

Article

INFLUENCE OF ABSCISIC ACID, *ASCOPHYLLUM NODOSUM* AND *ALOE VERA* ON THE PHENOLIC COMPOSITION AND COLOR OF GRAPE BERRY AND WINE OF 'CABERNET SAUVIGNON' VARIETY

INFLUÊNCIA DE ÁCIDO ABCÍSICO, *ASCOPHYLLUM NODOSUM* E *ALOE VERA* NA COMPOSIÇÃO FENÓLICA E NA COR DA UVA E DO VINHO DA VARIEDADE 'CABERNET SAUVIGNON'

Isabela Leticia Pessenti^{1,*}, Ricardo Antonio Ayub², José Luis Marcon Filho³, Fábio Chaves Clasen⁴, César Valmor Rombaldi⁴, Renato Vasconcelo Botelho⁵

¹Unicesumar; St Des. Westphalen, 60, Zip Code 84010-000, Ponta Grossa/Paraná, Brazil.

²UEPG; Av. General Carlos Cavalcanti, 4748, Zip Code 84030-900, Ponta Grossa/Paraná, Brazil.

³Vinicola Legado, Rod. Eng. Raul Azevedo de Macedo, 5800, Zip Code 83648-000, Campo Largo/Paraná, Brazil.

⁴UFPEL, Campus Universitário, Capão do Leão, Zip Code 96160-000, Pelotas/Paraná, Brazil.

⁵Unicentro, Street Padre Salvatore Renna, 875, Zip Code 85015-430, Guarapuava/Paraná, Brazil.

* Corresponding author: Tel.: +55 49 9 9956 5512; e-mail: isabela.pessenti@unicesumar.edu.br.

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SUMMARY

In viticulture, various techniques can be used to improve productivity, tolerance to biotic or abiotic stress, the quality of grapes and wines such as the use of plant regulators and biostimulants. Thus, the objective of this work was to evaluate the effect of application of abscisic acid (S-ABA), *Ascophyllum nodosum* (*A. nodosum*) seaweed extract and *Aloe vera* (*A. vera*) gel on phenolic composition and chromatic characteristics of grapes from the 'Cabernet Sauvignon' variety. The experiment was conducted in a commercial vineyard in Campo Largo - Paraná, in two consecutive seasons, 2017/18 and 2018/19, involving the following treatments: 1) control; 2) (S-ABA) 400 mg/L; 3) S-ABA 600 mg/L; 4) *A. vera* gel 200 mL/L; 5) *A. vera* gel 400 mL/L; 6) seaweed extract 0.2 mL/L; 7) seaweed extract 0.4 mL/L. Two applications were performed with the seaweed extract and *A. vera* gel when the bunches were at *veraison* stage (50 and 75% of grape berries with coloration). Total anthocyanins content, total polyphenols content and activity of phenylalanine ammonia-lyase (PAL), polyphenoloxidase and peroxidase enzymes were assessed in the berries skin. Total anthocyanins, individual anthocyanins and total polyphenol contents as well as lightness, chroma and hue angle were analyzed in the corresponding wines. S-ABA increased the content of anthocyanins and total polyphenols, as well as the activity of PAL in the first season. *A. nodosum* (AN) seaweed extract increased the total polyphenol content, total anthocyanins content and PAL in the berry skin of 'Cabernet Sauvignon' variety. S-ABA increased the total polyphenol content and anthocyanins in wine, as well as the *A. nodosum*, in at least one of the evaluated seasons.

RESUMO

Na viticultura, várias técnicas podem ser utilizadas para melhorar a produtividade, a tolerância ao estresse biótico ou abiótico e a qualidade das uvas e dos vinhos, tais como o uso de reguladores vegetais e de bioestimulantes. Assim, o objetivo deste trabalho foi avaliar o efeito da aplicação de ácido abscísico, de extrato de alga *Ascophyllum nodosum* e de gel de *Aloe vera* na composição fenólica e nas características cromáticas de uvas da variedade 'Cabernet Sauvignon'. O experimento foi realizado numa vinha comercial em Campo Largo - Paraná, em duas safras consecutivas, 2017/18 e 2018/19, envolvendo os seguintes tratamentos: 1) testemunha; 2) S-ABA 400 mg/L; 3) S-ABA 600 mg/L; 4) *A. vera* 200 mL/L; 5) *A. vera* 400 mL/L; 6) *A. nodosum* 0,2 mL/L; 7) *A. nodosum* 0,4 mL/L. Realizaram-se duas aplicações para o extrato de alga e gel, quando os cachos estavam em fase de pintor (50 e 75% das uvas apresentando mudança de cor). Avaliou-se o teor de antocianinas totais e polifenóis totais e atividade das enzimas fenilalanina amônia-liase, polifenoloxidase e peroxidase na película das uvas. No vinho, foram analisados o teor de antocianinas totais, antocianinas individuais e teor de polifenóis totais, luminosidade, cromatismo e tonalidade. O S-ABA incrementou o teor de antocianinas e polifenóis totais, assim como a atividade da PAL na primeira safra. *A. nodosum* (AN) promoveu o aumento do teor de polifenóis totais, antocianinas totais e a PAL na película da uva da variedade 'Cabernet Sauvignon'. No vinho, o S-ABA incrementou polifenóis totais e antocianinas totais, assim como o *A. nodosum*, em pelo menos uma das safras avaliadas.

Keywords: *Vitis vinifera*, flavonoids, biosynthesis, enzymatic activity, winemaking.

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INTRODUCTION

The color of red grapes' skin results from the synthesis of anthocyanins and degradation of chlorophyll (Deng *et al.*, 2019). Anthocyanins are water-soluble flavonoid compounds, and their concentration and diversity determine the intensity and stability of color in both grape berries and wines (Sun *et al.*, 2019). The use of biostimulants and plant regulators have been proposed as strategies to improve crop yield and quality, and can also be used in viticulture (Frioni *et al.*, 2018).

