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Maintenance of post-harvest antioxidant quality in 'Niagara Rosada' grape using salicylic acid

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Abstract: Salicylic acid is a plant growth regulator used in grapes to maintain postharvest quality. 'Niagara Rosada', a table grape, although much appreciated for its flavor, has a short shelf life. We evaluated the influence of postharvest application of different doses of salicylic acid on the quality of 'Niagara Rosada' after harvest in an effort to control rates of berry drop and decay, as well as to maintain the quality of grape bunches during refrigerated storage. Freshly harvested bunches of 'Niagara Rosada' (*Vitis labrusca* x *V. vinifera*) were immersed in salicylic acid solutions at concentrations of 0.0, 0.28, 0.55, 0.83, and 1.10 g L^{-1}, and then refrigerated (5 ± 1 °C and 95 ± 5 % RH) for 20 days. Physical and chemical analyses of grapes were performed at 5-day intervals. Salicylic acid maintained the postharvest quality of 'Niagara Rosada' grapes throughout storage. The lowest concentration of salicylic acid (0.28 g L^{-1}) effectively induced the synthesis of phenolic compounds and improved the antioxidant capacities of both grapes and stems. High levels of salicylic acid (0.83 and 1.10 g L^{-1}) resulted in an increase in anthocyanin content in fruit and enzyme activities (peroxidase and superoxide dismutase) in stems, enhancing conservation and reducing levels of decay and berry drop.

Keywords: decay; berry drop; cold storage; phenolic compounds; enzymes.

Introduction

Throughout the world, vine management techniques have been researched in order to increase productivity and improve the postharvest quality of grapes, including those destined for the table. Table grape quality is dependent on both cultivar type and management practices employed from flowering to harvest. 'Niagara Rosada' vines are medium-vigor plants and are known to be resistant to various pests and diseases. 'Niagara Rosada' grapes are pink-film fruits covered with a waxy bloom, featuring mucilaginous pulp and a sweet foxed flavor that is appreciated by consumers. Due to their high degree of acceptance in the domestic market, these American table grapes have been cultivated in areas where diseases have caused serious damage to vines (Maia and Camargo, 2012).

In order to be acceptable to consumers, fruits must have high postharvest quality. Table grapes with darkened stems from tissue oxidation, softened texture, dehydrated berries, berry drop, or other undesirable features will be declined, and their economic value will depreciate. Several techniques have been used in pre- and postharvest table grapes to reduce the incidence of berry decay, decrease softening rates, prevent rotting during storage, and extend shelf-life. Among these techniques, the use of plant growth regulators, such as salicylic acid, is attractive since it can be safely and easily applied. Salicylic acid (SA) or 2-hydroxybenzoic acid, is a plant growth regulator with a phenolic structure that plays a crucial role in the regulation of fruit development, growth, and ripening (Pérez-Llorca et al., 2019), as well as in plant resistance to biotic and abiotic stresses (Hassoon and Abdulsattar

Abduljabbar, 2020). One of the most important functions of salicylic acid in plants is to stimulate the production of compounds that scavenge free radicals, called antioxidants (Hassoon and Abduljabbar, 2020). It is an important secondary metabolite produced by grapes and plays an essential role in the determination of berry quality, affecting color, flavor, astringency, and bitterness (Blanch et al., 2020). The effects of SA rely on the synthesis of phenolic compounds, especially the activity of the phenylalanine ammonia lyase enzyme (PAL). In addition, recent research has indicated that SA has the potential to improve physical properties such as size, weight, and fruit firmness.

Despite its great potential for use as a table grape, information regarding the effect of exogenous application of plant regulators on postharvest fruit quality in 'Niagara Rosada' is scarce. Several studies have indicated that the exogenous application of SA improves important postharvest characteristics, such as enhancing antioxidant capacity (Gomes et al., 2021; Wang et al., 2015). SA may induce the inhibition of catalase (CAT), a hydrogen peroxide scavenging enzyme, resulting in an increase in levels of H2O2, which acts as a second messenger activating defense-related genes (Chen et al., 1993). Exogenous SA in grape *Vitis vinifera* L. cv. Jingxiu promoted an increase in superoxide dismutase (SOD) and peroxidase (POD) activities, although there was an increase in hydrogen peroxide, and this effect was attributed to the high rate of H2O2 production compared to its degradation by the enzyme (Wang and Li, 2006).

Furthermore, SA is related to disease resistance and shelf life (Gomes et al., 2021) in horticultural crops. Important fruit quality characteristics (e.g., sweetness, firmness, and color), which depend on the cultivar used, as well as pre- and postharvest factors (Lo'ay et al., 2019; Xu et al., 2019), have not been previously described in 'Niagara Rosada'. Thus, the aim of this research was to evaluate the influence of exogenous application of salicylic acid on the postharvest of 'Niagara Rosada' grapes on physical-chemical properties, as well as to assess antioxidant compounds (enzymatic and non-enzymatic) of bunches during cold storage.

