	WW	10.65 ± 0.24 abc	12.85 ± 0.61 b	17.85 ± 0.46 abc	22.50 ± 0.44 ab
		WD	8.40 ± 0.60 f	10.45 ± 0.22 b	14.25 ± 1.19 def	22.70 ± 0.59 a
	CC	WW	10.55 ± 0.44 abcd	14.70 ± 0.19 b	18.85 ± 0.49 a	21.90 ± 0.13 ab
		WD	10.20 ± 0.29 abcd	12.05 ± 0.87 b	15.90 ± 0.98 cde	21.85 ± 0.26 ab
CL306	CS	WW	10.53 ± 0.47 abcd	13.40 ± 1.20 b	17.67 ± 0.52 abc	22.00 ± 0.53 ab
		WD	10.00 ± 0.46 abcde	13.27 ± 1.11 b	14.73 ± 0.37 def	22.00 ± 0.31 ab
	CC	WW	10.60 ± 0.31 abcd	15.00 ± 0.46 b	17.27 ± 1.14 abc	22.27 ± 0.58 ab
		WD	9.90 ± 0.32 abcde	13.40 ± 0.61 b	16.53 ± 0.53 bcd	21.13 ± 0.52 bc
VN31	CS	WW	11.10 ± 0.21 a	12.60 ± 0.47 b	18.20 ± 0.62 ab	22.25 ± 0.15 ab
		WD	9.50 ± 0.37 def	11.60 ± 0.80 b	15.95 ± 0.83 cd	21.65 ± 0.47 ab
	CC	WW	10.95 ± 0.13 ab	14.25 ± 0.63 b	19.30 ± 0.42 a	22.00 ± 0.36 ab
		WD	8.40 ± 0.80 f	12.30 ± 0.97 b	16.40 ± 1.25 bcd	22.30 ± 0.35 ab
1084	CS	WW	9.60 ± 0.54 cde	11.60 ± 0.62 b	13.80 ± 0.94 ef	18.55 ± 1.19 de
		WD	8.73 ± 0.18 ef	11.47 ± 1.12 b	13.00 ± 0.20 f	17.73 ± 0.47 ef
	CC	WW	10.78 ± 0.27 ab	13.05 ± 0.70 b	18.15 ± 0.57 ab	19.75 ± 0.26 cd
		WD	9.85 ± 0.31 bcde	10.80 ± 0.62 b	14.50 ± 0.33 def	17.10 ± 0.44 f
P(CL)			ns	ns	***	***
P(T/CO_2/RH)			ns	ns	***	ns
P(I)			***	ns	***	*
P(CL x T/CO_2/RH)			*	ns	ns	ns
P(CL x I)			ns	ns	ns	ns
P(T/CO_2/RH x I)			ns	ns	ns	ns
P(CL)			ns	ns	ns	ns

Table S3. Total free amino acid content (nmol mg^{-1} pulp FW), relative abundance of amino acids grouped according to their precursor (ketoglutarate, pyruvate, aspartate, shikimate, phosphoglycerate) (%) and relative abundance of individual amino acids (%) at maturity in berries of the Tempranillo clones grown under two T/CO_2/RH conditions: current situation (CS: 24 °C/14 °C, 400 µmol mol^{-1}, and 45 %/65 % RH) and climate change (CC: 28 °C/18 °C, 700 µmol mol^{-1}, and 33 %/53 % RH), combined with two irrigation regimes: well-watered (WW) and water deficit (WD) at maturity. Probability values (P) for the main effects of clone, $P_{(CL)}$; T/CO_2/RH regime, $P_{(T/CO_2/RH)}$; irrigation regime, $P_{(I)}$; and their interactions, $P_{(CL \times T/CO_2/RH)}$, $P_{(CL \times I)}$, $P_{(T/CO_2/RH \times I)}$ and $P_{(CL \times T/CO_2/RH \times I)}$. ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant.

