



## Research article

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

Rodrigo Alonso <sup>a, b, \*</sup>, Federico J. Berli <sup>a</sup>, Rubén Bottini <sup>a</sup>, Patricia Piccoli <sup>a</sup><sup>a</sup> Laboratorio de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza, Facultad de Ciencias Agrarias, CONICET-Universidad Nacional de Cuyo, Almirante Brown 500, M5528AHB, Chacras de Coria, Mendoza, Argentina<sup>b</sup> Catena Institute of Wine, Bodega Catena Zapata, Cobos s/n, M5509, Agrelo, Mendoza, Argentina

## ARTICLE INFO

## Article history:

Received 1 February 2015

Accepted 27 March 2015

Available online 8 April 2015

## Keywords:

ABA

Drought

Secondary metabolism

Ultraviolet-B

*Vitis vinifera*

## 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  $\alpha$ -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.

© 2015 Elsevier Masson SAS. All rights reserved.

## 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  $0.40 \text{ W m}^{-2}$  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  $-1 \text{ MPa}$  (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;  $\Psi_s$ , stem water potential.

\* Corresponding author. Laboratorio de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza, Facultad de Ciencias Agrarias, CONICET-Universidad Nacional de Cuyo, Almirante Brown 500, M5528AHB, Chacras de Coria, Mendoza, Argentina.

E-mail addresses: [ralonso@fca.uncu.edu.ar](mailto:ralonso@fca.uncu.edu.ar), [ralonso@catenazapata.com](mailto:ralonso@catenazapata.com) (R. Alonso).

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 (Doupsis 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 grapevine 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 ( $\Psi_s$ ) 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.

$\Psi_s$  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,  $\pm$ -*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

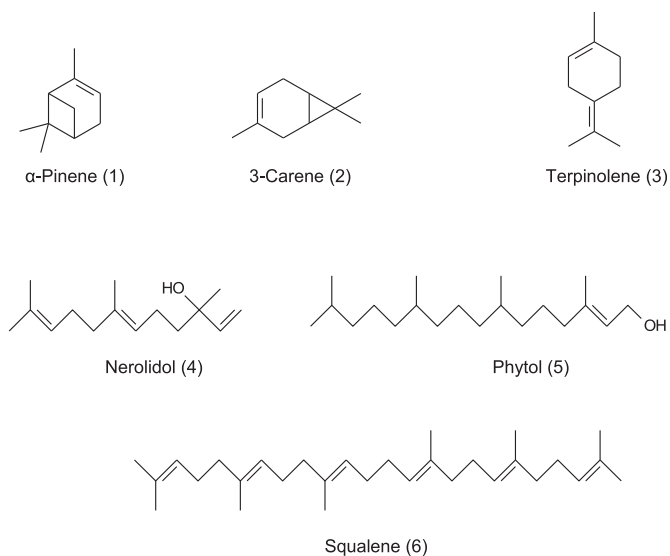


Fig. 1. Chemical structures of  $\alpha$ -pinene, 3-carene, terpinolene, nerolidol, phytol and squalene identified by GC-EIMS in grapevine leaves.

spectrometer (GC–MS; Clarus 500, PerkinElmer, Shelton, CT, USA) according to Gil et al. (2012).

#### 2.4. Statistical analysis

The effects of UV-B ( $P_{UV-B}$ ), moderate water deficit ( $P_D$ ), ABA application ( $P_{ABA}$ ) and their interactions were determined with multifactorial ANOVA and Fisher's LSD test ( $P \leq 0.05$ ), software InfoStat (version 2014; Grupo InfoStat, Córdoba, Argentina).