Results and Discussion

Physical, chemical and biochemical characteristics of berries

Throughout storage, there was an increase in TA and a decrease in pH in the fresh berries (Fig. 1A and 1C). However, SA did not significantly affect the levels of titratable acidity (TA) or pH (Fig. 1B and 1D), and the differences observed were exclusively based on the duration of storage. Other studies demonstrate that exogenous SA does not influence the content of TA and pH, as described by Gomes et al. (2021) in 'Niagara Rosada' grapes treated with different doses of SA and by Alrashdi et al. (2017) in 'El-Bayadi' table grapes. On the other hand, in seedless grapes ('Superior Seedless'), Lo'ay (2017) found a decrease in TA in response to SA. Thus, it is possible that the acid content may be dependent on the genotype, also influenced by the culture method, among other biotic and abiotic factors. This may explain the slight changes that were observed in 'Niagara Rosada' grapes throughout storage that occurred independently of SA application.

Salicylic acid treatment effectively maintained soluble solids (SS) content in 'Niagara Rosada' grapes throughout storage (Fig. 1E), and a significant interaction between SA level and duration of storage was observed. The SA dose affected SS levels of grapes subjected to all durations of storage, except grapes assessed five days postharvest. A concentration of 0.28 g L^{-1} SA was found to be sufficient for maintaining SS content. This result is significant because 'Niagara Rosada' is a table grape, and the SS content is crucial for the flavor of this cultivar. It is worth mentioning that grapes subjected to storage and SA treatments had SS content greater than the minimum quantity required by Brazilian legislation for table grapes (14° Brix) (Brasil, 2018).

Berry drop, decay, and weight loss were not significantly affected by SA treatment (Fig. 2B, 2D, and 2F). The results clearly indicated that the highest incidence of berry drop, decay, and weight loss occurred in 'Niagara Rosada' grapes after 20 days of storage. During this time, stems had darkened due to oxidative processes, and low enzymatic activity was observed, regardless of the SA treatment. After 20 days of storage, the grapes did not show commercial quality (visual quality) and should be discarded. In this study, we did not observe that any specific concentration of SA might influence berry drop, weight loss, or decay, as described by Gomes et al. (2021), who stated that 1 mmol L^{-1} SA in the pre-harvest was efficient in maintaining the quality of 'Niagara Rosada' grapes. The results obtained in this study may be attributed to the dosages used, and probably smaller doses of SA may be more efficient in decreasing berry drop, weight loss, and decay.

Although SA did not significantly influence the physical characteristics of grapes assessed, 0.28 g L^{-1} (2 mmol L^{-1}) SA promoted an enhancement in total phenolic compounds (221.75 mg 100 g-1) (Fig. 3A) and antioxidant capacity measured by FRAP (172.93 mmol Fe Kg -1) (Fig. 3C) after five days of storage. Higher levels of SA induced anthocyanin content (Fig. 3B). On the other hand, there was no influence on the levels of SA used in the antioxidant activity measured by the DPPH method (Fig. 3E). In accordance with our findings and previous studies (Gomes et al., 2021) in 'Niagara Rosada', it has been indicated that SA treatment of pre- and postharvest table grapes facilitates the maintenance of quality by minimizing loss of firmness and inducing the expression of antioxidant compounds, characteristics that have been associated with ripening processes and quality attribute loss. In our study, the highest levels of total phenolic compounds and anthocyanins were found in 'Niagara Rosada' grapes after treatment with 0.28 g L^{-1} SA (Fig. 3A and 3B), similar to that described by Alrashdi et al. (2017) in table grapes. This effect may be attributed to phenylalanine ammonia-lyase activity (PAL), whose product is phenylpropanoids (Chen et al., 2006), because the transcription of the PAL genes may be activated by SA (Kiselev et al., 2010).

Immersion of 'Niagara Rosada' grapes in high levels of SA (0.83 and 1.10 g L^{-1}) further enhanced anthocyanin content (15.4 mg 100 g^{-1} and 17.1 mg 100 g^{-1}, respectively) five days postharvest (Fig. 3B). Anthocyanin is principally responsible for grape skin coloration, which is a characteristic significantly correlated with grape quality. Increased levels of these compounds positively affect grape quality. Phenolic compounds, including anthocyanins, are secondary metabolites that influence grape qualities such as color, flavor, bitterness, and astringency, as well as the antimicrobial and antioxidant properties of fruits. Previous studies demonstrate that the application of SA induced the accumulation of phenolic compounds, such as flavonoids and anthocyanins (Gomes et al., 2021). In our study, SA exogenous increased the shelf life and the phenolic

