Ambiente & Água - An Interdisciplinary Journal of Applied Science
ISSN 1980-993X – doi:10.4136/1980-993X
www.ambi-agua.net
E-mail: ambi.agua@gmail.com

Gas exchange, growth and quality of guava seedlings under salt stress and salicylic acid

ARTICLES doi:10.4136/ambi-agua.2816

Received: 23 Nov. 2021; Accepted: 18 Apr. 2022

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ABSTRACT

Guava is a popular Brazilian fruit that is widely produced in Northeastern Brazil, a region with water sources that commonly have high concentrations of salts. Thus, searching for techniques that allow the management of these waters is extremely important for the expansion of irrigated agriculture. In this context, salicylic acid is a phytohormone that can contribute to reducing the effects of salt stress on plants. Given the above, this study evaluated the effect of foliar application of salicylic acid at different concentrations in the mitigation of salt stress on gas exchange, growth, and quality of 'Paluma' guava seedlings. The experiment was conducted in a greenhouse, in Campina Grande - PB, Brazil, using a randomized block design in a 5 × 5 factorial arrangement, corresponding to five levels of electrical conductivity of water (0.6, 1.5, 2.4, 3.3, and 4.2 dS m^{-1}) and five concentrations of salicylic acid (0 - Control; 0.8, 1.6, 2.4, and 3.2 mM), with four replicates and two plants per plot. Foliar application of salicylic acid at a concentration of up to 1.4 mM reduced the deleterious effects of salt stress on the instantaneous water use efficiency of 'Paluma' guava seedlings at 180 days after sowing. The concentrations of salicylic acid applied via foliar did not mitigate the harmful effects of irrigation water salinity on the growth and quality of 'Paluma' guava seedlings.

Keywords: Abiotic stress, *Psidium guajava* L., water scarcity.

Trocas gasosas, crescimento e qualidade de mudas de goiabeira sob estresse salino e ácido salicílico

RESUMO

A goiaba é uma fruta de grande aceitação pelos brasileiros e é largamente produzida no Nordeste do Brasil, região com a presença de fontes de água que comumente apresentam elevadas concentrações de sais. Assim, a busca por técnicas que permitam o manejo dessas águas é de extrema importância para expansão da agricultura irrigada. Neste contexto, o ácido salicílico é um fitohormônio que pode contribuir na diminuição dos efeitos do estresse salino nas plantas. Diante do exposto, objetivou-se com este estudo avaliar o efeito da aplicação foliar de ácido salicílico em diferentes concentrações na mitigação do estresse salino sobre as trocas gasosas, o crescimento e a qualidade de mudas de goiabeira 'Paluma'. O experimento foi conduzido em casa de vegetação, em Campina Grande – PB, utilizando-se o delineamento de blocos casualizados em arranjo fatorial 5 × 5, sendo cinco níveis de condutividade elétrica da água (0,6; 1,5; 2,4; 3,3 e 4,2 dS m^{-1}) e cinco concentrações de ácido salicílico (0 - Controle; 0,8; 1,6; 2,4 e 3,2 mM), com quatro repetições e duas plantas por parcela. A aplicação foliar de ácido salicílico na concentração de até 1,4 mM reduziu os efeitos deletérios do estresse salino sobre a eficiência instantânea no uso da água das mudas de goiabeira 'Paluma' aos 180 dias após o semeio. As concentrações de ácido salicílico aplicadas via foliar não mitigaram os efeitos nocivos da salinidade da água de irrigação sobre o crescimento e a qualidade das mudas de goiabeira 'Paluma'.

Palavras-chave: escassez hídrica, estresse abiótico, *Psidium guajava* L.

1. INTRODUCTION

Guava (*Psidium guajava* L.) is a tropical fruit found throughout Brazil, with emphasis on the cultivar Paluma for the great acceptance of its fruit by consumers, being consumed *in natura* or as processed products (Montes *et al.*, 2016). The fruit is easily found in open markets and supermarkets because it is the most cultivated in Brazil (Manica *et al.*, 2001; Dias *et al.*, 2012). The Northeast and Southeast regions of Brazil stand out as the largest guava producers in the country, respectively, accounting for 47.95 and 40.56% of the 22,128 hectares harvested. The state of Paraíba is responsible for 3.01% of guava production in the Northeast (IBGE, 2019).

The semi-arid region of Northeastern Brazil is characterized by high evapotranspiration rates, irregular rainfall and inadequate soil drainage, and well water most often has an electrical conductivity greater than 1.5 dS m^{-1}, standing out as a limiting factor for the production of various crops (Bezerra *et al.*, 2019). The salinity of irrigation water causes damage to agricultural production, inhibiting crop growth due to the reduction in water availability to plants because of a decrease in the osmotic potential of the soil solution, leading to stomatal closure and compromising transpiration and photosynthesis (Dias *et al.*, 2019).

Given the growing need to increase irrigated area studies that enable the use of saline water sources have become essential, especially in semi-arid regions (Silva *et al.*, 2021a). Thus, the use of elicitor substances such as salicylic acid (SA) has emerged as a promising alternative to minimize the harmful effects caused by biotic and abiotic stresses, including salinity (Nazar *et al.*, 2015).

Salicylic acid is a phenolic compound and acts as a growth regulator, playing an exclusive role in several physiological and biochemical processes, such as plant growth, floral induction, stomatal opening and closing, ion absorption, photosynthesis, and transpiration (Jini and Joseph, 2017; Silva *et al.*, 2020). Treatment with SA also reduces lipid peroxidation and may interact with other plant hormones to increase plant tolerance to salt stress (Souana *et al.*, 2020).

Some studies have reported that the exogenous application of SA increases the tolerance to salt stress in soursop (Silva *et al.*, 2020), almond (Mohammadi *et al.*, 2020), West Indian cherry (Dantas *et al.*, 2021), and tomato (Silva *et al.*, 2022). However, information on the use of salicylic acid in the production of guava rootstock under irrigation with saline water in the semi-arid region of the Northeast is scarce. In this context, this study evaluated the effect of foliar application of salicylic acid at different concentrations in mitigating the deleterious effects of salt stress on gas exchange, growth, and quality of 'Paluma' guava seedlings.

2. MATERIAL AND METHODS

The experiment was conducted from October 2020 to April 2021 in a greenhouse belonging to the Academic Unit of Agricultural Engineering of the Federal University of Campina Grande (UFCG), in Campina Grande, PB, Brazil (07°15'18" S latitude, 35°52'28" W longitude and an average altitude of 550 m). The greenhouse used was of the arch type, 30 m long and 21 m wide, with a ceiling height of 3.0 m, with a low-density polyethylene cover (150 microns). The data of air temperature (maximum and minimum) and mean relative humidity of air during the experimental period are presented in Figure 1.

