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

Marta Arrizabalaga-Arriazu1,2, Eric Gomès2, Fermín Morales3, Juan José Irigoyen1, Inmaculada Pascual1 and Ghislaine Hilbert2*

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

*Correspondence: Ghislaine Hilbert ghislaine.hilbert@inrae.fr

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

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

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

The Intergovernmental Panel on Climate Change (IPCC) has pointed out the ineluctable increase in the temperature worldwide and has identified climate change as an important threat to global food supply. Indeed, greenhouse gas (GHG) emissions have increased greatly during the last decades, affecting the equilibrium of biogeochemical cycles and, hence, the composition of the atmosphere (IPCC, 2013). Some of the consequences of the rise in the atmospheric concentration of GHG are at global climate level, as high levels of GHG block heat loss of the planet, thus contributing to the so-called "greenhouse effect" and to global warming. Another effect of the anthropogenic GHG emissions is the increase in the concentration of CO$_2$ in the atmosphere (IPCC, 2014). The IPCC has carried out different reports from scientific knowledges on climate change to determine the magnitude of these changes according to different scenarios. Some of the predictions for global mean temperature in 2100 show an increment between 2.2 ± 0.5 and 3.7 ± 0.7°C, and the estimations for future atmospheric CO$_2$ concentration are between 669.7 and 935.9 ppm (scenarios RCP 6.0 and RCP 8.5 of the IPCC) (IPCC, 2013).

Research on the response of grapevine to the abovementioned environmental factors has concluded that high temperature affects the phenology of grapevine, as well as grape berry development and ripening, hastening the latter (Jones and Davis, 2000; Duchêne and Schneider, 2005; Webb et al., 2007; Keller, 2010; Martínez-Lüscher et al., 2016a) and affecting both primary and secondary metabolisms. Berry sugar accumulation is altered under climate change conditions (Duchêne and Schneider, 2005; Kuhn et al., 2014), while malic acid degradation is enhanced by high temperatures (Orduña, 2010; Carbonell-Bejerano et al., 2013), and by its combination with elevated CO$_2$ (Salazar Parra et al., 2010; Martínez-Lüscher et al., 2016b). Secondary metabolism is also sensitive to high temperatures, particularly the flavonoid and aroma precursor pathways, as evidenced by transcriptomic, proteomic, and metabolomic approaches (Spayd et al., 2002; Tarara et al., 2008; Azuma et al., 2012; Rieth et al., 2014; Martínez-Lüscher et al., 2016a; Lecourieux et al., 2017, 2020; Arrizabalaga et al., 2018), thus affecting the balance of berry quality-related compounds at ripeness (Sadas and Moran, 2012; Kuhn et al., 2014). Whereas increased temperature has been consistently shown to reduce anthocyanin levels (Spayd et al., 2002; Mori et al., 2007; Tarara et al., 2008; Azuma et al., 2012), the effect of CO$_2$ on these compounds is more controversial, with some authors reporting no effect (Goncalves et al., 2009), while others reported an increase in anthocyanin levels (Kizildeniz et al., 2015). Finally, the decoupling in the accumulation of anthocyanins and sugars, thus leading to an imbalance between these two compounds at maturity, has been also described as a consequence of elevated temperature (Sadas and Moran, 2012; Kuhn et al., 2014).

The studies on the impact of multiple stress factors associated with climate change on grape development and composition remain limited due to their complexity. However, in the future, plants will be exposed to multiple elements of environmental change simultaneously, their combined effects being not always additive. Indeed, the impact of an increase in atmospheric CO$_2$ concentration on plant carbon metabolism, notably on photosynthesis and respiration processes, has been previously reported (Dusenge et al., 2019). Zhao et al. (2019) also demonstrated that photosynthesis of in vitro grape plantlet was promoted by elevated CO$_2$ concentration. These modifications may directly affect primary and secondary plant metabolism, thus leading to substantial changes in fruit composition. However, Dusenge et al. (2019) showed that some effects of increased CO$_2$ concentration on carbon plant metabolism could be minimized by elevated temperatures and highlighted the interest to study these climatic parameters together, both separately and in combination.

Regarding grapevine, previous studies on the effect of elevated CO$_2$ and elevated temperature indicated that the combination of these two factors can affect plant phenology, thus hastening grape ripening (Salazar-Parra, 2011; Martínez-Lüscher et al., 2016b) and decreasing malic acid concentration (Leibar et al., 2010). Nevertheless, the authors reported also a decrease in grape total anthocyanin concentration at maturity. Therefore, in order to avoid detrimental wine traits, it is important to determine how grape characteristics will be affected by the abovementioned climate change-related factors, acting not only individually but also combined, and to investigate approaches to mitigate the undesired effects, ensuring the sustainability of this crop. Among the strategies proposed to adapt viticulture to a changing environment, the genetic improvement and adaptation of elite and autochthonous varieties are very relevant to keep their intrinsic varietal values and typicity (Cunha et al., 2009; Keller, 2010; Carbonell-Bejerano et al., 2019). Accordingly, the selection of grapevine varieties and clones within the same variety with longer ripening periods has been suggested as a valuable tool to exploit in order to find accessions keeping current traits under future climate conditions (Duchêne et al., 2010).
Certain varieties used for wine production are tightly linked to specific production areas. This is the case of Tempranillo in Spain, Merlot in France, Sangiovese in Italy, or Fernão Pires in Portugal (Seo et al., 2003). Tempranillo requires warm, sunny days to reach full maturity but also cool nights to keep its natural acidity. Maturity occurs fairly early in comparison with Grenache, the variety commonly blended with Tempranillo. Nevertheless, the constant increase in temperature and CO₂ levels in the future could significantly shift the optimal maturation conditions in these areas, which would have a significant effect on berry quality. For these reasons, successfully exploiting the intravarietal diversity has the potential to face with the putative negative impacts of climate change, as it would allow to keep the culture of traditional varieties. The research done so far in Tempranillo includes the identification and characterization of a large number of clones (49 certified clones), which differ either in reproductive or vegetative traits (Ibáñez et al., 2015), making possible the research of clones better adapted to future climate conditions.

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

**MATERIALS AND METHODS**

**Plant Material: Origin and Development**

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

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

**Treatments and Plant Growth in Temperature Gradient Greenhouses**

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

An equal number of plants of each clone were placed in the first and the third module of four TGGs, leaving the central module free of plants. Half of the plants (those located in modules 1) grew at ambient temperature (T), corresponding to the ambient temperature outdoors, while the other half of the plants (those located in modules 3) grew under ambient plus 4°C warmer temperature (T + 4). Besides, the air CO₂ concentration was modified in two out of the four TGGs, resulting in half of the plants growing at current atmospheric CO₂ concentration (ca. 400 ppm; ACO₂) and the other half under increased air CO₂ concentration (700 ppm; ECO₂). Therefore, plants grew under four different CO₂ and temperature conditions:
(i) ambient temperature and ambient CO$_2$ ($T$/ACO$_2$), (ii) ambient temperature and elevated CO$_2$ ($T$/ECO$_2$), (iii) elevated temperature and ambient CO$_2$ ($T + 4$/ACO$_2$), and (iv) elevated temperature and elevated CO$_2$ ($T + 4$/ECO$_2$). The treatments were applied from fruit set (2016, May) to berry maturity (2016, September), which was considered to occur when the total soluble solid (TSS) content of the berries was ca. 22°Brix, each plant being measured individually (°Brix was determined on grape juice, after pressing entire grape berries, using a hand refractometer with temperature compensation). The number of plants per treatment and clone was 8.

