ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.28, n.2, e276236, 2024
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v28n2e276236

ORIGINAL ARTICLE

Formation of guava seedlings under salt stress and foliar application of hydrogen peroxide[1]

Formação de mudas de goiabeira sob estresse salino e aplicação foliar de peróxido de hidrogênio

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HIGHLIGHTS:

Application of hydrogen peroxide at up to concentration of 75 μM reduces the relative water content of leaf.
Gas exchange and synthesis of photosynthetic pigments are negatively affected by water salinity from 0.3 to 4.3 dS m^{-1}.
Hydrogen peroxide at concentrations of up to 75 μM does not attenuate the effects of salt stress on guava seedlings.

ABSTRACT: The occurrence of water with high concentrations of salts hinders the expansion of irrigated agriculture in the semi-arid region of the Brazilian Northeast, making it necessary to adopt strategies capable of minimizing the effects of salt stress on plants. In this context, the objective of present study was to evaluate the effects of hydrogen peroxide application on water relations, gas exchange, photosynthetic pigments, and growth of guava cv. Paluma under irrigation with saline waters in the seedling formation stage. A randomized block design was used, in a 5 × 4 factorial scheme, with three replicates, with five levels of electrical conductivity of water - ECw (0.3, 1.3, 2.3, 3.3, and 4.3 dS m^{-1}) and four concentrations of hydrogen peroxide – H_2O_2 (0, 25, 50, and 75 μM). Irrigation water salinity above 0.3 dS m^{-1} increased electrolyte leakage in the leaf blade and reduced the relative water content, synthesis of photosynthetic pigments, gas exchange, and growth of seedlings of guava cv. Paluma, at 80 days after sowing. Foliar application of hydrogen peroxide at concentrations of up to 75 μM reduces the relative water content in the leaf blade of guava seedlings and did not mitigate the effects of salt stress on guava plants in the seedling formation stage.

Key words: *Psidium guajava* L., salinity, acclimatization

RESUMO: A ocorrência de água com elevadas concentrações de sais dificulta a expansão da agricultura irrigada no semiárido do Nordeste brasileiro, tornando-se necessária a adoção de estratégias capazes de minimizar os efeitos do estresse salino sobre as plantas. Neste contexto, objetivou-se avaliar os efeitos da aplicação de peróxido de hidrogênio nas relações hídricas, nas trocas gasosas, nos pigmentos fotossintéticos e no crescimento de goiabeira cv. Paluma sob irrigação com águas salinas na formação de mudas. Foi utilizado o delineamento em blocos casualizados, em esquema fatorial 5 × 4, com três repetições, sendo cinco níveis de condutividade elétrica da água - CEa (0,3; 1,3; 2,3; 3,3 e 4,3 dS m^{-1}) e quatro concentrações de peróxido de hidrogênio – H_2O_2 (0; 25; 50 e 75 μM). A salinidade da água de irrigação acima de 0,3 dS m^{-1} aumentou o extravasamento de eletrólitos no limbo foliar, reduziu o conteúdo relativo de água, a síntese de pigmentos fotossintéticos, as trocas gasosas e o crescimento das mudas de goiabeira cv. Paluma, aos 80 dias após a semeadura. A aplicação foliar de peróxido de hidrogênio em concentrações de até 75 μM, reduz o conteúdo relativo de água no limbo foliar de mudas de goiabeira e não ameniza os efeitos do estresse salino em plantas de goiabeira na fase de formação de mudas.

Palavras-chave: *Psidium guajava* L., salinidade, aclimatação

• Ref. 276236 – Received 03 Jul, 2023
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• Accepted 02 Nov, 2023 • Published 24 Nov, 2023
Editors: Ítalo Herbet Lucena Cavalcante & Carlos Alberto Vieira de Azevedo

Introduction

Guava (*Psidium guajava* L.) is a tropical fruit belonging to the Myrtaceae family. In Brazil, the increase in the production of this fruit is associated with its fresh consumption as well as its use for various purposes through its industrialization, being commercially exploited as guava paste, jellies, fruits in syrup, purees, base for drinks, soft drinks, juices, and syrups (Onias et al., 2018).

However, the Brazilian Northeast, especially the semi-arid region, is characterized by high evapotranspiration rates and low precipitation, and the water sources in this region commonly have high levels of dissolved salts (Silva et al., 2021a), standing out as an abiotic stress factor for agricultural production (Lima et al., 2018; Silva et al., 2019). High levels of salts in water and/or soil can cause osmotic and ionic effects, causing damage to the cell membrane, stomatal closure, and decreases in photosynthetic efficiency, biosynthesis of photosynthetic pigments, and plant growth (Xavier et al., 2022; Pinheiro et al., 2022).

Among the strategies that have been employed to reduce salt stress in plants, foliar application of hydrogen peroxide (H_2O_2) stands out. It can act as a regulatory molecule in the defense system of plants against salt stress, as its characteristics allow it to cross membranes and spread among cellular compartments, which facilitates its signaling function (Silva et al., 2021a; Silva et al., 2024). At low concentrations, H_2O_2 causes a moderate stress condition, which results in the accumulation of signals in different parts of the plant (Silva et al., 2021a). Beneficial effects of H_2O_2 application have been observed in sour passion fruit (Andrade et al., 2019; Ramos et al., 2022), and soursop (Silva et al., 2021b; Capitulino et al., 2023). Thus, the objective of this study was to evaluate the effects of H_2O_2 on water relations, photosynthetic pigments, gas exchange, and growth of guava cv. Paluma under irrigation with saline waters in the seedling formation phase.

Material and Methods

The experiment was conducted from January to April 2022, under protected environment conditions (greenhouse) arch type, with low-density polyethylene cover of 150 microns, belonging to the Centro de Ciências e Tecnologia Agroalimentar (CCTA) of the Universidade Federal de Campina Grande (UFCG), in Pombal city, PB, Brazil, located by the geographical coordinates 6° 46' 8" S latitude and 37° 47' 45" W longitude and an altitude of 184 m.

The climate of the region, according to Köppen's climate classification adapted to Brazil, is characterized as "BSh", representing a hot and semi-arid climate (Alvares et al., 2014). The average annual temperature is 28 °C, with approximately 750 mm of rainfall per year and an average annual evaporation of 2000 mm. The data of average temperature and relative humidity of the air inside the greenhouse during the experimental period were collected daily with a thermohygrometer, as presented in Figure 1.