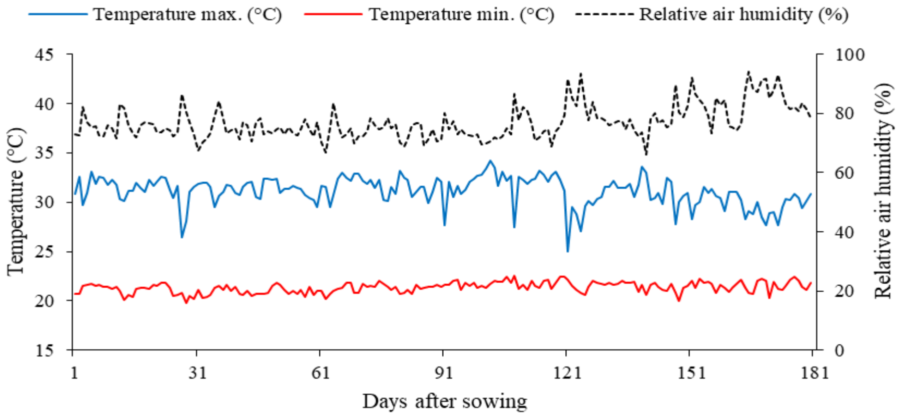

Figure 1. Mean values of air temperature (maximum and minimum) and relative humidity of air observed in the internal area of the greenhouse during the experimental period.

Treatments consisted of the combination of five levels of electrical conductivity of irrigation water - ECw (0.6 – Control, 1.5, 2.4, 3.3, and 4.2 dS m^{-1}) and five concentrations of salicylic acid - SA (0 – Control, 0.8, 1.6, 2.4, and 3.2 mM), distributed in randomized blocks in a 5 × 5 factorial arrangement with four replicates and two plants per plot. The levels of electrical conductivity of irrigation water were established considering the study conducted by Bezerra *et al.* (2019). The concentrations of SA were adapted according to Silva *et al.* (2020).

'Paluma' was the guava cultivar used in the experiment. It is a vigorous cultivar with easy propagation and tolerance to pests and diseases, especially rust (*Puccinia psidii* Wint.). The seeds used in the experiment were obtained from a guava orchard located in the experimental area of the Center of Science and Agri-Food Technology (CCTA), at the Pombal Campus belonging to UFCG, being manually extracted from the fruit pulp and subsequently air-dried in an open environment.

Irrigation waters with different electrical conductivities were prepared by dissolving NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ in local-supply water (ECw= 0.28 dS m^{-1}) following the equivalent ratio commonly found in the Brazilian Northeast of 7:2:1 for Na^+, Ca^{2+}, and Mg^{2+} (Medeiros, 1992); the quantities of salts were determined considering the relationship between ECw and the salt concentration (Richards, 1954), according to Equation 1.

$$Q = 10 \, x \, ECw \quad (1)$$

Where:

Q = Quantity of salts to be added ($mmol_c \, L^{-1}$);

ECw = Electrical conductivity of water (dS m^{-1})

Salicylic acid concentrations were obtained by dissolving the acid in 30% ethyl alcohol. The solution was always prepared on the days of biweekly application events, with the addition of the Wil fix spreader (0.5 mL L^{-1}) to assist in the fixation of SA on the leaves by breaking the surface tension. Spraying on the adaxial and abaxial sides was performed with a manual sprayer between 17:00 and 18:00 hours. To minimize the evaporation of the solution from the leaf surface, each plant before application was removed from the proximity of the others for spraying, avoiding cross-application of different concentrations of SA in each plot and returned to its location after spraying. Throughout the assay, a total of eight spraying operations were carried out with an average volume of 50 mL of SA applied per plant in each event.

The seedlings were grown in plastic bags with dimensions of 10 × 20 cm, filled with 1.6 kg of the substrate in the proportion of 3:1 (v/v basis) of a soil classified as *Neossolo Regolítico* (Entisol) with sandy loam texture, from the municipality of Lagoa Seca, PB, collected at 0-20 cm depth (A horizon), whose chemical and physical characteristics are shown in Table 1.

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

Chemical characteristics								
pH (H_2O)	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
(1:2.5)	(g dm^{-3})	(mg dm^{-3})	cmol$_c$ kg^{-1}					
6.50	8.10	79.00	0.24	0.51	14.90	5.40	0.00	0.90
Chemical characteristics				Physical characteristics				
EC_{se}	CEC	SAR_{se}	ESP	Particle-Size Fraction (g kg^{-1})			Moisture (dag kg^{-1})	
(dS m^{-1})	(cmol$_c$ kg^{-1})	(mmol L^{-1})$^{0.5}$		Sand	Silt	Clay	FC[1]	PWP[2]
2.15	21.44	0.16	3.08	572.7	100.7	326.6	25.91	12.96

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+} and H^+ extracted with 0.5 M calcium acetate at pH 7.0; ESP- Exchangeable sodium percentage; ECse – Electrical conductivity of saturation extract; SAR_{se} – Sodium adsorption ratio of soil saturation extract; [1]Field capacity tension of 33.42 kPA; [2]Permanent wilting point tension of 1519.50 kPa.

On the day prior to sowing, the soil moisture content was increased to the level corresponding to the maximum retention capacity with water of lowest level of electrical conductivity (ECw = 0.6 dS m^{-1}). Sowing was performed by placing 4 seeds per bag distributed equidistantly at a depth of 2 cm. After sowing, irrigation was carried out daily at 16 h, applying in each bag the volume corresponding to that obtained by the water balance, determined by Equation 2:

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

Where:

VI - volume of water to be used in the next irrigation event (mL);

Va - volume applied in the previous irrigation event (mL);

Vd - volume drained (mL); and

LF = leaching fraction (0.15).

Seedling emergence began at 20 days after sowing (DAS). After establishment of emergence, fertilization with nitrogen, phosphorus and potassium began as recommended by Novais *et al.* (1991), applying the equivalent to 100, 300, and 150 mg kg-1 of soil of N, P_2O_5, and K_2O, respectively, with the local-supply water (0.28 dS m^{-1}) split into nine applications from 40 DAS at intervals of 15 days up to 160 DAS. Applications with micronutrients were performed at the concentration of 2.5 g of Ubyfol® L^{-1} [(N (15%); P_2O_5 (15%); K_2O (15%);

Ca (1%); Mg (1.4%); S (2.7%); Zn (0.5%); B (0.05%); Fe (0.5%); Mn (0.05%); Cu (0.5%); Mo (0.02%)] through the leaves on their adaxial and abaxial sides, applied every two weeks to meet micronutrient needs.

Salicylic acid concentrations began to be applied at 67 DAS, when the plants showed uniform growth, and the other applications were performed every two weeks until 165 DAS. Irrigation with the different levels of water salinity started at 75 DAS, at daily intervals.