Seaweed-based extracts have been used as a sustainable tool to improve plant abiotic stress tolerance and increase fruit quality (Calvo *et al.*, 2014; Santaniello *et al.*, 2017), in particular the biosynthesis of flavonoids (Cai *et al.*, 2012; Fan *et al.*, 2014; Santaniello *et al.*, 2017). However, studies on the effect of these extracts on secondary metabolism compounds, fundamental to grape and wine quality, are still scarce. Salvi *et al.* (2019) tested the application of *Ascophyllum nodosum* (AN) in 'Sangiovese' variety, and the results indicated that the seaweed extract improved the ecophysiological characteristics, as well as increased the content of total sugars and polyphenols in dry and hot years.

Application of edible coatings, such as *Aloe vera* (AV), has proved to be a promising tool to improve the quality and extend the storage and post-harvest life of various fruits (Chrysargyris *et al.*, 2016). AV-based coatings modify the internal atmosphere of gases, reduce moisture loss and respiration rate, decrease oxidative darkening and reduce the proliferation of microorganisms in fruits, maintaining the physicochemical characteristics, such as color and firmness (Koushesh Saba and Emamifar, 2016).

The introduction of acid (S)-cis-abscisic (S-ABA) as an active ingredient in a commercial plant growth regulator (ProTone®) has become widely studied in *V. vinifera* under temperate conditions (Koyama *et al.*, 2018). Neto *et al.* (2017) observed an increase in the total content of anthocyanins, regardless of the doses of S-ABA applied to the 'Ruby' variety. Yamamoto *et al.* (2015) found an increase in total anthocyanin content in 'Isabel' grape berries (*V. labrusca*). Rufato *et al.* (2014) reported an increase of 48% and 80% in anthocyanin content resulting from the application of 600 and 800 mg/L of S-ABA, respectively. The maximum total concentration of polyphenols was associated with 600 mg/L in 'Isabel' grapes. Pessenti *et al.* (2019, 2020) also found an increase in anthocyanin content in 'Primitivo' and 'Malbec' varieties with the use of S-ABA at *veraison* stage.

In *V. vinifera* L., the main anthocyanins are cyanidin, peonidin, malvidin, petunidin, delphinidin, with a predominance of malvidin-3-glucoside (Dai *et al.*, 2011). The contents and the anthocyanin profile of

red grapes depend on the grapevine variety, stage of maturation of the berries, environmental factors and viticultural practices (Ollé *et al.*, 2011).

According to Seymour *et al.* (2013), flavonoids are produced through the phenylpropanoid pathway, starting with phenylalanine ammonia-lyase (PAL), from the shikimic acid route, through the non-oxidative deamination of L-phenylalanine into cinnamic acid. This is a very important reaction in regulating the carbon flow of this pathway, as it is the key regulatory enzyme for the synthesis of most phenolic compounds in the berry.

The synthesis and accumulation of phenolic compounds in the grapes are greatly influenced by several conditions, including light, air temperature, altitude, soil type, water availability, nutritional status, and disease incidence (Downey *et al.*, 2006; Dai *et al.*, 2011). Furthermore, knowledge about the enzymatic activity can be used to improve the quality of the grape and wine. This study aimed to determine the effects of pre-harvest application of different concentrations of S-ABA, *A. nodosum* seaweed extract and *A. vera* gel on the phenolic composition and enzymatic activities in the berry skin, and the phenolic composition and chromatic characteristics of the 'Cabernet Sauvignon' wine.

MATERIALS AND METHODS

Experimental design

The experiment was carried out in a commercial vineyard located in Campo Largo, Paraná, Brazil (25° 40'31"S, 49° 49'64"O; 840 m above sea level) with the 'Cabernet Sauvignon' variety (*Vitis vinifera* L.) for two consecutive seasons (2017/18 and 2018/19). The 7-year-old vines, grafted onto 'Paulsen 1103' rootstock, were planted with 3.0 x 1.5 m spacing and conducted in simple Guyot. According to Alvares *et al.* (2013), using the Köppen classification, the climate of the site is Cbf - temperate humid climate with summer temperate and well-defined winter and summer season; Table I shows the data of the National Institute of Meteorology for this site. Rainfall is well distributed every month of the year. The soil is classified as a dystrophic Red Latosol of clayey texture.

The experimental design was based on randomized blocks, with seven treatments, four replicates and an experimental plot consisted of three plants, being evaluated the central plant. The treatments were as follows: 1) control (water); 2) S-ABA 400 mg/L (ABA400); 3) S-ABA 600 mg/L (ABA600); 4) *Aloe vera* gel 200 mL/L (AV200); 5) *A. vera* gel 400 mL/L (AV400); 6) seaweed extract *A. nodosum* 0.2 mL/L (AN02); 7) seaweed extract *A. nodosum* 0.4 mL/L (AN04). The applications of aqueous solutions of S-ABA (Valent BioSciences Corporation, Libertyville, IL, USA) were performed directly on

the bunches, by using a costal spray up to the point of runoff, when 50% of the berries were colored (*veraison* stage). The extracts of *A. nodosum* and

Aloe vera gel were applied twice, when 50% and 75% of the berries were colored, respectively, with an interval of two weeks.

Table 1

Climatic information of the experimental site provided by the National Institute of Meteorology

	Growing-season (August – February)	
	2017/2018	2018/2019
Accumulated Precipitation (mm)	940	1040
Minimum temperature (°C)	12	11
Average temperature (°C)	19	20
Maximum temperature (°C)	29	30

Harvest and winemaking

In both seasons, the harvest was carried out in mid-February. The overall average soluble solids content was 18° Brix and 22° Brix, in the first season (2017/18) and in the second season (2018/19), respectively. After the harvest, the bunches were stored in plastic boxes (20 kg) in a cold chamber (10 – 12 °C) for 24 hours. Twenty-four hours after the harvest, the bunches of each plot were manually destemmed; a sample of 500 g for each repetition. Then the berries were manually crushed and placed in mini fermentation tanks (500 mL) at 21 °C. Subsequently, 10 g/hL of potassium metabisulfite was added and, after one hour, *Saccharomyces cerevisiae* yeast (10 g/100 L) was added. The alcoholic fermentation was monitored by measuring the density at 20 °C and weighing daily until stabilization, at which time the grape pomace was reassembling. After stabilization, the grape pomace was removed with the aid of voil bags.

