ANTHOCYANIN AND *TRANS*-RESVERATROL ACCUMULATION IS ASSOCIATED WITH ABSCISIC ACID AND METHYL JASMONIC ACID IN BERRY SKIN OF *Vitis vinifera* L. cvs. MALBEC, BONARDA, SYRAH, CABERNET SAUVIGNON, and PINOT NOIR

LA ACUMULACIÓN DE ANTOCIANINAS Y TRANS-RESVERATROL ESTÁ ASOCIADA AL ÁCIDO ABSCÍSICO Y ÁCIDO METIL JASMÓNICO A NIVEL DE HOLLEJOS DE BAYAS DE VID (VITIS VINIFERA L.) EN LAS VARIEDADES MALBEC, BONARDA, SYRAH, CABERNET SAUVIGNON Y PINOT NOIR

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Abstract:

Red grapes contain significant amounts of phenolic compounds, known to contribute to wine quality and to provide important health benefits. Berry skin phenolics can be elicited by plant hormones. The aim of this work was to increase the content of anthocyanins and *trans*-resveratrol in five red varieties cultured in Argentina: Malbec (M), Bonarda (B), Syrah (S), Cabernet Sauvignon (CS), and Pinot Noir (PN), in two different growing regions: Santa Rosa (SR) and Valle de Uco (VU), by applying a post-veraison hormonal treatment with abscisic acid (ABA) and methyl jasmonate (MeJA). Anthocyanins and *trans*-resveratrol contents were assessed using an HPLC. Between October and February mean maximum temperatures in SR were 10 % higher than in VU, however, there were no differences in mean minimum temperatures. In the hotter region (SR), in all varieties there was about a 100% increase of total anthocyanins by ABA treatment. In the coldest region (VU), ABA treatment increased total anthocyanins in B, CS, M and S at around 40 %. However, MeJA did not affect the total anthocyanins content. *Trans*-resveratrol content was significantly increased in M, PN and S by ABA and only in PN by MeJA in SR. Also, in VU was significantly increased in M and S by ABA, while it was increased in B, CS and S with MeJA. ABA showed a temperature mitigation effect in the hotter region on anthocyanins content, additionally showing a previously non-described effect increasing *trans*-resveratrol content. MeJA had a better performance on trans-resveratrol content in VU but not in all varieties. Future studies include winemaking to test their beneficial health properties in models of study on rats, a psychotropic level.

Keywords: Abscicic acid, methyl jasmonate, anthocyanins, *trans*-resveratrol.

1. Introduction

Viticulture is the main agro-industrial activity in Mendoza province. In the past few decades, national consumption and exportation of wines have steadily increased (INV, 2012). Part of this increment is most likely due to scientific evidence that demonstrates a tight correlation between a mild but regular red wine consumption and cardiovascular health in populations where wine accompanies everyday meals as a habit (Renaud and de Lorgeril, 1992). The observed health benefits of wine over atherosclerosis, hypertension, certain types of cancer, diabetes type II, neurological disorders and metabolic syndrome are mostly conferred by the presence of certain phenolic compounds that possess antioxidant activity (Kanner et al., 1994, Guilford and Pezzuto, 2011). In general, phenolics in grape berries are mainly located in skin and seeds, and these contents have a highly important role in determining the final oenological quality of red wines (Waterhouse, 2002; Rodriguez Montealegre et al., 2006). In grapes and wines the most abundant and important polyphenolic compounds are: anthocyanins (ANT), stilbenes (i.e. *trans*-resveratrol (T-RES) and derivatives), flavanols (e.g. quercetin), and flavan-3-ols (catechins and proanthocyanidins) (Waterhouse, 2002). Content in grapes is very variable and it depends on several genetic, management and environmental factors (Siemann and Creasy, 1992; Du et al., 2012). The vineyard region also has a very important role (Berli et al., 2011). 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 (Jones and Davis, 2000). Hormones such as abscisic acid (ABA) induces ANT synthesis in grapevine while jasmonates (MeJA) stimulate ANT and T-RES biosynthesis (Jiang and Joyce, 2002; Berli et al., 2011, Larronde et al., 2003). Currently there exist numerous local trials regarding biosynthesis stimulation and content of certain phenolic compounds in several red grapevine varieties grown in Mendoza (Deis et al., 2011; Berli et al., 2011; Alonso et al., 2016). ABA has proven to have positive effects on grapes growing under high temperature conditions, countering the decrease of anthocyanins in the interaction with this environmental factor (Malovini et al., 2014, 2016). MeJA induces RES accumulation on Malbec (Durán et al., 2016). There are no previous works assessing and/or quantifying the different phenolic compounds induced by hormones such as ABA and MeJA on five red varieties, located in productive regions with dissimilar climate conditions.