				Relative abundance (%)																						
			Total amino acid	**Ketoglutarate**	Proline	Arginine	Glutamic acid	Glutamine	Gaba	Histidine	**Pyruvate**	Alanine	Valine	Leucine	**Aspartate**	Threonine	Aspartic acid	Asparagine	Isoleucine	Methionine	Lysine	**Shikimate**	Tyrosine	Phenylalanine	**Phosphoglycerate**	Serine	Glycine
RJ43			**221.1**	**74.5**	30.61	21.63	2.93	15.13	3.65	0.57	**8.90**	7.17	1.00	0.72	**12.5**	7.53	2.19	2.07	0.43	0.17	0.10	**0.93**	0.47	0.46	**3.16**	2.66	0.50
CL306			**233.4**	**73.1**	30.26	16.85	3.30	18.96	2.97	0.71	**9.63**	7.22	1.31	1.11	**13.5**	7.18	2.73	2.50	0.75	0.25	0.06	**0.92**	0.45	0.46	**2.92**	2.61	0.31
VN31			**223.1**	**74.0**	34.48	13.92	3.29	18.98	2.89	0.47	**9.42**	7.50	1.10	0.82	**12.6**	7.21	2.51	2.03	0.51	0.20	0.10	**0.96**	0.50	0.47	**3.02**	2.74	0.28
1084			**118.5**	**68.4**	35.42	21.87	2.10	5.01	3.40	0.61	**9.60**	7.17	1.21	1.22	**18.5**	10.09	2.31	5.13	0.65	0.19	0.15	**0.82**	0.50	0.32	**2.64**	2.17	0.47
CS			**229.2**	**72.9**	31.12	18.07	2.94	16.65	3.35	0.77	**8.98**	7.16	0.99	0.83	**14.3**	7.75	2.44	3.32	0.49	0.19	0.10	**0.93**	0.45	0.49	**2.91**	2.32	0.59
CC			**167.9**	**72.2**	34.45	19.17	2.84	12.19	3.14	0.41	**9.74**	7.38	1.29	1.07	**14.2**	8.29	2.39	2.54	0.66	0.21	0.11	**0.88**	0.51	0.37	**2.97**	2.77	0.21
WW			**144.7**	**73.0**	35.85	20.27	2.99	9.79	3.73	0.37	**9.05**	7.40	0.89	0.76	**13.6**	8.44	2.20	2.47	0.33	0.11	0.10	**1.02**	0.55	0.47	**3.30**	2.85	0.45
WD			**253.3**	**72.1**	29.67	16.94	2.79	19.13	2.74	0.80	**9.70**	7.14	1.41	1.16	**14.9**	7.60	2.64	3.39	0.83	0.29	0.11	**0.79**	0.41	0.38	**2.57**	2.24	0.34
RJ43	CS	WW	**172.8**	**73.6**	36.00	19.02	2.87	10.75	4.50	0.43	**9.06**	7.72	0.76	0.58	**12.4**	8.01	1.60	2.35	0.22	0.12	0.09	**1.18**	0.59	0.59	**3.80**	2.85	0.96
		WD	**313.7**	**74.4**	29.01	16.06	3.48	21.72	3.45	0.69	**8.57**	6.97	0.94	0.66	**13.6**	7.08	2.78	3.09	0.43	0.19	0.06	**0.81**	0.36	0.44	**2.59**	2.05	0.54
	CC	WW	**118.3**	**76.0**	27.45	34.93	2.63	7.45	3.47	0.05	**7.76**	6.37	0.79	0.60	**11.7**	8.35	1.70	1.20	0.27	0.08	0.11	**0.97**	0.55	0.42	**3.59**	3.34	0.25
		WD	**279.6**	**74.2**	29.99	16.51	2.76	20.61	3.18	1.13	**10.21**	7.63	1.53	1.05	**12.2**	6.68	2.69	1.62	0.82	0.28	0.13	**0.75**	0.37	0.38	**2.65**	2.42	0.23
CL306	CS	WW	**231.1**	**73.9**	32.81	17.70	3.11	16.29	3.40	0.55	**8.85**	7.48	0.76	0.62	**12.7**	7.33	2.50	2.49	0.23	0.10	0.08	**1.22**	0.55	0.66	**3.33**	2.81	0.51
		WD	**246.5**	**72.5**	27.12	14.17	3.22	25.08	2.35	0.52	**9.99**	7.05	1.55	1.39	**14.1**	6.31	2.50	3.77	1.06	0.39	0.04	**0.86**	0.35	0.51	**2.63**	2.21	0.41
	CC	WW	**112.4**	**71.9**	36.50	17.80	4.36	9.37	3.64	0.28	**9.76**	8.14	0.93	0.69	**13.6**	9.16	2.52	1.46	0.31	0.10	0.07	**1.09**	0.63	0.46	**3.60**	3.49	0.11
		WD	**343.4**	**74.0**	24.61	17.75	2.50	25.09	2.50	1.50	**9.93**	6.21	1.99	1.74	**13.5**	5.91	3.41	2.28	1.41	0.40	0.06	**0.50**	0.27	0.23	**2.15**	1.93	0.21