Two replicates were joined together forming composite samples, resulting in 3 replications. Thereafter, slow fermentation took place, during which the mass was measured daily. After racking, malolactic fermentation occurred, and was determined by paper chromatography (OIV, 2015). Subsequently, potassium metabisulfite (10g/100 L) was added, and tartaric stabilization occurred in a cold chamber for 30 days with temperature control (0 °C). After tartaric stabilization, manual bottling, labeling and storage in a refrigerated environment (-20° C) were performed.

Phenolic compounds of grape berry and wine

The assessment of chromatic characteristics - lightness, chroma and hue angle -, total anthocyanins content and total polyphenol content analyses were performed both in the berry skin samples and in the wine. The total polyphenol content was determined according to the method of Singleton and Rossi (1965) with modifications. Results were expressed in mg equivalent of gallic acid/L.

Total anthocyanin quantification was performed as described by Lee and Francis (1972) with adaptations. The content of 1 g of grape skin (or 1 mL of wine) was removed from the ultrafreezer (-20 °C) and macerated in porcelain crucibles with 10 mL of extracting solution. Absorbance was measured at 535 nm using a spectrophotometer UV 1650 PC (Shimadzu, Kyoto, Japan), and the values were expressed in mg of anthocyanins per 100 g of plant material. Quantification of the total anthocyanins content was based on the following equation: $(VA \cdot FD) / 98.2$; VA is the absorbance value and FD is the dilution factor.

The chromatic characteristics (CIELab) of wine were determined by using a colorimeter, chroma meter CR-400/410 model (Minolta, Osaka, Japan), and three measurements were performed. L* represents the lightness of a surface (L*=100 corresponds to white; L*=0 corresponds to black); a* represents the color hue from green (a<0) to red (a>0), and b* represents the color hue from yellow (b<0) to blue (b>0). The values of a* and b* were used to calculate the chroma C, through the formula $C = (a^{*2} + b^{*2})^{1/2}$, and the hue angle h* (Carreño *et al.*, 1995).

Enzyme activities of the grape berry

Four bunches of grapes were collected per plot for the enzymatic analysis. The berry skin was frozen in liquid nitrogen in order to stop all metabolic reactions. These samples were stored in a freezer at -20°C for further analysis. The enzymatic extract obtained was used in the analysis of peroxidase (POD), polyphenoloxidase (PPO) and phenylalanine ammonia-lyase (PAL) activities.

The specific activity of peroxidase (POD) (E.C. 1.11.1.7) was determined according to the methodology of Urbanek *et al.* (1991) with adaptations. The unit of activity is equivalent to an increase by 0.01 times the amount of enzyme to 1 g of fresh weight/min (Fu *et al.*, 2011). The specific activity of polyphenoloxidase enzyme (PPO) (E.C1.10.3.2) was determined according to the method of Gaillard *et al.* (1993), with

modifications. A unit of PPO activity is equivalent to an increase in 0.001 times the amount of enzyme to 1 g of fresh weight/min (Kose *et al.*, 2011). The activity of phenylalanine ammonia-lyase enzyme (PAL) (E.C. 4.3.1.5) was determined according to Rodrigues *et al.* (2006). A unit of PAL activity is equivalent to an increase of 0.01 times the amount of enzyme to 1 g of fresh weight/h (Fu *et al.*, 2011).

Individual wine anthocyanins

The analysis of individual anthocyanins was performed by liquid chromatography coupled to mass spectrometry (LC-ESI-QTOF-MS/MS) in a UFLC Prominence system (Shimadzu, Japan) coupled to an Impact HD mass spectrometer (Bruker Daltonics, Bremen, Germany). These compounds were determined by the methods of Hoffmann *et al.* (2016) and Triches *et al.* (2020), with modifications. Anthocyanins were separated using C18 pre-column (2.0 x 4 mm) and Bidentate C18 column (100 x 2.1 mm, MicroSolv Technology Corporation, Leland, NC, USA), and 0.1% formic acid solution was in water (mobile phase A) and 0.1 % formic acid in acetonitrile (mobile phase B). The elution gradient used was as follows: 0-2 min, 10% B; 2-15 min, 10-75% B; 15-21 min 90% B; 21-30 min, 10% B. Other chromatographic conditions were: flow rate of 0.2 mL/min; injection volume of 10 μ L; column temperature of 40 $^{\circ}$ C. The mass spectrometer operated in ESI positive mode, with spectra acquired over a mass range from 50 m/z to 1200. The characteristics were: capillary voltage at 3.5 kV; drying gas temperature, 180 $^{\circ}$ C; drying gas flow, 8.0 L/min; nebulization gas, 2 bar; RF collision, 150 Vpp; transfer time of 70 μ s and pre-pulse storage, 8 μ s. The intensity of the collision energy was adjusted for automatic MS/MS experiments according to the m/z ratios: m/z 100, 15 eV; m/z 500, 35 eV; m/z 1000, 50 eV, and using nitrogen as collision gas.

Data were analyzed using data analysis 4.2 software (Bruker Daltonics, Bremen, Germany). The elemental composition of each compound was selected according to the exact masses and isotopic pattern using the Smart Formula tool (Bruker Compass DataAnalysisTM), which provides a list of possible molecular formulas combining the exact mass and isotopic distribution reflected in their error values (ppm) and mSigma, respectively.

Anthocyanins were characterized in terms of the fragmentation pattern in the UV/VIS (220-800 nm) and compared with data from databases (Metlin, MassBank, Kegg) and with the literature (Delgado de la Torre *et al.*, 2015; Willemsse *et al.*, 2015). The calibration curve was made with pelargonidin standard (0.2 to 30 μ g/mL) in methanol. The anthocyanins present in the samples were quantified

using the peak area of pelargonidin and its concentration. The results were expressed in mg/L.

