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Hydrogen peroxide application methods in guava seedlings grown under salt stress

Métodos de aplicação de peróxido de hidrogênio em mudas de goiabeira cultivadas sob estresse salino

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Highlights

Water salinity above 0.3 dS m^{-1} increases electrolyte leakage in the leaf blades.
H_2O_2 increases total chlorophyll in guava plants irrigated with 2.1 dS m^{-1} water.
Seedlings grown under 3.5 dS m^{-1} water salinity exhibit acceptable quality.

Abstract

The guava plant is of great socioeconomic importance in Northeastern Brazil. However, in this region, irrigation water often contains high salt concentrations that compromise plant growth. Therefore, strategies to mitigate the deleterious effects of salt stress on plants are necessary, such as the exogenous application of hydrogen peroxide. Nonetheless, studies on its application are still scarce. This study aimed to evaluate different methods of hydrogen peroxide application in guava plants irrigated with saline water during the seedling formation phase. The experiment was conducted under greenhouse conditions at the CCTA/UFCG in Pombal - PB, Brazil. It followed a randomized complete block design in a 5 × 4 factorial arrangement consisting of five levels of irrigation water electrical conductivity (ECw) (0.3, 1.1, 1.9, 2.7, and 3.5 dS m^{-1}) and four hydrogen peroxide (H_2O_2) application methods (M_1 – no peroxide application, M_2 – seed soaking, M_3 – foliar spray, and M_4 – seed soaking + foliar spray), all at a

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concentration of 20 μM, with three replicates and two plants per plot. Water salinity above 0.3 dS m^{-1} inhibited growth, total chlorophyll content, and dry matter accumulation in guava cv. Paluma seedlings. Foliar spray application of H_2O_2 mitigated the effects of salt stress on the number of leaves, leaf area, and dry matter in guava plants. H_2O_2 at a concentration of 20 μM reduced electrolyte leakage regardless of the application method. Guava seedlings irrigated with water up to 3.5 dS m^{-1} showed acceptable quality for field transplantation.
Key words: Antioxidant enzymes. Elicitors. *Psidium guajava* L. Saline water.

Resumo

A goiabeira tem grande importância socioeconômica no Nordeste brasileiro, porém nesta região é comum as águas destinadas para irrigação conterem elevadas concentrações de sais que comprometem o crescimento das plantas, sendo necessário o uso de estratégias que atenuem os efeitos deletérios do estresse salino sobre as plantas, tais como a aplicação exógena de peróxido de hidrogênio, porém escassos são os estudos sobre sua forma de aplicação. Dessa forma, objetivou-se avaliar métodos de aplicação do peróxido de hidrogênio em goiabeira irrigadas com águas salinas na fase de formação de mudas. O experimento foi realizado sob condições de casa de vegetação no CCTA/UFCG, em Pombal – PB. O delineamento experimental foi em blocos casualizados, em esquema fatorial 5 × 4, sendo cinco níveis de condutividade elétrica da água de irrigação - CEa (0,3; 1,1; 1,9; 2,7 e 3,5 dS m^{-1}) e quatro métodos de aplicação de peróxido de hidrogênio - H_2O_2 (M_1 - sem aplicação de peróxido, M_2 - aplicação via embebição das sementes, M_3 - aplicação por pulverização foliar e M_4 - aplicação via embebição das sementes + pulverização foliar) com peróxido na concentração de 20 μM, com três repetições e duas plantas por parcela. A salinidade da água a partir de 0,3 dS m^{-1} inibiu o crescimento, os teores de clorofila total e fitomassa seca das mudas de goiabeira cv. Paluma; O método de aplicação de H_2O_2 via pulverização foliar minimizou o efeito do estresse salino sobre o número de folhas, área foliar e fitomassa seca da goiabeira; H_2O_2 na concentração de 20 μM reduziu o extravasamento de eletrólito, independentemente do método de aplicação. As mudas de goiabeira irrigadas com água de até 3,5 dS m^{-1} apresentaram qualidade aceitável para transplantio no campo.
Palavras-chave: Águas salinas. Elicitores. Enzimas antioxidantes. *Psidium guajava* L.

Introduction

Guava (*Psidium guajava* L.) is known for producing fruits with a pleasant aroma and flavor, as well as high nutritional value. The fruits can be consumed fresh or processed, contributing to job creation and income generation (Angulo-López et al., 2021). Additionally, guava leaves contain phenolic compounds such as isoflavonoids, gallic acid, catechin, epicatechin, rutin, naringenin, and kaempferol, which exhibit anti-inflammatory and antioxidant properties (Shamili et al., 2021).

In Brazil, approximately 47.2% of the guava cultivation area is located in the Northeast region, with the states of Pernambuco (35.9%) and Bahia (8.4%) being the main producers in this area (Instituto Brasileiro de Geografia e Estatística [IBGE], 2025). However, the semiarid region is characterized by high evapotranspiration rates, irregular rainfall, and water sources with high salt concentrations, which limit the expansion of irrigated agriculture and contribute to soil salinization (G. S. de Lima et al., 2023).

Excess salts in water and/or soil are among the main factors limiting crop development due to the reduction in the osmotic potential of the soil solution. This triggers additional stresses, such as water and oxidative stress, which cause damage through nutritional imbalances, ionic toxicity, membrane disruption, reduced cell division and expansion, and the interruption of key metabolic processes (Shamili et al., 2021; Wani et al., 2020). These effects restrict the growth and productivity of several crops, including guava (S. S. da Silva et al., 2024).

Xavier et al. (2022), studying the growth and quality of guava seedlings under water salinity (ECw: 0.6, 1.5, 2.4, 3.3, and 4.2 dS m^{-1}), observed that increasing ECw negatively impacted the parameters evaluated. Similarly, S. S. da Silva et al. (2024) found that irrigation water salinity above 0.3 dS m^{-1} increased electrolyte leakage in the leaf blade, reducing relative water content, photosynthetic pigment synthesis, and seedling growth in guava cv. Paluma.

