

## Abscisic acid and methyl jasmonic acid module anthocyanins and *trans*-resveratrol accumulation in berry skin of five red *Vitis vinifera* cvs. in two contrasting viticultural regions of Mendoza-Argentina

### El ácido abscísico y metil jasmonato modulan la acumulación de antocianinas y *trans*-resveratrol en hollejos de bayas de cinco cultivares tintos de *Vitis vinifera* en dos regiones vitícolas contrastantes de Mendoza, Argentina

Emiliano Malovini <sup>1</sup>, Celeste Arancibia <sup>2</sup>, Martín Durán <sup>3</sup>, Ariel Fontana <sup>4</sup>,  
María Inés de Rosas <sup>5</sup>, Leonor Deis <sup>5</sup>, Raquel Gargantini <sup>6</sup>, Rubén Bottini <sup>7</sup>,  
Bruno Cavagnaro <sup>5</sup>, Liliana Martínez <sup>5\*</sup>

Originales: *Recepción:* 05/07/2019 - *Aceptación:* 06/11/2019

#### ABSTRACT

Berry skins from red grape cultivars contain significant amounts of polyphenols that contribute to wine quality and provide health benefits. These compounds can be elicited by plant hormones. The aim of this work was to increase the content of anthocyanins (ANT) and *trans*-resveratrol (*T*-RES) by application of abscisic acid (ABA) and methyl jasmonate (MeJA) in five red *V. vinifera* cvs. (Bonarda, Malbec, Syrah, Cabernet Sauvignon, and Pinot Noir), in two Argentinean contrasting growing regions (Santa Rosa and Valle de Uco). Results showed positive and differential effects of ABA and MeJA on the total ANT content for the diverse cultivars with changes in the proportions of blue and red ANT. ABA increased total ANT in both viticultural region, while MeJA had a positive effect only in Santa Rosa. Also, ABA and MeJA induced an accumulation of *T*-RES in different cultivars, regardless of the region; *T*-RES accumulation elicited by ABA was not previously described. This work brings out the possibility to use these hormones as practical tools to produce high-quality red wines in two contrasting viticultural regions.

#### Keywords

elicitors • phenolic compounds • grapevine • plant hormones

- 1 Becario Posdoctoral. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Almirante Brown 500. Chacras de Coria. Mendoza. M5528AHB. Argentina.
- 2 Becario Doctoral. Universidad Nacional de Cuyo-Corporación Vitivinícola Argentina (COVIAR).
- 3 Becario Doctoral. Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias.
- 4 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Instituto de Biología Agrícola de Mendoza (IBAM). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Grupo de Bioquímica Vegetal.
- 5 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Laboratorio de Fisiología Vegetal. Instituto de Biología Agrícola (IBAM) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). \*lmartinez@fca.uncu.edu.ar
- 6 Instituto Nacional de Vitivinicultura (INV). Mendoza. Argentina. Av. San Martín 430. Mendoza. Argentina.
- 7 Universidad Juan A. Maza. Área de Ciencia y Técnica. Lateral Sur del Acceso Este 2245. Guaymallén. Mendoza. 5519. Argentina.



## RESUMEN

Los hollejos de las uvas tintas contienen cantidades significativas de polifenoles que contribuyen a la calidad del vino y proporcionan beneficios para la salud. Estos compuestos pueden ser elicitados por hormonas vegetales. El objetivo de este trabajo fue aumentar el contenido de antocianinos (ANT) y *trans*-resveratrol (*T*-RES) mediante la aplicación de ácido abscísico (ABA) y jasmonato de metilo (MeJA) en 5 *V. vinifera* cvs. (Bonarda, Malbec, Syrah, Cabernet Sauvignon y Pinot Noir), en dos contrastantes regiones vitícolas argentinas (Santa Rosa y Valle de Uco). Los resultados mostraron un efecto positivo y diferencial de ABA y MeJA en el contenido total de ANT para los diversos cultivares, con cambios en las proporciones de ANT azul y rojo. ABA aumentó los ANT totales en ambas regiones vitícolas, mientras que MeJA tuvo un efecto positivo solo en Santa Rosa. Además, ABA y MeJA indujeron una acumulación de *T*-RES en diferentes cultivares, independientemente de la región; la acumulación de *T*-RES provocada por ABA ha sido previamente reportada. Este trabajo pone de manifiesto la posibilidad de utilizar estas hormonas como herramientas prácticas para producir vinos tintos de alta calidad en dos regiones vitícolas contrastantes.

**Palabras clave**

elicitores • compuestos fenólicos • vid • hormonas vegetales

## INTRODUCTION

Wine is a traditional beverage that has been associated with both healthy and harmful effects. Scientific evidence has demonstrated a tight correlation between a mild but regular red wine consumption and a healthy cardiovascular system in populations where wine accompanies everyday meals as a habit (28); benefits are mostly conferred by the presence of certain phenolic compounds that possess antioxidant activity (14). Phenolic compounds (mainly located in berry skin and seeds) have also an important role in determining the final oenological quality of red wines (29), being red wine known to contain 10-fold more phenolic compounds than white wine.

The biological potential of the wide range of chemical of polyphenols compounds in wine has been examined in extensive reviews (6, 24). In general, anthocyanins (ANT) are excellent antioxidants since they are easily oxidized under stress circumstances, having a protective effect on human health, regarding degenerative and chronic diseases (6). On the other hand, *trans*-resveratrol (*T*-RES) (a stilbene), is the most examined phenolic compound over the past decade due to its nutritional and medicinal value. *T*-RES exerts a plethora of biological functions, especially as a cardiovascular protective, antiplatelet, antioxidant, anti-inflammatory, blood glucose-lowering, anticancer, antiaging, neuroprotective, and anti-obesity compound (24).