Statistical analysis

Data normality was tested by the Kolmogorov-Smirnov test at 5% significance level. Variance analysis (ANOVA) was used for the treatment of the data from each season, and the means were compared using the Scott Knott test, at the level of 95% probability. Principal Component Analysis (PCA) was performed using all variables in order to analyze the relationship between the analyzed characteristics. The software used was R 4.0.3 (R Core Team, 2020), using the ExpDes.pt and FactoMiner package.

RESULTS AND DISCUSSION

Effects of plant regulators and biostimulants on phenolic compounds, color and enzymatic activity of grape skin

Table II shows the results of total anthocyanins content, total polyphenol content and phenylalanine ammonia-lyase (PAL), polyphenoloxidase (PPO) and peroxidase (POD) activities in the berries skin of 'Cabernet Sauvignon' in the two seasons (2017/18 and 2018/19). The ABA600 (S-ABA 600 mg/L) treatment was significantly higher than the others for the total anthocyanins content in the first season (2017/18), while the two tested doses of *A. nodosum* (AN02 0.2 mL/L; AN04 0.4 mL/L) were higher than the other treatments in the second season (2018/19). Regarding the total polyphenol content, the treatments ABA600 and AN02 obtained the highest values, followed by the treatments ABA400 (S-ABA 400 mg/L), AV400 (*A. vera* 400 mL/L) and AN04, in the first season. In 2018/19, AN04 treatments were statistically higher, followed by ABA600, differing from the other treatments.

The AV400 treatment obtained the highest value for PAL activity in the first season, followed by AV200 (*A. vera* 200 mL/L) treatment and the doses of *A. nodosum*, differing statistically from the other treatments. In the second season, the AN02 treatment was higher, followed by ABA600 treatment, differing from the other treatments. The control obtained the lowest value for this enzymatic activity.

In the first season, POD activity was significantly higher for the control when compared to the other treatments. However, in the second season, the AV200 treatment was superior to the other treatments, followed by doses of S-ABA and *A. nodosum*. The control and the AV400 treatment presented lower values than the others. For the POD activity, significant differences were observed only in the first season; the AN04 treatment was statistically lower than the others.

Table II

Total anthocyanins content (mg/100 g), total polyphenol content (mg GAE/100 L), PAL (U/g/h), POD (U/g) and PPO (U/g) of 'Cabernet Sauvignon' berries treated in pre-harvest of 2017/18 and 2018/19 seasons with different products

Cabernet Sauvignon	Total Anthocyanins (mg/100 g)		Total Polyphenols (mg GAE/100 L)		PAL (U/g/h)		POD (U/g)		PPO (U/g)
	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18
	TEST	118.43 b	47.51 b	1184.12 c	963.61 c	941.47 c	1933.84 d	33.75 a	0.75 c
ABA400	177.29 b	64.31 b	1466.17 b	861.05 c	979.64 c	2945.29 c	4.70 b	9.30 b	185.50 a
ABA600	278.10 a	51.06 b	1712.33 a	1532.84 b	769.72 c	4732.82 b	3.60 b	14.20 b	193.50 a
AV200	145.21 b	55.72 b	1271.30 c	509.76 c	1297.70 b	3905.85 c	4.64 b	23.95 a	176.00 a
AV400	157.33 b	46.20 b	1368.74 b	737.84 c	3225.19 a	3002.54 c	12.85 b	3.45 c	197.00a
AN02	123.82 b	100.62 a	1599.52 a	732.84 c	2315.52 b	5769.72 a	4.95 b	11.75 b	152.00 a
AN04	140.12 b	97.45 a	1499.60 b	2102.07 a	2340.96 b	3346.05 c	3.50 b	8.70 b	43.50 b

GAE: gallic acid equivalents; TEST: control; ABA400: S-ABA 400 mg/L; ABA600: S-ABA 600 mg/L; AV200: *A. vera* 200 mL/L; AV400: *A. vera* 400 mL/L; AN02: *A. nodosum* 0.2 mL/L and AN04: *A. nodosum* 0.4 mL/L¹. Average values followed by the same letter in the column are not significantly different by the Scott Knott test ($p < 0.05$).

Figure 1 shows the results of Principal Component Analysis for phenolic compounds and enzymatic activities of 'Cabernet Sauvignon' berries in the 2017/2018 season. The two Principal Components explain 78.3 % of the total variation. PC1 includes phenolic characteristics with a positive score, and enzymatic activities with a negative score. In addition, PC2 shows PAL activity with a negative score, and PPO and POD activities with positive scores.

Correlation between treatments with S-ABA and anthocyanin content and total polyphenols was observed. AV200 treatment is more correlated with POD, and *A. nodosum* was correlated with PAL.

Figure 2 presents the results of Principal Component Analysis for phenolic compounds and enzymatic activities of 'Cabernet Sauvignon' berries in the 2018/2019 season. The two Principal Components explain 79.2 % of the total variation. PC1 presents all phenolic and enzymatic characteristics with positive score. PC2 separates the phenolic characteristics from the enzymatic activities, which exhibit positive and negative scores, respectively.

Treatment AN04 was positively correlated with total polyphenols, as well as ABA600 for total anthocyanins. PAL was positively correlated with ABA600 and AN02 doses. The treatments ABA400 and AV400 and the control were negatively correlated with all variables.

In 'Cabernet Sauvignon', treatments with S-ABA (400 and 600 mg/L) promoted a positive increase in the total anthocyanins content in 2017/2018 season. Similarly, Koyama *et al.* (2014, 2018, 2019) and Yamamoto *et al.* (2015), verified an increase in the total anthocyanins contents in the berries and in the juice of the 'Isabel' grapevine variety. Sun *et al.* (2019) applied S-ABA in the 'Merlot' variety and reported increased values of anthocyanin in the grape berry.

