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Characterization of phenolic composition of *Vitis vinifera* L. 'Tempranillo' and 'Graciano' subjected to deficit irrigation during berry development

M. Niculcea[1)], L. Martinez-Lapuente[2)], Z. Guadalupe[2)], M. Sánchez-Díaz[1)], B. Ayestarán[2)] and M. C. Antolín[1)]

[1)] Grupo de Fisiología del Estrés en Plantas (Dpto. de Biología Ambiental), Unidad Asociada al CSIC (EEAD, Zaragoza, ICVV, Logroño), Facultades de Ciencias y Farmacia, Universidad de Navarra, Pamplona, Spain
[2)] Instituto de Ciencias de la Vid y del Vino (Universidad de La Rioja, Gobierno de La Rioja y CSIC), Logroño, Spain

Summary

The response of phenolic composition of skins from *Vitis vinifera* L. 'Tempranillo' and 'Graciano' grapes to water-deficit irrigation during berry growth and ripening was evaluated. The study was carried out using container-grown grapevines grown under controlled conditions in a greenhouse. Two irrigation treatments were imposed: control (well-watered) and sustained deficit irrigation (SDI). Twenty-eight phenolic compounds, including anthocyanins, flavonols and monomeric flavan-3-ols (catechins) as well as phenolic acids derivatives have been identified in the extracts prepared from the berry skins at physiological maturity. For both varieties, water deficit reduced leaf area and leaf area to crop mass ratio, and decreased berry size. However, there were no changes in juice total soluble solids, pH or total polyphenolic content. Water deficit resulted in decreased must titratable acidity in 'Graciano' berries. In 'Tempranillo', water limitation reduced total anthocyanins and flavonols, and increased hydroxycinnamic acids. In 'Graciano', water deficit resulted in increased flavonols and reduced catechins. Altogether, we concluded that under water-deficit irrigation, 'Graciano' grapes presented a differential composition of phenolic compounds that could result in improved fruit quality.

K e y w o r d s : Anthocyanins, flavonols, fruiting cuttings, hydroxycinnamic acids, sustained deficit irrigation.

Introduction

Phenolic substances are of profound significance to both the technological and nutritional value of grapes and wines (Downey *et al.* 2006). Grape and wine phenolics include two main classes: flavonoid and nonflavonoid compounds. Flavonoids, the most important family among grape phenols, include anthocyanins, flavan-3-ols and flavonols. Nonflavonoids include hydroxycinnamic acids and stilbenes. Flavonoids are regarded as one of the most important determinants of quality in red grapes and wines (Downey *et al.* 2006). In recent year's flavonoid compounds have also attracted attention for their potential health benefits (Pezzuto 2008). Anthocyanins are the main agents responsible for the colour of red grapes and the wines produced from them, and the individual anthocyanin content and composition in grape berry skins is an important factor determining wine quality because they have different characteristics with regard to colour or stability (reviewed by He *et al.* 2010). Flavan-3-ols exist as non-glycosylated monomers (catechin), dimers, and polymers (proanthocyanidins or condensed tannins). Flavan-3-ols play an important role in the taste and conservation of wine (Waterhouse 2002) and are also responsible for the bitterness, astringency and structure of wines (Gawell 1998, Peleg *et al.* 1999). Although colourless, flavonols seem to contribute to both bitterness and to wine colour as copigments (Boulton 2001, Hufnagel and Hofmann 2008), whereas that phenolic acids can participate in copigmentation (Schwarz *et al.* 2005).

The production of grapevine secondary metabolites can be impaired or magnified by abiotic stress factors such as drought. Thus, it has been demonstrated in grape that a moderate water restriction is useful to improve organoleptic quality of wine (Sivilotti *et al.* 2005, Chalmers *et al.* 2010). Changes in vine water status affect polyphenol amounts, indicating that cultural practices can be used to influence composition. Water deficit can enhance accumulation of anthocyanins through the stimulation of anthocyanin hydroxylation (Chaves *et al.* 2010). Indeed, Castellarin *et al.* (2007b) showed that water deficits accelerated anthocyanin accumulation and increased the expression of many genes responsible for the biosynthesis of anthocyanins. Besides changes in anthocyanin contents, its individual composition changed in response to plant water status (Esteban *et al.* 2001, Castellarin *et al.* 2007a, b, Bindon *et al.* 2008, Ollé *et al.* 2011). However, the effects of water deficits on flavonol and proanthocyanidin concentrations were smaller than on anthocyanins, showing only a moderate effect on flavonols (Grimplet *et al.* 2007, Castellarin *et al.* 2007b) and proanthocianidins (Chalmers *et al.* 2010, Ollé *et al.* 2011).