### 3. Results and discussion

#### 3.1. Gas exchange and photosynthesis

Table 1 shows that +D effectively reduced  $\Psi_s$  and hampered  $g_s$  and  $P_n$ , while UV-B and ABA did not affect  $\Psi_s$ . The combination of +UV-B and +D significantly reduce  $g_s$  and  $P_n$ . In Berli et al. (2013)  $P_n$  and  $g_s$  measured at veraison were reduced by exposure to UV-B, affecting growth, but these results suggest that combining +UV-B with moderate water deficit can increase the impairment of gas exchange and photosynthesis. Martínez-Lüscher et al. (2015) observed in grapevines similar results in  $g_s$ , water deficit conditions had larger effects than UV-B, although the combination of UV-B with water deficit did not significantly affect  $P_n$ . This difference with our results may be due to different experimental system employed. ABA applications in plants under UV-B make  $g_s$  unresponsive to +D. Additionally, +ABA in well watered treatments did not impair  $P_n$ . The latter suggests that ABA has additional indirect effects on photosynthesis via stimulation of carbohydrate transport as it has been shown in wheat (Travaglia et al., 2007) and even in grape (Moreno et al., 2011). Chlorophyll and MDA (an oxidative damage indicator) accumulation in leaves were not affected by treatments (data not shown) indicating that  $P_n$  in +UV-B/+D combined treatment was reduced because of lower stomatal opening and/or lower efficiency in the photosynthetic process and not due to chlorophyll decrease or cellular damage.

**Table 1**

Net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ) and stem water potential ( $\Psi_s$ ) of grapevines exposed to combined UV-B, water deficit and ABA treatments assessed at harvest. +UV-B: solar UV-B; –UV-B: minus UV-B; +ABA: plus ABA; –ABA: minus ABA; +D: moderate water deficit; –D: well watered. Values are means ( $n = 5$ ) and different letters indicate statistically significant differences between treatments at the  $P \leq 0.05$  level according to Fisher's LSD test.

Treatments	$P_n$ $\mu\text{mol m}^{-2} \text{s}^{-1}$	$g_s$ $\text{mmol m}^{-2} \text{s}^{-1}$	$\Psi_s$ MPa
<b>+UV-B</b>			
+D/+ABA	9.85 abc	235.6 bc	–1.0 b
–D/+ABA	10.92 ab	234.1 bc	–0.7 a
+D/–ABA	7.96 c	163.8 c	–1.0 b
–D/–ABA	12.16 a	346.8 a	–0.7 a
<b>–UV-B</b>			
+D/+ABA	9.01 bc	179.9 c	–1.0 b
–D/+ABA	11.90 a	312.9 ab	–0.7 a
+D/–ABA	11.31 ab	186.6 c	–1.0 b
–D/–ABA	12.02 a	304.5 ab	–0.7 a
<b>ANOVA</b>			
$P_{(UV-B)}$	0.1817	0.8179	0.8153
$P_{(ABA)}$	0.4563	0.5958	0.9832
$P_{(D)}$	0.0048	0.0002	<0.0001
$P_{(UV-B \times ABA)}$	0.2537	0.7730	0.9719
$P_{(UV-B \times D)}$	0.5807	0.4803	0.8216
$P_{(ABA \times D)}$	0.7168	0.0853	0.9898
$P_{(UV-B \times ABA \times D)}$	0.0695	0.0482	0.5543

#### 3.2. UV-absorbing compounds

The UVAC accumulation in leaves at harvest was significantly increased by +UV-B (Table 2). The UVAC were not responsive to +D and +ABA combined in the absence of +UV-B. UVAC protect leaf tissue of high UV-B levels by absorbing in this wavelength region (Burchard et al., 2000) and by scavenging free radicals so acting as effective antioxidants (Blokhina et al., 2003). Increases in UVAC have been reported in grapevine leaves as acclimation responses at high altitude UV-B levels (Berli et al., 2013). In grapevine leaves UVAC are mainly phenols, such as flavonols (quercetin and kaempferol) and hydroxycinnamic acids (caffeic acid, p-coumaric acid and ferulic acid; Berli et al., 2010). Doupis et al. (2011) and Martínez-Lüscher et al. (2015) working with cv. Soultanina and cv. Tempranillo, respectively, found that after long-term exposure to UV-B the accumulation of UVAC in grape leaves was not altered by water deficit. It has been shown that exogenous ABA increases internal concentration of ABA (Li et al., 2011), even in grapevine leaves (Berli et al., 2010), where ABA application stimulates production of phenolics. Therefore, we expected increase in UVAC content by ABA application. However, as it can be seen in Table 2, +UV-B signal is indispensable for triggering UVAC.

