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Salicylic acid does not alleviate salt stress on physiological indicators and growth of guava

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Abstract

In the semi-arid region, the quality of water for irrigation stands out as a limiting factor for the expansion of agriculture. Thus, it is extremely important to search for alternatives that minimize the effects of salt stress on plants. Foliar application of salicylic acid stands out among these strategies. In this context, the objective of this study was to evaluate the gas exchange, photosynthetic pigments, and growth of guava as a function of irrigation water salinity and exogenous application of salicylic acid in the post-grafting stage. The experiment was conducted under greenhouse conditions, in a randomized block experimental design, in a 2 × 4 facto rial scheme, corresponding to two levels of electrical conductivity of irrigation water (0.6 and 3.2 dS m^{-1}) and four concentrations of salicylic acid (0; 1.2; 2.4 and 3.6 mM), with three replicates. Irrigation with water of 3.2 dS m^{-1} caused reductions in transpiration, CO_2 assimilation rate, instantaneous water use efficiency and instantaneous carboxylation efficiency of guava, at 150 days after transplanting. Water with electrical conductivity of 3.2 dS m^{-1} reduced the growth in stem diameter and the absolute and relative growth rates, as well as the relative water content, and chlorophyll *a* and *b* contents of guava plants. The interaction between water salinity levels and salicylic acid concentrations did not influence the physiological indices and growth of guava in the post-grafting phase.

Keywords: *Psidium guajava* L., Tolerance, Phytohormone

Introduction

Belonging to the Myrtaceae family, guava (*Psidium guajava* L.) is a fruit crop that stands out for its high nutritional value and its various forms of commercialization; it can be consumed fresh or processed in the form of guava paste, jams, pastes, fruit in syrup, puree, base for beverages, soft drinks, juices, and syrups (Oliveira et al., 2015).

In Brazil, guava production in 2021 was 552,393 tons in an area of 22,353 hectares, with an average yield of 24,953 kg/ha. The Northeast region stands out in the states of Pernambuco, Bahia, and Ceará with a production of 198,754, 46,836, and 22,062 tons respectively. In the same year, Paraíba obtained a production of 2,366 tons with an average yield of 7,170 kg/ha (IBGE, 2022).

Despite its production potential in the Northeast region, rainfall irregularities and high temperatures of this region hamper the expansion of cultivation, and irrigation is the practice that enables cultivation throughout the year (Souza et al., 2016; Machado; Serralheiro, 2017). However, most water sources in the semi-arid region contain high salt concentrations, and the excessive accumulation of soluble salts in the soil affects the availability of water to plants, leading to reduction of osmotic potential and ionic toxicity, besides decreasing plant growth and development, from morphological, structural and metabolic changes in the plant (Lima et al., 2015; Souza et al., 2017).

Plants grown under irrigation with saline water can undergo damage in physiological, biochemical, and molecular processes (Silva et al., 2016; Soares et al., 2018). In plants, salt stress alters the ionic homeostasis, causing osmotic pressure, ionic toxicity (Bonifácio et al., 2018) and also causes an increase of reactive oxygen species at the subcellular level, which damages the cellular components and cause degradation of chlorophyll and

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lipid peroxidation of the membrane, thus decreasing its fluidity and selectivity (Taibi et al., 2016).

The search for alternatives that enable the use of saline water in agriculture has become constant in the semi-arid region of northeastern Brazil. Among the strategies that can mitigate the effects of salt stress on guava plants, exogenous application of salicylic acid stands out. It is a phenolic compound that acts in the signaling and activation of certain genes which act as a defense mechanism of the plant under biotic and abiotic stresses (Methenni et al., 2018; Silva et al., 2018). However, it should be considered that its effect depends on several factors such as stage of development, species, mode of application and concentration (Poór et al., 2019).

Although there are already studies with salicylic acid as an elicitor of abiotic stresses, research with the use of this substance in guava is still incipient. Thus, it is essential to conduct studies that seek to evaluate salicylic acid as an alternative in the mitigation of salt stress in guava in the post-graft formation stage. In this context, the objective of this study was to evaluate the gas exchange, photosynthetic pigments, and growth of guava as a function of irrigation water salinity and exogenous application of salicylic acid.

Material and Methods

The experiment was carried out from April to August 2020 under conditions of a greenhouse, belonging to the Center for Technology and Natural Resources of the Federal University of Campina Grande, PB, Brazil, located at the local coordinates 07°15'18" S latitude, 35°52'28" W longitude and average altitude of 550 m. The maximum and minimum temperature and relative humidity of the air data during the experiment are shown in (**Figure 1**).

A randomized block experimental design was used in a 2 × 4 factorial arrangement, whose treatments resulted from the combination of two factors: two levels of electrical conductivity of irrigation water - ECw (0.6 and 3.2 dS m^{-1}) and four concentrations of salicylic acid - SA (0; 1.2; 2.4 and 3.6 mM), with three replicates. The highest level of ECw was established based on studies conducted by (Bezerra et al., 2019) with guava cv. Paluma, whereas salicylic acid (SA) concentrations were determined according to a study conducted by (Silva et al., 2020) with the soursop crop (*Annona muricata* L.).

