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Scientific Article

Ascorbic acid as an elicitor of salt stress on the physiology and growth of guava

Ácido ascórbico como elicitor do estresse salino no crescimento e aspectos fisiológicos de goiabeira

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ABSTRACT - The objective of this study was to evaluate the physiology and growth of guava cv. Paluma as a function of irrigation with saline water and foliar application of ascorbic acid. The study was conducted using a randomized block experimental design in a 5 × 4 factorial scheme, whose treatments were formed by combining five levels of electrical conductivity of irrigation water - ECw (0.3, 1.2, 1.9, 2.6, and 3.3 dS m^{-1}), associated with four concentrations of ascorbic acid - AsA (0, 30, 60, and 90 mM), with three replicates and one plant per plot. Stem diameter, crown volume, vegetative vigor index, photosynthetic pigment contents, photochemical efficiency, electrolyte leakage, and relative water content were evaluated. Ascorbic acid at concentration of 60 mM increased electrolyte leakage and relative water content in plants under ECw of 3.2 and 2.9 dS m^{-1}, respectively. AsA concentration of 90 mM stimulated the biosynthesis of chlorophyll *a*, chlorophyll *b,* and total chlorophyll of guava plants grown under water salinity of 3.3 dS m^{-1}. Salinity above 0.3 dS m^{-1} reduced chlorophyll *a* fluorescence, crown volume, and vegetative vigor index of guava. Foliar application of 30 mM ascorbic acid increased the quantum efficiency of photosystem II up to ECw of 2.5 dS m^{-1}. The beneficial effect of ascorbic acid was obtained under ECw of 0.3 dS m^{-1}.

Keywords: *Psidium guajava* L.. Salinity. Enzymatic substrate.

RESUMO - Objetivou-se com esse trabalho avaliar a fisiologia e o crescimento da goiabeira cv. Paluma em função da irrigação com águas salinas e aplicação foliar de ácido ascórbico. A pesquisa foi desenvolvida utilizando o delineamento experimental de blocos casualizados, em esquema fatorial 5 × 4, cujos tratamentos foram construídos pela combinação de cinco níveis de condutividade elétrica da água de irrigação – CEa (0,3; 1,2; 1,9; 2,6 e 3,3 dS m^{-1}), associados a quatro concentrações de ácido ascórbico - AsA (0, 30, 60 e 90 mM), com três repetições e uma planta por parcela. Foi avaliado o diâmetro de caule, o volume da copa, o índice de vigor vegetativo, os teores de pigmentos fotossintéticos, a eficiência fotoquímica, o extravasamento de eletrólitos e o conteúdo relativo de água. Ácido ascórbico na concentração de 60 mM aumentou o extravasamento de eletrólitos e o conteúdo relativo de água das plantas sob CEa de 3,2 e 2,9 dS m^{-1}, respectivamente. A concentração de 90 mM de AsA estimulou a biossíntese de clorofila *a*, *b* e *total* das plantas de goiabeira cultivadas sob salinidade da água de 3,3 dS m^{-1}. A salinidade acima de 0,3 dS m^{-1} reduziu a fluorescência da clorofila *a*, o volume de copa e o índice de vigor vegetativo da goiabeira. A aplicação foliar de 30 mM de ácido ascórbico aumentou a eficiência quântica do fotossistema II até a CEa de 2,5 dS m^{-1}. O efeito benéfico do ácido ascórbico foi obtido sob CEa de 0,3 dS m^{-1}.

Palavras-chave: *Psidium guajava* L.. Salinidade. Substrato enzimático.

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INTRODUCTION

Belonging to the Myrtaceae family, guava (*Psidium guajava* L.) is a species native to Tropical America and distributed throughout the Brazilian territory, standing out among the main species commercially exploited in irrigated areas of the Brazilian Northeast. Its fruits can be consumed fresh and used for the production of juices, jellies, slices in syrup, fruit bars, dehydrated products, additives to other fruit juices (QUIRINO et al., 2018).

In Brazil, the regions with the highest production of guava are the Northeast, Southeast and South, with the states of Pernambuco, São Paulo, Bahia, Paraná and Rio de Janeiro standing out as the largest producers of this fruit crop. In the 2022 harvest, the national production was equivalent to 564,764 tons in an area corresponding to 22,630 hectares, with the Northeast region responsible for 281,524 tons (49.8%) and an average yield of 26,096 kg ha^{-1}. The State of Paraíba recorded an average production of 2,557 tons (IBGE, 2023).

Despite the prominence in national production, in the semi-arid region of the Brazilian Northeast, guava cultivation is conditioned on irrigation management, considering that the ideal range is 1,000 to 1,600 mm $year^{-1}$, well distributed throughout the year. In this region, the water used for irrigation has high levels of dissolved salts, both in surface and underground sources (LIMA et al., 2022), which can induce physiological changes and compromise plant growth and development (SILVA et al., 2024).

High concentrations of salts affect the electron transport system and photosynthetic efficiency and can also cause nutritional imbalances, altered levels of growth regulators, enzyme inhibition and metabolic disorders, which eventually lead to plant death (AHANGER et al., 2017). However, the intensity with which salt stress affects plants depends on other factors such as species, cultivar, types of salts, intensity and duration of stress, crop and irrigation management, edaphoclimatic conditions and phenological stage of the crop (SILVA et al., 2019; PINHEIRO et al., 2022).

One strategy that can mitigate salt stress on plants is to increase the cellular level of enzymatic substrates, such as ascorbic acid. It is a water-soluble antioxidant molecule that acts as a primary substrate in the cyclic pathway for the detoxification of free radicals such as O_2^-, $HO^\bullet$ and H_2O_2 and as a substrate of ascorbate peroxidase (APX), an essential enzyme of the ascorbate-glutathione pathway (SHARMA et al., 2019). This enzymatic substrate also acts to neutralize superoxide, singlet oxygen or superoxide radicals and as a secondary antioxidant during the reductive recycling of the oxidized form of α-tocopherol, another lipophilic antioxidant molecule (KURUTAS, 2016).

Foliar application of ascorbic acid may inhibit lipid peroxidation and decrease malondialdehyde in plant tissues, thus improving their antioxidant capacity (HASSAN et al., 2021). In this context, the objective of this study was to evaluate the effects of foliar application of ascorbic acid on the physiology and growth of guava cv. Paluma cultivated under irrigation with saline waters under the conditions of the semi-arid region of Paraíba, Brazil.