#### 3.3. Terpenes

The monoterpenes  $\alpha$ -pinene (1), 3-carene (2) and terpinolene (3), the sesquiterpene nerolidol (4), the diterpene phytol (5) and the triterpene squalene (6) were identified by GC–MS in leaves at harvest (for full mass spectra see Figs. 1–3 of Appendix A. Supplementary data). UV-B  $\times$  ABA  $\times$  D interact significantly for all terpenes (Table 2). In plants under the –UV-B/–D/–ABA combined treatment,  $\alpha$ -pinene and 3-carene were not detected; and terpinolene and nerolidol were found in very small amounts. The treatment that only received UV-B (+UV-B/–D/–ABA) showed the highest levels of monoterpenes. Our results from field experiments are consistent with those obtained by Gil et al. (2012) from *in vitro* grapevine leaves in which high UV-B increased  $\alpha$ -pinene, 3-carene, terpinolene and nerolidol contents, all of them with strong antioxidant properties. Additionally, Escoriaza et al. (2012) reported synthesis of nerolidol in response to fungal infection disease that affects grapevines. In agreement with our results and those of Gil et al. (2012), Pandey et al. (2014) suggested that increment of monoterpenes in plants of *Artemisia annua* under UV-B radiation, could be an acclimation mechanism adapted to minimize the UV-B-induced damage. Increase in several monoterpenes and sesquiterpenes were observed in eucalyptus leaves in responses to water deficit (Leicach et al., 2010), but to date there were no reports on grapevine leaves. The +ABA and +D treatments augmented the monoterpenes and nerolidol production. This may indicate that ABA signal mediates the terpene production, since +UV-B, +D and +ABA may increase endogenous ABA levels.

Phytol and squalene levels were higher under +UV-B/+D/+ABA combined treatment (UV-B  $\times$  ABA  $\times$  D significant interaction). Gil et al. (2012) found that phytol and squalene were augmented in *in vitro* grown grapevines under high UV-B, but little is known about their metabolic fate under stress conditions. Phytol could be acting as antioxidant because it has a moderate antioxidant activity (Ruberto and Baratta, 2000), or it may be a precursor of tocopherol, a potent antioxidant compound (Ischebeck et al., 2006). The possibility of phytol increase as the consequence of degradation of chloroplastic tissues may be ruled out since our data show that chlorophylls contents were unchanged (data not shown). Several studies have shown that plants under environmental signals, like high salinity, high UV-B levels and drought allocate squalene to produce triterpenes with high antioxidant capacity and to increase

**Table 2**

Biochemical parameters assessed at harvest in grapevine leaves exposed to combined UV-B, water deficit and ABA treatments. UVAC: UV-absorbing compounds, +UV-B: solar UV-B; –UV-B: minus UV-B; +ABA: plus ABA; –ABA: minus ABA; +D: moderate water deficit; –D: well watered. Values are means (n = 5) and different letters indicate statistically significant differences ( $P \leq 0.05$ ).

Treatments	UVAC $A_{305} \text{ g}^{-1} \text{ fr. wt}$	Monoterpenes			Sesquiterpene	Diterpene	Triterpene	Linolenic acid
		$\alpha$ -Pinene	3-Carene	Terpinolene	Nerolidol	Phytol	Squalene	
		$\text{ng mg}^{-1} \text{ fr. wt}$	$\text{ng mg}^{-1} \text{ fr. wt}$	$\text{ng mg}^{-1} \text{ fr. wt}$	$\text{ng mg}^{-1} \text{ fr. wt}$	$\text{ng mg}^{-1} \text{ fr. wt}$	$\text{ng mg}^{-1} \text{ fr. wt}$	$\text{ng mg}^{-1} \text{ fr. wt}$
<b>+UV-B</b>								
+D/+ABA	68.59 a	0.444 cd	3.02 c	107.3 c	103.4 c	1326.1 a	458.3 a	72.5 bc
–D/+ABA	64.54 ab	1.072 b	6.14 ab	165.4 b	221.3 b	381.6 cd	107.1 b	66.5 c
+D/–ABA	61.81 ab	1.092 b	4.88 bc	133.6 bc	214.6 b	154.2 d	21.7 b	31.4 d
–D/–ABA	70.10 a	1.706 a	6.96 a	227.8 a	206.1 b	975.6 ab	116.7 b	100.8 ab
<b>–UV-B</b>								
+D/+ABA	45.91 d	0.998 b	5.80 ab	140.3 bc	214.1 b	321.4 cd	64.3 b	60.1 c
–D/+ABA	56.52 bc	0.888 bc	5.27 ab	141.0 bc	285.0 a	121.3 d	36.4 b	31.9 d
+D/–ABA	55.17 bcd	1.146 b	5.67 ab	174.6 b	194.9 b	547.4 bcd	153.7 b	97.3 ab
–D/–ABA	50.36 cd	0.000 d	0.00 d	0.3 d	4.5 d	758.1 bc	71.2 b	110.3 a
<b>ANOVA</b>								
$P_{(UV-B)}$	<0.0001	0.0027	0.0177	0.0012	0.1940	0.0486	0.0258	0.3132
$P_{(ABA)}$	0.8360	0.2955	0.0922	0.5999	0.0001	0.3927	0.0766	0.0004
$P_{(D)}$	0.2840	0.7956	0.4119	0.5440	0.4861	0.9780	0.0244	0.0677
$P_{(UV-B \times ABA)}$	0.6450	0.0001	0.0003	0.0013	<0.0001	0.0095	0.0006	0.0002
$P_{(UV-B \times D)}$	0.8698	<0.0001	<0.0001	<0.0001	0.0001	0.9735	0.3936	0.0051
$P_{(ABA \times D)}$	0.7368	0.0160	0.0022	0.0091	<0.0001	0.0001	0.0094	0.0003
$P_{(UV-B \times ABA \times D)}$	0.0055	0.0226	0.0370	0.0004	0.0093	0.0081	0.0009	0.2244