The aim of this work is to evaluate, in berry skin, the accumulation of anthocyanin and *trans*-resveratrol on five red grapevine varieties cultivated in Argentina, over two different productive Cuyo Regions, with contrasting climate conditions, after direct spraying with ABA and MeJA.

Materials and Methods

The study was done in 2016 season. Commercial vineyards were selected in two different regions in Mendoza, Argentina. The first one in Santa Rosa (SR), located 90 km East from Mendoza city, at a 600 m.a.s.l. Altitude, and had higher average temperatures with warm nights, while the second vineyard was located in Valle de Uco (VU), specifically in Gualtallary locality, 90 km South-West from Mendoza city, at 1400 m.a.s.l. of altitude and had lower average temperatures, very cold nights and (Catania et al., 2007). In the growing season on study, the climatic data was obtained from nearby meteorological stations (Dirección de Contingencias Climáticas de Mendoza). In both vineyard locations homogenous and healthy plants from five red varieties, comprising Bonarda (B), Cabernet Sauvignon (CS), Malbec (M), Pinot Noir (PN) and Syrah (S), were selected. In SR vineyards were flood irrigated, with an overhead trellises, spur pruned, training system. In VU vineyards were drip irrigated with a vertical shoot position (VSP), spur pruned, training system. A randomized complete block experimental design with five replicates was used, where the experimental unit was a plant and each block comprising one row. Two independent hormone treatments were performed. Abscicic acid (ABA) treatment was done from veraison, from which bunches were sprayed with 1 mM ABA solution plus Tween 20 0.1 %, as a surfactant, at three opportunities (10 days apart). Metil jasmonate (MeJA) treatment was done before harvest, when bunches were sprayed with 10 mM MeJA in 40 % ethanol-water mixture with Tween 20 0.1 %, as a surfactant. For both hormone treatments, their respective controls were carried out by spraying bunches in control plants with their corresponding vehicle solution. The last application of ABA coincided with the only application of MeJA and the samples were collected four days later (Durán et al., 2016). After harvest, grapes from the modal size of each cultivar were chosen at random and kept at -80 ºC prior to processing. Phenolics were extracted from berry skins (after manually separating skin and pulp tissue), macerating ground skin tissue in a methanol-HCl 0.1 % mixture at 20 ºC for 48 hr covered from light. Extracts were filtrated by means of 0.45 µm cellulose acetate membranes, and anthocyanins (ANT) and *trans*-resveratrol (*T*-RES) contents were assessed by HPLC-DAD (SPD-M10AVP, Shimadzu, Chromatography Lab, Instituto Nacional de Vitivinicultura - INV) according to the official OIV methodology proposed by Otteneder (2004). ANT data was analyzed by bifactorial ANOVA (InfoStat v.2016 software, Grupo Infostat, FCA-UNC, Argentina). *T*-RES data was analyzed by GLM taking in account heteroscedasticity among cultivars. Means differences were analyzed by DGC test (Di Rienzo et al., 2002).

Results and Discussion

For the studied season, there were climatic differences between the two growing regions under study. From October to February the mean maximum temperatures in SR were 10 % higher than in VU, however, there were no differences in mean minimum temperatures (Table 1) in contrast to what was proposed by Catania et al., (2007). Mean relative humidity, expressed in percentage, was lower in SR (62.03 ± 0.95 %) than in VU (67.66 ± 0.98 %).

Since agronomic growing conditions among the assessed regions were not controlled during the trial, Variety x Region interaction effect was not evaluated. In terms of overall mean per region, SR presented berries with lower ANT content in each variety. In the studied season, In SR, B had the highest berry skin ANT content while PN had the lowest. Likewise, in VU, B and M were the two varieties showing the highest ANT contents, opposite to CS and PN which were the ones with the lowest contents. S had an intermediate ANT content in both growing regions (Figure 1). ABA significantly increased total ANT content in all varieties in SR, nearly doubling it in B (92 %), CS (100 %) and M (91 %; Figure 1.A). In VU, ABA had effect on every variety except for PN, and generated a mean increment of 40 % (B = 58 %, CS = 48 %, M = 26 % and S = 19 %) (Figure 1.B). This shows a possible mitigating effect of the ABA in warm conditions over anthocyanin content, as published previously in field trials on Malbec vines, in which the temperature was manipulated in the vineyard (Malovini, et al., 2014). MeJA had no effect on total ANT content in either of the studied growing regions, in contrast with de Rosas (2014).

Regarding T-RES content in berry skin, overall mean per region was lower in SR compared to VU in all the assessed varieties. Moreover, B was the one with the lowest T-RES contents in both regions. In SR, ABA increased the content of this compound in M, PN and S, and only in M and S in VU (Figure 2.C and 2.D). In contrast, in CS it had a negative effect, significantly decreasing T-RES content. No previous similar reports were found that show these effects. MeJA significantly increased the overall mean T-RES content in both regions; however in SR this effect was only significant in PN (Figure 2.A). In contrast, in VU this increment was observed in B (300 %), CS (43 %) and S (91 %; Figure 2.B).