Plant growth was evaluated at 104 and 180 DAS through stem diameter (SD), measured with a digital caliper, plant height (PH), measured with a graduated ruler, and total leaf area (LA), obtained according to Lima *et al.* (2012) as shown in Equation 3:

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

Where:

LA = total leaf area (cm^2);

L = leaf midrib length (cm).

The relative growth rates in plant height (RGR_{PH}), stem diameter (RGR_{SD}) and leaf area (RGR_{LA}) in the period from 104 to 180 DAS were obtained according to Benincasa (2003), as shown in Equation 4:

$$RGR = \frac{(lnA_2 - lnA_1)}{(t_2 - t_1)} \quad (4)$$

Where:

RGR = relative growth rate;

A_1 = plant growth at time t_1;

A_2 = plant growth at time t_2;

$t_2 - t_1$ = time difference between evaluations; and

ln = natural logarithm.

At 180 DAS, gas exchange was measured through 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}), internal CO_2 concentration – *Ci* (μmol m^{-2} s^{-1}), instantaneous carboxylation efficiency - *CEi* [(μmol m^{-2} S^{-1}) (μmol mol^{-1})$^{-1}$] and instantaneous water use efficiency - *WUEi* [(μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]. Gas exchange was determined on the third leaf counted from the apex using the portable photosynthesis meter LCPro+ from ADC BioScientific Ltda. The obtained data were then used to quantify water use efficiency (*WUEi*) (A/E) [(μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$] and instantaneous carboxylation efficiency (*CEi*) (A/Ci) [(μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$].

In the last evaluation (at 180 DAS), dry mass of leaf (LDM), stem (StDM), root (RDM), total dry mass (TDM) and Dickson quality index (DQI) of the seedlings were evaluated. The dry mass accumulation of each plant was obtained by oven drying for 48 hours and subsequent weighing on a semi-analytical scale, and TDM was obtained by the sum of dry mass of leaf, stem and root. Shoot dry mass (ShDM) was quantified by the sum of LDM and StDM.

Seedling quality was determined using the Dickson Quality Index - DQI (Dickson *et al.*, 1960), according to Equation 5:

$$DQI = (TDM)/[\left(\frac{PH}{SD}\right) + \left(\frac{ShDM}{RDM}\right)] \quad (5)$$

Where:

DQI= Dickson quality index;
PH = plant height (cm);
SD = stem diameter (mm);
TDM = total dry mass (g per plant),
ShDM = shoot dry mass (g per plant); and
RDM = root dry mass (g per plant).

The data obtained were subjected to analysis of variance by the F test, and when significance was observed, linear and quadratic polynomial regression analysis was performed for water salinity levels and salicylic acid concentrations ($p \leq 0.05$), using the statistical program SISVAR-ESAL version 5.6 (Ferreira, 2019).

3. RESULTS AND DISCUSSION

The interaction between saline levels (NS) and salicylic acid concentrations significantly influenced transpiration (E), instantaneous carboxylation efficiency (*CEi*), and instantaneous water use efficiency (*WUEi*), at 180 DAS (Table 2). On the other hand, saline levels significantly affected ($p \leq 0.01$) all gas exchange variables, except *WUEi*. The salicylic acid concentrations analyzed in isolation did not significantly influence the gas exchange variables of 'Paluma' guava.

Table 2. Summary of the analysis of variance for stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), CO_2 assimilation rate (*A*), transpiration (*E*), instantaneous carboxylation efficiency (*CEi*) and instantaneous water use efficiency (*WUEi*) of ‘Paluma’ guava irrigated with different levels of salinity and subjected to exogenous application of salicylic acid at 180 days after sowing.

Source of variation	DF	Mean squares					
		gs	*Ci*	*A*	*E*	*CEi*	*WUEi*
Salinity level (SL)	4	0.004^{**}	4094.12^{*}	7.04^{*}	1.30^{**}	0.000248^{**}	0.98^{ns}
Linear regression	1	0.009^{**}	10522.22^{*}	2.90^{ns}	3.25^{**}	0.000165^{*}	1.91^{ns}
Quadratic regression	1	0.004^{**}	1487.14^{ns}	13.37^{*}	1.35^{**}	0.000477^{**}	1.38^{ns}
Salicylic acid (SA)	4	0.001^{ns}	2427.70^{ns}	0.49^{ns}	0.06^{ns}	0.000016^{ns}	0.53^{ns}
Linear regression	1	0.000^{ns}	582.60^{ns}	0.00^{ns}	0.21^{ns}	0.000014^{ns}	0.24^{ns}
Quadratic regression	1	0.002^{*}	6528.32^{*}	0.68^{ns}	0.01^{ns}	0.000008^{ns}	1.21^{ns}
Interaction (SL × SA)	16	0.001^{ns}	1181.05^{ns}	2.41^{ns}	0.14^{*}	0.000078^{**}	1.99^{*}
Blocks	3	0.001^{*}	3901.04^{ns}	1.83^{ns}	0.04^{ns}	0.000054^{ns}	1.55^{ns}
Residue	72	0.000	1653.02	2.33	0.07	0.000027	1.01
CV %		38.63	14.64	48.10	21.92	38.39	39.14

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

The stomatal conductance of 'Paluma' guava seedlings was negatively affected by the increase in the electrical conductivity of the irrigation water up to 3.2 dS m^{-1} (Figure 2A). It is observed that plants irrigated with ECw of 3.2 dS m^{-1} obtained the lowest gs value (0.0488 mol H_2O m^{-2} s^{-1}), corresponding to a reduction of 40.0% (0.0325 mol H_2O m^{-2} s^{-1}), in relation to plants irrigated with ECw of 0.6 dS m^{-1}. Stomatal closure with the increase in ECw is a response to the osmotic stress caused by excess salts, being an important strategy against dehydration, maintaining a high cell water potential (Dias *et al.*, 2020). Reduction in stomatal conductance of plants due to the increase in ECw was also observed by Dias *et al.* (2019), in a study with West Indian cherry cv. 'BRS 366 Jaburu' subjected to water salinity (0.8 and 3.8 dS m^{-1}).