synthesis of membrane sterols that protect the cytosol (Douglas, 1985; Gil et al., 2012; Nasrollahi et al., 2014).

### 3.4. Linolenic acid

Table 2 shows that linolenic acid in grapevine leaves was highest in –UV-B/–D/–ABA combined treatment and lowest in +UV-B/+D/–ABA combined treatment. Linolenic acid is a major polyunsaturated fatty acid of membrane lipids, and the degree of fatty acid unsaturation is an important factor for membrane fluidity related to adaptation to adverse environmental conditions (Upchurch, 2008). In concordance with our results, it has been reported that water deficit and UV-B reduce the degree of fatty acids unsaturation (Bettaieb et al., 2009; Hamrouni et al., 2001).

## 4. Conclusions

Solar high altitude UV-B increased the accumulation of UV-absorbing compounds, while moderate water deficit mainly affected gas exchange and photosynthesis (the impact of moderate water deficit was greater when was combined with exposure to solar UV-B). The monoterpenes  $\alpha$ -pinene, 3-carene and terpinolene and the sesquiterpene nerolidol were augmented by UV-B, moderate water deficit or sprayed ABA. The triterpene squalene and the diterpene phytol were significantly higher in the treatment with combinations of water deficit, solar UV-B and ABA applications (possibly as an acclimation mechanism that modifies membrane fluidity under environmental signals). Two application of ABA on leaves and berries, at veraison and 15 days after, were enough to activate compounds with antioxidant and antifungal properties, which may increase the plant defensive mechanism against environmental signals, both biotic and abiotic.

### Author's contribution

R. Alonso and F.J. Berli conducted the experiment and carried out the physiological, biochemical and statistical analysis. R. Bottini and P. Piccoli collaborated in experiment design and in decided strategies. R. Alonso wrote the body of the paper and all authors reviewed, edited and approved the final version of the manuscript.

### Acknowledgments

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (PIP GI 2010–2012 to P. P.), Secretaría de Ciencia y Técnica de la Nación (PID 2007 0149 to R.B.), Secretaría de Ciencia y Técnica de la Universidad Nacional de Cuyo and Catena Institute of Wine (Bodegas Catena Zapata). The authors thank the technical assistance of L. Bolcato in GC–MS determinations.

### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.plaphy.2015.03.011>.