The experimental design was randomized blocks, in a 5 × 4 factorial arrangement, whose treatments consisted of five levels of electrical conductivity of irrigation water – ECw (0.3, 1.3, 2.3, 3.3, and 4.3 dS m^{-1}) and four concentrations of hydrogen peroxide – H_2O_2 (0, 25, 50, and 75 μM), with three replicates and two plants per plot. Electrical conductivity of irrigation water levels were defined based on a study conducted by Xavier et al. (2022), while H_2O_2 concentrations were based on an assay conducted by Veloso et al. (2020).

Seeds of the guava cultivar Paluma were used in the present study. Seedlings were formed in polyethylene bags with dimensions of 15 cm × 30 cm, filled with the mixture in a ratio of 2:1:1 (volume basis) of a sandy loam Entisol, sand, and organic matter (aged cattle manure). The soil came from the rural area of the municipality of São Domingos, PB (6° 48' 50" S latitude and 37° 56' 31" W longitude, at an altitude of 190 m), collected at 0-20 cm depth. Physical and chemical characteristics of the soil obtained according to the methodologies of Teixeira et al. (2017) are shown in Table 1.

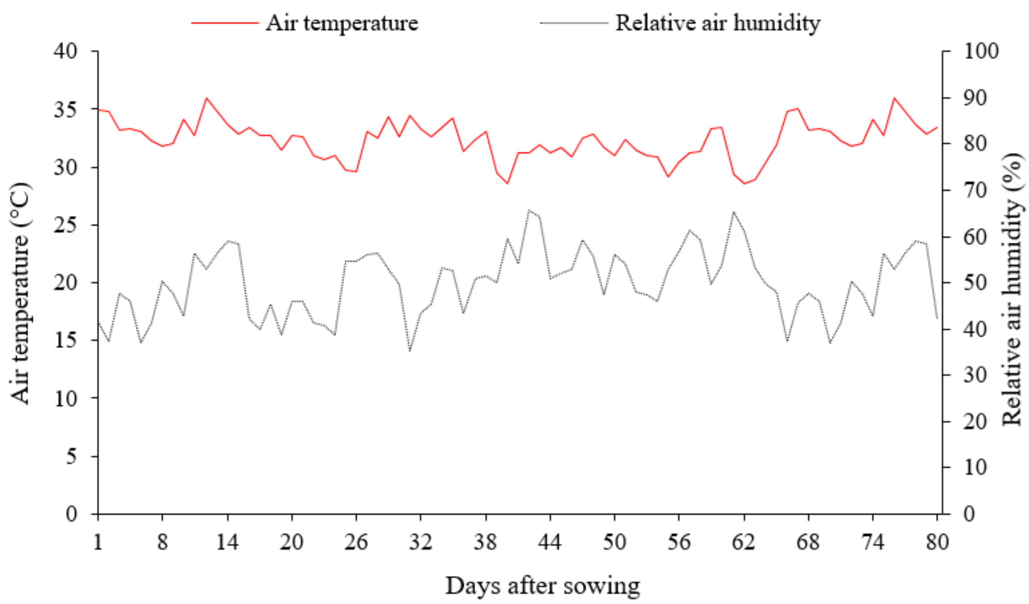


Figure 1. Mean air temperature and relative air humidity recorded during the experimental period inside the greenhouse

Table 1. Chemical and physical characteristics of the soil (0-20 cm depth) used in the experiment, before application of the treatments

Chemical characteristics								
pH (H_2O) (1:2.5)	OM (g kg^{-1})	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
			($cmol_c$ kg^{-1})					
8.53	3.10	77.30	0.56	0.20	5.08	5.11	0	0

Chemical characteristics				Physical characteristics				
EC_{se} (dS m^{-1})	CEC ($cmol_c$ kg^{-1})	SAR_{se} (mmol L^{-1})$^{0.5}$	ESP (%)	Particle-size fraction (g kg^{-1})			Moisture (dag kg^{-1})	
				Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
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^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; Al^{3+}+H^+ extracted with 0.5 M CaOAc at 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; [1,2] referring to the moisture content in the soil corresponding to field capacity and permanent wilting point

Fertilization with nitrogen, phosphorus, and potassium was carried out via fertigation, as recommended by Cavalcanti (2008) considering the nutritional requirements of the crop and the contents of the elements in the soil. Urea (45% N), monoammonium phosphate (50% P_2O_5 and 11% N), and potassium sulfate (50% K_2O) were used as sources of nitrogen, phosphorus, and potassium, respectively, and applied fortnightly. Fertilization with micronutrients was carried out weekly, starting 10 days after sowing (DAS), applying 1.0 g L^{-1} solution 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) through the leaves.

The solutions with adequate concentrations of H_2O_2 were prepared by dissolving H_2O_2 in distilled water. The applications were performed every 15 days, starting at 27 DAS. Applications with an average volume of 50 mL of solution per plant, were carried out by spraying so as to fully wet the leaves (abaxial and adaxial sides), using a 2 L spray bottle (high-pressure manual sprayer), from 5 p.m. During application, the plants were isolated to avoid drift among the different treatments.

Irrigation with saline water started at 30 DAS. The different levels of electrical conductivity of the water were obtained by the addition of adequate amounts of iodine-free NaCl to water from the municipal supply system of Pombal, PB (ECw = 0.3 dS m^{-1}), and the quantity was determined considering the relationship between ECw and the concentration of salts (Richards, 1954), according to Eq. 1:

$$C \approx 10 \times \text{ECw} \qquad (1)$$

where:

C - concentration of NaCl ($mmol_c$ L^{-1}); and,
ECw - electrical conductivity of water (dS m^{-1}).

Before sowing, the volume of water needed to raise the soil moisture content to the level corresponding to field capacity was determined, and water was applied according to the established treatments. Ten days after emergence, irrigation was performed daily at 5 p.m., applying to each bag the volume corresponding to that obtained by the water balance, using Eq. 2:

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

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 after previous irrigation event (mL); and,
LF - leaching fraction of 0.10, applied at an interval of 15 days.

Treatment effects on the crop were measured at 80 DAS, by the electrolyte leakage in the leaf blade (%EL), relative water content (RWC), photosynthetic pigments, gas exchange, and growth. Electrolyte leakage in the leaf blade was quantified according to Scotti-Campos et al. (2013); 5 leaf discs with area of 113 mm^2 were collected, washed with distilled water to remove other electrolytes adhered to the leaves, and placed in a beaker containing 50 mL of bidistilled water and hermetically sealed with aluminum foil.

