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Effect of Abscisic Acid on Yield, Anthocyanins, Polyphenols and Procyanidins in Grape *cv.* Cabernet Franc (*Vitis vinífera* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author HR designed and conducted the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MLMN and AZG managed the analyses of the study. Authors DJC and JAVQ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In recent years the wine industry in Mexico has increased its production as a result of exports and recognition for its wine quality abroad. An actual problem related to lack of berry pigmentation at harvest time was found in the cultivar Cabernet Franc. Its consequences involves in a delay on the

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biochemistry of maturity components and its impact in the wine elaboration. Abscisic acid (ABA) is a plant hormone involved in various stages of fruit maturity; however, little is known on its direct role in berry pigmentation and other fruit maturity components. On this basis and with the purpose to study the effect on yield, pigmentation and fruit quality, ABA was applied on the cv. Cabernet Franc.
Study Design: A completely randomized block design was used with four replicates per treatment. The data were analysed with the statistical program 'RStudio' for Windows version 10. , The Tukey (*P* < *0.05*) test was applied for the analysis of variance and comparison of means.
Place and Duration of Study: The experiment was conducted at the vineyard San Lorenzo, Casa Madero in Parras de la Fuente, Coahuila, Mexico and Horticulture lab in Universidad Autónoma Agraria Antonio Narro in Saltillo, Coahuila, Mexico, during the period of June - December 2017.
Methodology: When berries on rachis reached the veraison stage, a first spray of ABA was applied on the bunches using a 10 L back sprayer. The treatments were Control (H2O); 200, 400, 600 and 800 mg L^{-1} of ABA. The second spray at same doses was applied 14 days after first application.
Results: It was observed that ABA at any doses did not affect the weight, length, pH, soluble solids and acidity in fruits nor in yield. ABA at any concentration provoked a significant increment in the levels of anthocyanins, polyphenols and procyanidins in the harvested fruits.
Conclusion: It can be concluded that ABA as a hormone treatment can be used at the veraison stage in Cabernet Franc to improve the contents of anthocyanins, polyphenols and procyanidins in berries.

Keywords: Grape; Cabernet Franc; abscisic acid; berry pigmentation; procyanidins.

1. INTRODUCTION

Mexico is a significant wine producer destined to national, USA and Europe markets. In 2017, the wine vine plantation reached approximately 44 thousand tons, of which 25% were exported [1,2]. Cabernet Franc is a wine grape cultivar which is gaining interest among grape growers in Parras de la Fuente Coahuila as a result of its high-quality wine in colour and aromatic characteristics found in countries such as Chile and Argentina [3]. In certain Mexican area as well as in other countries such as Australia, USA and Italy, obtaining an adequate red colour for fruit at harvest is a major concern. Colour deficiency may be attributable to current climate changes that indicate higher temperatures, with longer periods of drought and annual and seasonal variability of rainfall [3]. The north-eastern region of Mexico is vulnerable to climate change where drought is accentuated [4]. As a result of increasing diurnal temperatures, the most suitable regions for grape cultivation could be at higher latitude areas [5]. The phenology and maturation of grape berries are affected by climate change [1,2]. Temperature above 30ºC would increase the total soluble solids because the berry ripens at this temperature [6]. Temperature above 35ºC would decrease the synthesis of monomeric anthocyanins which will affect skin colour [6]. These temperatures decrease synthesis of berry skin anthocyanins [7] and therefore cause a reduction in berry colour [8]. The accumulation of anthocyanins in the grape skin has been shown to be stimulated by exogenous application of sugar [9]. Plant growth regulators (PGRs) have proven to improve antioxidants content in fruits from several horticultural species. Based on the above, the present study was conducted to evaluate the effect of abscisic acid (ABA) on yield, anthocyanins and other grape berry quality parameters of Cabernet Franc.

