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Original Article

NPK fertilization modulates enzyme activity and mitigates the impacts of salinity on West Indian cherry

Fertilização com NPK modula a atividade enzimática e atenua os impactos da salinidade na aceroleira

A. M. da Silva Filho[a*], H. R. Gheyi[a], A. S. de Melo[b], P. C. C. Silva[c], T. I. da Silva[d], S. I. Bonou[a], R. S. de Alencar[b], G. F. Dias[b], C. F. de Lacerda[e] and R. L. de S. Ferraz[b]

[a] Universidade Federal de Campina Grande, Programa de Pós-Graduação em Engenharia Agrícola, Campina Grande, PB, Brasil
[b] Universidade Estadual da Paraíba, Programa de Pós-Graduação em Ciências Agrárias, Campina Grande, PB, Brasil
[c] Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, Maués, AM, Brasil
[d] Universidade Federal do Recôncavo da Bahia, Cruz das Almas, BA, Brasil
[e] Universidade Federal do Ceará, Programa de Pós-Graduação em Engenharia Agrícola, Fortaleza, CE, Brasil

Abstract

Salt stress causes several physiological and biochemical disorders and impairs plant growth. However, adequate fertilization can improve the nutritional status and may reduce significantly the harmful effects caused by salt stress. From this perspective, this study aimed to evaluate the impact of different combinations of nitrogen, phosphorus and potassium fertilization on the antioxidant activity and accumulation of organic and inorganic solutes in West Indian cherry leaves, in the second year of production. The experimental design was in randomized blocks, with treatments distributed in a 10 × 2 factorial arrangement corresponding to ten fertilization combinations (FC) of NPK (FC1: 80-100-100%, FC2:100-100-100%, FC3:120-100-100%, FC4:140-100-100%, FC5:100-80-100%, FC6:100-120-100%, FC7:100-140-100%, FC8:100-100-80%, FC9:100-100-120%, and FC10:100-100-140% of the recommendation) and two levels of electrical conductivity of irrigation water (ECw) (0.6 and 4.0 dS m^{-1}), with three replications. The multivariate analysis showed that irrigation with water of different electrical conductivities (0.6 and 4.0 dS m^{-1}) resulted in different responses concerning the enzyme activity, production of organic compounds, and accumulation of inorganic solutes in the leaves. Under irrigation with low salinity water, there was greater accumulation of K^+, soluble carbohydrates, and proline, and lower activity of antioxidative enzymes, especially SOD and APX. Under high salinity water, greater enzyme activity and higher concentrations of Na^+ and Cl^- were observed. The results indicate that the response of West Indian cherry to salinity was more towards redox homeostasis than osmotic homeostasis through the accumulation of compatible solutes. Fertilization combination FC5 (100-80-100% corresponding to 200, 24 and 80 g $plant^{-1}$ of NPK) modulates the enzyme activity of SOD and APX attenuating the impacts of salinity, being an efficient combination to preserve redox homeostasis in West Indian cherry plants grown under salt stress.

Keywords: *Malpighia emarginata*, salt stress, fertilization management, antioxidant system.

Resumo

O estresse salino causa distúrbios fisiológicos e bioquímicos que prejudicam o crescimento vegetal. Entretanto, acredita-se que a fertilização mineral adequada pode melhorar o estado nutricional e reduzir os danos causados pelo estresse salino. Dessa forma, o objetivo deste estudo foi avaliar os impactos de diferentes combinações de fertilização com nitrogênio, fosforo e potássio sobre o acúmulo de solutos (orgânicos e inorgânicos) e atividade antioxidante em folhas de aceroleira cultivada sob estresse salino no segundo ano de produção. O delineamento experimental foi em blocos casualizados com os tratamentos distribuídos em esquema fatorial 10 × 2, correspondendo a 10 combinações de adubação (FC) com nitrogênio, fósforo e potássio (FC1: 80-100-100%; FC2:100-100-100%; FC3:120-100-100%; FC4:140-100-100%; FC5:100-80-100%; FC6:100-120-100%; FC7:100-140-100%; FC8:100-100-80%; FC9:100-100-120% e FC10:100-100-140% da recomendação, referente ao segundo ano de cultivo) e dois níveis de condutividade elétrica da água (ECw) utilizada na irrigação (0,6 e 4,0 dS m^{-1}), com três repetições. A análise multivariada mostrou que irrigação com água de diferentes condutividades elétricas (0,6 e 4,0 dS m^{-1}) apresentou processos distintos em relação à atividade enzimática, produção de compostos orgânicos e acúmulo de solutos inorgânicos nas folhas. Sob irrigação com água de baixa salinidade houve maior acúmulo de K^+, carboidratos solúveis e prolina, e menor atividade de enzimas antioxidativas, especialmente SOD e APX. Sob alta salinidade se observou maior atividade enzimática e maiores concentrações de Na^+ e Cl^-. Os resultados indicam que a resposta da acerola à salinidade foi mais no sentido da homeostase redox do que da homeostase osmótica por meio do acúmulo de solutos compatíveis

*e-mail: manoel.silva@estudante.ufcg.edu.br
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com o metabolismo. A combinação de adubação FC5 (100-80-100% da recomendação correspondente a 200, 24 e 80 g planta^{-1} de NPK) modula a atividade enzimática da SOD e APX, atenuando os impactos da salinidade, sendo uma combinação eficiente para preservar a sua homeostase redox nas aceroleiras sob estresse salino.

Palavras-chave: *Malpighia emarginata*, estresse salino, manejo de adubação, sistema antioxidante.

1. Introduction

West Indian cherry (*Malpighia emarginata* D. C.) is a fruit from Central and Northern South America that is widely cultivated in Northeastern Brazil (Santos and Lima, 2020). Almost 70 thousand tons were produced in Brazil in 2017, with the State of Pernambuco leading the national production, with approximately 30.5% of the entire Brazilian output, followed by the States of Ceará (10.8%) and Sergipe (7.75%) (IBGE, 2017). The Northeast region of Brazil has some edaphoclimatic features considered hostile for most crops due to natural and/or anthropogenic factors, with soil and irrigation water salinity representing two of the main factors that most compromise plant production (Pessoa et al., 2022).

Salinity changes plant metabolism, negatively affecting osmotic, ionic, nutritional, and redox homeostasis (Shah et al., 2017; Silva et al., 2021). Furthermore, the increase in salt concentration in the rhizosphere reduces the free energy of water and, consequently, the water potential, hindering water and nutrient uptake by plants. As a defense process, plants trigger an osmotic adjustment mechanism that consists of accumulating organic and inorganic solutes and, consequently, maintaining the water potential gradient in the soil-plant system. Soluble proteins, soluble carbohydrates, glycine betaine, and amino acids are among the most studied organic solutes (Azevedo Neto et al., 2020; Munns et al., 2020).