MATERIAL AND METHODS

The experiment was carried out from September 2022 to August 2023 under field conditions in the experimental area belonging to the 'Rolando Enrique Rivas Castellón' farm, at the Center for Sciences and Agri-Food Technology - CCTA of the Federal University of Campina Grande - UFCG, in São Domingos, PB, Brazil, located by the coordinates: 06º 48' 50" S latitude and 37º 56' 31" W longitude, at an altitude of 190 m. Data of maximum and minimum temperature, relative humidity and rainfall during the experiment are presented in Figure 1.

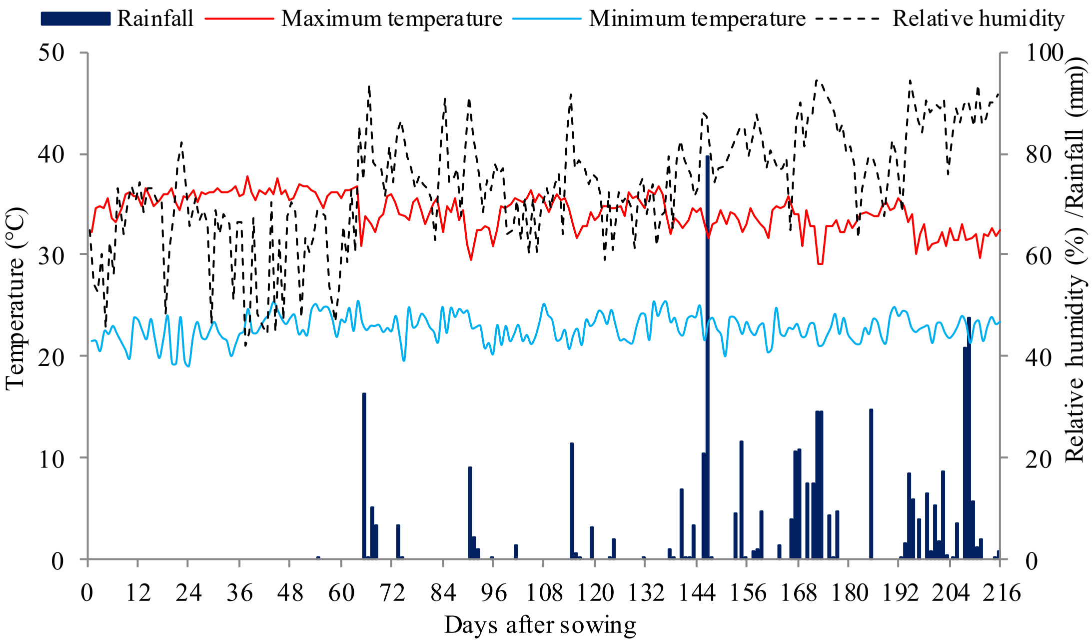


Figure 1. Meteorological data during the experimental period.

A randomized block design was used, in a 5 × 4 factorial scheme, whose treatments consisted of the combination of two factors: five levels of electrical conductivity of irrigation water (0.3, 1.2, 1.9, 2.6 and 3.3 dS m^{-1}), associated with four concentrations of ascorbic acid (AsA) (0, 30, 60 and 90 mM), with 3 replicates and one plant per plot. The levels of electrical conductivity of irrigation water were established based on a study conducted by Bezerra et al. (2019) with guava cv. Paluma, considering the threshold level of 0.3 dS m^{-1} identified by Ferreira et al. (2023) in the seedling formation phase. Ascorbic acid concentrations were adapted from study conducted by Hassan et al. (2021) with barley (*Hordeum vulgare* L.).

The guava cultivar used in the experiment was Paluma, which stands out for its vigor, easy propagation and tolerance to pests and diseases, especially rust (*Puccinia psidii* Wint.) (PINTO; SOUSA; RAMOS, 2004). The guava seedlings were grown in pots adapted as drainage lysimeters with 60 L capacity for plant cultivation; each lysimeter was perforated at the base to allow drainage, and connected to a transparent drain of 16 mm in diameter. The end of the drain was wrapped with a non-woven geotextile (Bidim OP 30) to avoid clogging by soil material. A plastic bottle was placed below each drain to collect drained water in order to estimate water consumption by the plant.

The lysimeters were filled with a 0.5-kg layer of crushed stone followed by 80 kg of sandy loam *Neossolo Regolítico* material (Entisol - Psamment), properly pounded to break up clods and homogenized, collected from the 0-20 cm layer. Before starting the experiment, physical and chemical characteristics (Table 1) of the soil were determined using methodologies proposed by Teixeira et al. (2017).

Table 1. Chemical and physical characteristics of the soil used in the experiment before the application of the treatments.

Chemical characteristics								
pH (H_2O)	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
(1:2.5)	g kg^{-1}	(mg kg^{-1})	------------------------------------- $cmol_c$ kg^{-1} -------------------------------------					
6.01	0.21	0.53	0.12	0.05	3.00	2.44	0.00	0.69
------------- Chemical characteristics -------------				----------------------- Physical characteristics -----------------------				
EC_{se}	CEC	SAR_{se}	ESP	Particle-size fraction (g kg^{-1})			Moisture (dag kg^{-1})	
(dS m^{-1})	$cmol_c$ kg^{-1}	(mMol $L^{-1})^{0.5}$	%	Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
0.71	6.25	0.61	0.80	756.50	200.10	43.40	33.57	5.01

pH – Hydrogen potential, OM – Organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl, pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc, pH 7.0; Al^{3+}+H^+ extracted with 0.5 M CaOAc, pH 7.0; EC_{se} - Electrical conductivity of saturated paste extract; CEC - Cation exchange capacity; SAR_{se} - Sodium adsorption ratio of saturated paste extract; ESP - Exchangeable sodium percentage; Superscripts [1 and 2] refer to the moisture contents in the soil corresponding to field capacity and permanent wilting point.

