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Research article

Controlled water deficit during ripening affects proanthocyanidin synthesis, concentration and composition in Cabernet Sauvignon grape skins

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ABSTRACT

The influence of controlled water deficit on the phenolic composition and gene expression of *VvLAR2*, *VvMYBPA1*, *VvMYBPA2* and *VvMYB4a* in Cabernet Sauvignon grape skins throughout ripening was investigated. The assay was carried out on own-rooted *Vitis vinifera* plants cv. Cabernet Sauvignon in a commercial vineyard from *veraison* until commercial harvest. Three irrigation regimes were used from *veraison* until harvest with the following treatments: T1: 3.6 mm day^{-1}; T2: 1.8 mm day^{-1} and T3: 0.3 mm day^{-1}. The content of total phenols and total anthocyanins in grape skins increased during ripening, but water deficit did not produce differences among treatments in the total anthocyanin concentration. Proanthocyanidins (PAs) decreased throughout ripening, although approximately 25 days after *veraison* (DAV), their content slightly increased. This effect was more pronounced in the most restrictive treatment (T3). A similar pattern was observed in the transcript abundance of *VvLAR2*, *VvMYBPA1* and *VvMYB4a*. PAs separation revealed differences in concentration but not in the proportion among fractions among the irrigation treatments. Additionally, controlled water deficit increased the mean degree of polymerization and the flavan-3-ol polymeric concentration in grape skins throughout ripening but with no effects on the extent of PAs galloylation. Our results suggest that the water status of Cabernet Sauvignon grapevines affects the gene expression for proteins involved in the synthesis of PAs, increasing their concentration and also their composition, with further evidence for the efficacy of a convenient, controlled water deficit strategy for grapevine cultivation.

1. Introduction

Phenolic compounds are considered the major determinant of the quality of red wines because of their involvement in the main sensory attributes of wine, such as colour, due to anthocyanins, and their body, mouthfeel, bitterness and astringency, which are conferred by proanthocyanidins (PAs). The sensory properties influenced by PAs depend not only on their concentration but also their composition and mean degree of polymerization (Brossaud et al., 2001; Vidal et al., 2003; Chira et al., 2012). Grape skins and seeds contain PAs, but the skins contain procyanidins and prodelphinidins, and are characterized by a lower proportion of galloylation and a higher mean degree of polymerization (mDP) compared with seeds (Downey et al., 2003; González-Manzano et al., 2004; Chira et al., 2015).

In many viticultural regions, regulated-deficit irrigation is a common practice because of its well-known effect on wine (Chaves et al., 2010; Roby et al., 2004; Acevedo-Opazo et al., 2010; Casassa et al., 2015; Zarrouk et al., 2012; Bonada et al., 2015; Kyraleou et al., 2016). In fact, deficit irrigation leads to a reduction in the size of the berries, resulting in a higher skin to pulp ratio, producing an effect of concentration of compounds (Kennedy et al., 2002). More importantly, water deficit stimulate the secondary metabolism in

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berries. Water restrictions at the time of *veraison* induce both a transient advancement of grape berry sugar accumulation, and an increase in the abscisic acid (ABA) concentration in the berries. Both sugars and ABA are signals for gene expression and protein synthesis involved in the phenylpropanoid pathway in berry skins, leading to the accumulation of flavanols, flavonols and anthocyanins (Pastenes et al., 2014; Castellarin et al., 2007; Deluc et al., 2009; Villalobos-González et al., 2016). However, it is still a matter of debate as to what extent the severity of water restriction affects not only the concentration but also, most importantly, the composition of phenolic compounds in grape berries, especially proanthocyanidins.

The phenylpropanoid pathway is tightly controlled by diverse transcription factors that require precise spatiotemporal coordination between plant development and environmental conditions (Cavallini et al., 2015). As for PAs, synthesis requires diverse genes, such as leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) that produce the flavan-3-ol monomers for the formation of proanthocyanidins polymers (Bogs et al., 2005). At the same time, their synthesis is modulated by transcription factors, among which the two well-characterized proteins *MYBPA1* and *MYBPA2* correspond to positive regulators (Bogs et al., 2007), while *MYB4a* and *MYB4b* are also necessary but act as negative regulators of the general phenylpropanoid pathway (Cavallini et al., 2015). Some studies have focused on the general phenylpropanoid pathway in grape berry skins, reporting effects on the concentration of PAs (Castellarin et al., 2007; Deluc et al., 2009; Matus et al., 2009; Genebra et al., 2014). However, few studies have examined the impact of water irrigation levels on the composition of grape berry PAs.

In the present study, we have assessed the effect of controlled water deficit, from weak to moderate water stress and from veraison until harvest, on the synthesis and composition of proanthocyanidins, as well as the expression of secondary metabolism related-genes in Cabernet Sauvignon grape berry skins throughout ripening.