For most glycophyte, cellular osmotic adjustment under salt stress preferably occurs through the accumulation of inorganic solutes (Lacerda et al., 2003; Zeng et al., 2015), notably sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions. The increase in the concentration of these ions can increase the cell's water uptake capacity under saline conditions. However, Na^+ and Cl^- are considered phytotoxic at higher concentrations. Thus, their accumulation can cause several negative effects on plant cells (Lacerda et al., 2003; Zeng et al., 2015; Azevedo Neto et al., 2020).

Environmental stresses can increase the production and accumulation of reactive oxygen species (ROS) in plants, especially under severe stress conditions. This increase is mainly due to the imbalance between the production and consumption of ROS. One of the sources of this imbalance is the excess of energy and electrons accumulated in plants under stress conditions (Shah et al., 2017). In response to each secondary stress, many plants increase the activity of antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD), which can mitigate, at least partially, the harmful effects of these free radicals (Silva et al., 2021).

In this scenario, many studies have been conducted to find viable solutions to mitigate the effects of salinity on fruit species, e.g., fertilization formulations using nitrogen, phosphorus, and potassium (NPK) in sugar apple (*Annona squamosa* L. - Souza et al., 2023b) and tomatoes (*Solanum lycopersicum* L. - Ahamed and Abdalla, 2019), among other crops. Furthermore, assays conducted with NPK fertilization alone indicate that this practice improves the carbohydrate metabolism, osmotic regulation, and the antioxidant capacity of plants (Hasanuzzaman et al., 2020; Dias et al., 2022). Silva et al. (2022) observed that the combined application of NPK (100-125-125% of the recommendation) associated with the electrical conductivity of irrigation water (ECw) of 3.0 dS m^{-1} in case of sugar apple increased the average mass and fruit diameter. However, each species responds differently depending on their nutrient requirements and nutritional status, especially when exposed to salinity. From this perspective, this study introduces a novel approach to mitigate salt stress in West Indian cherry (*Malpighia emarginata*) with NPK fertilization, aiming at evaluating the effects of different NPK combinations on the accumulation of solutes (organic and inorganic) and the antioxidant activity in plants grown under salt stress in the second year of production.

2. Material and Methods

The experiment was conducted in a greenhouse at the Department of Agricultural Engineering of the Federal University of Campina Grande, in the municipality of Campina Grande, Paraíba, Brazil (7º 12' 51" S, 35º 54' 23" W, elevation of 550 m a.s.l.). The air temperature (maximum and minimum) and the mean relative air humidity during the experimental period in the second year of production were measured inside the greenhouse (Figure 1).

The assay started on March 2, 2020, corresponding to the first year of cultivation, and was structured in a randomized block design. The treatments were distributed in a 2 × 10 factorial arrangement with three replicates and one plant per experimental plot. Two electrical conductivity levels of irrigation water (ECw – 0.6 and 4.0 dS m^{-1}) associated with ten fertilization combinations (FC) of NPK were studied (FC1 = 80-100-100%, FC2 = 100-100-100%, FC3 = 120-100-100%, FC4 = 140-100-100%, FC5 = 100-80-100%, FC6 = 100-120-100%, FC7 = 100-140-100%, FC8 = 100-100-80%, FC9 = 100-100-120%, and FC10 = 100-100-140% of the fertilization recommended by Cavalcante (2008) for the first year of cultivation). The NPK fertilization combination referring to treatment FC2 (100-100-100%) corresponded to the application of 100, 60, and 60 g plant^{-1} year^{-1} of N, P_2O_5, and K_2O, respectively. The saline water treatments were based on the study conducted by Silva et al. (2020).

The seedlings were transplanted to 200-L drainage lysimeters whose bottom was covered with geotextile fabric and filled with 1.0 kg gravel (9.5 to 19 mm size) and 230 kg of soil classified as Entisol (United States, 2014), collected (0-0.20 m depth) in the municipality of Riachão do Bacamarte – PB (7° 15' 34" S and 35° 40'

1" W, elevation of 192 m a.s.l.). The physicochemical attributes were determined according to the methodologies recommended by Teixeira et al. (2017), and the results are presented in Table 1.

The water used for irrigation was prepared by adding NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ to local tap water (ECw = 0.38 dS m^{-1}), maintaining the equivalent proportion of 7:2:1 using the relationship between the ECw and the concentration of salts ($mmol_c\ L^{-1} \approx$ ECw x 10) reported by Richards (1954). This proportion of Na:Ca:Mg is commonly found in water sources of Northeastern Brazil (Silva Junior et al., 1999). The details regarding the conduction and management of the assay adopted in the first year of production are described in Souza et al. (2023a).

At the end of the first year of cultivation, the West Indian cherry plants were subjected to 15 days of water stress, after which they were pruned on March 5, 2021, thus beginning the second year of production. The electrical conductivity levels of water and the NPK fertilization combinations used in the second year were the same as in the first year. However, the NPK doses were adopted according to the recommendation of Cavalcante (2008) for the second year of production, consisting of, respectively, 200, 30, and 80 g $plant^{-1}$ $year^{-1}$ of N, P_2O_5, and K_2O for the FC2 combination (100-100-100% of NPK) and the levels of the other combinations were adjusted accordingly.

Irrigation with the water of respective salinity levels began 15 days after pruning (DAP) by adopting a two-day (every other day) irrigation schedule. Water was applied to each lysimeter according to treatments, maintaining soil moisture close to field capacity. The volume of water was estimated by the water balance in the soil, i.e., the real water requirement of the plants, determined by Equation 1:

$$VI = \frac{\left(V_a - V_d\right)}{\left(1 - LF\right)} \tag{1}$$

where:
VI = water volume used in the irrigation event (mL), Va = water volume applied in the previous irrigation event (mL), Vd = water volume drained after the previous irrigation event (mL), and LF = leaching fraction (0.10) of the total water volume used in the period, to avoid excessive salt accumulation in the soil and was applied at an interval of 90 days.

The NPK dose was split into 24 equal applications, via topdressing, at 15-day intervals. The sources used were

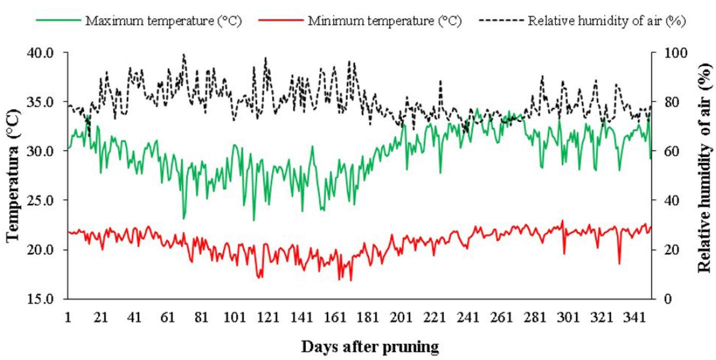

Figure 1. Daily maximum and minimum temperatures and mean relative air humidity observed inside the greenhouse during the experimental period (March 5, 2021 to February 17, 2022) in the second year of production of West Indian cherry (*Malpighia emarginata*).