### References

- Balint, G., Reynolds, A.G., 2013. Impact of exogenous abscisic acid on vine physiology and grape composition of Cabernet Sauvignon. *Am. J. Enol. Vitic.* 64, 74–87.
- Begg, J.E., Turner, N.C., 1970. Water potential gradients in field tobacco. *Plant Physiol.* 46, 343–346.
- Berli, F.J., Alonso, R., Bressan-Smith, R., Bottini, R., 2013. UV-B impairs growth and gas exchange in grapevines grown in high altitude. *Physiol. Plant.* 149, 127–140.
- Berli, F.J., Fanzone, M., Piccoli, P., Bottini, R., 2011. Solar UV-B and ABA are involved in phenol metabolism of *Vitis vinifera* L. increasing biosynthesis of berry skin polyphenols. *J. Agric. Food Chem.* 59, 4874–4884.
- Berli, F.J., Moreno, D., Piccoli, P., Hespagnol-Viana, L., Silva, M.F., Bressan-Smith, R., Cavagnaro, J.B., Bottini, R., 2010. Abscisic acid is involved in the response of grape (*Vitis vinifera* L.) cv. Malbec leaf tissues to ultraviolet-B radiation by enhancing ultraviolet-absorbing compounds, antioxidant enzymes and membrane sterols. *Plant Cell Environ.* 33, 1–10.
- Berli, F.J., D'Angelo, J., Cavagnaro, B., Bottini, R., Wuilloud, R., Silva, M.F., 2008. Phenolic composition in grape (*Vitis vinifera* L. cv. Malbec) ripened with different solar UV-B radiation levels by capillary zone electrophoresis. *J. Agric. Food Chem.* 56, 2892–2898.
- Bettaieb, I., Zakhama, N., Wannas, W.A., Kchouk, M., Marzouk, B., 2009. Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. *Sci. Hortic.* 120, 271–275.
- Blokhina, O., Virolainen, E., Fagerstedt, K.V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.* 91, 179–194.
- Burchard, P., Bilger, W., Weissenböck, G., 2000. Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet induced chlorophyll fluorescence measurements. *Plant Cell Environ.* 23, 1373–1380.
- Chaves, M.M., Zarrouk, O., Francisco, R., Costa, J.M., Santos, T., Regalado, A.P., Rodrigues, M.L., Lopes, C.M., 2010. Grapevine under deficit irrigation: hints from