Management strategies to cope with abiotic stresses, particularly salinity, are essential for the sustainability of irrigated agriculture. Among these strategies, the exogenous application of hydrogen peroxide (H_2O_2) has shown promise in mitigating salt stress in various crops (A. A. R. da Silva et al., 2019, 2021a,b). H_2O_2 acts as a signaling molecule in salt stress responses and, when applied at low concentrations, activates the plant defense system via antioxidant enzymes. This minimizes the harmful effects of salinity and promotes the accumulation of soluble proteins, soluble carbohydrates, and NO_3^-, while reducing Na^+ and Cl^- levels in plant tissues (Liu et al., 2020).

In fruit trees, the effects of H_2O_2 vary depending on the application method and the species. S. S. da Silva et al. (2024), evaluating foliar application of H_2O_2 to mitigate salinity stress in guava seedlings, did not observe a reduction in the effects of stress. In contrast, A. A. R. da Silva et al. (2019, 2021a), studying soursop and passion fruit seedlings, respectively, reported mitigation of salt stress when H_2O_2 was applied via seed imbibition at a concentration of 30 μM. However, studies on H_2O_2 application methods (foliar and seed soaking) in guava are still incipient. Therefore, the objective of this study was to evaluate hydrogen peroxide application methods in guava plants irrigated with saline water during the seedling formation stage.

Material and Methods

The experiment was conducted in a greenhouse at the Academic Unit of Agricultural Sciences, Federal University of Campina Grande, Pombal, Paraíba, Brazil (coordinates: 6º47'20" S, 37º48'01" W, at an altitude of 194 m). The region's climate is classified as BSh, a hot semiarid climate with an average annual temperature of 28 °C and rainfall of approximately 750 mm $year^{-1}$ (Alvares et al., 2014).

The experimental design was a randomized complete block in a 5 × 4 factorial arrangement, consisting of five levels of irrigation water electrical conductivity

(ECw) (0.3, 1.1, 1.9, 2.7, and 3.5 dS m^{-1}) and four hydrogen peroxide (H2O2) application methods (M_1 – no H_2O_2 application, M_2 – seed soaking, M_3 – foliar spray, and M_4 – seed soaking + foliar spray), all using a concentration of 20 μM, with three replicates and two plants per plot. The ECw levels were based on the work of Nobre et al. (2023), and the H_2O_2 concentration was based on Souza et al. (2019).

Guava seeds of the Paluma cultivar were harvested from ripe, healthy, and well-developed fruit collected from the orchard at the Rolando Enrique Rivas Castellón Experimental Farm, part of UFCG/CCTA, Pombal Campus - PB. The seeds were separated from the fruit and dried at room temperature. For sowing, three seeds were placed in each bag at a depth of 1 cm in the substrate. Black polyethylene bags with a capacity of 2 dm³, perforated for water drainage, were arranged on benches 0.8 m above the ground and filled with air-dried substrate composed of soil and sand in a 2:1 volume ratio, respectively. The soil was collected from an agricultural area in Pombal - PB, at a depth of 0-20 cm. Its physical, water, and chemical properties were determined according to the methodology described by Teixeira et al. (2017), with the following characteristics: pH (H_2O) = 8.53; organic matter = 3.10 g kg^{-1}; P = 77.30 mg kg^{-1}; K^+ = 0.56 $cmol_c$ kg^{-1}; Na^+ = 0.20 $cmol_c$ kg^{-1}; Ca^{+2} = 5.08 $cmol_c$ kg^{-1}; Al^{3+} = 0.00 $cmol_c$ kg^{-1}; H^+ = 0.00 $cmol_c$ kg^{-1}; electrical conductivity of the saturation extract = 0.46 dS m^{-1}; cation-exchange capacity = 10.95 $cmol_c$ kg^{-1}; sodium adsorption ratio of the saturation extract = 1.02 (mmol $L^{-1})^{0.5}$; exchangeable sodium percentage = 1.83%; sand = 775.70 g kg^{-1}; silt = 180.90 g kg^{-1}; clay = 43.40 g kg^{-1}; moisture 33.42 kPa[1] = 12.45 dag kg^{-1}; moisture 1519.5 kPa[2] = 5.00 dag kg^{-1}.

Fertilization with nitrogen, potassium, and phosphorus was carried out as top dressing, following the recommendations of Novais et al. (1991). A total of 0.21 g of urea, 0.71 g of potassium chloride, and 0.86 g of monoammonium phosphate were applied per bag, equivalent to 100, 150, and 300 mg kg^{-1} of N, K_2O, and P_2O_5, respectively. These were divided into three equal applications at 15-day intervals via fertigation, with the first application occurring at 25 days after sowing (DAS). Micronutrient fertilization was performed at 45 DAS using the commercial product Dripsol Micro®, applied as a foliar spray at a concentration of 0.5 g L^{-1}. The product contained 1.2% magnesium, 0.85% boron, 3.4% iron, 4.2% zinc, 3.2% manganese, 0.5% copper, and 0.06% molybdenum.

Seeds for treatments M_2 (soaking) and M_4 (soaking + foliar spray) were pretreated by immersion in 500 mL of 20 μM hydrogen peroxide for 24 h in the dark, following the protocol described by Souza et al. (2019), which also defined the soaking duration. The remaining seeds were subjected to heat treatment by immersion in water at 90 °C for 1 min. For treatments M_3 (foliar spray) and M_4 (soaking + foliar spray), hydrogen peroxide was applied manually to the foliage using a spray bottle at 40, 55, 70, 85, and 100 DAS, always at approximately 17:00. Both the abaxial and adaxial surfaces of the leaves were sprayed to ensure full wetting. A 1 M stock solution of hydrogen peroxide was diluted in distilled water to achieve a final concentration of 20 μM, with the solution prepared on the day of each application.

Irrigation water was prepared by dissolving sodium chloride (NaCl) to adjust the salinity levels of the municipal water supply (0.3 dS m^{-1}) from Pombal - PB. The relationship between ECw and salt concentration (mg L^{-1} = 640 × ECw) was used, as recommended by Richards (1954). After preparation, the water was stored in 60 L plastic tanks, properly sealed to prevent evaporation. During the experiment, the electrical conductivity of the irrigation water was monitored weekly using a portable conductivity meter.