Conclusion

A greater effect of ABA on the ANT content in all varieties in the warm region (SR) compared to the colder region (VU) was observed, showing a positive effect as a possible mitigator of the high temperature consequences on grapevine berry phenolic compounds. It also demonstrated a previously undescribed effect increasing T-RES content. MeJA had no effect on ANT content, only increasing T-RES content in VU. Future works will be carried out to confirm these results. Then with the Variety x Region x Hormone interactions that yield the highest amounts of ANT and T-RES, wines will be elaborated to study its psychotropic effects on an anxiety-depression rat model.

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References

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.

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): 4874–84.

CATANIA C.D., AVAGNINA S., MONTE D., MONTE R.F., TONIETTO J., 2007. Caracterização climática de regiões vitivinícolas Ibero-Americanas. Bento Gonçalves : Embrapa Uva e Vinho, 2007. 64p. ISBN 978-85-89921-04-6

DE ROSAS M.I. 2014. Elicitación de la expresión génica de antocianinas y trans-resveratrol en bayas de vid. Doctoral thesis PROBIOL – National University of Cuyo, 155p

DEIS L., CAVAGNARO B., BOTTINI R., WUILLOUD R., FERNANDA SILVA M., 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.

DI RIENZO J. GUZMAN 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.

DU B., HE B., SHI P., LI F., 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.
DURÁN M.F., MALOVINI E., FONTANA A., ARANCIBIA C., BOTTINI R., MARTINEZ 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. 13-18 de Junio 2016, Verona-Italia
GUILFORD J.M., PEZZUTO J.M., 2011. Wine and Health: A Review. Am. J. Enol. Vitic. 62(4): 471–486.
JIANG Y., JOYCE D.C., 2002. ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. : 171–174.
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.
KANNER J., FRANKEL J.E., GRANIT R., GERMAN B., KINSELLATSS J.E., 1994. Natural Antioxidants in Grapes and Wines. Journal of Agricultural and Food Chemistry. 42: 64–69.
LARRONDE F., GAUDILLERE J.P., KRISA S., DECENDIT A., DEFFIEUX G., MERILLON J.M., 2003. Airborne Methyl Jasmonate Induces Stilbene Accumulation in Leaves and Berries of Grapevine Plants. American Journal of Enology and Viticulture. 1: 63–66.
MALOVINI E., DEIS L., SARI S., DE ROSAS M.I., DURAN M., ARANCIBIA C., PONCE M.T., MARTINEZ 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 Phisiology and Biotechnology. 13-18 de Junio 2016, Verona-Italia.
MALOVINI E., SARI S., DE ROSAS M.I., DURAN M., GOMEZ 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, 9-14 Noviembre, Mendoza-San Juan, Argentina.
OTTENEDER H., MARX R., 2004. Method-performance study on the determination of nine characteristic anthocyanins in red wine by HPLC. Bull. l'OIV 77(877–78): 254–275.
RENAUD S., DE LORGERIL M., 1992. Wine, Alcohol, Platelets, and the French paradox for coronary heart disease. The Lancet, 339(8808), 1523-1526.
RODRIGUEZ MONTEALEGRE R., PECES ROMERO R., CHACON VOZMEDIANO L., MARTINEZ GASCUEÑA J., GARCIA 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: 687–693.
SIEMANN E.H., CREASY L.L., 1992. Concentration of the Phytoalexin Resveratrol in Wine. American Journal of Enology and Viticulture. 43(1): 49–52.
WATERHOUSE, A.L., 2002. Wine phenolics. Annals of the New York Academy of Sciences. 957: 21–36.

Table 1. Temperature and relative humidity differences between the two regions studied. Values are growing season means (October to February). Meteorological stations were located in Las Catitas (33° 15' 56" S; 68° 03' 28" O, Santa Rosa - SR) and El Peral (33° 20' 48,2" S; 69° 9' 27,7" O, Valle de Uco - VU) . Different letters indicate significant differences between regions determined by DGC test (p-value <0.05).

