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# Ascorbic acid as attenuator of salt stress effects on the morphophysiology of guava in the post-grafting phase

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## ABSTRACT

Guava cultivation in the Brazilian semi-arid region is constrained by the scarcity of high-quality water, which necessitates the use of saline water for irrigation. In this context, the application of antioxidant substances, such as ascorbic acid (AsA), has emerged as a promising strategy to mitigate the deleterious effects of salt stress. This study aimed to evaluate the effects of foliar AsA application on the morphophysiology of guava plants in the post-grafting phase when irrigated with saline water. The experiment tested five levels of water electrical conductivity—EC<sub>w</sub> (0.9, 1.5, 2.1, 2.7, and 3.3 dS m<sup>-1</sup>) and four concentrations of AsA (0, 200, 400, and 600 mg L<sup>-1</sup>). Salt stress, induced by water with conductivity from 0.9 dS m<sup>-1</sup> onwards, adversely affected gas exchange, photosynthetic pigments, and growth parameters of the guava plants. Conversely, foliar application of AsA demonstrated beneficial effects both as an isolated factor—improving relative water content and the CO<sub>2</sub> assimilation rate—and through its interaction with salinity. The importance of this mitigating action was particularly evidenced by the significant interaction in attenuating chlorophyll *a* degradation and, most notably, in promoting vegetative vigor, even in plants subjected to the highest level of salt stress (3.3 dS m<sup>-1</sup>), at 150 days after transplanting. The 600 mg L<sup>-1</sup> dosage of ascorbic acid (AsA) proved to be the most effective. The results indicate that foliar AsA application is a promising tool for managing salt stress, as it contributes to improving the physiology and growth of guava trees.

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Non-enzymatic compound; *Psidium guajava* L.; salinity; semi-arid region

## Introduction

Belonging to the Myrtaceae family, guava (*Psidium guajava* L.) is a fruit crop that stands out for its high nutritional value, being a source of vitamin A, B complex, phosphorus, potassium, iron, calcium and fiber, widely used in the agroindustry for

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juices, jellies, sweets and also consumed fresh (Onias et al. 2018). In 2023, Brazil produced 582,832 tons of guava in an area of 22,533 hectares, with the Northeast standing out as the main producing region, responsible for 281,524 tons, and the states of Pernambuco, São Paulo, and Bahia being the largest producers (IBGE 2024).

The semi-arid region of the Brazilian Northeast is characterized by high evaporation rates, high temperatures, low relative humidity, and irregular rainfall, factors that result in significant losses of water resources in both quantitative and qualitative terms, and the occurrence of water sources with high concentrations of dissolved salts is common (Lacerda et al. 2021). High concentrations of salts in water and/or soil stand out as one of the main limiting factors for the cultivation of salt stress-sensitive species, such as guava, due to restrictions in the absorption of water and nutrients by plants (Ferreira et al. 2023).

Under stress conditions, physiological disorders occur in plants, such as stomatal closure, chlorophyll degradation, and changes in photosystem functioning (Lima et al. 2018; Souto et al. 2024), besides growth inhibition due to the reduction in cellular turgor pressure caused by osmotic and ionic effects (Lima et al. 2020). High levels of salts in water cause several types of stress, including oxidative stress, resulting from the high generation of reactive oxygen species (ROS), which can inhibit chlorophyll synthesis, affect cellular components, and cause lipid peroxidation of membranes (Silva et al. 2021).

In this context, several studies have already been carried out, highlighting the deleterious effects of salinity in guava cultivation, addressing its impacts on morphological and physiological aspects (Xavier et al. 2022), production, and post-harvest quality (Bezerra et al. 2018; Lacerda et al. 2022). However, research has been conducted evaluating the effects of salt stress on plants propagated by seeds and cuttings. In this context, it is necessary to conduct research to identify alternatives capable of attenuating or mitigating the effects of salt stress on guava in the post-grafting phase.

It should be considered that elicitor substances have been employed as a strategy to mitigate the deleterious effects of salt stress on plants, and foliar application of non-enzymatic compounds, such as ascorbic acid (AsA) has stood out (Fatah and Sadek 2020). AsA is a water-soluble molecule, has antioxidant properties and can neutralize oxygen superoxide radicals due to its role as a primary substrate in cyclic pathways (Gaafar et al. 2020), in addition to protecting lipids and proteins against oxidative damage caused by salinity (Hassan et al. 2021).

Several studies have been conducted to evaluate the effects of foliar application of AsA on the mitigation of salt stress, as highlighted by Kanwal et al. (2024), who found that foliar application of AsA mitigated salt stress effects by increasing the synthesis of photosynthetic pigments and enzymatic and non-enzymatic activities in pea plants (*Pisum sativum* L.). Beneficial effects of foliar application of AsA were also observed by Elsidigg et al. (2022) in sorghum (*Sorghum bicolor* (L.) Moench) and Hassan et al. (2021) in barley (*Hordeum vulgare* L.). However, research addressing the effects of foliar application of AsA on fruit species, such as guava, in the post-grafting phase is still incipient in the literature.

In this study, we hypothesize that foliar application of AsA improves stomatal regulation and the energy efficiency of the electron transport chain. This tends to be

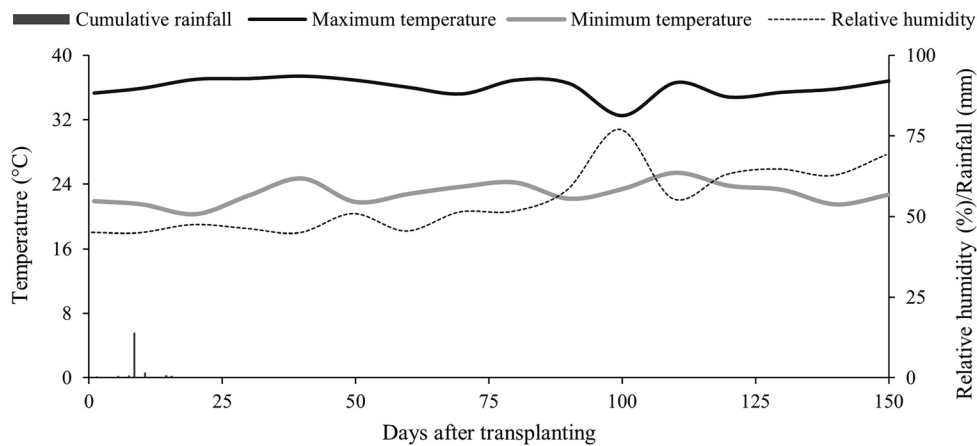
achieved by preserving photosynthetic pigments and ribulose-1,5-bisphosphate carboxylase activity, which maintains guava tree photosynthesis at levels that promote plant growth. These effects tend to be elucidated by the relationship presented between the study variables through univariate analysis and correlation between variables in the main component analysis.

Considering the economic and social importance of guava in the Brazilian fruit growing scenario, it is essential to establish strategies for water salinity management, considering that it is a common problem in the semi-arid region of Northeast Brazil, justifying the adoption of low-cost strategies, such as the application of AsA, whose effects are already observed under conditions of abiotic stress in plants. Thus, this study aimed to evaluate the effects of foliar application of ascorbic acid on the morphophysiology of guava subjected to irrigation with saline waters in the post-grafting phase in the semi-arid region of Northeast Brazil.

Materials and methods

Site description and experimental design

The research was conducted from September 2023 to February 2024 in drainage lysimeters under field conditions at the “Rolando Enrique Rivas Castellón” experimental farm, belonging to the Center for Sciences and Agri-Food Technology—CCTA of the Federal University of Campina Grande—UFCG, in São Domingos, Paraíba, at the coordinates 06°48’50” latitude (S) and 37°56’31” longitude (W), at an altitude of 190 m. According to Köppen’s climate classification adapted to Brazil, the climate is “BSh” type, which represents a hot and semi-arid climate (average annual temperature of 28 °C, rainfall around 750 mm year<sup>-1</sup> and average annual evaporation of 2000 mm) (Álvares et al. 2013). Data on maximum and minimum temperature, relative humidity, and rainfall are presented in Figure 1.



**Figure 1.** Data on average maximum and minimum temperature, cumulative rainfall, and relative humidity during the period from 15 September 2023 to 12 February 2024.

The experiment was carried out in a randomized block design, using the split-plot scheme, with five levels of electrical conductivity of water—ECw (0.9, 1.5, 2.1, 2.7, and 3.3 dS m<sup>-1</sup>) as the plots and four concentrations of ascorbic acid—AsA (0, 200, 400, and 600 mg L<sup>-1</sup>) as the subplots, with three replicates and one plant per plot. The ECw levels were established according to a study conducted by Bezerra et al. (2018). Ascorbic acid concentrations were determined based on a study conducted by Gaafar et al. (2020).

Grafted guava seedlings obtained from the São Francisco seedlings nursery in Petrolina, PE, were used in this study. The cultivar BRS Guaraçá was used as rootstock, and the cultivar Paluma was used as scion. The production of a grafted guava seedling begins with the rootstock's development for 4–8 months until it reaches the ideal diameter for grafting. After grafting, the seedling requires ~5 months (150 days) for healing and hardening-off, at which point it is ready for field transplanting. The cultivar BRS Guaraçá is a hybrid plant, which has characteristics of guava (*P. guajava* L.) and Brazilian guava (*Psidium guineense*) and stands out for its tolerance to root-knot nematodes (EMBRAPA 2010). The cultivar Paluma has an intense red pulp, elliptical-shaped and light green-colored leaves, and berry-type fruits, with a piriform shape (Medina et al. 1991). The material under study is classified as moderately sensitive to salinity, according to the criteria of Ayers and Westcot (1976). More specifically, research by Bezerra et al. (2018) establishes a salinity threshold for irrigation water at 1.42 dS m<sup>-1</sup> for cultivar Paluma.