Grapevine grows well in arid and semi-arid environments because it has relatively high drought tolerance (Chaves *et al.* 2010) but physiological and metabolic responses of berries to water deficit appear to be dependent on the cultivar (Deluc *et al.* 2009). Spain hosts a large number of native *Vitis vinifera* varieties. However, most of those genotypes remain uncharacterized, which limits their ability to improve berry quality traits. *V. vinifera* L. 'Tem-

Correspondence to: Dr. M. C. Antolín, Grupo de Fisiología del Estrés en Plantas (Dpto. de Biología Ambiental), Unidad Asociada al CSIC (EEAD, Zaragoza, ICVV, Logroño). Facultades de Ciencias y Farmacia, Universidad de Navarra, c/ Irunlarrea 1, 31008, Pamplona, Spain. Fax: +34-948-425-649. E-mail: cantolin@unav.es

pranillo' is a representative red wine Spanish grapevine variety with early ripening widely cultivated in northern and central Spain where it is the main variety in half of the Denominations of Origin (Cervera *et al.* 2002). 'Graciano' is a Spanish variety autochthonous in Rioja and Navarra (Northern Spain) that is used for its intense red colour, powerful aroma and high acidity. 'Graciano' is traditionally used as a blending partner for 'Tempranillo' based wines to increase their quality (García Marino *et al.* 2011, Ferrer Gallego *et al*. 2011). To date, there have been relatively few studies that have investigated the evolution of phenolic composition during ripening in 'Graciano' (Monagás *et al.* 2003, Núñez *et al.* 2004) and its response to water deficit is poorly known. Sustained deficit irrigation (SDI) is a technique that could potentially be readily adopted by the wine grape industry if adequate yields and berry compositional attributes met (Ortega-Farias *et al.* 2012). Therefore, the aim of this study was to analyze phenolic composition during berry development and ripening of berries by characterizing the response of 'Tempranillo' and 'Graciano' to sustained water-deficit irrigation. Potted vines were used to assure the imposition of a similar water stress pattern in both cultivars.

Material and Methods

Plant material: Dormant *Vitis vinifera* (L.) cuttings of 'Tempranillo' and 'Graciano' 400-500 mm long and 8-10 mm in diameter were selected in the winter of 2011. The cuttings were propagated by a technique that ensured that the formation of adventitious roots preceded bud burst using steps originally outlined in Mullins (1966) with some modifications described in Ollat *et al.* (1998) and Antolín *et al.* (2010). In short, the cuttings were rooted in a heat-bed (25 °C) inside a cool-room (5 °C) for 30 d. Rooted cuttings were planted in 10 L plastic pots containing a soil-peat (1:1, v/v) substrate. After rooting, cuttings were transferred to a greenhouse with a 25/20 °C and 70/80 % RH (day/night) regime. They were illuminated for 15 h with natural daylight supplemented with metal halide lamps (POWERSTAR, HQI-TS, 400W/D, PRO, Osram, Augsburg, Germany), providing a minimum photosynthetic photon flux density (PPFD) of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the level of the inflorescence. Bud-break took place one week after the rooted cuttings had been transferred to these conditions. Careful control of vegetative growth before flowering improves the partitioning of stored carbon towards the roots and the reproductive structures. Thus, only one flowering stem was allowed to develop on each plant during growth. After berry set (end of May), growth conditions in the greenhouse were changed to a 25/15 °C and 60/80 % RH (day/night) regime with a PPFD of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the level of the inflorescence. A nutrient solution provided mineral nutrition in accordance with grapevine requirements (Ollat *et al.* 1998).