Before sowing, the substrate was irrigated to field capacity using local water (0.3 dS m^{-1}). After sowing, irrigation was applied daily, and the volume was determined by weighing lysimetry. The volume lost by evapotranspiration was replenished according to Eq. 1, with the addition of a leaching fraction (LF) of 0.10 every 20 days to prevent excessive salt accumulation in the root zone. Irrigation with the respective saline water treatments began at 31 DAS.

$$Vi = [(Wi - Wf) \times 1] \quad (1)$$

Where:

Vi = volume of water applied per container (L);

Wi = initial weight of the container (bag) after irrigation (kg);

Wf = final weight of the container after 24 h (kg);

Constant 1 = specific mass of water.

Crop management included manual weeding and superficial soil scarification, following the technical recommendations for guava seedling development provided by Costa and Lima (2008).

At 120 DAS, the following parameters were evaluated: number of leaves (NL), leaf area (LA), plant height (PH), stem diameter (SD), total chlorophyll content (Clot), relative water content (RWC), electrolyte leakage (EL), leaf dry biomass (LDB), stem dry biomass (SDB), root dry biomass (RDB), and total dry biomass (TDB). Plant height was measured from the collar to the apical bud of the main shoot using a ruler graduated in centimeters. Stem diameter was measured with a digital caliper and expressed in millimeters. Leaf area was calculated by measuring the length of the main vein of leaves with fully open blades, as recommended by L. G. S. Lima et al. (2012), using Eq. 2.

$$LA = \sum^{0.3205} \times L^{2.0412} \quad (2)$$

Where:

LA = leaf area (cm²);

L = length of the main leaf vein (cm).

Total chlorophyll content (Clo *t*) was measured using the CCM-200 plus instrument (Opti-Science, Inc., USA) on a fully expanded leaf from the third pair of leaves in the middle third of the plant. To assess relative water content (RWC), three fully expanded leaves were selected, from which eight 113-mm² discs were extracted. The discs were immediately weighed on an analytical balance to obtain fresh weight (FW), then immersed in distilled water in sealed plastic bags for 24 h. Afterward, excess surface water was removed with paper towels, and the discs were weighed again to determine turgid weight (TW). Finally, the discs were dried in a forced-air oven at 65 °C ± 3 °C until reaching constant weight and then weighed on an analytical balance to obtain dry weight (DW), following the method of G. S. de Lima et al. (2015), as shown in Eq. 3.

$$\mathrm{RWC} = \frac{(\mathrm{FW}-\mathrm{DW})}{(\mathrm{TW}-\mathrm{DW})} \times 100 \quad (3)$$

Where:

RWC = relative water content (%);

FW = fresh weight of leaf discs (g);

TW = turgid weight of leaf discs (g);

DW = dry weight of leaf discs (g).

To evaluate electrolyte leakage (EL) in the leaf blade, eight 113-mm² discs were removed from three additional fully expanded leaves and placed in beakers containing 50 mL of distilled water, covered with aluminum foil. The beakers were kept at 25 °C for 24 h, after which the initial electrical conductivity (Ci) was measured. They were then placed in a forced-air circulation oven at 80 °C for 90 min. Immediately afterward, the final electrical conductivity (Cf) was measured. Electrolyte leakage (%) was calculated according to Eq. 4, as proposed by Scotti-Campos (2013):

$$\mathrm{EL} = \frac{Ci}{Cf} \times 100 \quad (4)$$

Where:

EL = electrolyte leakage (%);

Ci = initial electrical conductivity (dS m^{-1});

Cf = final electrical conductivity (dS m^{-1}).

The plants were then collected and taken to the laboratory for biomass accumulation analysis. The leaves, stems, and roots were separated and placed in properly labeled Kraft paper bags. These were placed in a forced-air circulation oven at 65 °C ± 3 °C until they reached constant weight. The following parameters were recorded: leaf dry biomass (LDB), stem dry biomass (SDB), and root dry biomass (RDB). These values were used to calculate total dry biomass (TDB) by summing LDB, SDB, and RDB. The root/shoot ratio (R/S) was calculated by dividing RDB by SDB. Dry biomasses were measured using an analytical balance, and results were expressed in g $plant^{-1}$. Seedling quality was determined using the Dickson Quality Index (DQI), as proposed by Dickson et al. (1960), according to Eq. 5:

$$\mathrm{DQI} = \frac{(\mathrm{TDB})}{(\mathrm{PH/SD}) + (\mathrm{SDB/RDB})} \quad (5)$$

Where:

DQI = Dickson's seedling quality index;

TDB = total dry biomass (g);

PH = plant height (cm);

SD = stem diameter (mm);

SDB = shoot dry biomass (g);

RDB = root dry biomass (g).

The data were analyzed using the F-test. When significant differences were detected, regression analyses (linear and polynomial) were conducted for the 'salinity level' factor, and Tukey's test ($p \leq 0.05$) was applied for the comparison of hydrogen peroxide application methods, using SISVAR statistical software (D. F. Ferreira, 2019).

Results and Discussion

The analysis of variance summary (Table 1) showed a significant interaction ($p \leq 0.01$) between salinity level (SL) and hydrogen peroxide application method (M) for leaf number, leaf area, total chlorophyll content, and relative water content in guava cv. Paluma. For the isolated effect of salinity level, significant effects were observed for plant height, stem diameter, and electrolyte leakage. The application methods of H_2O_2 had a significant effect only on electrolyte leakage in the leaf blade.

Table 1
Summary of analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), total chlorophyll (Clo *t*), relative water content (RWC), and electrolyte leakage (EL) of guava cv. Paluma subjected to irrigation water salinity levels and hydrogen peroxide application methods, at 120 days after sowing

Source of variation	DF	Mean square						
		PH	SD	NL	LA	Clo *t*	RWC	EL
Salinity level (SL)	4	259.04**	4.58**	84.60**	46449.83**	66.76**	1437.70**	91.41**
Linear regression	1	936.32**	17.98**	210.67**	128456.10**	73.22**	5461.96**	285.42**
Quadratic regression	1	31.72^{ns}	1.15^{ns}	118.33**	25754.36**	180.67**	264.27**	40.36*
H_2O_2 application method (M)	3	53.71^{ns}	0.42^{ns}	87.97**	85615.46**	3.58^{ns}	8435^{ns}	100.82**
SL × M	12	22.53^{ns}	0.72^{ns}	22.87**	33284.84**	26.37**	98.04**	6.81^{ns}
Block	2	80.67	1.60	23.21	1755.74	123.91	9.03	12.98
Residual	38	22.14	0.56	2.55	222.31	8.99	31.98	12.39
CV (%)		10.49	18.91	9.61	3.85	19.74	8.06	20.33

DF - degrees of freedom; CV (%) - coefficient of variation; ns, **, * - Not significant and significant at $p \leq 0.01$ and $p \leq 0.05$, respectively.

