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Flavonoid and amino acid profiling on *Vitis vinifera* L. cv Tempranillo subjected to deficit irrigation under elevated temperatures

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ABSTRACT

Throughout the southern Mediterranean regions of Europe, projected climate warming combined with severe droughts during the growing season may alter grape metabolism, thus modifying the nutritional value of berries and the quality of wines. This study investigated the effects of pre- and post-veraison drought under elevated temperatures on berry skin metabolism of two Tempranillo clones (CL).

Experimental assays were performed on fruit-bearing cuttings from CL-1089 and CL-843 of *Vitis vinifera* (L.) cv. Tempranillo subjected to two temperature regimes (24/14 °C or 28/18 °C (day/night)) combined with three irrigation regimes during berry ripening: (i) water deficit from fruit set to veraison (early deficit, ED); (ii) water deficit from veraison to maturity (late deficit, LD); and (iii) full irrigation (FI). At 24/14 °C, the LD treatment performed better than the ED treatment. Differences were attenuated at 28/18 °C and responses were modulated by type of clone. Elevated temperatures induced the accumulation of hexoses and amino acids in berries. ED at 24/14 °C reduced anthocyanins and flavonols, which may decrease the antioxidant properties of fruits. In contrast, the levels of these secondary metabolites did not decrease when LD was applied. Our results suggest that the adaptation of grapevines for climate change might be plausible with the optimization of timing of water deficit and the appropriate selection of clones.

1. Introduction

Grapes are one of the most important crops in Europe. According to the official dataset of the Statistics Division of Eurostat, in 2014 Europe produced 22.6 million tons, with Spain being one of the greatest producers (29.6% of European production) (Eurostat Statistics Division, 2014). The most distinctive characteristic of the South Mediterranean European climate is the concentration of rainfall in the winter half-year but future climate projections for the this region predict seasonal temperatures with higher rates of warming in summer and autumn (IPCC, 2014; Spinoni et al., 2015).

Abiotic stresses such as drought and high temperatures reduce grapevine yield due to their great impact on berry growth and ripening (Kuhn et al., 2014). Berry skin (exocarp) is the site for the synthesis of major compounds and defines grape berry quality, which mainly depend on sugars, organic acids, amino acids, phenolic compounds and aroma precursors (Castellarin et al., 2012; Darriet et al., 2012). This very active skin metabolism deeply influences the final characteristics of the berry and thus the understanding of metabolic responses to environmental constraints has both scientific and practical importance. The projected warming trends due to global climate change specially affect grapevine physiology. Harvest occurs sooner (Sadras and Petrie, 2011), berry sugar content (and alcohol in the wine) tends to increase (Petrie and Sadras, 2008) and phenolic and aromatic ripeness are delayed (Teixeira et al., 2013), which results in an imbalance between berry sugar accumulation and phenolic ripening (Sadras and Morán, 2012). Regarding drought, some studies have reported that application of regulated deficit irrigation during berry ripening has a significant impact on berry metabolism, which influences flavor and the quality characteristics of grapes and wines (Deluc et al., 2009). Indeed, water deficit improved accumulation of phenolic compounds, especially anthocyanins (Niculcea et al., 2014, 2015; Kyraleou et al., 2016) due to direct effects on flavonoid gene expression and metabolism (Castellarin et al., 2007). Thus, water deficit irrigation could enhance the nutraceutical value of berries since water restriction can induce the accumulation of anthocyanins (Kyraleou et al., 2016). Anthocyanins are the most important group of water-soluble pigments in plants, and they are regarded as important components in human nutrition due to their antioxidant capacities (Stintzing and Carle, 2004) and anti-carcinogenic effects against several types of cancer cells (You et al., 2011). However,

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expected benefits of water deficit may not be achieved under projected future warming conditions (Bonada et al., 2015).

The adaptation of grapevines grown in south Mediterranean Europe to a climate change scenario might require a selection of new grapevines varieties. Many studies have reported a broad clonal diversity in grapevine varieties for precocity of the phenological cycle, yield, berry composition, skin phenolic compounds and disease resistance (Revilla et al., 2009; van Leeuwen et al., 2013), and for response to environmental changes (Berdeja et al., 2015; Torres et al., 2016). Therefore, the aim of this study was to investigate the impact of pre- and post-veraison deficit irrigation under elevated temperatures on berry metabolism of two Tempranillo clones.