2. MATERIALS AND METHODS

2.1 Experimental Site, Material, and Design

The study was conducted in the Casa Madero vineyard located in Parras (102°11´W and 25°26´N, at 1,533 MSL) de la Fuente Coahuila, Mexico and in the laboratories of Universidad Autónoma Agraria Antonio Narro, in Saltillo, Coahuila, Mexico. Twelve-year-old vines of Cabernet Franc grafted on SO_4 rootstock were selected for the study. The site is located in Parras Coahuila, Mexico. Vines were spaced 1.5 x 2.5 m trained to a spindle bush system, oriented to a north-south direction and watered with drip irrigation. Plant fertilisation, pest control, and vineyard management were conducted according to the standard recommended practices during the growing season. A completely randomized block design was used with five treatments and four replicates per treatment. The treatments were; Control (H2O); ABA (Protone SG®, Valent BioSciences) at concentrations of 200, 400, 600 and 800 mg L^{-1}. All treatments were applied directly to tagged grape bunches with a 10 L backpack sprayer at veraison stage. Veraison was established when 50% of the berries in the bunch were soft, which corresponded to 51.11 ± 0.77% of berry softening. The second application with the same treatments was applied 14 days after the first application. Grapes were harvested 47 days after the second application of ABA when grape bunches reached approximately 85% of pigmentation. Four bunches per vine from each treatment were collected and weighed. The rachis length was determined on site. Bunches were transported under refrigerated conditions to the Postharvest Laboratory at Universidad Autónoma Agraria Antonio Narro and stored at -20°C until required for analysis. The results obtained were analysed using the statistical program 'RStudio' for Windows version 10 and the data were subjected to a comparison of means with the Tukey ($P< 0.05$) test.

2.2 Horticultural Parameters

2.2.1 Length of bunches

The length of rachis was measured at harvest time using a Staley Power Lock tape scale 0 to 8 m/26' [10].

2.2.2 Yield

At harvest time, when control berries reached 25% of °Brix, yield per plant and bunch weight were determined using a Sinberline scale with a capacity of 5 kg [10].

2.3 Quality Parameters

2.3.1 Total soluble solid content (°Brix)

The total soluble solid content was measured from a 10 mL fruit juice samples with a manual temperature- compensated refractometer (ATC-1E, Atago, Tokyo, Japan) and the results were expressed as % of °Brix [10].

2.3.2 Determination of pH

The pH of grapes was determined from a 10 mL juice sample extracted from grape berries by filtering it with gauze and using a Hanna Hi98130 potentiometer [10].

2.3.3 Acidity

The titratable acidity was obtained by titrating 10 mL of juice from a representative sample of grape with 0.1 N NaOH until neutralisation of organic acids at a pH of 8.2, adding 1% phenolphthalein as an indicator. In this study, the results were expressed as a percentage of tartaric acid equivalents on w/v basis [10].

2.4 Antioxidants

2.4.1 Polyphenols

The total polyphenol content was determined using the method of Wong-Paz et al. [11]. Five grams of homogenized berries were placed in a 250 mL flask containing 50 µL of 80% methanol solution. The extraction process was conducted in an ultrasonic bath (Brason B series 5510). The methanolic solution containing polyphenols was filtered on Whatman No.1 paper. The filtrate was collected in a volumetric flask, where after 20 µL of sodium carbonate (7.5 w/v) was added. The suspension was left to stand for 5 min. after which it was diluted in 125 µL of distilled water. The samples were calibrated using a Jenway spectrophotometer (Model 6320D) at a wavelength of 790 nm. The total polyphenol content was expressed in equivalents of gallic acid standard per gram of dry weight according to the calibration curve developed with concentrations of 0, 100, 200, 300, 400, 500 and 600 mg L^{-1}.

