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Research article

Changes in grapevine DNA methylation and polyphenols content induced by solar ultraviolet-B radiation, water deficit and abscisic acid spray treatments

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ABSTRACT

Environment and crop management shape plant's phenotype. Argentinean high-altitude vineyards are characterized by elevated solar ultraviolet-B radiation (UV−B) and water deficit (D) that enhance enological quality for red winemaking. These signals promote phenolics accumulation in leaves and berries, being the responses mediated by abscisic acid (ABA). DNA methylation is an epigenetic mechanism that regulates gene expression and may affect grapevine growth, development and acclimation, since methylation patterns are mitotically heritable. Berry skins low molecular weight polyphenols (LMWP) were characterized in field grown *Vitis vinifera* L. cv. Malbec plants exposed to contrasting UV-B, D, and ABA treatments during one season. The next season early fruit shoots were epigenetically (methylation-sensitive amplification polymorphism; MSAP) and biochemically (LMWP) characterized. Unstable epigenetic patterns and/or stochastic stress-induced methylation changes were observed. UV-B and D were the treatments that induced greater number of DNA methylation changes respect to Control; and UV-B promoted global hypermethylation of MSAP epiloci. Sequenced MSAP fragments associated with UV-B and ABA showed similarities with transcriptional regulators and ubiquitin ligases proteins activated by light. UV-B was associated with flavonols accumulation in berries and with hydroxycinnamic acids in the next season fruit shoots, suggesting that DNA methylation could regulate the LMWP accumulation and participate in acclimation mechanisms.

1. Introduction

The ability of an organism to express diverse phenotypes in different environments is called phenotypic plasticity, phenomenon that plays a key role in acclimatization processes in response to different stressors (Kelly et al., 2011). Phenotypic plasticity is particularly relevant for plants, since they cannot escape from stressful and unfavorable environmental conditions (Schlichting and Smith, 2002), thereby impacts on evolution, ecology and agriculture performance in a scenario of changing climate (Nicotra et al., 2010). The term describes developmental effects on morphological characters, but now is more broadly used, including physiological, biochemical and short-term gene expression changes (Atkinson and Urwin, 2012), as well as genetic and epigenetic modifications (Jablonka and Raz, 2009).

Epigenetics refers to heritable and reversible modifications in gene expression without changes in the DNA sequence. It includes mechanisms as DNA methylation, histone post-translational modifications, and small RNAs which can stabilize specific gene expression patterns in response to the environment (Henderson and Jacobsen, 2007). Thus, the relation between epigenetics and phenotypic plasticity has raised considerable scientific interest (Mirouze and Paszkowski, 2011; Zhang et al., 2013), especially in crops asexually propagated like grapevine where the winemaking quality is associated with terroir, which in turn involves plant material (genotype), environmental conditions and management characteristics (Van Leeuwen et al., 2004).

DNA methylation is the best understood epigenetic mechanism and, in higher plants, it participates in various biological processes, generating heritable phenotypes that increase the adaptation of plants to various abiotic and biotic factors (Johannes et al., 2009; Reinders et al., 2009). For example, differential DNA methylation patterns have been identified in response to drought and salinity, so affecting gene expression and phenotypes (Chinnusamy and Zhu, 2009). DNA

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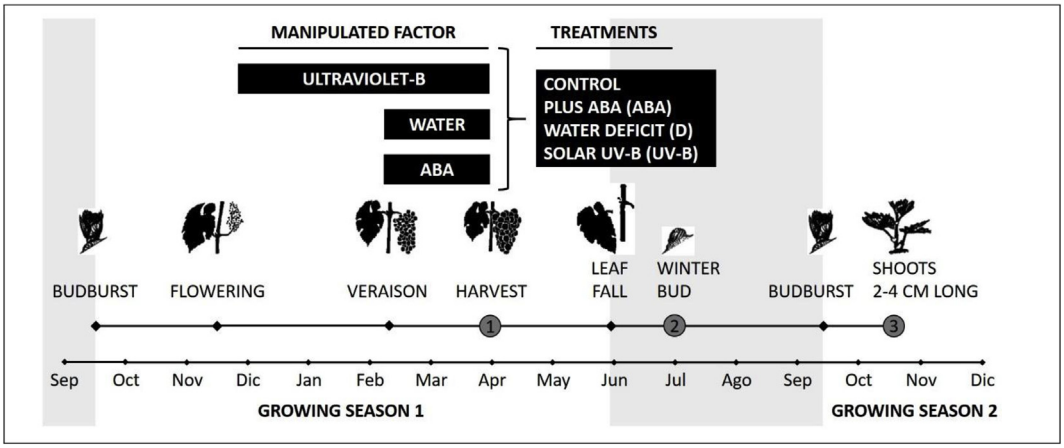

Fig. 1. Timeline schematic representation of grapevine growth stages and experimental conditions. Solar ultraviolet-B, water and abscisic acid were manipulated to set four treatments with combined factors: Control; plus ABA (ABA); water deficit (D) and solar UV-B (UV−B). Circle 1: measurements of berry biochemical characteristics in the growing season in which the treatments were applied; Circle 2: collection of treated canes in the field; Circle 3: evaluation of fruit shoot DNA methylation patterns and phenolic profile in the growing season 2.

methylation patterns are mitotically and/or meiotically heritable (Richards, 2006), and the findings describing the inheritance of DNA methylation have positioned it as a key component in evolutionary processes, as well as a tempting promise to use this additional source of phenotypic variation in plant breeding programs (Rodríguez López and Wilkinson, 2015). Thus, the environmental and management history can shape the expression of different genome regions, so controlling plant's phenotype.

