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# Hydrogen peroxide in the induction of tolerance of guava seedlings to salt stress

Peróxido de hidrogênio na indução de tolerância de mudas de goiabeira ao estresse salino

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## Highlights \_

Salt stress limits the water content and leaf gas exchange in guava. Guava cv. Paluma is classified as sensitive to salt stress. H<sub>2</sub>O<sub>2</sub> does not alleviate the deleterious effects of salt stress on guava seedlings.

## Abstract \_

Guava cultivation plays a significant role in the socioeconomic development of the Brazilian semi-arid region, contributing to employment opportunities and income generation. However, this region often faces the challenge of high levels of dissolved salts in water sources, necessitating strategies to mitigate the adverse effects of salt stress on plants. This study aimed to assess the impact of foliar application of hydrogen peroxide on gas exchange, photochemical efficiency, growth, and quality of guava seedlings subjected to salt stress. The experiment was conducted under greenhouse conditions in Pombal - PB, Brazil, following a randomized block design. The treatments comprised a 5 × 4 factorial arrangement, representing five levels of electrical conductivity in the irrigation water (ECw: 0.3, 1.3, 2.3, 3.3, and 4.3 dS m<sup>-1</sup>) and four concentrations of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>: 0, 25, 50, and 75  $\mu$ M). Each treatment was replicated four times, with two plants per plot. Irrigation with water having an electrical conductivity of 0.3 dS m<sup>-1</sup> significantly inhibited gas exchange, photochemical efficiency, and growth of guava seedlings after 91 days of emergence. Foliar application of hydrogen peroxide at concentrations up to 75  $\mu$ M did

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not alleviate the effects of salt stress during the seedling formation phase of guava plants. Guava cv. Paluma was found to be sensitive to water salinity during the seedling formation phase, with a threshold level of 0.3 dS m<sup>-1</sup> and a decrease in the growth rate of 11.48% per unit increase in salinity. **Key words:** *Psidium guajava* L. Abiotic stress. Reactive oxygen species.

#### Resumo -

A goiabeira é uma cultura importante no cenário socioeconômico da região semiárida brasileira, sendo uma fonte de geração de emprego e renda. Contudo, nesta região é comum a ocorrência de fontes hídricas com altos teores de sais dissolvidos e assim são necessárias estratégias que visem minimizar os efeitos deletérios do estresse salino nas plantas. Neste contexto, objetivou-se com este trabalho avaliar os efeitos da aplicação foliar com peróxido de hidrogênio nas trocas gasosas, eficiência fotoquímica, crescimento e qualidade de mudas de goiabeira sob estresse salino. O experimento foi conduzido sob condições de casa de vegetação em Pombal, Paraíba, utilizando-se o delineamento de blocos casualizados, com tratamentos arranjados em esquema fatorial 5 × 4, referentes a cinco níveis de condutividade elétrica da água - CEa (0,3; 1,3; 2,3; 3,3 e 4,3 dS m<sup>-1</sup>) e quatro concentrações de peróxido de hidrogênio – H<sub>2</sub>O<sub>2</sub> (0, 25, 50 e 75 µM) com quatro repetições e duas plantas por parcela. A irrigação com água de condutividade elétrica a partir de 0,3 dS m<sup>-1</sup> inibiu as trocas gasosas, eficiência fotoquímica e crescimento de mudas de goiabeira, aos 91 dias após a emergência. A aplicação foliar de peróxido de hidrogênio em concentrações de até 75 µM não amenizou os efeitos do estresse salino em plantas de goiabeira na fase de formação de mudas. A goiabeira cv. Paluma é classificada como sensível a salinidade da água na fase de formação de mudas, sendo o nível limiar de 0,3 dS m<sup>-1</sup> e diminuição por aumento unitário de 11.48%.

Palavras-chave: Psidium guajava L. Estresse abiótico. Espécie reativa de oxigênio.

### Introduction \_\_\_\_

Guava (*Psidium guajava* L.) is renowned for its high nutritional value and pleasant taste, offering various uses in the agro-industry, including fresh consumption, ice cream, pulp, jellies, and juices (Arango et al., 2020). Among the prominent cultivars, cv. Paluma stands out due to its intense red pulp, light-green elliptical leaves, and pear-shaped berry-type fruits.

