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Gibberellic acid in the 'Cabernet Sauvignon' grape: effects on grape cluster morphology and wine quality

Ácido giberélico na uva 'Cabernet Sauvignon': efeito na morfologia do cacho e na qualidade do vinho

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ABSTRACT

'Cabernet Sauvignon' is a commonly produced and consumed variety of grapes in Brazil, although late sprouting and flowering negatively affect wine production, hindering the production of elegant and balanced wines, especially in high-altitude regions of Santa Catarina. Therefore, this study sought to evaluate the effects of gibberellic acid (GA_3) on reducing and eliminating 'Cabernet Sauvignon' grape berry seeds and evaluate the physicochemical parameters of the wine produced in *Serra Catarinense*. The experiments were conducted in a commercial vineyard. The treatments were 0.0, 40.0, and 80.0 mg L^{-1} of GA_3 in the 2019/20 season and 0.0, 20.0, and 40.0 mg L^{-1} of GA_3 in the 2020/21 season. The applications were performed in full bloom. The characteristics evaluated were cluster weight, berry diameter, berry mass, number of seeds per berry, seed mass, peel:pulp ratio, cluster length, cluster compaction index, rachis mass, and berries per cluster. In both harvests, the zero dose of GA_3 was related to greater cluster compaction, berry and cluster mass, and seeds per berry. In the 2019/20 harvest, 80 mg L^{-1} of GA_3 was correlated to must total soluble sugar, wine acidity, must acidity, color parameter at 520 nm, and color intensity. In the last harvest, the color parameters were more correlated with the highest GA_3 dose, showing that this growth regulator increased free anthocyanins and total polyphenols. Moreover, 20–80 mg L^{-1} of GA_3 reduced the number and size of seeds but increased the tannin content in one harvest. Bunch compaction, cluster weight, diameter, and berry weight were reduced using GA_3. Lastly, 20 and 40 mg L^{-1} of GA_3 increased the total polyphenols and monomeric anthocyanins in the wine.

KEYWORDS: tannins; growth regulator; berry.

RESUMO

A variedade 'Cabernet Sauvignon' é uma das mais produzidas e consumidas no brasil, apesar disso a brotação e floração tardia trazem problemas na produção de vinhos dessa variedade, ocasionando características que dificultam a produção de vinhos elegantes e equilibrados, em especial nas regiões de altitude de Santa Catarina. Este trabalho teve como objetivo avaliar os efeitos do ácido giberélico na diminuição e eliminação das sementes nas bagas de uva 'Cabernet Sauvignon' e avaliar os parâmetros físico-químicos do vinho produzidos na Serra Catarinense. Os experimentos foram realizados em um vinhedo comercial da cv. Cabernet Sauvignon, enxertados em porta-enxerto 'Paulsen 1103', com 20 anos de idade. Os tratamentos foram: 0,0 mg L^{-1} de ácido giberélico (AG_3); 40,0 mg L^{-1} de AG_3; e 80,0 mg L^{-1} de AG_3, na safra 2019/20; e 0,0 mg L^{-1} de AG_3; 20,0 mg L^{-1} de AG_3; e 40,0 mg L^{-1} de AG_3, na safra 2020/21. As aplicações foram realizadas na plena floração. As características avaliadas nas uvas foram peso do cacho, diâmetro da baga, massa de bagas, número de sementes por baga, massa de sementes, número de sementes por cachos, comprimento de cacho, índice de compactação de cacho, massa da ráquis e bagas por cacho. Nas duas safras, a dose zero de AG_3 está relacionado com maior compactação do cacho, massa de baga e de cacho, sementes por baga. Por outro lado, na safra 2019/20 a dose de 80 mg L^{-1} de AG_3 apresentou correlação com SST do mosto, acidez do vinho, acidez do mosto, além do parâmetro de cor a 520 nm e intensidade de cor. Na última safra, os parâmetros de coloração foram mais correlacionados com a dose maior de AG_3, evidenciando que este fitorregulador aumentou as antocianinas livres e consequentemente os polifenóis totais. O AG_3 nas doses de 20 a 80 mg L^{-1},

reduziram o número e o tamanho de sementes, entretanto aumentaram o teor de taninos no vinho em uma das safras. A compactação do cacho, bem como peso de cacho, diâmetro e peso de bagas foram reduzidas com o uso do AG_3. O AG_3 nas doses de 20 e 40 mg L^{-1} aumentaram o teor de polifenóis totais e antocianinas monoméricas totais no vinho.