Polyphenol content in grapes is very variable and it depends on several genetic, environmental and management factors (8). Among the environmental factor, the vineyard location has a very important role (3). For example, high altitude vineyards have a wider temperature range and grapes are richer in polyphenolic compounds than grapes from vineyards located in lower altitude and warmer regions (19). Due to the important properties of these compounds, there is an increasing interest in producing grapes or wines with higher contents, which have more nutraceutical value. Plant hormones, such as abscisic acid (ABA), play an important role in plant physiological and biochemical processes. Exogenous applications of ABA induce the accumulation of ANT in grape berries by enhancing the transcription of anthocyanins synthesis related genes, thus improving the color of the fruit (17). On the other hand, jasmonates like methyl jasmonic acid (MeJA) stimulate *T*-RES biosynthesis (3, 18). Several local studies regarding content and biosynthesis stimulation of certain phenolic compounds have been conducted in red grapevine cultivars grown in Mendoza (1, 3, 5) and, in this context, ABA has shown positive effects on grapes growing under high temperature conditions, countering the decrease of ANT caused by this environmental factor (22, 23). In addition, MeJA has significantly increased *T*-RES accumulation, by exacerbating the expression of genes whose products are involved in its synthesis, such as

several members of the Stilbene Synthase (*VvSTS*) multigenic family, transcription factors *VvMYB14* and *VvMYB15.2*, and Phenylalanine ammonia-lyase (*VvPAL*) gene in Malbec (10). Currently, there are no previous works assessing the different phenolic compounds induced by hormones such as ABA and MeJA simultaneously in more than one variety and located in contrasting viticultural regions with dissimilar climate conditions, trellis systems, irrigation methods and soil depth, among other factors, and although the impact of plant hormones and high temperatures alone has been widely studied, their combined effect with other vineyard factors on plants remains poorly understood.

In Argentina, the vineyards involved in the production of red wine, account for almost 50% of the total country vineyards, distributed across irrigated arid areas. Mendoza, is one of the most favorable viticultural locations in Argentina that produces premium red wines and its vast viticultural area comprises great differences that could play a role in the modulation of ABA and MeJA exogenous application on berries. The aim of this work was to evaluate the accumulation of ANT and *T-RES* in the berry skin of five red *V. vinifera* cvs., after direct spraying with ABA and MeJA, in two different Argentinean viticultural regions.

## MATERIALS AND METHODS

The study was conducted during 2016 season, in commercial vineyards of two contrasting viticultural regions of Mendoza-Argentina. One of the vineyards was located in Santa Rosa (68°03'28" W and 33°15'56" S; 590 m a. s. l.), in East Mendoza. These vineyards consisted in a spur-pruned overhead trellis system (vine spacing: 4 m x 4.5 m), flood irrigated. All East Mendoza viticultural region show high average daily temperatures and warm nights (4), deep soils, and overhead is the predominant trellis system. The other vineyard was located in Gualtallary (69°15'37" W and 33°23'51" S; 1450 m a. s. l.), Valle de Uco, a region located in South West Mendoza. These vineyards were trained in a spur-pruned vertical shoot position system (vine spacing: 2.2 m x 1.4 m), drip irrigated. In comparison to Santa Rosa, this region shows lower average temperatures and very cold nights (4), shallow soils, and vertical shoot position system predominates.

Climatic differences were detected between the two growing regions by the analysis of daily data from nearby meteorological stations provided by Dirección de Contingencias Climáticas de Mendoza. Temperatures from January to March (2016) were significantly higher in Santa Rosa than in Valle de Uco region while relative humidity was lower in Santa Rosa than in Valle de Uco (table 1, page 454). According to these data, Santa Rosa was considered to be warmer and drier than Valle de Uco during the period when the experiment was conducted.

Vineyards from both locations were 10-12 years old, not covered by anti-hail net and managed according to the standard viticultural practices for each region and cultivar. In both locations Bonarda, Cabernet Sauvignon, Malbec, Pinot Noir and Syrah own-rooted *Vitis vinifera* cvs. were selected. Within a plot, 20 plants of each variety, were chosen randomly from a set of plants comprising only those with trunk perimeter of the media of the block  $\pm 1$  standard deviation. A randomized complete block experimental design with five replicates was used, where the experimental unit was a plant and each block was composed of a row. Two independent hormone treatments were performed. An aqueous solution (distilled water) of 1 mM ABA plus Tween 20 0.1% and was applied directly on the berries with a handheld sprayer until runoff, at three opportunities (10 days apart), starting at veraison, according to Malovini (2017). According to Durán (2016), MeJA treatment consisted of 10 mM MeJA in 40% ethanol-water solution with Tween 20 0.1%, and was sprayed on the berries together with the last ABA application. ABA control solution consisted of distilled water and Tween 20 0.1%, while MeJA control solution consisted of 40% ethanol-water solution with Tween 20 0.1%. Bunches from all cultivars were harvested 4 days after the last ABA and the MeJA application. In the laboratory, grapes from the modal size of each variety were randomly chosen and kept at - 80°C. For phenolics extraction, samples consisted of 1 g of fresh berry skins (after being manually separated from the pulp), macerated in methanol-HCl 0.1% mixture at 4°C for 48 h in the dark and then filtered through a 0.45  $\mu$ m cellulose acetate membrane.