Guava plant height and stem diameter decreased by 22.11% and 32.70%, respectively, with increasing irrigation water salinity (Figures 1A and 1B). This reduction in growth reflects the limitation of water and nutrient uptake caused by osmotic and ionic effects, which interfere with cell turgor pressure and consequently hinder plant development (Rodrigues et al., 2023). Similar findings were reported by J. T. A. Ferreira et al. (2023) in guava cv. Paluma seedlings under salt stress (0.3 to 4.3 dS m^{-1}) and H_2O_2 application, where growth inhibition was observed even at ECw levels as low as 0.3 dS m^{-1}.

According to the breakdown of application methods at each irrigation salinity level, a reduction in the number of leaves and leaf area was observed with increasing ECw in plants without H_2O_2 application (M_1) and those treated via seed soaking in H_2O_2 (M_2). However, in plants treated with foliar spray (M_3) and seed soaking + foliar spray (M_4), the highest leaf numbers were recorded at ECw levels of 1.8 and 1.7 dS m^{-1}, reaching 22.96 and 21.09 leaves, respectively (Figure 1C). Similarly, the largest leaf areas (596.85 and 511.94 cm²) were observed at ECw levels of 1.2 and 2.2 dS m^{-1} for M_3 and M_4, respectively (Figure 1D).

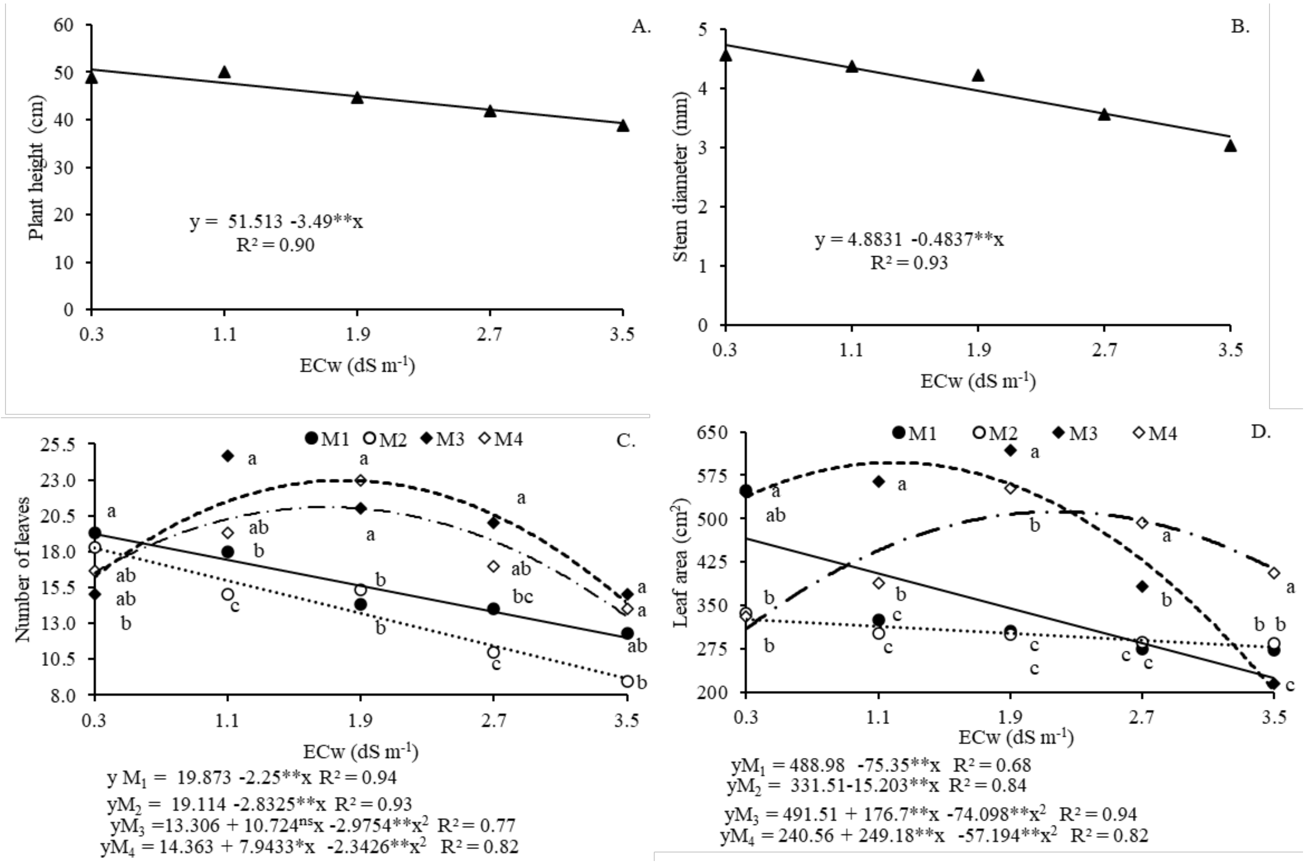

Figure 1. Plant height (A) and stem diameter (B) under irrigation water salinity (ECw), and number of leaves (C) and leaf area (D) of guava cv. Paluma, as a function of the interaction between irrigation water salinity and hydrogen peroxide application methods, 120 days after sowing.
M_1 – no H_2O_2 application; M_2 – application via seed soaking; M_3 – application via foliar spray; and M_4 – application via seed soaking + foliar spray. Means followed by the same lowercase letter indicate no significant difference between H_2O_2 application methods at each irrigation water salinity, according to Tukey's test ($p \leq 0.05$).