VN31	CS	WW	**202.8**	**75.5**	33.77	16.40	3.44	17.97	3.34	0.56	**8.46**	7.23	0.71	0.52	**11.9**	7.42	2.37	1.76	0.20	0.11	0.07	**1.07**	0.48	0.59	**3.06**	2.64	0.42
		WD	**375.0**	**75.5**	27.56	13.45	2.98	28.12	2.26	1.09	**8.74**	6.52	1.27	0.94	**12.8**	5.75	2.90	2.97	0.76	0.26	0.16	**0.74**	0.36	0.38	**2.25**	2.00	0.25
	CC	WW	**120.9**	**70.6**	40.10	14.50	3.65	8.49	3.82	0.06	**10.84**	8.98	1.03	0.84	**13.8**	9.13	2.62	1.48	0.38	0.12	0.10	**1.06**	0.64	0.42	**3.64**	3.48	0.17
		WD	**193.8**	**74.5**	36.49	11.32	3.08	21.34	2.14	0.17	**9.66**	7.29	1.39	0.98	**11.7**	6.54	2.15	1.91	0.70	0.32	0.05	**0.98**	0.51	0.48	**3.14**	2.84	0.30
1084	CS	WW	**122.2**	**70.2**	32.09	24.58	1.97	6.33	4.19	1.06	**8.84**	7.11	0.86	0.87	**17.1**	9.95	2.28	4.25	0.36	0.10	0.13	**0.85**	0.45	0.40	**3.02**	2.06	0.96
		WD	**156.3**	**66.1**	29.62	23.50	2.45	6.35	2.96	1.24	**9.76**	7.24	1.22	1.30	**20.9**	10.32	2.68	6.64	0.82	0.28	0.12	**0.72**	0.41	0.31	**2.54**	1.97	0.57
	CC	WW	**90.4**	**72.2**	47.45	15.94	2.26	3.15	3.41	0.00	**8.95**	6.42	1.24	1.30	**15.6**	8.04	2.16	4.50	0.63	0.14	0.11	**0.79**	0.53	0.27	**2.45**	2.30	0.15
		WD	**114.7**	**64.6**	31.08	23.88	1.81	4.54	2.95	0.29	**10.89**	7.94	1.51	1.44	**21.2**	12.12	2.20	5.50	0.84	0.27	0.23	**0.87**	0.60	0.28	**2.52**	2.30	0.21
$P_{(CL)}$			***	***	ns	*	***	***	*	ns	**ns**	ns	ns	***	***	***	ns	***	*	ns	*	**ns**	ns	**	**ns**	**	ns
$P_{(T/CO_2/RH)}$			**	**ns**	ns	ns	ns	ns	ns	**	**ns**	ns	**	*	**ns**	ns	ns	**	*	ns	ns	**ns**	*	***	**ns**	**	***
$P_{(I)}$			***	**ns**	**	ns	ns	***	***	***	**ns**	ns	***	***	**	**	*	***	***	***	ns	***	***	**	***	***	ns
$P_{(CL \times T/CO_2/RH)}$			**ns**	**ns**	ns	ns	ns	ns	ns	***	**ns**	ns	ns	ns	**ns**	ns	ns	ns	ns	ns	ns	**ns**	ns	ns	**ns**	ns	ns
$P_{(CL \times I)}$			**ns**	**	ns	ns	*	ns	ns	ns	**ns**	ns	ns	*	***	***	ns	ns	*	ns	ns	*	*	ns	*	*	ns
$P_{(T/CO_2/RH \times I)}$			**ns**	**ns**	ns	ns	**	ns	ns	*	**ns**	ns	ns	ns	**ns**	ns	ns	ns	ns	ns	ns	**ns**	ns	ns	**ns**	ns	ns
$P_{(CL \times T/CO_2/RH \times I)}$			*	**ns**	ns	ns	ns	ns	ns	*	**ns**	ns	ns	ns	**ns**	**	ns	ns	ns	ns	ns	**ns**	ns	ns	**ns**	ns	ns

Figure S1. Bunch weight at maturity of Tempranillo clones grown under two T/CO2/RH conditions: current situation (CS; 24 °C/14 °C, 400 µmol mol^{-1}, and 45 %/65 % RH) and climate change (CC; 28 °C/18 °C, 700 µmol mol^{-1}, and 33 %/53 % RH) combined with two irrigation regimes: well-watered (WW) and water deficit (WD). Results (values are means ± SE) are represented according to the T/CO_2/RH and irrigation regimes, considering each clone individually (n = 5-8). Means with letters in common are not significantly different according to LSD test (P > 0.05). All probability values for the interactions of factors (P(CL x T/CO_2/RH), P(CLxI), P(T/CO_2/RH x I) and P(CL x T/CO_2/RH x I)) were statistically not significant (P > 0.05).