2.4.2 Procyanidins

The extraction and total procyanidin content were carried out using the technique of Porter et al. [12]. One g of whole berry tissue sample was placed in a test tube with screw cap and 5 mL of 85% methanol solution was added to it. The suspension was left to stand for 12 hours at 25°C, and then filtered through a Whatman No. 1 filter paper. An aliquot of 250 µL from the solution was transferred into a test tube, adding 1.5 µL of 5% HCL-butanol and allowed to stand for an hour at 90°C. The sample was calibrated at an absorbance wavelength of 550 nm using a spectrophotometer. Results were expressed as equivalents of procyanidin B1 (Sigma-Aldrich) obtained from a calibration curve values 0, 100, 200, 300, 400, 500 and 600 mg L^{-1}.

2.4.3 Total anthocyanins

The extraction and total anthocyanin contents were conducted using the method of AOAC [10]. An amount of 2.5 g berry skin were ground and incubated for 24 hours in 3N HCl and 85% methanol solution, stored at a temperature of

8°C in complete darkness. The samples were filtered and stored at 7°C until required for analysis. Calibration of the equipment was made by placing a 2 mL cell of 30% hydrogen peroxide and 4 mL of extraction solution in a Jenway spectrophotometer (Model 6320D). Four mL of sample was placed into a cell, adding 2 mL of hydrogen peroxide and read at an absorbance of 525 nm. The content of anthocyanin was calculated using the following formula:

$$\text{anthocyanins (mg /100g)} = \frac{\text{50x \% absorbance 525nm}}{\text{extractor solution 0.405x sample weight}}$$

3. RESULTS AND DISCUSSION

3.1 Horticultural Parameters

It was revealed that ABA at any dose did not affect berry weight and grape bunch length (Table 1). The weight of the berries; although a tendency to be higher with ABA at 600 mg L^{-1} when compared to the control samples, the effect was not statistically significant (*P< 0.05*). This result is supported by the findings of Peppi et al. [13] and Ovalle [14] who found that the application of ABA at the veraison stage did not modify the final weight of berries and bunch length of Flame Seedless and Pinot Noir. These parameters were within the same range as in Cabernet Franc.

The yield per vine (Table 1) in Cabernet Franc did not show any significant difference between treatments with ABA and control; even the higher value of 3.16 kg at 200 mg L^{-1}, was not significantly different from the control (*P< 0.05).* Other grape wine cultivars such as Pinot Noir showed similar behavior when ABA was sprayed at veraison stage, resulting in similar values in yield to those found in the present study [13,14].

The weight of grape berries is determined by the number, volume and density of the cells [15]. The final weight is largely depending on the cell division before anthesis and cell elongation [15]. In addition but to a lesser extent, cell division after anthesis as well as an increment in the concentration of solutes also contributes in this physiological process [15]. The results obtained in the present phenotypic parameters are of significant value, with the known fact that ABA did not cause adverse effects in Cabernet Franc grape berries. It is well known that ABA is a hormone that causes a reduction in cell division and elongation in fruits of horticultural crops [9,16].

3.2 Soluble Solids, pH and Acidity

The effect of different concentrations of ABA (Table 2) on pH, acidity and soluble solids in matured berries of Cabernet Franc did not differ significantly among the treatments as well as the control sample.

Table 1. Effect of abscisic acid on the length (cm), weight (g) of grape bunches and yield (kg/vine) of cabernet Franc

Treatments (ABA mg L^{-1})	Bunch length (cm)^{ns}	Bunch weight (g)^{ns}	Yield per vine (kg)^{ns}
200	14.84±0.8a^{YZ}	186.56±2.1a	3.160±0.39a
400	13.81±0.5a	203.75±5.2a	2.787±0.65a
600	15.56±1.1a	231.25±7.3a	2.787±0.47a
800	15.68±0.3a	179.68±2.4a	2.685±0.47a
Control	15.21±0.3a	206.25±3.1a	2.880±0.51a

^{ns}: not significant, ^{yz}: The same letters in the same column indicate no significant differences in the content of the parameters measured among the different treatments (Tukey P<0.05); Average of four bunches per treatment