PALAVRAS-CHAVE: taninos; regulador de crescimento; baga

INTRODUCTION

The 'Cabernet Sauvignon' vine (*Vitis vinifera L.*) originated in Bordeaux (France) in 1929, as a result of crossbreeding between 'Cabernet Franc' and 'Sauvignon Blanc' (BOWERS & MEREDITH 1997). This grape variety is cultivated in various countries and adapts well to different edaphoclimatic conditions. In some cases, it presents late sprouting and maturation, and compared to other *vinifera* varieties, it stands out given its varietal characteristics. Moreover, this cultivar's physical and chemical composition favors the production of quality wines with good aging potential (ROBERTO et al. 2005). It has medium-sized clusters with small berries, sugar levels that can exceed 20° Brix, and an average acidity of 120 mEq L^{-1}, which is suitable for quality winemaking, with wines characterized by a reddish color and accentuated violet reflections (RIZZON & MIELE 2002).

Wines produced with this variety usually have 2-methoxy-3-isobutylpyrazine among their volatile compounds, a substance responsible for referring to the vegetable aroma of green bell pepper. This methoxypyrazine is generally present in wine from vineyards located in regions of higher altitudes and lower temperatures during maturation, such as *Serra Catarinense* (FALCÃO et al. 2007).

In 2000, the 'Cabernet Sauvignon' variety began to be produced in Santa Catarina State (southern Brazil) to produce fine wines. However, due to climate characteristics, including low temperatures at the end of maturation because of the region's high altitude, the maturation process of this variety is delayed (FELIPPETO et al. 2016, WURZ et al. 2019); in fact, sometimes the phenolic maturation is not even completed, resulting in highly astringent wines.

The phenolic compounds are substances of secondary metabolisms with important functions for the plants, possessing immense chemical diversity in various plant functions (TESZLÁK et al. 2005, KENNEDY 2008, RIENTH et al. 2021). In grape skins and seeds, phenolic compounds are present at higher concentrations, conferring wines a higher quality and characteristics such as flavor and color (KENNEDY 2008). Tannins, also called proanthocyanidins, are polymers responsible for astringency, which is the sensation caused by the reaction of wine tannins with proteins in saliva, causing a momentary loss of lubrication in the mouth (TYAGI et al. 2022, KENNEDY 2008). These wines then need to go through the aging process, in which the transformation of astringent tannins takes place, making these astringents softer, leading to wine with a "softness" characteristic, and thereby becoming more palatable (McRAE & KENNEDY 2011). The wine maturation process is slow and costly for winemakers; it is usually carried out using oak barrels, a process that, if it were faster, could improve profitability by making it possible to offer the product in less time.

This study aimed to evaluate the effects of gibberellic acid (GA_3) on reducing and eliminating 'Cabernet Sauvignon' grape berry seeds and evaluate the physicochemical parameters of the wine produced in *Serra Catarinense*.

MATERIAL AND METHODS

The experiments were carried out in a commercial vineyard in São Joaquim (Santa Catarina State, southern Brazil; 28°15'30"S and 49°57'21"W, 1250 m) that produces 'Cabernet Sauvignon' grapes, which were grafted on 'Paulsen 1103' rootstock, in the 2019/20 and 2020/21 growing seasons. The vineyards were planted in 2005. The climate in the region is classified as humid mesothermal (Cfb) according to the Köppen classification (PEEL et al. 2007), and the soil is a *Cambissolo Húmico* (EMBRAPA 2004). The weather conditions during the experiment were monitored by the automatic meteorological station of São Joaquim (28º16'S, 49º56'W, 1410 m), and data were collected from the National Institute of Meteorology database. The climate parameters monitored were average air temperature (ºC), relative air humidity (%), and rainfall (mm).

The evaluated treatments were 0.0, 40.0, and 80.0 mg L^{-1} of gibberellic acid (GA_3) for the 2019/20 season and 0.0, 20.0, and 40.0 mg L^{-1} of GA_3 for the 2020/21 season. The GA_3 doses in the second season were reduced due to the results of the first one. The experiments were conducted in a randomized block design, with three blocks and five plants per block, considering only the three plants inside each block for evaluation. A plant growth regulator (ProGibb 400®) was used as a source of GA_3, which contains 400 g kg^{-1}

of GA_3. In both seasons, GA_3 applications were made by immersing the clusters for five seconds in solutions with different GA_3 concentrations in the phenological stage of full bloom (BBCH 65) in all tree clusters of each block (LORENZ et al. 1995); the applications were made on 11/08/2019 (2019/20 season) and 11/13/2020 (2020/21 season).