The beakers were kept at a temperature of 25 °C for 120 min, and the initial electrical conductivity (Ci) was determined; subsequently, the beakers were placed in the oven with forced air ventilation and subjected to a temperature of 90 °C for 150 min, and then the final electrical conductivity (Cf) was measured. Electrolyte leakage was determined according to Eq. 3:

$$\%\text{EL} = \frac{\text{Ci}}{\text{Cf}} \times 100 \qquad (3)$$

where:

%EL - electrolyte leakage (%);
Ci - initial electrical conductivity (dS m^{-1}); and,
Cf - final electrical conductivity (dS m^{-1}).

RWC was determined using 8 discs collected from fully expanded leaves of each plant, weighed on a scale with a precision of 0.001 g; to determine leaf turgid mass (TM), the collected leaves were immersed in distilled water for 24 hours, wiped and then weighed, while dry mass was obtained by drying the discs in an oven at 65 °C. RWC was obtained according to Weatherley (1950), using Eq. 4:

$$\text{RWC} = \frac{(\text{FM} - \text{DM})}{(\text{TM} - \text{DM})} \times 100 \qquad (4)$$

where:

RWC - relative water content (%);
FM - leaf fresh mass (g);
TM - leaf turgid mass (g); and,

DM - leaf dry mass (g).

Readings of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) contents were performed by spectrophotometry at wavelengths of 470, 645, and 663 nm, and the amounts were calculated through Eqs. 5, 6, 7 and 8, according to the methodology proposed by Arnon (1949), in which A is the absorbance.

$$\text{Chl a} = 12.21A_{663} - 2.81A_{646} \tag{5}$$

$$\text{Chl b} = 20.13A_{646} - 5.03A_{663} \tag{6}$$

$$\text{Chl T} = 17.3A_{646} + 7.18A_{663} \tag{7}$$

$$\text{Car} = \frac{\left(1000A_{470} - 1.82\text{Chl a} - 85.02\text{Chl b}\right)}{198} \tag{8}$$

Gas exchange was evaluated based on stomatal conductance - gs (mol H_2O m^{-2} s^{-1}), transpiration - E (mmol H_2O m^{-2} s^{-1}), CO_2 assimilation rate - A (μmol CO_2 m^{-2} s^{-1}), and internal CO_2 concentration - Ci (μmol CO_2 m^{-2} s^{-1}). These data were then used to estimate the instantaneous water use efficiency - WUEi (A/E) [(μmol CO_2 m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$] and the instantaneous carboxylation efficiency - CEi (A/Ci) [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-2} s^{-1})$^{-1}$] under photosynthetic photon flux density of 1,200 μmol m^{-2} s^{-1} and airflow of 200 mL min^{-1}. Readings were performed between 7 and 10 a.m., using an infrared gas analyzer - IRGA (Infrared Gas Analyser, LCpro - SD model, from ADC Bioscientific, UK).

The absolute and relative growth rates in plant height (AGR_{PH}, RGR_{PH}), stem diameter (AGR_{SD}, RGR_{SD}), and leaf area (AGR_{LA}, RGR_{LA}) in the period 49 to 80 DAS were obtained according to Benincasa (2003), using Eqs. 9 and 10:

$$\text{AGR} = \frac{A_2 - A_1}{t_2 - t_1} \tag{9}$$

where:

AGR - absolute growth rate;
A_2 - plant growth at time t_2;
A_1 - plant growth at time t_1; and,
$t_2 - t_1$ - time difference between evaluations

$$\text{RGR} = \frac{\left(\ln A_2 - \ln A_1\right)}{\left(t_2 - t_1\right)} \tag{10}$$

where:

RGR - relative growth rate;
A_2 - plant growth at time t_2;
A_1 - plant growth at time t_1;
$t_2 - t_1$ - time difference between evaluations; and,
ln - natural logarithm.

The data were subjected to the normality of distribution test (Shapiro-Wilk) at 0.05 probability level and later analysis of variance was performed at 0.05 or 0.01 probability level. In cases of significant effect, linear and quadratic regression analyses were performed using the statistical program SISVAR-ESAL version 5.6. Due to the heterogeneity observed in A, WUEi, and CEi, the data were transformed to $\sqrt{x}$.

Results and Discussion

There were significant differences between water salinity levels (ECw) at $p \leq 0.05$ for relative water content (RWC) and at $p \leq 0.01$ for electrolyte leakage in the leaf blade (%EL), chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl T) contents (Table 2). Peroxide concentrations significantly ($p \leq 0.05$) affected RWC. There was no significant effect of the interaction between the factors (ECw × H_2O_2) on any of the variables analyzed at 80 days after sowing. The significant effects observed for the block variation source on RWC, Chl a, Chl b, Car, and Chl T may be related to the distribution of luminosity inside the greenhouse.

The salinity of irrigation water increased the electrolyte leakage in the leaf blade of guava seedlings (Figure 2A), by 16.23% per unit increase in ECw. When comparing plants irrigated with ECw of 4.3 dS m^{-1} to those grown under the lowest water salinity level (0.3 dS m^{-1}), an increase of 61.93% was observed in %EL. Despite the increase in electrolyte leakage in the leaf blade of seedlings, the maximum value obtained in this study (14.79%) in plants cultivated under ECw of 4.3 dS m^{-1} is much lower than 50%, indicating that there was no occurrence of injury to leaf tissues (Sullivan, 1971). In addition, the increase in %EL in the leaf blade stands out as a mechanism to prevent tissue dehydration, due to the reduction of the osmotic component caused by the increase in salinity (Fioreze et al., 2013).

Irrigation water salinity reduced the relative water content of guava seedlings cv. Paluma (Figure 2B) by 1.49% per unit increase in ECw. When comparing plants irrigated with water of 4.3 dS m^{-1} to those subjected to ECw of 0.3 dS m^{-1}, a decrease of 6.00% was observed in RWC. Reduction of RWC in the leaf tissues is associated with the osmotic effect resulting from the salt stress caused by irrigation water, which leads to disturbances in plant water balance, due to the difficulty in water absorption (Lima et al., 2020).