Grapevine (*Vitis vinifera* L.) is a worldwide economically important fruit crop that has been proposed as an experimental model to study the role of epigenetic variability in plant responses to stresses and phenotypic plasticity in perennial crops (Fortes and Gallusci, 2017). Grapevines possessed high phenotypic plasticity, since the same clone shows variability even among the berries within a cluster (Keller, 2010; Dal Santo et al., 2013). The phenotypic plasticity takes special relevance from an oenological point of view, since berry and wine composition can be associated with a wine region or specific vineyard, a goal pursued worldwide because of its commercial relevance. Therefore, there is increasing interest in generating scientific basis for better understanding of how individual factors of the terroir influences vine/wines (Anesi et al., 2015). In addition to be perennial, grapevines are propagated asexually and the environmental conditions presented in one season could determine the quality of the next vintage (Boss et al., 2003). That is, the inflorescences are determined at two developmental stages, during the period of inflorescence primordia development in one year and throughout bud-burst in the following season. In this sense, it is important to evaluate the epigenetic changes environmentally induced during one season and mitotically inherited in the next vintage.

Argentinean high-altitude vineyards are characterized by environmentally elevated solar ultraviolet B (UV−B) radiation (Berli et al., 2010) and water deficit; both conditions that can enhance oenological quality for red winemaking (Teixeira et al., 2013). We previously found that *Vitis vinifera* cv. Malbec, the iconic cultivar for red wine in Argentinean viticulture, exhibited physiological and biochemical particularities in response to environmental signals. Exposing grapevines to contrasting solar UV-B radiation, moderate water deficit regimes (D) and spraying abscisic acid (ABA), differentially elicited leaf and berries acclimation mechanisms, thus influencing fruit quality and fruit yield (Alonso et al., 2015, 2016).

It was proposed that epigenomic data should be included in the analysis of the terroir (Fabres et al., 2017), but despite the scientific and commercial importance of grapevine, there are few studies to understand the molecular mechanism of how plant genomes can be epigenetically shaped by growing conditions (Xie et al., 2017). The present work evaluated whether UV-B, D and ABA treatments induce grapevine DNA methylation changes in the Malbec genome, correlated this epigenetic mechanism with the variability observed in polyphenols metabolism, and studied to what extent this epigenetic characteristic is inherited as part of an acclimation mechanism.

2. Materials and methods

2.1. Plant material and experimental design

The experiment was conducted during two consecutive growth seasons using *Vitis vinifera* cv. Malbec vines grown in a high altitude vineyard in Gualtallary, Mendoza, Argentina (69°15′37″W and 33°23′51″S; 1450 m a.s.l.). In 2012–2013 (season 1), two UV-B environments were set by using a polyester cover that filtered ~80% of solar UV-B to minimize UV-B, but also 18% of UV-A and 12% of PAR (−UV−B); and in order to reduce environmental differences generated by the polyester cover, a polyethylene that transmitted most of the solar radiation (~90% of UV-B, 87% of UV-A and 87% of PAR; *UV-B treatment*). Plastics were positioned 2.5 m above ground level, covering the entire grapevine canopy from two weeks after flowering until harvest, as previously detailed (Berli et al., 2011). Erythemally-weighted solar UV-B irradiances, solar UV-A irradiances and PAR fluence rates were measured with PMA2102 UV-B detector, PMA2110C UV-A detector (Solar Light Company, Glenside, PA) and LI-190SA quantum sensor (LI-COR, Lincoln, NE), respectively. From here on, wherever mention "UV-B irradiances", we are really referring to "erythemally-weighted UV-B irradiances". Meteorogical data registered in the vineyard for season 1 (UV−B and UV-A irradiances, PAR fluence rates, daily air temperatures and rainfall) is presented in Supplementary Material (Table S1).

Vines grew with no soil water restriction until veraison (berries begin to get redness) and then, two irrigation regimes were set to obtain well-watered (-D) and moderate water deficit (*D*) treatments until harvest. A drip irrigation system plus black polyethylene films covering the ground to avoid rainfall water inputs were used. The aerial part of plants was sprayed at veraison (and repeated once 15 days after), with 1 mM ABA (*ABA treatment*; ± *-cis*, *trans*-abscisic acid, Kelinon Agrochemical Co., Beijing, China) or H_2O (-ABA) solutions, both containing 0.1% v/v of Triton X-100 as surfactant. A total of 4 treatments combining UV-B, D and ABA regimes were analyzed (Fig. 1): i) *Control* (−UV−B/-D/-ABA); ii) *ABA* (−UV−B/-D); iii) *D* (−UV−B/-ABA) and iv) *UV-B* (-D/-ABA). It is important to highlight that in the evaluated assays the term control did not represents the natural environmental conditions and was used as the treatment where each single factor was turned