Table 1. Chemical and physical attributes of the soil (0-20 cm depth) used in the experiment

Chemical attributes							
pH_{H2O}	O.M.	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	$Al^{3+} + H^+$
1:2.5	g dm^{-3}	mg dm^{-3}	$cmol_c\ kg^{-1}$				
6.5	8.1	79	0.24	0.51	14.90	5.40	0.90

....................Chemical attributes						..........................Physical attributes				
EC_e	CEC	SAR_e	ESP	SB	V	Particle fraction - g kg^{-1}			Moisture content - dag kg^{-1}	
dS m^{-1}	$cmol_c\ kg^{-1}$	$(mmol\ L^{-1})^{0.5}$	%	$cmol_c\ kg^{-1}$	%	Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
2.15	21.95	0.16	2.3	21.05	95.9	572.7	100.7	326.6	25.91	12.96

pH – potential of hydrogen; O.M. – organic matter: Walkley-Black Wet digestion; Ca2+ and Mg2+ - extracted with 1 M KCl at pH 7.0; Na+ and K+ - extracted with NH4OAC 1 M at pH 7.0; Al3+ + H+ - extracted with CaOAc 0.5 M at pH 7.0; ECe – Electrical conductivity of the saturated paste extract; CEC – Cation exchange capacity; SARe – Sodium adsorption ratio of the saturated paste extract; ESP – Exchangeable sodium percentage; SB – Sum of exchangeable bases (K+ + Ca2+ + Mg2+ + Na+); V – Base saturation% ([SB/CEC] × 100); 1–2 – Referring to field capacity and the permanent wilting point of soil, respectively.

calcium nitrate (15.5% N), monoammonium phosphate (60% P_2O_5 and 12% N), and potassium sulfate (51,5% K_2O). Foliar fertilization with micronutrients was performed every 15 days on the adaxial and abaxial leaf surfaces with a solution containing 1.0 g L^{-1} of Dripsol® (Mg = 1.1%, Zn = 4.2%, B = 0.85%, Fe = 3.4%, Mn = 3.2%, Cu = 0.5%, and Mo = 0.05%). Crop management practices, such as weeding, hoeing, soil scarification, and phytosanitary control were performed, whenever necessary during the experimental period.

At 30 DAP (04/04/2021), the plants were divided into four quadrants (North, South, East, and West) to establish new branches. Subsequently, at 304 DAP (03/01/2022 - period of full flowering and fruiting), two pairs of younger and fully expanded leaves were collected from each quadrant of the middle-third region of the canopy, which were immediately stored in a container at 4 °C for 30 minutes. Then, the material was taken to the laboratory and washed with distilled water to eliminate any contaminating waste from the leaf surface, after which it was stored in an ultra-freezer at a minimum temperature of -20 °C. Half of the material was lyophilized to prepare the extract for the analyses of organic and inorganic solutes, whereas the other half was macerated for enzyme activity analysis.

2.1. Determination of inorganic solutes

The extracts of the leaf samples were prepared in deionized water, following the methodology described by Azevedo Neto et al. (2020). For that purpose, 10 mL of deionized water was added to 0.1 g of a dry leaf powder obtained by grinding. The tubes were heated at 95 °C in a water bath for one hour and then centrifuged at 5,000 × g for five minutes. Then, the supernatant was filtered using quantitative filter paper. The concentration of Na^+ and K^+ were determined by flame photometry (Faithfull, 2002) with a Q498M2 photometer (Quimis, Diadema, SP, BR). The Cl^- concentration was determined by a UV-VIS spectrophotometer, model 2000 UV (Bel Engineering, Piracicaba, SP, BR) (Gaines et al., 1984), using a solution of thiocyanate of mercury in absolute methanol and 20.2% iron nitrate.

2.2. Determination of organic solutes

To determine the organic solutes, the extract was obtained by macerating, in a mortar, 0.1 g of lyophilized leaf in 6 mL of a 0.1 M potassium phosphate buffer solution (pH 7.0) containing EDTA (0.1 mM). The extract was filtered in a fine nylon fabric and centrifuged at 10,000 × *g* for 15 min. The supernatant was stored in a freezer and subsequently used to determine the organic solutes (soluble carbohydrates, free amino acids, soluble proteins, and free proline).

The soluble carbohydrates were determined by the phenol-sulfuric acid method using spectrophotometry at 490 nm (Dubois et al., 1956). The total free amino acids were determined by spectrophotometry at 570 nm by the ninhydrin method (Yemm et al., 1955). Concentration of free proline was determined by spectrophotometry at 520 nm by the acid ninhydrin method (Bates et al., 1973). Soluble proteins were determined by spectrophotometry at 595 nm using the protein-dye binding method (Bradford, 1976).

2.3. Determination of the activity of antioxidant enzymes

Enzyme activity (superoxide dismutase, catalase, and ascorbate peroxidase) was determined using 200 mg of leaf tissue macerated in 2 mL of potassium phosphate buffer solution (50 mM, pH 7.5) with addition of ascorbic acid (0.1 mM), EDTA (0.1 mM) and polyvinylpyrrolidone (3%). Subsequently, the extract was centrifuged at 10,000 × *g* at a temperature of 4 ºC for 20 min using a Mikro200r refrigerated centrifuge. The supernatant was aspirated and transferred to 2-mL centrifuge tubes, which were maintained in an ultra-freezer at -20 °C until the moment of the analyses.

The activity of superoxide dismutase (SOD) was determined based on the ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) by the enzyme present in the extract (Beauchamp and Fridovich, 1971). Catalase (CAT) activity was quantified according to Kar and Mishra (1976) based on the consumption of hydrogen peroxide (H_2O_2) by the enzyme present in the extract. The activity of ascorbate peroxidase (APX) was determined by measuring the reduction in absorbance at 290 nm after adding H_2O_2 to the reaction medium (Nakano and Asada, 1981).

2.4. Data analysis

The data obtained were tested for normality of distribution (Shapiro-Wilk's test). Next, the data were subjected to principal component analysis (PCA), and the treatments were plotted with the first two components (PC1 and PC2). The number of groups was defined based on the dispersion of the first two principal components (PC1 and PC2) by cluster analysis. Pearson's correlation coefficient ($p \leq 0.05$) was used to determine correlations between the variables belonging to enzyme activity and to the concentration of organic and inorganic solutes. Analysis of variance was performed for organic solutes and enzyme activity ($p \leq 0.05$). In case of significant effect, the means were compared using the F-test for the electrical conductivity of irrigation water and the Scott-Knott's test for the fertilization combinations (NPK). The statistical analyses were performed using the software R v.4.0.1 (R Core Team, 2022).