Experimental design: Two water management treatments were applied at fruit set (11 d after anthesis, DAA) [Eichhorn and Lorenz (E-L) phenological stage 27] (Coombe 1995). In the control treatment, pots were maintained at 80 % of pot capacity. Water-deficit treatment plants received 50 % of the water given to control plants (sustained deficit irrigated, SDI). The resulting soil conditions during the experiments are shown in Fig. 1. Soil water potential (Ψ) was monitored using a granular matrix sensor (Watermark Soil Moisture Sensor, Irrometer Co, Riverside, CA, USA) placed within each pot. Pot capacity was previously assessed by determining the amount of water retained after allowing water to freely drain through the holes at the bottom of the pot. The surface of the plant containers was covered with quartz stones during the experiments to minimize water loss due to evaporation. Watering was done by hand, providing a fixed volume of nutrient solution or deionised water so that the different treatments were supplied with the same amount of nutrients during water deficit.

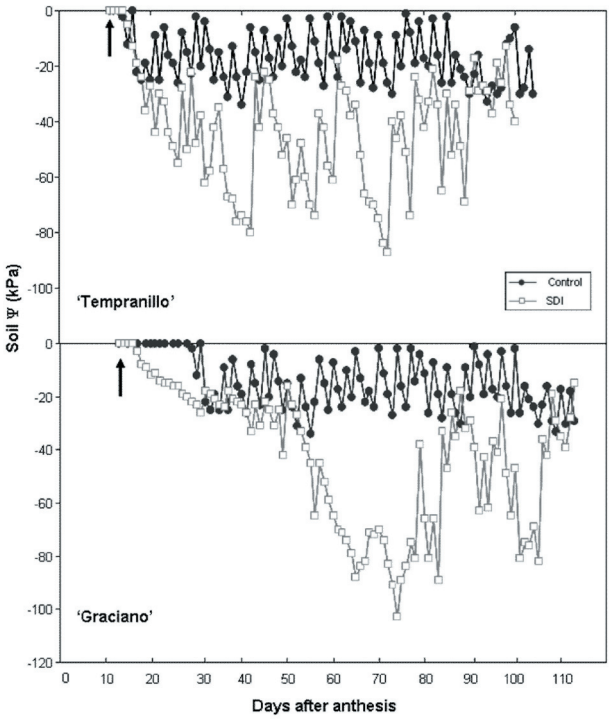

Fig. 1: Soil water potential (Ψ) measured from fruit set to harvest in pots subjected to different irrigation treatments: full irrigation (control) or sustained deficit irrigation (SDI). Values represent means (n = 5). Arrow indicates start of water treatments.

Berry samples were collected at three stages of development: (1) pea size, corresponding to berries 7 mm in diameter [E-L phenological stage 31] (Coombe 1995) [27 DAA) for the two water treatments]; (2) veraison, corresponding to berries that began to colour and enlarge (approximately 9°Brix) [E-L phenological stage 35] (60 and 65 DAA for water deficit and well-watered treatments, respectively); and (3) harvest, corresponding to commercially ripe berries (approximately 22 °Brix) [E-L phenological stage 38] (100 and 105 DAA in well-watered and water-deficit treatments, respectively). There were five plants for each treatment and sampling-time combination from two biological replicates. Ninety to 100 berries from each treatment (45-50 berries per biological replicate) were counted, weighed, and frozen at -80 °C for further analysis.

Plant water status: Pre-dawn leaf water potential (Ψ_{pd}) was measured with a SKYE SKPM 1400 pressure chamber (Powys, UK) on five fully expanded leaves per treatment at each sampling date just prior to irrigation. Ten berries from each treatment were collected, weighed, and subsequently 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.

Yield and fruit quality: Bunches from each treatment type were weighed and all berries from each bunch were separated and weighed to obtain yield. Berries were weighed individually, mean berry mass was determined and berries were separated into skin and flesh. Berry volume was calculated using the definition of a sphere ($4/3\ \pi r^3$, where r is the radius of the sphere). Leaf area was measured with a portable area meter (model LI-3000, Li-Cor, Lincoln, NE, USA) and the source to sink ratio was calculated as the quotient between total leaf area and bunch mass.