The foliar application (M_3) and foliar application combined with soaking (M_4) in H_2O_2 mitigated the harmful effects of salt stress on leaf number and area up to ECw levels of 1.8 and 1.7 dS m^{-1} for leaf number, and 1.2 and 2.2 dS m^{-1} for leaf area, respectively. The higher efficacy of M_3 and M_4 may be attributed to differences in absorption efficiency between seeds and leaf tissues, potentially enhancing H_2O_2 uptake. As a metabolic signal, H_2O_2 stimulates the absorption of water and essential nutrients (especially N, P, and K), promoting root development and maintaining ionic homeostasis under saline conditions (Farouk & Amira, 2018). This explains the mitigation of stress damage observed with foliar and combined applications. In a similar study, M. E. Ferreira et al. (2025) reported reduced salt stress effects and increased plant height and leaf area in passion fruit seedlings treated with foliar application of 15 μM H_2O_2 under ECw levels ranging from 0.3 to 3.5 dS m^{-1}.

In guava plants without H_2O_2 application, the total chlorophyll content decreased by 41.39% (from 8.22 mg g^{-1} FW) when comparing the lowest (0.3 dS m^{-1}) to the highest (3.5 dS m^{-1}) salinity levels. In contrast, plants treated with M_2, M_3, and M_4 showed increased total chlorophyll content up to ECw levels of 1.8, 2.1, and 1.6 dS m^{-1}, respectively, with estimated values of 18.79, 19.15, and 17.65 mg g^{-1} FW, respectively, followed by reductions at higher salinity levels (Figure 2A). When comparing treatments at each salinity level, M_1-treated seedlings had higher chlorophyll content than M_2-treated seedlings at 0.3 dS m^{-1}. However, at 1.9 dS m^{-1}, the opposite was observed, indicating the beneficial effect of H_2O_2 under moderate salinity. No significant differences were found between treatments at other ECw levels.

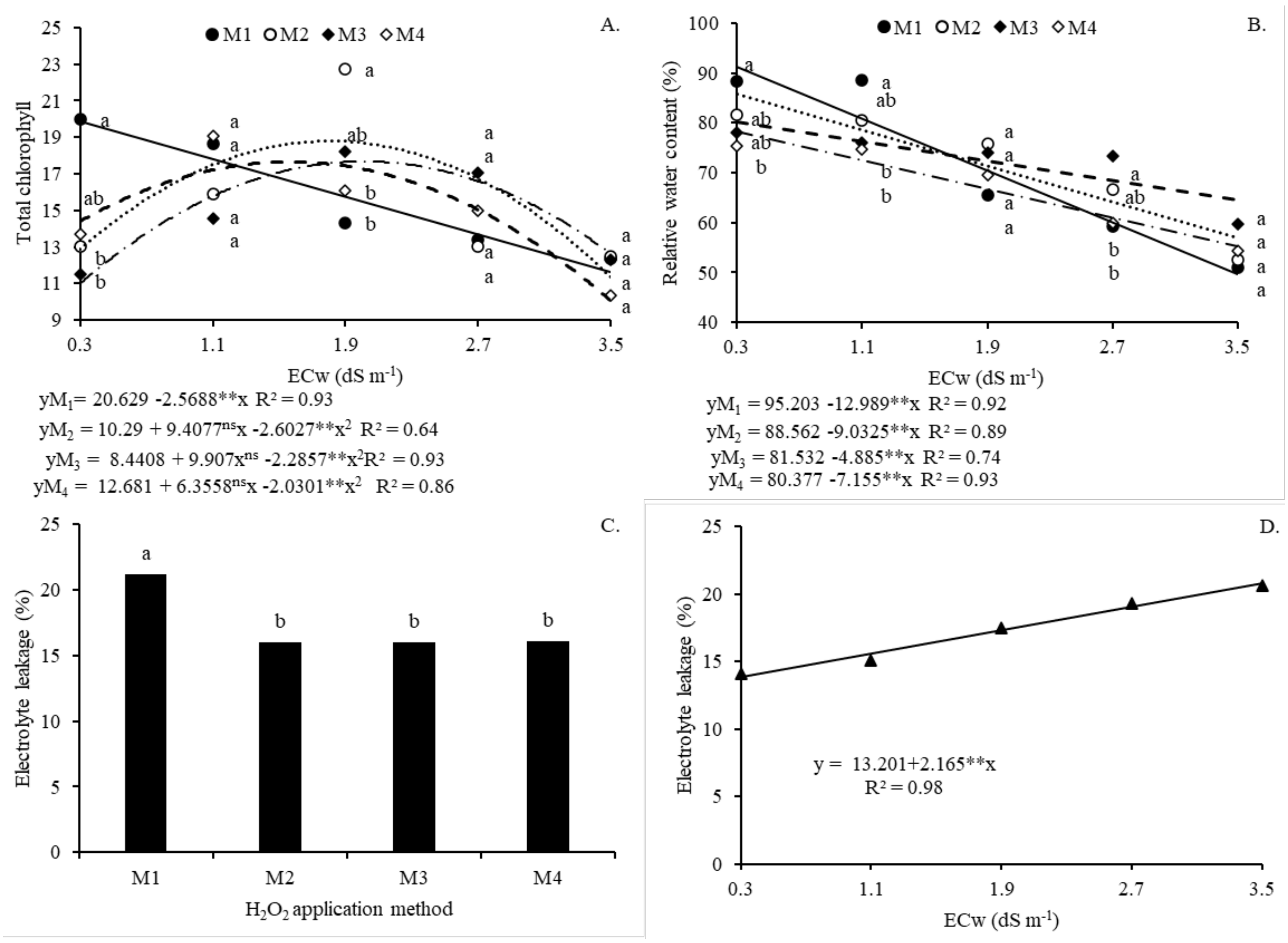

Figure 2. Total chlorophyll (A) and relative water content (B) as a function of the interaction between irrigation water salinity (ECw) and hydrogen peroxide application methods (C) and electrolyte leakage of guava cv. Paluma under irrigation water salinity (D), 120 days after sowing.
M_1 – no H_2O_2 application; M_2 – application via seed soaking; M_3 – application via foliar spray; and M_4 – application via seed soaking + foliar spray. Means followed by the same lowercase letter indicate no significant difference between H_2O_2 application methods within each irrigation water salinity, according to Tukey's test ($p \leq 0.05$).

The reduction in total chlorophyll content observed in plants without H_2O_2 application may be attributed to increased activity of chlorophyllase, the enzyme responsible for chlorophyll degradation. This likely results from the enhanced translocation of chloride ions, which replace nitrate due to the high salt concentration in the plant (Giordano et al., 2021). In contrast, plants subjected to H_2O_2 application may have experienced inhibited chlorophyllase activity or enhanced chlorophyll biosynthesis, even under salt stress. This is because H_2O_2 can mitigate the effects of salinity, promoting the maintenance of photosynthetic pigments and preserving the functioning of the photosynthetic machinery (Veloso et al., 2023).