Conducting the experiment

Plastic pots adapted as lysimeters with 60 L capacity were used for cultivating the plants. Two holes were drilled at the base of the pots and connected to transparent drains of 16 mm in diameter. The end of the drain inside the lysimeter was wrapped with a non-woven geotextile (Bidim OP 30) to prevent clogging by soil material. Two containers were placed below each drain for collecting drained water in order to estimate the water consumption by the plants.

Table 1. Chemical and physical characteristics of the soil used, in the 0–40 cm layer.

Chemical characteristics								
pH H <sub>2</sub> O	OM	P	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H <sup>+</sup>
(1:2.5)	g kg <sup>-1</sup>	(mg kg <sup>-1</sup> )	cmol <sub>c</sub> kg <sup>-1</sup>					
8.53	3.10	77.30	0.56	0.20	5.08	5.11	0	0
Chemical characteristics				Physical characteristics				
EC <sub>se</sub>	CEC	SAR <sub>se</sub>	ESP	Particle-size fraction (g kg <sup>-1</sup> )			Moisture (dag kg <sup>-1</sup> )	
(dS m <sup>-1</sup> )	cmol <sub>c</sub> kg <sup>-1</sup>	(mmol L <sup>-1</sup> ) <sup>0.5</sup>	%	Sand	Silt	Clay	33.42 kPa <sup>a</sup>	1519.5 kPa <sup>b</sup>
0.46	10.95	1.02	1.83	775.70	180.90	43.40	12.45	5.00

pH: hydrogen potential; OM: organic matter: Walkley-Black Wet Digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1 M KCl, pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted with 1 M NH<sub>4</sub>OAc, pH 7.0; Al<sup>3+</sup>+H<sup>+</sup> extracted with 0.5 M CaOAc, pH 7.0; EC<sub>se</sub>: electrical conductivity of the saturation extract; CEC: cation exchange capacity; SAR<sub>se</sub>: sodium adsorption ratio of the saturation extract; ESP: exchangeable sodium percentage.

<sup>a,b</sup>Referring to moisture contents in the soil corresponding to field capacity and permanent wilting point, respectively.

The pots were filled with a 0.5-kg layer of crushed stone, followed by 80 kg of *Neossolo Flúvico Eutrófico típico* (Fluvent) with a sandy loam texture, from the Experimental Farm, whose chemical and physical characteristics (Table 1) were obtained according to the methodology of Teixeira et al. (2017).

Fertilization with nitrogen, phosphorus, and potassium was carried out according to the recommendation of Cavalcanti, Lima Júnior, and Lima (2008) considering the nutritional requirements of the crop at the different stages of development and the potential fertility of the soil. Urea (45% N), potassium sulfate (50% K<sub>2</sub>O), and monoammonium phosphate (50% P<sub>2</sub>O<sub>5</sub>; 11% N) were used as sources of nitrogen, potassium, and phosphorus/nitrogen, respectively, applied fortnightly via fertigation. Micronutrients were foliar applied weekly, starting at 10 DAT, using 0.5 g L<sup>-1</sup> during the vegetative period and 1.0 g L<sup>-1</sup> in the other phases 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).

The irrigation water of the treatment with the lowest level of electrical conductivity (0.9 dS m<sup>-1</sup>) came from an artesian well located in an area of the Experimental Farm of CCTA/UFCG, whose chemical composition is presented in Table 2; the other ECw levels were prepared by dissolving iodine-free NaCl in the well water. Irrigation water was prepared considering the relationship between ECw and salt concentration (Richards 1954), according to Eq. (1):

$$Q \approx 10 \times (ECw - 0.9) \tag{1}$$

Where:

Q = Sum of cations (mmol<sub>c</sub> L<sup>-1</sup>); and ECw = Desired electrical conductivity of water (dS m<sup>-1</sup>).

Irrigation was applied with a localized drip system, using 32-mm-diameter PVC pipes in the main line and 16-mm-diameter low-density polyethylene pipes in the lateral lines, with drippers with flow rate of 10 L h<sup>-1</sup>. Two pressure-compensating drippers (GA 10 Gripa model) were installed in each plant, each one 15 cm away from the stem. Plants were irrigated daily, at 7:00 a.m., with local-supply water, applying to each container the volume corresponding to that obtained by the water balance, determined by Eq. (2):

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

Where:

VI = Volume of water to be applied (mL); Va = volume applied in the previous irrigation event (mL); Vd = Volume drained (mL); and LF = leaching fraction of 0.10 applied every 20 days.

**Table 2.** Chemical characteristics of the water with the lowest salinity used in the experiment.

Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	HCO <sub>3</sub> <sup>-</sup>	CO <sub>3</sub> <sup>2-</sup>	Cl <sup>-</sup>	EC (dS m <sup>-1</sup> )	pH	SAR (mmol L <sup>-1</sup> ) <sup>0.5</sup>
(mmol <sub>c</sub> L <sup>-1</sup> )									
0.15	0.43	0.65	0.30	0.33	0.00	0.62	0.90	7.07	1.21

EC: electrical conductivity; SAR: sodium adsorption ratio.

**Table 3.** Total water consumption of guava trees cv. Paluma, grafted onto BRS Guaraçá rootstock under different salinity levels of irrigation water, at 150 days after transplanting (DAT).

Saline level (dS m <sup>-1</sup> )	Water consumption	
	L plant <sup>-1</sup>	mm plant <sup>-1*</sup>
0.9	797.9	4070.91
1.5	792.7	4044.39
2.1	722.5	3686.22
2.7	664.7	3391.32
3.3	649.5	3313.77

\*Water depth calculated based on the surface area of the lysimeter (0.196 m<sup>2</sup>).

Water consumption was recorded at each irrigation event. At the end of 150 days after transplanting (DAT), these values were summed to determine the total consumption for each salinity level of the irrigation water, as presented in Table 3.

The different AsA concentrations were applied by foliar spraying after dissolution in water (EC<sub>w</sub> = 0.3 dS m<sup>-1</sup>), at 15-day intervals, starting at 5 p.m. At the time of applications, the plants were isolated with a plastic structure to avoid drifting between the different treatments. The average volume of spray applied per plant was 50 mL.

### Analyzed variables

Evaluations were performed at 150 days after transplanting (DAT), a time point corresponding to the end of the vegetative phase. The selection of this developmental stage was strategic, as it represents the plant's peak vegetative growth and physiological potential before metabolic resources are reallocated to reproductive sinks (i.e., flowers and fruits). This approach allows for a precise assessment of treatment effects on the plant's vegetative framework, a critical growth stage often overlooked in studies that focus solely on the yield components of guava. During this period, growth, relative water content (RWC), electrolyte leakage (EL) in the leaf blade, gas exchange, and photochemical efficiency of the plants were evaluated.

Growth was determined based on: stem diameter, measured with a digital caliper at 3 positions (scion, grafting point, and rootstock); crown diameter ( $D_{\text{Crown}}$ ), obtained through the average of the crown diameter observed in the planting row direction (RD) and interrow direction (IRD); crown volume ( $V_{\text{Crown}}$ ), obtained based on plant height (H), RD and IRD, using Eq. (3); and VVI, obtained according to Portella et al. (2015), as presented in Eq. (4):

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

Where:

$V_{\text{Crown}}$ —crown volume (m<sup>3</sup>); H—plant height (m); RD—crown diameter in the row direction (m); and IRD—crown diameter in the interrow direction (m).

$$VVI = \frac{[H + D_{\text{Crown}} + (D_{\text{RS}} \times 10)]}{100} \quad (4)$$

Where:

VVI—vegetative vigor index; H—plant height (m);  $D_{\text{Crown}}$ —crown diameter (m); and  $D_{\text{RS}}$ —rootstock stem diameter (m).

Relative water content (RWC) was determined using three fully expanded leaves of each plant, after collecting 8 disks with area of 1.54 cm<sup>2</sup> and weighing them on a 0.001 g precision scale to determine the fresh weight of leaves (FW); for turgid weight (TW), the disks were immersed in distilled water for 24 h, and after excess water was removed from the surface, the disks were weighed and the values were recorded; dry weight (DW) was obtained after drying the disks in an oven in forced air circulation at a temperature of 65 °C for a period of 48 h, according to the methodology of Weatherley (1950), using Eq. (5):

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100 \quad (5)$$

Where:

RWC—Relative water content (%); FW—Disk fresh weight (g); TW—Disk turgid weight (g); and DW—Disk dry weight (g).