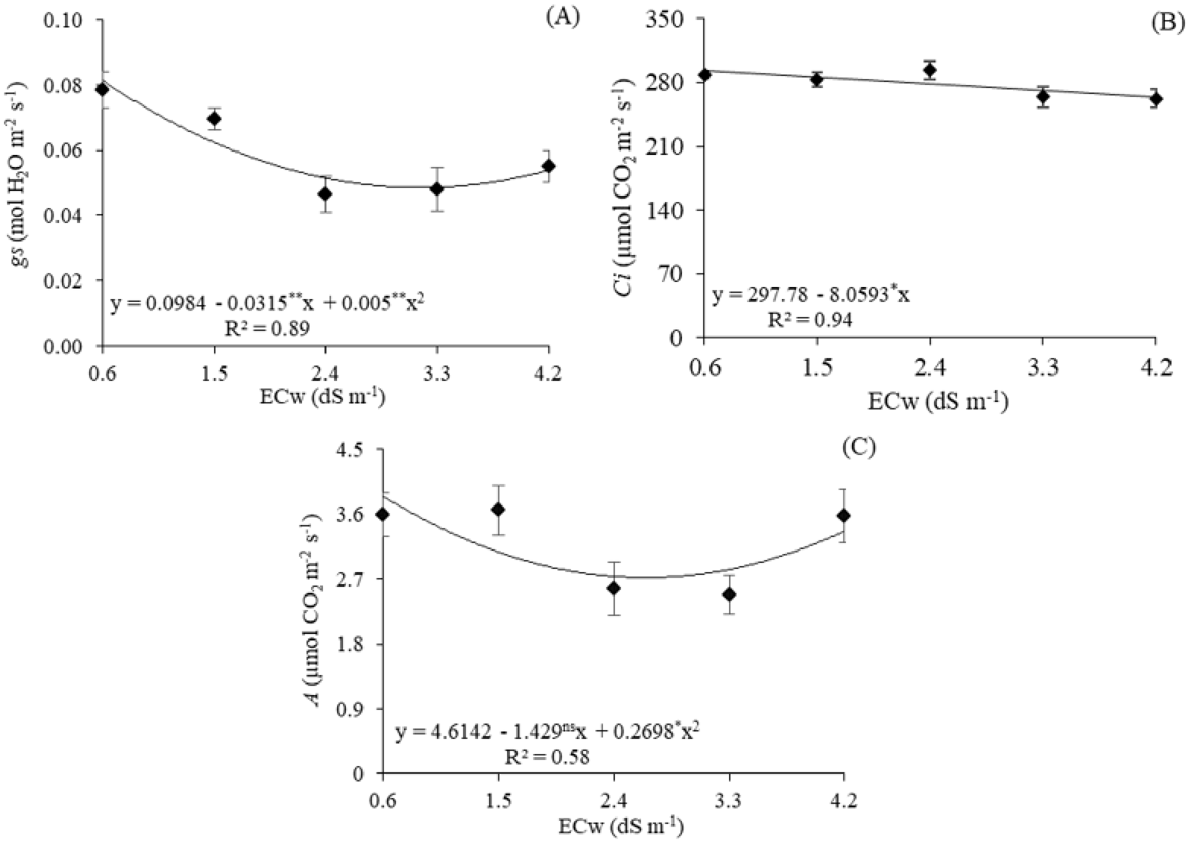

Figure 2. Stomatal conductance - *gs* (A), internal CO_2 concentration - *Ci* (B), and CO_2 assimilation rate - *A* (C) of 'Paluma' guava seedlings, as a function of the levels of irrigation water salinity - ECw and exogenous application of salicylic acid, at 180 days after sowing.
Vertical bar represents the standard error of the mean (n=4); *, ** respectively not significant, significant at $p \leq 0.05$ and $p \leq 0.01$.

The internal CO_2 concentration of the 'Paluma' guava was linearly reduced with the increase in the electrical conductivity of the irrigation water (Figure 2B), with a reduction of 2.71% per unit increase in ECw. When comparing the Ci of plants irrigated with water of higher salinity (4.2 dS m^{-1}) to those cultivated under the lowest salinity level (0.6 dS m^{-1}), a reduction of 10.0% is observed (29.01 µmol CO_2 m^{-2} s^{-1}). Corroborating the present study, Lacerda *et al.* (2022) in a study carried out with 'Paluma' guava under saline stress (0.6 and 3.2 dS m^{-1}) found a reduction of 9.51% (25.43 µmol CO_2 m^{-2} s^{-1}) in the Ci of plants irrigated with ECw of 3.2 dS m^{-1} compared to those cultivated under ECw of 0.6 dS m^{-1}. The reduction in *Ci* can be seen as a consequence of stomatal closure and is one of the main mechanisms responsible for the reduction in CO_2 assimilation rate (Lima *et al.*, 2021).

Analyzing the regression equation in Figure 2C, referring to the CO_2 assimilation rate, it appears that the guava seedlings had a reduction in A when irrigated with ECw of up to 2.65 dS m^{-1}. When comparing seedlings irrigated with ECw of 2.65 dS m^{-1} to plants cultivated under ECw of 0.6 dS m^{-1}, a reduction of 25.3% (0.92 µmol CO_2 m^{-2} s^{-1}) was observed. Reduction in CO_2 assimilation rate is directly related to stomatal closure, leading to a consequent reduction in leaf transpiration and a decrease in the internal CO_2 concentration in leaves (Altuntas *et al.*, 2018). Another factor to be considered that leads to reduction in *A* is the increase in mesophyll resistance to the entry of atmospheric CO_2 caused by salinity, which can also reduce enzymatic activities that are related to photosynthetic carbon metabolism (Soares *et al.*, 2021).

The increase in salicylic acid concentrations increased the transpiration of 'Paluma' guava seedlings when irrigated with ECw of up to 3.7 dS m^{-1} (Figure 3A). However, the maximum value of E (1.78 mmol H_2O m^{-2} s^{-1}) was recorded in plants irrigated with ECw of 0.6 dS m^{-1} and sprayed with a concentration of 3.2 mM of SA, corresponding to an increase of 21.9% (0.39 mmol H_2O m^{-2} s^{-1}) compared to those grown under the same ECw (0.6 dS m^{-1}) and without SA application (0 mM). Similar results were obtained by Silva *et al.* (2021a) in soursop plants under saline stress (ECw ranging from 0.8 to 4.0 dS m^{-1}), the authors found that foliar application of salicylic acid at a concentration of 1.4 mM promoted an increase in E regardless of the electrical conductivity of irrigation water.

According to Dantas *et al.* (2021), the increase in transpiration due to the application of salicylic acid may be related to its role in the regulation of stomatal opening, promoting the entry of water and CO_2 into the cells. In addition, under stressful conditions, salicylic acid helps to protect and increase the activity of antioxidant enzymes, increasing plant tolerance (Rajeshwari and Bhuvaneshwari, 2017).