The relative water content (RWC) of guava plants declined across all H_2O_2 application methods, with reductions of 45.52% (M_1), 33.67% (M_2), 19.52% (M_3), and 29.27% (M_4) when comparing the lowest and highest ECw levels (0.3 and 3.5 dS m^{-1}) (Figure 2B). It is evident that although RWC decreased in all treatments as salinity increased, the most pronounced reduction (45.52%) occurred in the absence of H_2O_2 application (M_1), while the smallest reduction was observed in plants treated with foliar application (M_3).

When analyzing the treatments by salinity level, it was found that at ECw levels of 0.3 and 1.1 dS m^{-1}, plants treated with M_1 had higher RWC than those treated with M_3. However, at 2.7 dS m^{-1}, the opposite was observed: plants under M_3 had higher RWC than those under M_1. These results suggest that H_2O_2 application attenuates the effects of salt stress on RWC, possibly by reducing Na^+ uptake in favor of K^+, which helps maintain better leaf water status (P. C. C. Silva et al., 2023).

Regardless of the application method, H_2O_2 reduced electrolyte leakage in the leaf blades of guava cv. Paluma. Compared to the absence of application (control), methods M_2, M_3, and M_4 led to reductions in EL of 32.33%, 32.83%, and 31.84%, respectively (Figure 2C). H_2O_2 may have altered the regulation of membrane protein activity, including ion channels and membrane transporters, which control ion movement across cell membranes. By modulating these proteins, cells can adjust membrane permeability, thereby reducing electrolyte leakage into surrounding tissues (Pan et al., 2021).

An increase in irrigation water salinity led to greater electrolyte leakage in guava plants, with a 49.96% increase observed when comparing plants irrigated with water at 0.3 dS m^{-1} to those at 3.5 dS m^{-1} (Figure 2D). This increase is attributed to the phytotoxic effects of excess Na^+ and Cl^- in the leaf tissue. The accumulation of these ions alters membrane composition, leading to rupture (Wani et al., 2020). Similar results were reported by S. S. da Silva et al. (2024), who found that electrolyte leakage in guava seedlings increased by 61.93% when comparing plants irrigated with ECw of 4.3 dS m^{-1} to those irrigated with the lowest salinity level (0.3 dS m^{-1}).

There was a significant interaction between the factors (SL × M) on the dry biomass of leaves, stems, roots, shoots, and total biomass, as well as on the root-to-shoot ratio, indicating that both factors simultaneously influenced the dry matter accumulation of guava cv. Paluma plants at 120 days after transplanting. The Dickson Quality Index was significantly affected only by the salinity factor (Table 2).

Table 2
Summary of analysis of variance for leaf (LDB), stem (SDB), root (RDB), and total (TDB) dry biomass, root/shoot ratio (R/S) and Dickson seedling quality index (DQI) of guava cv. Paluma subjected to irrigation water salinity levels and hydrogen peroxide application methods, at 120 days after sowing

Source of variation	DF	Mean square					
		LDB	SDB	RDB	TDB	R/S	DQI
Salinity level (SL)	4	2.77**	1.67**	17.12**	40.14**	1.33**	0.39**
Linear regression	1	5.77**	1.47*	36.35**	93.19**	2.23**	1.08**
Quadratic regression	1	3.64**	4.22**	15.64**	62.69**	0.01*	0.46**
H_2O_2 application method (M)	3	0.54*	0.50^{ns}	2.76*	4.68*	0.76*	0.02^{ns}
SL × M	12	1.25**	1.22**	2.49**	9.80**	0.84**	0.06^{ns}
Block	2	0.33	0.23	0.28	0.77	0.10	0.08
Residual	38	0.14	0.22	0.65	1.17	0.19	0.02
CV (%)		20.21	35.38	18.05	14.19	28.01	26.80

DF - degrees of freedom; CV (%) - coefficient of variation; ns, **, * - Not significant and significant at $p \leq 0.01$ and $p \leq 0.05$, respectively

The highest accumulation of leaf dry biomass (LDB) (2.85 g plant^{-1}) (Figure 3A) was observed in seedlings treated with the M_3 method and irrigated with water at an ECw of 1.9 dS m^{-1}. Compared to the highest salinity level tested (3.5 dS m^{-1}), this represented a significant reduction of 56.83% in LDB. In the absence of H_2O_2 (M_1), LDB decreased progressively with increasing salinity, with a reduction of 58.48% between ECw levels of 0.3 and 3.5 dS m^{-1}. On the other hand, methods M_2 and M_4 led to increases in LDB up to ECw values of 1.4 and 1.6 dS m^{-1}, respectively, with maximum values of 1.95 and 2.40 g plant^{-1}. In the breakdown by salinity level, plants under M_1 showed the highest LDB at ECw levels of 0.3 and 3.5 dS m^{-1}. In contrast, at ECw levels of 1.9 and 2.7 dS m^{-1}, the M_3 method resulted in significantly higher LDB values than M_1.

Stem dry biomass (SDB) accumulation (Figure 3B) peaked at 2.58 g plant^{-1} in seedlings treated with M_3 and irrigated with water at an ECw of 1.96 dS m^{-1}. When compared to seedlings irrigated at 3.5 dS m^{-1}, this represented a 58.71% reduction. Under M_2, the highest SDB was 1.72 g at ECw 1.93 dS m^{-1}, followed by a 51.59% decrease at the highest salinity level. In the control treatment (without H_2O_2 application), SDB decreased by 72.73% between ECw levels of 0.3 and 3.5 dS m^{-1}. The M_4 method did not fit the tested mathematical models. In the breakdown of H_2O_2 application methods by salinity level, M_1 showed the highest SDB at 0.3 dS m^{-1}. However, at ECw levels of 1.9 and 2.7 dS m^{-1}, M_3 significantly outperformed M1 in stem biomass accumulation.