2. Material and methods

2.1. Plant material and growth conditions

Dormant 400–500 mm long *Vitis vinifera* (L.) cuttings from two clones of Tempranillo were collected during the winter of 2014 from an experimental vineyard of the Institute of Sciences of Vine and Wine (Logroño, Spain) (Denomination of Origin Rioja, North of Spain) (latitude: 42°28′12″N; longitude: 2°26′44″W, altitude: 384 mamsl). A brief description of the selected clones is presented in Table 1. Cuttings from each clone were selected to obtain fruit-bearing cuttings according to the steps originally outlined by Mullins (1966) with some modifications described in Ollat et al. (1998) and Morales et al. (2016). Briefly, cuttings were rooted in a heat-bed (27 °C) and then kept in a cool room (4 °C). One month later, the cuttings were planted in 6.5 l plastic pots containing a mixture of vermiculite-sand-light peat (2.5:2.5:1, v:v:v) and transferred to greenhouses, which were adapted to simulate climate change conditions (more details in Morales et al., 2014). Experiments were made with potted vines to ensure that both clones were subjected to the same temperature conditions and similar water stress patterns. Previous research has demonstrated that fruit-bearing cuttings are a meaningful and useful model system to study the response of berry ripening to environmental factors (Morales et al., 2016). Initial growth conditions were a 25/15 °C and 50/90% relative humidity (day/night) regime and natural daylight (photosynthetic photon flux density, PPFD, was on average 850 μmol m^{-2} s^{-1} at midday) supplemented with high-pressure sodium lamps (SON-T Agro Phillips, Eindhoven, Netherlands) to extend the photoperiod up to 15 h and ensure a minimum PPFD of 350 μmol m^{-2} s^{-1} at the level of the inflorescence. Until fruit set, plants were watered twice per day with the nutrient solution reported by Ollat et al. (1998) alternated with water to maintain the soil water content at 80% of pot capacity.

Table 1
Summary of the agronomic characteristics of the Tempranillo clones used in this study. Data provided by Institute of Sciences of Vine and Wine (Logroño, Spain) were collected and averaged over the 2009–2012 period from plants grown in field.

	CL-1089	CL-843
City of origin (region)	Bargota (Navarra) (Latitude: 42°33′40″N; longitude: 2°18′43″W, altitude: 587 mamsl).	Oyón (Álava) (Latitude: 42°30′21″N; longitude: 2°26′11″W, altitude: 435 mamsl).
Agronomic classification		
Reproductive cycle	Short	Long
Yield	High	High
Reproductive cycle		
Fruit set-veraison (days)	52	61
Veraison-maturity (days)	33	56
Yield components		
Yield (kg vine^{-1})	21.91	12.65
Bunch mass (g bunch^{-1})	154	199
Berry mass (g)	2.05	1.50

2.2. Experimental design

We established a two-factorial experiment where two temperature regimes were combined with three water regimes. At fruit set (Eichhorn and Lorenz (E-L) growth stage 27) (Coombe, 1995) fruit-bearing cuttings (36 plants per clone) were divided into two groups that were exposed to different temperature regimes: 24/14 °C (day/night) and 28/18 °C (day/night). At this stage, plants have 4–5 fully expanded leaves. The 24/14 °C temperature regime was selected according average temperatures recorded in La Rioja (1981–2010) (AEMET, 2016) during the growing season. The 28/18 °C temperature regime was selected according to predictions of a rise of 4.0 °C at the end of the present century (IPCC, 2014). Both temperature regimes were maintained until berries ripened (21 − 23°Brix) (E-L 38 stage).

Within each temperature regime, plants were divided into three groups that were subjected to different irrigation programs. Two deficit irrigation strategies were compared with full irrigation (FI). In the FI treatment, pots were maintained at 80% of pot capacity from fruit set to harvest. In the deficit irrigation treatments, plants received 50% of the water given to FI plants from fruit set (E-L 27 stage) to onset of veraison (E-L 35 stage) (early deficit, ED) or from onset of veraison (E-L 35 stage) to maturity (E-L 38 stage) (late deficit, LD). Volumetric soil water content was monitored with an EC 5 water sensor (Decagon Devices, Inc., Pullman, WA, USA) placed within each pot. There were three replicates for each treatment.

Non-destructive determinations were made at four stages of berry development: 1) when berries began to soften (E-L 34 stage, green berries); 2) when berries began to colour and enlarge (E-L 35 stage, veraison); 3) seven days after veraison (E-L 36 stage); and 4) fourteen days after veraison (E-L 37 stage). At fruit maturity (E-L 38 stage), plants were harvested separately based on sugar level (21 − 23°Brix) from berry subsamples (2–3 berries) taken weekly. Length of phenological phases was recorded as the number of days from fruit set (E-L 27 stage) to veraison (E-L 35 stage), and from veraison (E-L 35 stage) to maturity (E-L38 stage).

2.3. Plant measurements

Predawn leaf water potential (Ψ_{pd}) was measured with a SKYE SKPM 1400 pressure chamber (Skye Instruments Ltd, Llandrindod, Wales, UK) on three fully expanded leaves per treatment at each sampling date just prior to irrigation.

The evolution of epidermal levels of flavonols and anthocyanins was estimated *in situ* by using a handheld, non-destructive fluorescence-based proximal Multiplex3™ sensor (Force-A, Orsay, France) as described by Agati et al. (2013). At maturity, ten berries from each treatment were collected and weighed. Mean fresh berry mass was determined and then, berries were separated into skin and flesh (including seeds). Subsequently, each berry fraction was oven-dried at 80 °C until constant mass was reached. Berry water content was calculated as 100*(FM-DM)/FM, where FM is fresh mass and DM is dry mass. The relative skin mass was calculated as the quotient between skin FM and total berry FM expressed as a percentage. The rest of the berries were counted, weighed and frozen at −80 °C for further analysis.

2.4. Sugars, organic acids and amino acids profiles in berry skins

Samples of 5–10 berries per plant were separated into skin and flesh. Skins were powdered separately in an MM200 ball grinder (Retsch, Haan, Germany) and then, they were freeze dried in a Vir Tis Bench Top K lyophilizer (SP Scientific, Warminster, Philadelphia, PA, USA). Skins from each plant were used to analyse primary and