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Research article

Cultivar specific metabolic changes in grapevines berry skins in relation to deficit irrigation and hydraulic behavior

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ABSTRACT

Deficit irrigation techniques are widely used in commercial vineyards. Nevertheless, varieties respond differently to water availability, prompting the need to elucidate the physiological and molecular mechanisms involved in the interactions between genotypes and their environment.

In the present study, the variability in berry metabolism under deficit irrigation was investigated in the field on Shiraz and Cabernet Sauvignon (CS), known for their hydraulic variability. Berry skin metabolite profiling of the two cultivars was performed by parallel GC–MS and LC-MS at four development stages.

Under similar irrigation, the cultivars differed in stomata regulation. In response to water deficit, CS exhibited lessened loss in berry weight and milder metabolic alteration of berry-skin primary metabolites, as compared with Shiraz. The metabolic stress responses were shown to depend on berry phenology. Characteristic metabolic changes included a decrease in amino acids and TCA cycle intermediates from veraison onward. In contrast, water deficit induced the accumulation of stress-related metabolites such as: proline, beta-alanine, raffinose, nicotinate and ascorbate, to a greater extent in Shiraz. Polyphenol metabolism in response to water stress also underwent significant changes, unique to each cultivar.

Results suggest a link between the vine hydraulics and water-deficit driven changes in the berry skin metabolism, with significant consequences on the metabolic composition of the fruit.

1. Introduction

Grapevines are traditionally grown without artificial irrigation in the Mediterranean semi-arid regions. In recent years, however, irregular precipitation, desertification related processes, introductions to new arid growth areas and the development of inexpensive and reliable irrigation equipment led to an increase in the number and extension of irrigated vineyards (Cifre et al., 2005). The ability to manipulate water availability of plants has led to the development of several deficit irrigation techniques, which were shown to improve grape quality without significantly affecting quantity (Chaves et al., 2010). Nevertheless the intimate and complex interaction between genotype, environment, and molecular regulation of metabolism limits our predictive capability regarding the effect of water deficit on wine quality. As a result, today more than ever before, it is relevant to understand the physiological and molecular mechanisms governing the interactions between berry quality and the environment in which the vine is grown.

It is generally accepted that grapevine (*Vitis vinifera*) cultivars possess significant variability in their hydraulic behavior, a feature reflected in the cultivar-specific responses to water deficit (Schultz and Stoll, 2010). Aquaporins expression (Vandeleur et al., 2009), stomata regulation through hormonal balance (Hopper et al., 2014;

Abbreviations: CS, Cabernet Sauvignon; GC–MS, gas chromatograph mass spectrometer; LC-MS, liquid chromatograph mass spectrometer; ET_{pan}, pan based evapotranspiration; K_i, irrigation factor; Ψ_{PD}, pre-dawn water potential; g_s, stomata conductance; θ, soil volumetric water content; TA, titratable acidity; PCA, principal component analysis; WD, water deficit; GHB, butanoate 2,4 dehydroxy; GST, glutathione *S*-transferase; UFGT, flavonoid 3-*O*-glucosyltransferase.

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Soar et al., 2006), and their interaction (Shatil-Cohen et al., 2011) were suggested as possible control mechanisms. Additionally, hydraulic conductance and embolism in respect to xylem architecture could present a significant contribution to the phenomena (Hochberg et al., 2014). In a recently published study (Hochberg et al., 2013a, 2013b), Shiraz and Cabernet Sauvignon (CS) presented significantly different hydraulic behavior and metabolic responses in the leaf. Under similar irrigation treatments in both cultivars, tighter stomata regulation of CS resulted in improved water balance and milder metabolic perturbation. As compared with leaves, berry metabolism determines the organoleptic properties of the wine, thus a similar hydraulics-mediated mitigating phenomenon in the berry would translate into economic gain.

In the wine industry, the distinct and recognizable characteristics of wine are usually attributed to differences in the chemical composition of flavonoid compounds and serves as a signature to a given variety (Downey et al., 2006; Rimando and Suh, 2008). Phenylalanine-derived polyphenols accumulate mainly in the skin of grape berries, during the course of fruit development, and comprise mainly flavonols, flavan-3-ols (flavanols) and anthocyanins (Bogs et al., 2005, 2006; Downey et al., 2004). The extent of accumulation of phenolic compounds in grape berries varies among cultivars, developmental stages, growing regions and horticultural practices in relation to irradiation, nutrient, and temperature interventions (Downey et al., 2006; Jackson and Lombard, 1993)). Recently, Degu et al. (2014) showed the occurrence of differential regulation in the berry polyphenol metabolism of Shiraz and CS grown in an arid environment. Deluc et al. (2009) have revealed significant differences in the flavonoid metabolism in responses to water deficit of white (Chardonnay) and red (Cabernet Sauvignon) cultivars. Several studies have explored flavonoids response to stress (Castellarin et al., 2007a, 2007b; Deluc et al., 2010; Deluc et al., 2009), emphasizing the power of novel omics tools to describe complicated biological interactions. Nevertheless, flavonoid synthesis and degradation in response to stress and in respect to cultivar hydraulic variability, is yet to be elucidated.