3. Results and Discussion

The multidimensional space of the original variables was reduced to two principal components (PC1 and PC2). The eigenvalues and the percentage of variation explained by each component jointly represented 54.63% of the total variation, with PC1 explaining 38.56% of the variance and PC2 16.07% of the total variance (Table 2).

Three groups were formed by the combination of treatments. Group 1 was formed by all NPK combinations and plants irrigated with the electrical conductivity of 0.6 dS m^{-1} (EC0.6), as well as by combination FC10 with the electrical conductivity of 4.0 dS m^{-1} (EC4). The second group was formed by the NPK combinations FC1, FC3, FC4,

Table 2. Eigenvalues, percentage of the total variance explained, multivariate analysis of variance (MANOVA), and correlation coefficients (r) between original variables and principal components in West Indian cherry (Malpighia emarginata) plants grown under salt stress (ECw) and NPK fertilization combinations during the second year of production and 304 days after pruning (DAP).

	Principal components	
	PC1	**PC2**
Variance	4.24	1.77
% of variance	38.56	16.07
Cumulative variance (%)	38.56	54.63

PCs	**Cl^-**	**Eigenvectors**									
		Na^+	**K^+**	**Na^+/K^+**	**CAR**	**PRT**	**AMI**	**PRO**	**SOD**	**CAT**	**APX**
PC1	0.359	0.442	-0.299	0.458	-0.277	0.287	0.073	-0.036	0.213	0.038	0.404
PC2	-0.236	0.070	-0.021	0.028	0.093	0.343	0.578	0.063	0.072	0.620	-0.290

PCs - principal components: PC1 - principal component 1; PC2 - principal component 2. Inorganic solutes: Cl^-, Na+, K+, Na+/K+; Organic solutes: CAR – soluble carbohydrates, PRT – soluble proteins, AMI – free amino acids, and PRO – free proline; Enzyme activity: SOD - superoxide dismutase, CAT – catalase, and APX – ascorbate peroxidase.

FC5, and FC8 with EC4 and group 3 was formed by FC2, FC4, FC6, FC7, and FC9 with EC4 (Figure 2).

The soluble protein, Na^+ concentrations, Na^+/K^+ ratio, and SOD activity were highly related to combinations FC4EC4 (140-100-100% of the NPK level and ECw of 4 dS m^{-1}) and FC9EC4 (100-100-120% of the NPK recommendation and ECw of 4 dS m^{-1}). The Cl^- concentration and the APX activity were more related to combination FC7EC4 (100-140-100% of the NPK recommendation and ECw of 4 dS m^{-1}). Such combinations belong to the same group. The amino acid content and CAT activity were more related to combinations FC1EC4 (80-100-100% of the NPK recommendation and ECw of 4 dS m^{-1}), FC3EC4 (120-100-100% of the NPK recommendation and ECw of 4 dS m^{-1}), FC5EC4 (100-80-100% of the NPK recommendation and ECw of 4 dS m^{-1}), with these treatments belonging to the same group. The contents of amino acids and K^+ concentration were more related to combinations FC9EC0.6 (100-100-120% of the NPK recommendation and and ECw of 0.6 dS m^{-1}) and FC10EC0.6 (100-100-140% of the NPK recommendation and ECw of 0.6 dS m^{-1}). The proline content had a small contribution for the principal component analysis.

The activity of the SOD enzyme had a positive correlation with the Na^+ concentration and the Na^+/K^+ ratio, as well as the activity of the APX enzyme with the Cl^- concentration. CAT activity was positively related to the content of amino acids (Amino), as well as the concentration of K^+ with the proline content. The K^+ concentration was negatively correlated with the concentration of Cl^-, Na^+, and the Na^+/K^+ ratio, whereas the proline content was negatively correlated with the Cl^- concentration and APX activity. The protein content was positively correlated with the content of amino acids and the activity of the SOD, CAT, and APX activities (Figure 3).

Nitrogen and potassium play crucial role in protein synthesis in plants. Nitrogen is a principal component of amino acids, the building blocks of proteins, whereas potassium acts as an essential ion in osmotic regulation and nutrient transport (Taiz et al., 2017; Santos et al., 2022). The high Na^+/K^+ ratio in combinations FC4EC4 and FC9EC4 might indicate an ionic imbalance that affects nutrient uptake, directly impacting protein synthesis.

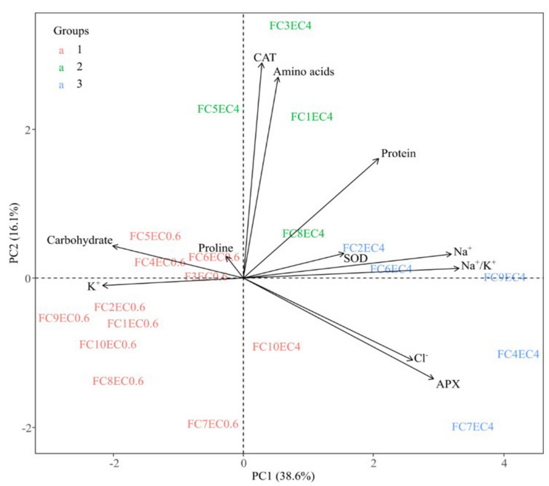

Figure 2. Principal component analysis with clusters for the combination between the electrical conductivity of irrigation water (ECw) and NPK fertilization combinations (FC) in West Indian cherry (*Malpighia emarginata*) plants during the second year of production 304 days after pruning (DAP). PC1 - Principal component 1; PC2 - Principal component 2; FC - Fertilization combination with nitrogen, phosphorus, and potassium (NPK), namely: FC1 = 80-100-100%, FC2 = 100-100-100%, FC3 = 120-100-100%, FC4 = 140-100-100%, FC5 = 100-80-100%, FC6 = 100-120-100%, FC7 = 100-140-100%, FC8 = 100-100-80%, FC9 = 100-100-120%, and FC10 = 100-100-140% of the recommendation proposed by Cavalcante (2008) for the cultivation of West Indies cherry in the second year; EC4 - electrical conductivity of irrigation water (4.0 dS m^{-1}) and EC0.6 (0.6 dS m^{-1}); Inorganic solutes: Cl^-, Na^+, K^+, Na^+/K^+; Organic solutes: soluble carbohydrates, soluble proteins, free amino acids, and free proline; Enzyme activity: SOD - superoxide dismutase, CAT – catalase, and APX – ascorbate peroxidase (APX).

