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Research article

Malbec grape (*Vitis vinifera* L.) responses to the environment: Berry phenolics as influenced by solar UV-B, water deficit and sprayed abscisic acid

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A B S T R A C T

High-altitude vineyards receive elevated solar ultraviolet-B (UV-B) levels so producing high quality berries for winemaking because of induction in the synthesis of phenolic compounds. Water deficit (D) after veraison, is a commonly used tool to regulate berry polyphenols concentration in red wine cultivars. Abscisic acid (ABA) plays a crucial role in the acclimation to environmental factors/signals (including UV-B and D). The aim of the present study was to evaluate independent and interactive effects of high-altitude solar UV-B, moderate water deficit and ABA applications on Vitis vinifera cv. Malbec berries. The experiment was conducted during two growing seasons with two treatments of UV-B (+UV-B and −UV-B), watering (+D and −D) and ABA (+ABA and −ABA), in a factorial design. Berry fresh weight, sugar content, fruit yield, phenolic compounds profile and antioxidant capacity (ORAC) were analyzed at harvest. Previous incidence of high UV-B prevented deleterious effects of water deficit, i.e. berry weight reduction and diminution of sugar accumulation. High UV-B increased total phenols (mainly astilbin, quercetin and kaempferol) and ORAC, irrespectively of the combination with other factors. Fruit yield was reduced by combining water deficit and high UV-B or water deficit and ABA. Two applications of ABA were enough to induced biochemical changes increasing total anthocyanins, especially those with higher antioxidant capacity.

1. Introduction

Enhancement in the synthesis of secondary metabolites like phenolics is part of the plant's response to environment, but also phenolics in grape berries are important for wine quality (Cheynier et al., 1998; Ghaste et al., 2015). Phenolics include flavonoids such as anthocyanins, flavonols (quercetin, kaempferol), dihydroxyflavonols (e.g. astilbin) and flavanols (catechins, epicatechins and tannins), as well as non-flavonoids, such as stilbenes (resveratrol), hydroxycinnamic and hydroxybenzoic acids (Garrido and Borges, 2013). Phenolics attract pollinators and seed dispersers, and protect plants from pathogens (Dixon and Paiva, 1995; Solovchenko and Schmitz-Eiberger, 2003; Braidot et al., 2008). Additionally, they accumulate in response to several biotic and abiotic signals, mainly as antioxidants so reducing oxidative damages (Dixon and Paiva, 1995; Braidot et al., 2008).

As altitude increases and the atmosphere thins more solar ultraviolet-B (UV-B) radiation (280–315 nm) reaches the surface (Frohnmeyer and Staiger, 2003). High-altitude vineyards in Mendoza, Argentina, receive higher levels of erythemally weighted UV-B irradiance than those at lower altitudes (Berli et al., 2010), producing high quality berries for winemaking by inducing synthesis of phenolics in the berry skin (Berli et al., 2011, 2015).

Previous reports have shown that berry composition in phenolics is characterized by a differential sensitivity to water stress

Abbreviations: ABA, abscisic acid; D, water deficit; DAF, days after flowering; EC, epicatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; FW, fresh weight; GC, gallocatechin; GCG, gallocatechin gallate; HPLC-MWD, high performance liquid chromatography-multiple wavelength detector; K-3-glc, kaempferol-3-glucoside; LMWP, low molecular weight phenols; ORAC, oxygen radical absorbance capacity; Q-3-glc, quercetin-3-glucoside; UV-B, ultraviolet-B.

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that is dependent on the cultivar (Medrano et al., 2003; Koundouras et al., 2006; Niculcea et al., 2014). Although in varieties for red wines such as Cabernet Sauvignon, Syrah and Merlot the effects of water deficit on berry phenolics is widely studied (Lovisolo et al., 2010), there are few studies in Malbec (Shellie and King, 2013; Shellie and Bowen, 2014). Irrigation shortage in the vineyard is a tool that regulates skin polyphenols in red grapevines (Kennedy et al., 2002; Lovisolo et al., 2010), and some authors (Ojeda et al., 2002; Castellarin et al., 2007) have shown that such increase is consequence of decreases in berry size (which increases the pulp-skin ratio), but also through augments in the expression of genes for the synthesis. However, the effect of water stress in the berry is genotype dependent (Niculcea et al., 2014), and polyphenol reductions have been reported (Zarrouk et al., 2012).

The plant growth regulator abscisic acid (ABA) plays a crucial role not only in fruit development and ripening, but also in the plant's adaptive responses to biotic and abiotic stresses (Leng et al., 2014; Cohen et al., 2015). In grapevine, it is well known that berry skin ABA levels increase during veraison (Wheeler et al., 2009; Berli et al., 2011), and also that ABA applications increase phenolics in grape berries (Koyama et al., 2010; Balint and Reynolds, 2013), even in the cv. Malbec (Berli et al., 2015).