**Table 1.** Temperature and relative humidity differences between the two studied regions. Values are means of growing season (January-March). Meteorological stations were located in Las Catitas (33°15'56" S; 68°03'28" W, Santa Rosa) and El Peral (33°20'48.2" S; 69°9'27.7" W, Valle de Uco).

**Tabla 1.** Diferencias de temperatura y humedad relativa entre las dos regiones estudiadas. Los valores son los medios para la temporada de cultivo (enero-marzo). Las estaciones meteorológicas se ubicaron en Las Catitas (33°15'56" S; 68°03'28" O, Santa Rosa) y El Peral (33°20'48,2" S; 69°9'27,7" O, Valle de Uco).

Different letters indicate significant differences between regions by DGC test ( $p < 0.05$ ).

Letras diferentes indican diferencias significativas entre regiones determinadas por la prueba DGC ( $p < 0,05$ ).

Season	Meteorological Station	Temperature			Relative humidity	
		n	Maximum (°C)	Mean (°C)	Mean (%)	
2016	Santa Rosa	111	31.23 ± 0,45 a	22.08 ± 0.41 a	15.63 ± 0.42 a	66.78 ± 1.16 b
	Gualtallary	105	29.22 ± 0,45 b	20.96 ± 0.41 b	14.03 ± 0.40 a	72.41 ± 1.16 a

ANT and *T-RES* contents were assessed by HPLC-DAD (SPD-M10AVP, Shimadzu, and Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany) according to the official OIV methodology proposed by Otteneder (2004). Total ANT content was computed as the sum of individual ANT (delphinidin-3-glucoside, malvidin-3-glucoside, petunidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, malvidin-3-O-(6"-acetyl)glucoside, peonidin-3-O-(6"-acetyl)glucoside, malvidin-3-O-(6"-*p*-coumaroyl)glucoside and peonidin-3-O-(6"-*p*-coumaroyl)glucoside) and it was expressed as a mg of malvidin-3-O-glucoside equivalent per gram of fresh berry skin weight. Also, ANT chemical profile as red (cyanidin-3-glucoside and peonidin-3-glucoside) and blue (delphinidin-3-glucoside, malvidin-3-glucoside, and petunidin-3-glucoside) content was analyzed. *T-RES* content was expressed as  $\mu\text{g g}^{-1}$  of fresh berry skin weight. At harvest, soluble solids ( $^{\circ}$  Brix) were measured with a portable automatic refractometer (Atago®), and also pH and berry size were assessed. Data were analyzed using mixed linear models (MLM) with factorial structure, several alternative correlation structures were evaluated, as well as different structures of residual variance. Since natural pH and  $^{\circ}$  Brix variations between the different cultivars and regions, at harvest, could influence ANT and *T-RES* content, these variables were considered as co-variables in the MLM. The best models were selected using the Akaike (AIC) and Schwarz (BIC) information criteria (InfoStat v.2016 software, Grupo Infostat, FCA-UNC, Argentina). When significant differences were found between treatments, means were compared using DGC method involving a comparison based on multiple hierarchical conglomerates, ( $p < 0.05$ ) (7).

## RESULTS AND DISCUSSION

Anthocyanins and resveratrol are important factors that determine berry color and nutraceutical properties, relevant aspects for producing good quality red wines. Numerous reports have shown that exogenous applications of ABA and MeJA can improve these parameters in grape berry skin (9, 12, 26, 30, 31). In this trial, we examined the effect of these plant hormones in five different *V. vinifera* red cultivars in two contrasting viticultural regions.

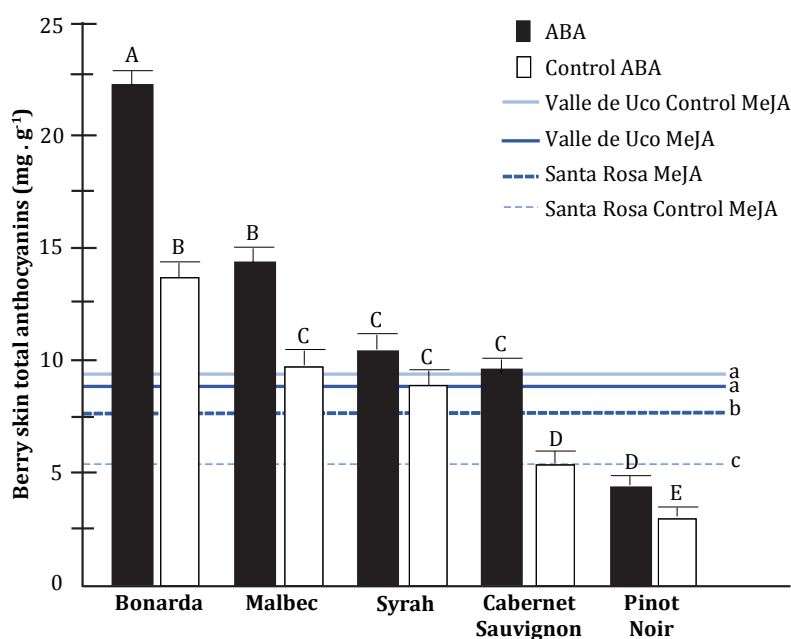
ABA treatment increased total ANT concentration in all cultivars, except in Syrah (ABA treatment x Cultivar  $p < 0.0001$ ) (figure 1, page 455). Such increments ranged from 35% in Malbec and 46% in Pinot Noir, to 73 and 70% in Bonarda and Cabernet Sauvignon. Since Syrah is known to have an anisohydric behavior (16), this could be due to deficient ABA receptors, and it could also explain the lack of response to ANT accumulation after ABA sprayed. On the other hand, the analysis revealed an interaction between cultivars and the viticultural region ( $p < 0.0001$ ). Regardless of the many differences that characterize both regions, it could be expected that, given the lower temperatures in Valle de Uco, all cultivars would accumulate more total ANT, when compared to Santa Rosa. However, not all cultivars behaved in such a way. In Valle de Uco, Malbec showed a two-fold accumulation, Pinot Noir 53% and Syrah 40%, compared to Santa Rosa. Bonarda and Cabernet Sauvignon seemed to be more plastic cultivars; there were no differences in this variable between the two regions.