Guava production in Brazil in 2021 reached 552,393 tons in 2021, with a planted area of 22,137 hectares and an average yield of 24,953 kg ha<sup>-1</sup>. The Northeast region stands as the largest producer, with the state of Paraíba exhibiting lower yields than the national average, producing 2,366 tons with an average yield of 7,170 kg ha<sup>-1</sup> (Instituto Brasileiro de Geografia e Estatística [IBGE], 2022).

The semi-arid region of Northeastern Brazil presents soil-climatic characteristics such as high temperatures evaporation, low relative humidity, and irregular rainfall distribution, leading to challenges in the availability and quality of water resources (J. B. dos Santos et al., 2016; G. P. Santos et al., 2018; Soares et al., 2018). Additionally, the region commonly encounters water sources with high concentrations of dissolved salts (G. S. de Lima et al., 2019a).

Under stress conditions, plants experience physiological disorders. including stomatal closure, degradation of photosynthetic pigments (Lima et al., 2018; Pinheiro et al., 2022), and alterations in fluorescence signals (Dias et al., 2021a,b). Excessive salt levels also induce oxidative stress by generating reactive oxygen species (ROS), which can hinder chlorophyll synthesis, impair cellular components, and cause lipid peroxidation of membranes (Andrade et al., 2022).

Previous studies have explored the use of saline water in guava production. For instance, Bezerra et al. (2018) found that an electrical conductivity of 2.15 dS m<sup>-1</sup> in the saturation extract limited gas exchange and plant growth. E. M. da Silva et al. (2017), in their investigation of Paluma guava trees during the seedling formation phase under saline water irrigation, observed inhibition of photosynthetic pigments in leaves due to increased salinity in the irrigation water. However, most studies evaluating the effects of salt stress on guava have primarily focused on nutritional management using different fertilizer sources. Another alternative approach to mitigate salt stress is the use of elicitors such as hydrogen peroxide  $(H_2O_2)$ , as demonstrated by Capitulino et al. (2022) and Veloso et al. (2020) in soursop, Andrade et al. (2022) in sour passion fruit, and Silva et al. (2020) in guava.

Given the socioeconomic significance of guava cultivation in the Brazilian fruit industry, it is crucial to conduct research focused on studying the effects of salt stress during the seedling formation phase. This knowledge is vital for establishing guava cultivation in areas affected by water and soil salinity issues, particularly in the semiarid region of Northeast Brazil. Thus, this study aimed to evaluate the impact of foliar application with hydrogen peroxide on gas exchange, photochemical efficiency, growth, and quality of guava seedlings under salt stress.

### Material and Methods \_\_\_\_

This study was conducted from January to April 2022 in a greenhouse at the Agrifood Science and Technology Center (CCTA), Federal University of Campina Grande (UFCG), located in Pombal, Paraíba (PB), Brazil, The greenhouse is situated at coordinates 6°46' 8" latitude (S) and 37°47'45" longitude (W), 184 m above sea level. The climate of the region, according to the Köppen climate classification adapted to Brazil, is characterized as "BSh", representing a hot and semi-arid climate. The average annual temperature is 28 °C, with approximately 750 mm of rainfall per year and an average annual evaporation of 2000 mm. Figure 1 shows the temperature and relative humidity data recorded during the experimental period.



**Figure 1.** Variation in temperature (°C) and relative humidity in air (%) during the seedling growth period. Pombal, 2022.

The experiment involved seedlings of guava cv. Paluma and followed a randomized block design. The treatments consisted of a 5 × 4 factorial arrangement, combining five levels of electrical conductivity in the water (ECw: 0.3, 1.3, 2.3, 3.3, and 4.3 dS m<sup>-1</sup>), based on the study by Bezerra et al. (2018), and four concentrations of hydrogen peroxide ( $H_2O_2$ : 0, 25, 50, and 75 µM), based on the work by A. A. R. da Silva et al. (2021). The experiment included four replications, with two plants per plot.