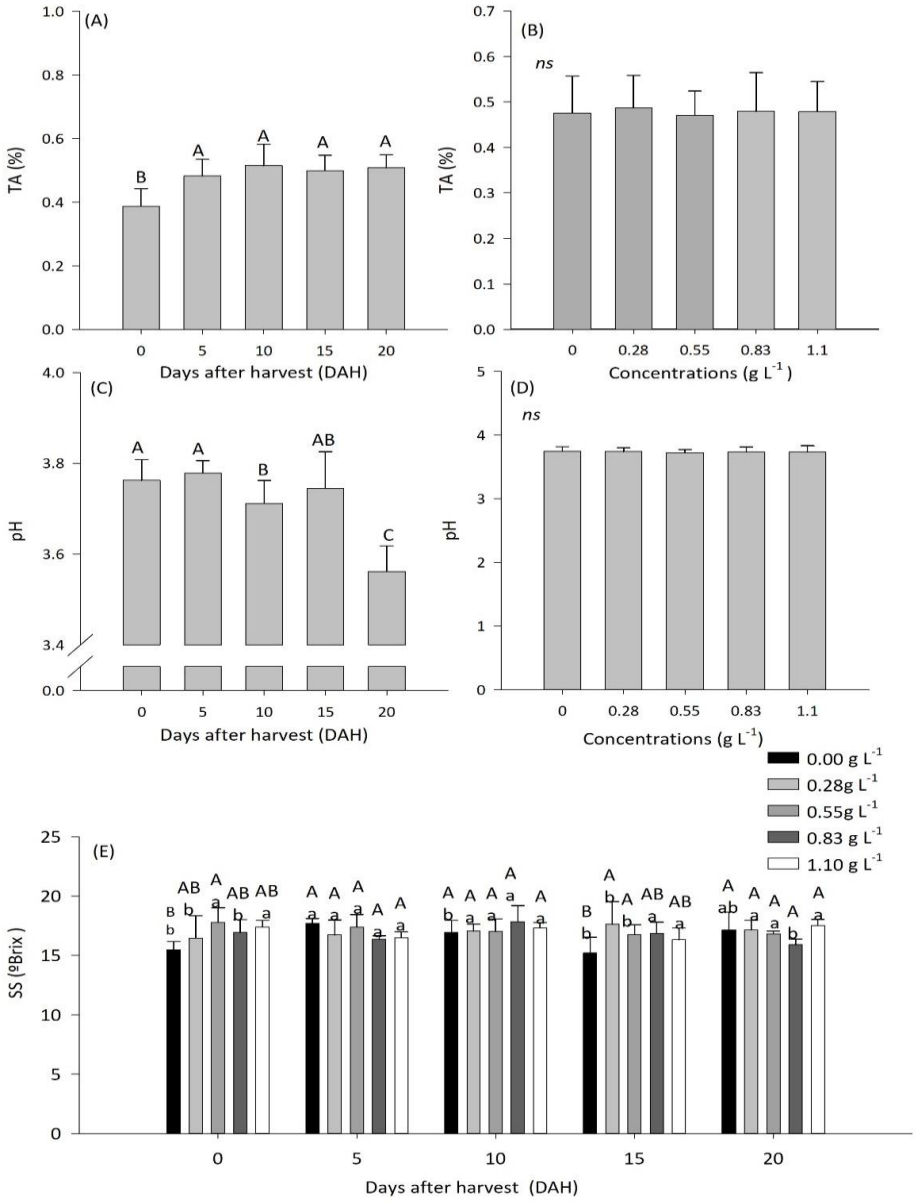

Figure 1. Titratable acidity, pH and soluble solids of 'Niagara Rosada' grape must treated with different concentrations of salicylic acid in the post-harvest and stored under refrigerate conditions. Capital letters compare the days after treatment and lower case the concentrations of SA. (Tukey test, $p \leq 0.05$). *NS*: no significant.

compounds, which can influence the final quality of the product.

Chlorophyll and antioxidant enzymes (SOD, CAT and POD) in stem

At harvest (day 0), stems were green and resistant to berry drop (Fig. 2A, 4, and 5A). Throughout storage, total chlorophyll content and catalase (CAT) activity levels decreased significantly, regardless of the SA dose applied. Chlorophyll levels are often considered an indicator of senescence in green tissues, such as stems. The reductions in chlorophyll content and CAT activity may be due to oxidation reactions, which were accompanied by visible darkening and made plants less resistant. Additionally, berry drop and decay increased with the duration of storage (Fig. 2A and 2E). Effective control of rachis browning is necessary to control postharvest decay in table grapes. SA may inhibit oxidative damage and decrease catalase activity, besides increasing the levels of peroxide, which act as second messengers in the activation of defense-related genes, as described by Wang and Li (2006) in grapevines sprayed with a 100 µmol L^{-1} solution of SA. In contrast, H2O2 may be metabolized by peroxidases, enzymes that play important roles in plant detoxification, which use phenolic compounds as substrates (Simões et al., 2020).

In stems, the highest peroxidase (POD) activity was observed after grapes were stored for 10 days, while the highest superoxide dismutase (SOD) activities occurred after 5 and 15 days of storage (Fig. 5B and 5D). Increased antioxidant enzyme and non-enzymatic compounds (e.g., phenolic compounds) often make these plants more tolerant to different stressful