The irrigation water of the treatment with the lowest level of electrical conductivity (0.3 dS m^{-1}) came from the supply system of the municipality of São Domingos, PB. As sodium is an ion that is normally present at high concentration in the waters of the semi-arid region, only Na^+ in the form of chloride was used, dissolved in the local-supply water, with quantity determined considering the relationship between ECw and the concentration of salts (RICHARDS, 1954), according to Equation 1:

$$Q \approx 10 \times ECw \qquad (1)$$

Where:
Q = Sum of cations ($mmol_c$ L^{-1}); and,
ECw = Electrical conductivity of water (dS m^{-1}).

After preparation and calibration of the ECw using a portable conductivity meter, the waters were stored in 200 L plastic pots, one for each ECw level, respectively. Irrigation with waters of different levels of electrical conductivity was carried out at 2-day intervals, applying to each lysimeter the volume corresponding to that obtained by the water balance, determined according to Equation 2:

$$VI = \frac{(Va - Vd)}{(1 - LF)} \qquad (2)$$

Where:
VI = volume of water to be used in the irrigation event (mL);
Va = volume applied in the previous irrigation event (mL);
Vd = volume drained (mL); and,
LF = leaching fraction of 0.15 applied every 15 days.

Fertilization with nitrogen, phosphorus and potassium was carried out according to the recommendation of Cavalcanti et al. (2008) considering the nutritional requirements of the crop and the contents of the elements in the soil. The sources used were urea (45% N), potassium sulfate (50% K_2O) and monoammonium phosphate (50% P_2O_5 and 11% N). Fertilization began at 15 DAT and was split to be applied every ten days with values of 4.44 g of P_2O_5, 5.55 g of N and 2.39 g of K_2O per plant. Fertilization with micronutrients was carried out weekly via foliar application, starting at 20 DAT, on the adaxial and abaxial surfaces, considering the nutritional requirements of the crop at the concentration of 1 g L^{-1} of Dripsol Micro® (1.2% magnesium, 0.85% boron, 3.4% iron, 4.2% zinc, 3.2% manganese, 0.5% copper and 0.06% molybdenum).

Cultural practices and phytosanitary treatments were carried out, adopting control measures when necessary. Formative pruning was carried out so as to guide the plant in order to obtain well-distributed branches with balanced and airy architecture, thus allowing greater penetration of light and favoring ventilation inside the crown.

The plants were grown with a single stem up to 50 cm height, when the terminal bud was eliminated. From the shoots that emerged, four well-distributed branches were left, in the direction of the four cardinal points. After reaching maturity, the main branches were pruned to remain 30 cm long.

Foliar applications of ascorbic acid started at 90 days after transplanting, carried out 72 h before irrigation with saline water to induce stress signaling in guava plants. Subsequently, the applications were carried out every 15 days, until the beginning of the flowering stage, at 5 p.m. The concentrations of ascorbic acid were obtained by dissolving it in distilled water, which was prepared on the day of each application. Foliar applications were performed by spraying the abaxial and adaxial surfaces of the leaves, in order to wet them completely.

A surfactant (Tween 20 at 0.025%) was added to the spray solutions to break the surface tension of the water and favor the absorption of ascorbic acid into the leaves. The plants were isolated with a structure made of plastic tarpaulin during the applications of ascorbic acid to prevent the solutions from drifting. During the experiment, average volumes of 154.8, 165.18 and 152.22 mL of ascorbic acid were applied per plant at concentrations of 30, 60 and 90 mM, respectively.

At 216 days after transplanting, growth in stem diameter (SD), crown volume (V_{Crown}), vegetative vigor index (VVI), contents of photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids), chlorophyll *a* fluorescence, electrolyte leakage and relative water content in the leaf blade were evaluated. SD was measured at 4 cm height from the collar of the plant.

Crown volume (V_{Crown}) was calculated from the plant height (H), crown diameter in the row direction (RD) and interrow direction (IRD), using Equation 3:

$$V_{Crown} = \left(\frac{\pi}{6}\right) \times H \times RD \times IRD \quad (3)$$

Where:
V_{Crown} – crown volume (m^3);
H – plant height (m);
RD – crown diameter in the row direction (m); and
IRD – crown diameter in the interrow direction (m).

VVI was measured from plant height (H), DM_{crown} and rootstock stem diameter, using Equation 4, proposed by Portella et al. (2016):

$$VVI = \frac{H + DM_{crown} + SD \times 10}{100} \quad (4)$$

Where:
VVI – vegetative vigor index;
H – plant height (m);
DM_{crown} – crown diameter (m); and
SD – stem diameter (m).

Contents of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids) were quantified according to Arnon (1949). Chlorophyll *a* fluorescence was measured by initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v) and quantum efficiency of photosystem II (F_v/F_m) in leaves pre-adapted to the dark using leaf clips for 30 min, between 07:0 and 10:0 a.m., in the median leaf of the intermediate productive branch of the plant in order to ensure that all first acceptors were oxidized, that is, the reaction centers were opened, using a pulse-modulated fluorometer, OS5p model from Opti Science.

Electrolyte leakage (EL%) was determined after collection of five leaf discs with an area of 1.54 cm^2 each from two leaves of the middle third of the plant, which were washed and stored in an Erlenmeyer® flask containing 50 mL of distilled water. After being closed with aluminum foil, the Erlenmeyer® flasks were stored at a temperature of 25 °C for 90 min, then the initial electrical conductivity of the medium (ECi) was measured using a benchtop conductivity meter (MB11, MS Techonopon®). Subsequently, the Erlenmeyer® flasks were subjected to a temperature of 90 °C for 90 min in a drying oven (SL100/336, SOLAB®) and, after cooling of their content, the final electrical conductivity (ECf) was determined. The percentage of electrolyte leakage in the leaf blade was expressed as the percentage of initial electrical conductivity relative to the electrical conductivity after treatment for 90 min at 90 ºC, according to Scotti-Campos et al. (2013), making adaptations, as shown in Equation 5:

$$EL = \frac{ECi}{ECf} \times 100 \quad (5)$$

Where:
EL – electrolyte leakage in the leaf blade (%);
ECi - initial electrical conductivity (dS m^{-1}); and,
ECf - final electrical conductivity (dS m^{-1}).