The guava seedlings were grown in polyethylene bags measuring  $15 \times 30$  cm. The bags were filled with a mixture composed of Regosols (Entisol - Psamments) with sandy loam texture, sand, and tanned bovine manure in a ratio of 2:1:1 (volume basis). The soil and sand were autoclaved before mixing with cattle manure to prevent nematode issues. The soil was obtained from the rural area of São Domingos - PB, collected from a depth of 0-20 cm. Table 1 describes the physical and chemical characteristics of the soil, determined following the methodologies of Teixeira et al. (2017).

#### Table 1

Chemical and physical characteristics of the soil used in the experiment, before the implementation of treatments. Pombal, 2022

Chemical characteristics										
pH H <sub>2</sub> O)	ОМ	Р	K⁺	Na⁺	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H⁺		
(1:2.5)	g kg⁻¹	mg kg⁻¹	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	CEC	SARse	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⁻¹	(mmol L <sup>-1</sup> ) <sup>0.5</sup>	%	Sand	Silt	Clay	33.42 kPa <sup>1</sup>	1519.5 kPa <sup>2</sup>		
0.46	10.95	1.02	1.83	775.70	180.90	43.40	12.45	5.00		

pH - potential of hydrogen; OM - organic matter, Walkley-Black wet digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1M KCl, pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted using 1M NH<sub>4</sub>OAc, pH 7.0; Al<sub>3</sub><sup>+</sup>+H<sup>+</sup> extracted using 0.5 M CaOAc, pH 7.0; ECse - electrical conductivity of the saturation extract; CEC - cation exchange capacity; SARse - sodium adsorption ratio of the saturation extract; ESP - exchangeable sodium percentage; <sup>1,2</sup> referring to the moisture content in the soil corresponding to field capacity and permanent wilting point.

Nitrogen, phosphorus, and potassium fertilization followed the recommendations of Cavalcanti (2008), considering the nutritional requirements of the crop and the levels of the elements in the soil. Nitrogen was applied using urea (45% N) and monoammonium phosphate (50% P<sub>2</sub>O<sub>5</sub> and 11% N); potassium was supplied as potassium sulfate (50% K<sub>2</sub>O); and phosphorus was applied as (50% P<sub>2</sub>O<sub>5</sub> monoammonium phosphate and 11% N). Micronutrients were applied weekly via foliar spray starting from 10 days after emergence using Dripsol Micro® at a concentration of 1.0 g  $L^{-1}$  (containing 1.2%) magnesium, 0.85% boron, 3.4% iron, 4.2% zinc, 3.2% manganese, 0.5% copper, and 0.06% molybdenum).

Different levels of water salinity were achieved by adding NaCl according to the predetermined treatments. The water source used was from the Pombal - PB supply system, which had an electrical conductivity of 0.3 dS  $m^{-1}$ . The amount of NaCl was calculated

based on the relationship between the electrical conductivity of water (ECw) and salt concentration (Richards, 1954), using Eq. 1:

SC  $\approx$  10  $\times$  ECw.....(1)

in which SC refer to sum of cations  $\left(\text{mmol}_{\text{c}} \ \text{L}^{\text{-1}}\right)$ ; and

ECw refer to electrical conductivity of water (dS m<sup>-1</sup>).

After preparing the water, ECw was checked and, if necessary, adjusted before use.

Before sowing, the volume of water required to raise the soil moisture to field capacity was determined by applying water based on the established treatments. Ten days after emergence, irrigation was performed daily, at 17h00, applying the volume determined by the water balance in each bag. The volume of water applied in each irrigation event was calculated using Eq. 2:

VI =	(Vp - Vd)		(2)
V 1	(1 - LF)	••••••	(4)

in which VI refer to volume of water to be used in the irrigation event (mL);

Vp: volume applied in the previous irrigation event (mL);

Vd: volume drained in the previous irrigation event (mL); and

LF: leaching fraction of 0.10.

The different concentrations of hydrogen peroxide were applied as a foliar spray by diluting it in distilled water. The application was performed every 15 days at 17h00min to minimize light exposure, as hydrogen peroxide degrades rapidly in the presence of light (Ramos et al., 2022). At the time of application, the plants were isolated to prevent drift between different treatments, and an average volume of 50 mL of spray solution per plant was used.