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

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

Berry Analyses Preparation
Analyses were carried out by doing pools of berries (3–10 berries per bunch, see above, from two or three different plants per pool, four pools per treatment and clone). Berries were manually peeled and separated into skin, pulp, and seeds. Fresh skins, pulps, and seeds were weighed and the data obtained were used to determine the relative skin mass (relation between skin fresh weight and total berry fresh weight, expressed as a percentage). The pulp was crushed to obtain the must, which was centrifuged and used for sugar, malic acid, and amino acid analyses. The skin was freeze-dried in an Alph1-4, lyophilizer (CHRIST, Osterode, Germany), weighed to calculate the water content, and ground into powder using an MM200 ball grinder (Retsch, Haan, Germany) for carrying out the anthocyanin analyses.

Total Soluble Solids, Sugar, Malic Acid, and Amino Acid Profile Analyses
Total soluble solids content in the must was measured using a Handheld Digital Refractometer (RD110). Sugar (glucose and fructose) concentration was determined enzymatically using an automated absorbance microplate reader (Ebx800UV, BioTek Instruments, Inc., Winooski, VT, United States) using the Glucose/Fructose kit from BIOSENTEC (Toulouse, France) according to the manufacturer. Malic acid was determined with a Bran and Luebbe TRAACS 800 autoanalyzer (Bran & Luebbe, Plaisir, France) as described in detail in Bobeica et al. (2015). For the amino acid analysis, samples were derivatized with the AccQFluor Reagent (6-aminquinolyl-N-hydroxy-succinimidyl-carbamate, Waters, Milford, MA, United States) (Cohen and Michaud, 1993) as described by Hilbert et al. (2015). The products of this reaction were analyzed with an UltiMate 3000 UHPLC system (Thermo Electron SAS, Waltham, MA, United States) equipped with an FLD-3000 Fluorescence Detector (Thermo Electron SAS, Waltham, MA, United States). Amino acids were separated using as eluents sodium sodium acetate buffer (eluent A, 140 mM at pH 5.7), acetonitrile (eluent B), and water (eluent C) at 37°C and 0.5 ml min$^{-1}$ through an AccQTag Ultra column, 2.1 mm $\times$ 100 mm, 1.7 µm (Waters, Milford, MA, United States) according to Habran et al. (2016). The concentration and identification of each compound was determined through a chromatographic analysis as described in Pereira et al. (2006), using an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Samples were loaded alternated with control samples as in Torres et al. (2017).

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

**Statistical Analysis**

The data were statistically analyzed using R (3.5.1), carrying out a three-way ANOVA (clone, temperature, and CO\(_2\) concentration) and a Fisher's least significant difference (LSD) was carried out as a post hoc test when statistically significant differences were found (\(P < 0.05\)).

**RESULTS**

**Phenological Development**

In general, elevated CO\(_2\) had a higher impact on grape phenology in the period comprised between fruit set to mid-veraison, whereas ripening (mid-veraison to maturity) was more affected by elevated temperature (Figure 1A). The number of days elapsed between fruit set and mid-veraison was slightly, but significantly, shortened by ECO\(_2\), and especially when it was combined with \(T + 4\) (Figure 1A). However, this hastening effect of CO\(_2\) was nullified between mid-veraison and maturity, whereas the increase of temperature reduced significantly this period in up to 5 days. Also, the duration from mid-veraison to maturity was affected significantly by the clone identity, mainly because 1084 needed more time to reach maturity, regardless of the growing condition (Figure 1B). Although significant interactions among factors were not detected, it is worth pointing out the significant difference in the elapsed days between mid-veraison and maturity of T3 plants grown under \(T + 4/ACO_2\) compared with \(T/ACO_2\), as maturity was reached 12 days earlier when \(T + 4\) was applied.

**Berry Characteristics**

Berry diameter differed significantly among clones at the onset of veraison, mid-veraison, and maturity (Table 1). It was also modified by temperature, decreasing under \(T + 4\) 2 weeks after mid-veraison and at maturity. By contrast, the CO\(_2\) level did not markedly affect this berry characteristic. The only noteworthy interaction among the parameters was at mid-veraison, when the effect of ECO\(_2\) was different depending on the temperature regime and the clone (triple interaction).

In general, berries from all the studied clones presented similar relative skin mass throughout the experiment except at maturity, when 1084 had significantly lower values than CL306, T3, and VN31 (Table 2). Relative skin masses were higher 1 and 2 weeks after mid-veraison in grapes developed under \(T + 4\) compared with those grown at \(T\). Grapes under
ECO2 had a lower relative skin mass than ACO2 at the onset of veraison, but higher 1 and 2 weeks after mid-veraison. At maturity, the T3 clone was the most affected one by the increase in temperature of T + 4, especially when combined with ACO2.

### Malic Acid

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

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

---

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

| Berry diameter (mm) | Onset of veraison | Mid-veraison | 1 week after mid-veraison | 2 weeks after mid-veraison | Maturity |
|---------------------|-------------------|-------------|--------------------------|---------------------------|---------|
| RJ43 | 10.51 ± 0.18ab | 10.78 ± 0.23bc | 11.36 ± 0.21b | 13.19 ± 0.19a | 12.71 ± 0.17ab |
| CL306 | 10.11 ± 0.16b | 10.57 ± 0.13c | 11.36 ± 0.19b | 12.07 ± 0.19a | 12.33 ± 0.20b |
| T3 | 10.86 ± 0.20a | 11.11 ± 0.19b | 11.63 ± 0.23ab | 12.40 ± 0.22a | 13.11 ± 0.17a |
| VN31 | 10.77 ± 0.15a | 11.19 ± 0.13ab | 13.00 ± 1.25a | 13.81 ± 1.07a | 13.07 ± 0.16a |
| 1084 | 10.86 ± 0.23a | 11.60 ± 0.19a | 12.10 ± 0.20ab | 12.16 ± 0.30a | 12.88 ± 0.25a |
| T | 10.75 ± 0.12a | 11.18 ± 0.13a | 11.85 ± 0.13a | 13.39 ± 0.60a | 13.14 ± 0.13a |
| T + 4 | 10.49 ± 0.13a | 10.92 ± 0.13a | 11.93 ± 0.52a | 12.07 ± 0.15b | 12.50 ± 0.10b |
| ACO2 | 10.60 ± 0.10a | 11.07 ± 0.12a | 12.16 ± 0.51a | 12.42 ± 0.11a | 12.68 ± 0.13a |
| ECO2 | 10.64 ± 0.14a | 11.03 ± 0.13a | 11.62 ± 0.15a | 13.03 ± 0.63a | 12.96 ± 0.13a |

Results (values are means ± SE) are shown according to clone identity (n = 16), temperature regime (n = 40), CO2 concentration (n = 40), and the combined factors (n = 4). Means with letters in common within the same stage and factor (clone, temperature, CO2, or their interactions) are not significantly different according to the least significant difference (LSD) test (P > 0.05). Probability values (P) for the main effects of clone, P_CL, temperature, P_T; CO2, P_CO2, and their interactions, P_CL × T, P_CL × CO2, P_T × CO2, and P_CL × T × CO2. ***P < 0.001; **P < 0.01; *P < 0.05; ns, not significant.
TABLE 2 | Relative skin mass (%) in grape berries of the five Tempranillo clones (RJ43, CL306, T3, VN31, and 1084) grown under four temperature/CO₂ regimes: ambient temperature (T) or ambient temperature + 4°C (T + 4), combined with ambient CO₂ (ca. 400 ppm, ACO₂) or elevated CO₂ (700 ppm, ECO₂).