Superoxide dismutase (SOD) is a critical antioxidant enzyme to combat oxidative stress in plants (Melo et al.,

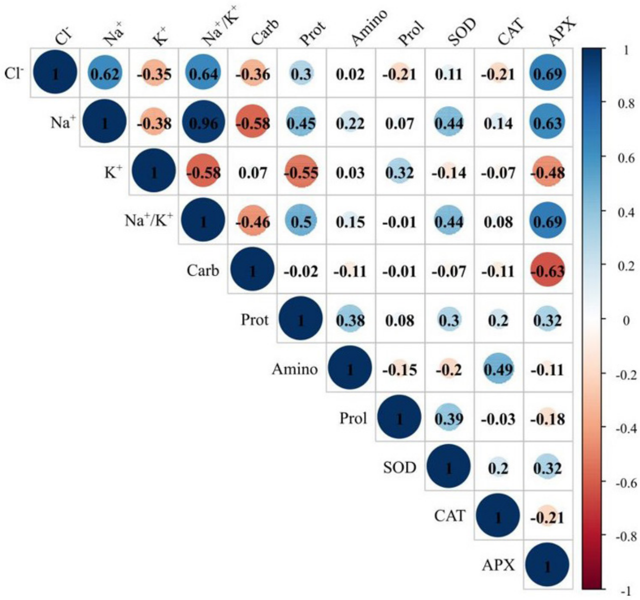

Figure 3. Pearson's correlation for the concentration of organic and inorganic solutes and enzyme activity in West Indian cherry (*Malpighia emarginata*) plants grown under salt stress (ECw) and NPK fertilization combinations (FC) during the second year of production 304 days after pruning (DAP). Significant correlations (p≤0.05) are highlighted in circles.

2022). The strong positive relationship between SOD activity and the Na^+ concentration and the Na^+/K^+ ratio (Figure 3) in combinations FC4EC4 and FC9EC4 suggests that these conditions lead to increased oxidative stress in plants (Figure 2). Excess Na^+ and ionic imbalance can lead to the production of free radicals, stimulating SOD activity as a defense mechanism.

Peroxidase activity (APX) is related to the detoxification of hydrogen peroxide (H_2O_2), a toxic by-product of cellular metabolism (Melo et al., 2022). The close relationship between APX activity and the Cl^- concentration in combination FC7EC4 suggests that the excess of this ion can trigger the production of H_2O_2, resulting in higher APX activity in order to mitigate the oxidative stress. Furthermore, catalase (CAT) is another antioxidant enzyme that catalyzes the decomposition of H_2O_2 into oxygen and water. The strong positive relationship between CAT activity and the contents of amino acids (Figure 3) in combinations FC1EC4, FC3EC4, and FC5EC4 (Figure 2) suggests that the adequate concentration of amino acids can stimulate the antioxidant defense in plants. Amino acids can serve as co-factors for antioxidant enzymes.

Potassium is vital for ionic regulation in plant cells (Taiz et al., 2017). The negative correlation between K^+ and Na^+ concentration and the Na^+/K^+ ratio (Figure 3) indicates that, under conditions of higher concentrations of Na^+, plants could resist to maintain the K^+ balance in cells, which has direct implications on water and nutrient transport.

The summary of the analysis of variance shows that the treatments did not affect ($p>0.05$) the content of soluble carbohydrates. On the other hand, the soluble proteins had a significant effect ($p\leq0.01$) only of the ECw factor. Additionally, the interaction between ECw and FC affected ($p\leq0.01$) the concentrations of free amino acids and proline in the leaves of West Indian cherry (Table 3).

Salt stress increased on average 8.56% the content of soluble proteins in the leaves (Figure 4). This type of stress usually reduces the protein content in plant leaves, as observed in tomatoes by Ali et al. (2021). However, in tolerant plants, prolonged salt stress can increase the expression of genes related to protein synthesis as an adaptation mechanism in response to stress (Han et al., 2019). The accumulation of different soluble proteins can be considered a vital strategy to regulate plant growth and development under salt stress (Athar et al., 2022).

Han et al. (2019) observed in apple (*Malus domestica* Borkh.) cultivar 'Royal Gala' grown under stress conditions, there was a positive regulation in the expression of proteins related to energy metabolism and antioxidant defense in the leaves, providing plants with the ability to tolerate salt stress. It should be noted that the increase in the leaf protein content due to salinity can vary between species and depend on factors such as the intensity and duration of salt stress. Furthermore, these proteins are directly involved with the acquisition of new phenotypes that can adopt to the salt-stressed environment, contributing to vital metabolic processes. Thus, the contribution of specific protein is more important for salt tolerance mechanisms than the protein content (Athar et al., 2022).

The highest content of free amino acids in non-stressed plants were found in treatments FC5 and FC6, which showed significant differences in relation to the other treatments. On the other hand, under salt stress conditions (ECw= 4 dS m^{-1}), the highest values were observed in combinations FC3, FC5, and FC8 (Figure 5).

Table 3. Summary of the analysis of variance for the contents of organic solutes – soluble carbohydrates (CAR), soluble proteins (PRT), free amino acids (AMI), and free proline (PRO) present in the leaves of West Indian cherry (Malpighia emarginata) plants irrigated with salinized water (ECw) and fertilized with different combinations of nitrogen, phosphorus, and potassium (NPK) during the second year of production 304 days after pruning (DAP).

Sources of variation	DF	Mean squares			
		CAR	PRT	AMI	PRO
Electrical conductivity of water – ECw	1	20766.15^{ns}	13.55^{**}	180.75^{**}	0.02^{ns}
Fertilization combinations – FC	9	7548.18^{ns}	0.89^{ns}	126.21^{**}	0.86^{**}
Interaction (Ecw × FC)	9	1666.16^{ns}	0.71^{ns}	104.05^{**}	0.89^{**}
Block	2	14455.67^{ns}	1.84^{ns}	4.02^{ns}	0.15^{*}
Residual	38	5660.36	1.71	4.97	0.04
CV (%)		12.47	11.33	11.69	10.02

DF – Degrees of freedom; CV – coefficient of variation; ns, *, **, not significant and significant at p ≤ 0.05 and p ≤ 0.01, respectively.

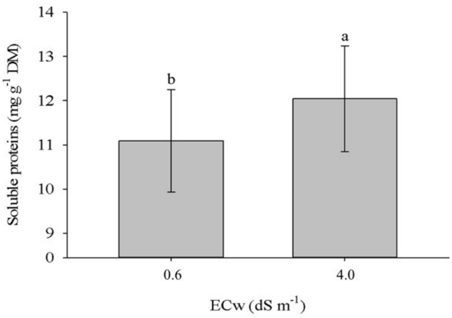

Figure 4. Content of soluble proteins in leaves of West Indian cherry (*Malpighia emarginata*) as a function of irrigation water salinity (ECw) during the second year of production 304 days after pruning (DAP). Means of three replications ± standard error. Means followed by the same letters do not differ by F test (p < 0.05)