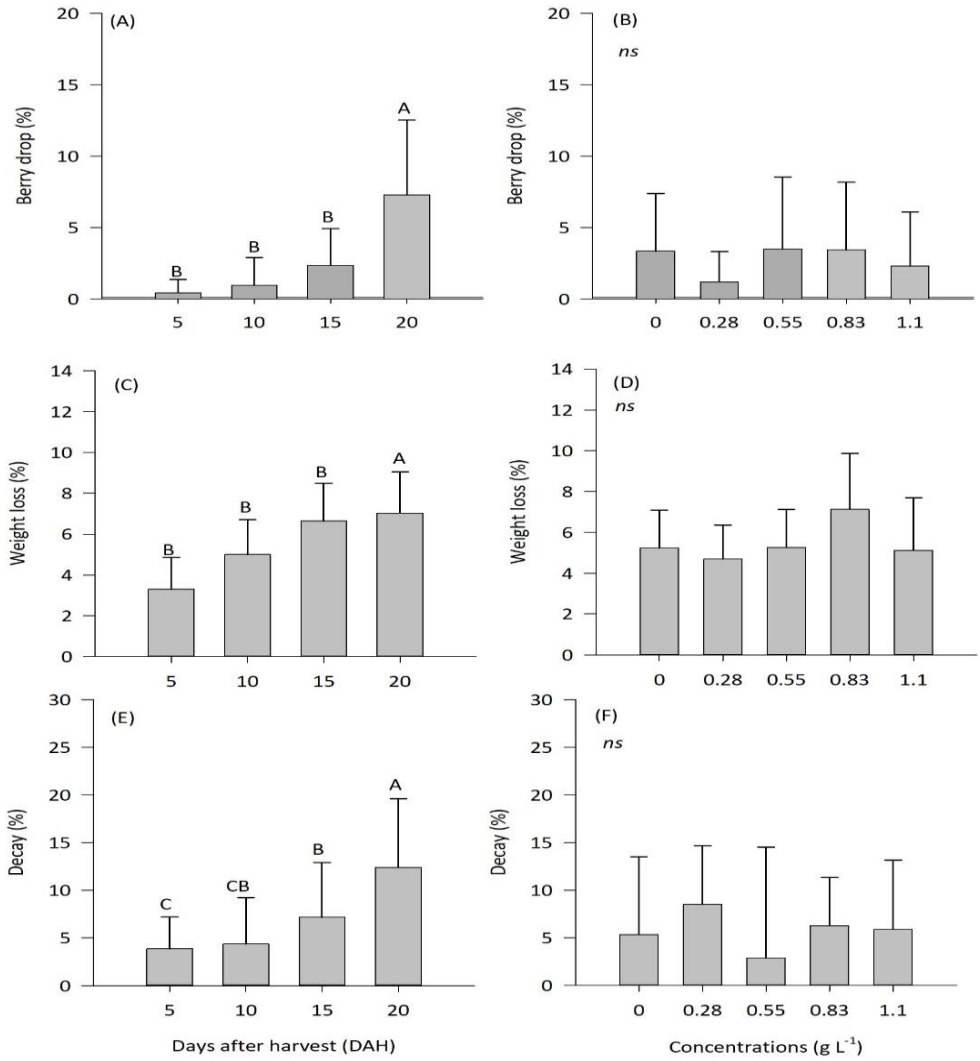

Figure 2. Berries drops, weight loss and incidence of decay of 'Niagara Rosada' grapes treated with different concentrations of salicylic acid in the post-harvest and stored under refrigerated conditions. Capital letters compare the days after treatment and lower case the concentrations of SA. (Tukey's test, $p \leq 0.05$). *NS*: no significant.

conditions, such as low humidity and elevated temperatures (Shah Jahan et al., 2019). These are important biochemical and physiological characteristics for grapes growing under adverse environmental conditions (Schultz & Stoll, 2010).

Increased POD and SOD activities that occurred in response to SA application may be related to the low percentages of berry drop and decay, respectively. These effects may be due to the fact that fruits treated with 0.28 g L^{-1} SA had elevated POD activity and reduced levels of berry drop. Plants treated with 0.55 g L^{-1} SA showed an increase in SOD activity and a reduced incidence of decay (Fig. 2E and 5E). These results may be important because they demonstrate a possible relationship between peroxidase/berry drop and SOD/decay. Antioxidant enzymes (e.g., POD and SOD) are commonly studied in postharvest plants because they are involved in plant resistance (Zhou et al., 2009). Studies indicate that in grapes, SA may induce antioxidant enzyme activities, such as POD and SOD, which may delay fruit deterioration and promote an increase in antioxidant capacity (Xu et al., 2019). Application of elicitors such as SA in citrus induced defense-related enzyme activities (PAL; cinnamate-4-hydroxylase, C4H; hydroxycinnamoyl-CoA ligase, 4CL; and polyphenoloxidase, PPO) and stimulated the accumulation of phenolic acids and lignin in fruits inoculated with fungi (*P. italicum* and *P. digitatum*) for a short period, which may be correlated with decreases in infection incidence and lesion diameter (Zhou et al., 2018).