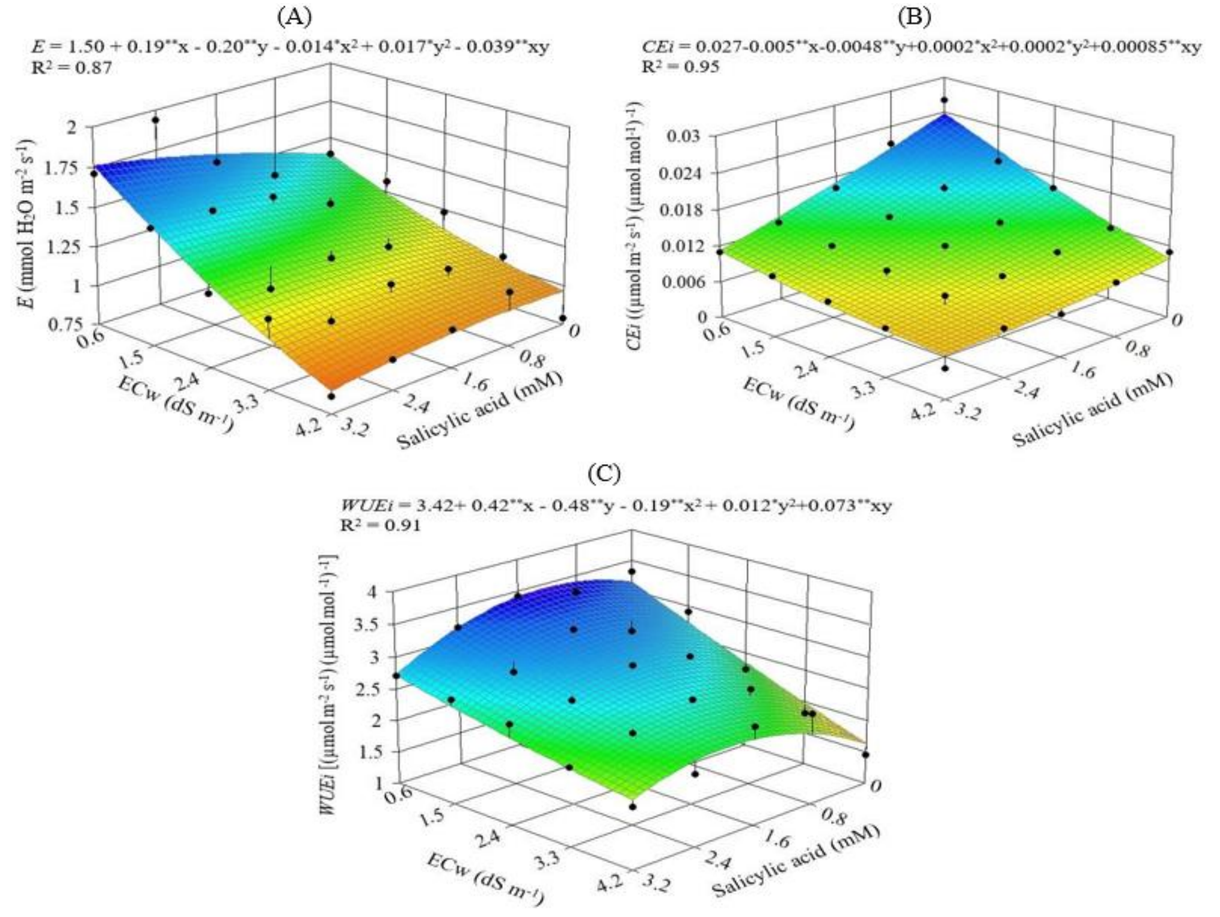

Figure 3. Response surface for transpiration - *E* (A), instantaneous carboxylation efficiency -*CEi* (B) and instantaneous water use efficiency - *WUEi* (C) as a function of the interaction between water salinity - ECw and salicylic acid - SA concentrations in guava cv. 'Paluma' at 180 days after sowing. X and Y - SA concentration and ECw, respectively; *, ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. Point and vertical lines represent the mean and the standard error (n=4).

The instantaneous efficiency of carboxylation was negatively affected by the increase in the electrical conductivity of the irrigation water, regardless of the salicylic acid concentration.

The guava plants irrigated with ECw of 0.6 dS m^{-1} and submitted to a concentration of 0 mM of SA, stood out with the highest *CEi* value [0.0242 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$]. The lowest *CEi* value [0.0078 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] was recorded in plants irrigated with ECw of 4.2 dS m^{-1} and subjected to a concentration of 3.2 mM SA. The decrease in instantaneous carboxylation efficiency due to salt stress may be associated with metabolic restrictions in the Calvin cycle and the occurrence of non-stomatal factors that act on the photosynthetic apparatus, such as inhibition of RuBisCO enzyme activity due to the reduction in the availability of ATP and NADPH (Lima *et al.*, 2020).

Foliar application of SA up to an estimated concentration of 1.4 mM promoted an increase in *WUEi*, regardless of the electrical conductivity of the irrigation water (Figure 3C). According to the regression equation, it appears that plants irrigated with ECw of 0.6 dS m^{-1} and submitted to a concentration of 1.4 mM of SA reached the highest *WUEi* value [3.42 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$], corresponding to an increase of 8.9% [0.28 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] in relation to plants irrigated with the same ECw and without SA application (0 mM). On the other hand, the lowest *WUEi* value [1.62 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] was obtained from plants irrigated with ECw of 4.2 dS m^{-1} and without application of SA (0 mM).

Agami *et al.* (2019), in research carried out with wheat plants under water stress, also found that salicylic acid (0.1 mM) was able to increase the efficiency of water use, even in plants under stress. Salicylic acid is an endogenous phenolic-type regulator, which regulates the physiological and biochemical processes of plants to alleviate the deleterious effects caused by various stresses, including saline stress (Ghassemi-Golezani *et al.*, 2018).

According to the summary of the analysis of variance (Table 3), it can be seen that the interaction between the factors under study (SL × SA) did not significantly affect any of the analyzed variables. The saline levels analyzed in isolation significantly influenced all the variables under study. Furthermore, salicylic acid concentrations promoted a significant effect ($p \leq 0.05$) for RDM, TDM, and DQI.

The relative growth rates in plant height (RGR_{PH}), stem diameter (RGR_{SD}), and leaf area (RGR_{LA}) were negatively affected by the increase in the electrical conductivity of the irrigation water (Figure 4). A decreasing linear effect can be observed, with decreases per unit increment of ECw of 2.16, 9.09, and 6.15% in RGR_{PH}, RGR_{SD}, and RGR_{LA}, respectively. Comparison of 'Paluma' guava seedlings irrigated with an ECw of 4.2 dS m^{-1} with those grown under an ECw of 0.6 dS m^{-1}, indicated a reduction of 7.87% (0.0011 cm cm^{-1} day^{-1}) in the RGR_{PH}, 34.62% (0.0036 nm mm^{-1} day^{-1}) in the RGR_{SD} and 23% (0.0043 cm^2 cm^{-2} day^{-1}) in the RGR_{LA}, in the period from 104 to 180 DAS.