Electrolyte leakage in the leaf blade (EL) was evaluated to determine the capacity for cell membrane disruption under water stress conditions. Leaves were collected from the middle third of the plant to obtain 10 leaf disks with area of 113 mm<sup>2</sup>, and washed with distilled water to remove other adhered electrolytes, which were placed in beakers with 50 mL of bidistilled water and the beakers hermetically sealed with aluminum foil. The beakers were kept at a temperature of 25 °C for 90 min, when the initial electrical conductivity (Ci) was measured. Subsequently, the beakers were taken to an oven with forced air ventilation and subjected to a temperature of 80 °C for 24 h, after which the final electrical conductivity (Cf) was measured again according to Scotti-Campos et al. (2013). EL was then determined using Eq. (6):

$$\text{EL (\%)} = \frac{Ci}{Cf} \times 100 \quad (6)$$

Where:

EL = Electrolyte leakage in the leaf blade; Ci = Initial electrical conductivity (dS m<sup>-1</sup>); and Cf = Final electrical conductivity (dS m<sup>-1</sup>).

Contents of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were quantified according to Arnon (1949). In the extracts from the disks removed from the leaves, the concentrations of chlorophyll and carotenoids in the solutions were determined using an spectrophotometer at absorbance wavelength (ABS) (470, 646, and 663 nm), using Eqs. (7)–(9):

$$\text{Chl } a = 12.21 \text{ ABS}_{663} - 2.81 \text{ ABS}_{646} \quad (7)$$

$$\text{Chl } b = 20.13 \text{ ABS}_{646} - 5.03 \text{ ABS}_{663} \quad (8)$$



$$\text{Car} = (1000 \text{ABS}_{470} - 1.82 \text{Chl } a - 85.02 \text{Chl } b) / 198 \quad (9)$$

Where:

Chl *a*—Chlorophyll *a*; Chl *b*—Chlorophyll *b*; and Car—Total carotenoids.

The values obtained for chlorophyll *a*, chlorophyll *b*, and carotenoid contents in the leaves were expressed in  $\text{mg g}^{-1}$  of fresh matter (FM).

Chlorophyll *a* fluorescence was evaluated using a modulated-pulse fluorometer (OS5p model from Opti Science), through initial fluorescence ( $F_0$ ), maximum fluorescence ( $F_m$ ), variable fluorescence ( $F_v$ ) and quantum efficiency of photosystem II ( $F_v/F_m$ ); this protocol was performed after adapting the leaves to the dark for 30 min, using clips of the instrument, to ensure that all the first acceptors were oxidized, that is, ensuring that reaction centers were open.

Gas exchange was evaluated based on stomatal conductance— $g_s$  ( $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ),  $\text{CO}_2$  assimilation rate— $A$  ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ), transpiration— $E$  ( $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and intercellular  $\text{CO}_2$  concentration— $C_i$  ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ) with a portable infrared carbon dioxide analyzer (IRGA), “LCPro+” model from ADC BioScientific Ltda. These data were then used to calculate the instantaneous water use efficiency— $WUE_i$  ( $A/g_s$ ) [ $(\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}) (\text{mol H}_2\text{O m}^{-2} \text{s}^{-1})^{-1}$ ] and instantaneous carboxylation efficiency— $CE_i$  ( $A/C_i$ ) [ $(\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}) (\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1})^{-1}$ ]. Readings were taken between 7:00 and 10:00 a.m. under natural conditions of air temperature and  $\text{CO}_2$  concentration, selecting a fully developed leaf free of any visible damage.

### Statistical analyses

The data were subjected to analysis of variance, using the F test at 0.05 and 0.01 probability levels, and in cases of significance, polynomial regression analysis was performed for the levels of electrical conductivity of water and concentrations of ascorbic acid, using SISVAR software (Ferreira 2019). The response surfaces were plotted using SIGMA PLOT software, version 12.5. The correlation between the variables was obtained by plotting a two axis Principal Component Analysis (PCA), which was generated using packages in the R statistical software (R Core Team 2024).

### Results

Irrigation water salinity levels caused significant effects on the relative water content (RWC), electrolyte leakage (EL), stomatal conductance ( $g_s$ ), internal  $\text{CO}_2$  concentration ( $C_i$ ),  $\text{CO}_2$  assimilation rate ( $A$ ), and instantaneous water use efficiency ( $WUE_i$ ) of guava at 150 days after transplanting (Table 4). Ascorbic acid concentrations significantly affected the relative water content in the leaf blade (RWC),  $\text{CO}_2$  assimilation rate ( $A$ ), and instantaneous water use efficiency ( $WUE_i$ ). On the other hand, the interaction between the factors ( $\text{SL} \times \text{AsA}$ ) significantly influenced transpiration ( $E$ ). There was no significant effect of the sources of variation on the instantaneous carboxylation efficiency ( $CE_i$ ) of guava at 150 days after transplanting (DAT).

The relative water content (Figure 2(A)) in the leaf blade of guava decreased linearly with the increase in water salinity levels, by 6.51% per unit increment in ECw. When

**Table 4.** Summary of the analysis of variance for relative water content (RWC), electrolyte leakage (EL), stomatal conductance ( $g_s$ ), internal  $\text{CO}_2$  concentration ( $C_i$ ),  $\text{CO}_2$  assimilation rate ( $a$ ), instantaneous water use efficiency ( $WUE_i$ ), transpiration ( $E$ ), and instantaneous carboxylation efficiency ( $CE_i$ ) of guava cultivated under water salinity (SL) and concentrations of ascorbic acid (AsA), at 150 days after transplanting (DAT).

Sources of variation	DF	Mean squares							
		RWC	EL	$g_s$	$C_i$	$A$	$WUE_i$	$E$	$CE_i$
Salinity levels (SL)	4	914.56**	110.71**	20484.94**	6208.98*	81.19**	3.63*	4.80**	0.00014 <sup>ns</sup>
Linear regression	1	3527.44**	415.11**	66176.03**	23632.13**	292.34**	11.40**	5.44*	0.00001 <sup>ns</sup>
Quadratic regression	1	65.38 <sup>ns</sup>	14.63 <sup>ns</sup>	14672.02**	952.38*	26.84 <sup>ns</sup>	0.25 <sup>ns</sup>	2.25**	0.00003 <sup>ns</sup>
Residual 1	8	34.14	5.39	525.51	1825.53	13.62	0.97	0.14	0.0045
Ascorbic acid (AsA)	3	91.41*	10.94 <sup>ns</sup>	1429.50 <sup>ns</sup>	565.12 <sup>ns</sup>	32.29*	3.91*	2.86**	0.0031 <sup>ns</sup>
Linear regression	1	229.70**	23.21 <sup>ns</sup>	286.16 <sup>ns</sup>	50.43 <sup>ns</sup>	70.08**	5.85*	8.09**	0.002 <sup>ns</sup>
Quadratic regression	1	38.06 <sup>ns</sup>	7.51 <sup>ns</sup>	3390.01 <sup>ns</sup>	1135.35 <sup>ns</sup>	26.40 <sup>ns</sup>	5.09*	0.42 <sup>ns</sup>	0.0005 <sup>ns</sup>
Interaction (SL $\times$ AsA)	12	32.46 <sup>ns</sup>	9.77 <sup>ns</sup>	647.01 <sup>ns</sup>	537.79 <sup>ns</sup>	12.55 <sup>ns</sup>	2.27 <sup>ns</sup>	0.81**	0.0023 <sup>ns</sup>
Blocks	2	34.298 <sup>ns</sup>	7.65 <sup>ns</sup>	977.01 <sup>ns</sup>	0.20 <sup>ns</sup>	16.13 <sup>ns</sup>	0.28 <sup>ns</sup>	0.61**	0.0015 <sup>ns</sup>
Residual 2	30	24.45	4.43	1097.41	619.91	12.55	1.28	0.18	0.0015
CV 1 (%)		8.27	13.92	12.06	30.42	18.27	16.12	11.22	44.80
CV 2 (%)		7	12.61	17.42	17.73	13.63	18.50	12.58	26.19

DF: degrees of freedom; CV (%): coefficient of variation.

\*Significant at  $p \leq 0.05$ .

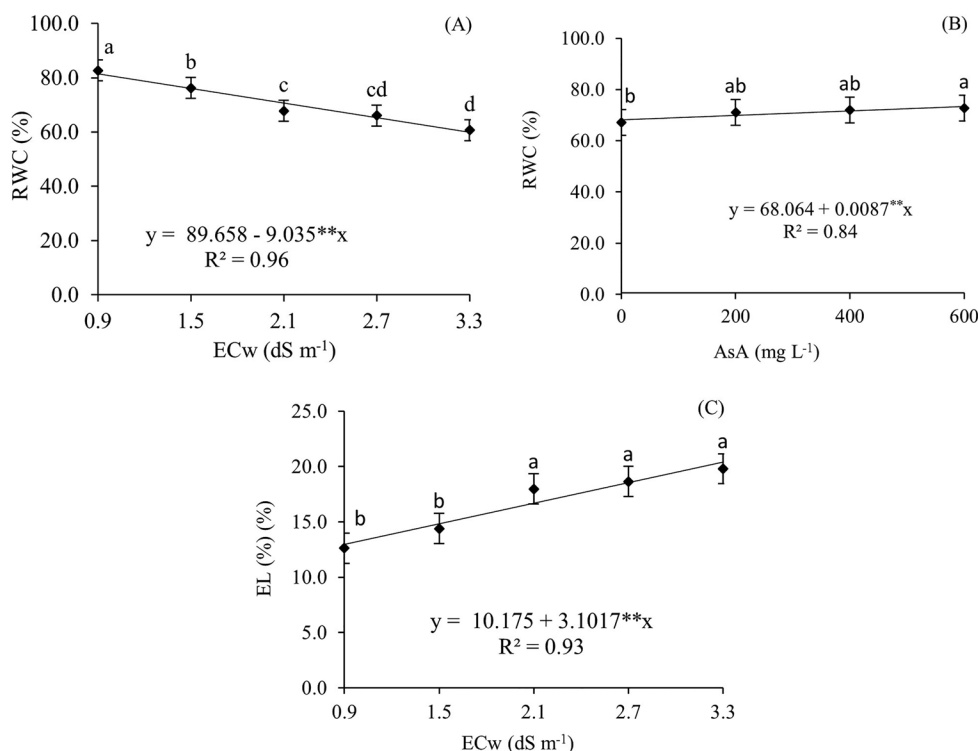
\*\*Significant at  $p \leq 0.01$  probability.