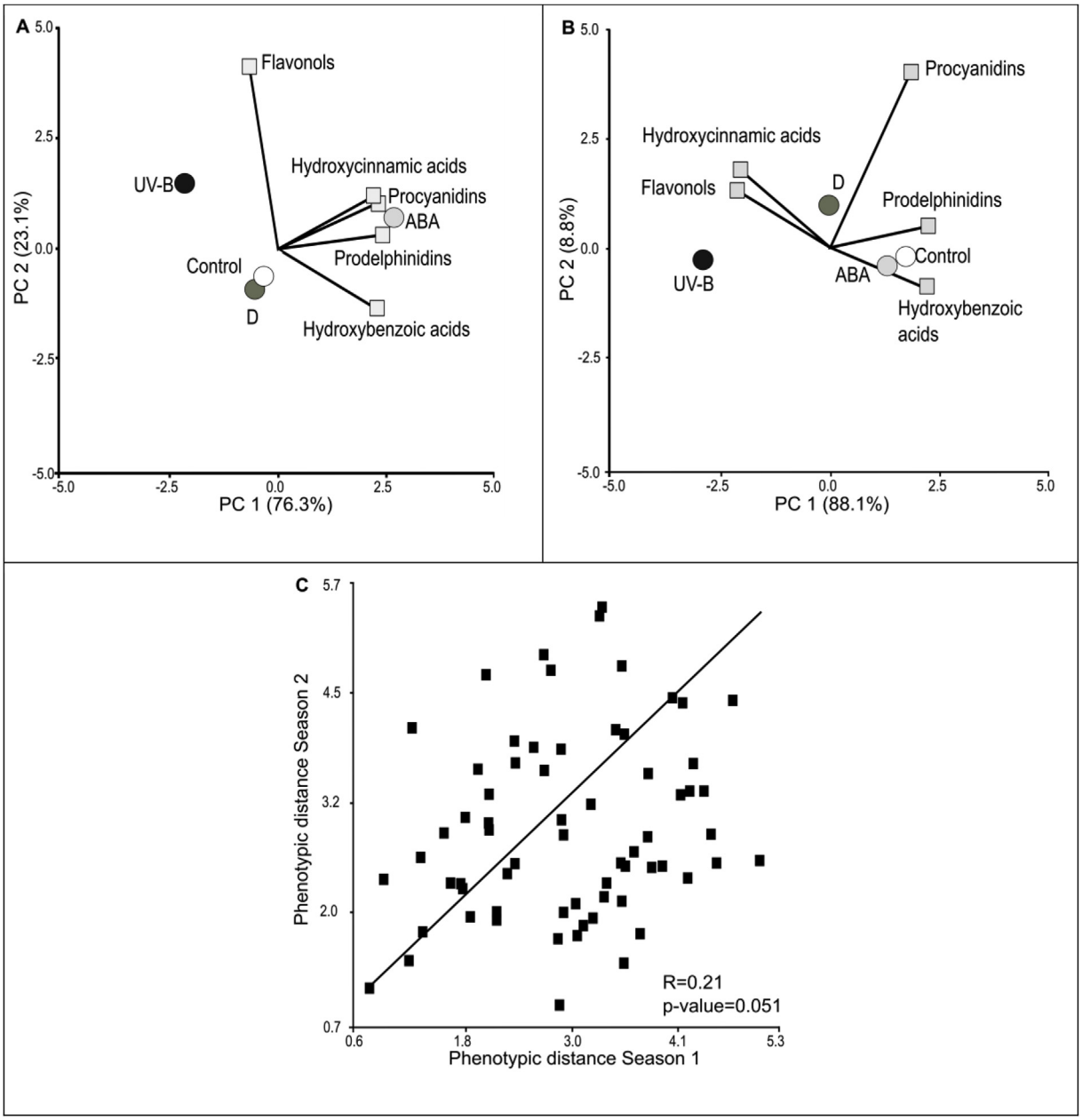

Fig. 2. Polyphenolic characterization. Biochemical variability observed in two consecutive growing seasons in vines cv. Malbec exposed to four different treatments: Control, ABA, D and UV-B. Phenols (flavonols, procyanidins, prodelphinidins, hydroxycinnamic acids and hydroxybenzoic acids) in berry skins at harvest during the growing season 1 in which the treatments were applied (A) and in the early fruit shoots in the growing season 2 (B). Mantel's test with Pearson correlation coefficient among phenotypic distance matrices from both growing seasons (C).

off (*i.e.* minus UV-B, well-watered and not sprayed with ABA). A randomized complete block design with 5 blocks was used (n = 5), and the experimental unit consisted of two plants. Stem water potentials at midday were measured fortnightly from veraison with a pressure chamber, and were lesser for D as compared with Control, UV-B and ABA, reaching up to −1.1 MPa (Fig. S1).