| Onset of veraison | Mid-veraison | 1 week after mid-veraison | 2 weeks after mid-veraison | Maturity |
|------------------|--------------|--------------------------|---------------------------|----------|
| RJ43             |              |                          |                           |          |
| CL306            |              |                          |                           |          |
| T3               |              |                          |                           |          |
| VN31             |              |                          |                           |          |
| 1084             |              |                          |                           |          |
| T + 4            |              |                          |                           |          |
| T + 4            |              |                          |                           |          |
| T + 4            | 15.19 ± 0.59a| 13.94 ± 0.48a            | 14.98 ± 0.47a             | 17.31 ± 1.01a |
| T + 4            | 16.56 ± 0.57a| 13.74 ± 0.46a            | 13.67 ± 0.42a             | 14.35 ± 0.77b |
| ECO₂             | 14.11 ± 0.33b| 14.57 ± 0.39a            | 15.17 ± 0.37a             | 16.56 ± 0.75a |
| RJ43 T ACO₂      | 17.79 ± 0.71ab| 15.33 ± 0.91abc          | 12.24 ± 0.35c             | 15.80 ± 1.19bdef |
| CL306 T ACO₂     | 16.64 ± 1.35abc| 12.91 ± 0.41abc          | 13.90 ± 0.29abc           | 14.07 ± 1.08abc |
| T + ACO₂         | 14.68 ± 0.44abc| 15.75 ± 0.86abc          | 12.55 ± 0.74bc            | 15.70 ± 0.98abc |
| T + 4 ACO₂       | 15.04 ± 1.59bcd| 13.94 ± 0.74abc          | 16.64 ± 3.13a             | 18.60 ± 1.64abc |
| CL306 T ECO₂     | 14.79 ± 0.81bcd| 14.46 ± 0.63abc          | 15.84 ± 1.35ab            | 22.58 ± 5.00a |
| T + 4 ECO₂       | 17.36 ± 1.15abc| 15.18 ± 1.05abc          | 14.48 ± 1.75abc           | 14.12 ± 1.50def |
| T + 4 ECO₂       | 12.05 ± 0.81bcd| 13.18 ± 0.74abc          | 13.26 ± 0.65abc           | 15.10 ± 0.29def |
| VN31 T ACO₂      | 17.32 ± 1.59abc| 14.92 ± 1.19abc          | 14.36 ± 0.85abc           | 13.66 ± 0.93bc |
| T + ACO₂         | 14.86 ± 0.75bcd| 13.44 ± 0.50abc          | 14.78 ± 0.53abc           | 15.82 ± 0.84abc |
| T + 4 ACO₂       | 15.43 ± 0.41bcd| 11.81 ± 2.22c             | 13.36 ± 1.07abc           | 14.09 ± 2.50abc |
| VN31 T ECO₂      | 14.10 ± 0.65bcd| 15.44 ± 1.42abc          | 16.65 ± 1.42a             | 19.57 ± 0.95ab |
| T + 4 ECO₂       | 15.59 ± 0.67bcd| 13.07 ± 0.38abc          | 12.93 ± 1.14abc           | 12.34 ± 0.35c |
| T + 4 ECO₂       | 14.78 ± 1.10bcd| 14.35 ± 1.08abc          | 15.50 ± 1.45abc           | 12.75 ± 0.50bc |
| ECO₂             | 13.55 ± 1.85c| 13.57 ± 0.82abc          | 15.31 ± 1.14abc           | 17.02 ± 0.96abc |

P_{(ICL)} ns ns ns ns *

P_{(T)} ns ns * ns

P_{(CO₂)} *** ns ns ns

Results (values are means ± SE) are shown according to the clone (n = 18), temperature (n = 40), CO₂ concentration (n = 40), and the combined factors (n = 4). Means with letters in common within the same factor and stage (clone, temperature, CO₂, or their interactions) are not significantly different according to the LSD test (P > 0.05). Probability values (P) for the main effects of clone, P_{(ICL)}; temperature, P_{(T)}; and CO₂, P_{(CO₂)}; **P < 0.001; ***P < 0.001; *P < 0.05; ns, not significant. All probability values for the interactions of factors [P_{(ICL × T)}; P_{(ICL × CO₂)}; P_{(T × CO₂)}; and P_{(ICL × T × CO₂)}] were statistically not significant (P > 0.05).

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

Sugars and Total Soluble Solids

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

Amino Acids

In general, total amino acid concentration reached the highest values 2 weeks after mid-veraison in clones RJ43, CL306, and VN31, whereas the concentration of amino acid continued increasing until maturity in T3 and 1084 (Figure 4A and Supplementary Tables 1A–E). Amino acid levels were similar among clones throughout berry development, except 2 weeks after mid-veraison, when 1084 had lower levels in comparison with the other clones. At this stage, and considering all the clones,
FIGURE 2 | Malic acid concentration of the five Tempranillo clones grown under four temperature/CO$_2$ regimes: ambient temperature ($T$) or ambient temperature + 4°C ($T$ + 4), combined with ambient CO$_2$ (ca. 400 ppm, ACO$_2$) or elevated CO$_2$ (700 ppm, ECO$_2$). Data (values are means ± SE, $n = 4$) are presented according to temperature ($T$ or $T$ + 4) and CO$_2$ regime (ACO$_2$ or ECO$_2$) (A) throughout ripening and (B) at maturity. Data are presented according to the temperature ($T$ or $T$ + 4) and CO$_2$ regime (ACO$_2$ or ECO$_2$) and considering each clone individually. Means with letters in common are not significantly different according to the LSD test ($P > 0.05$). Probability values ($P$) for the main effects of clone, $P_{(CL)}$; temperature, $P_{(T)}$; and CO$_2$, $P_{(CO2)}$. $***P < 0.001$; $*P < 0.05$; ns, not significant. All probability values for the interactions of factors [$P_{(CL \times T)}$, $P_{(CL \times CO2)}$, $P_{(T \times CO2)}$, and $P_{(CL \times T \times CO2)}$] were statistically not significant ($P > 0.05$).

the $T$ + 4 treatment reduced total amino acid concentration compared with the $T$ treatment (from 15.9 ± 1.33 to 12.3 ± 1.18 µmol ml$^{-1}$, respectively, Supplementary Table 1D). Also, ECO$_2$ diminished the amino acid levels with respect to ACO$_2$ (from 17.1 ± 1.48 to 11.2 ± 0.82 µmol ml$^{-1}$, respectively) 2 weeks after mid-veraison (Supplementary Table 1D). At maturity, there were no significant effects of temperature and CO$_2$ (neither individually nor combined). However, $T$ + 4 tended to reduce the concentration of total amino acids (especially under ACO$_2$) in all the clones and ECO$_2$ tended to reduce the amino acid levels of CL306, T3, and VN31 at ambient temperature (Figure 4B and Supplementary Table 1E).

The relative abundance of the different amino acids varied among clones. Specifically, 1 and 2 weeks after mid-veraison and at maturity, the pyruvate and aspartate derivatives were more abundant in 1084 at the expense of α-ketoglutarate derivatives (except GABA and arginine, which increased) (Figure 4C and Supplementary Tables 1D,E). Considering all the clones as a whole, ECO$_2$ significantly reduced the proportion of α-ketoglutarate derivatives (although arginine and GABA tended to increase in the later stages of the ripening period at ECO$_2$), increasing the abundance of those originated from aspartate and pyruvate (2 weeks after mid-veraison and at maturity, respectively). Even though there were no significant interactions, there were two exceptions to this effect at maturity, as ECO$_2$ increased α-ketoglutarate derivatives in the grapes of clones RJ43 and 1084 exposed to $T$ and $T$ + 4, respectively. In addition, the rise in the relative abundance of pyruvate derivatives observed in the ECO$_2$ treatment at maturity was globally stronger at $T$ + 4 (Figure 4C and Supplementary Table 1E). Moreover, at the onset of veraison, ECO$_2$ strongly increased the relative abundance of phenylalanine in all clones and more specifically in CL306 and 1084 when combined with elevated temperature.