Principal component analysis (PCA)

In order to establish a descriptive model for grouping SA levels (treatments) as a function of storage time and variables analyzed, a multivariate statistical analysis of the dataset using PCA was conducted (Fig. 6). The PC1 axis accounted for 44.08% of the total variance observed. The dispersion of variables according to PC1 and PC2 revealed that the highest concentrations of SA were observed in table grapes stored in the cold for 5 days, which were grouped into PC2+ and PC1- groups. SA treatment resulted in increased levels of phenolic compounds, total monomeric anthocyanins, and antioxidant activity. This finding indicated that grapes treated with the

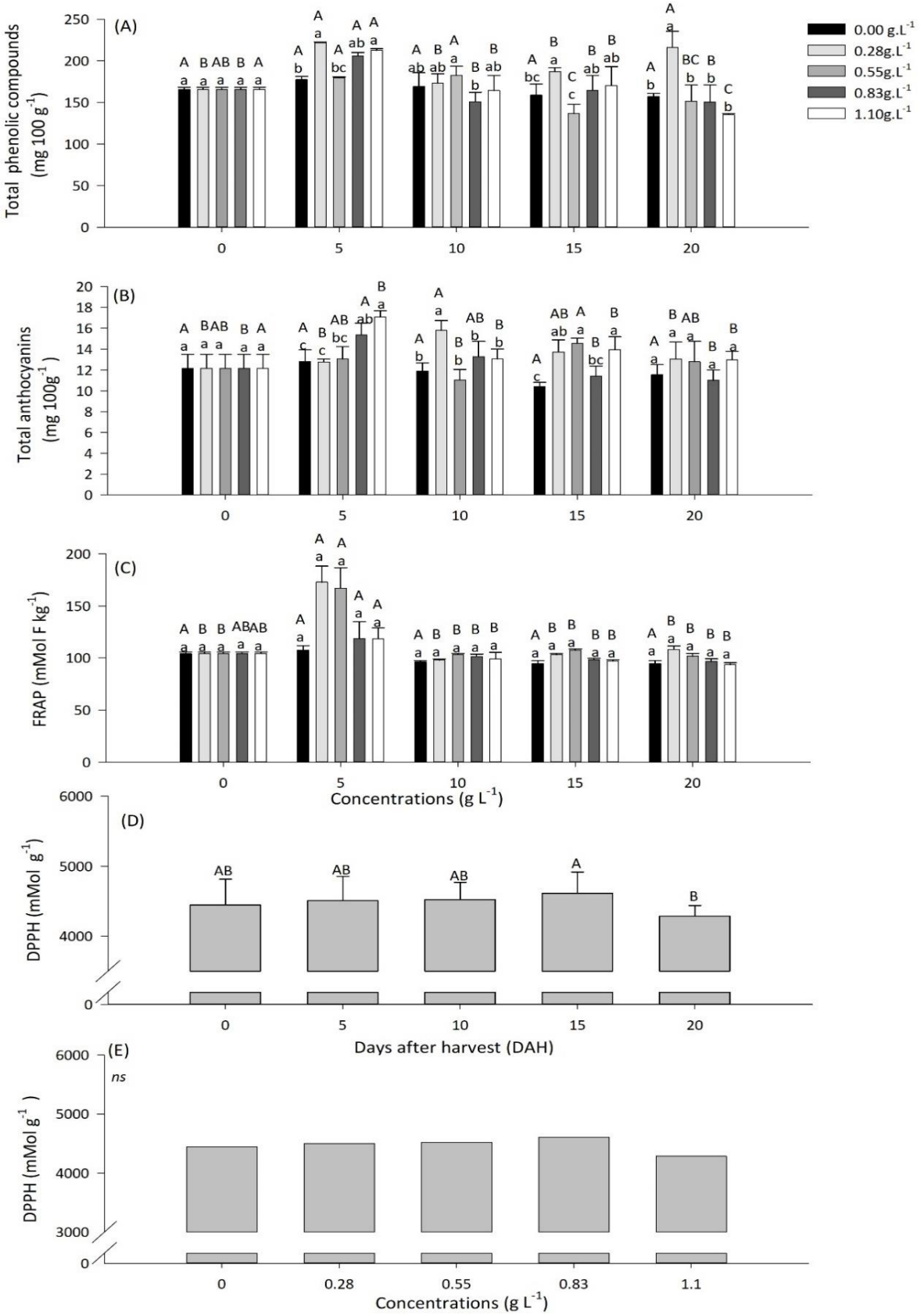

Figure 3. - Total phenolic compounds, total anthocyanins and antioxidant activity (FRAP and DPPH) of 'Niagara Rosada' grape treated with different concentrations of salicylic acid in the post-harvest and stored under refrigerated conditions. Capital letters compare the days after treatment and lower case the concentrations of SA. (Tukey's test, $p \leq 0.05$). *NS*: no significant.