In the 2013–2014 (season 2) in order to uniform the physiological conditions of sprouting, during winter time (point 2 in Fig. 1), two canes per experimental unit were collected in nylon bags and maintained at 4 °C during one month to break bud endo-dormancy. Then, canes were washed with running water, cut into 30 cm long (5 buds) cuttings, placed in 5 L plastic pots filled with moist perlite, and cultivated in a growth chamber with a 16/8 h photoperiod (400 μmol m^{-2} s^{-1} PAR), 25 ± 2 °C and ~50% RH. After one month under these conditions, bud burst was completed (point 3 in Figs. 1) and Figs. 2–4 cm long fruit shoots, stage 9 (Coombe, 1995) were sampled, immediately frozen with liquid nitrogen and kept at −75 °C for further biochemical and DNA methylation analysis (see 2.2 and 2.3 sections).

2.2. Phenotypic characterization

Low molecular weight polyphenols (LMWP) profile was evaluated during two consecutive growing seasons, in berry skins along season 1 (point 1 in Fig. 1), and in early fruit shoots in season 2 (point 3 in Fig. 1). Berries at harvest-ripe, stage 38 and 2–4 cm long fruit shoots, stage 9 (Coombe, 1995) were collected respectively and kept at −75 °C for further biochemical analysis. LMWP were analyzed as described in (Murcia et al., 2017) from shoot samples (containing two to three leaves and inflorescences). One gram of tissues fresh weight was homogenized in mortar and pestle with liquid N_2 and extracted with

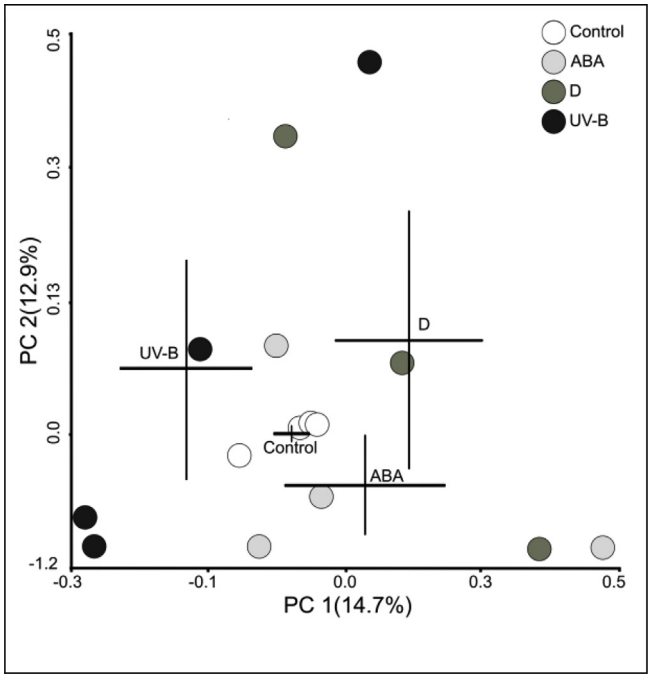

Fig. 3. Epigenetic variability representation. Principal coordinates analyses (PCoA) epigenetic distances based on methylation-sensitive amplification polymorphisms (MSAP) technique for vines cv. Malbec exposed to Control, ABA, D and UV-B treatments. Circles represent replicates of each treatment and crosses represent treatment group mean ± SEM. Percentage of the variability capture by each principal coordinate (PC) is shown in parenthesis.

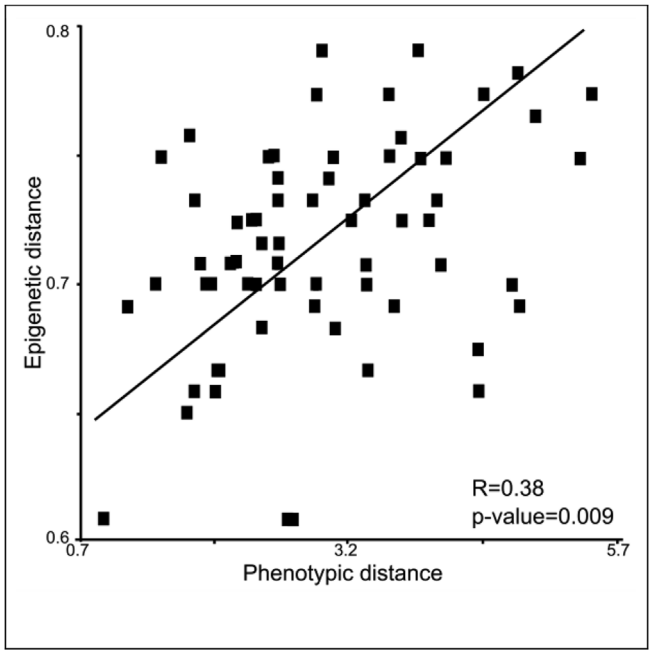

Fig. 4. Correlation analysis. Epigenetic and phenotypic variability association based on distance matrices analyzed through Mantel's test with Pearson correlation coefficient.

5 mL of 99:1 (v/v) MeOH:HCl solution and sonication for 5 min. Then, mixture was centrifuged, aliquots evaporated to dryness, reconstituted in the initial mobile phase, filtered and then analyzed by high-performance liquid chromatography with a photodiode array detector (HPLC-DAD; Dionex Ultimate 3000 system, Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany).