Total Skin Anthocyanins

Anthocyanin levels did not differ significantly in the early ripening period among clones, but 2 weeks after mid-veraison and at maturity, the concentration of total anthocyanins was lower in 1084, whereas RJ43 showed the highest values (Figures 5A,B). $T$ + 4 had a significant enhancing effect
on anthocyanins at the onset of veraison and 2 weeks after mid-veraison, regardless of the clone and CO$_2$ level, this effect disappearing at maturity. ECO$_2$ increased anthocyanin concentration at the onset of veraison and mid-veraison but reduced the levels 2 weeks after mid-veraison (Figure 5A). At maturity, although there were no significant interactions between factors, the $T + 4$/ECO$_2$ treatment seemed to have different effects depending on the clone. Whereas, in RJ43, the grapes exposed to $T + 4$/ECO$_2$ (climate change conditions) had significantly lower anthocyanin levels than those exposed to $T$/ACO$_2$ (current conditions), in CL306, the concentration of skin anthocyanins increased with climate change conditions ($T + 4$/ECO$_2$) (Figure 5B).

**Total Anthocyanins to TSS Ratio**

Clones showed different anthocyanin to TSS ratios at maturity regardless of the temperature and CO$_2$ regime, RJ43 and T3 having the highest values (Figure 6A). Considering the clones altogether, a significant interaction between temperature and CO$_2$ was observed (Figure 6B). Thus, the significant decrease of the ratio between anthocyanins and TSS under ACO$_2$ induced by $T + 4$, with respect to $T$, disappeared under ECO$_2$. When the temperature and CO$_2$ effects were analyzed for each clone independently, the growing conditions showed slightly different effects on the anthocyanin to TSS ratio (Figure 6C). In RJ43 and VN31, the impact of $T + 4$ on the ratio was more evident under ACO$_2$ conditions. ECO$_2$ strongly increased the ratio in CL306 plants at $T + 4$, while it did not have any effect at $T$. Finally, neither temperature nor CO$_2$ had a marked impact on the relationship between anthocyanins and TSS in T3 and 1084.

**DISCUSSION**

**Performance of Clones**

Clones showed different characteristics in all the studied parameters. The accession that differed the most from the others studied was 1084. It had an extremely long berry ripening period associated with a lower sugar accumulation rate, not even reaching the optimum sugar levels for wine production. The
FIGURE 4 | Amino acid concentration in berries of the five Tempranillo clones grown under four temperature/CO$_2$ regimes: ambient temperature (T) or ambient temperature + 4°C (T + 4), combined with ambient CO$_2$ (ca. 400 ppm, ACO$_2$) or elevated CO$_2$ (700 ppm, ECO$_2$). Data are presented according to temperature (T or T + 4) and CO$_2$ regime (ACO$_2$ or ECO$_2$) and considering each clone individually (values are means ± SE, n = 3–4). (A) Throughout ripening and (B) at maturity. Relative abundance of amino acids (C) grouped by their precursor. Means with letters in common are not significantly different according to the LSD test (P > 0.05). Probability values (P) for the main effects of clone, P$_{CL}$; temperature, P$_{T}$; and CO$_2$, P$_{CO_2}$; ***P < 0.001; *P < 0.05; ns, not significant. All probability values for the interactions of factors [P$_{CL}$×T, P$_{CL}$×CO$_2$, P$_{T}$×CO$_2$, and P$_{CL}$×T×CO$_2$] were statistically not significant (P > 0.05).

1084 accession also had berries with low relative skin mass and presented the lowest values of malic acid at maturity. Despite T3, VN31, and RJ43 having similar berry diameter to 1084 (indicating similar size), their malic acid values were higher than in 1084. This result suggests that the low concentration of malic acid in 1084 was not associated with a dilution effect due to high berry size. After veraison, malate is released from the vacuole and becomes available for catabolism through involvement in gluconeogenesis, respiration through the tricarboxylic acid (TCA) cycle, amino acid interconversions, and the production of secondary compounds such as anthocyanins and flavonols (Ruffner and Kliewer, 1975; Ruffner et al., 1976; Ruffner, 1982; Famiani et al., 2000; Ollat et al., 2002; Sweetman et al., 2009).

Probably, the longer ripening period of the 1084 accession, already seen in previous experiments (Arrizabalaga et al., 2018), contributed to higher malate breakdown, thus reaching a lower malic acid concentration at maturity. Interestingly, clones differed in the moment to reach the amino acidic maturity, earlier in RJ43, CL306, and VN31 than in T3 and 1084. The amino acid profile at maturity was also different among clones, 1084 showing a reduced proportion of α-ketoglutarate and increased abundance of pyruvate and aspartate derivatives. The higher concentration of isoleucine, leucine, and valine (aromatic precursors) in 1084 might have a positive effect on the final wine aroma (Valdés et al., 2019).

The 1084 accession also presented a lower concentration of skin anthocyanins, compared with the other clones. The results are in agreement with our previous work (Arrizabalaga et al., 2018) and may be related to the slow sugar accumulation rate observed in 1084. Dai et al. (2014), using an experimental system allowing the long-term in vitro culture of grape berries, reported an induction in total anthocyanins by rising sugar concentrations in the culture medium, as well as a negative correlation between phenylalanine and total anthocyanin levels. In the present study, phenylalanine was similar in 1084, and even higher 2 weeks after mid-veraison, compared with the other clones. Therefore, the lower anthocyanin accumulation may be a consequence of a limitation in the biosynthetic enzymatic activity rather than to a limitation of its precursor, as suggested by Dai et al. (2014). Also, it is possible that α-ketoglutarate availability is lower in 1084; thus, biosynthetic steps that use α-ketoglutarate as reducing power for anthocyanin accumulation (i.e., hydroxylases) become limiting. Indeed, α-ketoglutarate level was reported to be one of the metabolic drivers of anthocyanin accumulation in grape cells (Soubeyrand et al., 2018). In opposition to the results obtained by Roby and Matthews (2008), the anthocyanin content in berries was not systematically positively correlated to the relative skin mass, except for 1084. These discrepancies may be related to the fact that in our case anthocyanins were measured in dry skin and Roby and Matthews (2008) did it in the whole berry. In
addition, both 1084 and T3 had some of the largest diameters. However, the relative skin mass of this two clones differed significantly, T3 having a higher value and 1084 the lowest. These results indicate a lack of effect of berry size on the relative skin mass already observed by Barbagallo et al. (2011). These berry parameters are important as they are considered to determine the solutes extraction during the maceration process (Walker et al., 2005; Matthews and Nuzzo, 2007; Roby and Matthews, 2008; Barbagallo et al., 2011).