<sup>ns</sup>Not significant.

comparing the RWC of plants that received EC<sub>w</sub> of 3.3 dS m<sup>-1</sup> to that of plants cultivated under water salinity of 0.9 dS m<sup>-1</sup>, a reduction of 16.62% was observed. Ascorbic acid (AsA) promoted a positive effect on the RWC of guava (Figure 2(B)), with an increase of 0.012% per unit increase in AsA concentration, equivalent to an increase of 7.66% between plants cultivated under concentrations of 0 and 600 mg L<sup>-1</sup>. Regarding electrolyte leakage (Figure 2(C)), the increase in EC<sub>w</sub> levels promoted a linear increase of 30.48% per unit increase in EC<sub>w</sub>. In relative terms, there was an increase in EL of 57.40% between plants irrigated with EC<sub>w</sub> of 0.9 and 3.3 dS m<sup>-1</sup>.

Irrigation water salinity negatively affected the stomatal conductance of guava (Figure 3(A)), which changed from 319.08 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> at EC<sub>w</sub> of 0.9 dS m<sup>-1</sup> to 158.21 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> at EC<sub>w</sub> of 3.3 dS m<sup>-1</sup>, with a loss of 50.41% in  $g_s$ . A similar behavior was observed for the internal CO<sub>2</sub> concentration (Figure 3(B)), as water salinity of 3.3 dS m<sup>-1</sup> resulted in a decrease of 32.39% compared to the value obtained in plants irrigated with water of 0.9 dS m<sup>-1</sup>.

Effects of water salinity on CO<sub>2</sub> assimilation rate (Figure 4(A)) caused losses of 2.60 per unit increase in EC<sub>w</sub> level. Similar results were found by Bezerra et al. (2018) in a study with guava irrigated with water of different levels of electrical conductivity, in which they concluded that EC<sub>w</sub> above 2.15 dS m<sup>-1</sup> resulted in a reduction in the CO<sub>2</sub> assimilation rate of plants. However, with the increase in water salinity level, guava plants showed greater water use efficiency (Figure 4(C)), with a value of 6.728 at EC<sub>w</sub> of 3.3 dS m<sup>-1</sup>, which was 18.37% higher than that obtained in plants irrigated with water of 0.9 dS m<sup>-1</sup>.



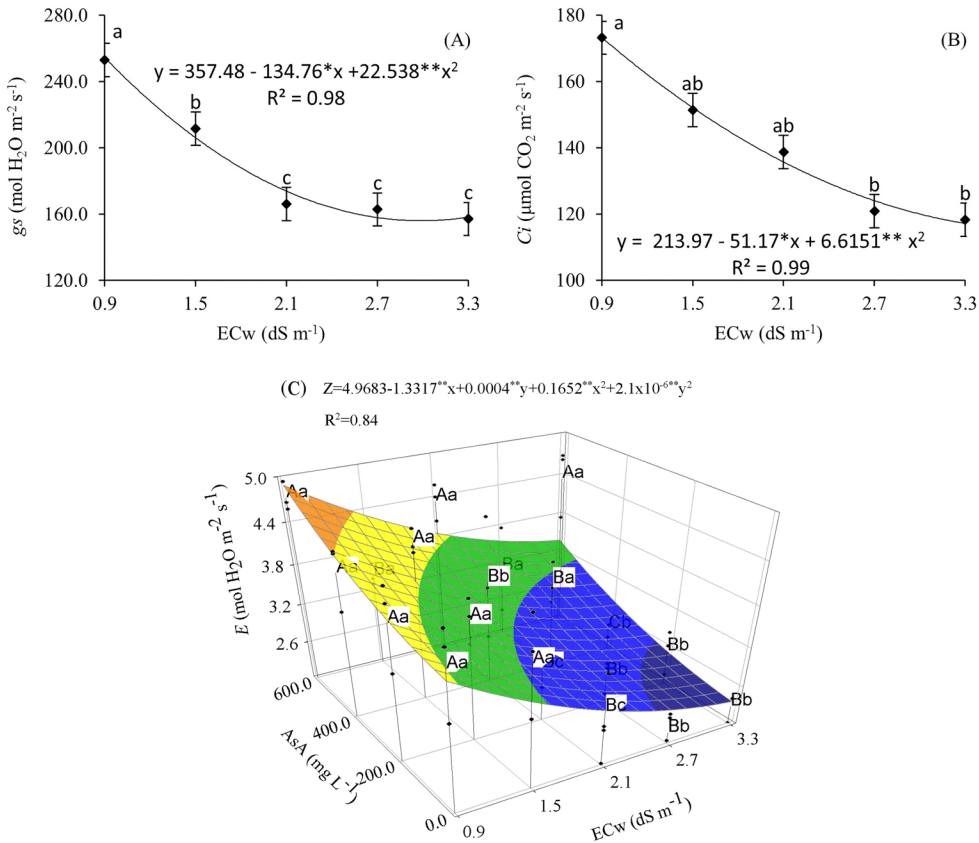
**Figure 2.** Relative water content—RWC in the leaf blade of guava, as a function of the levels of electrical conductivity of water—ECw (A) and the concentrations of ascorbic acid—AsA (B), and electrolyte leakage—EL (C), as a function of the ECw levels, at 150 days after transplanting (DAT).

**\*\*Significant at  $p \leq 0.01$  by the  $F$  test; Means followed by different letters indicate a significant difference by the Tukey test ( $p \leq 0.05$ ).**

Guava transpiration decreased with the increase in the salinity level of irrigation water (Figure 3(C)), with the lowest value obtained at ECw of 3.3 dS m<sup>-1</sup> and without application of AsA (2.36 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), whereas the application of AsA resulted in increases in  $E$ , whose maximum value was obtained in plants irrigated with water of 0.9 dS m<sup>-1</sup> and under AsA concentration of 600 mg L<sup>-1</sup> (4.90 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), a behavior that is maintained with the foliar application of 600 mg L<sup>-1</sup> of AsA in plants grown under ECw of 3.3 dS m<sup>-1</sup>, increasing  $E$  by 29.97% compared to those that did not receive AsA application.

Benefits of AsA as a single factor were also observed for relative water content (Figure 2(B)) and CO<sub>2</sub> assimilation rate (Figure 4(B)), with the concentration of 600 mg L<sup>-1</sup> resulting in gains of 7.21% in RWC and 12.65% in  $A$  compared to control plants. However,  $WUE_i$  increased up to a concentration of 230 mg L<sup>-1</sup> (Figure 4(D)), resulting in a value of 6.56 (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), corresponding to an increase of 4.85% compared to plants without AsA application.

There was a significant effect of irrigation water salinity levels on the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) and initial fluorescence ( $F_0$ ) of guava at 150 days after transplanting (Table 5). The concentrations of ascorbic acid (AsA) and the interaction between the factors (SL × AsA) significantly

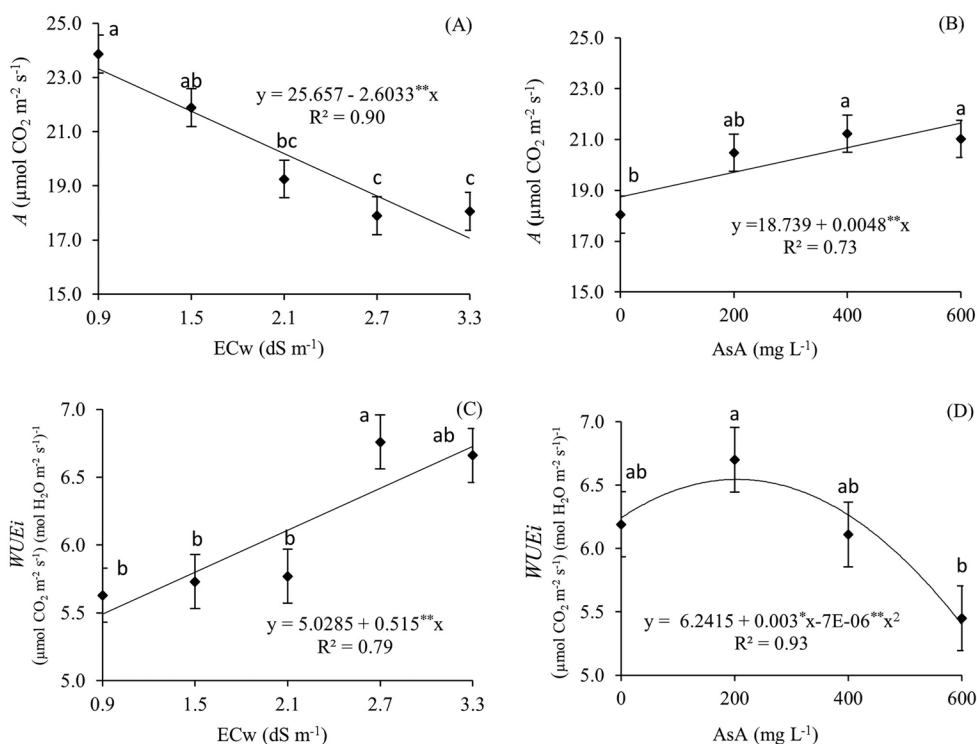


**Figure 3.** Stomatal conductance— $g_s$  (A) and internal  $\text{CO}_2$  concentration— $C_i$  (B) of guava as a function of the levels of electrical conductivity of water— $\text{ECw}$ , and transpiration— $E$  (C) as a function of the interaction between the levels of electrical conductivity of water— $\text{ECw}$  and concentrations of ascorbic acid— $\text{AsA}$ , at 150 days after transplanting (DAT).