Meteorological Station	**Temperature**				**Relative humidity**
	n	***Maximum (ºC)***	***Mean (ºC)***	***Minimum (ºC)***	***Mean (%)***
Las Catitas (SR)	111	31.24 ± 0.36 *a*	22.91 ± 0.26 *a*	14.09 ± 0.32 *a*	62.03 ± 0.95 *b*
El Peral (VU)	105	28.91 ± 0.38 *b*	20.84 ± 0.27 *b*	13.51 ± 0.33 *a*	67.66 ± 0.98 *a*

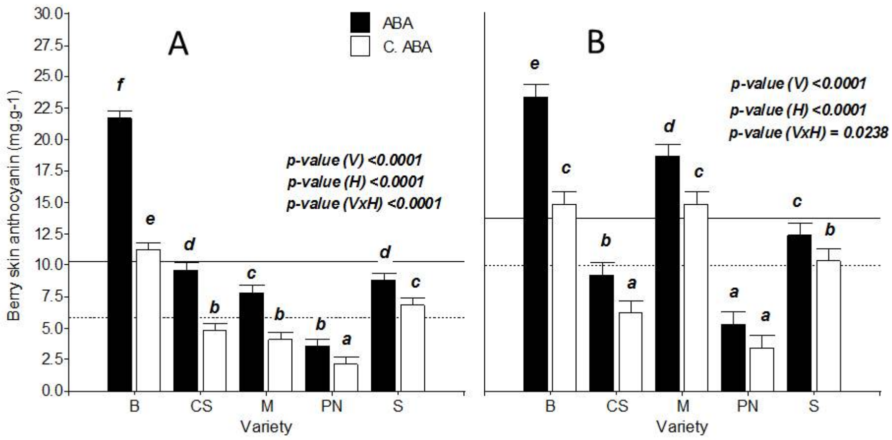

Figure 1. Total anthocyanin content in grape berry skins treated with abscisic acid (ABA). Mean values for each of the five varieties in study are shown for both growing regions: Santa Rosa (A) and Valle de Uco (B). Varieties are: Bonarda (B), Cabernet Sauvignon (CS), Malbec (M), Pinot Noir (PN) and Syrah (S). Bars indicate standard error (SE), n =5. Different letters indicate significant differences between treatments, determined by DGC test (p-value <0.05). For each Region: p-value (V), Variety effect, p-value (H), Hormonal treatment effect, p-value (VxH), Variety x Hormonal treatment interaction effect. Horizontal lines represent the overall means for Hormonal treatment in each particular region, solid line refers to ABA treatment and dot line refers to control treatment.

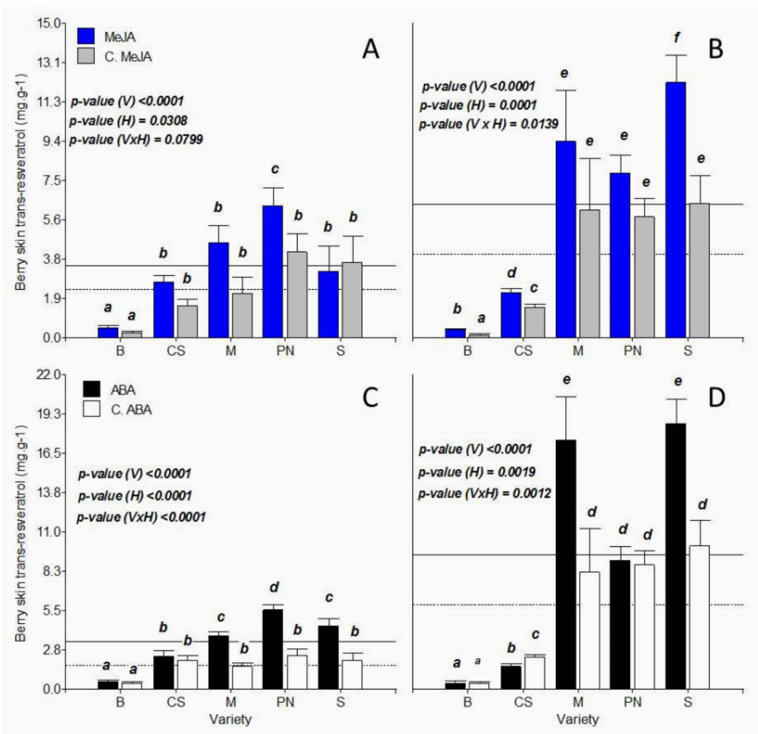

Figure 2. *T*-RES content in berry skin of the five varieties in study at two different growing regions. (A) Berry skin *T*-RES content in grapes treated with methyl jasmonic acid (MeJA) in Santa Rosa. (B) Berry skin *T*-RES content in grapes treated with MeJA in Valle de Uco. (C) Berry skin *T*-RES content in grapes treated with abscisic acid (ABA) in Santa Rosa. (D) Berry skin *T*-RES content in grapes treated with ABA in Valle de Uco. Bars indicate standard error (SE), ABA: n = 5; MeJA: n = 4. Different letters indicate significant differences between treatments determined by DGC test (p-value <0.05). For each Region: p-value (V), Variety effect, p-value (H), Hormonal treatment effect, p-value (VxH), Variety x Hormonal treatment interaction effect. Horizontal lines represent the overall means for hormonal treatment in each particular region, solid line refers to hormonal treatments (ABA or MeJA) and dot line refers to their respective control treatments.