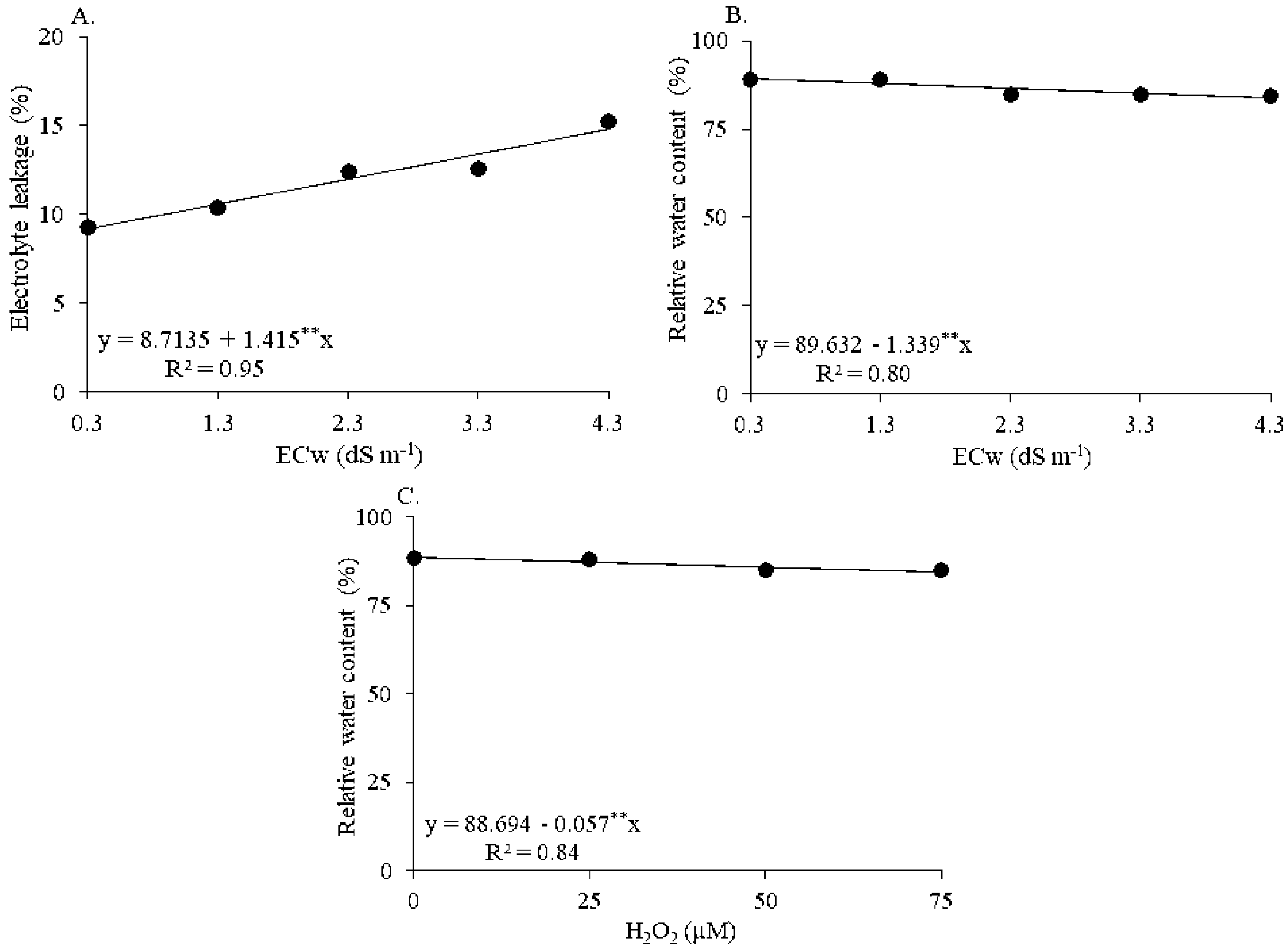
Foliar application of H_2O_2 linearly reduced the RWC of guava seedlings cv. Paluma (Figure 2C), by 1.61% per 25 μM increase in H_2O_2 concentration. When comparing the RWC of plants that received 75 μM of H_2O_2 to that of plants grown under 0 μM, a decrease of 4.81% was observed. Exogenous application of low concentrations of H_2O_2 favors acclimatization for the plant to minimize the problems caused by salt stress (Andrade et al., 2019). However, high concentrations of H_2O_2 can induce oxidative stress, causing lipid peroxidation, damage to cell membranes, protein degradation, DNA double-strand breakage, and cell death (Rutschow et al., 2011). Unlike the results obtained in the present study, Silva et al. (2024), observed that foliar application of H_2O_2 at concentrations of up to 10 μM increased the relative water content in the leaf blade of soursop plants.

The photosynthetic pigments were reduced with an increase in irrigation water salinity (Figure 3). For the contents of

Table 2. Summary of the analysis of variance for the electrolyte leakage in the leaf blade (%EL), relative water content (RWC), chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Car), and total chlorophyll (Chl T) contents of guava seedlings grown under different electrical conductivities of the irrigation water (ECw) and exogenous application of hydrogen peroxide (H_2O_2), at 80 days after sowing (DAS)

Source of variation	DF	Mean squares					
		%EL	RWC	Chl a	Chl b	Car	Chl T
Electrical conductivity of water (ECw)	4	63.17^{**}	66.89^{*}	102.87^{**}	11.42^{**}	175.50^{ns}	179.50^{**}
Linear regression	1	240.38^{**}	215.52^{**}	358.94^{**}	44.45^{**}	51.94^{ns}	655.62^{**}
Quadratic regression	1	0.91^{ns}	9.75^{ns}	8.17^{**}	0.21^{ns}	250.85^{ns}	5.76^{**}
Hydrogen peroxide (H_2O_2)	3	1.08^{ns}	60.21^{*}	3.42^{ns}	0.96^{ns}	134.74^{ns}	7.54^{ns}
Linear regression	1	1.00^{ns}	152.72^{**}	0.04^{ns}	0.26^{ns}	263.61^{ns}	0.53^{ns}
Quadratic regression	1	1.83	0.22^{ns}	9.07^{ns}	2.57^{ns}	122.98^{ns}	21.34^{ns}
Interaction (ECw × H_2O_2)	12	3.37^{ns}	21.83^{ns}	9.25^{ns}	3.35^{ns}	129.85^{ns}	23.19^{ns}
Blocks	2	3.71^{ns}	308.31^{**}	40.11^{*}	28.84^{**}	168.65^{**}	137.01^{**}
Residual	38	2.69	20.32	7.72	2.52	142.21	18.30
CV (%)		13.70	5.21	15.19	27.40	18.23	17.76