Total polyphenol content was correlated with S-ABA treatments in one of the studied seasons. Pessenti *et al.* (2019, 2020) reported that the anthocyanin and polyphenol contents increased in the grape skin of 'Malbec' and 'Primitivo' varieties with S-ABA 400 or 600 mg/L. For the 'Alachua' table grape and 'Noble' wine, S-ABA treatments also increased the accumulation of anthocyanins and total phenolic compounds (Sandhu *et al.*, 2011).

Sun *et al.* (2019) showed that PAL participates in the flavonoid route, acting as a precursor. These authors found higher expression of this enzyme with 600 mg/L of S-ABA, which increased the contents of polyphenols and total anthocyanins. In this work, the doses of *A. nodosum* were correlated in both seasons with the PAL activity, contributing to the increase of flavonoids' biosynthesis.

Froni *et al.* (2018) conducted an experiment in which *A. nodosum* extract was applied to 'Cabernet Franc' and 'Pinot Noir' grapes. It was found an increase in the levels of anthocyanins and total polyphenols in relation to the control. Similar results were found in this study with the application of *A. nodosum*. These authors point out that the application at the beginning of maturation (*veraison* stage), which promoted the increase of anthocyanins, may be related to the phenylpropanoid route induced by higher sugar content, generating an increase in secondary metabolism, including anthocyanins and other flavonoids. Viencz *et al.* (2020) observed an increase of phenolic compounds of 'Irati' plums, with positive linear effect according to the doses of the product containing the seaweed extract *A. nodosum*.

The PAL, PPO and POD showed higher activity with treatments with *A. nodosum* at one season (Table II). Figures 1 and 2 show a positive correlation of *A. nodosum* with the doses of PAL. Fan *et al.* (2011), observed higher phenolic content associated with the higher doses of *A. nodosum* extract in spinach, which was assigned to higher antioxidant capacity due to increased activity of antioxidant enzymes.

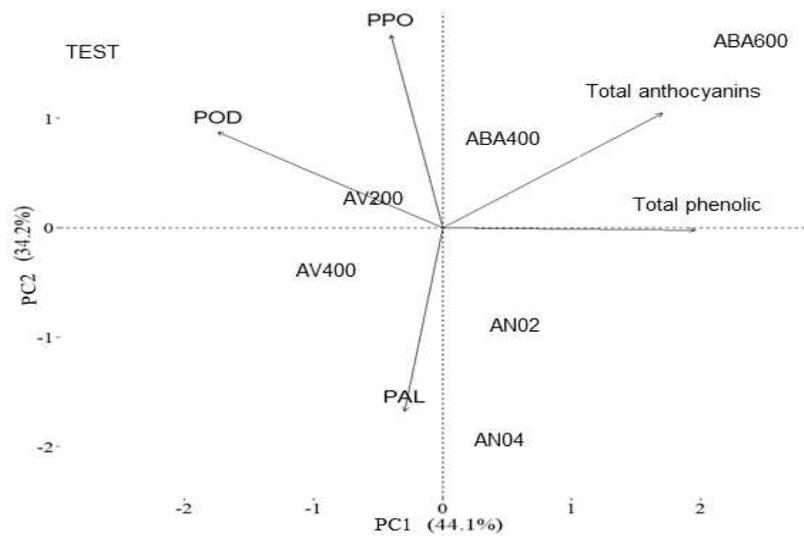


Figure 1. Principal Component Analysis for phenolics and enzymatic activities of 'Cabernet Sauvignon' grapes in 2017/18. PAL (Phenylalanine Ammonia-Liase), PPO (Polyphenoloxidase), POD (Peroxidase). TEST: control; ABA400: S-ABA 400 mg/L; ABA600: S-ABA 600 mg/L; AV200: *A. vera* 200 mL/L; AV400: *A. vera* 400 mL/L; AN02: *A. nodosum* 0.2 mL/L and AN04: *A. nodosum* 0.4 mL/L.

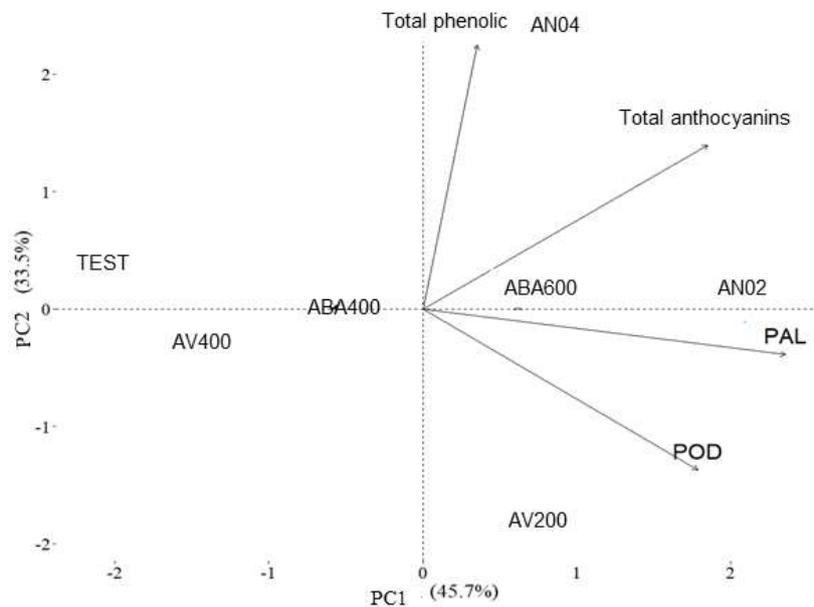


Figure 2. Principal Component for phenolics and enzymatic activities of 'Cabernet Sauvignon' grapes in 2018/19. PAL (Phenylalanine Ammonia-Liase), POD (Peroxidase). TEST: control; ABA400: S-ABA 400 mg/L; ABA600: S-ABA 600 mg/L; AV200: *A. vera* 200 mL/L; AV400: *A. vera* 400 mL/L; AN02: *A. nodosum* 0.2 mL/L and AN04: *A. nodosum* 0.4 mL/L.