2.3. Methylation-sensitive amplified polymorphism (MSAP)

DNA methylation patterns can vary according to different phenological growth stages, so we decided to uniform the physiological conditions at 2–4 cm long fruit shoots for molecular analysis. DNA was extracted from 15 mg of frozen tissues grounded to powder in mortar and pestle with liquid N_2 using the DNeasy Plant Kit (Qiagen, Valencia, CA, USA) according to the manufacturer instructions, in 4 biological replicates per treatment. Isolated DNA was spectrophotometrically quantified and 100 ng μL^{-1} working solutions were prepared for each analyzed sample. MSAP procedure was completed according to (Cara et al., 2013). The pre-selective amplification was performed with *Eco*RI: 5′-GACTGCGTACCAATTCA-3′ and *Hpa*II-*Msp*I: 5′-ATGAGTCTCGATC GGA-3′ primers. The selective amplification reactions were performed using fluorescence labeled **Eco*RI primers. The following three selective **Eco*RI + 3/*Hpa*II-*Msp*I + 3 primer combinations were used: *AAG/AAT, *ACG/ATG and *ACA/AAT. The final amplification products were separated with an ABI PRISM 3130 sequencer (Applied Biosystems, California, USA). For each primer combination, generated electropherograms were visualized using GeneMapper Software v3.7 (Applied Biosystems, California, USA) and fragments among 100 and 600 pb were manually scored (Cara et al., 2014). Three different methylation patterns (codified from 0 to 3 according to the presence/absence of fragments in *Eco*RI/*Hpa*II and *Eco*RI/*Msp*I profiles for each evaluated plant) were established as indicated in Supplementary Material (Fig. S2). Epiloci with missing data were excluded from the analyses. Then, the methylation patterns (epiallele) were transformed to binary epigenetic data matrices according to the presence (1) or absence (0) of the particular epiallele (Cara et al., 2013). Epialleles that turned out singletons (*i.e.* MSAP patterns observed only in one of the analyzed samples) were excluded to prevent biased parameter estimates (Bonin et al., 2004).

2.4. Identification of MSAP fragments with altered methylation

MSAP fragments with differential methylation patterns respect to the consensus epigenotype (see below in 2.5 section) were sequenced on an ABI PRISM 3130 sequencer (Applied Biosystems, California, USA) in order to identify altered genome regions. Two unlabeled selective *Eco*RI + 3/*Hpa*II-*Msp*I + 3 primer combinations were used: AGC/AAT and AGC/ATC. Amplified fragments were separated in a polyacrylamide denaturing gel, gel isolated, eluted, re-amplified, cloned and sequenced following the descriptions of (Gimenez et al., 2016). The obtained sequences were analyzed using the BLASTn packages of the GenBank at the NCBI; http://www.ncbi.nlm.nih.gov.

2.5. Data analyses

ANOVA was performed with InfoStat Software version 2009 (Grupo InfoStat, Córdoba, Argentina), by using Fisher's LSD and 0.05 of significance level, and previously checking data normality and homoscedasticity. Principal component analyses (PCA) throughout biplot graphics and standardized (centered and variance-scaled) were used to evaluate the biochemical characteristics. The matrix for the analysis consisted of 4 classification cases (Control, ABA, D and UV-B), and 5 variables (groups of LMWP: flavonols, hydroxycinnamic acids, hydroxybenzoic acids, procyanidins and prodelphinidins).

Global plant methylation status for each treatment was computed as the observed percentage of methylated, hemimethylated, and unmethylated loci (Cara et al., 2013). The proportion of epialleles at each methylation pattern was evaluated by a generalized linear model, with log as link function for Binomial distribution. Epigenetic polymorphism was studied through a principal coordinates analysis (PCoA) with InfoStat Software. Additionaly, the Sorensen-Dice coefficient was calculated to obtain the distance matrices (Sneath and Sokal, 1973). To evidence the epigenetic variability induced by the treatments, a

consensus state was inferred based on the MSAP epialleles observed in plants from the Control as proposed by Verhoeven et al. (2010). Succinctly, this analysis assumes that the consensus state is the starting state of all assayed plants in the experiment and that any deviations have been induced by the evaluated treatments. The consensus state (present or absent) was determined for 413 epialleles that were either monomorphic or had only one deviating observation (among 4 replicate individuals) in the Control (Table S2). From the total epialleles analyzed, 75 were excluded as the consensus state could be not established. The frequency of treatment-induced methylation changes were analyzed with a GLM model, with the marker and the individual plant effect set as random factors. Afterwards, the presence/absence scores of 413 epialelles were evaluated against the consensus epigenotype. Mantel's test with Pearson correlation coefficient were used to analyze the correlation among phenotypic distance matrices from both growing seasons and to compare the epigenetic and phenotypic distance matrices obtained from the analyses performed in season 2.