*, **, ns - Significant at $p \leq 0.05$, $p \leq 0.01$, and not significant by F test, respectively

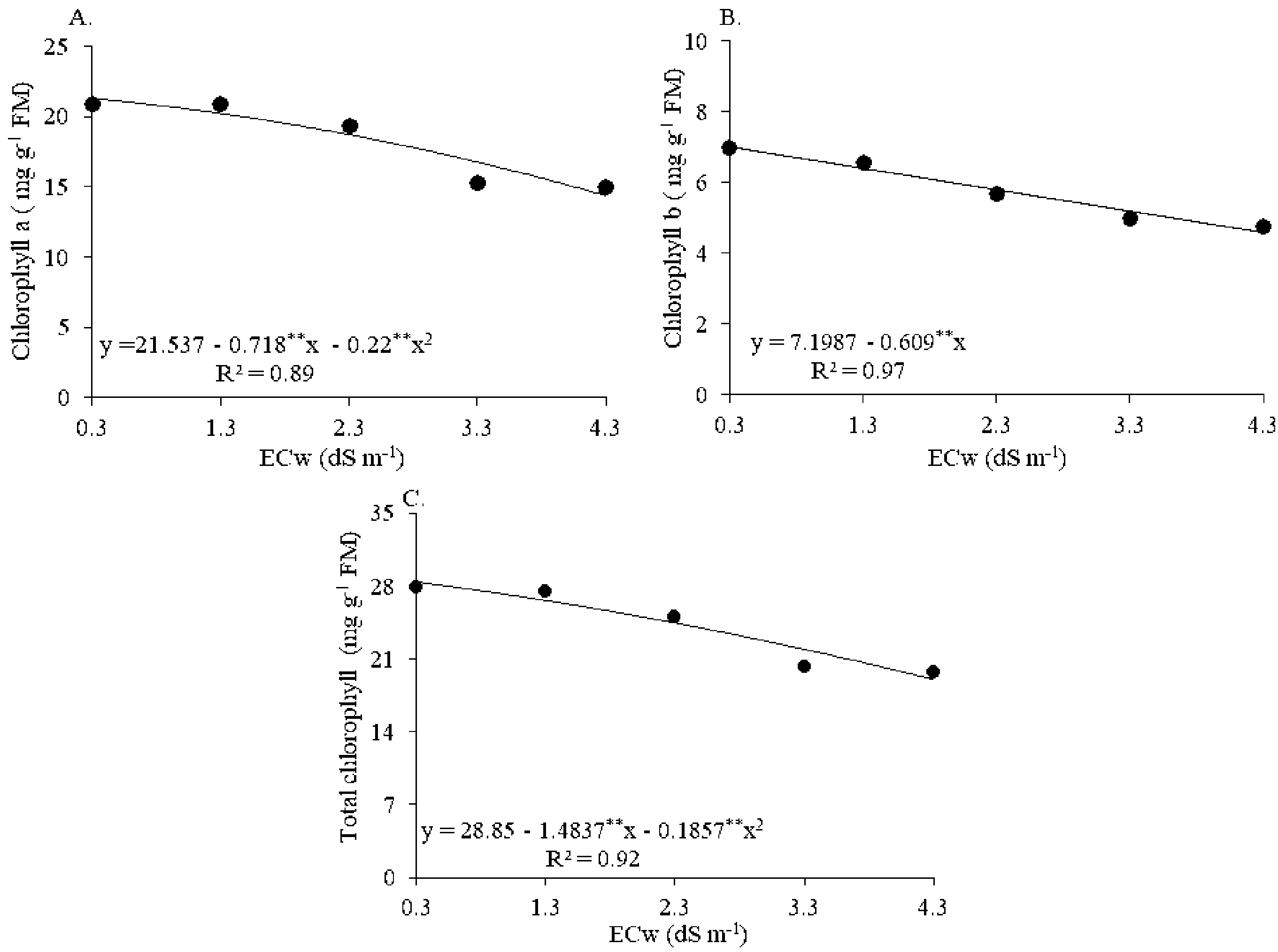


** - Significant at $p \leq 0.01$ by the F test

Figure 2. Electrolyte leakage (A) and relative water content – RWC (B) in the leaf blade of guava seedlings cv. Paluma, as a function of the salinity of irrigation water – ECw, and relative water content as a function of concentrations of hydrogen peroxide – H_2O_2 (C), at 80 days after sowing (DAS)

chlorophyll a (Figure 3A) and total chlorophyll (Figure 3C), the maximum values of 21.30 and 28.38 mg g^{-1} FM, respectively, were obtained in plants irrigated with 0.3 dS m^{-1} water, followed by a reduction from this ECw level on, with the minimum estimated values (14.38 and 19.03 mg g^{-1} FM) obtained under water salinity of 4.3 dS m^{-1}. As for chlorophyll b (Figure 3B), a linear decrease of 8.45% per unit increase in ECw, and when comparing the Chl b of plants irrigated with the highest salinity level (4.3 dS m^{-1}) to the value of those subjected to the lowest level (0.3 dS m^{-1}), a decrease of 34.72% (2.43 mg g^{-1} FM) was observed in the Chl b of guava seedlings cv. Paluma.

The reduction in the synthesis of photosynthetic pigments of guava seedlings cv. Paluma in this study results from the stress caused by the increase in water salinity, which may have stimulated the activity of the enzyme chlorophyllase, which acts in the degradation of photosynthesizing pigment molecules,



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

Figure 3. Chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C) contents of guava seedlings cv. Paluma, as a function of the salinity of irrigation water – ECw, 80 days after sowing (DAS)

or from the photooxidation caused by oxidative stress (Dias et al., 2019). In addition, excess salts can also induce damage to chloroplasts and therefore provoke imbalance and loss of activity of pigmentation proteins.