At 91 days after emergence, various parameters were evaluated to assess the effects of the treatments on guava seedlings. These included relative water content (RWC) and electrolyte leakage (%EL) in the leaf blade, chlorophyll a fluorescence, gas exchange parameters, and growth. The evaluation was conducted once when the seedlings reached a suitable quality for transplanting in the field. Stem diameter (SD) was measured at 5 cm from the neck of the plant using a digital caliper. Plant height (PH) was measured from the stem to the insertion of the apical meristem. Determining SD and PH is important for assessing the quality of seedlings using the Dickson Quality Index (Dickson et at., 1960), and allows establishing the ideal time for grafting. The number of leaves (NL) was determined by counting leaves longer than 3 cm. Leaf area (LA) was calculated using the method described by Lima et al. (2012) with Eq. 3.

$$LA = \sum 0.3205 \times L^{2.0412}$$
 (3)

in which LA: the total leaf area (cm<sup>2</sup>); and L: the length of the leaf main vein (cm).

To evaluate the accumulation of stem dry biomass (SDB), leaf dry biomass (LDB), and root dry biomass (RDB), plants from each treatment were harvested. The different plant parts, including leaves, stems, and roots, were separated and placed in paper bags. They were then dried in a forced-air oven at a constant temperature of 65 °C until a constant weight was achieved. After drying, the materials were weighed using a precision scale, which provided the values for LDB, SDB, and RDB. The sum of these values represented the total dry biomass (TDB) of the plants. Shoot dry biomass was calculated by adding the values of LDB and SDB.

The relative water content (RWC) was determined using three fully expanded leaves from each plant. These leaves were weighed on a scale with a precision of 0.001 g. To determine the fresh weight of the leaves (FW), the collected leaves were immersed in distilled water for 24 h, dried, weighed, and the values were recorded. The dry matter weight was obtained after drying in a forced-air oven for 48 h, following the methodology of Weatherley (1950). The RWC was calculated using the following equation (Eq. 4):

RWC (%) =  $\frac{FW - DW}{TW - DW} \times 100....(4)$ 

in which RWC: relative water content (%);

FW: fresh weight (g); and

DW: dry weight (g); TW: turgid weight (g)

The water saturation deficit was determined according to the methodology described by Taiz et al. (2017), using Eq. 5



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

in which WSD: water saturation deficit (%); FW: fresh weight (g);

TW: turgid weight (g); and

DW: dry weight (g).

Chlorophyll а fluorescence was evaluated using а pulse-modulated fluorometer (Opti Science, model OS5p). This assessment was based on the measurements of initial fluorescence (F<sub>a</sub>), maximum fluorescence (Fm). variable fluorescence (Fv), and guantum efficiency of photosystem II (Fv/Fm). The protocol involved adapting the leaves in the dark for 30 min and using tweezers contained in the equipment to ensure that all acceptors were oxidized and reaction centers were open.

Gas exchange parameters, including stomatal conductance (*gs*, mol  $H_2O$  m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> assimilation rate (*A*, µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), transpiration (*E*, mmol  $H_2O$  m<sup>-2</sup> s<sup>-1</sup>), and intercellular CO<sub>2</sub> concentration (*Ci*, µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), were evaluated using a portable infrared carbon dioxide analyzer (IRGA) model "LCPro+" by ADC BioScientific Ltd. Additionally, the intrinsic water use efficiency (iWUE) (*A/gs*) [(µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (mol  $H_2O$  m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>] was quantified using the obtained data. Readings were taken between 07h00 and 10h00 under natural air temperature and CO<sub>2</sub> concentration conditions.

Seedling quality was assessed using the Dickson quality index (Dickson et at., 1960), according to Eq. 6.

$$DQI = \frac{(TDB)}{(PH/SD) + (ShDB/RDB)}$$
(6)

in which DQI: Dickson quality index;

PH: plant height (cm); SD: stem diameter (mm); TDB: total dry biomass (g); ShDB: shoot dry biomass (g); and RDB: root dry biomass (g).

The tolerance of guava plants to salt stress during the seedling formation phase was measured based on the relative accumulation of total dry biomass per plant. The plateau model with a linear decrease, as proposed by Maas and Hoffman (1977), was employed. Model parameters were adjusted by minimizing the squared errors using the Microsoft Excel Solver tool, following Bione et al. (2021).