A subsample of 25 berries was crushed for determination of total soluble sugars, pH and titratable acidity. Soluble solids were analysed using a temperature-compensating refractometer (Zuzi model 315, Auxilab, Spain) and were expressed as °Brix. Must pH was measured with a pH meter (Crison Instruments, Barcelona, Spain) standardised to pH 7.0 and 4.0. Finally, titratable acidity was measured by titration with NaOH, and was expressed as g tartaric acid·L^{-1}.

Analysis of phenolic compounds: Berry samples were homogenized using an Ultra Turrax grinder mixer (IKA, Staufen, Germany) at 5,500 g for 1 min. Thereafter, the homogenate (1 g) was transferred into a pretared centrifuge tube and 10 mL of 50 % v/v aqueous ethanol (pH 2.0) was added (Iland *et al.* 2004). Maceration was then allowed for 1 h in an ultrasonic bath (Bandelin, Berlin, Germany). The tube was then centrifuged at 2,300 g for 15 min (Sorvall RC 6 Plus, Du Pont, BH, Germany) and the supernatant was named as the extract. Hydroalcoholic extracts (2 mL) were directly fractionated by gel permeation chromatography (GPC) on a TOYOPEARL® gel HP-50F column (Tosoh, Montgomeryville, PA, USA) using the method described by Guadalupe *et al.* (2006). A first fraction (F1) was eluted with ethanol/water/trifluoroacetic acid (55:45:0.05 v/v/v) and a second fraction (F2) was recovered by elution with acetone/water (60:40 v/v). The two fractions were taken to dryness under vacuum and then the first fraction was used to quantify monomeric phenolics and the second one was used to determine the proanthocyanidin content. All samples were fractionated in duplicate.

Monomeric phenolic compounds in F1 fractions were analyzed by HPLC-DAD on an Agilent modular 1100 liquid chromatograph (Waldbronn, Germany) equipped with a G1313A injector, a G1311A HPLC quaternary pump, an online G1379A degasser, a G1316A oven, a G1315B photodiode array detector, and Agilent Chemstation software. A Kromasil 100-C18 reverse phase column (5-µm packing, 200 x 4.6 mm i.d.), protected with a guard column of the same material (Teknokroma, Barcelona, Spain) and thermo-regulated at 30 °C, was used. Individual phenolic compounds were identified by HPLC-MS. Anthocyanins, hydroxycinnamic acids, flavonols, and flavan-3-ols were determined as indicated by Guadalupe and Ayestarán (2008). Each measurement was run in triplicate.

Statistical analysis: Data of each variety were submitted separately to one-way analysis of variance (ANOVA). Data of percentages were transformed with an arcsine method before ANOVA. The pH data were transformed to H^+ concentrations before running ANOVA. Significant difference was statistically considered at the level of $P < 0.05$. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows XP (SPSS Inc., Chicago, IL, USA). All values shown in the figures are means ± standard errors (SE).

Results and Discussion

Water status plant growth and yield: For both grapevine varieties water-deficit treatment provoked significant differences in water status at veraison, as indicated by the decrease of leaf Ψ_{pd} in comparison to well-irrigated plants. However, differences between treatments were not observed at harvest (Fig. 2). Plants subjected to SDI had reduced vegetative growth, as shown by the marked decrease in leaf area, and decreased yield (Tab. 1). Although significant reductions in the leaf area-to-crop mass ratio were obtained in SDI plants, values remaining around 9-10 $cm^2 \cdot g^{-1}$ were close to optimal values (between 6.2 and 10 $cm^2 \cdot g^{-1}$) of grapevine vigour reported by Smart and Robinson (2006).