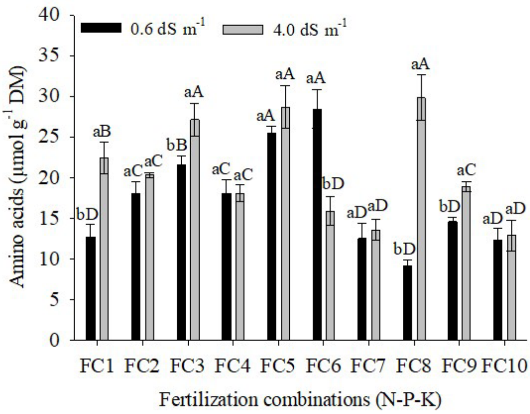

Figure 5. Content of free amino acids in leaves of West Indian cherry (*Malpighia emarginata*) plants grown under salt stress (ECw) and NPK fertilization combinations (FC) during the second year of production 304 days after pruning (DAP). FC1 = 80-100-100%, FC2 = 100-100-100% (control), FC3 = 120-100-100%, FC4 = 140-100-100%, FC5 = 100-80-100%, FC6 = 100-120-100%, FC7 = 100-140-100%, FC8 = 100-100-80%, FC9 = 100-100-120%, and FC10 = 100-100-140% of the recommended N-P-K level for the second year of production. Means followed by the same uppercase letters indicate no significant differences between fertilization combinations (Scott-Knott's test, p ≤ 0.05) for the same salinity level, and the same lowercase letters in the same fertilization combination indicate no significant differences between water salinity levels (Fisher's test, p ≤ 0.05). Means of three replications ± standard error.

In leaves of plants grown without salt stress, the content of free amino acid increased by 40.8 and 56.9% for combinations FC5 and FC6, respectively, and decreased by 29.8, 30.9, 49.1, 19.3, and 32% in combinations FC1, FC7, FC8, FC9, and FC10, respectively, in comparison to FC2 under the recommended fertilization (Figure 5). While under salt stress conditions, on average 40.6% increase was observed for combinations FC3, FC5, and FC8 and in combinations FC6, FC7, and FC10 on average 30.5% decrease occurred in the content of free amino acids compared to FC2 plants (Figure 5). Overall, the results show that, under both conditions (absence and presence of salt stress), under combination FC5 the content of amino acids increased, whereas in the combinations FC7 and FC10 it reduced compared to the other combinations.

Salt stress increased the concentration of free amino acids in the leaves when plants were subjected to fertilization combinations FC1 (77%), FC3 (25%), FC8 (224%), and FC9 (29%), compared to non-stressed plants. On the other hand, salinity reduced the content of free amino acids in plants subjected to combination FC6 (44%). This increase under salt stress is due to the acclimation mechanism of plants to tolerate salt stress. It should be noted that amino acids are considered one of the main organic solutes related to osmotic adjustment in plants under stress (Hasanuzzaman and Fujita., 2022). For Silva et al. (2019a), amino acids such as proline and γ-aminobutyric acid (GABA) could also be related to the consumption of reactive oxygen species. Furthermore, the increase in amino acid concentration could be related to the fact that nitrogen fertilization increases the activity of nitrate reductase, facilitating its rapid conversion into N precursors for the production of amino acids necessary for protein synthesis (Ahanger et al., 2019).

The highest content of proline in non-stressed plants were found in combination FC10, which showed a significant difference in relation to the other treatments. On the other hand, under stress conditions (ECw= 4 dS m^{-1}), the highest values were found in combinations FC2 and FC6 (Figure 6).

In fertilization combination FC10 the free proline content decreased 47.8% in plants grown under salt stress. Under the same conditions, only combination FC6 had a free proline content similar to the fertilizer combination as per the recommended level (FC2). Other combinations that showed reductions compared to FC2 in the free proline concentration under salt stress, were FC3 (18%), FC1, FC7, and FC9 (35% on average), and FC4, FC5, FC8, and FC10 (47% on average) (Figure 6).

Irrigation water salinity (ECw= 4.0 dS m^{-1}) increased the proline content by 38.1, 47.5, 49.4%, and 33% in combinations FC2, FC3, FC6, and FC7, respectively and decreased the content by 23.7, 16.9, and 47.8% in combinations FC5, FC9, and FC10, respectively compared to plants irrigated without stress (ECw= 0.6 dS m^{-1}) (Figure 6). The expressive increase observed under combination FC10 in non-stressed plants could be due to the increase in the K supply. Weimberg et al. (1982) stated that, below the threshold concentration, the increase in KCl application increased the concentration of proline in sorghum leaves (*Sorghum bicolor* (L.) Moench), thus improving osmotic adjustment in response to the salt.

Combination FC6 was effective to reduce the effects of salinity on the accumulation of free proline, resulting in contents similar to the combination of the recommended level (FC2). According to Bargaz et al. (2016), the increase in P supply in the soil can expressively increase the concentration of proline in common bean hybrids (*Phaseolus vulgaris* L.) grown under different salinity levels. It should be noted that proline plays a key role in osmoprotection, acting in the resilience of plants under abiotic stress conditions (Melo et al., 2022). Under salt stress, proline accumulation is an adaptative response of plants, which is due to the maintenance of osmotic balance to protect cell structures from damage (El Moukhtari et al., 2020). Therefore, the results found in the present study reinforce the importance of adequate fertilization management to mitigate the deleterious effect of salinity on West Indian cherry plants.

The effect of interaction between the ECw and FC factors was significant ($p \leq 0.01$) for the antioxidant enzyme activity in West Indian cherry leaves (Table 4). The highest activity of superoxide dismutase (SOD) in non-stressed plants was found in treatments FC8 and FC9, which showed significant differences compared to the other combinations (Figure 7). On the other hand, under stress conditions (ECw= 4 dS m^{-1}), higher values were observed in combinations FC2, FC3, FC6, and FC7, compared to the other combinations (Figure 7). Plants grown without salt stress and under combinations FC8 and FC9 showed higher SOD activity (on average 6%), whereas combinations FC3, FC4, FC6 and FC7 showed lower activity (on average 7.7%) compared to combination FC2. In plants subjected to salt stress, combinations FC1, FC4, FC5, FC8, and FC10 showed

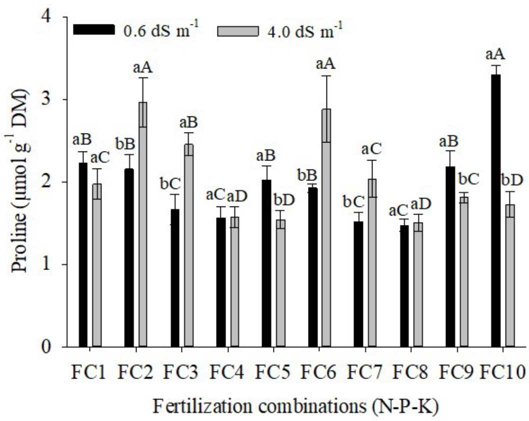

Figure 6. Content of free proline in leaves of West Indian cherry (*Malpighia emarginata*) grown under salt stress (ECw) and combinations of NPK fertilization during the second year of production 304 days after pruning (DAP). See Figure 5 for details of the FC treatments. Means followed by the same uppercase letters indicate no significant differences between fertilization combinations (Scott-Knott's test, $p \leq 0.05$) for the same salinity level, and the same lowercase letters in the same fertilization combination indicate no significant differences between water salinity levels (Fisher's test, $p \leq 0.05$). Means of three replications ± standard error.