Taskos *et al.* (2019) reported an increase of 21% of anthocyanins, 26% in yield and 35% in the number of berries in 'Merlot' grapes treated with *A. nodosum*. Santaniello *et al.* (2017) applied AN

solution in *Arabidopsis thaliana* and observed an increase in the contents of anthocyanins and total polyphenols, along with the increase of POD activity.

Sogvar *et al.* (2016) observed that anthocyanin content increased in strawberries submitted to AV application during storage. The same authors found that the total polyphenol content increased in strawberries submitted to the application of *A. vera* gel during storage. Castillo *et al.* (2010) performed the pre-harvest application with *A. vera* gel in 'Autumn Royal' grapes, and did not find significant differences for chemical characteristics, such as soluble solids, pH and titratable acidity, but it was observed that the color change by the higher hue angle value.

Effects of plant regulators and biostimulants on the phenolic compounds and color of wine

The phenolic and chromatic characteristics of 'Cabernet Sauvignon' wine in the 2017/2018 and 2018/2019 seasons are presented in Table III. The treatments with the two doses of *S*-ABA, AV400 and AN02 significantly increased the total anthocyanins content in the first season, followed by the other treatments. In the second season, ABA400 treatment and the two doses of *A. nodosum* and *A. vera* gel induced higher levels of total anthocyanins than ABA600 and control.

Regarding the total polyphenol content, in the first season, the treatments ABA400 and AV400 promoted higher averages than the other treatments. In the 2018/19 season, the AV400 treatment had higher effect on the total polyphenol content, followed by treatments with the two doses of *A. nodosum* and AV200.

For L*, the AV200 treatment was statistically superior to the other treatments, followed by the control, AN04, AN02, ABA600, AV400 and ABA400, in the first season. In the second season, the control was associated with the highest L* value, followed by AN04, AV400, AV200, AN02, and finally, the two doses of *S*-ABA.

For h*, in the first season, the control, AV400 and AN04 obtained higher averages, followed by ABA600, AN02, AV400 and ABA400. In the second season, ABA600 treatment obtained the lowest mean, differing significantly from the other treatments.

For C, the results were similar to those of h* in the first season, with higher averages for the control, AV400 and AN04. In the second season, the control and AN04 treatments were higher, followed by the other treatments, and ABA600 obtained the lowest average.

Figure 3 shows the results of Principal Component Analysis for the phenolic and chromatic characteristics of 'Cabernet Sauvignon' wine in the 2017/2018 season. PC1 and PC2 explain 96.4% of the total variation. PC1 includes chromatic characteristics with a positive score, and phenolic characteristics with a negative score. PC2 separate all characteristics with a positive score, except for the total polyphenol content.

Figure 4 presents the Principal Component Analysis (PCA) for the phenolic and chromatic characteristics of 'Cabernet Sauvignon' wine in the 2018/2019 season. PC1 and PC2 explain 86.1 % of the total variation. PC1 includes chromatic characteristics and phenolic compounds with a positive score. PC2 separate all phenolic characteristics, with a positive score, from the chromatic characteristics, with a negative score. A positive correlation between phenolics and the treatments of *A. nodosum* and AV400 was observed. The ABA600 treatment was negatively correlated with anthocyanins and polyphenols. The chromatic characteristics were positively correlated with the AV200 treatment.

Table III

Total anthocyanins (mg/100g), total polyphenols (mg GAE/100 L), lightness, chroma and hue angle of 'Cabernet Sauvignon' wine resulting from the treatments performed in the 2017/18 and 2018/19 seasons

Cabernet Sauvignon	Total Anthocyanins (mg/100 g)		Total Polyphenols (mg GAE/100 L)		L*		H*		C	
	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
	TEST	90.52 b	41.030 b	861.05 b	130.28 d	19.53 b	24.01 a	32.70 a	34.02 a	61.80 a
ABA400	100.40 a	50.30 a	1727.71 a	150.79 d	14.01 g	19.14 f	27.35 d	32.32 a	52.57 d	60.16 b
ABA600	116.90 a	44.50 b	1255.92 b	89.25 d	17.45 e	13.69 g	31.12 b	26.69 b	58.20 b	52.67 c
AV200	75.15 b	51.01 a	1173.87 b	235.4 c	19.93 a	20.31 d	32.35 a	34.60 a	61.57 a	60.41 b
AV400	112.93 a	49.28 a	1796.94 a	545.60 a	16.09 f	20.93 c	29.76 c	33.03 a	55.89 c	62.36 b
AN02	105.80 a	52.13 a	1135.41 b	404.64 b	17.58 d	19.71 e	31.05 b	32.63 a	58.34 b	61.36 b
AN04	86.96 b	57.63 a	948.28 b	286.69 c	19.35 c	23.09 b	32.47 a	33.56 a	61.90 a	65.01 a

GAE: gallic acid equivalents; TEST: control; ABA400: *S*-ABA 400 mg/L; ABA600: *S*-ABA 600 mg/L; AV200: *A. vera* 200 mL/L; AV400: *A. vera* 400 mL/L; AN02: *A. nodosum* 0.2 mL/L and AN04: *A. nodosum* 0.4 mL/L¹. Average values followed by the same letter in the column are not significantly different by the Scott Knott test (p<0.05).