It seems most likely that the ANT accumulation is due to an upregulation of CHI, F3'H, DFR, and UFGT genes and the VvMYBA1 and VvMYBA2 transcription factors, as Koyama *et al.* (2018) demonstrated in *Vitis vinifera* x *Vitis labrusca* hybrid after elicitation with ABA. In this experiment, since no triple interaction between hormonal treatment, cultivar and regions was found, the two significant interactions mentioned above have additive effects. Overall, ABA effect on total ANT was not affected by the viticultural region ( $p > 0.05$ ). This seems to indicate that ABA could turn into a good agronomic tool to be used in both regions. On the other hand, the application of MeJA was effective to induce the accumulation of total ANT only in Santa Rosa ( $p$  MeJA treatment x Region = 0.0289) (figure 1). One explanation for this phenomenon could be an effect of environmental factors, as previous studies have shown that the season had a significant effect on ANT accumulation in response to exogenous application of MeJA (10, 26). In Santa Rosa, when the hormone was applied, the difference with total ANT content observed in the control plants of Valle de Uco, was notoriously smaller (figure 1). This could be helpful to achieve wines of higher ANT content in a region where it is normally lower, like Santa Rosa.

Regarding ANT chemical profile, ABA increased red ANT in all cultivars, while blue ANT were increased in all, except on Syrah (ABA treatment x Cultivar for blue ANT  $p < 0.0001$  and for red ANT  $p = 0.0001$ ) (figure 2, page 456). This data suggested that the observed increment in total ANT with ABA application did not occur under an equal distribution of the two types of ANT. Bonarda, Malbec and Cabernet Sauvignon experienced a higher accumulation of the red than the blue type. The proportional increment of red over blue in Bonarda was 45%, 38% in Malbec and 19% in Cabernet Sauvignon. Interestingly, in Pinot Noir, the increment on total ANT responded to a higher relative increment of blue over red (43%), which has not been previously described and could be a desirable enological trait for blending. Finally, Syrah only had a significant increment of 44% in red ANT after ABA application.

Different capital letters indicate significant differences between ABA and Control ABA treatments by DGC test ( $p < 0.05$ ). Dashed lines represent means of the interaction of MeJA treatments and the viticultural regions; different lowercase letters next to the lines indicate significant differences by DGC test ( $p < 0.05$ ).

Letras mayúsculas diferentes indican diferencias significativas entre el tratamiento ABA y el Control ABA, determinadas por la prueba DGC ( $p < 0,05$ ). Las líneas discontinuas representan las medias de las interacciones de los tratamientos MeJA y las zonas vitícolas; letras minúsculas diferentes junto a las líneas indican diferencias significativas, determinadas por la prueba DGC ( $p < 0,05$ ).

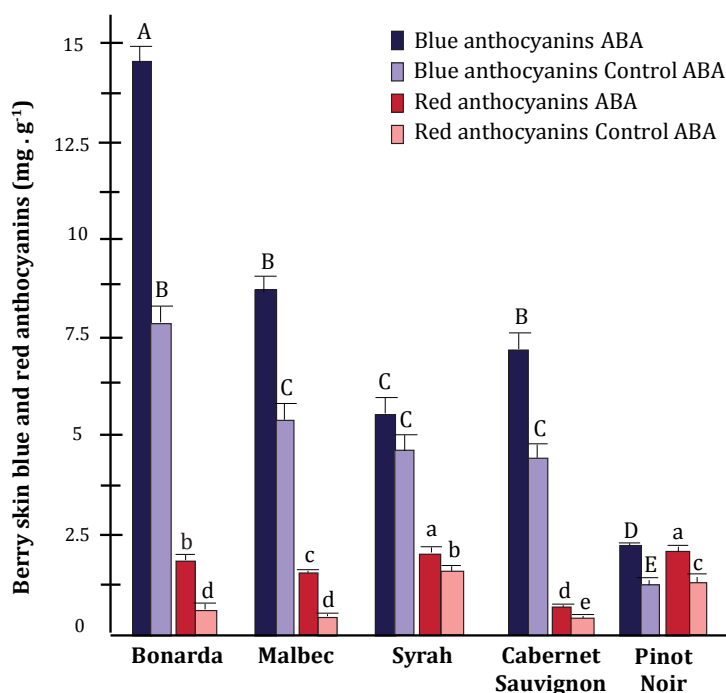


**Figure 1.** Bar graph for the interaction of hormonal treatments (ABA) and cultivars, and the interaction of hormonal treatments (MeJA) and the viticultural region, on total anthocyanins content in berry skin.

**Figura 1.** Gráfico de barras para la interacción entre el tratamiento hormonal (ABA) y los cultivares de vid, y la interacción del tratamiento hormonal (MeJA) con la región vitícola, sobre el contenido del total de antocianos en hollejo.