The grapes were harvested on 03/11/2020 and 03/05/2021 and sent for laboratory analyses: A) cluster weight: obtained by weighing 10 clusters per repetition on an analytical scale (Marte) and measured in grams (g); B) berry diameter: obtained by transversely measuring 30 berries per repetition using a digital caliper (Zaas) and measured in centimeters (cm); C) berry mass: obtained by weighing 100 berries per repetition and measured in grams (g); D) number of seeds per berry, which were obtained by counting the number of seeds of 100 berries per repetition and expressed in seed per berry; E) seed mass: obtained by weighing 30 seeds per repetition on an analytical scale and measured in grams (g); F) cluster length: obtained by measuring 10 bunches per repetition using a digital caliper, and measured in centimeters (cm); G) cluster compaction index: obtained by the formula [(cluster mass)/(cluster length)²] proposed by TELLO & IBANEZ (2014); H) rachis mass: measured by weighing the rachis of 10 bunches per block on an analytical scale and measured in g; I) berries per cluster: obtained by counting the number of berries in 10 clusters per repetition and expressed in berries $bunch^{-1}$; and J) peel:pulp ratio: measured by dividing the peel mass by the pulp mass of 30 berries.

The grapes underwent microvinification from the destemming onwards, while automatic equipment carried out berry crushing. While obtaining the must, antioxidant SO_2 was added at a concentration of 25 mg L^{-1} (potassium metabisulphite, EVER®) and pectinolytic enzymes 0.02 mg L^{-1} (Lafazyn extract Laffort®). The musts were fermented separately according to each treatment using yeasts (*Saccharomyces cerevisiae*) in a proportion of 0.25 g L^{-1} (Myco Ferm IT Fruity Flavor). The maceration time was five days with two daily pumping-overs.

Fermentation was conducted under controlled temperature conditions (20 °C) and monitored daily by a specific gravity tester densimeter. After the alcoholic fermentation, the wines were clarified using bentonite in the proportion of 3 g L^{-1}. For this experiment, we chose not to do the malolactic fermentation, adding 50 mg L^{-1} of SO_2 for preservation (potassium metabisulfite, EVER®). Thus, the treatments proceeded to cold tartaric stabilization (7 days at 0 °C). Afterward, they were bottled in dark 750-mL bottles and sealed with cork stoppers.

Thirty days later, the wines were analyzed regarding enological parameters of total acidity (mEq L^{-1}), pH, alcohol by volume (% vol), residual sugar (g L^{-1}), density, volatile acidity (mEq L^{-1}), free sulfur dioxide (mg L^{-1}) and total sulfur dioxide (mg L^{-1}), according to the methods of the International Organization of Vine and Wine (OIV 2020).

The wines were characterized by a UV-Vis spectrophotometer for phenolic composition and color parameters. The total polyphenol content of the wine was determined using the Folin-Ciocalteu method, as described by SINGLETON & ROSSI (1965), by colorimetric reaction and absorbance reading at 760 nm. Results were expressed in mg L^{-1} gallic acid. The color parameters were determined according to GLORIES (1984) from the absorbance of the sample at 420, 520, and 620 nm. Total monomeric anthocyanins were determined by the differential pH method according to GIUSTI & WROLSTAD (2001).

The total polyphenol index was also determined by direct reading at 280 nm of the sample diluted at 1% from the total polyphenol index; tannin content was indirectly calculated according to the method of RIZZON (2010). Total tannins (g L^{-1}) were analyzed by the method described by RIZZON (2010) based on the property of monomeric or polymerized proanthocyanidins to generate anthocyanins by heating in an acid medium.

The results obtained were tested for the normality of the errors using the Shapiro-Wilk test, and those which did not show normality were transformed using the formula $\sqrt{x} + 0.5$. The data were submitted to analysis of variance ($p<0.05$) and regression test using the SISVAR software (FERREIRA 2014). All data were submitted to principal component analysis (PCA) using the PAST 4.0b software to provide an overview of the results. Before the PCA, the data matrix was auto-scaled for each variable to obtain the same weight for all variables (mean = 0 and variance = 1).

RESULTS AND DISCUSSION

The GA_3 can have different effects depending on the phenological stage of application. If applied before flowering, it increases the length of the cluster, when applied during flowering, it reduces the fruit set and, when applied after flowering, increases the size of berries in seedless grapes (GIANFAGNA 1995, SANTOLALLA & ESCOBEDO 2018, GAO et al. 2020). In the two evaluated years, GA_3 did not significantly

affect cluster length and rachis mass (Table 1), although it reduced the number of berries per cluster, reaffirming that there is no effect in increasing cluster length when applied during flowering, despite reducing the number of berries per cluster.

Cluster weight was reduced in the two evaluated years with GA_3, with a greater magnitude in the first year due to higher GA_3 doses (Table 1). The reduction in the cluster mass resulted from a berry diameter reduction in the 2019/20 season and a reduction in berry mass in both seasons. The PCA showed a positive correlation of berry mass with dose zero of GA_3 (Figure 1). The GA_3, when applied after flowering, unlike in this study, increases the berry size and, consequently, cluster weight. TYAGI et al. (2022) found greater diameter and weight of berries when GA_3 was applied to 'Sangiovese' grape with 8.7 mm diameter. The effect of a plant hormone on the plant depends on factors such as concentration, type, age, tissue sensitivity, and interaction with other hormones (TAIZ & ZEIGER 2013).