X and Y—electrical conductivity of water— $\text{ECw}$  and concentration of ascorbic acid— $\text{AsA}$ , respectively; \* and \*\* significant at  $p \leq 0.05$  and  $0.01$ , respectively, by the  $F$  test. Uppercase letters compare  $\text{ECw}$  levels and lowercase letters compare  $\text{AsA}$  concentrations (Tukey's test,  $p \leq 0.05$ ).

influenced only the  $\text{Chl } a$  contents. The variables maximum fluorescence ( $F_m$ ), variable fluorescence ( $F_v$ ), and quantum efficiency of photosystem II ( $F_v/F_m$ ) were not significantly affected by the sources of variation tested.

The increase in irrigation water salinity compromised the chlorophyll  $a$  content of guava plants (Figure 5(A)), with greater severity in those grown without  $\text{AsA}$  application, for which the  $\text{ECw}$  of  $3.3 \text{ dS m}^{-1}$  resulted in the lowest  $\text{Chl } a$  value,  $15.01 \text{ mg g}^{-1} \text{ FM}$ . Foliar application of  $\text{AsA}$  promoted increments in  $\text{Chl } a$  contents, with the point of maximum gain established under irrigation with water of  $0.9 \text{ dS m}^{-1}$  and  $\text{AsA}$  application of  $600 \text{ mg L}^{-1}$  ( $29.79 \text{ mg g}^{-1} \text{ FM}$ ), corresponding to an increase of 14.63% compared to those that did not receive  $\text{AsA}$  application at the same salinity level. Similar studies were conducted by Silva et al. (2024) with soursop under irrigation with water of different salinity levels ( $\text{ECw}$  ranging from  $0.8$  to  $4.0 \text{ dS m}^{-1}$ ), in which they found a decrease of 44.64% in the  $\text{Chl } a$  contents of plants grown



**Figure 4.** CO<sub>2</sub> assimilation rate—A of guava as a function of the levels of electrical conductivity of water—ECw (A) and concentrations of ascorbic acid—AsA (B), and instantaneous water use efficiency—WUEi as a function of the ECw levels (C) and concentrations of AsA (D), at 150 days after transplanting (DAT).

\* and \*\* significant at  $p \leq 0.01$  and  $0.01$ , respectively, by the  $F$  test. Means followed by different letters indicate a significant difference by the Tukey test ( $p \leq 0.05$ ).

under ECw of  $4.0 \text{ dS m}^{-1}$  compared to those that received the lowest water salinity level ( $0.8 \text{ dS m}^{-1}$ ).

Chlorophyll *b* contents were also negatively affected by irrigation water salinity (Figure 5(B)), with losses of 12.48% per unit increase in ECw, which resulted in a value of  $13.32 \text{ mg g}^{-1} \text{ FM}$  at ECw of  $3.3 \text{ dS m}^{-1}$ , 33.76% lower than that found in plants irrigated with water of  $0.9$  ( $20.11 \text{ mg g}^{-1} \text{ FM}$ ). This result differed from the one observed for carotenoid contents (Figure 5(C)), whose highest value was found in plants under irrigation with ECw of  $3.3 \text{ dS m}^{-1}$  ( $6.27 \text{ mg g}^{-1} \text{ FM}$ ), with a gain of 20.41% compared to that obtained in plants under irrigation with ECw of  $0.9 \text{ dS m}^{-1}$ .

Irrigation water salinity altered the initial fluorescence of guava (Figure 5(D)), whose value at ECw of  $3.3 \text{ dS m}^{-1}$  ( $0.0375$ ) was 24.32% higher than that obtained in plants irrigated with ECw of  $0.9 \text{ dS m}^{-1}$ . Similar responses were reported by Sá et al. (2021), who evaluated the initial fluorescence of custard apple (*Annona squamosa* L.) under salt stress (ECw between  $0.8$  and  $3.0 \text{ dS m}^{-1}$ ) and found a 7.0% increase in the initial fluorescence of plants when irrigated using water with the highest salinity level.

There was a significant effect of irrigation water salinity levels on the crown volume ( $V_{\text{Crown}}$ ) and crown diameter ( $D_{\text{Crown}}$ ) of guava at 150 days after transplanting (Table 5).

**Table 5.** Summary of the analysis of variance for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (Car), initial fluorescence ( $F_0$ ), maximum fluorescence ( $F_m$ ), variable fluorescence ( $F_v$ ), and quantum efficiency of photosystem II ( $F_v/F_m$ ) of guava cultivated under water salinity (SL) and concentrations of ascorbic acid (AsA), at 150 days after transplanting (DAT).

Sources of variation	DF	Mean square						
		Chl <i>a</i>	Chl <i>b</i>	Car	$F_0$	$F_m$	$F_v$	$F_v/F_m$
Salinity levels (SL)	4	204.96**	90.36*	3.33**	0.00008*	492.81 <sup>ns</sup>	492.91 <sup>ns</sup>	0.014 <sup>ns</sup>
Linear regression	1	6.20 <sup>ns</sup>	345.95**	12.14**	0.000400**	0.0008 <sup>ns</sup>	0.0002 <sup>ns</sup>	0.008 <sup>ns</sup>
Quadratic regression	1	1.86 <sup>ns</sup>	11.06*	1.08 <sup>ns</sup>	0.000080**	703.75 <sup>ns</sup>	703.97 <sup>ns</sup>	0.032 <sup>ns</sup>
Residual 1	8	2.46	2.54	0.16	0.000018	491.96	492.15	0.019
Ascorbic acid (AsA)	3	61.39**	1.60 <sup>ns</sup>	1.51 <sup>ns</sup>	0.000010 <sup>ns</sup>	492.30 <sup>ns</sup>	492.43 <sup>ns</sup>	0.006 <sup>ns</sup>
Linear regression	1	141.50**	2.69 <sup>ns</sup>	8.32*	0.000011 <sup>ns</sup>	98.18 <sup>ns</sup>	98.21 <sup>ns</sup>	0.0044 <sup>ns</sup>
Quadratic regression	1	0.18 <sup>ns</sup>	0.12 <sup>ns</sup>	0.16 <sup>ns</sup>	0.000019 <sup>ns</sup>	493.25 <sup>ns</sup>	493.46 <sup>ns</sup>	0.014 <sup>ns</sup>
Interaction (SL × AsA)	12	16.20**	2.40 <sup>ns</sup>	0.60 <sup>ns</sup>	0.000002 <sup>ns</sup>	491.71 <sup>ns</sup>	491.74 <sup>ns</sup>	0.005 <sup>ns</sup>
Blocks	2	0.58*	2.25 <sup>ns</sup>	0.03 <sup>ns</sup>	0.000002 <sup>ns</sup>	492.17 <sup>ns</sup>	492.15 <sup>ns</sup>	0.005 <sup>ns</sup>
Residual 2	30	1.78	2.17	0.46	0.000011	492.26	492.18	0.007
CV 1 (%)		7.19	9.53	7.35	13.58	733.40	741.46	17.60
CV 2 (%)		6.12	8.81	12.38	10.42	733.63	742.36	18.88

DF: degrees of freedom; CV (%): coefficient of variation.

\*Significant at  $p \leq 0.05$ .