Table 2. Effect of abscisic acid on pH, tartaric acid (%) and °Brix (%) in Cabernet Franc grapes

Treatments (ABA mg L^{-1})	pH ^{ns}	Tartaric acid (%) equiv.^{ns}	° Brix (%)^{ns}
200	3.928± 0.52a^{YZ}	0.325± 0.65a	25.675± 2.72a
400	3.925± 0.37a	0.363± 0.72a	26.000± 4.35a
600	3.900± 0.45a	0.380± 0.47a	25.750± 1.79a
800	4.013± 0.49a	0.358± 0.91a	25.925± 3.82a
Control	3.935± 0.49a	0.335± 0.81a	25.100± 2.98a

^{ns}: not significant, ^{yz}: The same letters in the same column indicate no significant differences in the content of the parameters measured among the different treatments (Tukey P<0.05); Average of four bunches per treatment

In a previous research, Sandhu et al. [17] found that the application of abscisic acid did not alter the mass, soluble solids and pH of berries in the cultivars Noble and Alachua. Cutipa [18] also reported that abscisic acid did not modify pH and soluble solids in Red Globe. During the period of grape maturation, plant metabolism is characterized by greater photosynthesis, whereas sugar degradation by plant respiration is less than during the growth period of berries. The increase in sugar in the tissue may be due to photosynthesis, reserved in the stems or by transformation of malic acid [19].

The soluble solid content consistently increased throughout the process of berry ripening [20, 21, 22]. There is a rapid accumulation of sugar in the berries from the veraison stage due to the migration of photosynthetic products through the grape bunches. Additionally, sugar reserves such as fructose are also mobilised through the shoots and root system [20].

3.3 Anthocyanins

The effect of exogenous application of ABA on the anthocyanin content was significantly different from the control treatment, i.e. ABA treatments significantly increased the levels of anthocyanins in Cabernet Franc grape skin (Fig.1). All four treatments resulted in an increase of anthocyanin content above 21%. However significant differences among the treatments were not evident.

The results of this study corroborate the findings of Peppi et al. [13]. They reported that the application of ABA at 400 mg L^{-1} to grape cultivar Crimson resulted in an accumulation of anthocyanins in the epidermis of the fruit. The use of abscisic acid produced higher levels of anthocyanins and intensity of colour in grape variety Isabel [23]. The increment of anthocyanin in grape berries due to ABA as reported by various researchers [13,23] and results reported in this paper, contribute to consider ABA as a berry skin anthocyanin promoter [24,25].

The colour of grape berry skin depends on the anthocyanin synthesis pathway [26]. Anthocyanins are synthesised from the precursors phenylalanine and acetate through the path of phenyl-propanoids [27,28,29]. Studies on Kyoho grape cultivar have shown that ABA stimulates the expression of genes involved in the path of the phenylpropanoids when applied at the veraison stage. The increase in anthocyanins follows the increase of enzymes synthesis involved in secondary metabolism [30]. Anthocyanins are synthesised during veraison; a stage which corresponds to change in colour [31]. It accumulates in the vacuoles of tissue, particularly in the first four cell layers in the hypodermis of the berries; and in some cases, in the mesocarp and seeds [32]. The development of colour in the berry skin is also affected by factors such as diurnal temperature, light intensity, berry quality, leaf surface area, shoot length, carbohydrates, mineral nutrition and plant hormones [33,34,35].