Table 1. Morphological and physicochemical characteristics of 'Cabernet Sauvignon' grapes when Gibberellic acid is applied in different doses during flowering in the 2019/20 and 2020/21 seasons.

	Gibberellic acid (mg L^{-1})									
	2019/2020					2020/21				
	0	40	80	*p-value*	R^2	0	20	40	*p-value*	R^2
Cluster mass (g)	143.8 ±20.7*	64.3 ±12.9	64.5 ±10.7	*0.0003*	*0.75*	92.0 ±8.25*	68.9 ±7.33	75.9 ±6.70	*0.0005*	*0.46*
Berry diameter (cm)	1.28 ±0.04*	1.20 ±0.04	1.15 ±0.09	*0.0145*	*0.98*	1.31 ±0.04^{ns}	1.28 ±0.03	1.28 ±0.02	*0.0945*	-
Berry mass (g)	1.25 ±0.08*	0.91 ±0.09	0.68 ±0.05	*0.0000*	*0.97*	1.37 ±0.12*	1.28 ±0.09	1.27 ±0.08	*0.0799*	*0.86*
Seed $berry^{-1}$	1.38 ±0.13*	1.16 ±0.18	0.85 ±0.07	*0.0106*	*0.99*	1.15 ±0.06^{ns}	0.90 ±0.16	0.93 ±0.21	*0.1209*	-
Seed mass (g of 100 berries)	4.41 ±0.17*	3.62 ±0.76	2.70 ±0.37	*0.0091*	*0.99*	3.37 ±0.41^{ns}	3.42 ±0.24	2.99 ±0.25	*0.2864*	-
Cluster length (cm)	15.6 ±1.51^{ns}	16.00 ±1.51	17.2 ±2.30	*0.4426*	-	16.0 ±1.12^{ns}	16.4 ±0.78	16.6 ±0.87	*0.7626*	-
Cluster compaction index	0.60 ±0.16*	0.26 ±0.07	0.22 ±0.03	*0.0020*	*0.82*	0.35 ±0.03*	0.26 ±0.03	0.28 ±0.04	*0.0092*	*0.55*
Rachis mass (g)	7.48 ±2.15^{ns}	4.55 ±0.42	4.74 ±0.97	*0.1541*	-	3.07 ±0.90^{ns}	2.74 ±0.52	2.82 ±0.29	*0.8251*	-
Berries per cluster	107.0 ±23.0*	65.2 ±10.9	87.2 ±9.36	*0.0310*	*0.22*	77.7 ±4.98*	58.4 ±5.21	58.3 ±7.73	*0.0466*	*0.75*
Peel:pulp ratio	-	-	-			0.24 ±0.01*	0.22 ±0.01	0.20 ±0.03	*0.0688*	*0.97*
pH	3.10 ±0.04^{ns}	3.14 ±0.00	3.10 ±0.00	*0.1800*	-	2.97 ±0.02*	3.03 ±0.01	3.04 ±0.01	*0.0065*	*0.82*
Total soluble solids (° Brix)	21.9 ±0.12*	21.8 ±0.00	22.0 ±0.00	*0.0494*	*0.43*	19.6 ±0.67^{ns}	20.3 ±0.38	20.1 ±0.31	*0.1099*	-
Total acidity (mEq L^{-1})	127.3 ±1.53*	124.7 ±0.58	128.3 ±0.58	*0.0104*	*0.07*	95.0 ±1.00*	85.3 ±2.08	81.0 ±3.46	*0.0051*	*0.95*

* Significant linear regression ($p<0.05$): Cluster mass: $y = -0.3966x + 130.55$ (season: 2019/20); $y = -0.1607x + 87.005$ (s: 20/21); Berry diameter: $y = -0.0006x + 1.2704$ (s: 19/20); Berry mass: $y = -0.0028x + 1.2283$ (s: 19/20); $y = -0.001x + 1.3533$ (s: 20/21); Berry seeds: $y = -0.0026x + 1.3917$ (s: 19/20); Seed mass (g of 100 berries): $y = -0.0086x + 4.4346$ (s: 19/20); Cluster compaction index: $y = -0.0019x + 0.5495$ (s: 19/20); $y = -0.0007x + 0.3339$ (s: 20/21); Berries per cluster: $-0.0988x + 96.375$ (s: 19/20); $y = -0.1942x + 74.542$ (s: 20/21); Total acidity: $y = 0.005x + 126.28$ (s: 19/20); $y = -0.14x + 94.111$ (s: 20/21); Total soluble solids: $y = 0.0007x + 21.822$ (s: 19/20); Peel:pulp ratio: $y = -0.0004x + 0.2413$ (s: 20/21); pH: $y = 0.0007x + 2.9789$ (s: 20/21). ^{ns} = non-significant difference; R^2 = coefficient of determination; ± Standard deviation.