Different capital letters indicate significant differences between treatments, for blue anthocyanin type determined by DGC test ( $p < 0.05$ ) and different lowercase letters indicate significant differences between treatments, for red anthocyanin type determined by DGC test ( $p < 0.05$ ).

Letras mayúsculas diferentes indican diferencias significativas entre los tratamientos para las antocianos azules, determinadas por la prueba DGC ( $p < 0,05$ ) y las letras minúsculas diferentes indican diferencias significativas entre los tratamientos para las antocianos rojos, determinadas por la prueba DGC ( $p < 0,05$ ).



**Figure 2.** Bar graph for the interaction of ABA treatments and cultivars on blue and red anthocyanin contents in berry skins.

**Figura 2.** Gráfico de barras para la interacción de los tratamientos de ABA con los cultivares sobre el contenido en hollejo de antocianos azules y rojos.

On the other hand, MeJA did not show any influence on the blue ANT accumulation in berry skin, neither as a simple effect, nor in interaction with the other tested factors ( $p > 0.05$ ). Regarding red ANT in grape skin, the accumulation of these compounds was affected by the triple interaction of the assessed factors (MeJA treatment x Region x Cultivar  $p = 0.009$ ) (table 2, page 457). In Syrah and Pinot Noir, MeJA did not influence red ANT levels in plants of Valle de Uco, and when it was applied in Santa Rosa, these polyphenols reached comparable levels to those found in Valle de Uco. Cabernet Sauvignon showed no differences in red ANT levels between treatments, nor between regions. Finally, these compounds were not affected by MeJA applications on Bonarda and Malbec. They only showed a difference attributable to the region where they grew, an effect previously seen by other researchers (32).

Regarding *T-RES* content in berry skin, the effectiveness of both hormonal treatments was not influenced by the viticultural region ( $p > 0.05$ ). ABA and MeJA showed an interaction with cultivars (MeJA treatment x Cultivar  $p = 0.0043$  and ABA treatment x Cultivar  $p = 0.0007$ ) (figure 3, page 457). The effect on *T-RES* accumulation triggered by the exogenous application of ABA, contradicts Wang *et al.* (2016) study. In both control treatments, Malbec, Syrah and Pinot Noir had the highest values, while Cabernet Sauvignon showed medium values, and Bonarda the lowest. This observation in Bonarda is coincident with the low *T-RES* observed in Bonarda wines from Mendoza-Argentina (11). ABA only increased the accumulation of this polyphenol in Malbec by 93% and in Syrah by 48%. Methyl jasmonate, on the other hand, only induced a significantly higher accumulation in Bonarda (150%) and Cabernet Sauvignon (53%).

On the other hand, Pinot Noir did not respond to hormonal applications. In this work, induction values described are comparable to those previously found in similar studies on Malbec (9), Syrah (12), Tempranillo, Graciano and Monastrel (13, 26). Finally, on the whole, grapes accumulated more *T-RES* in Valle de Uco than in Santa Rosa ( $p$  ABA treatment x Cultivar  $< 0.0001$  and  $p$  MeJA treatment x Cultivar  $< 0.0001$ ) (data not shown). This has an additive effect to the one on *T-RES* accumulation given by the interactions detected between ABA and MeJA treatments x Cultivar.

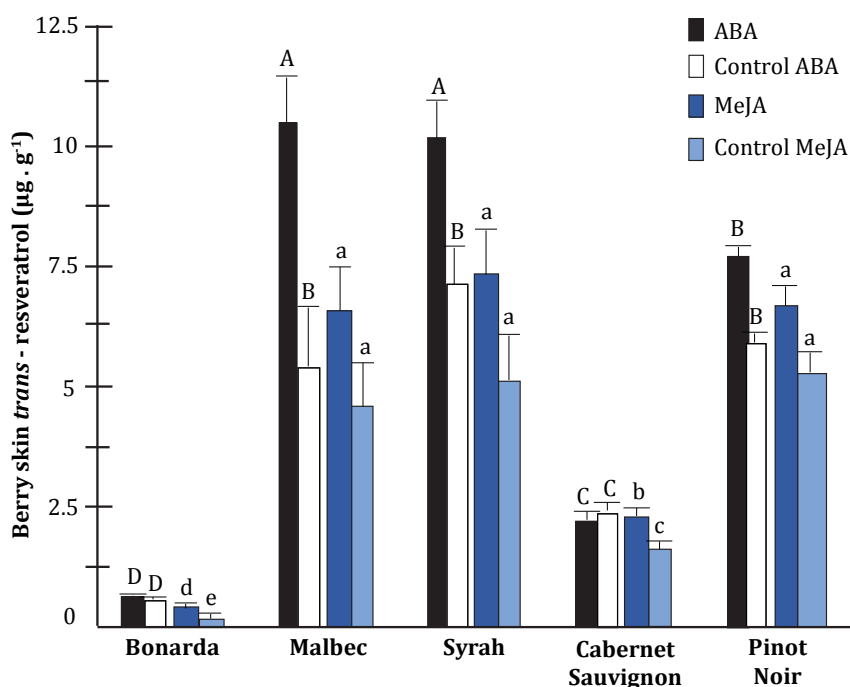
**Table 2.** Table of the interaction among cultivars, viticultural region and MeJA treatments on red anthocyanins (mg. g<sup>-1</sup>) in berry skin.

**Tabla 2.** Tabla de la interacción entre cultivares, zona vitícola y tratamiento de MeJA sobre los antocianos rojizos (mg. g<sup>-1</sup>) en hollejo.