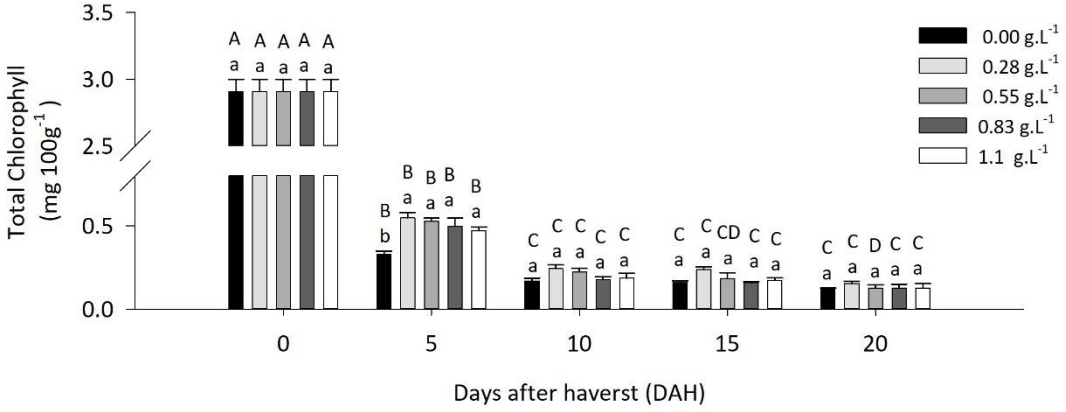

Figure 4. Total chlorophyll in 'Niagara Rosada' grape stem treated with different concentrations of salicylic acid in the post-harvest and stored under refrigerated conditions. Capital letters compare the days after treatment and lower case the concentrations of SA. (Tukey's test, $p \leq 0.05$).

highest concentrations of SA were of the greatest quality, due to the higher content of bioactive compounds, mainly anthocyanins, responsible for the color of the berries (Fig. 3B). The monomeric anthocyanins present in red grapes are responsible for berry color, and intra- or intermolecular interactions between anthocyanins and other organic chemicals, especially phenolics, have the potential to further alter berry color.

Materials and Methods

Experimental area, cultivation conditions and experimental design

Experiments were conducted using grapes produced during the 2017/2018 cycle in São Manuel, São Paulo, Brazil (22°44'S, 48°34'W, at an altitude of 740 m). The climate of the farm, according to the Köppen classification, is of the *Cfa* type, classified as humid temperate mesothermal with concentrated rains occurring from November to April. The average annual rainfall in the area is 1,465 mm, with average minimum and maximum temperatures of 14.5°C and 27.1°C, respectively.

'Niagara Rosada' grapevines (*Vitis labrusca* x *V. vinifera*) grafted onto 'IAC 766' rootstocks [*Vitis riparia* x (*V. cordifolia* x *V. rupestris*)] were spaced at 2.0 x 0.8 m intervals, in their fourth productive cycle. The vines were trained on a unilateral single cordon with three vertical catch wires. During production pruning, one bud was identified for each productive branch, followed by the application of 5% hydrogenated cyanamide. After sprouting, one productive branch per bush was maintained. A drip irrigation system was used to ensure optimal soil moisture in the field. Bunches were harvested as soon as the soluble solid (SS) content reached 14° Brix and achieved an intense and uniform pink color.

Experimental design and treatments

A completely randomized experimental design was employed, consisting of a randomized block with three replicates of five vines each (totaling 15 vines) randomly selected per rootstock (a total of 30 vines throughout the vineyard, all of the same cultivar, age, and vigor). Plants were subdivided into plots, with each subjected to aqueous solutions containing different doses of salicylic acid (SA) (0.0; 0.28; 0.55; 0.83; and 1.10 g L^{-1}). Six repetitions of two bunches each from every subplot were evaluated at 0, 5, 10, 15, and 20 days after harvest. Aqueous solutions of SA (Sigma Aldrich Co., USA) were prepared by dissolving SA in 100 mL of ethanol before adding water to reach a final volume of 25 L. SA treatments were applied immediately after harvest by immersing bunches in SA solutions for 45 minutes. Bunches were then dried and stored in expanded polystyrene trays under refrigerated conditions (5 ± 1 °C and 95 ± 5 % RH) until evaluations were performed.

Weight loss, berries drops and decay

Weight loss was determined by daily weighing of the bunches on the evaluation days, and the results were expressed as percentage of weight loss. Berry drop was determined cumulatively on the days evaluated, by lightly shaking the bunches twice, recording the number of berries that dropped, and weighing them. Results were expressed as a percentage using the following equation (Eq. 1):

Berry drop (%) = TWB × 100/IWB (Eq. 1)

Where IWB represents the initial weight of the bunch (day 0) and TWB is the total weight of berries dropped on the day that the evaluation was performed.

Decay was also determined cumulatively throughout each day evaluated. Therefore, the berries that had decayed were removed from the bunches, weighed, and the results were expressed as a percentage using the following equation (Eq. 2):

Decay (%) = TWDB × 100 / IWB (Eq. 2)

Where IWB indicates the initial weight of the bunch (day 0), and TWDB represents the total weight of decayed berries identified on the day the evaluation was performed.

Physical-chemical properties of must

The physicochemical characteristics of the must were determined by assessing 60 berries per experimental plot. The must was obtained by pressing the berries, and the soluble solid (SS) content was determined via direct refractometry