The data were subjected to analysis of variance using the F-test at a significance level of 0.05 and 0.01. In cases of significance, polynomial regression analysis was performed for water salinity levels and hydrogen peroxide concentrations using SISVAR software (Ferreira, 2019).

#### **Results and Discussion** \_\_\_\_

There was a significant impact of irrigation water salinity levels on the relative water content (RWC), water saturation deficit (WSD),  $CO_2$  assimilation rate, and water use efficiency (iWUE) of guava seedlings at 91 days after emergence (DAE). However, the concentrations of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and the interaction between salinity levels and H<sub>2</sub>O<sub>2</sub> did not have a significant effect on the evaluated variables. Leaf transpiration and intercellular  $CO_2$  concentration were not significantly influenced by the tested sources of variation.

The relative water content in the leaf blade showed a linear decrease with increasing water salinity (Figure 2A), at a rate of 3.03% per unit increment of electrical conductivity of the irrigation water (ECw). Comparing RWC in relative terms, there was a reduction of 12.24% in plants cultivated under an ECw of 4.3 dS m<sup>-1</sup> relative to those receiving water with a conductivity of 0.3 dS m<sup>-1</sup>. This reduction in leaf water content is attributed to the decrease in the osmotic

potential of the soil solution, which restricts water and nutrient uptake by the plants. The increase in the electrical conductivity of water also leads to stomatal closure, resulting in decreased transpiration and water absorption (G. S. de Lima et al., 2019b). Xavier et al. (2022a) reported similar findings in a study on guava seedlings under irrigation with saline water (ECw: 0.6 to 4.2 dS m<sup>-1</sup>), in which the salinity level of 4.2 dS m<sup>-1</sup> reduced RWC at 180 days after sowing.



**Figure 2.** Relative water content (RWC, A), water saturation deficit (WSD, B), net  $CO_2$  assimilation rate (*A*, C), and instantaneous water use efficiency (iWUE, D) of guava seedlings as a function of different irrigation water electrical conductivities (ECw) 91 days after emergence. \*, \*\* - significant at p ≤ 0.05 and at p ≤ 0.01 by the F test, respectively. The reduction in leaf water content resulted in a linear increase in the water saturation deficit of guava plants (Figure 2B), at a rate of 13.52% per unit increment of ECw. Plants exposed to the water salinity level of  $4.3 \, dS \, m^{-1}$  exhibited a 51.99% increase in WSD compared with those grown under an ECw of 0.3  $dS \, m^{-1}$ . This reflects the difficulty of water absorption by the roots due to osmotic stress and becomes a limiting factor for maintaining leaf water status. These findings support the observations of G. S. de Lima et al. (2019b), who reported that excessive sodium in irrigation water resulted in the greatest water saturation deficit in the castor bean crop.

Regarding the  $CO_2$  assimilation rate (Figure 2C) and iWUE (Figure D), the maximum estimated values of 21.71 and 7.01, respectively, were obtained under ECw levels of 1.20 and 1.10 dS m<sup>-1</sup> at 91 DAE. From these ECw levels, the values decreased, reaching minimum values of 13.35 and 4.44 under a water salinity of 4.2 dS m<sup>-1</sup>. This behavior of iWUE may be associated with a reduction in cellular osmotic potential, where water absorption and assimilation processes are altered in an attempt to maintain metabolic processes and repair damage caused by salinity (Xavier et al., 2022b). The  $CO_2$  assimilation rate can also be influenced by damage caused by oxidative stress (Silva et al., 2019).

Irrigation water salinity levels significantly affected the fluorescence (Fv) and quantum efficiency of photosystem II (Fv/Fm) of the guava plants. However, the concentrations of  $H_2O_2$  and the interaction between salinity levels and  $H_2O_2$  did not have a significant influence on the measured variables.

Variable fluorescence (Figure 3A) decreased linearly with increasing levels of electrical conductivity in the irrigation water. Specifically, fluorescence decreased by 3.11% per unit increase in ECw, resulting in a decline of 12.45% between plants subjected to ECw of 0.3 and 4.3 dS m<sup>-1</sup>. This decline in fluorescence can be attributed to the plant's reduced ability to absorb light energy, which directly affects electron flow in the transport chain (Dias et al., 2021).