Relative water content (RWC) was determined after collecting two leaves from the middle third of the main branch to obtain five 12 mm diameter discs from each leaf. Immediately after collection, the discs were weighed, and fresh mass (FM) was obtained. Soon after, the samples were placed in beakers, immersed in 50 mL of distilled water and left to rest for 90 min. After this period, excess water was removed from the discs using paper towels, to obtain the turgid mass (TM) of the samples, which were placed to dry in an oven with forced air circulation at a temperature of 65 ± 3 ºC and kept until reaching constant weight, and the dry mass (DM) of the samples was obtained. Relative water content in the leaf blade (RWC) was determined according to Weatherley (1950) using Equation 6:

$$RWC = \frac{(FM - DM)}{(TM - DM)} \times 100 \quad (6)$$

Where:
RWC - relative water content (%);
FM - fresh mass of the discs (g);
TM - turgid mass of the discs (g); and
DM- dry mass of the discs (g).

The data were analyzed for normality (Shapiro-Wilk test), and then subjected to analysis of variance by the F test ($p \leq 0.05$); when significant, polynomial regression analysis was performed for the levels of electrical conductivity of the water and concentrations of ascorbic acid, using the statistical software SISVAR ESAL.

RESULTS AND DISCUSSION

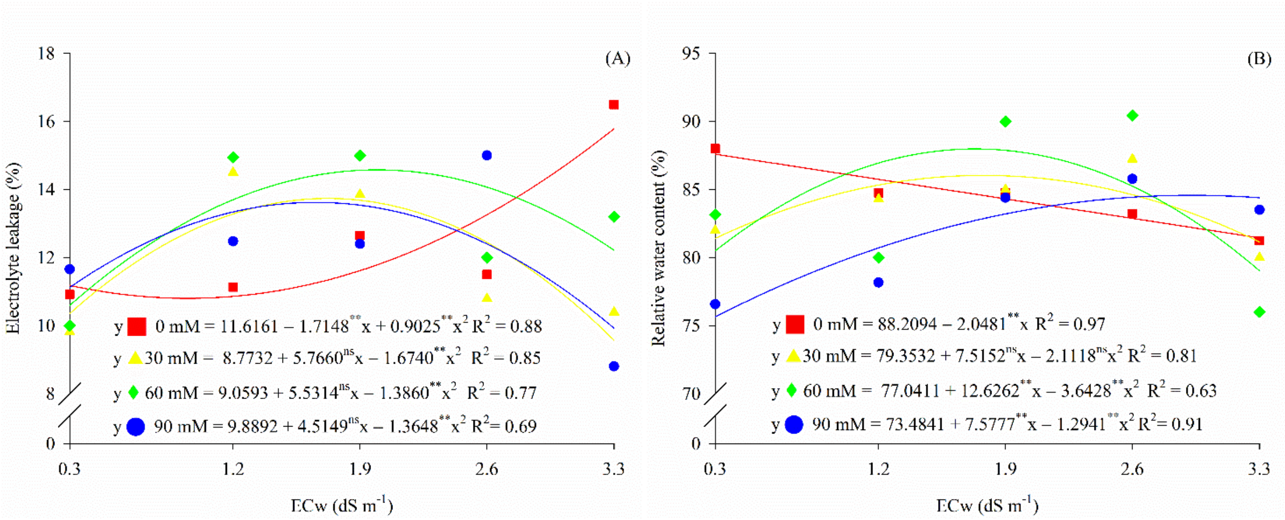
There was a significant effect for the interaction between the factors salinity levels (SL) and foliar application of ascorbic acid (AsA) on the electrolyte leakage (EL%), relative water content (RWC%), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *T*) and carotenoids of guava plants cv. Paluma, at 216 days after transplantation (Table 2).

Table 2. Summary of the analysis of variance for electrolyte leakage (EL%), relative water content in the leaf blade (RWC%), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *T*) and carotenoid (Car) contents of guava plants cv. Paluma, subjected to salinity levels of irrigation water and ascorbic acid concentrations at 216 days after transplanting.

Sources of variation	DF	Mean squares					
		EL%	RWC%	Chl *a*	Chl *b*	Chl *T*	Car
Salinity level (SL)	4	16.52**	27.44**	32.0**	3.05**	53.18**	3.75**
Linear regression	1	4.06*	26.28**	45.86**	4.66**	79.7**	0.28*
Quadratic regression	1	28.2**	4.71ns	1.22ns	2.66**	7.50**	3.20**
Ascorbic acid (AsA)	3	2.06ns	27.29**	7.85ns	5.76**	27.0**	0.17ns
Linear regression	1	0.29ns	59.18**	6.75ns	5.20**	23.80**	0.24ns
Quadratic regression	1	0.010ns	21.67**	4.39ns	2.70**	13.98*	0.18ns
Interaction (SL × AsA)	12	16.0**	54.87**	8.89**	0.77**	12.9**	2.42**
Blocks	2	0.88ns	0.46	1.75ns	0.36ns	0.57ns	0.029ns
Residual	38	0.84	2.01	3.09	0.14	3.17	0.064
CV (%)		7.50	1.70	10.1	8.82	8.27	4.25

**; * and ns, significant at $p \leq 0.11$ and $p \leq 0.05$ and not significant; DF – degrees of freedom.

For electrolyte leakage (EL%), the increase in the electrical conductivity of irrigation water promoted an increase, with the highest estimated value (15.78%) in plants subjected to 0 mM of AsA and ECw of 3.3 dS m^{-1} (Figure 2A). On the other hand, plants cultivated under concentrations of 30, 60 and 90 mM obtained maximum values of 13.72, 14.57 and 13.62%, under irrigation with water of 1.8, 2.0 and 1.7 dS m^{-1}, respectively. The increase in electrolyte leakage due to the increase in salinity is an indication that the accumulation of salts in the soil tends to reduce cell membrane integrity (FERREIRA et al., 2023) and possibly occurred due to ionic effects, triggering changes in the plant's nutritional balance, reducing the levels of Ca^{+}, which is an essential element for cell wall formation, as observed in guava by Silva et al. (2024).