With fewer berries in the cluster, the cluster compaction index was lower using GA_3. This effect has already been reported elsewhere with 'Sauvignon Blanc' and 'Pinot Noir' grapes (SILVA et al. 2018, 2019), and is important in improving cluster aeration and reducing conducive factors to mold incidence in the clusters.

In the first season (2019/2020), the GA_3 at both doses (40 and 80 mg L^{-1} of GA_3) reduced the seed $berry^{-1}$, as well as decreased seed mass, evidencing the effect of exogenous GA_3 in causing apyrenia (Table 1). However, there was a reduction in the 2020/21 season without a significant difference. The GA_3 applied in the pre-flowering and flowering stages inhibits pollen tube penetration into the ovary tissue, even if the stigma has been pollinated with pollen grains with high germination capacity (OKAMOTO & MIURA 2005). CHENG et al. (2013) reported that seed abortion caused by GA_3 is partly due to increased cell damage by reduced enzymatic antioxidant activity and reactive oxygen species accumulation.

SANTOLALLA & ESCOBEDO (2018) analyzed 'Red Globe' grapes and found a reduction in the number of seeds when GA_3 was applied between the beginning of panicle growth and up to 20% of flowering. GAO et al. (2020) found no alteration in 'Cabernet Franc' and 'Cabernet Sauvignon' grape seeds with GA_3. Nevertheless, unlike our study, in which it was applied in full bloom, GAO et al. (2020) applied GA_3 before flowering. The peel:pulp ratio decreased with GA_3, which can be explained by the reduction in pulp growth stimulated by gibberellin to the detriment of a lower stimulus to epidermis cell growth.

The physicochemical characteristics of the grape must, submitted to different doses of GA_3 during flowering, are listed in Table 1. In the 2019/20 season, the total soluble solids and total acidity of the must slightly increased, and higher levels were observed in musts that received 80 mg L^{-1} of GA_3. In the 2020/21 season, GA_3 did not significantly interfere with the total soluble solids content. Notably, the grapes from the 2020/21 season applied with the GA_3 showed lower acidity than the control treatment, regardless of the dosage. As a result, the pH of the 2020/21 season grapes was higher using GA_3. These results demonstrate that new studies must be carried out due to the difference in acidity and soluble solids between seasons. By applying GA_3 to the berry, TYAGI et al. (2022) found no change in the total acidity in 'Sangiovese' grapes, although the researchers found a reduction in sugar content. In 'Bordô' grapes, applying 100 mg L^{-1} of GA_3 14 days after full bloom increased sugar content and reduced acidity (CHIAROTTI et al. 2011), indicating the different effects of GA_3 between cultivars and application times.

The chemical composition of wines obtained from 'Cabernet Sauvignon' grapes applied with GA_3 is listed in Table 2. The physicochemical standards of the wines are in accordance with the identity and quality standards established by Brazilian legislation (BRASIL 2018). Residual sugar content in wine in both seasons was higher with GA_3 applications (Table 2), as the GA_3 likely made it difficult for yeasts to consume sugars during fermentation. However, further research is needed to confirm this. Regardless of the vintage, the wines are classified as dry, according to Brazilian legislation, considering their residual sugar concentration.

Table 2. Chemical characteristics of 'Cabernet Sauvignon' wine produced with grapes when Gibberellic acid was applied in different concentrations during flowering in the 2019/20 and 2020/21 seasons.