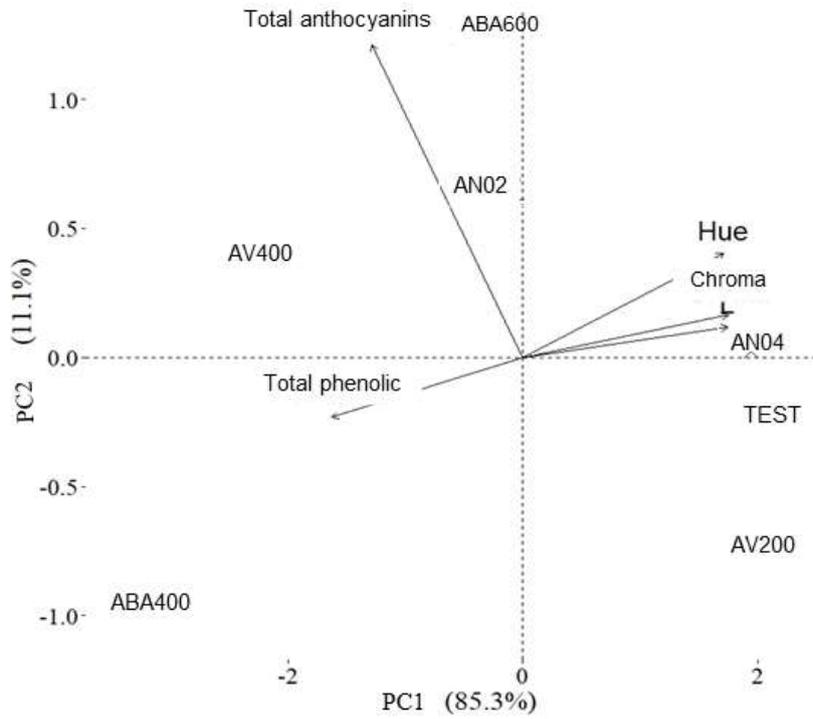


Figure 3. Principal Component Analysis for the phenolics and chromatic characteristics of 'Cabernet Sauvignon' wine in 2017/18. TEST: control; ABA400: S-ABA 400 mg/L; ABA600: S-ABA 600 mg/L; AV200: *A. vera* 200 mL/L; AV400: *A. vera* 400 mL/L; AN02: *A. nodosum* 0.2 mL/L and AN04 *A. nodosum* 0.4 mL/L.

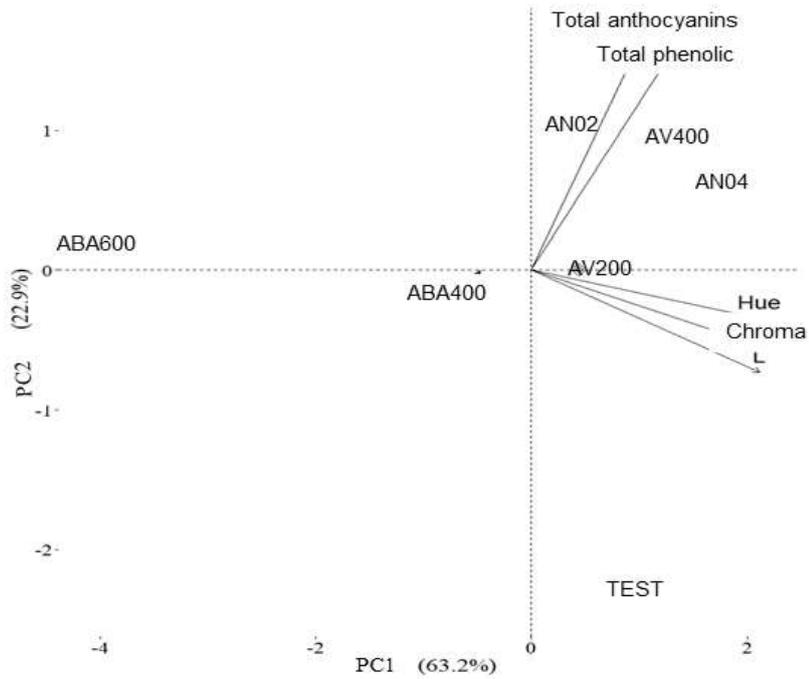


Figure 4. Principal Component Analysis for the phenolics and chromatic characteristics of 'Cabernet Sauvignon' wine in 2018/19. TEST: control; ABA400: S-ABA 400 mg/L; ABA600: S-ABA 600 mg/L; AV200: *A. vera* 200 mL/L; AV400: *A. vera* 400 mL/L; AN02: *A. nodosum* 0.2 mL/L and AN04 *A. nodosum* 0.4 mL/L.

Individual anthocyanins in wine

Table IV shows the results for individual anthocyanins of wine in the 2017/2018 season. The treatments with ABA400 and AV400 were significantly superior for the compounds: Malv3G, Peon3G, Delp3,6AcG, Peo3,6AcG and for

Petu3pcG. For AN04, the anthocyanins that showed the highest levels were cyanidin-3-glycoside, peonidin-3-glycoside, delphinidin-3-glycoside, petunidin-3-glycoside and delphinidin-3,6-acetyl-glycoside. For the AN02 treatment, petunidin-3-glycoside was the only compound with significantly higher content.

Table IV

Individual anthocyanins of 'Cabernet Sauvignon' wine resulting from the treatments performed in 2017/18

Cabernet Sauvignon	Individual Anthocyanins (mg/L)							
	Cyan3G	Malv3G	Peon3G	Delp3G	Petu3G	Delp3,6AcG	Peon3,6AcG	Petu3pcG
TEST	0.771 c	7.397 b	1.162 c	0.974 c	0.839 a	0.819 a	1.079 b	0.759 b
ABA400	0.779 b	7.799 a	1.339 a	0.992 b	0.829 b	0.847 a	1.160 a	0.762 a
ABA600	0.760 d	7.193 c	1.244 b	0.918 e	0.834 b	0.830 a	1.080 b	0.759 b
AV200	0.778 b	4.776 d	0.886 d	1.008 b	0.820 c	0.779 c	0.852 d	0.755 c
AV400	0.782 a	7.246 c	1.327 a	1.057 a	0.840 a	0.822 a	1.049 c	0.760 b
AN02	0.777 b	7.431 b	1.135 c	0.997 b	0.837 a	0.804 b	1.033 c	0.759 b
AN04	0.770 c	7.305 e	0.849 d	0.950 d	0.818 c	0.777 c	0.767 e	0.756 c

Cyan3G: Cyanidin-3-Glucoside; Malv3G: Malvidin-3-Glucoside; Peonidin-3-Glucoside: Peon3G; Delp3G: Delphinidin -3-Glucoside; Petu3G: Petunidin-3-Glucoside; Delp3,6AcG: Delphinidin-3,6-Acetyl-Glucoside; Peon3,6AcG: Peonidin-3,6-Acetyl-Glucoside; Petu3pcG:Petunidin-3-(p-coumaril)-Glucoside

Figure 5 shows the results of Principal Component Analysis for individual anthocyanins in 'Cabernet Sauvignon' wine in 2017/2018 season. The two Principal Components explain 91.1 % of the total variation. PC1 includes all individual anthocyanins with a positive score. In addition, PC2 shows cyanidin and delphinidin with a positive score, and other anthocyanins with a negative score.