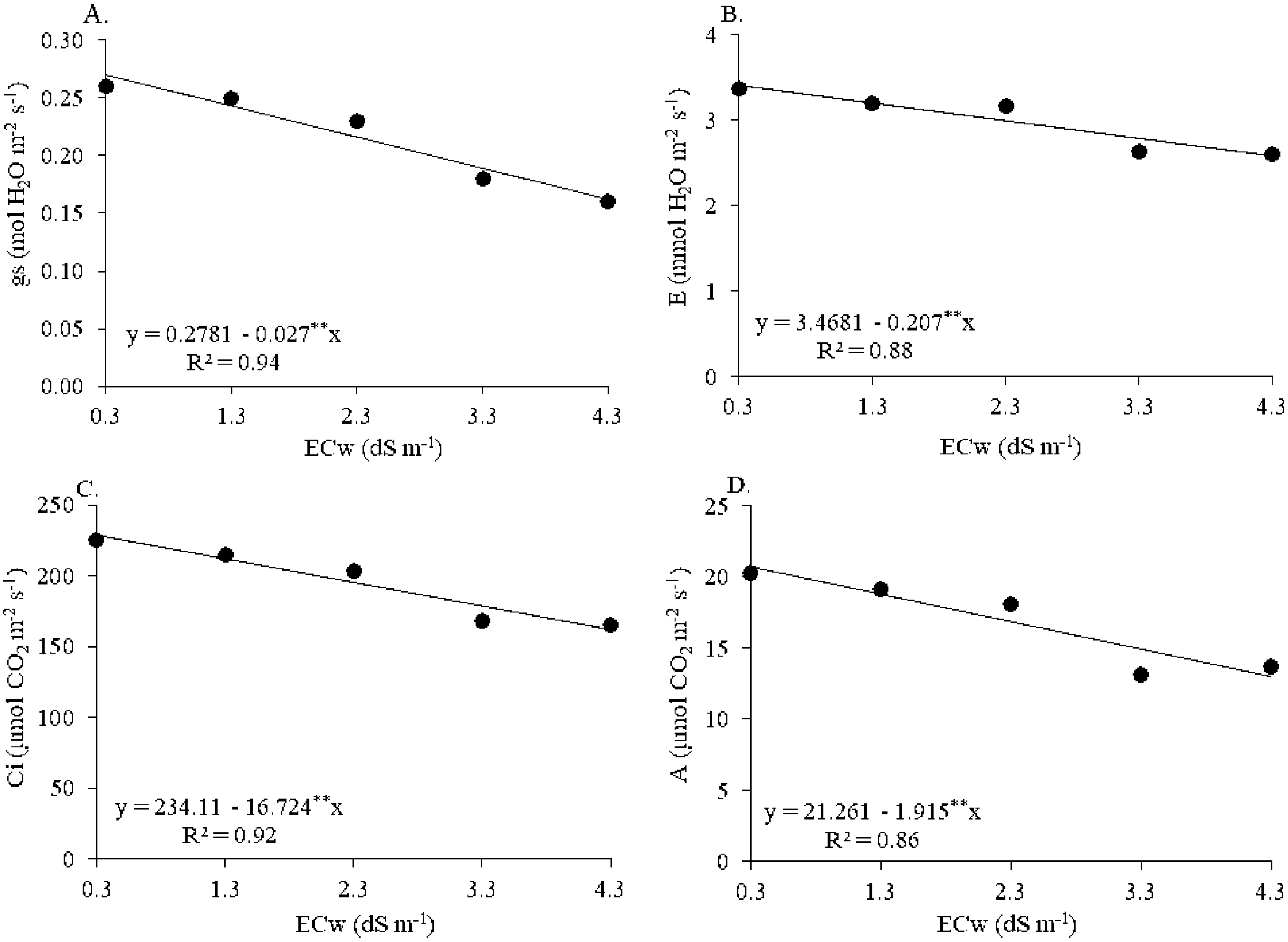
According to the summary of the analysis of variance, there was a significant effect ($p \leq 0.01$) of the ECw levels of irrigation water on stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci), and CO_2 assimilation rate (A) (Table 3). For the H_2O_2 factor, as well as for the interaction between the studied factors (ECw × H_2O_2), no significant effect was found on the gas exchange of guava seedlings at 80 DAS.

The stomatal conductance (gs) of guava seedlings was reduced linearly with the increase in electrical conductivity of water (Figure 4A), with a decrease of 9.70% per unit increase in ECw. When comparing the gs of plants subjected to ECw of 4.3 dS m^{-1} to the value of plants that received 0.3 dS m^{-1}, a decrease of 40.0% (0.108 mol H_2O m^{-2} s^{-1}) was observed. The decrease in water absorption caused by the increase in salinity

Table 3. Summary of the analysis of variance for stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci), CO_2 assimilation rate (A), instantaneous water use efficiency (WUEi), and instantaneous carboxylation efficiency (CEi) of guava seedlings grown under different electrical conductivities of irrigation water (ECw) and exogenous application of hydrogen peroxide (H_2O_2), 80 days after sowing (DAS)

Source of variation	DF	Mean squares					
		gs[1]	E	Ci	A[1]	WUEi[1]	CEi[1]
Electrical conductivity of water (ECw)	4	0.021^{**}	1.46^{**}	9054.97^{**}	127.11^{**}	2.45^{ns}	0.0004^{ns}
Linear regression	1	0.080^{**}	5.20^{**}	33567.07^{**}	439.87^{**}	6.11^{ns}	0.00102^{ns}
Quadratic regression	1	0.001^{ns}	0.04^{ns}	70.72^{ns}	0.13^{ns}	0.02^{ns}	0.00001^{ns}
Hydrogen peroxide (H_2O_2)	3	0.001^{ns}	0.10^{ns}	254.77^{ns}	7.69^{ns}	0.07^{ns}	0.0004^{ns}
Linear regression	1	0.002^{ns}	0.19^{ns}	530.67^{ns}	11.06^{ns}	0.14^{ns}	0.0008^{ns}
Quadratic regression	1	0.001^{ns}	0.05^{ns}	36.81^{ns}	8.07^{ns}	0.04^{ns}	0.0001^{ns}
Interaction (ECw × H_2O_2)	12	0.003^{ns}	0.23^{ns}	612.50^{ns}	18.63^{ns}	4.90^{ns}	0.0005^{ns}
Blocks	2	0.008^{ns}	0.29^{ns}	8490.9^{**}	1.22^{ns}	0.98^{ns}	0.0022^{ns}
Residual	38	0.004	0.19	1068.45	24.79	3.89	0.0009
CV (%)		14.32	14.57	16.71	16.10	17.89	17.79

*, **, ns - Significant at $p \leq 0.05$, $p \leq 0.01$, and not significant by F test, respectively; [1]Statistical analysis performed after data transformation to $\sqrt{x}$



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

Figure 4. Stomatal conductance – gs (A), transpiration – E (B), internal CO_2 concentration – Ci (C), and CO_2 assimilation rate – A (D) of guava seedlings cv. Paluma, as a function of the salinity of irrigation water - ECw, at 80 days after sowing (DAS)

was reflected in the relative water content and increased the water saturation deficit in the leaf blade. In addition, it reduced gs to minimize water loss to the atmosphere. Another factor that may have contributed to the partial closure of stomata and consequently to a decrease in transpiration, internal CO_2 concentration, and CO_2 assimilation rate was the occurrence of high temperatures (on average 32.57 °C) and low relative humidity of air (on average 49.67%), according to data presented in Figure 1.