2. Materials and methods

2.1. Chemical reagents and equipment

Methylcellulose (1500 cP, viscosity at 20 g/L) and a standard of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin and (−)-epicatechin-3-*O*-gallate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). PVDF membranes of 0.45- and 0.22-μm pore size were acquired from Millipore (Billerica, MA, USA). Anhydrous sodium sulphate, agarose, potassium metabisulfite, vanillin, ethyl acetate, lithium chloride, diethyl ether, sodium hydroxide, hydrochloric acid, sulfuric acid, methanol, ethanol, acetone, ascorbic acid, phloroglucinol and sodium acetate were purchased from Merck (Darmstadt, Germany). Sodium phosphate dibasic anhydrous and potassium phosphate monobasic were acquired from J.T. Baker (Phillipsburg, NJ, USA). All reagents were of analytical grade or higher. Sep-Pak Plus Environmental tC_{18} cartridges (900 mg) and Sep-Pak Plus Short tC_{18} cartridges (400 mg) were obtained from Waters (Milford, MA, USA). DNAse I amplification grade and SuperScript™ III First-Strand synthesis were supplied by Invitrogen™ (USA). LightCycler® FastStart DNA Master SYBR Green I was obtained from Roche (Switzerland). Nitrogen gas was supplied by Indura S.A (Santiago, Chile). Liquid nitrogen was supplied by Linde (Santiago, Chile). Ultrapure water was obtained from a Purelab Ultra MK2 purification system (Elga, St. Albans, UK).

Phloroglucinolysis studies were performed using a 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1315B photodiode array detector (DAD), a G1311A quaternary pump, a G1313A autosampler, a G1322A degasser and a G1316A thermostatted column compartment with a reverse phase LiChro Cart 100 RP-18 column (5 μm, 4.0 mm i.d x 250 mm; Agilent Technologies). RNA measurements were made using an Epoch microplate reader (Biotek, Winooski, VT, USA). Real time PCR analysis was performed using a LightCycler® 96 system (Roche, Switzerland). Absorbance values were measured using a UV-1601 UV−Visible spectrophotometer (Shimadzu, Kyoto, Japan).

2.2. Experimental site, irrigation treatment and berry sampling

The assay was carried out on 12-year-old own-rooted *Vitis vinifera* plants cv. Cabernet Sauvignon, in the organic commercial vineyard Haras de Pirque winery, located in the Maipo Valley in central Chile (33°42′30″S, 70°36′13″W), during the 2014 growing season. The historical average yield for this site was approximately 8 tons ha^{-1}. The trellising system contains vertically trained vines pruned using a double Guyot method, with drip irrigation in north-south oriented rows, planted at 2.5 m between rows and 1.5 m between plants. The site has deep colluvial soil with clay loam texture. The canopy management of the vineyard is typical for vineyards located in this region, and the experimental site experiences a warm semi-arid Mediterranean climate. The 2014 season had typical warm conditions during the period from January to April on the study site, with an average maximum temperature of 28.9 °C and an average minimum temperature of 7.1 °C. No rain occurred throughout the experiment. The average ETp for the period was 3.7 mm day^{-1}.

The experimental design consisted of completely randomized blocks with five replicates, each of which consisted of seven consecutive plants. Each block corresponded to one row, and blocks were separated by one row. Each treatment was separated by at least five plants in the row. Treatments and replicates were randomly distributed in the experimental area. To reduce the xylem water potential of vines, irrigation was suspended from 25 days before veraison to 10 days before veraison, i.e., the time when irrigation treatment started. The irrigation treatments were applied weekly until five days before last sampling date and were established by means of a combination of drip emitters with different water volumes, resulting in three different treatments, T1: 3.6 mm day^{-1}; T2: 1.8 mm day^{-1} and T3: 0.3 mm day^{-1}. The application of irrigation treatments resulting in the following average values for midday stem water potential throughout the season: T1, $\Psi = -0.8$ MPa; T2, $\Psi = -0.9$ MPa and T3, $\Psi = -1.0$ MPa. As a reference, midday stem water potential values higher than −0.6 MPa are considered no water deficit, while range values of −0.6 to −0.9 and −0.9 to −1.1, are considered weak water deficit and weak to moderate water deficit, respectively (Van Leuween et al., 2009). Plant water status was monitored weekly by measuring midday stem water potential using a pressure chamber. For this process, leaves were enclosed in aluminium plastic bags for 90 min at midday. Berries were sampled for chemical analysis on the following dates: −3, 13, 27, 41 and 60 days after *veraison* (DAV). Samples of 50 berries per replicate were randomly collected from five to seven clusters in each plant throughout the ripening period and immediately weighed, frozen, and stored at −80 °C until processing. The following physical and chemical variables were assessed: weight of 50 berries, skin weight of 50 berries, titratable acidity (g tartaric acid L^{-1}) and total soluble solid (°Brix) in berry juice, by means of a temperature-compensated refractometer (RHB-32ATC) (OIV, 2012). All analyses were performed in quintuplicate. The *veraison* date (8 February 2014) was determined by visual observation and berry firmness. All grapes were harvested at the commercial harvest date, which occurred on the same day for all treatments. This corresponded to the last sampling date (60