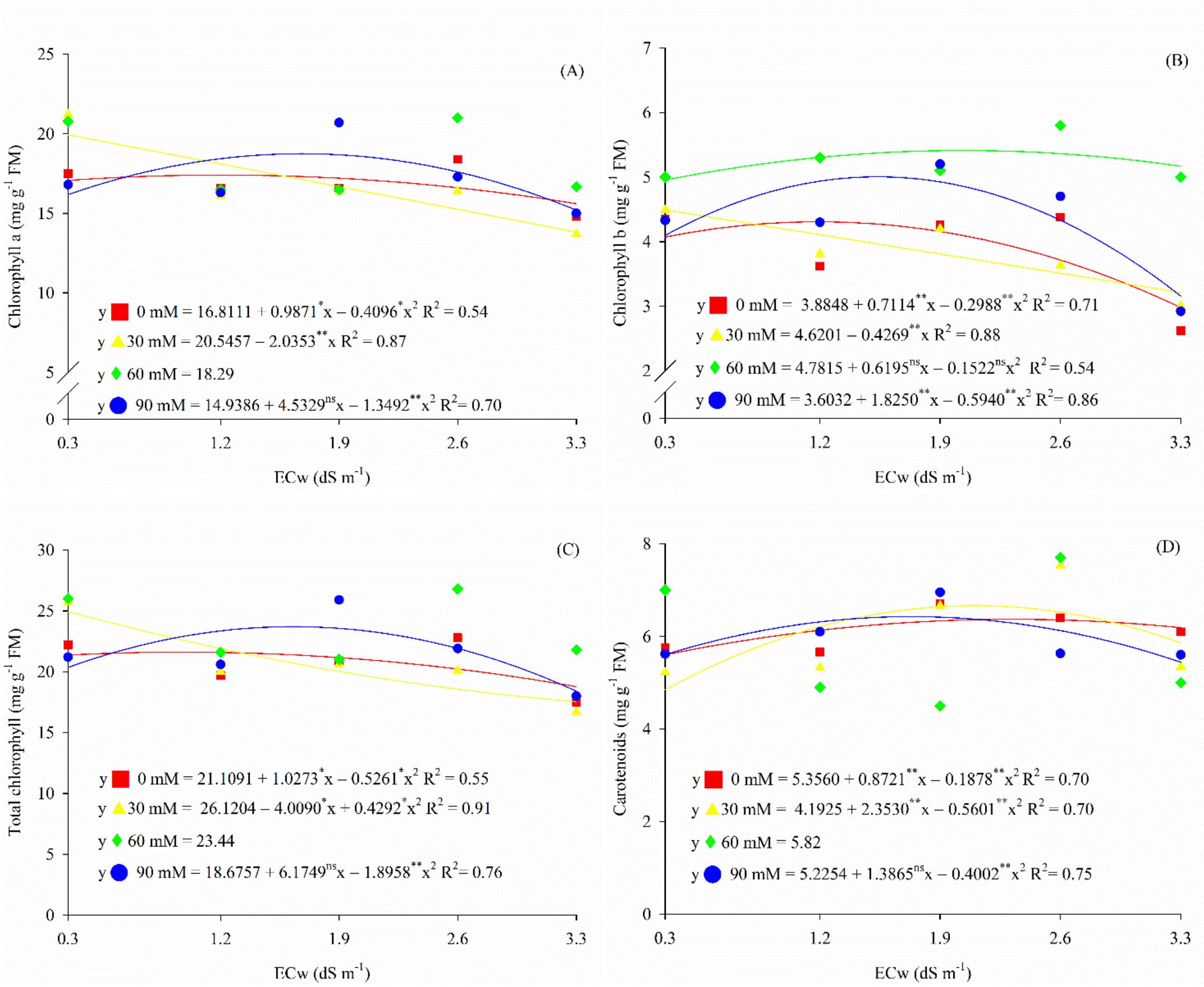
ns, * - Not significant $p > 0.05$, Significant $p \leq 0.01$ by the F test, respectively

Figure 2. Electrolyte leakage (A) and relative water content (B) in the leaf blade of guava plants cv. Paluma, as a function of levels of electrical conductivity of the water – ECw and ascorbic acid concentrations, at 216 days after transplanting.

The relative water content (RWC) in the leaves of plants subjected to AsA concentration of 0 mM decreased linearly by 2.32% per unit increment in ECw, according to the mathematical model (Figure 2B). When comparing the RWC of plants cultivated with ECw of 3.3 dS m^{-1} to that of plants that received 0.3 dS m^{-1}, a decrease of 7.0% was observed. The reduction in RWC with the increase in ECw levels occurs due to osmotic effects, restricting the capacity of water absorption by the roots, limiting the maintenance of the water status of guava plants, as observed by Ferreira et al. (2023).

Foliar applications of the AsA concentrations of 30, 60 and 90 mM promoted increments in RWC even with the increase in salt stress, resulting in maximum estimated values of 86.03, 88.97 and 84.57% in plants irrigated using water with ECw of 1.8, 1.7 and 2.9 dS m^{-1}, respectively, which indicates the beneficial effect of ascorbic acid in the reduction of the osmotic effects caused by salt stress. This may be related to its physiological functions, acting as a modulator of the functions of reactive oxygen species (ROS), as a primary substrate in the cyclic pathway in enzymatic detoxification (EL-BELTAGI et al., 2022), and can help maintain the water content in the leaves, even under salt stress conditions.

Foliar application of ascorbic acid at a concentration of 30 mM caused a decrease in chlorophyll *a* contents (Figure 3A), equal to 9.91% per unit increment in ECw, i.e., a reduction of 30.62% in the contents between plants grown under ECw of 0.3 and 3.3 dS m^{-1}. On the other hand, plants subjected to concentrations of 0 and 90 mM reached the maximum estimated values of 17.41 and 18.75 mg g^{-1} FM, under irrigation using water with salinity of 1.2 and 1.7 dS m^{-1}, respectively. The occurrence of this effect indicates that ascorbic acid can stimulate the biosynthesis of chlorophyll in plants under stress conditions, due to its action as an antioxidant compound that contributes to the regulation of ROS activity, reducing the oxidative stress effect on the biosynthesis of pigments, maintaining the plant's ability to perform photosynthesis, even under stress conditions (HAMIDI et al., 2024).



*, ** - Significant at $p \leq 0.05$ and ≤ 0.01 by the F test, respectively

Figure 3. Chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C) and carotenoid (D) contents of guava plants cv. Paluma, as a function of levels of electrical conductivity of the water and ascorbic acid concentrations, at 216 days after transplanting.