The aim of the present study was to compare independent and interactive effects of high-altitude solar UV-B, moderate water deficit and ABA applications on berries of *V. vinifera* cv. Malbec, the most cultivated variety of Argentina. We hypothesize that i) phenols increase as defense mechanisms of tissues against UV-B and water deficit, which in turn enhance the quality for red winemaking; and ii) ABA is involved in signaling of these defense mechanisms, whereby ABA application increase phenols.

2. Materials and methods

2.1. Plant material and experimental design

The experiment was conducted during the 2011–2012 and 2012–2013 growing seasons using Malbec vines grown in a high altitude vineyard in Gualtallary, Mendoza, Argentina (69°15′37″ W and 33°23′51″ S) at 1450 m a.s.l., as it is described in Alonso et al. (2015). Briefly, a low UV-B treatment (-UV-B) was set by using a polyester cover that absorbed 78% of UV-B and 18% of ultraviolet-A radiation (UV-A); and a close to ambient UV-B treatment (+UV-B) was established with a low-density polyethylene that transmitted 90% of UV-B and 87% of UV-A. Even though for technical reasons we were not able to completely exclude UV-B in −UV-B treatment, and thus plants received a 22% of ambient solar UV-B, Pontin et al. (2010) found that low UV-B irradiance induced grape plants morphogenic responses (purportedly modulated by UVR8; Jenkins, 2009) while high fluence rate UV-B doubled the number of genes modulated by low fluence UV-B (probably induce by ROS; Hideg et al., 2013). The UV-B treatments were given from 15 days after flowering (DAF) until harvest at 142 DAF. Vines were maintained with no soil water restriction until veraison (84 DAF), and then two irrigation regimes were set, a well-watered treatment (-D) and a moderate water deficit treatment (+D), maintaining stem water potentials at midday of −0.7 and −1.0 MPa, respectively. Additionally, the aerial part of plants was sprayed at veraison and repeated once 15 days after, with 1 mM ABA (+ABA; ±-*cis*, *trans*-abscisic acid, Kelinon Agrochemical Co., Beijing, China) or water (-ABA) solutions containing 0.1% v/v of Triton X-100 as tensioactive. In summary, a total of 8 combined treatments were performed: (i) +UV-B/+D/+ABA; (ii) +UV-B/-D/+ABA); (iii) +UV-B/+D/-ABA; (iv) +UV-B/-D/-ABA; (v) -UV-B/+D/+ABA; (vi) -UV-B/-D/+ABA; (vii) -UV-B/+D/-ABA; and (viii) -UV-B/-D/-ABA.

A randomized complete block design with a 2 × 2 × 2 factorial arrangement of treatment and 5 blocks was used, and the experimental unit consisted of two selected plants for the two consecutive seasons.

2.2. UV-B irradiance

Measurements of erythemal UV-B were performed in the vineyard (in a wide open area) on day 5 (±2 days because clear days without cloud cover were chosen) of the months of December to April in seasons 2011–2012 and 2012–2013 (Fig. 1), with a radiometer and erythemally-weighted UV-B detector (PMA2200 and PMA2102, Solar Light Company Inc., Glenside, PA, USA).

2.3. Berry sampling, berry growth and fruit yield

At harvest (142 DAF), 30 berries per experimental unit were collected into nylon bags (5 berries per cluster; 2 from the top, 2 from the middle and 1 from the bottom). Samples were kept on dry ice to prevent enzyme degradation and dehydration, taken to the laboratory where berry fresh weight (FW) was determined before storage at −20 °C. After berry sampling, all clusters were harvested, weighed and yield per plant was calculated.

2.4. Berry skin phenolics extraction and sugar accumulation

Fifteen berries per experimental unit were defrosted at room temperature (25 ± 2 °C) and peeled by hand. Phenols extraction from berry skins, pulps relative concentration of sugars (°Brix) and

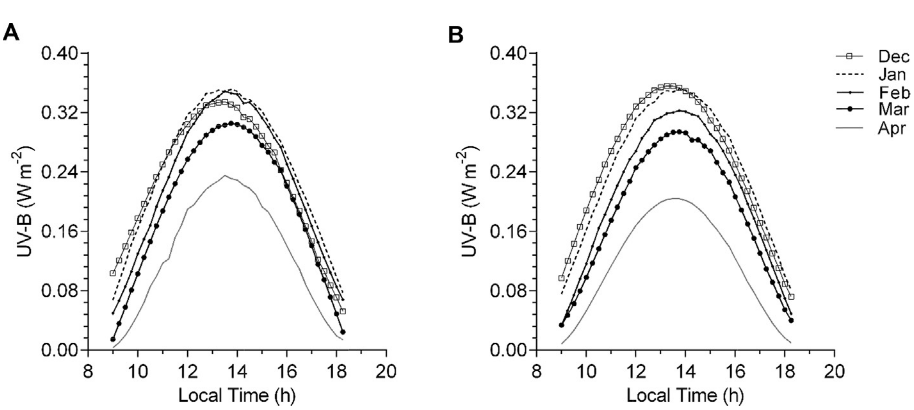

Fig. 1. Erythemal UV-B irradiance (W m^{-2}) measured in the vineyard on day 5th, from December to April, in season 2011–2012 (**A**) and season 2012–2013 (**B**).