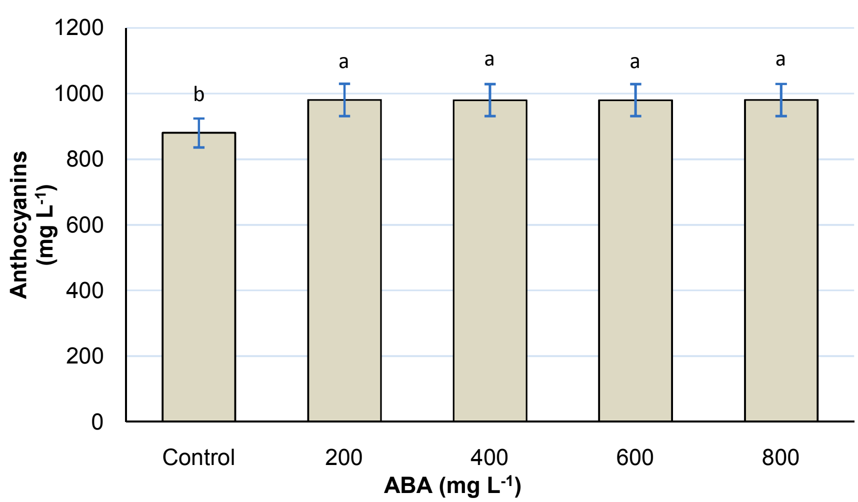

Fig. 1. Effect of ABA on the anthocyanin content of Cabernet Franc grapes
Different letters indicate the statistically significant differences (P<0.05)

3.4 Polyphenols

The two treatments of ABA applied at veraison and 14 days later, resulted in an increment in the content of polyphenols in berries when compared to control fruits (Fig. 2). This effect was significant at any concentration of the hormone ($P< 0.05$). The treatment with ABA at 800 mg L^{-1} resulted in the highest content of polyphenols in the tissue. This treatment was 7-fold higher than the control sample in the content of polyphenols. By comparing the increment in these compounds among ABA treatments, a concentration scale response in the content of polyphenols was observed in grape berries.

It has been reported that the application of ABA induces the synthesis of phenolic compounds in grape berries [36,37]. Rufato et al. [23] applied different doses of ABA to Isabel grapes and found that the concentration of 800 mg L^{-1} resulted in a 95% increment in the content of polyphenols in berries. These results support the findings in this research with Cabernet Franc. Studies conducted in Chile, had determined that an application of ABA in Carmenere grapes five days before the veraison stage, increased the content of sugars and anthocyanins and decreased the acidity in the berries; an effect which is associated with an increasing concentration of phenolic compounds [38]. Grape is a non-climacteric fruit and therefore once berries are separated from the bunch, there is no increase in the level of polyphenols in these organs [26]. Total phenolic compounds increased during the berry maturation process [39, 40]. In this study, the use of ABA did not change the content of soluble solids, acidity and pH when it stimulated a higher content of polyphenols in berries. This valuable expertise would be an alternative to be used by grape growers in Parras, Coahuila Mexico.

3.5 Procyanidins

Figure 3 illustrates the effect of ABA on the content of procyanidins. ABA at any concentration resulted in a significant increase in the levels of procyanidins in ripe grapes. The application of 600 mg L^{-1} treatment resulted in the highest level of procyanidins in grape berries. The dose of 800 mg L^{-1} produced less procyanidins than the 600 mg L^{-1} dose. This physiological response may support the hormonal postulate which indicates that a high concentration in cell tissue could cause a supra-optimal behaviour and therefore a decrease in the synthesis of certain organic compounds [16].

Procyanidins contribute to the astringency of wine; therefore, the levels in grape berries directly affect the quality of wine [25]. Limited information is available on the effect of ABA on the procyanidin synthesis [38]. Condensed tannins are derived from flavan-3-ol units, identified as procyanidins [41]. They result from the hydrolysis of anthocyanins at high temperature [42]. The proanthocyanidins exist in