Grape cultivar	Viticultural region	Treatment	Means ± E.E.
Bonarda	Valle de Uco	MeJA	1.25 ± 0.29 B
Bonarda	Valle de Uco	Control Me JA	1.25 ± 0.29 B
Bonarda	Santa Rosa	MeJA	0.33 ± 0.07 C
Bonarda	Santa Rosa	Control MeJA	0.4 ± 0.07 C
Malbec	Valle de Uco	Control MeJA	1.06 ± 0.16 B
Malbec	Valle de Uco	MeJA	0.96 ± 0.16 B
Malbec	Santa Rosa	Control MeJA	0.5 ± 0.05 C
Malbec	Santa Rosa	MeJA	0.48 ± 0.05 C
Syrah	Valle de Uco	MeJA	1.27 ± 0.19 B
Syrah	Valle de Uco	Control MeJA	1.46 ± 0.19 B
Syrah	Santa Rosa	MeJA	2.29 ± 0.26 A
Syrah	Santa Rosa	Control MeJA	1.26 ± 0.26 B
Cabernet Sauvignon	Valle de Uco	MeJA	0.36 ± 0.06 C
Cabernet Sauvignon	Valle de Uco	Control MeJA	0.49 ± 0.06 C
Cabernet Sauvignon	Santa Rosa	MeJA	0.39 ± 0.04 C
Cabernet Sauvignon	Santa Rosa	Control MeJA	0.31 ± 0.04 C
Pinot Noir	Valle de Uco	MeJA	1.99 ± 0.29 A
Pinot Noir	Valle de Uco	Control MeJA	1.84 ± 0.29 A
Pinot Noir	Santa Rosa	MeJA	2.81 ± 0.32 A
Pinot Noir	Santa Rosa	Control MeJA	0.88 ± 0.32 B

Different letters represent significant differences by DGC test ( $p < 0.05$ ).  
Letras diferentes indican diferencias significativas entre regiones determinadas por la prueba DGC ( $p < 0,05$ ).

Different capital letters indicate significant differences between ABA and Control ABA treatments and cultivars by DGC test ( $p < 0.05$ ). Different lowercase letters indicate significant differences between MeJA and Control MeJA treatments and grape cultivars by DGC test ( $p < 0.05$ ).  
Letras mayúsculas diferentes indican diferencias significativas entre los tratamientos ABA y Control ABA y los cultivares de vid, determinadas por la prueba DGC ( $p < 0,05$ ).  
Letras minúsculas diferentes indican diferencias significativas entre los tratamientos MeJA y Control MeJA y los cultivares de vid, determinadas por la prueba DGC ( $p < 0,05$ ).



**Figure 3.** Bar graph for the interaction of hormonal treatments and cultivars on *T*-RES content in berry skin.

**Figura 3.** Gráfico de barras para la interacción de los tratamientos hormonales y los cultivares sobre el contenido de *T*-RES.



The higher levels of erythematous weighted UV-B irradiance received in Valle de Uco, due to its altitude, could also be the cause of a higher *T-RES* content as it has been found to enhance PAL activity (1, 2). However, differences in trellis or irrigation systems that could potentially play a role in the ANT accumulation cannot be discarded. For example, it is possible that the higher vigor of vines in Santa Rosa conferred by their architecture, the irrigation method and the climate of the region lead vines to a different utilization of photosynthates compared to vines of Valle de Uco; this intricate interaction of factors is hard to tell apart. Even so, under the given experimental vineyards' conditions, the influence of the different thermal parameters between Santa Rosa and Valle de Uco are conspicuous and they might be playing the major role in the accumulation of *T-RES* and polyphenols in general.

In this study, pH and °Brix were evaluated to determine its influence on the analyzed polyphenols. They were used as co-variables in the GLMs and the analyses showed no correlation with total ANT, ANT chemical profile or *T-RES*. Regarding these parameters, Hiratsuka *et al.* (2001) suggested that the reason for ABA promoting the coloration of grapes might be due to the increase of soluble sugar content caused by this hormone. This would provide more substrate for the final production of ANT and promote the activation of ANT synthase or the expression of related genes. In this experiment, none of the hormonal treatments modified this parameter ( $p > 0.05$ ). Similar results were previously described in Syrah berries (27). Regarding pH, berries sprayed with MeJA had a higher pH ( $3.92 \pm 0.03$ ) than those sprayed with control solution ( $3.87 \pm 0.02$ ) ( $p = 0.0001$ ). Portu *et al.* (2018) found that MeJA applications on Graciano cultivar resulted in more acidic berries, while Fernández-Marín (2014) found that this hormone did not affect the acidity of Syrah grapes. Apparently, MeJA has an interaction with *Vitis* ssp. genotypes and, possibly, with the season (26). ABA did not modify pH values ( $p > 0.05$ ), in accordance with the results showed by Sun *et al.* (2019) on *V. vinifera* cv. Merlot berries. Finally, none of the hormonal treatments influenced berry size ( $p > 0.05$ ).

The results of this experiment show that ABA is a valuable agronomic tool for increasing ANT in grapes, in two contrasting Argentinean viticultural regions, Valle de Uco and Santa Rosa. Meanwhile, the application of MeJA only increased these compounds in Santa Rosa. These increases responded, at least in part, to changes in the blue and red ANT ratios induced by the hormones. In addition, the use of MeJA and ABA constitutes a promising innovation for both regions. It could increase wine's nutraceutical value by augmenting the contents of *T-RES*. In addition, this study has led to another experiment, where red wines with enhanced ANT and *T-RES* contents obtained with these hormones, are being studied for their psychotropic effects on animal models. This contributes to understanding the influence of wine consumption on human health. Also, current studies in similar contrasting regions are being carried out, including transcriptomic analysis of ANT and *T-RES* regulation mediated by ABA and MeJA.