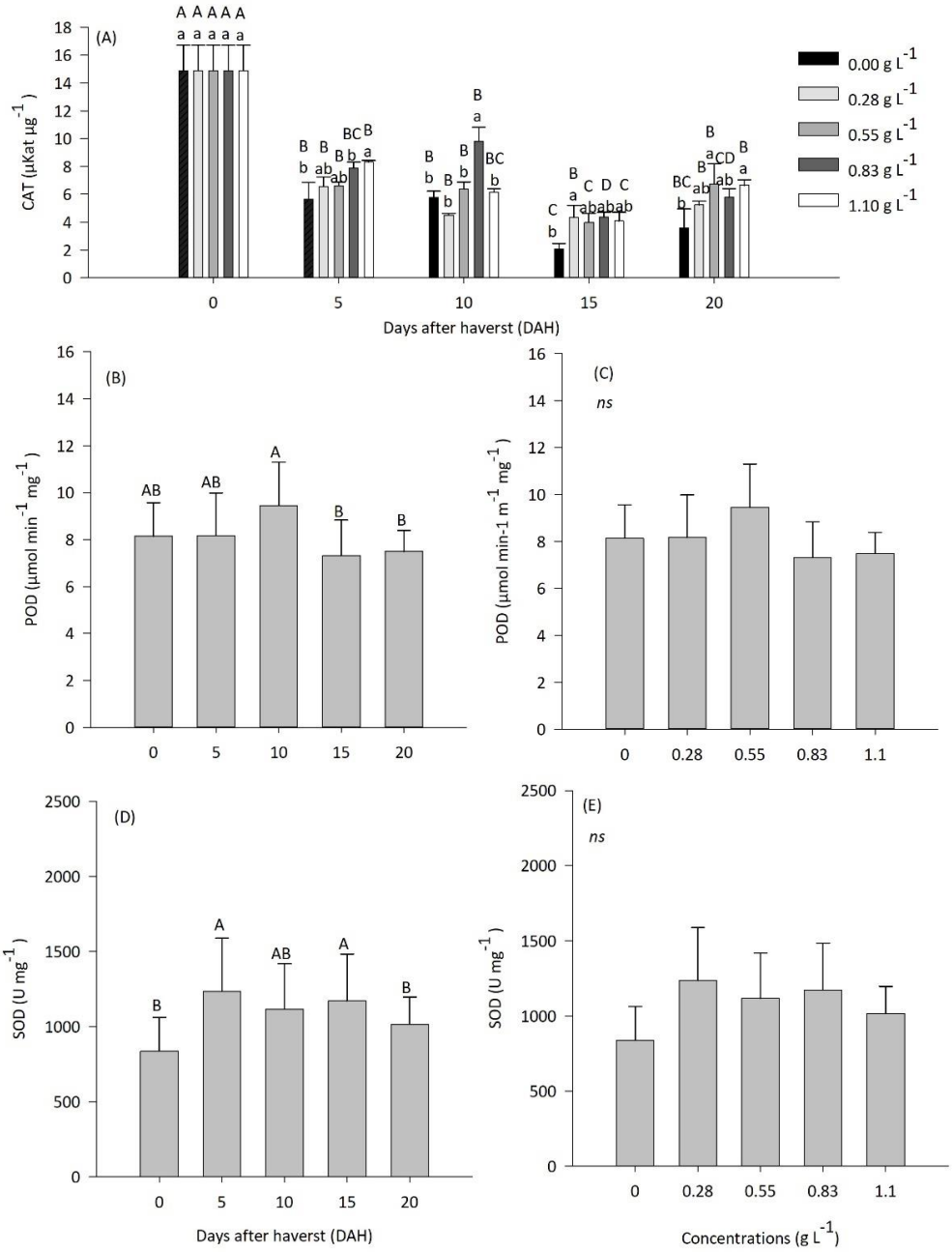

Figure 5. Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities in 'Niagara Rosada' grape stem treated with different concentrations of salicylic acid in the post-harvest and stored under refrigerated conditions. Capital letters compare the days after treatment and lower case the concentrations of SA. (Tukey's test, $p \leq 0.05$). *NS*: no significant.

using an Atago® digital refractometer. The results were expressed in degrees Brix. pH was measured using a pH meter (Micronal B-274), and titratable acidity (TA) was determined by titration (expressed as % tartaric acid). These analyses were performed according to the procedures outlined by the Adolfo Lutz Institute (Zenebon et al., 2008).

Phenolic compounds and antioxidant activity of grape berries

Total levels of monomeric anthocyanins were determined using the pH-differential method (Giusti and Wrolstad, 2001), and the total monomeric anthocyanin content was expressed as cyanidin-3-glycoside equivalents per mg fresh weight. Total phenolic compounds were determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965), and the results were expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW).

DPPH method is based on the scavenging of free radicals from DPPH (2,2-diphenyl-1-picrylhydrazyl) and was conducted according to Brand-Williams et al. (1995), with the results expressed in µmol equivalent TEAC per gram of fresh weight.

