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Research article

Acclimation mechanisms elicited by sprayed abscisic acid, solar UV-B and water deficit in leaf tissues of field-grown grapevines

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ABSTRACT

The independent and interactive effects of solar ultraviolet-B radiation (UV-B), moderate water deficit and sprayed abscisic acid (ABA) on leaves gas exchange and biochemical aspects of field-grown grapevines of the cv. Malbec were investigated in a high altitude vineyard (1450 m a.s.l.). Two UV-B treatments (ambient solar UV-B or reduced UV-B), two watering treatments (well watered or moderate water deficit) and two ABA treatments (no ABA and sprayed ABA) were given alone and combined in a factorial design. Gas exchange and photosynthesis were reduced by water deficit and highly impaired in the UV-B and water deficit combined treatment. UV-absorbing compounds were stimulated independently by UV-B. The monoterpenes α-pinene, 3-carene and terpinolene, and the sesquiterpene nerolidol were augmented by UV-B, water deficit or sprayed ABA. Levels of the triterpene squalene and the diterpene phytol were significantly higher in the treatment that combined UV-B, water deficit and ABA applications. Environment signals (solar UV-B and moderate water deficit) and sprayed ABA elicited mechanisms of acclimation by augmenting the content of terpenes with antioxidant and antifungal properties, thus enhancing the plant defensive mechanisms towards signals both biotic and abiotic.

1. Introduction

Viticulture is an activity of great importance in Argentina (http://www.inv.gov.ar), and the most reputed vineyards for red winemaking are located in Mendoza at high altitude (ca. 1500 m a.s.l.). This environment has relatively high solar ultraviolet-B (UV-B) radiation levels, with fluence rates that in summertime reach up to 0.40 W m^{-2} at noon (Berli et al., 2010). Detrimental effects of UV-B on plants have been reported (Jansen, 2002; Kakani et al., 2003), including impairments of growth and gas exchange in grapevines grown at high altitude (Berli et al., 2013). Grapevine perception of relatively high UV-B levels induces acclimation responses (Berli et al., 2010, 2013; Gil et al., 2012; Pontin et al., 2010). High solar UV-B also increases grape berry skin phenolics which improves the quality for red winemaking, although growth and fruit yield are reduced (Berli et al., 2011). Phenolics and terpenes are compounds with antioxidant properties that play a protective role against UV-B, and accumulation of these secondary metabolites have been observed in grapevine leaves in response to UV-B (Berli et al., 2010, 2013; Gil et al., 2012; Kolb et al., 2001).

It has been shown that effects of water deficit on grapevine depend on the plants phenological stage, the severity of the stress and the cultivar studied (Chaves et al., 2010; Ojeda et al., 2002). The maintenance of a stem water potential of about −1 MPa (a moderate water deficit) after veraison, the phenological stage in which berries begin to color and enlarge, have been proposed as a strategy to improve berry quality for red winemaking (Leeuwen et al., 2009). It is assumed that water deficit between veraison and maturity decreases berry sizes and, at the same time, both the skin to pulp ratio and the biosynthesis of phenolics increase (Ojeda et al., 2002). While the effects of water deficit in grapevines have

Abbreviations: ABA, abscisic acid; +ABA, plus ABA treatment; −ABA, minus ABA treatment; DAF, days after flowering; +D, moderate water deficit treatment; −D, well watered treatment; g_s, stomatal conductance; MDA, malondialdehyde; PAR, photosynthetic active radiation; P_n, net photosynthesis; UVAC, UV-A; ultraviolet-A radiation, UV-absorbing compounds; UV-B, ultraviolet-B radiation; +UV-B, solar UV-B treatment; −UV-B, minus UV-B treatment; Ψ_s, stem water potential.

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been widely studied (Chaves et al., 2010 and literature cited therein), there are only limited reports in relation to water deficit and UV-B interactions (Doupis et al., 2011; Martínez-Lüscher et al., 2015).

The phytohormone abscisic acid (ABA) regulates many physiological and biochemical acclimation processes, and some of them are common for different stress conditions (Creelman, 1989; Seki et al., 2002). Berry skin ABA levels increased markedly during veraison reaching the maximum after two weeks (Berli et al., 2011; Wheeler et al., 2009). Additionally, ABA biosynthesis in grapevine is induced by water deficit in leaves (Iacono et al., 1998) and berries (Deluc et al., 2009), but also by high UV-B in leaves (Berli et al., 2010; Gil et al., 2012). While there are several reports about the effect of ABA applications in the accumulation of phenolics in grape berries (Balint and Reynolds, 2013; Berli et al., 2011; Koyama et al., 2009), there are few reports regarding the effect of exogenous ABA on leaf physiology (Zhang and Dami, 2012). In previous work (Berli et al., 2010) we found that weekly ABA applications in grapevine leaves from bud-break to harvest improved tolerance to solar UV-B through increment of antioxidant enzymes activities, membrane-sterols that participate in structural defense, and the accumulation of phenolics and UV-absorbing compounds (UVAC).