**Global Response of Tempranillo Clones to Elevated Temperature**

The increment of temperature shortened the elapsed time between mid-veraison and maturity and reduced the size of berries at the end of the ripening process. These results agree with previous studies, in which berries stopped increasing their volume after being heat-treated at mid-veraison and mid-ripening (Kliewer, 1977; Roby and Matthews, 2008; Keller, 2010; Orduña, 2010) and high temperature accelerated the ripening process (Jones and Davis, 2000; Duchêne and Schneider, 2005; Keller, 2010; Arrizabalaga et al., 2018). Given that maturity was determined by the level of TSS (ca. 22 °Brix), the reduction of the time to reach this stage in the $T + 4$ treatment indicates a faster and more efficient accumulation of sugars under these conditions. In the present study, the lower berry size in the $T + 4$ may have contributed to the higher concentration of sugars observed. However, the effect of high temperatures on sugar accumulation varies among studies and conditions. Whereas elevated temperature has been reported to enhance sugar accumulation (Jones et al., 2005; Mosedale et al., 2016), in other cases, berry sugar content at harvest was not affected (Lecourieux et al., 2017), and sugar accumulation was stopped (Roby and Matthews, 2008; Greer and Weston, 2010), or even decreased (Carbonell-Bejerano et al., 2013; Greer and Weedon, 2013; Kuhn et al., 2014). These apparently contradictory results may be due to differences in the experimental procedures, which included more
Arrizabalaga-Artiazu et al. High Temperature and CO₂ Modify Berry Composition

It is well established that warm temperatures promote the decrease of organic acid levels in grape berries after mid-veraison, by accelerating malate degradation (Ordúña, 2010; Carbonell-Bejerano et al., 2013; Sweetman et al., 2014; Torres et al., 2016). Malic acid respiration is favored by heat, and the genes involved in its transmembrane transport display a marked regulation by temperature (Rienth et al., 2016). In addition, the enhancement of the anaplerotic capacity of the TCA cycle for amino acid biosynthesis by elevated temperatures has also been suggested (Sweetman et al., 2014; Lecourieux et al., 2017). In our work, although malic acid degradation was promoted by T + 4 from mid-veraison onward, the concentration of total amino acids tended to decrease in this treatment compared with T, and changes in the proportion α-ketoglutarate, pyruvate, or aspartate amino acid derivatives were not so obvious. These results may indicate that under the temperature conditions assayed, 4°C of difference between T and T + 4 vs. increases of 8 (Lecourieux et al., 2017) and 10°C (Sweetman et al., 2014) in the mentioned studies, the anaplerotic capacity of the TCA cycle for amino acid biosynthesis may not be markedly increased. Other pathways such as gluconeogenesis may have played a more important role in malate degradation, thus contributing to the differences in sugar accumulation observed between temperature regimes. Despite the limited changes in the amino acid profile, an increased proportion of proline and arginine was observed under high temperature, as previously reported by other authors (Lecourieux et al., 2017; Torres et al., 2017). Proline has a protective role in plants against abiotic stresses, including elevated temperature, whereas arginine is an important source of nitrogen during winemaking (Garde-Cerdán et al., 2011), despite being a precursor of putrescine, a compound with negative effects on human health (Guo et al., 2015).

Considering the clones altogether, high temperature significantly increased the concentration of anthocyanins 2 weeks after mid-veraison, but it did not affect the final anthocyanin levels at maturity. These results do not agree with previous studies that demonstrate that high temperature during ripening
Global Response of Tempranillo Clones to Elevated CO₂

High atmospheric CO₂ concentration slightly hastened grape phenology, but only from fruit set to mid-veraison, as reported by Martinez-Lüscher et al. (2016a) for red and white Tempranillo. Regarding fruit composition, the increase in malic acid concentration of grapes exposed to ECO₂ at the onset of veraison may indicate an enhancement of the organic acid biosynthesis at early berry development stages. These differences disappeared in later stages, when the degradation of malic acid took place, and the grapes ripened under ECO₂ conditions reaching even lower malic acid levels at maturity than those grown at ACO₂, possibly because of an accelerated breakdown. Similarly, Bindi et al. (2001), in a field experiment using a free air CO₂ enrichment (FACE) facility, observed that organic acid components were positively affected by increases in CO₂ concentration at the middle of the ripening season, an effect that almost completely vanished at maturity. On the other hand, ECO₂ significantly decreased the amino acid concentration at the onset of veraison and 2 weeks after mid-veraison. Elevated CO₂ may induce a priority in the storage of nitrogen-free compounds (i.e., carbohydrates) compared with nitrogen-containing compounds, such as amino acids and proteins (Myers et al., 2014). In our case, the concentration of sugars in the berries was not significantly altered by ECO₂ at these stages, so the decrease in amino acids could be a consequence of the inhibition of N assimilation as reported in different plant species (Serret et al., 2018). Similarly, in a previous study with the same clones and under similar growth conditions, berries exposed to ECO₂ showed lower N concentration in the berries (Arrizabalaga-Arriazu et al., 2020). The differences observed up to 2 weeks after veraison in the present study decreased at maturity, when the ECO₂ treatment showed only a slightly lower total amino acid concentration. At this stage, even though the relative abundance of some individual amino acids was significantly affected by ECO₂ conditions, the proportions of amino acid families according to their precursor were little modified, except a slightly reduced proportion of α-ketoglutarate derivatives, at the expense of those originated from pyruvate. The significant decrease in the concentration of glutamine under ECO₂ may involve a limitation for yeast growth during fermentation since glutamine, together with arginine, is one of the mayor sources of N for yeast (Zoecklein et al., 1999).

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

Response of Clones to Combined Elevated Temperature and CO₂

The analysis of the combined effects of high temperature and elevated CO₂ on the parameters analyzed indicates low interactive effects of these environmental factors on sugar accumulation and, thus, in the length of the ripening period, as ECO₂ did not modify the hastening effect induced by T + 4. Conversely, the increase in temperature and CO₂ showed additive effects enhancing malic acid degradation, a phenomenon
especially marked in the 1084 accession (probably due to its longer exposure to these conditions), but less evident in RJ43 and CL306. Such reduction of malic acid content and its impact on must acidity should certainly affect wine production, not only for its contribution to sourness and organoleptic properties but also because of its influence on wine microbiological stability. In addition, it might make the winemaking process more expensive in the future due to the need of acidifying the must for achieving a proper fermentation (Keller, 2010). The combined application of elevated temperature and high CO$_2$ did not seem to have a marked impact on the total level of amino acids at maturity, although a tendency to decrease the amino acid concentrations was observed as reported by Martínez-Lüscher et al. (2016a). This effect was more evident 2 weeks after mid-veraison, $T > 4$ and ECO$_2$ significantly reducing their concentration. These results suggest an impact of climate change delaying the amino acidic maturity of grapes, which seemed to be reached some weeks earlier in the $T$/ACO$_2$ treatment. Amino acids (excluding proline and hydroxyproline) are very important components of the yeast assimilable nitrogen (YAN), thus having implication for must fermentation. A decrease of total amino acids level could involve a lower N availability during the fermentation process. In the same way, a reduction in the concentration of shikimate and phosphoglycerate derivatives may have implications in the organoleptic properties of the wine produced from these grapes, since the shikimate route is involved in the biosynthesis of aromatic amino acids (tyrosine and phenylalanine) and the phenylpropanoid pathway (phenylalanine).

As for amino acids, combined elevated temperature and CO$_2$ did not significantly impact anthocyanin concentration at maturity when considering all the clones as a whole. However, the observed increase due to high temperature 2 weeks after veraison was offset by combination with elevated CO$_2$. Interestingly, a significant interaction between temperature and CO$_2$ was observed for the relationship between anthocyanin and sugar levels: the temperature-induced decrease in this ratio under ACO$_2$ was not observed under ECO$_2$. This result suggests that in a future climate change scenario, elevated CO$_2$ may, at least partially, mitigate the negative impact of high temperature on the imbalance between sugars and anthocyanins in ripe berries.