Table 4. Summary of the analysis of variance for the enzyme activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) in the leaves of West Indian cherry plants irrigated with salinized water (ECw), and combinations of nitrogen, phosphorus, and potassium (NPK) during the second year of production 304 days after pruning (DAP).

Sources of variation	DF	Mean squares		
		SOD	CAT	APX
Water electrical conductivity – ECw	1	2302.89^{**}	10.23^{**}	10.03^{**}
Fertilization combinations – FC	9	236.59^{**}	2.88^{**}	1.28^{**}
Interaction (ECw × FC)	9	285.85^{**}	1.91^{**}	1.22^{**}
Block	2	13.21^{ns}	0.002^{ns}	0.058^{ns}
Residual	38	16.38	0.003	0.046
CV (%)		2.22	6.23	12.55

DF – Degree of freedom; CV – coefficient of variation; ns, **, not significant and significant at $p \leq 0.01$, respectively.

the lowest activity. When analyzing the effect of salt stress within each combination, salinity increased SOD activity in most combinations (FC1, FC2, FC3, FC4, FC6, FC7, and FC9) (Figure 7). On the other hand, in combinations FC5 and FC10, salinity did not affect SOD activity while, in combination FC8, it was reduced by 6% (Figure 7).

The lower activity of SOD in plants grown without salt stress could be associated with the higher availability of N and P_2O_5. Silva et al. (2019b) reported that the increase in phosphate fertilization reduced the activity of SOD and other antioxidant enzymes starting at the estimated level of 150 kg ha^{-1} of P_2O_5 in sugarcane plants fertilized with 50, 100, 200, and 300 kg ha^{-1} of P_2O_5 before planting. Additionally, Liao et al. (2019) studied the effect of nitrogen fertilization (0 to 2.72 kg plant^{-1} year^{-1}, in the phases of germination, physiological fruit maturation, expansion of young fruits, and color change period) in the citrus cv. Huangguogan. The authors stated that fertilization applied at high concentrations can reduce the efficiency of SOD activity and, when performed adequately, improves enzyme activity in the combat against free radicals. This behavior could be related to the fact that the appropriate rate of N application improves the activity of antioxidant enzymes, increases the elimination capacity of ROS by mesophyll cells, and maintains cell stability, thus increasing plant growth (Yue et al., 2021). Under stress conditions, SOD represents the main defense line of plants and is responsible for eliminating $O_2^{\bullet-}$, which is the most abundant component of ROS (Elsawy et al., 2018). The increased activities of these enzymes are commonly associated with defense against oxidative damage, consequently, mitigating the harmful effects of salt stress.

The highest catalase activity (CAT) was found in non-stress plants, in treatment FC1, showing significant differences in relation to the other treatments. On the other hand, under stress conditions (ECw= 4 dS m^{-1}), the highest values were found in combinations FC1 and FC5 (Figure 8).

Different from the behavior observed for the SOD enzyme, the CAT activity in non-stressed plants in the FC1 combination was highest while in other treatments it was15.3 (FC5 – 0.6 dS m^{-1}) to 70.3% (FC2 – 0.6 dS m^{-1}) lower. In plants under stress there was an expressive increase in CAT activity in FC1, FC3, and FC5 combinations compared to other combinations which presented approximately 80% inferior activity. Comparing the respective saline and non-saline combinations, in combinations FC1, FC3, and FC5, water salinity (ECw= 4.0 dS m^{-1}) expressively increased CAT activity by 258.2, 384.6, and 325.9%, respectively while in combinations FC2, FC8, and FC10, these increases amounted to 140.7, 21.5, and 42%, respectively. In contrast, salinity did not affect the CAT activity in West Indian cherry leaves in the other combinations (Figure 8).

Catalase is directly related to the elimination of H_2O_2, mainly under salt stress conditions. Several authors have reported that catalase activity is crucial for the elimination of H_2O_2 in plants under stress conditions, due to its high K_m value (between 40 and 600 mM), indicating that CAT activity can increase linearly with increasing concentrations of H_2O_2 (Ahanger et al., 2019; Silva et al., 2021; Ali et al., 2021; Melo et al., 2022).

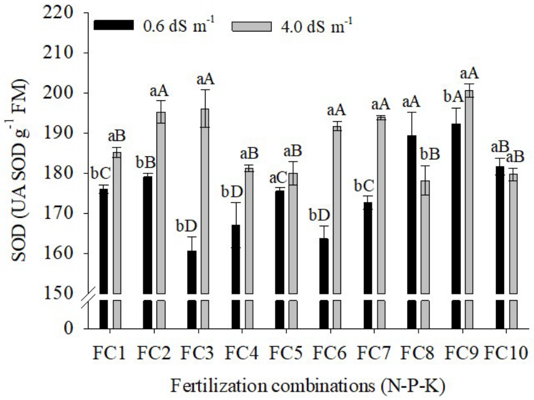

Figure 7. Superoxide dismutase activity (SOD) in leaves of West Indian cherry (*Malpighia emarginata*) grown under salt stress (ECw) and combinations of NPK fertilization during the second year of production 304 days after pruning. See Figure 5 for details of the FC treatments. Means followed by the same uppercase letters indicate no significant differences between fertilization combinations (Scott-Knott's test, p ≤ 0.05) for the same salinity level, and the same lowercase letters in the same fertilization combination indicate no significant differences between water salinity levels (Fisher's test, p ≤ 0.05). Means of three replications ± standard error.

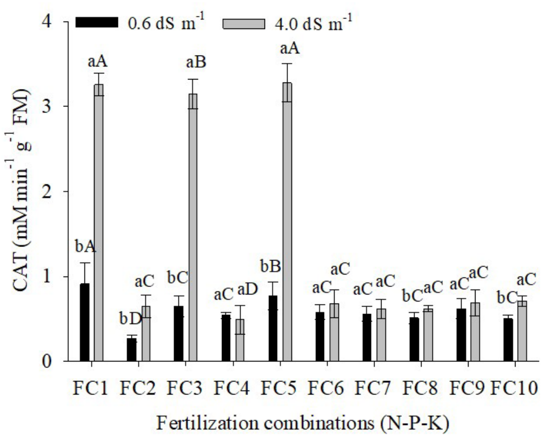

Figure 8. Activity of catalase (CAT) in leaves of West Indian cherry (*Malpighia emarginata*) grown under salt stress (ECw) and combinations of NPK fertilization during the second year of production 304 days after pruning (DAP). See Figure 5 for details of the FC treatments. Means followed by the same uppercase letters indicate no significant differences between fertilization combinations (Scott-Knott's test, p ≤ 0.05) for the same salinity level, and the same lowercase letters in the same fertilization combination indicate no significant differences between water salinity levels (Fisher's test, p ≤ 0.05). Means of three replications ± standard error.