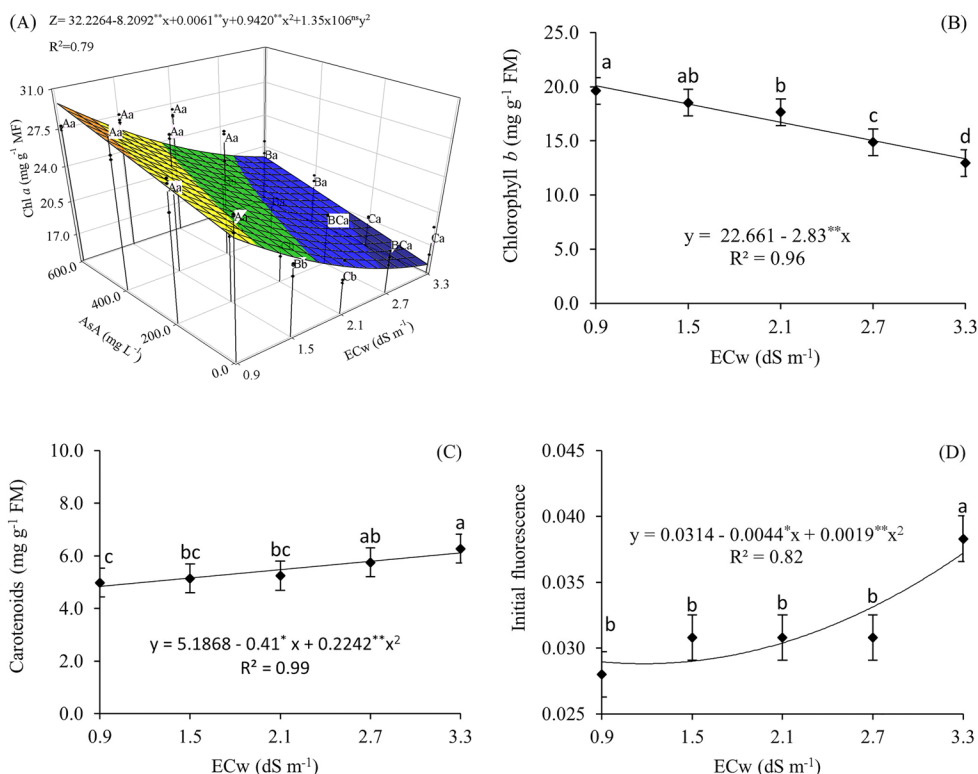
\*\*Significant at  $p \leq 0.01$  probability.

<sup>ns</sup>Not significant.

Ascorbic acid (AsA) concentrations caused a significant effect on the grafting point diameter ( $D_{GP}$ ) and vegetative vigor index (VVI). The interaction between the factors (SL × AsA) significantly influenced the scion diameter ( $D_{SC}$ ), grafting point diameter ( $D_{GP}$ ), rootstock diameter ( $D_{RS}$ ), and vegetative vigor index (VVI) of guava at 150 days after transplanting (DAT) (Table 6).

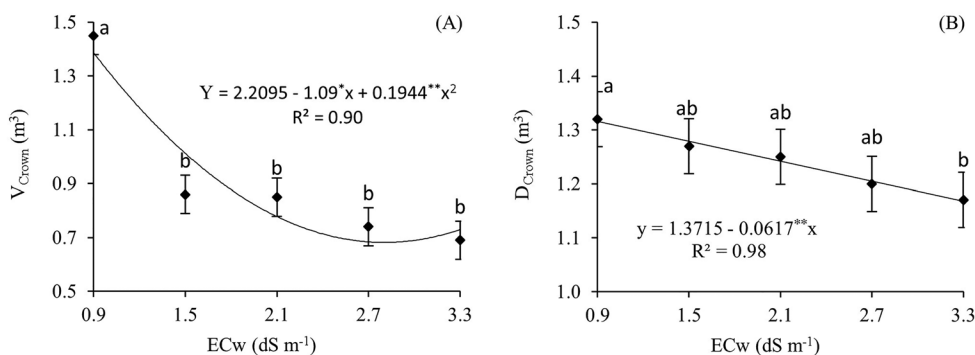
Guava crown volume decreased with the increase in irrigation water salinity (Figure 6(A)), from 1.385 m<sup>3</sup> in plants cultivated under ECw 0.9 dS m<sup>-1</sup> to 0.729 m<sup>3</sup> in plants irrigated with ECw of 3.3 dS m<sup>-1</sup>, resulting in a loss of 47.36% in  $V_{Crown}$ . These effects were also observed in crown diameter (Figure 6(B)), but with reductions that reached 14.85% when comparing the value obtained with irrigation of 3.3 dS m<sup>-1</sup> to that observed in plants cultivated with water of 0.9 dS m<sup>-1</sup>.

Scion diameter (Figure 7(A)), grafting point diameter (Figure 7(B)) and rootstock diameter (Figure 7(C)) decreased with the increase in irrigation water salinity level, with the lowest value observed in plants without AsA application and irrigated with ECw of 3.3 dS m<sup>-1</sup> (17.79, 20.49, and 17.43 mm, respectively), with the respective losses of 24.45, 11.79, and 17.47% compared to the values obtained in plants without AsA application and irrigated with ECw of 0.9 dS m<sup>-1</sup>. However, AsA application proved to be beneficial to guava stem diameter, with the highest values for  $D_{SC}$ ,  $D_{GP}$  and  $D_{RS}$  (23.98, 26.64, and 21.39 mm) obtained in plants irrigated with water of 0.9 dS m<sup>-1</sup> under AsA concentration of 600 mg L<sup>-1</sup>, with gains of 2.1, 12.80, and 1.68% compared to that obtained in plants without AsA application, respectively. It should be noted that at transplanting, seedlings were characterized by a mean height of 40 cm and a



**Figure 5.** Contents of chlorophyll *a*—Chl *a* (A) of guava as a function of the interaction between the levels of electrical conductivity of water—ECw and concentrations of ascorbic acid—AsA, and contents of chlorophyll *b*—Chl *b* (B), carotenoids—Car (C) and initial fluorescence (D), as a function of ECw levels, at 150 days after transplanting (DAT).

X and Y—electrical conductivity of water—ECw and concentration of ascorbic acid—AsA, respectively; \* and \*\* significant at  $p \leq 0.05$  and  $0.01$ , respectively, by the *F* test. Uppercase letters compare ECw levels and lowercase letters compare AsA concentrations (Tukey's test,  $p \leq 0.05$ ).



**Figure 6.** Crown volume— $V_{\text{Crown}}$  (A) and crown diameter— $D_{\text{Crown}}$  (B) of guava plants as a function of the levels of electrical conductivity of water—ECw, at 150 days after transplanting (DAT).

\* and \*\* significant at  $p \leq 0.05$  and  $0.01$ , respectively, by the *F* test. Means followed by different letters indicate a significant difference by the Tukey test ( $p \leq 0.05$ ).



**Table 6.** Summary of the analysis of variance for crown volume ( $V_{\text{Crown}}$ ), crown diameter ( $D_{\text{Crown}}$ ), scion diameter ( $D_{\text{SC}}$ ), grafting point diameter ( $D_{\text{GP}}$ ), rootstock diameter ( $D_{\text{RS}}$ ), and vegetative vigor index (VVI) of guava cultivated under water salinity levels (SL) and concentrations of ascorbic acid (AsA), at 150 days after transplanting (DAT).

Sources of variation	DF	Mean squares					
		$V_{\text{Crown}}$	$D_{\text{Crown}}$	$D_{\text{SC}}$	$D_{\text{GP}}$	$D_{\text{RS}}$	VVI
Salinity levels (SL)	4	1.120**	0.040*	47.74**	28.14*	25.99**	2.57**
Linear regression	1	3.240**	0.1591**	0.33 <sup>ns</sup>	34.36**	0.48 <sup>ns</sup>	0.0004 <sup>ns</sup>
Quadratic regression	1	0.795**	0.0002 <sup>ns</sup>	0.48 <sup>ns</sup>	7.12*	1.94 <sup>ns</sup>	0.003 <sup>ns</sup>
Residual 1	8	0.05	0.018	1.34	1.76	1.21	0.04
Ascorbic acid (AsA)	3	0.017 <sup>ns</sup>	0.0039 <sup>ns</sup>	3.80 <sup>ns</sup>	30.57**	0.80 <sup>ns</sup>	0.66**
Linear regression	1	0.02 <sup>ns</sup>	0.0007 <sup>ns</sup>	8.67*	42.33**	1.06 <sup>ns</sup>	1.18**
Quadratic regression	1	0.03 <sup>ns</sup>	0.0052 <sup>ns</sup>	0.03 <sup>ns</sup>	41.90**	1.33 <sup>ns</sup>	0.05 <sup>ns</sup>
Interaction (SL $\times$ AsA)	12	0.049 <sup>ns</sup>	0.0113 <sup>ns</sup>	4.06**	2.77**	5.79**	0.30**
Blocks	2	0.006 <sup>ns</sup>	0.0155 <sup>ns</sup>	2.81 <sup>ns</sup>	0.211 <sup>ns</sup>	0.60	0.009
Residual 2	30	0.05	0.014	1.41	1.63	2.08	0.05
CV 1 (%)		26.02	10.92	5.58	5.86	5.78	12.06
CV 2 (%)		26.34	9.60	5.73	5.64	7.75	14.16

DF: degrees of freedom; CV (%): coefficient of variation.

\*Significant at  $p \leq 0.05$ .

\*\*Significant at  $p \leq 0.01$  probability.