For the chlorophyll *b* contents (Figure 3B), foliar application of ascorbic acid had a mitigating effect on the damage caused by salt stress; in plants cultivated under concentrations of 60 and 90 mM, the maximum estimated values of 5.41 and 5.00 mg g^{-1} FM were obtained under irrigation with ECw of 2.0 and 1.5 dS m^{-1}. The concentration of 30 mM promoted the maximum estimated values (4.49 mg g^{-1} FM) in plants grown under water salinity of 0.3 dS m^{-1}. Total chlorophyll contents (Figure 3C) were higher in plants subjected to foliar application of ascorbic acid at concentrations of 30 and 90 mM, with the highest values (24.95 and 23.69 mg g^{-1} FM) obtained under water salinity of

0.3 and 1.7 dS m^{-1}, respectively. In plants subjected to AsA concentration of 0 mM, the highest value (21.6123.69 mg g^{-1} FM) was obtained under ECw of 1.0 dS m^{-1}.

The beneficial effect of foliar application of ascorbic acid on pigment production in plants under stress conditions has been reported for other species, such as in sweet pepper (*Capsicum annuum* L.), for which the application of up to 1.0 µM of ascorbic acid promoted an increase in chlorophyll and carotenoid contents under water stress conditions (KHAZAEI, ESMAIELPOUR; ESTAJI, 2020).

The carotenoid contents (Figure 3D) of guava plants grown under foliar application of 0, 30 and 90 mM of ascorbic acid reached the maximum estimated values (6.37, 6.66 and 6.42 mg g^{-1} FM) under ECw of 2.3, 2.1 and 1.7 dS m^{-1}, respectively. On the other hand, plants cultivated under the concentration of 60 mM showed an average Chl *T* content of 5.82 mg g^{-1} FM. The increase in carotenoid synthesis can be considered a photoprotection mechanism of plants, in order to increase antioxidant activity, aimed at reducing oxidative stress (HASSAN et al., 2021).

The interaction between the salinity levels and the foliar application of ascorbic acid had a significant effect ($p \leq 0.01$) on the initial (F_0), variable (F_v) and maximum (F_m) fluorescence and on the quantum efficiency of photosystem II (F_v/F_m) of guava plants cv. Paluma, at 216 days after transplantation (Table 3).

Table 3. Summary of the analysis of variance for the initial fluorescence (F_0), variable fluorescence (F_v), maximum fluorescence (F_m) and quantum efficiency of photosystem II (F_v/F_m) of guava plants cv. Paluma, subjected to salinity levels of irrigation water and ascorbic acid concentrations at 216 days after transplanting.

Sources of variation	DF	Mean squares			
		F_0	F_v	F_m	F_v/F_m
Salinity levels (SL)	4	0.00012**	0.022**	0.023**	0.049**
Linear regression	1	0.000023^{ns}	0.019**	0.0017**	0.0062**
Quadratic regression	1	0.000079^{ns}	0.012**	0.0010**	0.0078**
Ascorbic acid (AsA)	3	0.00014**	0.0074**	0.0087**	0.026**
Linear regression	1	0.000019^{ns}	0.00829**	0.009**	0.020**
Quadratic regression	1	0.00039**	0.00823**	0.012**	0.000022^{ns}
Interaction (SL × AsA)	12	0.00010**	0.014**	0.015**	0.0039**
Blocks	2	0.000026^{ns}	0.00083^{ns}	0.0008^{ns}	0.00014^{ns}
Residual	38	0.000020	0.00066	0.00066	0.00022
CV (%)		4.77	7.12	5.64	1.91

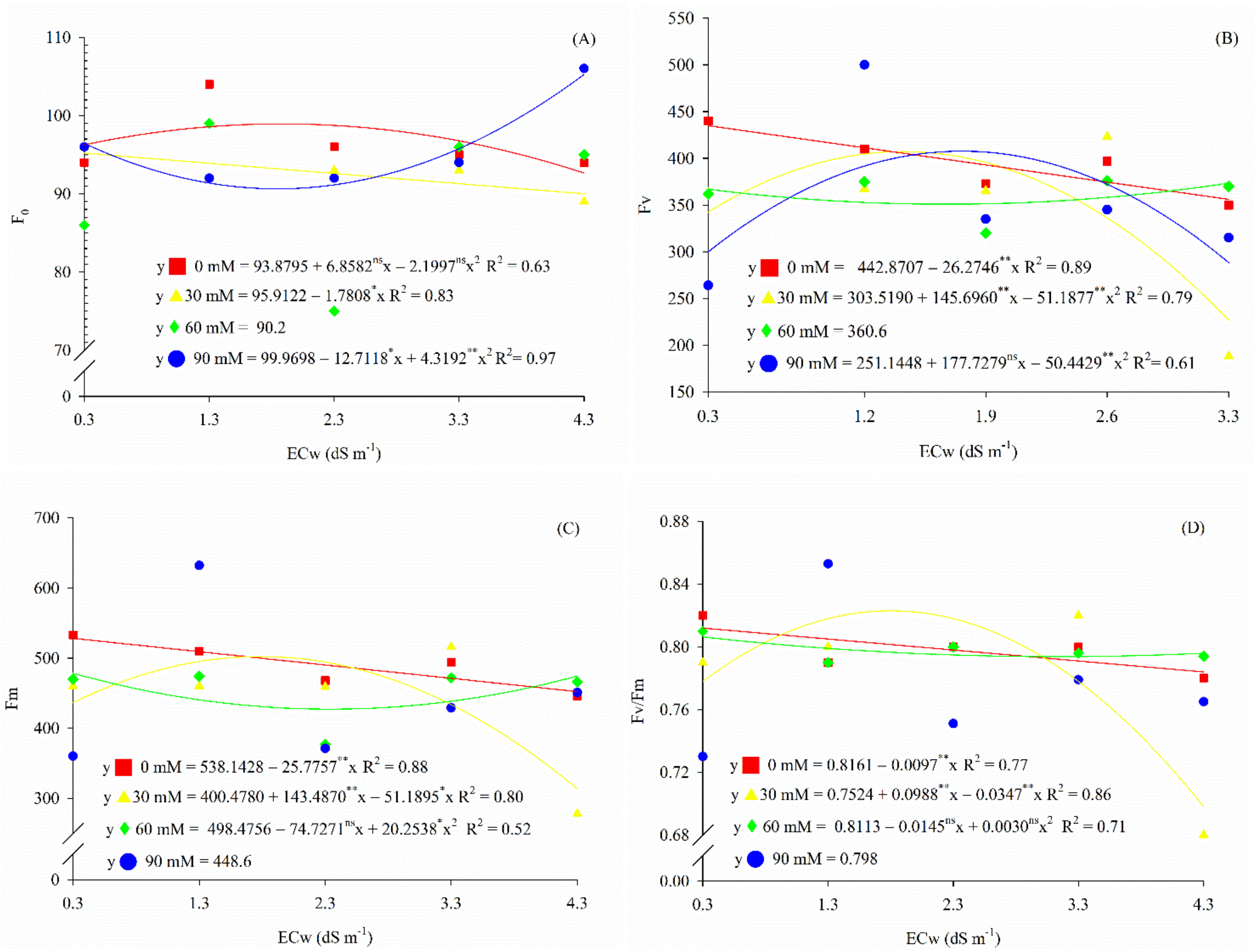
** and ^{ns,} significant at $p \leq 0.01$ and non-significant; DF – degrees of freedom.