3. Results

3.1. Polyphenolic characterization

LMWP assessed in ripe berry skins during season 1 were syringic acid and gallic acid (hydroxybenzoic acids), catechin, epicatechin and epicatechin gallate (procyanidins), gallocatechin, epigallocatechin gallate, gallocatechin gallate (prodelphinidins), caffeic acid and ferulic acid (hydroxycinnamic acids), quercetin-3-glucoside, kaempferol-3-glucoside and quercetin (flavonols) and astilbin (dihydroxyflavonol). UV-B increased flavonols 2.54-fold while ABA promoted hydroxycinnamic acids 1.36-fold, as compared to Control (Table 1). Based on Fig. 2A, the sum of components 1 and 2 of the PCA accounted for 99.4% of the total variation. UV-B was purportedly associated with superior levels of flavonols; ABA was related with high levels of hydroxycinnamic acids, but also with flavanols (procyanidins and prodelphinidins); meanwhile D did not induce variability respect to the Control.

In fruit shoots collected at early growth stages in season 2 the LMWP appraised were gallic acid (hydroxybenzoic acid), catechin (procyanidin), gallocatechin, epigallocatechin gallate, gallocatechin gallate (prodelphinidins), caftaric acid and p-coumaric acid (hydroxycinnamic acids), quercetin-3-glucoside, kaempferol-3-glucoside and quercetin (flavonols) and the stilbene polydatin. UV-B increased hydroxycinnamic acids 1.63-fold respect to Control, without effects of the other treatments (Table 1). PCA shows that 96.9% of the total variation may be explained by PC1 plus PC2 (Fig. 2B). UV-B was separated from the rest of the treatments and associated with high levels of hydroxycinnamic acids, but also with flavonols (Fig. 2B).

Fig. 2C shows a positive and statistically significant correlation among phenotypic distance matrices (based on LMWP profiles) from both growing seasons (season 1 *vs.* season 2).

3.2. Methylation analyses

A total of 301 fragments were considered epiloci, and were computed as 665 epialleles (alternative methylation patterns at determined epilocus). From the 665 epialleles, 171 were identified as singletons and excluded for further analyses. As well, 6 monomorphic epialleles were not considered in the PCoA analysis (*i.e.* 488 epialleles and MSAP markers were used).

Fig. 3 shows that the 4 replicates of Control had reduced epigenetic distance and grouped together, while ABA, D and UV-B treatments induced high dispersion in their replicates. In addition, ABA constitutes the group that presented smaller epigenetic distance respect to the Control, while D and UV-B separated respect to the Control along the first axis (which explain 14.7% of the total epigenetic variability), indicating epigenetic dissimilarities.

The Mantel tests showed a statistically significant correlation of LMWP data obtained from fruit shoots collected at early grow stages in season 2 with MSAP variability (Fig. 4).

The consensus epigenotype based on 413 epialleles showed that the proportion of changed epiloci differed significantly among groups (Table 2). Also, pairwise comparisons between the control group (consensus epigenotype) and individual treatments showed that UV-B induced more epigenetic changes (23%), followed by D (21%) and ABA (18%). When global methylation levels were evaluated, only UV-B induced changes respect to Control (Fig. 5). The percentage of unmethylated epialleles for UV-B was remarkably lower than that for Control (31% *vs.* 38%) and the percentage of methylated epialleles for UV-B was noticeably higher than in Control (44% *vs.* 36%). In addition, UV-B showed differences in the percentage of unmethylated and methylated epialleles respect to ABA.

Six out of 10 sequenced MSAP fragments matched with non-coding regions or showed no similarity; while three were related to different transcriptional regulators, and one to a protein that is activated by light (Table 3). Two of the sequenced fragments (UVB-1 and UVB-3) that presented DNA methylation patterns associated with UV-B treatment showed similarity to different components of the ubiquitin ligase complex. The third sequenced fragment associated with high UV-B radiation (UVB-2) presented a non-methylated pattern and matched with proteins that are activated by light, such as ATP synthase. The fragment associated to ABA (ABA-1) treatment showed similarity to the basic helix-loop-helix transcription factor bHLH63.

4. Discussion

The molecular bases of grapevine plasticity have been explored

Table 1
Polyphenolic characterization. Low molecular weight polyphenols (LMWP) in berries at harvest during the growing season 1 and in the early fruit shoots in the growing season 2 (μg g-1), for vines cv. Malbec exposed to Control, ABA, D and UV-B. Values are means ± SEM (n = 5) and different letters within each column and season indicate statistically significant differences (Fisher's LSD, p-value ≤ 0.05).

Treatment	Flavonols		Hydroxycinnamic acids		Hydroxybenzoic acids	Procyanidins	Prodelphinidins
Season 1							
Control	8.077 ± 0.528	b	4.877 ± 0.211	b	15.973 ± 0.899	14.010 ± 0.688	3.560 ± 0.293
ABA	13.147 ± 1.918	ab	6.633 ± 0.065	a	17.643 ± 0.253	17.853 ± 1.388	4.433 ± 0.113
D	8.100 ± 1.018	b	3.700 ± 0.387	c	16.457 ± 1.145	14.587 ± 2.880	3.443 ± 0.809
UV-B	20.553 ± 4.107	a	4.057 ± 0.295	bc	14.617 ± 0.162	13.960 ± 0.885	3.180 ± 0.495
p-value	*0.0164*		*0.0002*		*0.1058*	*0.3701*	*0.3755*
Season 2							
Control	13.997 ± 1.813		7.227 ± 0.645	bc	2.913 ± 1.275	36.880 ± 0.982	27.233 ± 1.801
ABA	16.910 ± 4.273		5.547 ± 0.302	c	2.610 ± 0.379	35.740 ± 2.934	26.967 ± 0.899
D	20.313 ± 1.679		9.393 ± 1.864	ab	2.067 ± 0.348	38.247 ± 6.276	24.737 ± 6.192
UV-B	24.013 ± 4.911		11.770 ± 0.748	a	1.350 ± 0.839	29.627 ± 6.301	18.630 ± 2.404
p-value	*0.2768*		*0.0160*		*0.5645*	*0.6033*	*0.3313*

Table 2
Methylation changes. Treatment effects on the probability that an epiallele deviates from the consensus epigenotype based on presence/absence scores of 413 epialleles in four (Control, UV-B and ABA) and three (D) replicates.