Similar results were observed by Bezerra *et al.* (2018a) in a study carried out with 'Paluma' guava under saline stress (ECw ranging from 0.3 to 3.5 dS m^{-1}), where they found that the increase in the electrical conductivity of irrigation water negatively affected the absolute and relative growth rates of plants. Reduction in growth of plant height and stem diameter is the result of changes in soil water potential caused by excess salts, which restricts water absorption, decreasing turgor pressure and cell activity of plants, by inhibiting cell expansion and elongation (Lopes *et al.*, 2019). Reduction in leaf area (Figure 4C) can be considered a mechanism to protect plants from salt stress, since it leads to a decrease in the absorption of water and toxic ions that would result in damage to essential biochemical processes (Dias *et al.*, 2020).

The increase in water salinity negatively affected the dry mass of leaf and stem accumulation of guava plants (Figure 5A and 5B), the reductions were 14.23% and 15.72% per unit increase, respectively. When comparing the LDM and StDM of plants irrigated with the highest salinity level (ECw=4.2 dS m^{-1}) to those of plants subjected to ECw of 0.6 dS m^{-1}, there were reductions of 56.01% and 62.48%, respectively.

Table 3. Summary of the analysis of variance for relative growth rates in plant height (RGR_{PH}), stem diameter (RGR_{SD}), and leaf area (RGR_{LA}) during the period 104 to 180 days after sowing (DAS), and dry mass of leaf (LDM), stem (StDM), and root (RDM), total dry mass (TDM) and Dickson quality index (DQI) of ‘Paluma’ guava plants irrigated with different levels of salinity and subjected to exogenous application of salicylic acid, at 180 days after sowing.

Source of variation	DF	Mean squares							
		RGR_{PH}	RGR_{SD}	RGR_{LA}	LDM	StDM	RDM	TDM	DQI
Salinity levels (SL)	4	0.000014^{**}	0.000047^{**}	0.000082^{*}	34.70^{**}	44.22^{**}	43.50^{**}	362.98^{**}	3.09^{**}
Linear regression	1	0.000019^{*}	0.000174^{**}	0.000232^{**}	137.35^{**}	173.99^{**}	671.79^{**}	1407.20^{**}	11.47^{**}
Quadratic regression	1	0.000000^{ns}	0.000001^{ns}	0.000008^{ns}	0.15^{ns}	1.39^{ns}	31.10^{**}	18.36^{ns}	0.47^{**}
Salicylic acid (SA)	4	0.000005^{ns}	0.000003^{ns}	0.000052^{ns}	2.43^{ns}	2.27^{ns}	0.66^{*}	14.04^{*}	0.07^{*}
Linear regression	1	0.000001^{ns}	0.000011^{*}	0.000132^{*}	0.65^{ns}	2.84^{ns}	2.02^{ns}	10.12^{ns}	0.01^{ns}
Quadratic regression	1	0.000017^{*}	0.000001^{ns}	0.000067^{ns}	4.89^{**}	1.54^{ns}	7.51^{**}	22.88^{*}	0.24^{**}
Interaction (SL × SA)	16	0.000002^{ns}	0.000003^{ns}	0.000043^{ns}	1.06^{ns}	0.69^{ns}	0.33^{ns}	4.29^{ns}	0.03^{ns}
Blocks	3	0.000024^{**}	0.000062^{**}	0.000025^{**}	4.77^{**}	14.07^{**}	1.13^{**}	25.77^{**}	0.01^{ns}
Residue	72	0.000004	0.000002	0.000025	1.09	1.02	0.24	4.19	0.02
CV %		14.81	17.00	30.11	24.51	24.68	18.70	20.20	19.25

^{ns}, *, ** respectively not significant, significant at $p \leq 0.05$ and $p \leq 0.01$; DF - degree of freedom; CV (%) - coefficient of variation.

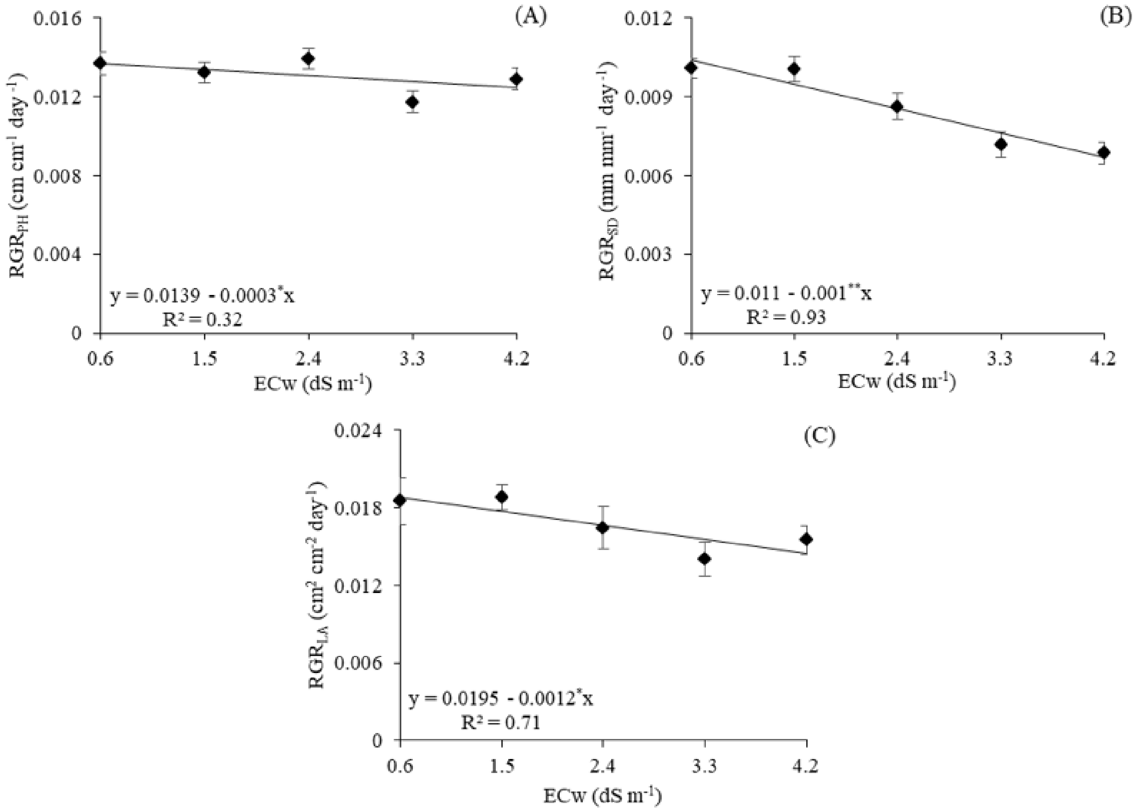

Figure 4. Relative growth rates in plant height - RGR_{PH} (A), stem diameter - RGR_{SD} (B) and leaf area - RGR_{LA} (C) of 'Paluma' guava, as a function of water salinity levels - ECw, during the period 104 to 180 days after sowing.
Vertical bar represents the standard error of the mean (n=4); *, ** respectively, significant at $p \leq 0.05$ and $p \leq 0.01$.