Berry characteristics: Berry size of potted grapevines was smaller than those obtained in field-grown grapevines (Fig. 2), and there was significant reductions of berry mass and berry volume in the SDI treatment, which reached 75 % and 68 % of the control berries in 'Tempranillo' and 'Graciano', respectively (Tab. 1). However, no significant differences in the relative skin mass were detected. In 'Tempranillo', SDI induced no changes in total soluble solids, juice pH, titratable acidity or total polyphe-

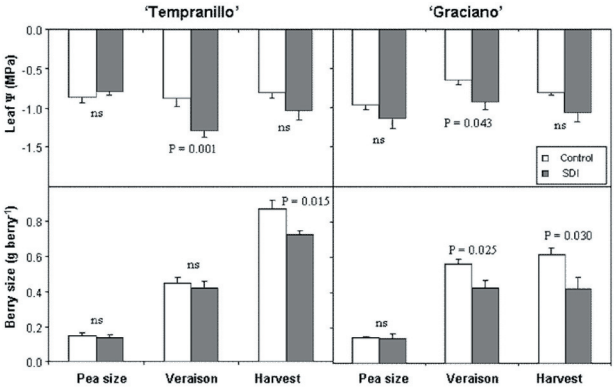

Fig. 2: Pre-dawn leaf water potential (Ψ_{pd}) and berry size of fruiting cuttings of 'Tempranillo' and 'Graciano' grapevines subjected to different irrigation treatments: full irrigation (Control), or sustained deficit irrigation (SDI). Values represent means ± SE (n = 5). Within each phenological stage and variety means were considered statistically different when $P < 0.05$. ns: not significant.

Table 1

Main plant and berry characteristics and composition recorded at harvest from fruiting cuttings of 'Tempranillo' and 'Graciano' subjected to different irrigation treatments during berry ripening: full irrigation (Control) or sustained deficit irrigation (SDI)

Measurement	Tempranillo		Graciano	
	Control	SDI	Control	SDI
Vine vigour				
Leaf area ($m^2 \cdot plant^{-1}$)	0.37 a	0.11 b	0.23 a	0.09 b
Yield ($g \cdot plant^{-1}$)	212.08 a	115.71 b	143,65 a	85.50 b
Leaf area/crop mass ($cm^2 \cdot g^{-1}$)	17.5 a	9.5 b	16.1 a	9.5 b
Berry characteristics				
Berry volume (mm^3)	969 a	735 b	667 a	455 b
Berry water content (g water$\cdot g^{-1}$ FM, %)	87 a	88 a	85 a	81 a
Relative skin mass (% of berry FM)	30.1 a	35.3 a	26.5 a	30.7 a
Fruit composition				
Total soluble solids (°Brix)	21.6 a	20.1 a	19.0 a	21.2 a
Juice pH	3.7 a	3.8 a	2.7 a	3.0 a
Titratable acidity ($g \cdot L^{-1}$)	5.8 a	4.7 a	7.4 a	4.6 b
Total polyphenols ($mg \cdot L^{-1}$)	2.56 a	2.51 a	3.16 a	3.22 a

Values represent means (n = 5). Within each file and variety, means followed by a different letter are significantly different ($P < 0.05$). FM indicates fresh mass.

nolic content but in 'Graciano' water deficit resulted in decreased must titratable acidity (Tab. 1). Some studies have reported increased concentration of polyphenols in water-stressed berries due to size reduction, increase in the relative skin mass ratio and/or stimulation of their biosynthetic pathways (ESTEBAN et al. 2001, SIVILOTTI *et al.* 2005, CASTELLARIN *et al.* 2007b). However, this seems not to be a general response due to differences in the cultivar studied and, in the timing, severity and duration of water deficit. In our case, no changes in polyphenol contents could have resulted from a reduction in polyphenolic biosynthesis that may be compensated by a concentration effect due to smaller berries (NICULCEA *et al*. 2013).

Berry phenolic composition: The results of the current study showed that berry skin anthocyanins were dominated by malvidin-3-glucosides regardless the variety and water irrigation applied (Tab. 2) as observed for a number of other grapevine varieties (MAZZA *et al.* 1999, KALLITHRAKA

Table 2

Effect of irrigation treatment (SDI and control) on anthocyanin derivatives determined at harvest in grape berries of fruiting cuttings of 'Tempranillo' and 'Graciano'