Treatments	Total cases[1]	Observed changes (%)	Adjusted changes (%)		Contrast 'Control *vs* treatment'
Control	1652	13	13 ± 1	c	
ABA	1652	19	18 ± 1	b	*0.0001*
D	1239	21	21 ± 1	a	*0.0001*
UV-B	1652	24	23 ± 1	a	*0.0007*

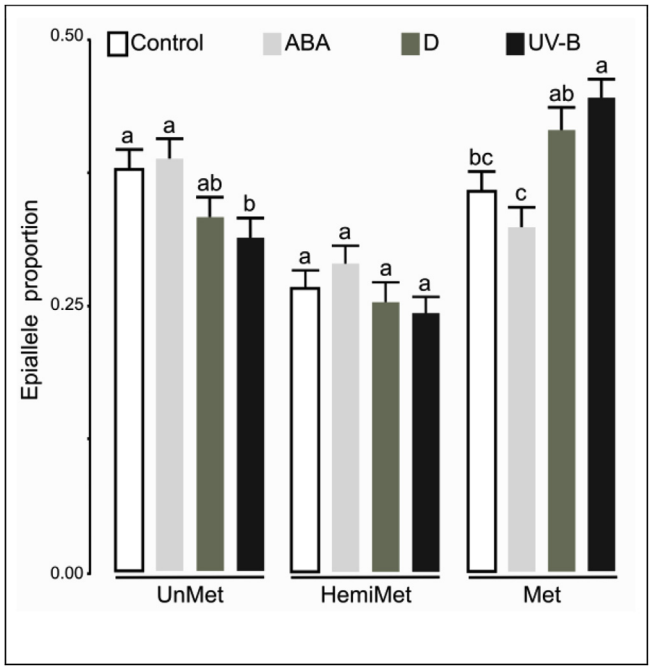

Fig. 5. Global methylation levels. Proportion of unmethylated (UnMet), hemimethylated (HemiMet) and methylated (Met) epialleles based on MSAP data for vines cv. Malbec exposed to Control, ABA, D and UV-B. Values are means ± SEM and different letters within each methylation pattern indicate statistically significant differences (Fisher's LSD, p-value ≤ 0.05).

mainly by comparing the berry transcriptome (Dal Santo et al., 2013, 2016) and by evaluation of sRNAs as epigenetic mechanisms (Fabres et al., 2017; Paim Pinto et al., 2016). In addition, the role of DNA methylation in grapevine, but has been explored associated to tissue culture-induced epigenetic variation (Schellenbaum et al., 2008; Baránek et al., 2015) and to differentiation of commercial clones (Imazio et al., 2002; Ocaña et al., 2013). However, there is a lack of information about how specific environment factors/signals induce changes in grapevine's epigenome and to what extent this generated epigenetic variability affects fruit composition. In the present study we explored the effects on phenotype and epigenome of two particular components considered within the terroir concept, i.e. UV-B, as an abiotic factor, and water restriction, as both an abiotic factor and a management practice. In addition, the variability induced by the stress-related hormone ABA was assayed.

The DNA methylation changes induced by three treatments were evaluated via three approximations: i) by comparing epigenetic distances within and among treatments, ii) by comparing the number of methylation changes per treatments respect to the control (were the single factors are turned off; *i.e.* minus UV-B, well-watered and not sprayed with ABA) and iii) by comparing the global methylation levels among treated and control plants. When the epigenetic distances were compared, dispersion between replicates was observed. These epiloci

Table 3
BLAST DNA similarity of isolated and sequenced MSAP fragments.

MSAP Fragment	Treatment/Methylation pattern		Similarity product, nucleotide accession	Similarity (%)	BLAST E score
UVB-1[a]	UV-B/3[b]	Ubiquitin ligase	*Vitis vinifera* RING-H2 finger protein ATL56-like, XM_010658428.2	96	5.00E-71
UVB-2	UV-B/1	Protein activated with light	*Pyrus communis* putative ATP synthase gamma chain mRNA, AY435422.1	95	3.00E-08
UVB-3	UV-B/1	Ubiquitin ligase complex	Predicted *Vitis vinifera* F-box/LRR-repeat MAX2 homolog A mRNA, XM_010658740.1	100	4.00E-46
ABA-1	ABA/2	Transcription factor	Predicted *Vitis vinifera* transcription factor bHLH63 mRNA, XM_002284077.2	100	5.00E-14

[a] Working designation.
[b] Methylation pattern according to Fig. S1.

with polymorphic methylation patterns, even within the control group, are possibly due to that in the Malbec genome certain genomic regions present unstable epigenetic patterns. This dispersion among replicates was more marked in treated plants, result that could be interpreted as stochastic stress-induced methylation changes. Similar results were obtained in asexual dandelions (*Taraxacum officinale*), where various stresses, most notably chemical induction of herbivore and pathogen defenses, induced random methylation changes (Verhoeven et al., 2010).