The stomatal regulation observed in this study through the partial closure of the stomata caused by the salt stress led to a reduction in the flow of water vapor to maintain the leaf water potential and avoid dehydration of the guard cells, which results in the restriction of the normal flow of CO_2 in leaf mesophyll cells, impairing plant transpiration (Lima et al., 2023). In addition, the regulation of stomata also contributes to reducing the absorption of toxic ions such as Na^+ and Cl^- (Dias et al., 2019).

The leaf transpiration (E) of guava seedlings decreased linearly with the increase in the salinity of irrigation water (Figure 4B), by 5.96% per unit increase in ECw. When comparing in relative terms, there was a reduction of 24.31% (0.828 mmol H_2O m^{-2} s^{-1}) between plants grown under irrigation water of 4.3 dS m^{-1} and those that received the lowest salinity level (0.3 dS m^{-1}). The reduction in gs also affects leaf transpiration, resulting in less water loss by the plant, which has difficulty absorbing it due to the reduction in soil water potential caused by excess salts (Pinheiro et al., 2022). Xavier et al. (2022), in a study with guava cv. Paluma under salt stress (ECw ranging from 0.6 to 4.2 dS m^{-1}) in the seedling formation stage, also found that leaf gas exchange was inhibited by salt stress, which led to reductions in gs and Ci at 180 days after sowing.

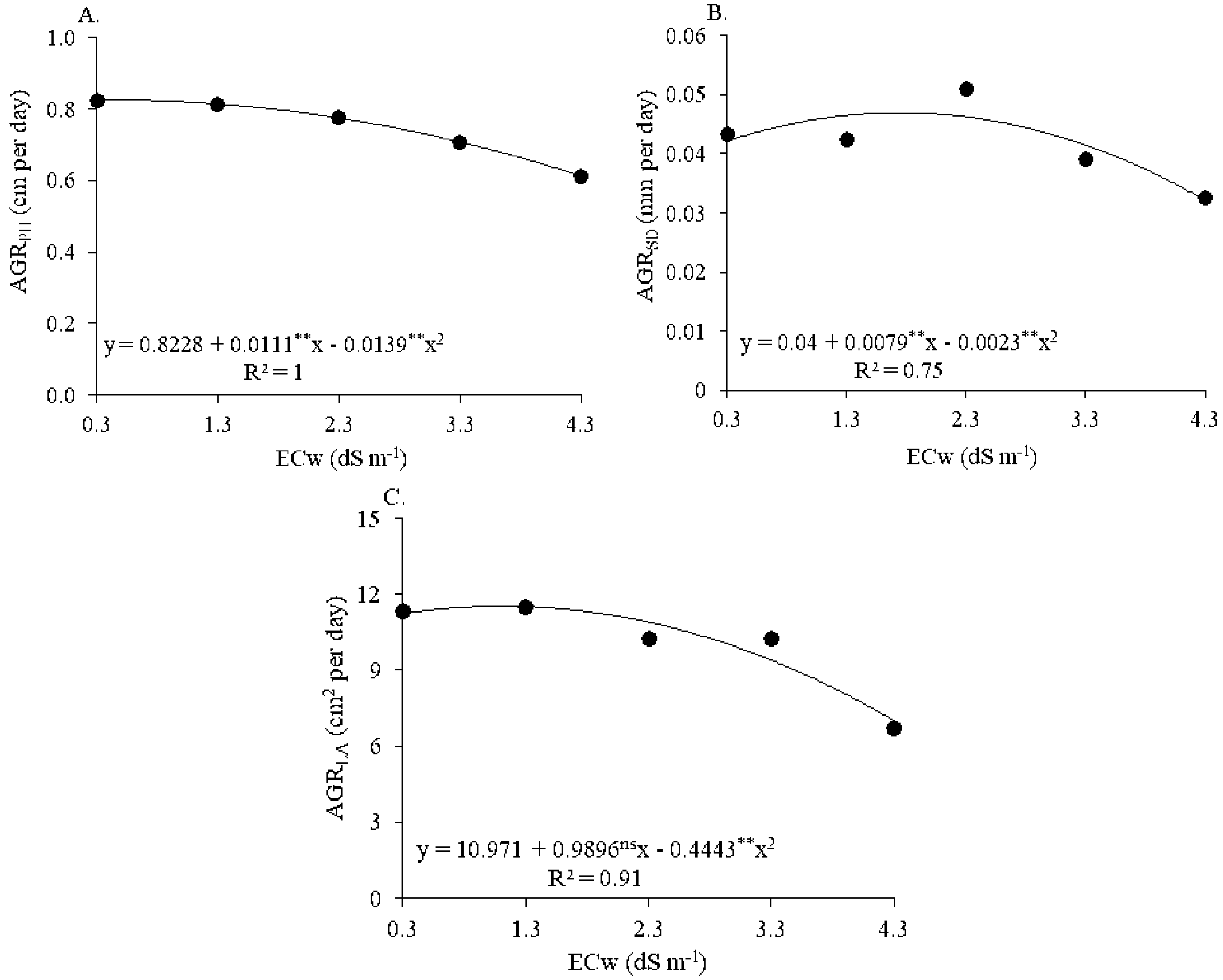
Water salinity also reduced the internal CO_2 concentration (Ci) of guava seedlings (Figure 4C), a decrease of 7.14% per unit increase in ECw. When comparing the Ci of plants irrigated with water of 4.3 dS m^{-1} to the value of those grown under ECw of 0.3 dS m^{-1}, a decrease of 29.20% (66.896 μmol CO_2 m^{-2} s^{-1}) was observed. The reduction in internal CO_2 concentration can be attributed to the partial closure of the stomata, which restricts CO_2 diffusion in the substomatal chamber, and to the inhibition of the activity of ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), which predisposes the photosynthetic apparatus to increased energy dissipation and negative regulation of photosynthesis (Oliveira et al., 2022).