		Tempranillo		Graciano	
		Control	SDI	Control	SDI
3-Monoglucosides ($mg \cdot kg^{-1}$)	Delphinidin	250.6 a	92.2 b	229.1 a	183.3 a
	Cyanidin	44.5 a	12.4 b	55.8 a	40.4 a
	Petunidin	170.5 a	70.9 b	173.0 a	154.3 a
	Peonidin	89.2 a	42.1 b	140.0 a	162.5 a
	Malvidin	486.3 a	335.0 b	530.5 a	623.8 a
% of total		86.7 a	72.2 b	83.4 a	77.9 b
3-Acetyl-glucosides ($mg \cdot kg^{-1}$)	Delphinidin	5.4 a	2.4 b	19.1 a	13.5 b
	Cyanidin	0.3 a	0.5 a	5.2 a	3.7 b
	Petunidin	4.5 a	2.7 b	1.6 b	2.1 a
	Peonidin	2.0 a	1.6 b	59.2 a	65.8 a
	Malvidin	15.4 a	16.0 a	10.1 a	11.8 a
% of total		2.5 b	3.4 a	7.0 a	6.9 a
3 *p*-Coumaroyl-glucosides ($mg \cdot kg^{-1}$)	Delphinidin	N.D.	N.D.	20.9 a	20.2 a
	Cyanidin	21.7 a	20.0 a	8.7 b	13.6 a
	Petunidin	3.1 b	7.3 a	19.7 a	24.7 a
	Peonidin	N.D.	N.D.	14.1 b	28.1 a
	Malvidin	88.8 b	13.8 a	63.4 b	10.9 a
% of total		10.8 b	24.4 a	9.6 b	15.2 a

Values represent means (n = 25). Within each file and variety, means followed by a different letter are significantly different ($P<0.05$). ND: not detected.

et al. 2009). However, anthocyanin qualitative profiles in well-watered and SDI plants were significantly different for some compounds because each grape cultivar is characterized by a distinct set of anthocyanins (He *et al*. 2010, Dai *et al.* 2011). In response to the SDI treatment, total anthocyanin concentration at harvest was decreased in 'Tempranillo' grapevines (Fig. 3). Consistent with our results, a reduction in the amount of anthocyanins has been reported elsewhere by Esteban *et al.* (2001). Application of different water treatments also resulted in changes of individual anthocyanin composition in both varieties (Tab. 2). In 'Tempranillo', the concentration of all glucosides derivatives at harvest was decreased by SDI, while increases of contribution of 3-*p*-coumaroyl and 3-acetyl-glucosides to total anthocyanins occurred in response to SDI (Tab. 2). Increased proportion of 3-*p*-coumaroyl-glucosides was due to increased derivatives of malvidin and petunidin. In 'Graciano', SDI treatment did no modified total concentration of anthocyanins and amounts of glucosides derivatives. In this variety, the contribution of 3-*p*-coumaroyl-glucosides to total anthocyanins increased and was due mainly to increased derivatives of cyanidin, peonidin and malvidin. In the same way, other authors have shown that degree of hydroxylation and methoxylation of the anthocyanins in grape berries may be altered by changes in irrigation management (Bindon *et al.* 2008). Thus, it has been reported that water deficit can enhance accumulation of anthocyanins, through the stimulation of anthocyanin hydroxylation that converts hydroxylated anthocyanins (cyanidin and delphinidin) into their methoxylated derivates (peonidin, petunidin and malvidin) through the differential regulation of flavonoid 3'-hydroxylase, flavonoid 3', 5'-hydroxylase and O-methyltransferase (Castellarin *et al.* 2007b), as showed previously in 'Cabernet Sauvignon' (Castellarin *et al.* 2007b, Deluc *et al.* 2009), 'Shiraz' (Ollé *et al.* 2011) and 'Tempranillo' (Santesteban *et al.* 2011). In 'Graciano' significant levels of 3-monoglucoside of delphinidin and its *p*-coumaroylated derivatives were detected (Tab. 3).