When the number of methylation changes was compared, the three treatments induced epigenetic methylation changes compared to control plants, showing that the Malbec grapevines experienced a genome-wide epigenetic reprogramming in response to those treatments. Some specific methylation changes were associated with specific treatments that affected coding sequences and could represent a programed response to a specific stress. The characterization of these Malbec genomic sequences in which treatments induced specific methylation changes showed promissory results. In arabidopsis, functional ubiquitin ligases proteins ORTH/VIM regulate DNA methylation (Kraft et al., 2008) and VIM deficiency resulted in strong DNA hypomethylation (Kim et al., 2014). Likewise, the ubiquitin ligase activity of ORTH/VIM animal homolog UHRF1 is required for maintenance of DNA methylation in mammals (Qin et al., 2015). The sequence associated with ABA treatment showed similarity with one of the largest families of transcription factors controlling a number of different biological processes (Carretero-Paulet et al., 2010). Further studies are required to demonstrate if the observed epigenetic changes altered gene expression of identified target genes.

The global methylation comparison showed that the UV-B induced hypermethylation in the Malbec genome; this result is consistent with those obtained for the cv. Shiraz that compared vineyards from three Australian regions located at different altitude (Xie et al., 2017). In arabidopsis, an interplay between DNA methylation and UV-B induced DNA damage repair have been characterized: ddm1 mutants, which lack the protein DECREASE IN DNA METHYLATION1 (DDM1) required for normal patterns of genomic DNA, accumulated more DNA damage after UV-B exposure than WT plants (Qüesta et al., 2013). Thus, the DNA hypermethylation observed could be the molecular mechanisms that underlay acclimation processes. However, other authors found that UV-B induce DNA hypomethylation in *Artemisia annua* (Pandey and Pandey-Rai, 2015), suggesting that hypermethylation by UV-B could be species dependent. The plants treated with moderate water restriction also showed a trend, although no statistically significant, to the hypermethylation respect to the control plants. Several abiotic stresses have demonstrated to induce higher methylation levels in different species (Kovar et al., 1997; Labra et al., 2002; Dyachenko et al., 2006; Zhong and Wang, 2007), which could indicate that the hypermethylation is a common genome response to abiotic stresses. One of the possible pathways conducting to the observed hypermethylation could be the RNA-directed DNA methylation controlled by sRNA (Tamiru et al., 2017), molecules that are considered within the epigenetic mechanisms and exert key biological roles in plants environmental plasticity (Borges and Martienssen, 2015). It has been suggested that epigenetic processes such as DNA methylation are an integral part of ABA-regulated processes (Chinnusamy et al., 2008). In the present work, the ABA doses and times assayed did not induced differences in the percentage of unmethylated, hemimethylated and methylated epialleles respect to Control, so the evidence available is still unclear.

A positive correlation between the epigenetic variability induced by treatments and the biochemical differences assessed in early fruit shoots was observed. This result suggests that both kinds of treatments, UV-B and D applied in one season could induce epigenetic variability that contribute to the biochemical composition in the next season. We previously found that ABA increased the levels of hydroxycinnamic acids (ferulic and caffeic acids) in leaves and berries of Malbec grapevines (Berli et al., 2010, 2011, 2015; Alonso et al., 2016). In the present study, a significant increase in hydroxycinnamic acid in ABA treatment respect to Control was observed, which is in concordance with previous experiment that applied ABA (Yamamoto et al., 2015). Hydroxycinnamic acids increases in light exposed berries compared with shaded ones (Sun et al., 2017), and the increments observed in early fruit shoots after UV-B treatment could be part of an acclimation mechanism that may operate as an epidermal UV-absorbing compound and/or as an antioxidant (Berli et al., 2010).

In summary, our results suggest that DNA methylation could regulate LMWP accumulations during and after UV-B treatment throughout the mitotic inheritance of this epigenetic mechanism and participate in acclimation grapevine processes. Considering that grapevines are exposed to multiple and simultaneous environmental signals in the vineyards, future studies should be focused on the analysis of different Malbec clones grown in different environments for various seasons. By assessing if these induced changes have a memory-effect will contribute to better understand the terroir-dependent quality traits in wines.

Conflicts of interest

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Carlos Marfil: Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Formal analysis, Writing – review & editing. **Verónica Ibañez:** Data curation, Methodology, Formal analysis, Writing – review & editing. **Rodrigo Alonso:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Anabella Varela:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Rubén Bottini:** Conceptualization, Funding acquisition, Writing – review & editing. **Ricardo Masuelli:** Conceptualization, Funding acquisition, Writing – review & editing. **Ariel Fontana:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Federico Berli:** Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Formal analysis, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.12.021.

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