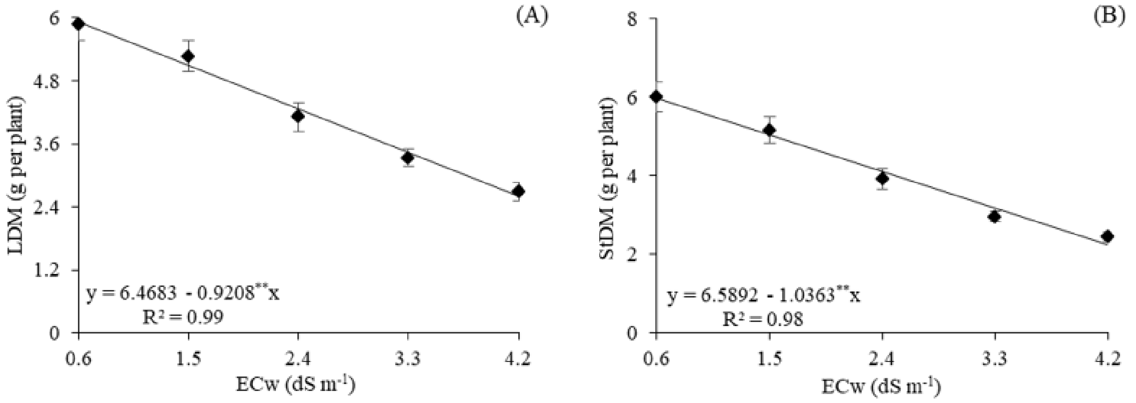

Figure 5. Dry mass of leaf - LDM (A) and stem - StDM (B) of 'Paluma' guava plants, as a function of water salinity levels - ECw, at 180 days after sowing.
Vertical bar represents the standard error of the mean (n=4); **, significant at $p \leq 0.01$.

The energy expenditure for maintaining metabolic activities induces changes in plant growth, as observed through the reduction in RGR_{PH}, RGR_{SD} and RGR_{LA}; and when plants absorb water with excess salts (mainly Na^+ and Cl^-), these ions get accumulated in cell tissues, causing stomatal closure, reduction in gas exchange and damage to photosynthetic apparatus, which results in lower CO_2 assimilation, nutritional imbalances, decrease in turgor, and reduction in cell expansion and division, causing lower growth and consequent biomass accumulation (Bonacina *et al.*, 2022).

Bezerra *et al.* (2018b) in a study evaluating the growth of grafted plants of 'Paluma' guava subjected to different levels of irrigation water salinity (ECw between 0.3 and 3.5 dS m^{-1}) and doses of nitrogen fertilization (70, 100, 130, and 160% of the recommended dose for the crop), found that salinity in irrigation water negatively affected the leaf area, stem diameter, and shoot dry mass of 'Paluma' guava plants.

The salinity of irrigation water caused reduction in the accumulation of root dry mass (Figure 6A), with decreases of 19.95% per unit increment in ECw. When the RDM of plants grown under electrical conductivity of 4.2 dS m^{-1} was compared to that of plants under the lowest salinity level (0.6 dS m^{-1}), there was a reduction of 81.65% (3.56 g per plant). It is also observed that water salinity caused a reduction in the total dry mass (Figure 6B), 16.32% per unit increase in ECw, and the TDM of plants irrigated with water of highest salinity (4.2 dS m^{-1}) was reduced by 65.17% (10.61 g per plant) compared to control treatment (0.6 dS m^{-1}).

The reduction in biomass may be associated with the deleterious effects of salinity on plants, which reduces water absorption capacity and causes immediate interference in CO_2 assimilation processes and energy diversion to other processes, such as osmotic adjustment, maintenance of basic metabolic processes and repair of damage caused by salt stress (Silva *et al.*, 2021b). In a study conducted by Souza *et al.* (2017), evaluating the influence of irrigation with different salinity levels (ECw between 0.3 and 3.5 dS m^{-1}) on 'Paluma' guava rootstock, the authors also verified a linear reduction in the RDM of plants with the increase in irrigation ECw.

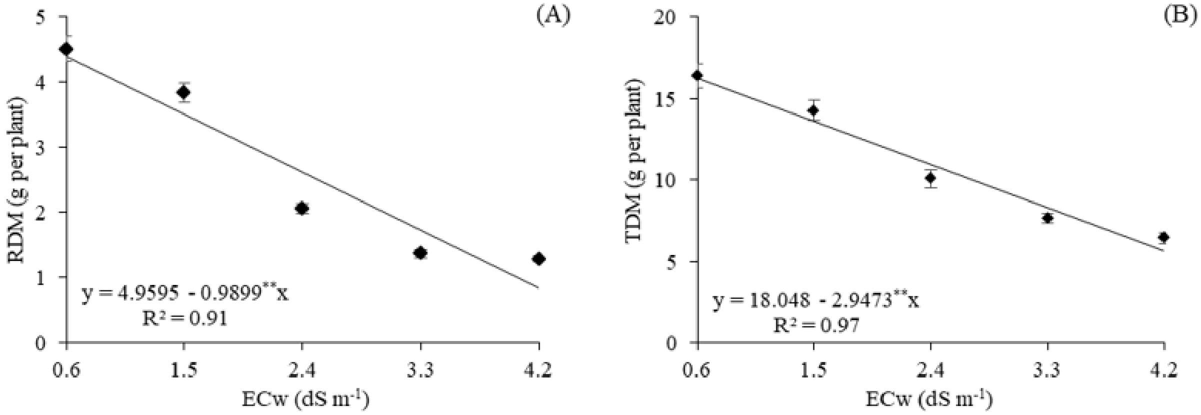

Figure 6. Root dry mass - RDM (A) and total dry mass - TDM (B) of 'Paluma' guava seedlings, as a function of water salinity levels - ECw, at 180 days after sowing.
Vertical bar represents the standard error of the mean (n=4); **, significant at $p \leq 0.01$.

Salicylic acid concentrations caused a significant effect on the RDM and TDM (Figure 7A and 7B) of 'Paluma' guava seedlings. According to the regression equation (Figure 7A), plants that did not receive exogenous application of SA (0 mM) obtained the highest value of RDM (2.87 g per plant), while those subjected to SA concentration of 1.84 mM had the lowest value (2.45 g per plant). Salicylic acid concentrations negatively influenced the production of total dry mass (Figure 7B). Plants subjected to the highest concentration of salicylic acid (3.2 mM) reduced their TDM by 66.13% (10.92 g per plant) compared to those in the control treatment (0 mM).