Here we present the metabolic response of Shiraz and CS under deficit irrigation. The cultivars were grown under two irrigation treatments and their berry skin was profiled for metabolic features using gas and liquid chromatography mass spectrometry. It was hypothesized that under similar deficit irrigation, improved water balance in CS will result in a milder metabolic alteration as compared with Shiraz.

2. Materials and methods

2.1. Plant material and irrigation treatments

The field experiment was conducted exactly as described in Degu et al. (2014). Five-year old commercial vines of two red wine grape varieties of *V. vinifera* L. (Shiraz and Cabernet Sauvignon – CS) grafted on Ruggeri rootstock were employed during the 2011 growing season. Both cultivars were planted 3 m between rows and 1.5 m between vines and were trained in a vertical shoot position (VSP) trellis system. The cultivars were grown side by side with similar orientation and soil property (measured as soil composition). The experiment tested the metabolic response of grape skins to two irrigation levels (control and 50% irrigation) regulated by different drippers in six plots per treatment. Following common practice in the area, irrigation was applied every four days based on evaporation from evaporation pan Class A (ET_{pan}) and was calculated using an irrigation factor (K_i) of 0.25 as follows: $ET_{pan} \times K_i$. The Negev area is hotter and drier as compared with common grape cultivation regions and as such ET_{pan} during the experiment period (June–August) was 7.5–14 mm/day, resulting in irrigation of 1.8–3.5 mm/day. Until fruit set, both treatments were irrigated similarly (a total of 80 mm) using emitters at a flow rate of 2.2 L/h. At fruit set, only in the low irrigation treatment (50%), drippers were switched to 1.2 L/h. Low flow drippers resulted in a 43% (for the duration of the experiment) and 56% (for the entire season) irrigation amounts in the 50% treatment as compared with the irrigation of the control treatment. Irrigation volume was monitored in every treatment using low flow water gauges (SF, Arad, Israel), independent from the main irrigation controller.

2.2. Water potential, stomatal conductance, volumetric soil water content and berry characterization

Pre-dawn leaf water potential (Ψ_{PD}, MPa) was measured using a pressure bomb chamber (Arimad 3000, Israel) at pre-dawn. Measurements were performed on fully expanded, mature leaf, from each plot. Immediately before excision, a plastic bag was placed over the leaf laminar. Each leaf was excised from the shoot using a scalpel blade and then placed into the pressure chamber with the petiole protruding from the chamber lid. The chamber was pressurized using a nitrogen tank, and Ψ_{PD} was recorded when the initial xylem sap was observed emerging from the cut end of the petiole.

Stomatal conductance (g_s) was measured using the SC-1 leaf porometer (Decagon, WA, USA). For every plot, fully expanded leaves on the sunny side of the vine were measured during mid-morning (9:00–10:00am).

To measure soil volumetric water content (θ), 2-3 sensors (5 TE, Decagon, WA, USA) per treatment were buried 10 cm away from the dripper and 15 cm below the surface of the soil. Measurements were taken every 15 min and stored in a data logger (Em-50, Decagon, WA, USA).

To determine the effect of water deficit on berry quality related parameters, a series of measurements were taken at 6 time points between fruit set and harvest. For each treatment, berry weight, Brix and titratable acidity (TA) were assayed from whole berries exactly as described in Degu et al. (2014).

2.3. Sampling and metabolite extraction

Samples were taken for metabolic analysis at four berry developmental stages in both cultivars: pre-veraison (June 19th); veraison (July 19th); post-veraison (Aug 9th) and harvest, Aug 24th for Shiraz and Sep 11th for CS. It should be noted that due to significant differences in berry maturation (according to Brix) the date of harvest is not the same for the two cultivars. At all sampling dates, 6 skin samples per plot, each composed of 6 berry skins, were collected at 8:00am, snap freezed immediately with liquid nitrogen and kept at −80°c until further analysis. Ahead of extraction samples were freeze dried in a lyophilizer (Virtis Gardiner, N.Y. R525, Model 10-MR-TR). The samples were extracted for parallel metabolite profiling (LC-MS and GC–MS), according to Weckwerth et al. (2004) and exactly as described in Degu et al., 2014. All chemicals were purchased from Sigma–Aldrich if not indicated otherwise. Frozen powder (70 mg) was incubated in a 1 ml pre-chilled methanol/water/chloroform extraction solution (2.5/1/1 v/v/v). Internal standards, (i.e. 0.2 mg/ml ribitol in water, 1 mg/ml ampicillin in water and 1 mg/ml corticosterone in methanol), were added into the extraction mixture to give a concentration of 0.86 μg ml^{-1}, 5.6 μg ml^{-1}, 7.16 μg ml^{-1}, respectively in the final injection. The mixture was then briefly vortexed, centrifuged for 2 min at 14,000 RPM (microcentrifuge 5417R) and the supernatant was decanted into the new tubes. The supernatant was mixed with 300 μl of chloroform (LC-MS grade) and 300 μl of UPLC-grade water and then centrifuged at 14,000 RPM for 2 min. After that, 100 μl of