- physiological and molecular data. *Ann. Bot.* 105, 661–676.
- Coombe, B.G., 1995. Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. *Aust. J. Grape Wine Res.* 1, 104–110.
- Creelman, R.A., 1989. Abscisic acid physiology and biosynthesis in higher plants. *Physiol. Plant.* 75, 131–136.
- Deluc, L.G., Quilici, D.R., Decendit, A., Grimplet, J., Wheatley, M.D., Schlauch, K.A., Merillon, J.M., Cushman, J.C., Cramer, G.R., 2009. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics* 10, 212.
- Douglas, T., 1985. NaCl effects on 4-desmethylsterol composition of plasma-membrane-enriched preparations from citrus roots. *Plant Cell Environ.* 8, 687–692.
- Doupis, G., Chartzoulakis, K., Beis, A., Patakas, A., 2011. Allometric and biochemical responses of grapevines subjected to drought and enhanced ultraviolet-B radiation. *Aust. J. Grape Wine Res.* 17, 36–42.
- Escoriza, G., Sansberro, P., Garcia-Lampasona, S., Gatica, M., Bottini, R., Piccoli, P., 2012. In vitro cultures of *Vitis vinifera* L. cv. Chardonnay synthesize the phytoalexin nerolidol upon infection by *Phaeoacremonium parasiticum*. *Phytopathol. Mediterr.* 52, 289–297.
- Gil, M., Pontin, M., Berli, F.J., Bottini, R., Piccoli, P., 2012. Metabolism of terpenes in the response of grape (*Vitis vinifera* L.) leaf tissues to UV-B radiation. *Phytochemistry* 77, 89–98.
- Hamrouni, I., Salah, H.B., Marzouk, B., 2001. Effects of water-deficit on lipids of safflower aerial parts. *Phytochemistry* 58, 277–280.
- Iacono, F., Buccella, A., Peterlunger, E., 1998. Water stress and rootstock influence on leaf gas exchange of grafted and ungrafted grapevines. *Sci. Hortic.* 75, 27–39.
- Ischebeck, T., Zbierzak, A.M., Kanwischer, M., Dörmann, P., 2006. A salvage pathway for phytol metabolism in *Arabidopsis*. *J. Biol. Chem.* 281, 2470–2477.
- Jansen, M.A.K., 2002. Ultraviolet-B radiation effects on plants: induction of morphogenic responses. *Physiol. Plant.* 116, 423–429.
- Kakani, V.G., Reddy, K.R., Zhao, D., Sailaja, K., 2003. Field crop responses to ultraviolet-B radiation: a review. *Agric. For. Meteorol.* 120, 191–218.
- Kolb, C.A., Käser, M.A., Kopecký, J., Zotz, G., Riederer, M., Pfündel, E.E., 2001. Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. *Plant Physiol.* 127, 863–875.
- Koyama, K., Sadamatsu, K., Goto-Yamamoto, N., 2009. Abscisic acid stimulated ripening and gene expression in berry skins of the Cabernet Sauvignon grape. *Funct. Integr. Genomics* 1–15.
- Leeuwen, C.V., Tregoat, O., Choné, X., Bois, B., Pernet, D., Gaudillère, J.P., 2009. Vine water status is a key factor in grape ripening and vintage quality for red bordeaux wine. How can it be assessed for vineyard management purposes? *J. Int. des Sci. de la Vigne du Vin* 43, 121–134.
- Leicach, S.R., Garau, A.M., Guarnaschelli, A.B., Yaber Grass, M.A., Sztarker, N.D., Dato, A., 2010. Changes in *Eucalyptus camaldulensis* essential oil composition as response to drought preconditioning. *J. Plant Interact.* 5, 205–210.
- Li, Y., Zhao, H., Duan, B., Korpelainen, H., Li, C., 2011. Effect of drought and ABA on growth, photosynthesis and antioxidant system of *Cotinus coggygria* seedlings under two different light conditions. *Environ. Exp. Bot.* 71, 107–113.
- Martínez-Lüscher, J., Morales, F., Delrot, S., Sánchez-Díaz, M., Gomès, E., Aguirreola, J., Pascual, I., 2015. Characterization of the adaptive response of grapevine (cv. Tempranillo) to UV-B radiation under water deficit conditions. *Plant Sci.* 232, 13–22.
- Moreno, D., Berli, F., Piccoli, P., Bottini, R., 2011. Gibberellins and abscisic acid promote carbon allocation in roots and berries of grape plants. *J. Plant Growth Regul.* 30, 220–228.
- Nasrollahi, V., Mirzaie-Asl, A., Piri, K., Nazeri, S., Mehrabi, R., 2014. The effect of drought stress on the expression of key genes involved in the biosynthesis of triterpenoid saponins in liquorice (*Glycyrrhiza glabra*). *Phytochemistry* 103, 32–37.
- Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A., Deloire, A., 2002. Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* 53, 261–267.
- Pandey, N., Pandey-Rai, S., 2014. Modulations of physiological responses and possible involvement of defense-related secondary metabolites in acclimation of *Artemisia annua* L. against short-term UV-B radiation. *Planta* 240 (3), 611–627.
- Pontin, M.A., Piccoli, P., Francisco, R., Bottini, R., Martínez-Zapater, J.M., Lijavetzky, D., 2010. Transcriptome changes in grapevine (*Vitis vinifera* L.) cv. Malbec leaves induced by ultraviolet-B radiation. *BMC Plant Biol.* 10.
- Ruberto, G., Baratta, M.T., 2000. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* 69, 167–174.
- Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., Satou, M., Akiyama, K., Taji, T., Yamaguchi-Shinozaki, K., Carninci, P., Kawai, J., Hayashizaki, Y., Shinozaki, K., 2002. Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant J.* 31, 279–292.
- Travaglia, C., Cohen, A.C., Reinoso, H., Castillo, C., Bottini, R., 2007. Exogenous abscisic acid increases carbohydrate accumulation and redistribution to the grains in wheat grown under field conditions of soil water restriction. *J. Plant Growth Regul.* 26, 285–289.
- Upchurch, R.G., 2008. Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnol. Lett.* 30, 967–977.
- Wheeler, S., Loveys, B., Ford, C., Davies, C., 2009. The relationship between the expression of abscisic acid biosynthesis genes, accumulation of abscisic acid and the promotion of *Vitis vinifera* L. berry ripening by abscisic acid. *Aust. J. Grape Wine Res.* 15, 195–204.
- Zhang, Y., Dami, I.E., 2012. Foliar application of abscisic acid increases freezing tolerance of field-grown *Vitis vinifera* Cabernet Franc grapevines. *Am. J. Enol. Vitic.* 63, 377–384.