Salicylic acid is an important compound capable of mitigating the effects of salt stress and is present in several physiological processes of plants (Silva *et al.*, 2018). In the present study, the negative effect of SA on TDM may be associated with the method of application and the concentration used, since its use as a mitigator of salt stress depends on other factors such as species and/or genotype and environmental factors, as well as abiotic factors (El-Esawi *et al.*, 2017).

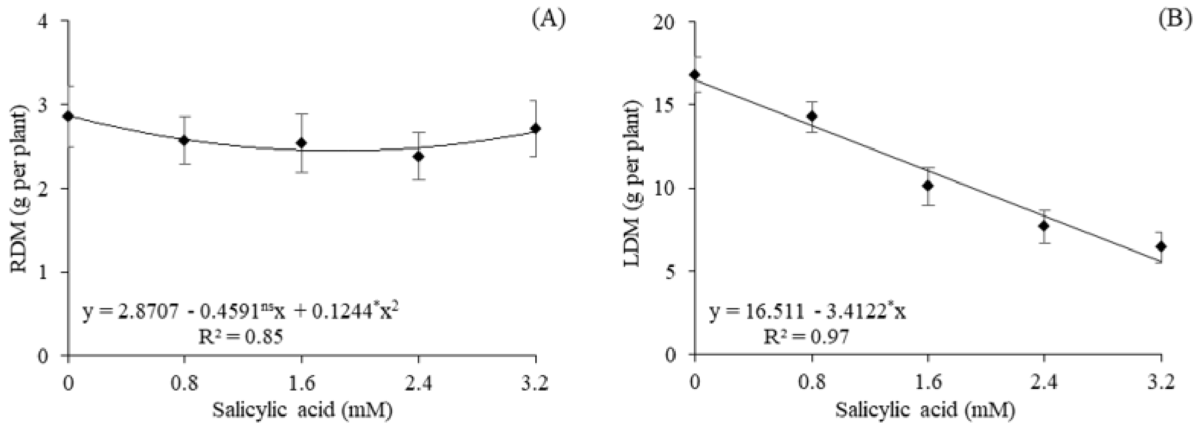

Figure 7. Root dry mass - RDM (A) and total dry mass - TDM (B) of 'Paluma' guava seedlings, as a function of salicylic acid concentrations – SA, at 180 days after sowing.
Vertical bar represents the standard error of the mean (n=4); ^{ns}, * respectively not significant and significant at $p \leq 0.05$.

Dickson's quality index (DQI) was reduced linearly with the increase in electrical conductivity levels of irrigation water (Figure 8A), by 18.58% per unit increase in ECw. Plants grown under water salinity of 4.2 dS m^{-1} reduced their DQI by 75.29% compared to those irrigated with ECw of 0.6 dS m^{-1}. DQI is an integrated morphological measure that, for relating robustness (plant height and stem diameter) to biomass distribution balance, is considered a good indicator of quality of seedlings to be used in the field (Lima *et al.*, 2021).

Lima *et al.* (2021), in a study evaluating the gas exchange, growth and quality of passion fruit cultivars 'BRS Sol do Cerrado' and 'Guinezinho' irrigated with saline waters (0.3 to 3.5 dS m^{-1}), also observed reductions in DQI of 44.83% and 62.18%, respectively, when comparing the lowest and highest levels of ECw.

Regarding the effects of SA concentrations on Dickson quality index (Figure 8B), it was verified that the maximum estimated value (0.8625) was obtained in plants subjected to SA concentration of 0 mM, while the lowest estimated value (0.7415) was obtained under the concentration of 1.82 mM according to the regression equation. The reduction in DQI with the increase in SA concentration may be related to the reduction observed in TDM (Figure 7B) since this is one of the variables used to determine the DQI. Despite the reduction in DQI, the values obtained in this study ranged from 0.31 to 1.27, which indicate seedlings with acceptable quality for transplanting to the field (Dickson *et al.*, 1960).

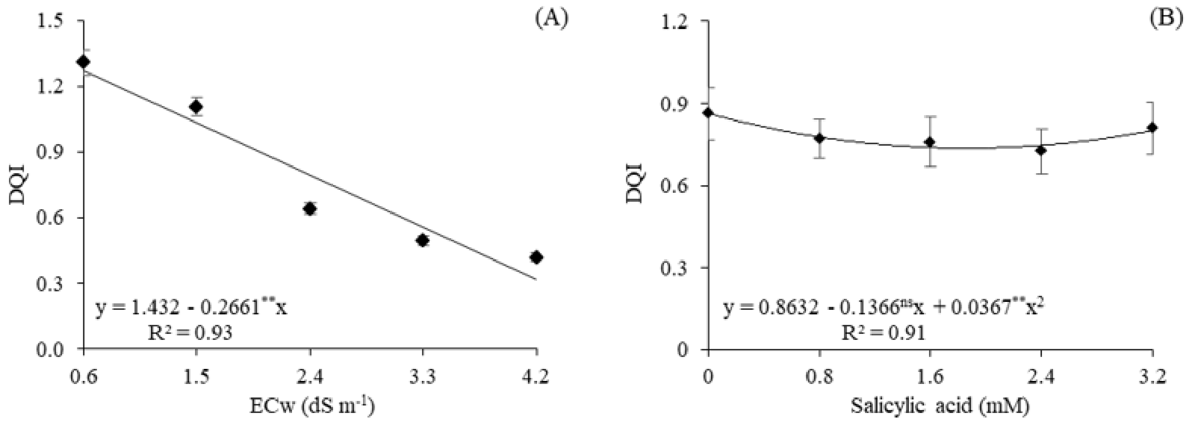

Figure 8. Dickson Quality Index (DQI) of 'Paluma' guava seedlings as a function of irrigation water salinity levels - ECw (A) and salicylic acid concentrations - SA (B) at 180 days after sowing.
Vertical bar represents the standard error of the mean (n=4); ^{ns}, **, respectively not significant and significant at $p \leq 0.01$.

In general, in the present study, it was observed that the foliar application of salicylic acid did not induce the tolerance of plants to salt stress, this fact may be related to the frequency of application (15 days), with only seven applications being carried out during the research. In addition, the beneficial effect of salicylic acid depends on several factors, including concentration, plant species, stage of crop development, and mode of application (Semida *et al.*, 2017); therefore, further research is needed to better understand the effects of SA on guava.

4. CONCLUSIONS

Foliar application of salicylic acid at a concentration of up to 1.4 mM reduces the deleterious effects of saline stress on the instantaneous water use efficiency of 'Paluma' guava seedlings at 180 days after sowing.

The transpiration of guava seedlings irrigated with electrical conductivity of up to 3.7 dS m^{-1} is benefited by the foliar application of salicylic acid at a concentration of 3.2 mM.

The concentrations of salicylic acid applied via foliar did not mitigate the harmful effects of irrigation water salinity on the growth and quality of 'Paluma' guava seedlings.

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