Regarding the CO_2 assimilation rate (A) of guava seedlings (Figure 4D), there was a linear decrease with the increase in ECw levels, equal to a 9.0% per unit increase in ECw. When comparing the CO_2 assimilation rate of plants irrigated with ECw of 4.3 dS m^{-1} to the values of plants subjected to water salinity of 0.3 dS m^{-1}, a reduction of 7.66 μmol CO_2 m^{-2} s^{-1} (37.02%) was observed. The decrease in A due to salt stress is usually related to the partial closure of the stomata, restricting the entry of CO_2 into the substomatal chamber (Lima et al., 2019), probably due to phytotoxic damage resulting from the accumulation of salts in the plant, leading to reduced RuBisCO activity and, consequently, carbon consumption in the Calvin cycle (Pan et al., 2021). Lacerda et al. (2022), when evaluating the morphophysiology of guava plants cv. Paluma subjected to irrigation with saline water (ECw of 0.8 and 3.2 dS m^{-1}), concluded that water salinity of 3.2 dS m^{-1} reduced their CO_2 assimilation rate in the post-grafting stage.

Table 4. Summary of the analysis of variance for absolute and relative growth rates in plant height (AGR_{PH}, RGR_{PH}), stem diameter (AGR_{SD}, RGR_{SD}), and leaf area (AGR_{LA}, RGR_{LA}) of guava seedlings grown under different electrical conductivities of irrigation water (ECw), and exogenous application of hydrogen peroxide (H_2O_2), during the period 49 to 80 days after sowing (DAS)

Source of variation	DF	Mean squares					
		AGR_{PH}	AGR_{SD}	AGR_{LA}	RGR_{PH}	RGR_{SD}	RGR_{LA}
Electrical conductivity of water (ECw)	4	0.1059^{**}	0.0005^{*}	45.4234^{*}	0.00001^{ns}	0.00006^{ns}	0.000118^{ns}
Linear regression	1	0.3370^{**}	0.0007^{**}	133.41^{ns}	0.000001^{ns}	0.000008^{ns}	0.000001^{ns}
Quadratic regression	1	0.3325^{**}	0.0008^{**}	33.17^{**}	0.000029^{ns}	0.000048^{ns}	0.000015^{ns}
Hydrogen peroxide (H_2O_2)	3	0.0245^{ns}	0.0002^{ns}	0.6731^{ns}	0.00001^{ns}	0.00001^{ns}	0.000006^{ns}
Linear regression	1	0.0458^{ns}	0.000033^{ns}	1.6383^{ns}	0.000048^{ns}	0.000001^{ns}	0.000083^{ns}
Quadratic regression	1	0.0040^{ns}	0.000007^{ns}	0.1118^{ns}	0.000001^{ns}	0.000002^{ns}	0.000002^{ns}
Interaction (ECw × H_2O_2)	12	0.0295^{ns}	0.0003^{ns}	9.3379^{ns}	0.00001^{ns}	0.00003^{ns}	0.000062^{ns}
Blocks	2	0.0891^{*}	0.0002^{ns}	170.1010^{**}	0.00001^{ns}	0.00001^{ns}	0.000327^{ns}
Residual	38	0.0240	0.0001	14.9472	0.00001	0.00002^{ns}	0.000069
CV (%)		20.76	30.57	38.63	20.87	41.35	32.95

*, **, ns - Significant at p ≤ 0.05, p ≤ 0.01, and not significant by F test, respectively



**, ns - Significant at p ≤ 0.01, and not significant by F test, respectively

Figure 5. Absolute growth rates in plant height – AGR_{PH} (A), stem diameter – AGR_{SD} (B), and leaf area – AGR_{LA} (C) of guava seedlings as a function of salinity of irrigation water – ECw, during the period 49 to 80 days after sowing (DAS)

The absolute growth rates in plant height (AGR_{PH}), stem diameter (AGR_{SD}), and leaf area (AGR_{LA}) were significantly affected by ECw levels (Table 4), while the effect on the relative growth rates (RGR_{PH}, RGR_{SD}, and RGR_{LA}) of these variables was not significant ($p > 0.05$). Peroxide concentrations and the interaction between factors (ECw × H_2O_2) did not significantly affect any of the variables measured.

The salinity of irrigation water inhibited the absolute growth rates of guava seedlings cv. Paluma (Figure 5), and the regression equations showed a quadratic fit. For AGR_{PH} (Figure 5A), the maximum estimated value of 0.8250 cm per day was obtained in plants irrigated with 0.4 dS m^{-1}, and from this water salinity level on, there was a reduction in their AGR_{PH}. As for AGR_{SD} (Figure 5B) and AGR_{LA} (Figure 5C), the maximum estimated values of 0.04678 mm per day and 11.521 cm^2 per day were reached in plants irrigated with 1.8 and 1.1 dS m^{-1}, respectively, decreasing from these levels of irrigation water salinity. The reduction in the growth of guava seedlings under salt stress results from the reduction in water potential caused by excess salts in the soil. This situation imposes on plants a higher energy expenditure to maintain metabolic activities (Lima et al., 2020), causing decline in meristematic activity and cellular elongation, as well as functional and metabolism disorders.

In general, the results obtained in this study show that foliar application of H_2O_2 at concentrations ranging from 0 to 75 μM did not influence, either individually or through the interaction with electrical conductivity of irrigation water, the water relations, gas exchange, and growth rates of guava in the seedling formation stage. It is worth pointing out that the effect of H_2O_2 on plants depends on several factors, including the frequency of application and the concentration used; that is, at higher concentrations, H_2O_2 can spread rapidly across the subcellular membrane, resulting in oxidative damage to the plasma membrane (Capitulino et al., 2023).

Conclusions

1. Irrigation water salinity from 0.3 to 4.5 dS m^{-1} increases electrolyte leakage in the leaf blade and reduces the relative water content, synthesis of photosynthetic pigments, gas exchange, and growth rates of guava seedlings cv. Paluma, 80 days after sowing.

2. Foliar application of hydrogen peroxide at concentrations of up to 75 μM reduces the relative water content in the leaf blade of guava seedlings.

3. Foliar application of hydrogen peroxide up to a concentration of 75 μM does not mitigate the effects of salt stress on guava plants during the seedling formation phase.

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