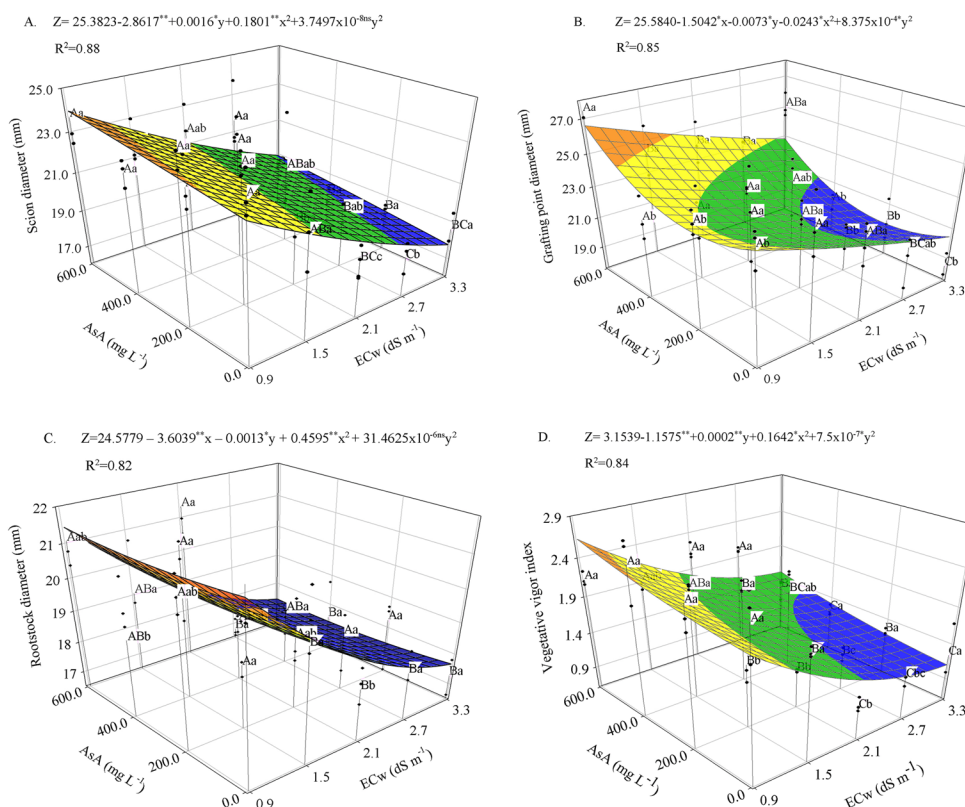
<sup>ns</sup>Not significant.

mean diameter of  $\sim 7$  mm across the graft union (scion and rootstock), conforming to the quality standards for field establishment.

Negative effects of water salinity were observed on the vegetative vigor index (Figure 7(D)), with more severe effects on plants without AsA application and under irrigation with the highest EC<sub>w</sub>, which showed a value of 1.122, with a loss of 50.01% compared to plants irrigated with water of 0.9 dS m<sup>-1</sup> (2.145). However, with the application of AsA, these effects were reduced under EC<sub>w</sub> of 3.3 dS m<sup>-1</sup>, with an increase of 34.75% in VVI under the application of the AsA concentration of 600 mg L<sup>-1</sup> (1.512). This AsA concentration resulted in the maximum value for this variable (2.635) when applied to plants irrigated with water of 0.9 dS m<sup>-1</sup>, with an increase of 17.37% compared to those that did not receive application of AsA under the same EC<sub>w</sub> level.

Principal component analysis (PCA) presents 71.52% of the original variation of the data in the first two axes (Figure 8), and the association between the variables is concentrated on axis 1, which represents 58.8% of the present variance. In this component, a strong correlation is observed between the variables relative water content (RWC,  $r=0.90$ ), gas exchange ( $g_s$ ,  $r=0.83$ ;  $C_i$ ,  $r=0.86$ ;  $E$ ,  $r=0.83$ ; and  $A$ ,  $r=0.77$ ), chlorophyll contents (Chl  $a$ ,  $r=0.85$ ; Chl  $b$ ,  $r=0.84$ ), stem diameters ( $D_{\text{SC}}$ ,  $r=0.91$ ;  $D_{\text{GP}}$ ,  $r=0.78$ ; and  $D_{\text{RS}}$ ,  $r=0.79$ ), crown volume and diameter ( $V_{\text{Crown}}$ ,  $r=0.88$ ;  $D_{\text{Crown}}$ ,  $r=0.71$ ), and this behavior was favored in the treatments of group 1, followed by group 2. In turn, the negative correlation occurred with the variables carotenoids (CAR = -0.62), electrolyte leakage (EL,  $r=-0.87$ ), and initial fluorescence ( $F_0$ ,  $r=-0.65$ ), which are strong in group 5, followed by group 4 of the treatments. For the components of axis 2, which explains 12.7% of the variance, there was a positive correlation between



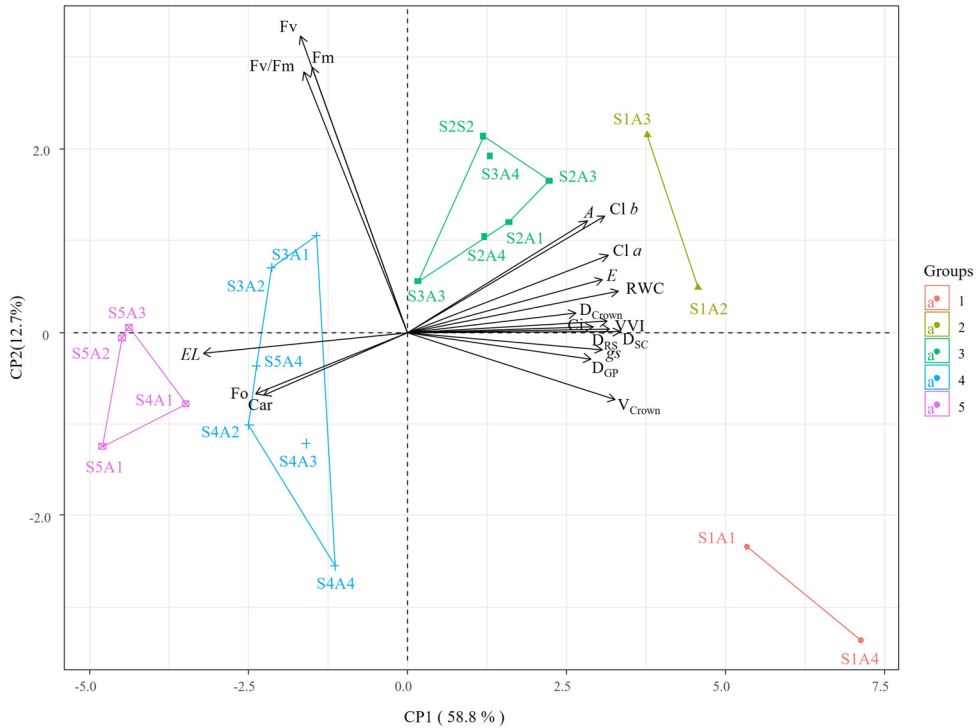


**Figure 7.** Scion diameter (A), grafting point diameter (B), rootstock diameter (C), and vegetative vigor index (D) of guava as a function of the interaction between the levels of electrical conductivity of water (ECw) and concentrations of ascorbic acid (AsA) at 150 days after transplanting (DAT). X and Y—electrical conductivity of water—ECw and concentration of ascorbic acid—AsA, respectively; \* and \*\* significant at  $p \leq 0.05$  and  $0.01$ , respectively, by the  $F$  test. Uppercase letters compare ECw levels and lowercase letters compare AsA concentrations (Tukey's test,  $p \leq 0.05$ ).

maximum (Fm,  $r=0.78$ ) and variable (Fv,  $r=0.88$ ) fluorescences and quantum efficiency of photosystem II (Fv/Fm,  $r=0.77$ ), and these variables were favored in the treatments of group 3.

## Discussion

The reductions observed in the relative water content of the guava leaf blade in the present study are a direct response to water limitation, which is the initial effect of salinity on plants. This saline stress impedes water absorption by reducing the osmotic potential of the soil solution due to salt accumulation near the roots (Liu, Jiang, and Yuan 2024), resulting in a lower solute flow in the xylem as the plant attempts to prevent water loss to the environment (Haworth et al. 2018; Silva et al. 2022). This physiological behavior manifests as an adjustment in gas exchange, specifically a decrease in stomatal opening, as recorded in our data. Similar findings were reported by Lacerda et al. (2022), who observed reductions in both RWC and stomatal conductance (gs) in cutting-propagated guava trees under irrigation water salinity of  $3.2 \text{ dS m}^{-1}$  at 150 DAT.



**Figure 8.** Two-dimensional projection of the scores of the principal components for the factors saline water irrigation strategies (S) and concentrations of ascorbic acid (A) and of the variables analyzed in the two principal components (PC1 and PC2).

S: saline water irrigation strategies, S1 (SE—plants under irrigation using water with low electrical conductivity throughout the cycle); S2 (VE—plants grown under salt stress in the vegetative stage); S3 (FL—flowering); S4 (FR—fruiting); S5 (VE/FL—salt stress in the vegetative and flowering stages, with irrigation with low salinity in the fruiting stage); S6 (VE/FR—plants subjected to salt stress in the vegetative and fruiting stages); A: ascorbic acid, A1 (0 mg L<sup>-1</sup>); A2 (200 mg L<sup>-1</sup>); A3 (400 mg L<sup>-1</sup>); A4 (600 mg L<sup>-1</sup>); RWC: relative water content (%); EL: electrolyte leakage (%) and initial fluorescence; Ci: intercellular CO<sub>2</sub> concentration (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>); gs: stomatal conductance (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>); A: CO<sub>2</sub> assimilation rate (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>); E: transpiration (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>); WUEi: instantaneous water use efficiency (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>); Chl a: chlorophyll a (mg g<sup>-1</sup> FM); Chl b: chlorophyll b (mg g<sup>-1</sup> FM); Car: carotenoids (mg g<sup>-1</sup> FM); D<sub>SC</sub>, D<sub>GP</sub> and D<sub>RS</sub>: scion diameter, grafting point diameter and rootstock diameter (mm), respectively; D<sub>Crown</sub>: crown diameter (m); V<sub>Crown</sub>: crown volume (m<sup>3</sup>); VVI: vegetative vigor index.