For the initial fluorescence (F_0), plants subjected to foliar application of ascorbic acid at concentrations of 0 and 90 mM obtained the maximum estimated values (99.21 and 105.03) under irrigation with ECw of 1.6 and 3.3 dS m^{-1}, respectively (Figure 4A). Under 30 mM, there was a decrease of 1.78% in the initial fluorescence per unit increment in ECw. Increase in F_0 is indicative of damage to the reaction centers of the PSII, which induces a reduction in the ability to transfer excitation energy from the antenna to the PSII (GALVÃO SOBRINHO et al., 2023). Possibly, as salinity increased, there was a greater accumulation of ROS, causing ascorbic acid to be used to promote the mediation and reduction of these compounds. Thus, foliar application of ascorbic acid can mediate the high reduction power of ROS, as well as their elimination, reducing the oxidation-reduction activities, under salt stress conditions (CHEN et al., 2021).

Variable fluorescence (Figure 4B) was higher (434.9) in plants subjected to AsA concentration of 0 mM under ECw of 0.3 dS m^{-1}, followed by decreases of 5.93% per unit increment in ECw levels. At concentrations of 30 and 90 mM, the maximum increments obtained (407.16 and 407.62) occurred in plants that received irrigation with water of 1.4 and 1.8 dS m^{-1}, respectively. The results obtained for F_v demonstrate that salt stress compromised the photosynthetic apparatus of guava plants, affecting the functioning of PSII and, consequently, the efficiency of their CO_2 absorption capacity.

For maximum fluorescence (Figure 4C), foliar application of 0 mM caused a linear decrease of 4.80% per unit increment in ECw levels. In relative terms, there was a 14.6% reduction in F_m between plants irrigated with water of 0.3 and 3.3 dS m^{-1}. For the concentrations of 30 and 60 mM, the maximum estimated values (501.44 and 477.88) were found in plants irrigated with water of 1.4 and 0.3 dS m^{-1}, respectively. The behavior observed in F_m, as well as in F_v, indicates that salt stress compromised the photosynthetic apparatus of guava plants, which may be associated with the degradation of chlorophyll pigments, caused by ionic toxicity and the reduction in the osmotic potential of the soil, resulting in water deficit (XAVIER et al., 2022).

Foliar application of ascorbic acid at concentration of 30 mM promoted the maximum estimated value of 0.823 in the quantum efficiency of photosystem II (Figure 4D) of plants grown under ECw of 2.5 dS m^{-1}, representing gains of 5.78% compared to plants subjected to the lowest salinity (0.3 dS m^{-1}). At the concentration of 60 mM, the maximum and minimum values (0.807 and 0.793) occurred at the salinity levels of 0.3 and 2.4 dS m^{-1}, respectively. At the concentration of 0 mM, there was a linear decrease of 1.19% per unit increase in ECw, while the concentration of 90 mM resulted in an average value of 0.798. Thus, it is possible to observe that the application of 30 mM of ascorbic acid was efficient in reducing the effect of salt stress, promoting improvements in the quantum efficiency of PSII. Under stress conditions, ascorbic acid can help maintain balance in the distribution of energy, since it can act as a cofactor of antioxidant enzymes, such as violaxanthin de-epoxidase, which is directly involved in the ability to dissipate energy (CHEN et al., 2023).



*, ** - Significant at $p \leq 0.05$ and ≤ 0.01 by the F test, respectively

Figure 4. Initial fluorescence – F_0 (A), variable fluorescence – $_{Fv}$ (B), maximum fluorescence – F_m (C) and quantum efficiency of photosystem II – F_v/F_m (D) of guava cv. Paluma, as a function of levels of electrical conductivity of the water and ascorbic acid concentrations, at 216 days after transplanting.

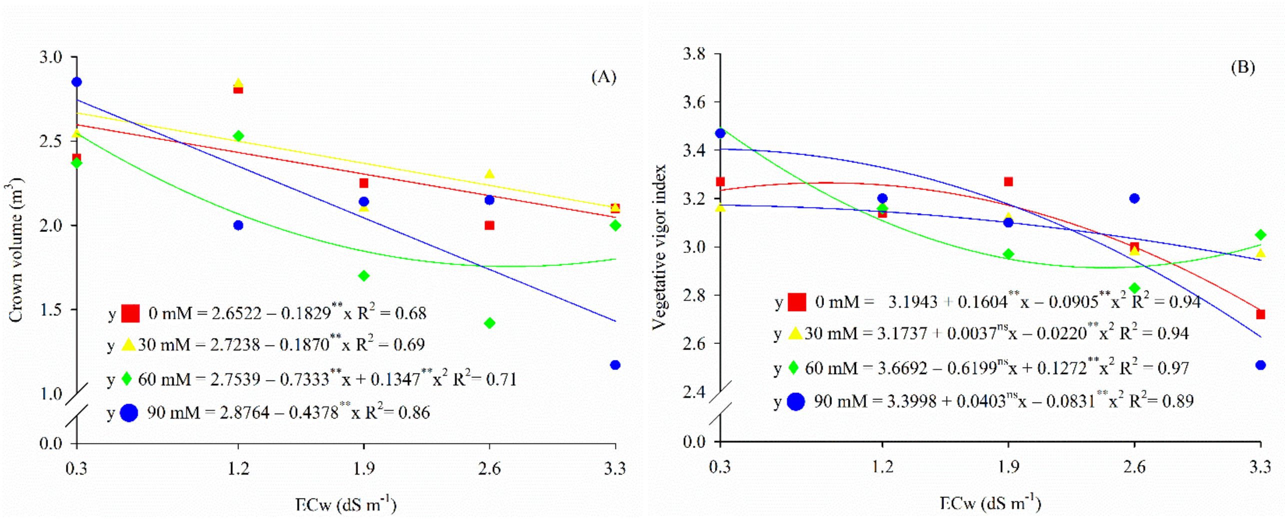
There was a significant effect of the interaction between the factors (SL × AsA) on the crown volume (V_{Crown}) and vegetative vigor index (VVI) of guava plants cv. Paluma, at 216 days after transplanting (Table 4), while stem diameter (SD) was not affected by the individual factors and the interaction.