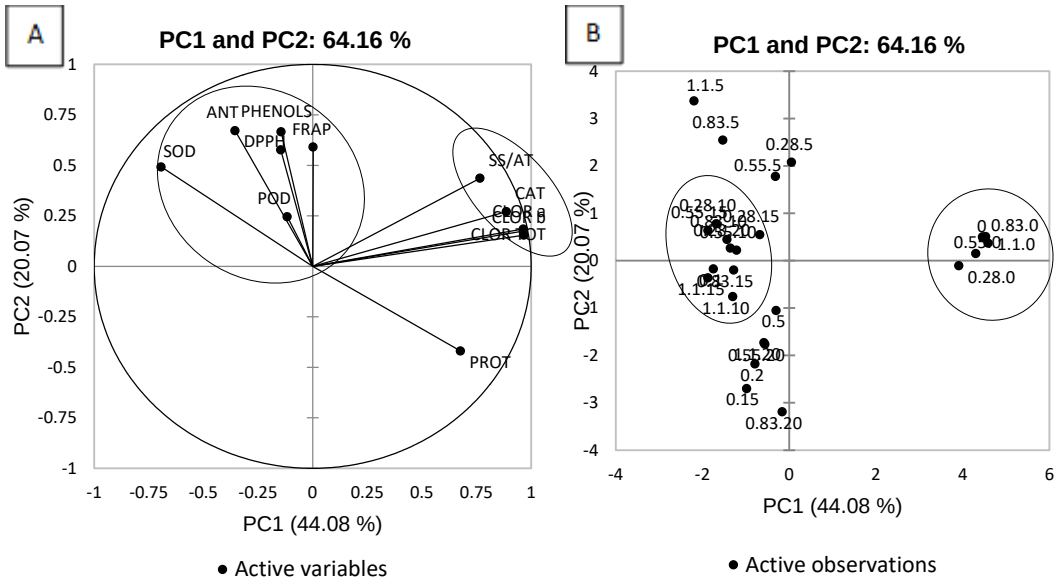

Figure 6. Two-dimensional projection (A) and scores (B) from physical-chemical, biochemical and enzymatic characteristics for the interaction between salicylic acid levels and evaluation days of the 'Niagara Rosada' table grape.
Note: 0.0 (dose 0 and 0 day); 0.5 (dose 0 and 5 day); 0.10 (dose 0 and 10 day); 0.15 (dose 0 and 15 day); 0.20 (dose 0 and 20 day); 0.28.0 (dose 0,28 g L^{-1} and 0 day); 0.28.5 (dose 0,28 g L^{-1} and 5 day); 0.28.10 (dose 0,28 g L^{-1} and 10 day); 0.28.15 (dose 0,28 g L^{-1} and 15 day); 0.28.20 (dose 0,28 g L^{-1} and 20 day). 0.55.0 (dose 0.55 g L^{-1} and 0 day); 0.55.5 (dose 0,55 g L^{-1} and 5 day); 0.55.10 (dose 0,28 g L^{-1} and 10 day); 0.55.15 (dose 0,55 g L^{-1} and 15 day); 0.55.20 (dose 0,55 g L^{-1} and 20 day). 0.83.0 (dose 0,83 g L^{-1} and 0 day); 0.83.5 (dose 0,83 g L^{-1} and 5 day); 0.83.10 (dose 0,83 g L^{-1} and 10 day); 0.83.15 (dose 0,83 g L^{-1} and 15 day); 0.83.20 (dose 0,83 g L^{-1} and 20 day). 1.10.0 (dose 1,10 g L^{-1} and 0 day); 1.10.5 (dose 1,10 g L^{-1} and 5 day); 1.10.10 (dose 1,10 g L^{-1} and 10 day); 1.10.15 (dose 1.10 g L^{-1} and 15 day); 1.10.20 (dose 1.10 g L^{-1} and 20 day).

The FRAP method is based on the iron ion reduction capacity, which changes from Fe3+ to Fe2+, and was performed following the protocol outlined by Benzie and Strain (1999). The results are expressed in mmol Fe per kg of fresh weight.

Quantification of proteins, enzyme activities and pigment content within stems

Extracts used to quantify protein and antioxidant enzyme levels were obtained from fresh materials. Stems were powdered in liquid nitrogen, and 2 mL of 0.1 mol L^{-1} potassium phosphate buffer at pH 6.8 with 100 mg of polyvinylpolypyrrolidone (PVPP) was added.

Superoxide Dismutase (SOD) activity was determined according to Giannopolitis and Ries (1977), with the results expressed in units of activity (UA) per milligram of protein. Peroxidase activity (POD) was determined according to Teisseire and Guy (2000), and the results were expressed in micromoles of purpurogalin per minute per milligram of protein. Catalase (CAT) activity was determined according to the methodology proposed by Peixoto et al. (1999), and the experimental results were expressed as micromoles of substrate decomposed per µKat per µg protein. Total soluble proteins were quantified according to Bradford (1976), and the results were used to calculate the levels of antioxidant enzymes.

Total chlorophyll levels were determined according to the method described by Sims and Gamon (2002), and absorbance values were converted to µg per 100 g FW.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), followed by the Tukey test at a significance level of 5 % to compare average values determined for samples subjected to different doses of SA and different durations of storage. Additionally, principal component analyses (PCA) were performed to characterize interactions between SA concentrations and storage durations using XLSTAT software, version 2017, by Addinsoft, France.

Conclusion

SA positively affects postharvest quality of 'Niagara Rosada' grapes when stored under cold conditions. Treatment of grapes with low SA doses (0.28 g L^{-1}) effectively improves fruit quality by increasing levels of total phenolic compounds. Furthermore, exogenous application of SA enhances the antioxidant capacity of berries, particularly on the 5th day of cold storage. In addition, the application of 0.83 and 1.10 g L^{-1} SA enhances anthocyanin content in berries and increases antioxidant enzyme activities (POD and SOD) in the stem, which are important features for enhancing preservation and reducing the incidence of decay in 'Niagara Rosada' grape bunches.

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