In Mendoza, *Vitis vinifera* cv. Malbec has found favorable ecological features for its development. Based on these antecedents, in this study we hypothesized that: 1) ABA applications at veraison and after 15 days induce biochemical changes in grapevine leaves that increase defense mechanisms against UV-B and water deficit; 2) biochemical and physiological changes in grapevines leaves elicited by UV-B are promoted differentially by moderate water deficit. Therefore, the present work compared independent and interactive effects of high-altitude solar UV-B, moderate water deficit and ABA applications on leaves gas exchange and biochemical aspects in field-grown grapevines (*V. vinifera* cv. Malbec).

2. Materials and methods

2.1. Plant material and experimental design

The experiment was carried out during 2013 growing season, in a commercial high altitude vineyard (1450 m a.s.l., 69°15′37″ W and 33°23′51″ S), Gualtallary, Mendoza, Argentina, as it is described in Berli et al. (2013). A minus UV-B treatment (−UV-B) was set by using a polyester cover that absorbed 78% of UV-B, 18% of ultraviolet-A radiation (UV-A) and 12% of photosynthetic active radiation (PAR) from the sunlight. A solar UV-B treatment (+UV-B) was established by covering the canopy with low-density polyethylene that transmitted 90% of UV-B, 87% of UV-A and 87% of PAR. Plastics were set up 2.5 m above ground level, covering the entire canopy, protected with anti-hail nets and replaced after breakdown or transmittance reduction. The transmittance spectral characteristics were reported previously (Berli et al., 2008, 2011). The UV-B treatments were given from 15 days before flowering, stage 23 (Coombe, 1995), mid-November, until harvest at 142 days after flowering (DAF), in early April. The +UV-B and −UV-B treated grapevines were maintained with no soil water restriction until veraison, 84 DAF, stage 35 (Coombe, 1995), mid-February, by using a drip irrigation system and with a black polyethylene film placed on the ground of the whole experiment to avoid rainfall inputs. Based on previous experiments with grapevine in pots, soil water content was indirectly monitored every two weeks by measurement of stem water potential (Ψ_s) at midday as described by Begg and Turner (1970). From veraison onwards, half the plants were given the well watered treatment (−D) in each UV-B regime by keeping them at field capacity (i.e. Ψ_s approximately −0.7 MPa), while in the other half, irrigation was restricted until harvest (+D; moderate water deficit). To increase the natural levels of ABA produced in berry skin at veraison, the aerial part of plants (including leaves and berries) was sprayed at veraison and repeated once 15 day after, with 1 mM ABA solutions (+ABA; plus ABA treatments, ±-*cis, trans*-abscisic acid, 90% purity, Kelinon Agrochemical Co., Beijing, China) or water (−ABA; minus ABA treatment), until runoff and in the late afternoon to minimize ABA photodegradation. Solutions contained 0.1% v/v of Triton X-100 and a minimum amount of ethanol (to initially dissolve the ABA). In summary, a total of 8 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. The experimental unit consisted of two selected plants (based in homogeneity) from six consecutive plants in the row. Two shoots per experimental unit were selected, marked and used for the non-destructive measurements, while the rest of the shoots were used for leaves sampling.

2.2. Photosynthesis, stomatal conductance and chlorophyll content at harvest

Photosynthesis (P_n) and stomatal conductance (g_s) were measured with a portable infrared gas analyzer (CIRAS-2, PP System, Amesbury, MA, USA), between 10:30 am and 12:00 pm, in fully expanded (10–12th from the apex) and sun-exposed leaves. Chlorophyll relative amount was measured with a portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, Osaka, Japan), considering all the leaves from selected shoots.

2.3. Sampling of leaves and biochemical parameters assessed

At harvest, three leaves (12–15th from the apex) per experimental unit were collected in nylon bags, immediately frozen with liquid nitrogen, transported to the laboratory and kept at −80 °C. UVAC and malondialdehyde (MDA) were determined as described in Berli et al. (2010). Terpenes (Fig. 1) and linolenic acid were evaluated using a gas chromatography–electron impact mass

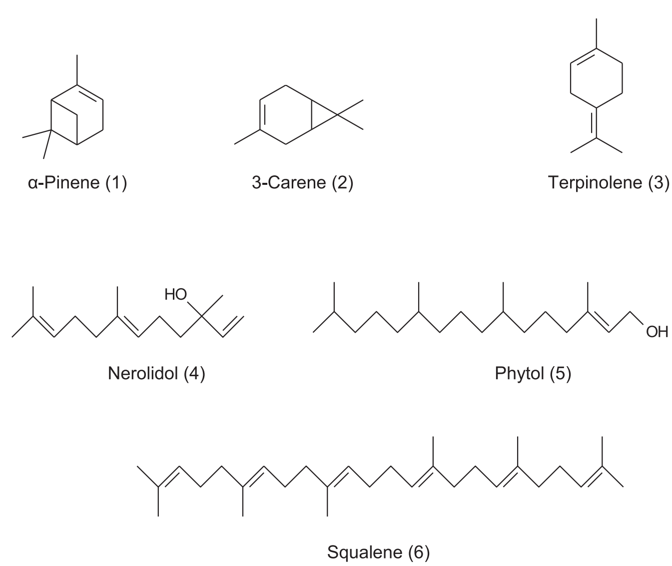

Fig. 1. Chemical structures of α-pinene, 3-carene, terpinolene, nerolidol, phytol and squalene identified by GC-EIMS in grapevine leaves.