In addition, regarding the anthocyanin content, there was a wide range of responses of the clones studied to climate change conditions. Total anthocyanin concentration at maturity was impacted by combined elevated temperature and CO$_2$, showing a decrease in RJ43, T3, and VN31 (stronger in the former), despite their difference in the reproductive cycle length (defined as intermediate, short, and long, respectively). Conversely, combined elevated temperature and CO$_2$ increased anthocyanin content in CL306 (characterized as a short reproductive cycle clone) and in 1084 (long reproductive cycle). Moreover, while in RJ43 and VN31 climate change conditions ($T + 4$/ECO$_2$) markedly reduced the anthocyanin to sugar ratio with respect to the current conditions ($T$/ACO$_2$) (differences statistically significant in RJ43), the balance between these two compounds was less affected in CL306, T3, and 1084. Grapevine is characterized by a pronounced sensitivity toward the environment, and the metabolic composition of the berries has been reported to show a broad phenotypic plasticity, offering advantages such as the range of different wines that can be produced from the same cultivar and the adaptation of existing cultivars to different growing regions (Keller, 2010). The present results suggest that clones of the same variety may perform differently under climate change conditions, some, such as RJ43, showing greater variation and others, such as 1084, offering more consistency. Unfortunately, we cannot directly associate these different performances with the length of their grape development period as we hypothesized originally, at least as far as anthocyanins and anthocyanin to sugar ratio are concerned. In this line, although 1084, with the longest length of the reproductive cycle, was one of the least affected in terms of anthocyanin and sugar balance, other clones that exhibited an early maturity (CL306 and T3) were not so affected either. A recent study has demonstrated differences in the berry phenotypic plasticity between grapevine varieties in response to changes in environmental conditions, Cabernet Sauvignon being less dependent on growth conditions, thus showing a limited transcriptomic plasticity associated with epigenetic regulation (Dal Santo et al., 2018). These authors also concluded that within-cultivar diversity may modulate gene expression in response to environmental cues. We suggest that clones within the same variety can also exhibit different degrees of phenotypic plasticity for grape composition and, consequently, different capacities of adaptation to climate change conditions. Recently, Tortosa et al. (2019) have reported that some clones of Tempranillo variety can perform better water use efficiency than others depending of the water availability conditions. Although the results obtained need to be considered in the light of the limitations of the study (i.e., 1 year experiment, potted plants, and controlled conditions), and they should be validated with studies in the field, the differences observed point toward the usefulness of the exploitation of the grapevine genotypic diversity in order to optimize the genotype–environment interaction and the adaptation of traditional varieties to the foreseen climate scenarios.

**CONCLUSION**

The projected increases in atmospheric CO$_2$ concentration and in average air temperature advanced grape maturity, reducing the elapsed time between fruit set and mid-veraison and between mid-veraison and maturity, respectively. High temperature hastened berry ripening, sugar accumulation, and malic acid breakdown, especially when combined with elevated CO$_2$. In contrast, climate change conditions seemed to delay amino acidic maturity. Even though the increase of temperature and high CO$_2$ concentration (both individually and combined) did not affect anthocyanin concentration, the clones studied showed different values for this parameter. The reduction of the ratio between anthocyanins and TSS under $T + 4$ was partially mitigated by ECO$_2$. Additionally, the study reveals the existence of a differential response of Tempranillo clones to the projected future temperature and CO$_2$ levels in terms of grape composition.
DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

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

FUNDING

This work was supported by the Ministerio de Economía y Competitividad of Spain (AGL2014-56075-C2-1-R), Fundación Universitaria de Navarra (2018), European Union (Erasmus+ grant to MA-A), Aquitaine Regional Council (AquimOb grant to MA-A), and Asociación de Amigos de la Universidad de Navarra (doctoral grant to MA-A). This work also supported by the metaprogramme Adaptation of Agriculture and Forests to Climate Change (AAFC) of the French National Institute for Agriculture, Food and Environment (INRAE), in the frame of LACCAVE-21 project.

ACKNOWLEDGMENTS

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

SUPPLEMENTARY MATERIAL

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

REFERENCES

Acevedo de la Cruz, A., Hilbert, G., Rivière, C., Mengin, V., Ollat, N., Bordenave, L., et al. (2012). Anthocyanin identification and composition of wild Vitis spp. accessions by using LC-MS and LC-NMR. Analyst. Chem. Acta 732, 145–152. doi: 10.1016/j.aca.2011.11.060

Arrizabalaga-Arriazu, M., Morales, F., Oyarzun, M., Delrot, S., Gomés, E., Irigoyen, J. J., et al. (2018). Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. Plant Sci. 267, 74–83. doi: 10.1016/j.plantsci.2017.11.009

Arrizabalaga-Arriazu, M., Morales, F., Irigoyen, J. J., Hilbert, G., and Pascual-I. (2020). Growth performance and carbon partitioning of grapevine Tempranillo clones under simulated climate change scenarios: elevated CO_{2} and temperature. J. Plant Physiol. 252:153226. doi: 10.1016/j.jplph.2020.153226

Azuma, A., Yakushiji, H., Koshita, Y., and Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. Planta 236, 1067–1080. doi: 10.1007/s00425-012-1650-x

Barbagallo, M. G., Guidoni, S., and Hunter, J. J. (2011). Berry size and qualitative characteristics of Vitis vinifera L. cv. Syrah. S. Afr. J. Enol. Viticult. 32, 129–136. doi: 10.21548/32-1-1372

Bindi, M., Fiább, L., and Miglietta, F. (2001). Free Air CO_{2} Enrichment (FACE) of grapevine (Vitis vinifera L.): II. Growth and quality of grape and wine in response to elevated CO_{2} concentrations. Eur. J. Agron. 14, 145–155. doi: 10.1016/S1161-0301(00)00093-9

Bobeica, N., Poni, S., Hilbert, G., Renaud, C., Gomés, E., Delrot, S., et al. (2015). Differential responses of sugar, organic acids and anthocyanins to source-sink modulation in Cabernet Sauvignon and Sangiovese grapevines. Front. Plant Sci. 6:382. doi: 10.3389/fpls.2015.00382

Carbonell-Bejerano, P., Royo, C., Mauri, N., Ibáñez, J., and Zapater, J. M. M. (2019). “Somatic variation and cultivar innovation in grapevine,” in Advances in Grape and Wine Biotechnology, eds A. Morata and I. Loira (London: IntechOpen), 1–22. doi: 10.5772/intechopen.86443

Carbonell-Bejerano, P., Marta María, E., Torres-Pérez, R., Royo, C., Liavetzky, D., Bravo, G., et al. (2013). Thermostolerance responses in ripening berries of Vitis vinifera L. cv Muscat Hamburg. Plant Cell Physiol. 54, 1200–1216. doi: 10.1093/pcp/pcp071

Cibrain, F., Jimeno, K., Sagüés, A., Rodríguez, M., Abad, J., Martínez, M. C., et al. (2018). TempraNA?: tempranillos con matrícula. Navarra Agraria 22, 12–20.

Cohen, S. A., and Michaud, D. P. (1993). Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolyzable amino acids via high-performance liquid chromatography. Anal. Biochem. 211, 279–287. doi: 10.1006/abio.1993.1270

Cohen, S. D., Tarara, I. M., and Kennedy, J. A. (2008). Assessing the impact of temperature on grape phenolic metabolism. Analyst. Chem. Acta 621, 57–67. doi: 10.1016/j.aca.2007.11.029

Conde, C., Silva, P., Fontes, N., Dias, A., Tavares, R., Sousa, M., et al. (2007). Biochemical changes throughout grape berry development and fruit and wine quality. Food 1, 1–22.