	Gibberellic acid (mg L^{-1})									
	2019/2020					2020/21				
	0	40	80	*p-value*	R^2	0	20	40	*p-value*	R^2
Relative density at 20 °C	992.0 ±0.82^{ns}	992.0 ±0.82	991.2 ±0.50	*0.3713*	-	995.8 ±0,41^{ns}	996.0 ±0.00	996.0 ±0.00	*0.3911*	-
Alcohol by volume (% vol)	11.9 ±0.13^{ns}	11.9 ±0.00	11.9 ±0.05	*0.8966*	-	11.6 ±0.12^{ns}	11.7 ±0.15	11.6 ±0.06	*0.1753*	-
Residual sugar (g/L glucose)	1.83 ±0.08*	1.87 ±0.05	2.07 ±0.10	*0.0222*	*0.88*	2.23 ±0.03*	2.55 ±0.18	2.45 ±0.10	*0.0008*	*0.47*
Total acidity (mEq/L)	114.7 ±4.67*	117.1 ±1.88	121.5 ±1.60	*0.0385*	*0.97*	111.0 ±3.69*	105.2 ±4.07	104.0 ±2.83	*0.0086*	*0.87*
pH	3.37 ±0.03*	3.28 ±0.04	3.23 ±0.02	*0.0035*	*0.98*	3.22 ±0.09^{ns}	3.13 ±0.41	3.27 ±0.09	*0.6290*	-
Volatile acidity (mEq/L)	9.75 ±2.75^{ns}	9.00 ±0.82	7.50 ±0.58	*0.2593*	-	6.67 ±1.37^{ns}	6.83 ±1.17	7.67 ±0.52	*0.2599*	-
Free SO_2 (mg/L)	38.4 ±4.13^{ns}	42.4 ±3.33	37.6 ±3.81	*0.3373*	-	32.0 ±11.1^{ns}	38.4 ±6.64	37.7 ±2.60	*0.3079*	-
Total SO_2 (mg/L)	123.6 ±6,18*	115.2 ±7.84	114.4 ±3.33	*0.0041*	*0.81*	99.6 ±31.6^{ns}	105.4 ±11.7	103.7 ±7.97	*0.8777*	-
Total polyphenols (mg/L of gallic acid)	2,246.0 ±143.3^{ns}	2,237.3 ±375,9	2,463.5 ±52,2	*0.3051*	-	1,236.5 ±41.9*	1,388.3 ±38.6	1,351.8 ±7.99	*0.0000*	*0.53*
TMA (mg/L malvidin-3-glycoside)	46.4 ±15.0^{ns}	129.6 ±135.0	210.1 ±54.8	*0.1308*	-	418.2 ±26.5*	488.8 ±19.2	477.4 ±5.40	*0.0000*	*0.61*
Color 420 nm	47.9 ±6.50^{ns}	41.8 ±21.5	49.1 ±29.1	*0.7688*		27.3 ±2.34*	31.6 ±2.34	38.3 ±0.43	*0.0000*	*0.98*
Color 520 nm	58.9 ±6.0*	37.6 ±13.3	65.2 ±25.0	*0.0152*	*0.04*	29.4 ±6.15*	31.7 ±2.88	37.3 ±1.38	*0.0107*	*0.94*
Color 620 nm	43.6 ±4.22*	22.9 ±7.42	36.9 ±15.9	*0.0078*	*0.10*	12.9 ±6.35^{ns}	14.8 ±1.20	16.6 ±2.14	*0.2790*	
Color intensity	150.4 ±16.7^{ns}	102.4 ±42.2	151.2 ±64.2	*0.0548*	-	69.5 ±14.1*	78.1 ±4.08	92.3 ±3.38	*0.0014*	*0.98*
Color tone	0.81 ±0.03^{ns}	1.06 ±0.20	0.75 ±0.49	*0.3681*	-	0.95 ±0.12^{ns}	1.00 ±0.02	1.03 ±0.04	*0.1800*	-

Color density	106.8 ±12.5^{ns}	79.5 ±34.8	114.3 ±49.5	*0.1152*	-	56.7 ±8.49*	63.2 ±5.22	75.6 ±1.34	*0.0002*	*0.97*
Total tannins (g/L)	1.73 ±0.11^{ns}	1.49 ±1.04	2.23 ±0.11	*0.2273*	-	1.18 ±0.28^{ns}	1.29 ±0.17	1.19 ±0.29	*0.6868*	-

* Significant linear regression ($p<0.05$): Residual sugar: y = 0.0012x + 1.8042 (season: 2019/20); y = 0.0023x + 2.2964 (s: 20/21); Total acidity y = 0.0343x + 114.33 (s: 19/20); y = -0.07x + 110.22 (s: 20/21); pH: y = -0.0007x + 3.3663 (s: 19/20); Total SO_2: 0.046x + 122.33 (s: 19/20); Color 420: y = 0.1104x + 26.849 (s: 20/21); Color 520: y = 0.0314x + 50.788 (s: 19/20); y = 0.0791x + 28.858 (s: 20/21); Color 620: y = -0.0335x + 37.825 (s: 19/20); Total polyphenols: y = 1.1523x + 1267.9 (s: 20/21); TMA: y = 0.5923x + 431.87 (s: 20/21); Color intensity: y = 0.2274x + 68.602 (s: 20/21); Color density: y = 0.1895x + 55.706 (s: 20/21); ^{ns} = non-significant difference; R^2 = coefficient of determination; TMA = total monomeric anthocyanins; ± Standard deviation.

The acidity of the wine and must evaluated at the harvest increased in the 2019/20 season and decreased in the 2020/21 season with GA_3 doses, which may be due to the effect of climatic conditions between the years. Wine pH in the 2019/20 season was reduced with the increase in GA_3 doses. The climatic data of the harvests are listed in Table 3. In the 2020/21 season, there was more precipitation (mm) than in the previous one (2019/20), which provided a higher relative humidity in most of the experiment period. Thus, it is likely that there was an interaction between GA_3 and climatic conditions of the crop that resulted in changes in acidity behavior.