## CONCLUSION

The phenolic composition of the main red grape cultivars treated with ABA and MeJA in two contrasting viticultural regions (Santa Rosa and Valle de Uco, Mendoza-Argentina) was reported for the first time. A positive effect of ABA and MeJA on the total ANT content was observed, at different magnitudes, for the diverse cultivars. The increment of these compounds responded to changes in their chemical profile. ABA increased total ANT regardless of the viticultural region, while MeJA had a positive effect only in Santa Rosa. On the other hand, ABA and MeJA induced an accumulation of *T-RES* in different cultivars, while the region had no effect on these treatments. *T-RES* accumulation elicited by ABA was previously non-reported.

The use of these practical tools would allow the industry to produce high-quality red wines with enhanced organoleptic and nutraceutical value, in the mentioned contrasting areas.



## REFERENCES

1. Alonso, R.; Berli, F. J.; Fontana, A.; Piccoli, P.; Bottini, R. 2016. Malbec grape (*Vitis vinifera* L.) responses to the environment: Berry phenolics as influenced by solar UV-B, water deficit and sprayed abscisic acid. *Plant Physiology and Biochemistry*. 109: 84-90.
2. Berli, F. J.; Moreno, D.; Piccoli, P.; Hespagnol-Viana, L.; Bressan-Smith, R.; Silva, M. F.; 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 and Environment*. 33: 1-10.
3. 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. *Journal of Agricultural and Food Chemistry*. 59(9): 4874-4884.
4. Catania, C. D.; del Monte, S. A.; Uliarte, E. M.; del Monte, R. F.; Tonietto, J. 2007. Caracterización climática de regiones vitivinícolas Ibero-Americanas. *Embrapa Uva e Vinho*.
5. Deis, L.; Cavagnaro, B.; Bottini, R.; Wuilloud, R.; Silva, M. F. 2011. Water deficit and exogenous ABA significantly affect grape and wine phenolic composition under in field and *in-vitro* conditions. *Plant Growth Regulation*. 65(1): 11-21.
6. De Rosas, M. I.; Deis, L.; Martínez, L.; Durán, M.; Malovini, E.; Cavagnaro, J. B. 2018. Anthocyanins in nutrition biochemistry and health benefits. In: Gargiulo, P. A. and Mesones Arroyo, H. L. (Eds.) *Psychiatry and Neuroscience Update. From Translational Research to a Humanistic Approach. Volume III*.
7. Di Rienzo, J. A.; Guzman, A. W.; Casanoves, F. 2002. A multiple-comparisons method based on the distribution of the root node distance of a binary tree. *Journal of Agricultural, Biological, and Environmental Statistics*. 7(2): 129-142.
8. Du, B.; He, B.-J.; Shi, P.-B.; Li, F.-Y.; Li, J.; Zhu, F.-M. 2012. Phenolic content and antioxidant activity of wine grapes and table grapes. *Journal of Medicinal Plants Research*. 6(17): 3381-3387.
9. Durán, M. F.; Malovini, E.; Fontana, A.; Arancibia, C.; Bottini, R.; Martínez L. 2016. Analysis of ripening heterogeneity and anthocyanin accumulation changes in *Vitis vinifera* cv. Malbec berries in response to methyl jasmonate. X International Symposium on Grapevine Physiology and Biotechnology. Verona. Italia.
10. Durán, M. F. 2019. Análisis transcriptómico y metabólico de la biosíntesis de trans-resveratrol y antocianinas en bayas de vid de los cultivares Malbec y Pixie elicitados con ácido metil jasmónico y ácido salicílico. Tesis Doctoral. PROBIOL - Universidad Nacional de Cuyo. 215p.
11. Fanzone, M.; Zamora, F.; Jofré, V.; Assof, M.; Gómez-Cordovés, C.; Peña-Neira, A. 2012. Phenolic characterisation of red wines from different grape varieties cultivated in Mendoza province (Argentina). *Journal of the Science of Food and Agriculture*. 92(3): 704-718.
12. Fernández-Marín, M. I.; Puertas, B.; Guerrero, R. F.; García-Parrilla, M. C.; Cantos-Villar, E. 2014. Preharvest methyl jasmonate and postharvest UVC treatments: Increasing stilbenes in wine journal. *Food Science*. 79(3): C310-C317.
13. Gil-Muñoz, R.; Fernández-Fernández, J. I.; Crespo-Villegas, O.; Garde-Cerdán, T. 2017. Elicitors used as a tool to increase stilbenes in grapes and wines. *Food Research International*. 98: 34-39.
14. Guilford, J. M.; Pezzuto J. M. 2011. Wine and Health: A Review. *American Journal of Enology and Viticulture* 62(4): 471-486.
15. Hiratsuka, S.; Onodera, H.; Kawai, Y.; Kubo, T.; Itoh, H.; Wada, R. 2001. ABA and sugar effects on anthocyanin formation in grape berry cultured *in vitro*. *Science Horticulture*. 90: 121-130.
16. Hugalde, I.; Bonada, M.; Vila, H. 2018. The phenomenon of cavitation in grapevine... Unravelling implicated mechanisms. *Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina*. 50(2): 33-47.
17. Jeong, S. T.; Goto-Yamamoto, N.; Kobayashi, S.; Esaka, M. 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. *Plant Science*. 167(2): 247-252.
18. Jiang, Y.; Joyce, D. C. 2003. ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. *Plant Growth Regulation*. 39: 171-174.
19. Jones, G. V.; Davis, R. E. 2000. Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. *American Journal of Enology and Viticulture*. 51(3): 249-261.
20. Koyama, R.; Roberto, S. R.; 1,\* de Souza, R. T.; Wellington, F. S.; Borges, W. F. S.; Anderson, M.; Waterhouse, A. L.; Cantu, D.; Matthew, W.; Fidelibus, M. W.; Barbara Blanco-Ulate, B. 2018. Exogenous abscisic acid promotes anthocyanin biosynthesis and increased expression of flavonoid synthesis genes in *Vitis vinifera* × *Vitis labrusca* table grapes in a subtropical region. *Frontiers in Plant Science*. 9: 323.
21. Malovini, E. 2017. Empleo del estrés hídrico y hormonas vegetales como alternativas para mitigar el efecto del incremento de temperatura sobre el cv. Malbec (*Vitis vinifera* L.) en Mendoza, Argentina. Tesis doctoral PROBIOL-Universidad Nacional de Cuyo. Mendoza. Argentina. 168 p.