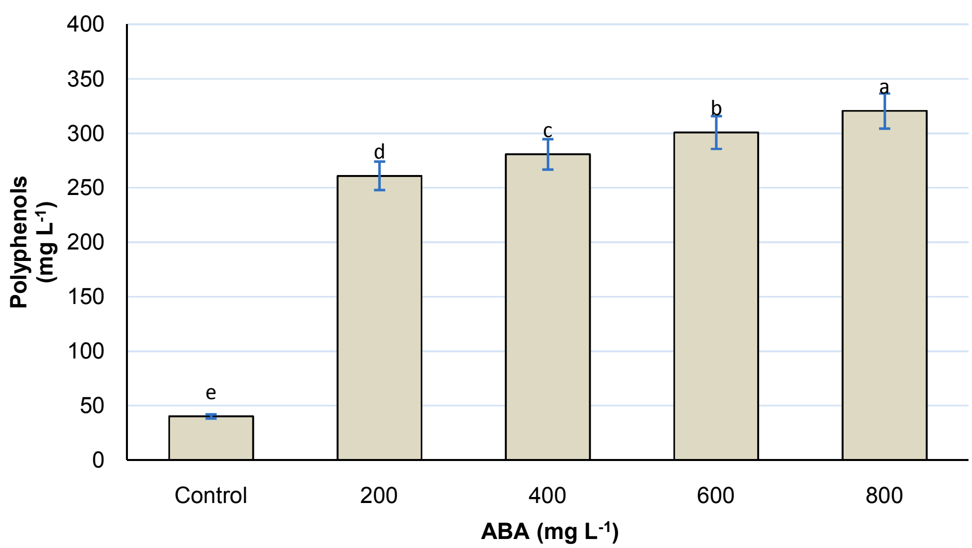

Fig. 2. Effect of ABA on the polyphenol content of Cabernet Franc grapes
Different letters indicate the statistically significant difference (P<0.05)

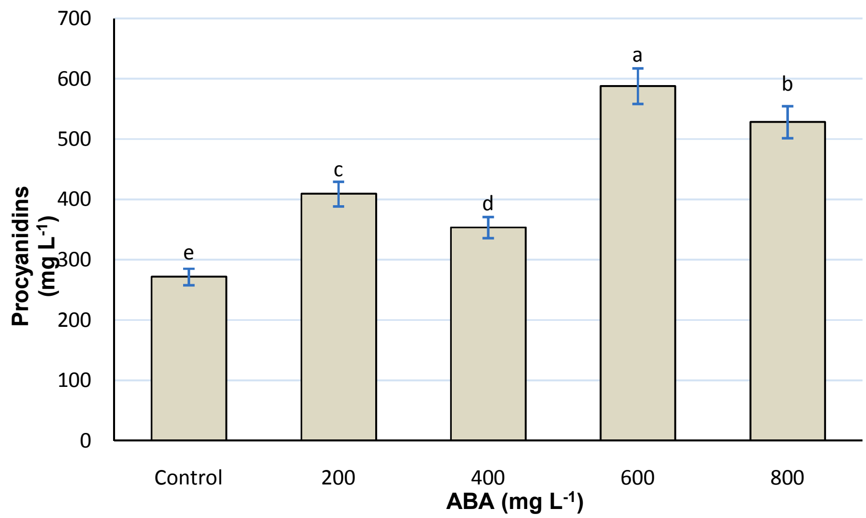

Fig. 3. Effect of ABA on the procyanidin content of Cabernet Franc grapes
Different letters indicate the statistically significant difference (P<0.05)

their flavanic form and are linked with compounds such as proteins and polysaccharides, forming a complex structure [40]. It has been reported that the application of ABA to grape vines, accelerates the ripening process of berries [34] through a self-catalytic role [42].

It can be depicted that the use of ABA as a hormone treatment is an alternative method to increase the levels of procyanidins in Cabernet Franc grapes.

4. CONCLUSIONS

It is revealed from the present study that the application of abscisic acid at veraison stage of Cabernet Franc grapes did not affect yield, length and weight of grape bunches, pH, acidity and soluble solids in berries. Abscisic acid at concentrations of 200, 400, 600 and 800 mg L^{-1} at veraison stage significantly increased the anthocyanin, total polyphenol and procyanidin contents in matured berries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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