Cunha, J., Santos, M. T., Carneiro, L. C., Fevereiro, P., and Eiras-Dias, J. E. (2009). Enrichment (FACE) of the five Tempranillo clones grown under four temperature/CO_{2} regimes: ambient temperature (T) and ambient temperature + 4°C (T + 4), combined with ambient CO_{2} (ca. 400 ppm, ACO_{2}) or elevated CO_{2} (700 ppm, ECO_{2}). Data are presented according to the temperature (T or T + 4) and CO_{2} regime (ACO_{2} or ECO_{2}) and considering each clone individually (values are means ± SE, n = 4). Means with letters in common are not significantly different according to LSD test (P > 0.05).

Carbonell-Bejerano, P., Royo, C., Mauri, N., Ibáñez, J., and Zapater, J. M. M. (2020). Long-term in vitro culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. J. Exp. Bot. 65, 4665–4677. doi: 10.1093/jxb/ert489
Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., et al. (2018). Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G × E) on the berry transcriptome. Plant J. 93, 1143–1159. doi: 10.1111/tp.13834

de Rosas, I., Ponce, M. T., Malovini, E., Deis, L., Cavagnaro, B., and Cavagnaro, P. (2017). Loss of anthocyanins and modification of the anthocyanin profiles in grape berries of Malbec and Bonarda grown under high temperature conditions. Plant Sci. 258, 137–145. doi: 10.1016/j.plantsci.2017.01.015

Downey, M. O., Dokoozlian, N. K., and Krsitc, M. P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. Am. J. Enol. Viticul. 57, 257–268.

Duchène, E., Huard, F., Dumas, V., Schneider, C., and Merdinoglu, D. (2010). The challenge of adapting grapevine varieties to climate change. Clim. Res. 41, 193–204. doi: 10.3354/cr008850

Duchène, E., and Schneider, C. (2005). Grapevine and climatic changes: a glance at the situation in Alsace. Agron. Sustain. Dev. 25, 93–99. doi: 10.1051/agro:2004057

Dusenge, M. E., Duarte, A. G., and Way, D. A. (2019). Plant carbon metabolism and temperature effects on photosynthesis, photorespiration and respiration. New Phytol. 221, 32–49. doi: 10.1111/nph.15283

EVERA (2009). Evaluación de Clones Comerciales de seis Variedades de vid en Navarra. 1995-2005. Pamplona: Desarrollo Rural y Medio Ambiente Gobierno de Navarra.

Famiani, F., Walker, R. P., Tecsi, L., Chen, Z., Proietti, P., and Leegood, R. C. (2000). EVENA (2009).

Gouot, J. C., Smith, J. P., Holzapfel, B. P., Walker, A. R., and Barril, C. (2019b). Cultural practice and increased temperature on grape berry metabolism. Food Chem. 313, 65–77. doi: 10.1016/j.foodchem.2019.01.150

Greer, D. H., and Weston, C. (2010). Heat stress affects flowering, berry growth, concentration.

Hilbert, G., Temsamani, H., Bordenave, L., Pedrot, E., Chaher, N., Cluzet, S., et al. (2016). Roostocks/Scion/Nitrogen interactions affectsecondary metabolism in grape berries of Malbec and Bonarda grown under high temperature conditions. Plant Sci. 221, 32–49. doi: 10.1111/nph.15283

Hjelm, G., Temsamani, H., Bordenave, L., Pedrot, E., Chaher, N., Cluzet, S., et al. (2015). Berry flesh and skin ripening features in Vitis vinifera cv. red and white Tempranillo. Yield and must quality in three consecu.

Jones, G. V., and Davis, R. E. (2000). Climate influences on grapevine phenology grape composition, and wine production and quality for Bordeaux, France. Am. J. Enol. Vitic. 51, 249–261.

Kiljeeper, W. M. (1977). Effect of high temperatures during the bloom-Set period on grape berry development. J. Exper. Bot. 28, 215–224.

Kuhn, N., Guan, L., Dai, Z. W., Wu, B.-H., Lauvergeat, V., Gomès, E., et al. (2014). Berry ripening: recently heard through the grapevine. J. Exp. Bot. 65, 4543–4559. doi: 10.1093/jxb/ert395

Kiljeeper, W. M. (1977). Effect of high temperatures during the bloom-Set period on grape berry development. J. Exper. Bot. 28, 215–224.

Leibar, U., Pascual, I., Morales, A., Aizpurua, A., and Unamunzaga, O. (2016). Grape yield and quality responses to simulated year 2100 expected climatic conditions under different soil textures. J. Sci. Food Agric. 97, 2633–2640. doi: 10.1002//jsfa.8806

Li, D., Zhang, X., Li, L., Aghdam, M. S., Wei, X., Liu, J., et al. (2019). Elevated CO2 delayed the chlorophyll degradation and anthocyanin accumulation in postharvest strawberry fruit. Food Chem. 285, 163–170. doi: 10.1016/j.foodchem.2019.01.150

Ljavezitsky, D., Carbonell-Bejerano, P., Grimpltet, J., Bravo, G., Flores, P., Fennoll, J., et al. (2012). Berry flesh and skin ripening features in Vitis vinifera as assessed by transcriptional profiling. PLoS One 7:e39547. doi: 10.1371/journal.pone.0039547

Martínez-Lüscher, J., Kizildeniz, T., Pascual, I., Mekni, I., Santesteban, H., Pascual, I., Morales, F., and Irigoyen, J. J. (2015). Effects of climate change including elevated CO2 concentration, temperature and water deficit on growth, water status, and yield quality of grapevine (Vitis vinifera L.) cultivars. Agric. Water Manag. 159, 155–164. doi: 10.1016/j.agwat.2015.06.015

Kiljeeper, W. M. (1977). Effect of high temperatures during the bloom-Set period on fruit-Set, ovule fertility, and berry growth of several grape cultivars. Am. J. Enol. Vitic. 28, 215–222.

Lebar, U., Pascual, I., Morales, A., Aizpurua, A., and Unamunzaga, O. (2016). Grape yield and quality responses to simulated year 2100 expected climatic conditions under different soil textures. J. Sci. Food Agric. 97, 2633–2640. doi: 10.1002//jsfa.8806

Li, D., Zhang, X., Li, L., Aghdam, M. S., Wei, X., Liu, J., et al. (2019). Elevated CO2 delayed the chlorophyll degradation and anthocyanin accumulation in postharvest strawberry fruit. Food Chem. 285, 163–170. doi: 10.1016/j.foodchem.2019.01.150

Ljavezitsky, D., Carbonell-Bejerano, P., Grimpltet, J., Bravo, G., Flores, P., Fennoll, J., et al. (2012). Berry flesh and skin ripening features in Vitis vinifera as assessed by transcriptional profiling. PLoS One 7:e39547. doi: 10.1371/journal.pone.0039547

Martínez-Lüscher, J., Kizildeniz, T., Pascual, I., Mekni, I., Santesteban, H., Pascual, I., Morales, F., and Irigoyen, J. J. (2015). Effects of climate change including elevated CO2 concentration, temperature and water deficit on growth, water status, and yield quality of grapevine (Vitis vinifera L.) cultivars. Agric. Water Manag. 159, 155–164. doi: 10.1016/j.agwat.2015.06.015

Kiljeeper, W. M. (1977). Effect of high temperatures during the bloom-Set period on fruit-Set, ovule fertility, and berry growth of several grape cultivars. Am. J. Enol. Vitic. 28, 215–222.