Table 3. Monthly averages of precipitation, average temperature, and relative humidity in the 2018/20 and 2020/21 seasons in São Joaquim, Santa Catarina State, southern Brazil.

Month	Precipitation (mm)		Average temperature (°C)		Average humidity (%)	
	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21
November	102.2	138.6	15.7	15.0	78.5	84.0
December	70.8	151.8	17.2	16.6	73.2	82.3
January	185	261.2	17.5	17.1	79.6	85.7
February	80.8	112.6	16.3	16.6	80.7	80.3
March	12.8	98	16.8	16.4	74.7	86.2

Regarding total polyphenols and total monomeric anthocyanins in wines, a significant increase in concentration was observed when using GA_3, being higher in the 2020/21 season (Table 2). Polyphenols are important because they act in the plant's defense against pathogens and stress and are important for human health (RIENTH et al. 2021). Changes in phenolic compounds and anthocyanins caused by GA_3 may affect wine color, antioxidant potential, and sensory quality (TESZLÁK et al. 2005). This result was reflected in the increased intensity and density of the wine color. TYAGI et al. (2022) found a reduction in the anthocyanin content in 'Sangiovese' grapes by applying GA_3 to the formed berry. Despite this study with 'Cabernet Sauvignon' grapes, the difference in the period of application may indicate a difference in the response to anthocyanin accumulation.

The initial hypothesis that using GA_3 could reduce seed number and size and consequently reduce tannin content in the wine was not confirmed. In the first and second years of evaluation, the tannin content did not differ from the control (Table 2). However, even with no significant difference, the total tannin content was higher when the grapes received 80 and 40 mg L^{-1} of GA_3 in 2019/20 and 2020/21, respectively. This means treatment with GA_3 to reduce tannin content cannot be recommended without further analyses. Most of the tannin extracted from the grape likely came from the skin and pulp and, to a lesser extent, from the seed. Furthermore, even though the GA_3 reduced seed number and size, the impact on tannin extraction in the winemaking process was greater with GA_3 than in the control group. In grapes, about 54% of extractable proanthocyanidins come from the skin, 30% from the seeds, and 15% from the pulp (BINDON et al. 2010). According to KENNEDY (2008), 64% of the tannin in 'Pinot Noir' wines came from the seed and 36% from the skin. In 'Sangiovese' grapes, GA_3 increased proanthocyanidin content, which is also known as condensed tannins, by 34% (TYAGI et al. 2022). Proanthocyanidins are produced by the phenylpropanoid route, an important secondary metabolism route in plants that derives from aromatic amino acids (TYAGI et al. 2021).

The PCA provided an overview of the variables compared to the treatments with GA_3, and PC1 and PC2 in the first season explained 53.2 and 46.8% of the overall variance, respectively (Figures 1A and 1B). In the second season, PC1 and PC2 explained 89.2 and 10.8% of the overall variance, respectively (Figures 1C and 1D). In both harvests, the zero dose of GA_3 is related to greater cluster compaction, berry and cluster

mass, and seeds per berry. Nonetheless, in the 2019/20 season, 80 mg L^{-1} of GA_3 was correlated with total soluble sugars of the must, wine acidity, must acidity, color (520 nm), and color intensity (Figures 1A and 1B). This dose (80 mg L^{-1} of GA_3) was on the opposite side of seed mass, seed $berry^{-1}$, berry mass, and berries per cluster, demonstrating that GA_3 reduces this 'Cabernet Sauvignon' grape characteristic, as also reported by SANTOLALLA & ESCOBEDO (2018). The acidity in the 2020/21 season, both in the must and wine, unlike the first harvest, correlated with the control. In the last season, the color parameters correlated more with the higher dose of GA_3, showing that this growth regulator increased free anthocyanins and, consequently, total polyphenols (Figures 1C and 1D). The primary component analysis also showed that both doses of GA_3 in the 2020/21 season increased the tannin content in the wine. It is likely that GA_3 stimulated some of the enzymes of the phenylpropanoid route in the 'Cabernet Sauvignon' grape, including leucoanthocyanidin reductase and anthocyanidin reductase, increasing tannin content.