It is important to point out that in plants under saline stress (ECw = 4.0 dS m^{-1}), during cellular respiration, molecular oxygen (O_2) accepts four electrons to produce two water molecules, however, it is only capable of accepting one electron at a time. This mechanism leads to generating ROS, capable of reacting with various biomolecules, altering them or leading to inactivation and consequent loss of biological activity (Belo and Souza, 2016). Furthermore, stress due to nitrogen deficiency (80% of the recommended dose) or excess (120% of the recommended dose) may have promoted greater catalase activity. This occurred to attenuate lipid peroxidation triggered by the activity of H_2O_2, which caused an increase in electrolyte leakage (Silva Filho et al., 2023a), which is justified by the activation of the antioxidative mechanism, indicated by the increase of the activity of the CAT to dismutate peroxide into water and oxygen. It should be added that this may have been complemented by the osmotic adjustment mechanism, indicated by the increase in the levels of amino acids (Figure 5) and proteins (Figure 4), which can also be justified by the decrease in the relative water content in the leaf (Silva Filho et al., 2023a) since the leaf tissues contained a greater amount of amino protein compounds. On the other hand, when nitrogen fertilization was excessive, there may have been synergism between the salts and an increase in salt stress, which may also have caused a homeostatic imbalance and compensation for the increase in amino acid and protein levels.

In plants fertilized with the FC5 combination, decreased amount of phosphorus applied to the soil (80% of the recommended dose) associated with saline stress may have increased H_2O_2 activity due to the instability of the phospholipid double layer and weakening of high-energy bonds, such as those found in adenosine triphosphate (ATP), which induced an increase in electrolyte leakage and a reduction in the quantum efficiency of photosystem II (Silva Filho et al., 2023b), as observed by Kaya et al. (2024) and Dissanayaka et al. (2021).

The highest ascorbate peroxidase (APX) activity in non-stressed plants was found in treatments FC5, FC7, and FC8, which showed significant differences in relation to the other treatments, while under salt stress conditions (ECw= 4 dS m^{-1}) the highest values were found in the FC4 and FC7 combinations (Figure 9).

Plants grown under combinations FC3, FC5, FC7, and FC8 without salt stress had higher APX activity (13.1, 50, 36.9, and 27%, respectively) than the FC2 combination with the recommended fertilization. On the other hand, under salt stress conditions, the FC4, FC7, and FC9 combinations had the higher activity (respectively, 53.4, 64.9, and 16%) while in combinations FC1, FC5, and FC10, there was a reduction of 21.8, 30.9, and 26.3%, respectively, compared to the FC2 combination. With the exception of combinations FC3 and FC5 salt stressed increased APX activity in all combinations varying from 26.4 (FC8) to 189.6% (FC9) compared to non-stressed plants (Figure 9).

Similar to CAT, the APX enzyme is also responsible for eliminating H_2O_2 in plants, but it has lower Km value (below 100 µM) than CAT. Therefore, APX has greater affinity for this substrate, reaching half the maximum speed with a lower concentration of hydrogen peroxide. (Nelson et al., 2019). In our study, the responses related to APX activity in West Indian cherry leaves under salt stress were apparently more representative. These results may indicate that, for this plant, the imbalance caused

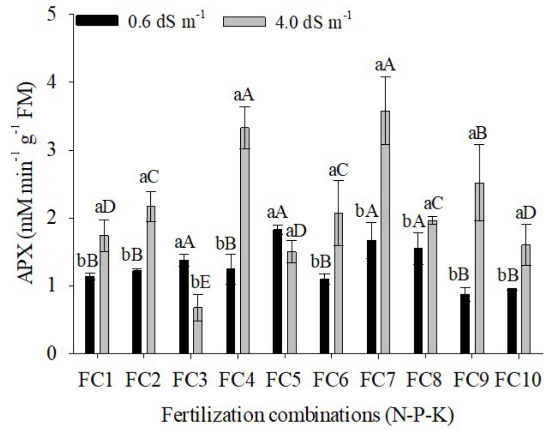

Figure 9. Ascorbate peroxidase (APX) activity in leaves of West Indian cherry (*Malpighia emarginata*) cultivated under salt stress (ECw) and combinations of fertilization with NPK during the second year of production 304 days after pruning (DAP). See Figure 5 for details of the FC treatments. Means followed by the same uppercase letters indicate no significant differences between fertilization combinations (Scott-Knott's test, $p \leq 0.05$) for the same salinity level, and the same lowercase letters in the same fertilization combination indicate no significant differences between water salinity levels (Fisher's test, $p \leq 0.05$). Means of three replications ± standard error.

by salinity under the studied conditions produces low concentrations of H_2O_2, which favors an increase in APX activity (Silva et al., 2021). Another possible explanation for this effect in West Indian cherry plants could be the high concentration of ascorbate in their leaves, the main reducing agent in the APX reaction (Deuner et al., 2008; Maruta and Ishikawa, 2017; Barros et al., 2019).

The results presented in this study corroborate those reported by Abid et al. (2020), who observed increased catalase activity in kiwi plants grown under salt stress conditions. Furthermore, these effects are related to the fact that nitrogen can improve the antioxidant capacity and salt tolerance (Ahanger et al., 2019). Additionally, in line with the results of this study, it has been reported that the application of nitrogen resulted in a notable increase in the levels of antioxidant enzymes in crops such as soybean (Borella et al., 2019), *Catharanthus roseus* (Misra and Gupta, 2006) and blueberry (*Vaccinium myrtillus* L. - Yañez-Mansilla et al., 2014).

4. Conclusions

Irrigation with water of different salinity (0.6 and 4.0 dS m^{-1}) results in distinct responses concerning enzyme activity and the production of soluble organic compounds. Under irrigation with low ECw, there was greater accumulation of soluble carbohydrates and proline, showing lower enzyme activity of SOD and APX. The opposite effect was observed in treatments of higher electrical conductivity water, with greater enzyme activity and lower concentration of proline and soluble carbohydrates. In treatments with higher ECw, enzyme activity was favored, probably in response to salinity-induced oxidative stress. Therefore, the response of West Indian cherry to salinity was more towards the search for redox homeostasis than osmotic homeostasis through the accumulation of solutes compatible with metabolism. Fertilization combination FC5 - 100-80-100% of NPK recommendation, corresponding to 200, 24, and 80 g plant^{-1} for the second year of production modulates the enzyme activity of SOD and APX, which mitigates the impacts of water salinity on West Indian cherry, constituting an efficient management practice to preserve its redox homeostasis under salt stress. New studies become imperative to elucidate the underlying molecular mechanisms and to understand the exact influence of the specific combination of fertilizers. These insights are crucial to improving fertilization practices, strengthening the salt tolerance of fruit plants, and promoting sustainable production in adverse conditions.

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