22. Malovini, E.; Sari, S.; De Rosas, M. I.; Durán, M.; Gómez, L.; Cobos, D. 2014. Aumento de temperatura, restricción hídrica y ácido abscísico sobre la composición enológica de vinos de *Vitis vinifera* cv. Malbec. 37th OIV Congress. Mendoza-San Juan, Argentina.
23. Malovini, E.; Deis, L.; Sari, S.; De Rosas, M. I.; Durán, M.; Arancibia, C.; Ponce, M. T.; Martínez, L.; Borgo, R.; Cavagnaro, J. B. 2016. Climate change on *Vitis vinifera* cv. Malbec, temperature increase and water restriction effect on the wines composition in a field study over three consecutive years. X International Symposium on Grapevine Physiology and Biotechnology. Verona-Italia.
24. Martínez, L.; Durán, M.; Malovini, E.; De Rosas, M. I.; Deis, L.; Cavagnaro, J. B. 2018. A very promising molecule resveratrol, induced synthesis and health benefits. In: Gargiulo, P. A. and Mesones Arroyo, H. L. (Eds.) Psychiatry and Neuroscience Update. From Translational Research to a Humanistic Approach. Volume III.
25. Otteneder, H.; Marx, R. 2004. Method-performance study on the determination of nine characteristic anthocyanins in red wine by HPLC. Bulletin d'OIV 77 (877-78): 254-275.
26. Portu, J.; López, R.; Santamaría, P.; Garde-Cerdán, T. 2018. Methyl jasmonate treatment to increase grape and wine phenolic content in Tempranillo and Graciano varieties during two growing seasons. Scientia Horticulturae. 240: 378-386.
27. Ramirez, H.; Mancera-Noyola, L., Zermeño-González, A.; Jasso-Cantú, D.; Villarreal-Quintanilla, J. A. 2019. Efecto del ácido abscísico sobre fenotipo y calidad del fruto en vid Shiraz. 2019. Ecosistemas y Recursos Agropecuarios. 6(16):153-158.
28. Renaud, S.; de Lorgeril, M. 1992. Wine, alcohol, platelets, and the French paradox for coronary heart disease. The Lancet. 339(8808): 1523-1526.
29. Rodríguez Montealegre, R.; Peces Romero, R.; Chacón Vozmediano, L.; Martínez Gascueña, J.; García Romero E. 2006. Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. Journal of Food Composition and Analysis. 19(6-7):687-693.
30. Ruiz-García, Y.; Romero-Cascales, I.; Gil-Muñoz, R.; Fernández-Fernández, J. I.; López-Roca, J. M.; Gómez-Plaza, E. 2012. Improving grape phenolic content and wine chromatic characteristics through the use of two different elicitors: Methyl jasmonate versus benzothiadiazole. Journal of Agriculture and Food Chemistry. 60(5): 1283-1290.
31. Sun, Y.; Qiaozhen, L.; Xi, B.; Dai, H. 2019. Study on the regulation of anthocyanin biosynthesis by exogenous abscisic acid in grapevine. Scientia Horticulturae 250: 294-301.
32. Urvieta, R.; Buscema, F.; Bottini, R.; Coste, B.; Fontana, A. 2018. Phenolic and sensory profiles discriminate geographical indications for Malbec wines from different regions of Mendoza, Argentina. Food chemistry. 265: 120-127.
33. Wang, J.; Wang, S.; Liu, G.; Edwards, E. J.; Duan, W.; Li, S.; Wang, L. 2016. The Synthesis and Accumulation of resveratrol are associated with veraison and abscisic acid concentration in beihong (*Vitis vinifera* × *Vitis amurensis*) berry skin. Frontiers in plant science. 7: 1605.

#### ACKNOWLEDGEMENTS

This paper is part of Emiliano Malovini postdoctoral CONICET fellow. We would like to thank Familia Zuccardi "La Agrícola S. A." and Doña Paula for facilitating their vineyards; to the Laboratory of Chromatography - INV, especially to Fanny Previde, Daniela Marmili and Jéscica Baldo and to the undergraduate and postgraduate students from UNCuyo, who participated in field and laboratory activities.

This work was funded by the National University of Cuyo (Research Program SeCTyP - UNCuyo, 2015-2019), Mendoza, Argentina.