**Figure 3.** Variable fluorescence (A) and quantum efficiency of photosystem II (Fv/Fm, B) of guava seedlings as a function of different irrigation water electrical conductivities (ECw) 91 days after emergence.

\*\* - significant at  $p \le 0.01$  by the F test.

Similarly, Fv/Fm (Figure 3B) decreased with increasing levels of ECw, exhibiting a reduction of 1.13% per unit increment of ECw. When comparing plants irrigated with water of 4.2 dS m<sup>-1</sup> and those subjected to the lowest ECw level (0.3 dS m<sup>-1</sup>), a decrease of 4.56% in Fv/Fm was observed. This reduction indicates damage to the photosynthetic apparatus caused by salinity, leading to compromised photosynthetic efficiency of leaves under ambient light conditions. The deleterious effect is attributed to lipid peroxidation as a response to the diversion of electron flux from CO<sub>2</sub> assimilation in plants (Andrade et al., 2022).

The salinity levels of irrigation water significantly affected the plant height (PH),

stem diameter (SD), number of leaves (NL), and leaf area (LA) of guava seedlings. However, the concentrations of  $H_2O_2$  and the interaction between salinity levels and  $H_2O_2$  did not have a significant effect on any of the measured variables.

Irrigation with saline water inhibited the growth of the guava plants in terms of PH (Figure 4A), SD (Figure 4B), NL (Figure 4C), and LA (Figura 4D) at 91 DAE. Plant height, SD, and NL decreased by 7.65, 4.89, and 6.03%, respectively, per unit increment in ECw. Plants subjected to the ECw of 4.2 dS m<sup>-1</sup> experienced reductions of 31.32, 19.86, and 24.59% in PH, SD, and NL, respectively, compared with those irrigated with an ECw of 0.3 dS m<sup>-1</sup>.



**Figure 4.** Plant height (A), stem diameter (B), number of leaves (C), and leaf area (D) of guava rootstocks as a function of different irrigation water electrical conductivities (ECw) 91 days after emergence.

\*, \*\* - significant at  $p \le 0.05$  and at  $p \le 0.01$  by the F test, respectively.

The decline in plant growth can be attributed to osmotic and ionic effects caused by excess of soluble salts in the root zone, resulting in decreased cell turgor and reduced cell expansion and growth as a consequence (G. S. de Lima et al., 2020a). Xavier et al. (2022b) also reported the negative effects of increasing electrical conductivity in irrigation water from 0.6 dS  $m^{-1}$  on the relative growth of PH and SD in guava plants during the seedling formation phase.

In terms of LA (Figure 4D), the maximum estimated value of 722.42 cm<sup>2</sup> was obtained in plants irrigated with an ECw of 0.6 dS m<sup>-1</sup>. In contrast, water salinity of 4.2 dS m<sup>-1</sup> resulted in a LA of 537.34 cm<sup>2</sup>. Comparing plants cultivated under ECw of 4.2 dS m<sup>-1</sup> with those subjected to the lowest water salinity level (0.6 dS m<sup>-1</sup>), a decrease of 185.07 cm<sup>2</sup> in LA was observed. The reduction of leaf area can be considered a tolerance mechanism aimed at reducing the transpiration area and maintaining the leaf water status of plants under salt stress. In addition, it provides a decrease in the absorption of water and toxic ions that would result in damage to essential biochemical processes (A. A. R. da Silva et al., 2021).

According to the results of the analysis of variance, irrigation water salinity levels had a significant effect on stem dry biomass (SDB), leaf dry biomass (LDB), root dry biomass (RDB), shoot dry biomass (ShDB), total dry biomass (TDB), and Dickson quality index (DQI) at 91 DAE. However, the concentrations of  $H_2O_2$  and the interaction between salinity levels and  $H_2O_2$  did not significantly affect any of the measured variables in the guava plants.

The accumulation of SDB (Figure 5A), LDB (Figure 5B), RDB (Figure 5C), ShDB (Figure 5D), and TDB (Figure 5E) decreased linearly with increasing water salinity levels, exhibiting reductions of 12.04, 9.89, 9.51, 10.79, and 10.52%, respectively. Guava plants grown under an ECw of 4.2 dS m<sup>-1</sup> had 50.0, 40.78, 39.16, 44.61, and 43.45% lower SDB, LDB, RDB, ShDB, and TDB, respectively, compared with those irrigated with water of 0.3 dS m<sup>-1</sup>.