The flavonol production begins before the veraison, and increases during berry ripening (Ivanova *et al.* 2011) (Fig. 3). Our results showed significant changes in the total concentration of flavonols at harvest in both varieties subjected to water deficit conditions (Fig. 3). Thus, SDI treatment reduced flavonol concentrations in 'Tempranillo' and this decrease was due mainly to decreased myricetin 3-*O*-glucoside (Tab. 3). By contrast in 'Graciano', flavonol accumulation was stimulated under SDI conditions because increased concentration of quercetin-3-*O*-glucoside, myricetin and isorhamnetin. The observed differential responses of anthocyanins and flavonols to water deficit irrigation could be a result of the little turnover of anthocyanins that once formed they are accumulated in the skin,

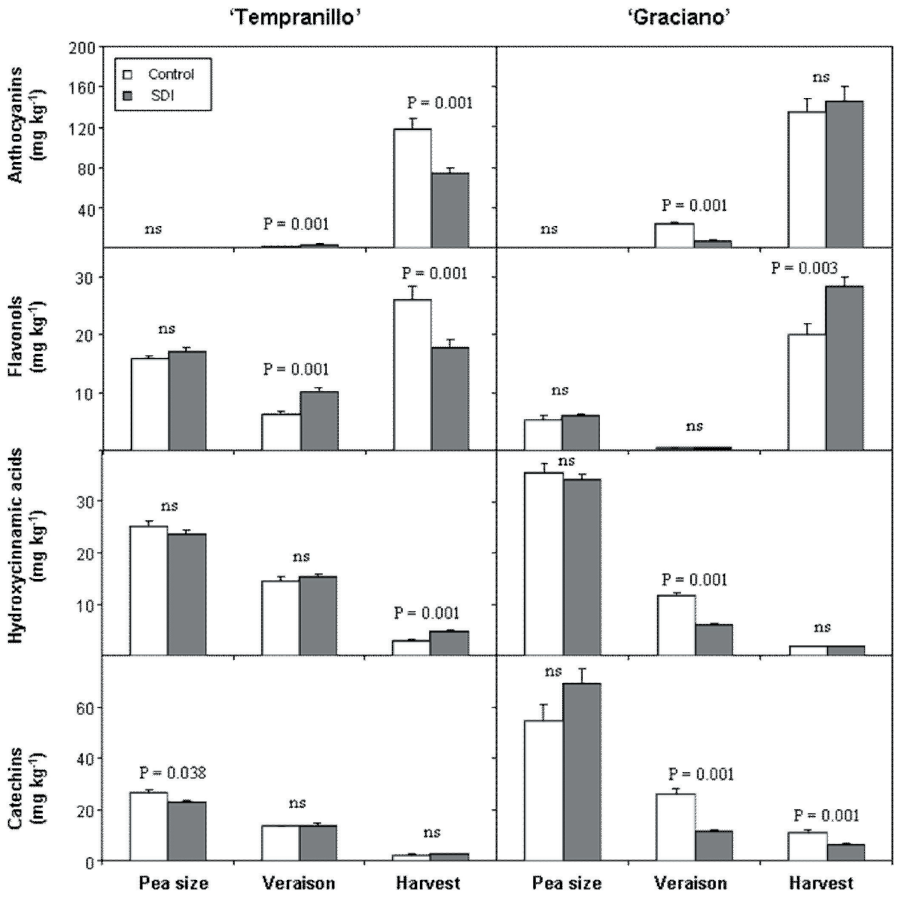

Fig. 3: Concentration of monomeric anthocyanins, flavonols, hydroxycinnamic acids and catechins at different stages of berry growth and ripening of 'Tempranillo' and 'Graciano' grapevines subjected to different irrigation treatments: full irrigation (Control), or sustained deficit irrigation (SDI). Values represent means ± SE (n = 25). Within each phenological stage and variety means were considered statistically different when $P < 0.05$. ns: not significant.