Lebar, U., Pascual, I., Morales, A., Aizpurua, A., and Unamunzaga, O. (2016). Grape yield and quality responses to simulated year 2100 expected climatic conditions under different soil textures. J. Sci. Food Agric. 97, 2633–2640. doi: 10.1002//jsfa.8806

Li, D., Zhang, X., Li, L., Aghdam, M. S., Wei, X., Liu, J., et al. (2019). Elevated CO2 delayed the chlorophyll degradation and anthocyanin accumulation in postharvest strawberry fruit. Food Chem. 285, 163–170. doi: 10.1016/j.foodchem.2019.01.150

Ljavezitsky, D., Carbonell-Bejerano, P., Grimpltet, J., Bravo, G., Flores, P., Fennoll, J., et al. (2012). Berry flesh and skin ripening features in Vitis vinifera as assessed by transcriptional profiling. PLoS One 7:e39547. doi: 10.1371/journal.pone.0039547

Martínez-Lüscher, J., Kizildeniz, T., Pascual, I., Mekni, I., Santesteban, H., Pascual, I., Morales, F., and Irigoyen, J. J. (2015). Effects of climate change including elevated CO2 concentration, temperature and water deficit on growth, water status, and yield quality of grapevine (Vitis vinifera L.) cultivars. Agric. Water Manag. 159, 155–164. doi: 10.1016/j.agwat.2015.06.015

Kiljeeper, W. M. (1977). Effect of high temperatures during the bloom-Set period on fruit-Set, ovule fertility, and berry growth of several grape cultivars. Am. J. Enol. Vitic. 28, 215–222.
anthocyanin and sugar accumulation. Austr. J. Grape Wine Res. 22, 87–95. doi: 10.1111/jgwr.12213
Matthews, M. A., and Nuzzo, V. (2007). Berry size and yield paradigms on grapes and wines quality. Acta Hort. 754, 423–436. doi: 10.17660/ActaHortic.2007.754.25
Mora, F., Pascual, I., Sánchez-Díaz, M., Aguirreolea, J., Irigoyen, J. I., Goicoechea, N., et al. (2014). Methodological advances: using greenhouses to simulate climate change scenarios. Plant Sci. 226, 30–40. doi: 10.1016/j.plantsci.2014.03.018
Mori, K., Goto-Yamamoto, M., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. J. Exp. Bot. 58, 1935–1945. doi: 10.1093/jxb/erm055
Mosedale, J. R., Abernethy, K. E., Smart, R. E., Wilson, R. J., and Maclean, I. M. D. (2016). Climate change impacts and adaptive strategies: lessons from the grapevine. Glob. Chang. Biol. 22, 3814–3828. doi: 10.1111/gcb.13406
Myers, S. S., Kloog, I., Huybers, P., Leakey, A. D. B., Bloom, A. J., Manso, F., et al. (2010). Effects of climate change scenarios on Tempranillo grapevine. Austr. J. Enol. Viticult. 53, 171–182. doi: 10.1038/nature13179
OIV (2017). 2017 World Viticulture Situation: OIV Statistical Report on World Vitiviniculture. Paris: OIV.
Ollat, N., Carde, J.-P., Gaudillère, J.-P., Carde, J.-P., Barrieu, F., Diakou-Verdin, P., and Moing, O. (2007). Microclimate influence on mineral and metabolic profiles of grape berries. J. Agric. Food Chem. 55, 6765–6775. doi: 10.1021/jf061013k
Rieth, M., Roby, G., and Matthews, M. A. (2008). Berry growth and development: a review. OENO One 36, 109–131.
Rieth, N., Geny, L., and Soyer, J.-P. (1998). Grapevine fruiting cuttings: validation of an experimental system to study grapevine physiology. I. Main vegetative characteristics. J. Sci. Vigne Vin 32, 1–9. doi: 10.1080/09261244.1998.11926000
Sefc, K. M., Steinkellner, H., Lefort, F., Botta, R., Da Câmara Machado, A., Borrego, J., et al. (2003). Evaluation of the genetic contribution of local wild vines to European grapevine cultivars. Am. J. Enol. Viticult. 54, 15–21.
Selvar, M. D., Youshi, S., Vicente, R., Pihilo, M. C., Ollat, N., Carde, J.-P., Gaudillère, J.-P., Barrieu, F., Diakou-Verdin, P., and Moing, O. (2018). Interactive effects of CO2 concentration and water regime on stable isotope signatures, nitrogen assimilation and growth in sweet pepper. Front. Plant Sci. 8:2180. doi: 10.3389/fpls.2017.02180
Soubeyrand, E., Colombié, S., Beauvoit, B., Dai, Z., Cluzet, S., Hilbert, G., et al. (2018). Constraint-based modeling highlights cell energy, redox status and α-ketoglutarate availability as metabolic drivers for anthocyanin accumulation in grape cells under nitrogen limitation. Front. Plant Sci. 9:421. doi: 10.3389/fpls.2018.00421
Torres, N., Goicoechea, N., Morales, F., and Antolín, M. C. (2016). Berry quality and antioxidant properties in Vitis vinifera cv. Tempranillo as affected by clonal variability, mycorrhizal inoculation and temperature. Crop. Past. Sci. 67, 961–977. doi: 10.1017/cp16038
Torres, N., Hilbert, G., Luquin, J., Goicoechea, N., and Antolín, M. C. (2017). Flavanoid and amino acid profiling on Vitis vinifera L. cv. Tempranillo subjected to deficit irrigation under elevated temperatures. J. Food Compost. Analys. 62, 51–62. doi: 10.1016/j.jfca.2017.05.001
Tortosa, J., Elahonda, J. M., Douthe, C., Pou, A., Garcia-Escudero, E., Toro, G., et al. (2019). The intra-cultivar variability on water use efficiency at different water status as a target selection in grapevine: Influence of ambient and genotype. Agric. Water Manag. 223:105764. doi: 10.1016/j.agwat.2019.05.032
Valdés, M. E., Talaverano, M. I., Moreno, D., Prieto, M. H., Mancha, L. A., Uriarte, D., et al. (2019). Effect of the timing of water deficit on the must amino acid profile of Tempranillo grapes grown under the semiarid conditions of SW Spain. Food Chem. 292, 24–31. doi: 10.1016/j.foodchem.2019.04.046
Vicente Castro, A. (2012). Respuesta Agronómica y Cualitativa de 4 Clones Certificados de Vitis vinifera L. cv. Tempranillo en la DO Arlanza. Available online at: http://uvadoc.uva.es/handle/10324/1677 (accessed September 1, 2020).
Vitis Navarra (2019). Tempranillo Para el XXI. Recuperando el Origen. Available online at: http://www.vitisnavarra.com/clones-exclusivos (accessed May 29, 2019).
Walker, R. R., Blackmore, D. H., Clingeleffer, P. R., Kerridge, G. H., Rühl, E. H., and Nichols, P. R. (2005). Shiraz berry size in relation to seed number and implications for juice and wine composition. Austr. J. Grape Wine Res. 11, 2–8. doi: 10.1111/j.1755-0238.2005.tb00273.x
Webb, L. B., Whetton, P. H., and Barlow, E. W. R. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. Austr. J. Grape Wine Res. 13, 165–175. doi: 10.1111/j.1755-0238.2007.b00247.x
Webb, L. B., Whetton, P. H., and Barlow, E. W. R. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. Austr. J. Grape Wine Res. 13, 165–175. doi: 10.1111/j.1755-0238.2007.b00247.x
Yamane, T., Jeong, S. T., Goto-Yamamoto, N., Koshita, Y., and Kobayashi, S. (2006). Effects of temperature on anthocyanin biosynthesis in grape berry skins. Am. J. Enol. Viticult. 57, 54–59.
Zoecklein, B. W., Fugelsang, K. C., Gump, B. H., and Nury, F. S. (1999). 
Wine Analysis and Production. New York, NY: Kluwer Academic 
Publishers.

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