In the absence of H_2O_2 (control), increasing ECw led to a 54.95% reduction in root dry biomass (RDB), with a maximum value of 3.49 g plant^{-1} at 0.3 dS m^{-1} (Figure 3C). In contrast, methods M_2, M_3, and M_4 induced maximum RDB accumulation at ECw levels of 1.16, 1.65, and 1.67 dS m^{-1}, with respective values of 5.13, 5.00, and 5.60 g plant^{-1}. As salinity increased beyond these levels, RDB values declined. In the treatment breakdown by salinity level, seedlings under M_1 at ECw 0.3 dS m^{-1} differed from the other treatments. However, at ECw 1.1 dS m^{-1}, M_1 had significantly lower values. At 2.7 dS m^{-1}, seedlings under M_4 had higher RDB than those treated with M_2. For the remaining ECw levels, no significant differences in RDB were observed among treatments.

The total dry biomass (TDB) of guava cv. Paluma seedlings without hydrogen peroxide application decreased by 60.05% (6.87 g plant^{-1}) with increasing electrical conductivity of irrigation water, up to 3.5 dS m^{-1} (Figure 3D). Similarly, for TDB, an increase was observed up to ECw values of 1.42, 1.81, and 1.55 dS m^{-1}, with corresponding maxima of 8.67, 10.60, and 9.83 g plant^{-1}. ECw levels above these thresholds led to a sharp decline. When analyzed by salinity level, the M_1 method resulted in statistically higher TDB at 0.3 dS m^{-1}. However, at higher salinities, M_4 (1.1 dS m^{-1}) and M_3 (1.9 and 2.7 dS m^{-1}) significantly outperformed M_1. At an ECw of 3.5 dS m^{-1}, no significant difference among treatments was observed.

In general, the reduction in dry matter accumulation in plants not treated with H_2O_2 occurs because salt stress induces both water imbalance (osmotic effect) and ion toxicity, inhibiting growth and impairing various physiological processes, thereby reducing biomass accumulation (Hajihashemi et al., 2021). Moreover, the plant may redirect its energy toward maintaining vital functions, limiting growth, reducing chlorophyll synthesis, and minimizing reactive oxygen species production through antioxidant enzyme activity (Liu et al., 2020).

However, the application of H_2O_2 led to an increase in dry matter under moderate salinity, suggesting that foliar-applied H_2O_2 may have acclimated plants to 2.7 dS m^{-1} by activating natural defense mechanisms in glycophytes, such as osmotic adjustment and organic solute accumulation (Ramos et al., 2022). A. A. R. da Silva et al. (2021b), investigating different H_2O_2 application methods (foliar, seed soaking, and foliar + soaking), found that seed soaking reduced the impact of salt stress on dry biomass production in soursop seedlings.

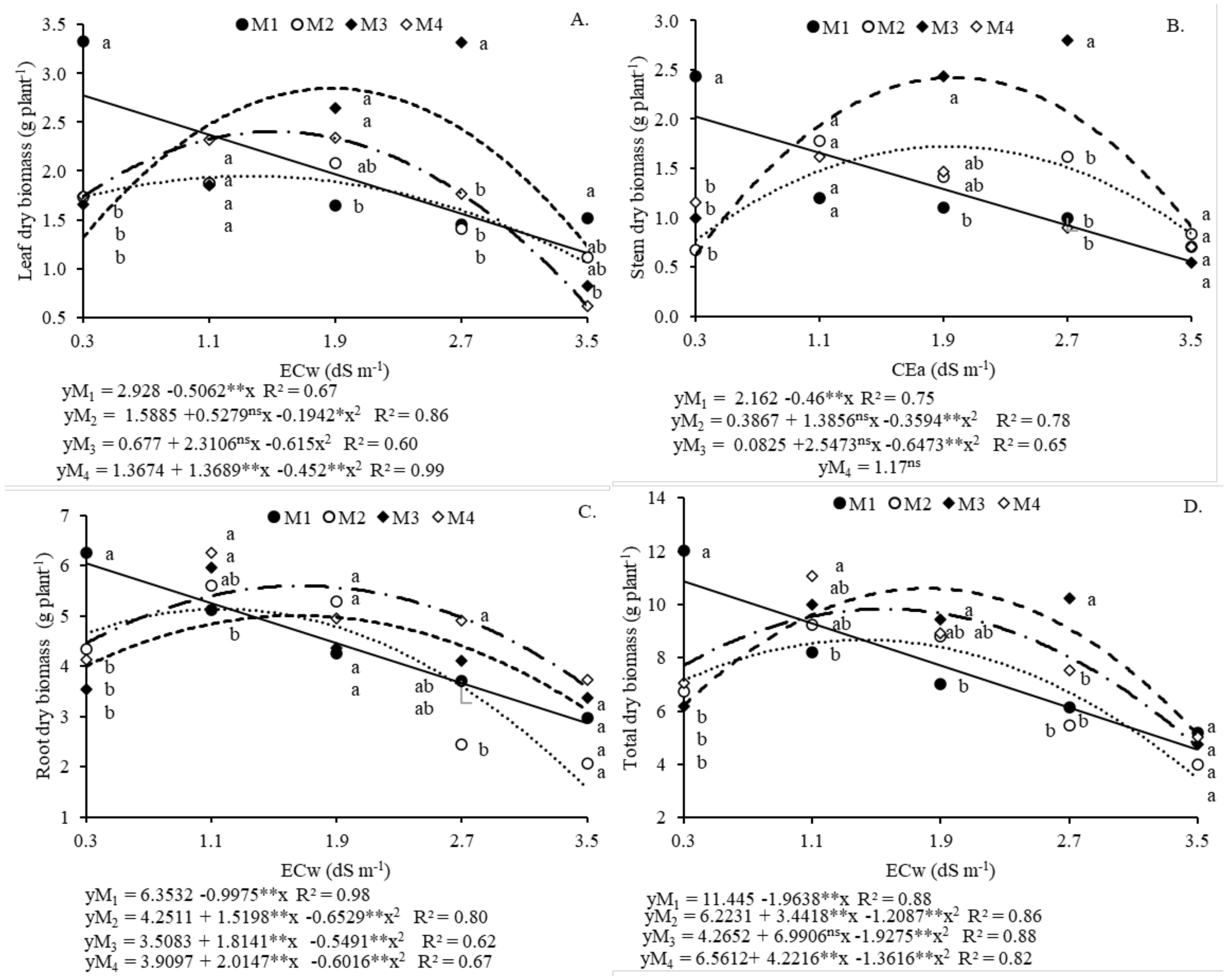

Figure 3. Leaf (LDB; A), stem (SDB; B), root (RDB; C), and total (TDB; D) dry biomass of guava cv. Paluma as a function of the interaction between irrigation water salinity (ECw) and hydrogen peroxide application methods, 120 days after sowing.
M_1 – no H_2O_2 application; M_2 – application via seed soaking; M_3 – application via foliar spray; and M_4 – application via seed soaking + foliar spray. Means followed by the same lowercase letter indicate no significant difference between H_2O_2 application methods within each irrigation water salinity, according to the Tukey's test ($p \leq 0.05$).