For crown volume (Figure 5A), it can be observed that the foliar application of ascorbic acid at concentrations of 0, 30 and 90 mM led to linear decreases of 6.98, 6.87 and 15.22% per unit increment in ECw, i.e., when comparing the V_{Crown} of plants irrigated with water of 3.3 dS m^{-1} to that of plants cultivated under 0.3 dS m^{-1}, there were reductions of 15.70, 15.43 and 32.88%, respectively. In plants subjected to 60 mM of AsA, the maximum and minimum estimated values were 2.54 and 1.75, respectively, under irrigation with ECw of 0.3 and 2.7 dS m^{-1}. The reduction in leaf area in plants under salt stress reflects a decrease in crown volume and can be considered an adaptation strategy to reduce water loss to the atmosphere and absorption of toxic ions (LIMA et al., 2020). In addition, excess salts in water and/or soil also contribute to inhibition of plant growth, due to the reduction in the water potential of the soil solution, inhibiting water absorption by the roots and causing decreased cell expansion (FERREIRA et al., 2023).

Table 4. Summary of the analysis of variance for stem diameter (SD), crown volume (V_{Crown}) and vegetative vigor index (VVI) of guava plants cv. Paluma, subjected to salinity levels of irrigation water and ascorbic acid concentrations at 216 days after transplanting.

Sources of variation	DF	Mean squares		
		SD	V_{Crown}	VVI
Salinity levels (SL)	4	30.59^{ns}	1.30^{**}	0.45^{**}
Linear regression	1	50.07^{ns}	4.45^{**}	1.71^{**}
Quadratic regression	1	0.72^{ns}	0.040^{ns}	0.0099^{ns}
Ascorbic acid (AsA)	3	6.81^{ns}	0.46^{**}	0.0020^{ns}
Linear regression	1	1.11^{ns}	0.60^{**}	0.0043^{ns}
Quadratic regression	1	18.66^{ns}	0.11^{ns}	0.0018^{ns}
Interaction (SL × AsA)	12	24.08^{ns}	0.34^{**}	0.10^{**}
Blocks	2	32.65^{ns}	0.010^{ns}	0.005^{ns}
Residual	38	23.19	0.019	0.0075
CV (%)		15.9	6.42	2.81

** and ^{ns}, significant at $p \leq 0.01$ and non-significant; DF – degrees of freedom.



** - Significant at $p \leq 0.01$ by the F-test, respectively

Figure 5. Crown volume (A) and vegetative vigor index (B) of guava plants cv. Paluma, as a function of levels of electrical conductivity of the water and ascorbic acid concentrations, at 216 days after transplanting.

The vegetative vigor index (Figure 5B) of guava plants behaved similarly to crown volume, as foliar application was beneficial under ECw of 0.3 dS m^{-1}, and AsA concentrations of 30, 60 and 90 mM promoted maximum values of 3.172, 3.496 and 3.404, respectively, obtained at ECw of 0.3 dS m^{-1}, decreasing with the increase in salinity levels, with reductions of 7.14, 13.90 and 22.81%, respectively, when compared with the values obtained under ECw of 3.3 dS m^{-1}.

At the concentration of 0 mM, the maximum and minimum values estimated were 3.265 and 2.738, found under irrigation with water of 0.9 and 3.3 dS m^{-1}, respectively. Under low salinity conditions, foliar application of AsA stimulated the vegetative vigor index of guava plants. Possibly, ascorbic acid may have stimulated the production of growth-enhancing compounds, such as metabolic regulators and bioactivators, which acted as stabilizers of cellular components, being able to increase resistance to stress conditions, as well as improving plant growth.

The reduction in the crown volume and vegetative vigor index of guava plants results from the deleterious effects caused by salt stress, affecting the growth and development of plants due to the osmotic effect, caused by the high concentration of soluble salts in the soil and/or water, restricting the absorption of water and nutrients, leading to water deficit and nutritional imbalance (LIMA et al., 2018).

Reductions in the crown volume and vigor index of guava plants, obtained in the present study, were also observed by Lacerda et. al (2022), who evaluated the morphophysiology and production of guava as a function of water salinity and found that plants cultivated with salinity level of 3.2 dS m^{-1} showed significant reductions in these parameters when compared to plants that received low-salinity water (0.6 dS m^{-1}).

CONCLUSIONS

Foliar application of ascorbic acid at concentration of 60 mM increases the electrolyte leakage and relative water content in the leaf blade of guava plants cv. Paluma cultivated under water salinity of 3.2 and 2.9 dS m^{-1}, respectively.

Foliar application of ascorbic acid at concentration of 90 mM increases the contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll of guava plants grown under water salinity of 2.8 dS m^{-1}.

There is a reduction in the chlorophyll *a* fluorescence indices of guava irrigated using water with electrical conductivity above 0.3 dS m^{-1}. However, foliar application of 30 mM ascorbic acid increases the quantum efficiency of photosystem II up to water salinity of 2.5 dS m^{-1}.

The beneficial effect of ascorbic acid application in guava is obtained under irrigation using water with electrical conductivity of 0.3 dS m^{-1}.

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