Table 3

Effect of irrigation treatment (SDI and control) on individual composition of flavonols and hydroxycinnamic acids determined at harvest in grape berries of fruiting cuttings of 'Tempranillo' and 'Graciano'

		Tempranillo		Graciano	
		Control	SDI	Control	SDI
Flavonols (mg·kg^{-1})	Myricetin 3-*O*-glucoside	20.5 a	12.4 b	ND	ND
	Quercetin 3-*O*-glucuronide + Quercetin 3-*O*-glucoside	5.4 a	5.3 a	ND	ND
	Quercetin 3-*O*-glucoside	ND	ND	8.8 b	13.1 a
	Myricetin	ND	ND	8.8 b	12.0 a
	Quercetin	ND	ND	1.4 a	1.6 a
	Isorhamnetin	ND	ND	1.0 b	1.5 a
Hydroxycinnamic acids (mg·kg^{-1})	*c*-caftaric acid	6.3 a	2.6 b	ND	ND
	t-caftaric acid	12.8 b	2.7 a	9.7 a	7.1 b
	c-coutaric acid	ND	ND	2.8 a	1.5 b
	t- coutaric acid	ND	ND	3.6 a	2.4 b
	Caffeic acid	3.8 b	16.8 a	0.5 a	0.5 a
	Coumaric acid	ND	ND	0.4 b	3.5 a
	Ferulic acid	ND	ND	ND	0.5

Values represent means (n = 25). Within each file and variety, means followed by a different letter are significantly different ($P < 0.05$). ND: not detected.

while flavonols are used for the synthesis of other compounds and are easily degraded (Adams 2006). Although it was reported that deficit irrigation has a moderate effect on flavonol synthesis in red cultivars (Grimplet *et al.* 2007, Sofo *et al.* 2012) our findings reinforced the idea that flavonol accumulation under water deficit conditions differed between grape varieties (Deluc *et al.* 2009). Thus, the predominant flavonols in 'Tempranillo' were myricetin 3-*O* glucoside and quercetin 3-*O* glucuronide + quercetin 3-*O* glucoside (Tab. 3). In contrast, the main flavonols found in 'Graciano' berries were quercetin 3-*O*-glucoside, myricetin, quercetin and isorhamnetin.

The highest amounts of hydroxycinnamic acids and catechins were found at pea size stage and then, they decreased until harvest (Fig. 3). Similar patterns for these compounds were reported in 'Cabernet Sauvignon' and 'Carménère' grapes (Obreque-Slier *et al.* 2010). At veraison, SDI treatment provoked a significant reduction of total hydroxycinnamic acids and catechins in 'Graciano', but no significant alterations were detected in 'Tempranillo'. In well-watered plants, *t*-caftaric acid was found to be the main hydroxycinnamic acid, accounting for 55 % of the total regardless of variety (Tab. 3). In 'Tempranillo', SDI treatment resulted in increased *t*-caftaric and caffeic acids. By contrast in 'Graciano', although water-deficit irrigation did not alter total hydroxycinnamic acids at harvest, its individual composition was modified. Thus, SDI treatment reduced concentrations of *t*-caftaric, *c*-coutaric and *t*-coutaric and increased concentrations of coumaric and ferulic acids (Tab. 3).

Overall, characterization of phenolic composition of 'Tempranillo' and 'Graciano' subjected to deficit irrigation conditions suggests that 'Graciano' could result in improved fruit quality. Specifically, the higher levels of peonidin-3-acetyl-glucoside found in 'Graciano' could confer a deep red color when transferred from grape to the corresponding wine that could represent a positive sensory parameter (Santos-Buelga and de Freitas 2009). In addition, the higher contribution of total acetylated anthocyanins also could constitute a positive qualitative index (Tab. 2), and the changes in hydroxycinnamic acid composition could be relevant because they are involved in browning reactions, and since they are precursors of volatile phenols in wine (Romeyer *et al.* 1983, Chatonnet *et al*. 1993).

Conclusion

This study provides evidence of a variety-dependent response of phenolic accumulation and composition to sustained deficit irrigation applied during berry growth and ripening. Thus, in 'Tempranillo' water deficit irrigation reduced contents of total anthocyanins and flavonols and increased hydroxycinnamic acids. In 'Graciano', water limitation resulted in increased flavonols and reduced monomeric flavan-3-ols (catechins). Under water deficit irrigation, 'Graciano' achieved higher contents of total anthocyanins and flavonols than 'Tempranillo', and analyses of monomeric phenolic compounds suggest that under these conditions, 'Graciano' could result in improved fruit quality. However, although pot experiments enable optimal control of grapevine irrigation, which is necessary to assure that both varieties experience the required water stress, extrapolations to field-grown grapevines should be made with due caution.

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