For the root/shoot ratio of guava seedlings under different H_2O_2 application methods and salinity levels, M_1 and M_3 did not show significant effects in the fitted mathematical models. In contrast, M_2 resulted in a 54.38% reduction in the root/shoot ratio between ECw levels of 0.3 and 3.5 dS m^{-1} (Figure 4A). Seedlings under M_4 showed the lowest ratio at 1.26 dS m^{-1}, followed by a 92.15% increase at 3.5 dS m^{-1}. Analyzing the H_2O_2 application methods within each salinity level At ECw levels of 2.7 and 3.5 dS m^{-1}, M_4-treated plants had higher root/shoot ratios than those treated with M_1 and M_2. The increase in root/shoot ratio under salinity is likely due to a greater reduction in shoot dry biomass compared to roots (A. A. R. da Silva et al., 2021b). The observed decline in this ratio with the soaking method suggests that this form of H_2O_2 application was not beneficial. Conversely, combining soaking and foliar spraying may have significantly enhanced salinity tolerance by boosting antioxidant defenses and modifying gene expression, sugar transport, and phytohormone activity (Chattha et al., 2022).

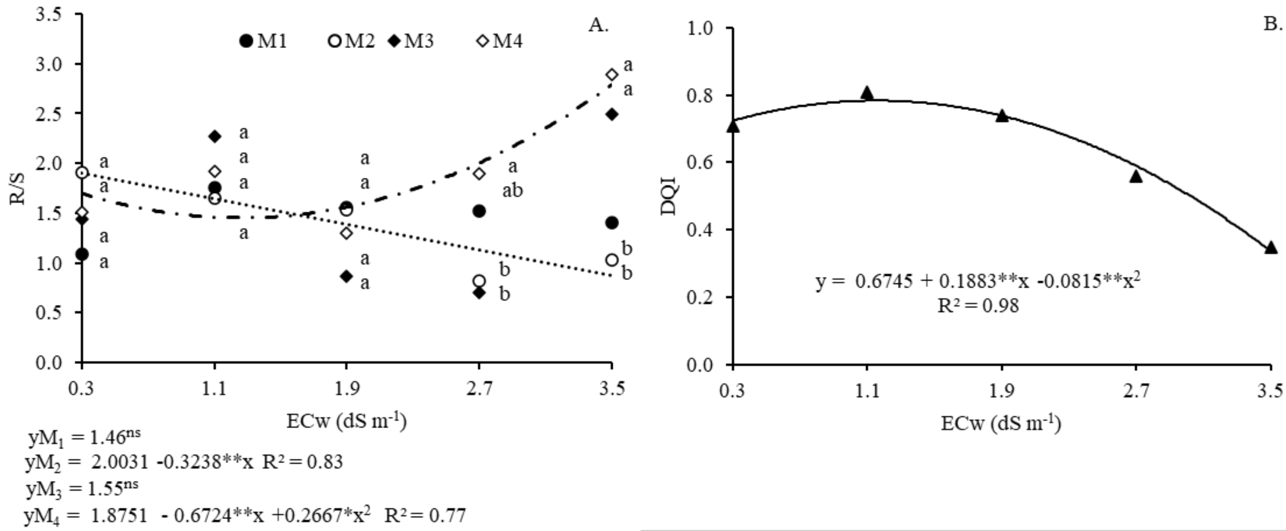

Figure 4. Root/shoot ratio - (R/S; A) as a function of the interaction between irrigation water salinity (ECw) and hydrogen peroxide application methods and Dickson quality index (DQI; B) of guava cv. Paluma as a function of irrigation water salinity, 120 days after sowing.
M_1 – no H_2O_2 application; M_2 – application via seed soaking; M_3 – application via foliar spray; and M_4 – application via seed soaking + foliar spray. Means followed by the same lowercase letter indicate no significant difference between H_2O_2 application methods within each irrigation water salinity, according to the Tukey's test ($p \leq 0.05$).

Regarding the Dickson Quality Index as a function of irrigation water salinity, the data fit a polynomial regression model, with a maximum estimated value of 0.78 at an ECw of 1.2 dS m^{-1}, followed by a decrease at higher salinity levels. In a study on guava seedlings under salt stress (ECw from 0.6 to 4.2 dS m^{-1}), Xavier et al. (2022) observed a 75.29% reduction in DQI with increasing ECw. J. T. A. Ferreira et al. (2023), assessing guava seedling quality, reported a DQI decrease from 0.49 (0.3 dS m^{-1}) to 0.20 (4.3 dS m^{-1}), which are lower than the values found in the present study. The DQI is a morphological indicator of seedling quality and hardiness, growth potential, and survivability. In this context, guava seedlings in this study showed a good quality index (0.33) even under ECw of 3.5 dS m^{-1} (Gallegos-Cedillo et al., 2021), as values above 0.28 indicate good vigor and robustness (Dikson et al., 1960).

Conclusions

Irrigation water salinity above 0.3 dS m^{-1} reduces growth, total chlorophyll content, and dry biomass of guava seedlings cv. Paluma.

Foliar application of H_2O_2 mitigates the effects of salt stress on the number of leaves, leaf area, and dry matter of guava seedlings.

Hydrogen peroxide at a concentration of 20 μM reduces electrolyte leakage in guava seedlings, regardless of the application method.

Guava seedlings irrigated with water at 1.2 dS m^{-1} exhibited excellent Dickson Quality Indices. Seedlings irrigated with water at 3.5 dS m^{-1} still showed acceptable quality for field transplantation.

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