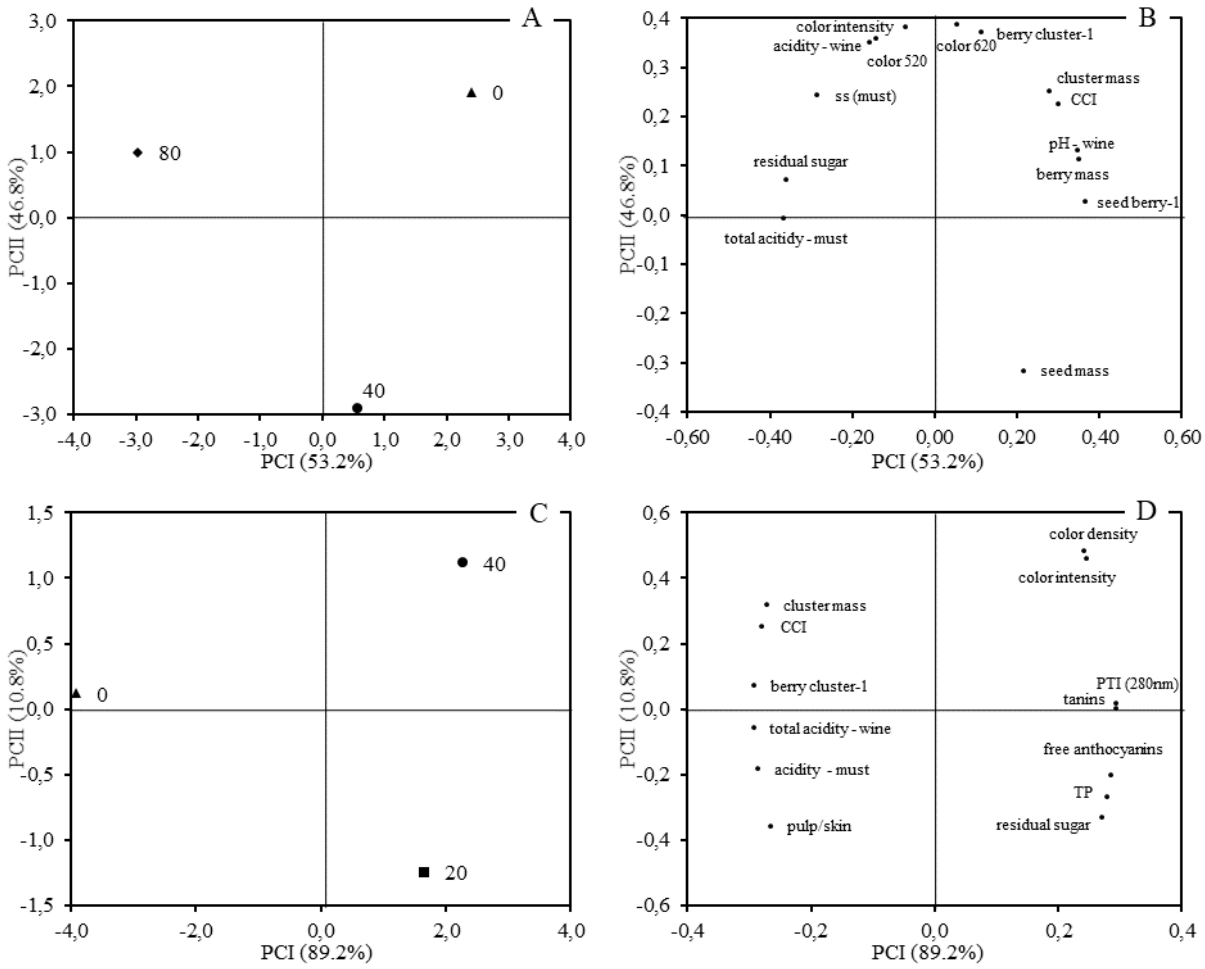

Figure 1. Principal component analysis showing the score plots (A – 2019/20 and C – 2020/21) and loadings of the variables (B - 2019/20 and D – 2020/21) of 'Cabernet Sauvignon' grape and wine submitted to pre-harvest gibberellic acid (GA_3) application (2019/20 season - [0] 0.0 mg L^{-1} of GA_3, [40] 40.0 mg L^{-1} of GA_3, and [80] 80.0 mg L^{-1} of GA_3; 2020/21 season - [0] 0.0 mg L^{-1} of GA_3, [20] 20.0 mg L^{-1} of GA_3, and [40] 40.0 mg L^{-1} of GA_3. TP: total polyphenol; TPI: total polyphenol index (280 nm); CCI: cluster compaction index.

CONCLUSION

After two years of investigating GA_3 application in 'Cabernet Sauvignon' grapes in *Serra Catarinense*, the following considerations are presented:

(1) Gibberellic acid applied to clusters in full bloom, in doses of 20-80 mg L^{-1}, reduces the number and size of seeds. However, this was not reflected in reducing the tannin content in the wine in the vintages studied.

(2) The cluster compaction, bunch weight, and berry weight and diameter were reduced with GA_3 use.

(3) The use of GA_3 in the flowering of 'Cabernet Sauvignon' grapes, at doses of 20 and 40 mg L^{-1}, increases the total polyphenols and total monomeric anthocyanins in the wine.

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