The reduction in plant biomass accumulation under salt stress conditions can be attributed to osmotic effects and the redirection of energy to maintain vital minimize lipid peroxidation, activities. inhibit chlorophyll synthesis, and reduce the synthesis of reactive oxygen species through the activity of antioxidant enzymes (G. S. de Lima et al., 2020b). Changes in photoassimilate partitioning reflect а decrease in plant growth and, consequently, in biomass accumulation. Similar findings were reported by Xavier et al. (2022b), who observed decreases in stem, root, and total dry biomass of guava seedlings with increasing electrical conductivity of irrigation water from 0.6 dS m<sup>-1</sup>.

Irrigation with saline water also negatively affected the DQI of guava seedlings (Figure 5F), exhibiting a decrease of 9.78% per unit increment in ECw. In relative terms, there was a 40.32% reduction in DQI between plants grown under an ECw of 4.3 dS m<sup>-1</sup> and those irrigated with the lowest salinity level (0.3 dS m<sup>-1</sup>). Despite the decline in DQI with increasing ECw levels, water with an electrical conductivity of up to 4.3 dS m<sup>-1</sup> can still be used to produce guava seedlings with acceptable quality for transplanting in



the field, as long as the DQI remains above 0.28 (Dickson et al., 1960). The Dickson quality index is an integrated morphological

variable that reflects robustness, balance, and biomass distribution in the plant (G. S. de Lima et al., 2021).



**Figure 5.** Stem dry biomass (SDB, A), leaf dry biomass (LDB, B), root dry biomass (RDB, C), shoot dry biomass (ShDB, D), total dry biomass (TDB, E), and Dickson Quality Index (DQI, F) of guava seedlings as a function of different irrigation water electrical conductivities (ECw) 91 days after emergence.

\*\* - significant at  $p \le 0.01$  by the F test.

In a study on guava plants during the seedling formation phase under salt stress (ECw ranging from 0.6 to 4.2 dS m<sup>-1</sup>), Xavier et al. (2022b) reported a 75.29% decrease in DQI with increasing electrical conductivity of the water. The threshold salinity level for irrigation water in guava cv. Paluma, determined using the plateau model followed by linear decay (Maas & Hoffman, 1977), was found to be 0.3 dS m<sup>-1</sup>, with a decrease of 11.48% per unit increase above this salinity level (Figure 6). However, irrigation with water of 2.91 dS m<sup>-1</sup> electrical conductivity resulted in a relative total dry biomass yield of 70%, while using

water with an electrical conductivity of 4.65 dS m<sup>-1</sup> provided a relative total dry biomass production of 50%. Based on the tolerance level defined by Maas and Hoffman (1977), considering the relative biomass production and the decrease per unit increase above the threshold level, guava cv. Paluma is classified as sensitive to salinity during the seedling formation phase. It is important to emphasize that the tolerance level may vary depending on the species and/or cultivar, soil and climate conditions, as well as irrigation and fertilization management.



**Figure 6.** Relative total dry biomass yield of guava cv. Paluma plants as a function of irrigation water electrical conductivity (ECw) as obtained by the plateau model of Maas and Hoffman (1977).

### Conclusions

Water salinity at a level of 0.3 dS m<sup>-1</sup> has detrimental effects on the relative water content in the leaf blade, growth, and the Dickson quality index of guava seedlings. Additionally, it leads to an increase in the water saturation deficit observed in the seedlings 91 days after emergence.

Irrigation with water having a conductivity higher than 0.3 dS m<sup>-1</sup> negatively impacts the variable fluorescence and quantum efficiency of photosystem II of guava seedlings.

The application of peroxide, up to a concentration of 75  $\mu$ M, does not alleviate the negative effects of salt stress in guava seedlings of the Paluma cultivar.

Guava cv. Paluma is classified as sensitive to water salinity during the seedling formation phase, with a threshold salinity level of 0.3 dS  $m^{-1}$ . Furthermore, it exhibits a reduction of 11.48% per unit increase in electrical conductivity.

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