The treatments ABA600, AN04 and AV200 were negatively correlated with individual anthocyanins, and the AV400 treatment was positively correlated with delphinidin and cyanidin. ABA400 treatment was correlated with other individual anthocyanins.

The positive effects of biostimulants on ecophysiology and their performance in secondary metabolism, demonstrated in some studies (Khan *et al.*, 2009; Fan *et al.*, 2011, 2014), are explained by the presence of bioactive compounds, such as peptides and phenolics. However, the mode of action of the biostimulating molecules present in algae extracts remains largely unknown. Few studies have been conducted on the effects of *A. nodosum* on ecophysiology and the partition of secondary metabolites in *V. vinifera* (Salvi *et al.*, 2019). Gutiérrez-Gamboa *et al.* (2020) evaluated the use of

low and high doses of *A. nodosum* in grapes 'Tempranillo Blanco', verifying that high concentrations of *A. nodosum* promoted the increase of leucoanthocyanidin reductase and anthocyanidin reductase, which are involved in the biosynthesis of anthocyanins.

Treatments with Aloe vera promoted an increase in the content of anthocyanins and total polyphenols in both seasons and with at least one dose. This effect also occurred in individual anthocyanins, such as cyanidin, delfinidin and delfinidin-3,6-glucoside.

The color of red wine is essentially due to the release of pigments from the skin of grape berries during the winemaking process. For most red wine grapes, anthocyanins are mainly located in the third or fourth layer of cells closest to the outer berry skin and little in pulp cells (He *et al.*, 2010; He *et al.*, 2012). Thus, the exogenous application of the products studied in this work seemed to positively affect the biosynthesis of flavonoids in the grape skin, increasing the content of polyphenols and anthocyanins, which were then released to the must/wine through the maceration stage during winemaking.

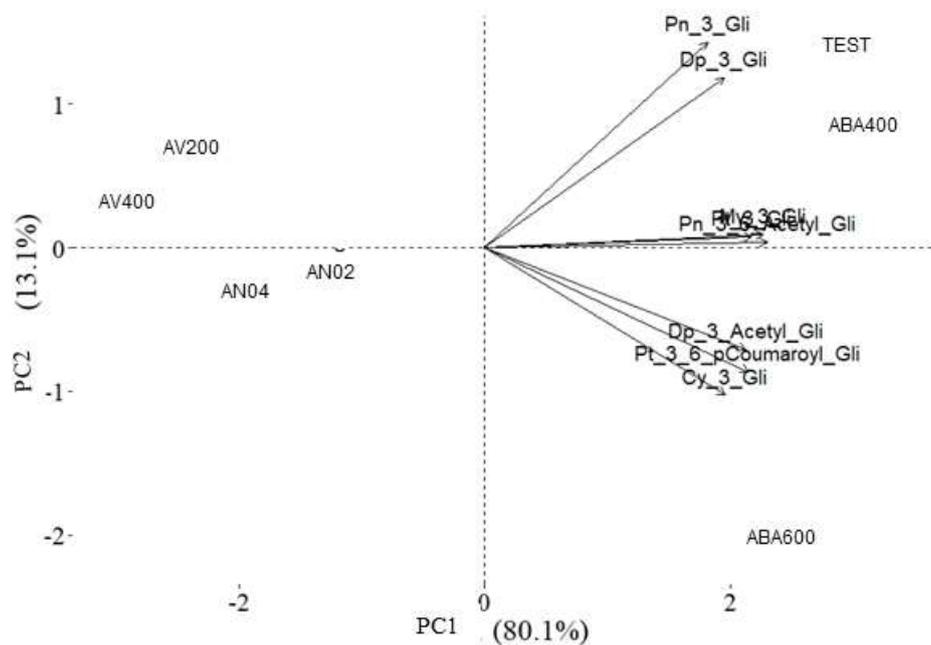


Figure 5. Principal Component Analysis for individual anthocyanins of 'Cabernet Sauvignon' wine in 2017/18. TEST; ABA400: 400 mg/L; ABA600: *S*-ABA 600 mg/L; AV200: *A. vera* 200 mL/L; AV400: *A. vera* 400 mL/L; AN02: *A. nodosum* 0.2 mL/L and AN04: *A. nodosum* 0.4 mL/L.

Several authors have studied the use of hormone regulators and biostimulants, such as seaweed extract and *Aloe vera* gel in red grapes. Gutiérrez-Gamboa and Moreno-Simunovic (2021) stated that the mechanisms of action involved in these processes are not fully understood. The use of seaweeds in viticulture allowed to improve grapevine productivity and to enhance grape and wine quality, mostly in terms of their phenolic composition. More studies are still needed to elucidate in detail the secondary metabolism of the grapes and its interference in the final quality of the wine.

CONCLUSIONS

In these experimental conditions, the application of *S*-ABA 600 mg/L promoted the increasing of phenolic content, chromatic characteristics and enzymatic activities of 'Cabernet Sauvignon' grapes. Regarding the wine composition, two doses of *S*-ABA increased the phenolic content and individual anthocyanins, confirming the results found in the grape skin.

A. vera 200 mL/L increased the phenolic content and the polyphenoloxidase activity in the 'Cabernet Sauvignon' grapes. In wine, the best results for monomeric anthocyanins and phenolic content were

associated with the treatment of *A. vera* with 400 ml/L. These results indicate that *Aloe vera* gel is a promising tool for viticulture, but further studies are needed.

Ascophyllum nodosum in pre-harvest had few influence on the phenolic compounds of the wines. Further studies are needed to characterize its influence on grape metabolism and wine quality.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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