The data from this study revealed that increased salts in the rhizosphere induced a restriction in stomatal opening, which, in turn, impaired transpirational flow and CO<sub>2</sub> diffusion into the substomatal chamber. This effect compromised leaf turgidity, crucial for maintaining the plant's metabolic activity (Haworth et al. 2021) and for the functionality of RuBisCO's carboxylase activity (Feng et al. 2020). The correlation between these variables and carbon assimilation in our study, along with the apparent lack of a salinity effect on carboxylation efficiency, reinforces the idea that damage to carbon assimilation is intimately linked to stomatal activity (Ou, Lin, and Chang 2014). Additionally, we detected non-stomatal effects, manifested by decreased chlorophyll content and initial fluorescence in guava. This observation is justified by the fact that salinity accentuates the synthesis of the enzyme chlorophyllase (Souto et al. 2024), which increases the energy demand for electron flow to plastoquinone and,

subsequently, to the electron transport chain, resulting in insufficient ATP and NADPH production for the Calvin cycle (Haworth et al. 2018).

Although salinity increased electrolyte leakage, the observed values remained below the 50% threshold considered critical for cell membrane integrity (Sullivan 1971). This suggests that the maintenance of high carotenoid levels may have favored the dissipation of excess energy as heat, as they act as reducing agents, protecting against oxidative damage (Souto et al. 2024). Indeed, our data indicate a positive correlation between increased carotenoid levels and salinity intensification. This increase occurred concomitantly with responses typically associated with oxidative damage, such as increased electrolyte leakage and initial fluorescence ( $F_0$ )—parameters that, in turn, showed a negative correlation with photosynthetic activity and plant growth.

This behavior of carotenoids, by dissipating excess energy, may have been crucial in mitigating the overheating of the photosynthetic area, especially given a potential reduction in transpiration, a notion supported by the maintenance of intrinsic water use efficiency in guava plants. It is noteworthy that under more severe salt stress conditions in semi-arid regions, studies frequently report excessive production of ROS that can overwhelm the protective capacity of leaf carotenoids (Alinia et al. 2024). Such a scenario would lead to cell membrane damage and compromise carbon assimilation, as demonstrated in custard apple (*Annona squamosa* L.) under  $3.0 \text{ dS m}^{-1}$  (Sá et al. 2021) and in guava (*P. guajava* L.) itself under  $6.15 \text{ dS m}^{-1}$  (Bezerra et al. 2018), a situation potentially more drastic than that observed in the present study.

Salt stress effects are visible in the crown and diameter of guava trees, associated with the restriction of photosynthetic activity and energy expenditure in metabolic regulation, in view of the impacts observed with the reduction of relative water content, which can affect enzymatic synthesis (Arif et al. 2020), especially the antioxidant activity of the plant (Liu, Jiang, and Yuan 2024). This is reinforced by the increase in initial fluorescence ( $F_0$ ) to maintain photochemical activity, given the lack of effects of salinity on the quantum efficiency of the photosystem (Shin et al. 2020), corroborating the idea that the flow of photoassimilates destined for meristematic activity is compromised to maintain plant homeostasis under accumulation of salts near the root system (Lu and Fricke 2023). In line with that, Hossain et al. (2022) demonstrate that plants under salt stress show an increase in the production of osmolytes, such as proline and glycine betaine, and antioxidants, such as ascorbate and superoxide dismutase, but growth losses are observed. The reductions found are similar to that obtained by Lacerda et al. (2022) in guava irrigated with water of up to  $3.2 \text{ dS m}^{-1}$ .

The exogenous application of ascorbic acid (AsA) proved to be an effective strategy for mitigating the physiological impacts of salt stress on guava plants in this study. The beneficial action of AsA is grounded in its role as a multifunctional non-enzymatic antioxidant molecule, capable of directly scavenging ROS and protecting vital cellular components, such as lipids and proteins, from oxidative damage (Fatah and Sadek 2020; Hassan et al. 2021). In our experiment, the most prominent effect of AsA application was the increase in leaf relative water content (RWC). This improvement in water status is a prerequisite for maintaining cell turgor, which in turn sustains stomatal opening and photosynthetic activity, even under stress conditions (Hasanuzzaman et al. 2023). Similar responses to AsA application have been reported in other crops, such

as by Noreen et al. (2021), with 200 ppm in barley, and by Fatah and Sadek (2020), with 100 and 200 ppm in sugar beet.

The mechanism underlying this protection is complex and multifaceted. As observed, AsA, produced in the cytosol, functions not only as an ROS scavenger but also as a primary substrate in the ascorbate-glutathione cycle, a crucial pathway for the detoxification of hydrogen peroxide with the aid of ascorbate peroxidase (Farag et al. 2020; Wu et al. 2024). Additionally, its versatility is manifested in its role as a cofactor for essential enzymes, including hydroxylases involved in cell wall formation (Celi et al. 2023) and violaxanthin de-epoxidase, which synthesizes zeaxanthin for protection against photoinhibition (Smirnoff 2018). Its function further extends to regulating plant hormone synthesis, thereby directly impacting plant development (Arábia et al. 2024; Zheng et al. 2022).

This capacity to maintain redox equilibrium is enhanced by its function as an exceptional electron donor within the photochemical apparatus. The remarkable stability of its oxidized form, the monodehydroascorbate radical, allows for the safe dissipation of excess electrons (Bielski 1982), which results in the preservation of thylakoid membrane integrity and the functionality of photosynthetic pigments (El-Beltagi et al. 2022; Hassan et al. 2021). This protection directly translates into a greater carbon assimilation capacity, which, in turn, underpins the observed gains in growth parameters, such as scion diameter and the vegetative vigor index. By mitigating oxidative damage, the plant can allocate energy and photoassimilates toward growth and development instead of expending them on cellular repair processes (Elsiddig et al. 2022). Our results corroborate the findings of Ibrahim (2013) in olive trees and Abdulrahman (2013) in almond seedlings, who also reported significant improvements in vegetative growth following AsA application.

In this context, the results of the present study are directly comparable to other research on guava under abiotic stress. Lacerda et al. (2025), for instance, observed that the application of 90 mM AsA to guava trees under water deficit increased the CO<sub>2</sub> assimilation rate and reduced electrolyte leakage, which reinforces our observation that AsA supports plant physiology and membrane integrity. Even more specifically, the work by Torres et al. (2025) on guava under salt stress demonstrated that 60 mM AsA also increased RWC, aligning with our findings. It is crucial to note, however, a divergence from the findings of Torres et al. (2025), who reported stimulated chlorophyll biosynthesis with a 90 mM AsA application under the highest salinity (3.3 dS m<sup>-1</sup>). In our study, despite observing some gains in chlorophyll *a* content, the overall benefits of AsA were less pronounced at this same stress level, as indicated by the Principal Component Analysis (PCA). This suggests that although AsA could not completely reverse the physical limitations imposed by severe stress, such as stomatal restriction, its biochemical function was preserved. This is consistent with its role in optimizing metabolic and energetic processes at the cellular level, thereby sustaining the carbon assimilation capacity even when stomatal limitations are not overcome (El-Beltagi et al. 2022).

This apparent divergence highlights that although the results of this study partially corroborate our initial hypothesis, the efficacy of AsA is complex. Its beneficial effects were notable, but the interaction with salinity proved to be a key factor, especially for growth. The PCA reinforced salinity as the predominant factor and indicated that under

the most severe stress ( $3.3 \text{ dS m}^{-1}$ ), AsA application did not significantly alter plant behavior compared to the untreated control. This suggests the existence of a saline stress threshold beyond which the exogenous application of AsA, at the tested concentrations, may not be sufficient to completely reverse the damage. Such findings underscore the need for future investigations to optimize application strategies, especially considering that the evaluation in the present study occurred at the end of the vegetative phase. The established relationship between AsA and plant hormones (Akram et al. 2017) suggests that its application could contribute to a more effective regulation of the flowering phase, as reported by Fatima et al. (2024) for sour passion fruit. This demonstrates the necessity of research aimed at understanding AsA's interaction with factors beyond basic physiology to maximize its protective potential in the cultivation of fruit trees like guava.

## Conclusions

Salt stress, induced by irrigation with water of increasing electrical conductivity, adversely affected the gas exchange, photosynthetic pigments, and growth parameters of guava plants in the post-grafting phase, starting from a water conductivity of  $0.9 \text{ dS m}^{-1}$ .

Foliar application of ascorbic acid (AsA) demonstrated beneficial effects both as an isolated factor—improving relative water content and the  $\text{CO}_2$  assimilation rate, and through its interaction with salinity. The importance of this mitigating action was evidenced by the significant interaction in attenuating chlorophyll *a* degradation and, most notably, in promoting vegetative vigor, even in plants subjected to the highest level of salt stress ( $3.3 \text{ dS m}^{-1}$ ), at 150 days after transplanting.

The  $600 \text{ mg L}^{-1}$  dosage of ascorbic acid (AsA) proved to be the most effective. The results indicate that foliar AsA application is a promising tool for managing salt stress, as it contributes to improving the physiology and growth of guava trees.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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