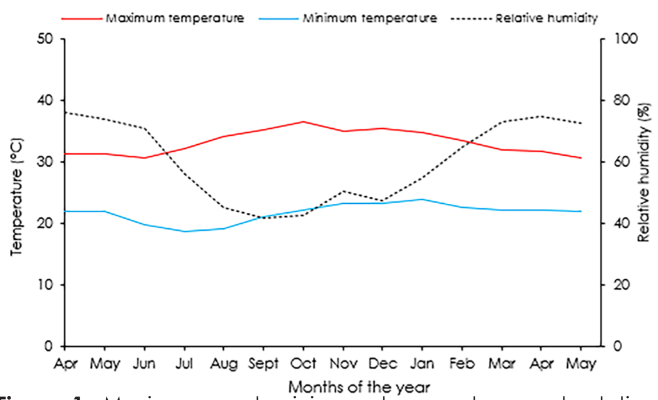


Figure 1. Maximum and minimum temperature and relative humidity of air during the experimental period.

Salicylic acid concentrations were prepared by dissolution in 30% of ethyl alcohol (95.5% purity) in distilled water, as it is a substance with low solubility in water at room temperature. In the preparation of the solution, the Wil fix adjuvant at the concentration of 0.5 mL L^{-1} of solution was used to reduce the surface tension of the drops on the leaf surface (adaxial and abaxial sides). Salicylic acid applications started at 45 days after transplanting (DAT) and extended up to the stage of full flowering (205 DAT). The frequency of application was 30 days and, during this period, an average value of 683.33 mL of the respective solution was applied per plant. The applications were performed at 17 hours and during the application; the plant was isolated using plastic curtains to pre-vent the solution from drifting.

Rootstocks in this study were seedlings of heirloom guava, coming from the seedling nursery located in Sousa-PB and the scion was the cv. Paluma. Grafted seedlings were acquired at the age of 70 days after grafting. The cultivar Paluma is a clone derived from the Rubi-Supreme guava, obtained from open pollination seeds (Cavalini, 2004); its fruits are suitable for both table consumption and industrialization, with mass between 140 and 250 g, longitudinal diameter of 8 to 10 cm, transverse diameter of 7 to 9 cm and intense red flesh.

Containers with capacity of 200 L adapted as drainage lysimeters were used. At the base of each lysimeter, a 16-mm-diameter drain was installed to drain excess water and connected to a container for collecting drained water and subsequently determining water consumption by plants. The tip of the drain inside the pot was wrapped with a nonwoven geotextile (Bidim OP 30) to prevent clogging by soil material.

The lysimeters were filled by placing a 1-kg layer of crushed stone n° 0, followed by 250 kg of a *Neossolo Regolítico* (Entisol) of sandy loam texture (0-20 cm depth), properly pounded to break up clods and coming from the rural area of the municipality of Lagoa Seca, PB. Its chemical and physical characteristics (**Table 1**) were obtained according to (Teixeira et al., 2017).

The water with the lowest electrical conductivity (0.6 dS m^{-1}) was obtained from the supply system of Campina Grande-PB, whereas the highest level of ECw

Table 1. Chemical and physical characteristics of the soil used in the experiment

Chemical characteristics								
pH (H_2O)	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
1:2.5	dag kg^{-1}	mg dm^{-3}	..................cmol$_c$ kg^{-1}..................					
6.5	8.1	79.00	0.24	0.51	14.90	5.40	0.00	0.90
.... Chemical characteristics...........				.... Physical characteristics..........................				
EC_{se}	CEC	SAR	ESP	Size fraction (g kg^{-1})			Water content (dag kg^{-1})	
dS m^{-1}	cmol$_c$ kg^{-1}	(mmol L^{-1})$^{0.5}$	%	Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
2.15	16.54	0.16	3.08	572.70	100.70	326.60	25.91	12.96

pH - Hydrogen potential, OM - Organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; Al^{3+}+H^+ extracted with 0.5 M CaOAc at pH 7.0; ECse - Electrical conductivity of saturation extract; CEC - Cation exchange capacity; SAR - Sodium adsorption ratio of the saturation extract; ESP - Exchangeable sodium percentage; [1,2] referring to field capacity and permanent wilting point, respectively.

(3.2 dS m^{-1}) was prepared by dissolving NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ salts, in the equivalent ratio of 7:2:1, respectively, in local-supply water (ECw = 0.6 dS m^{-1}) of the municipality of Campina Grande, PB, considering the relationship between ECw and salt concentration, according to (Richards, 1954), as shown in Eq. 1:

$$C \approx 10 \times ECw \quad (1)$$

Where:

C = concentration of salts to be applied (mmol$_c$ L^{-1}); and
ECw = electrical conductivity of water (dS m^{-1}).

Transplanting was performed at 20 days after acquiring the seedlings, to pit holes with dimensions of 20 × 20 × 20 cm and, before the transplanting, the seedlings were checked to see whether their roots were bounded. After transplanting, the seedlings were acclimated for 50 days, a period in which the plants were irrigated with water of electrical conductivity of 0.6 dS m^{-1}.

Formative pruning was carried out when the plants reached a height of 50 cm when the branch of apical dominance was cut to stimulate the production of lateral branches. With the emergence of the new branches, the secondary branches that were well distributed and balanced were selected, and then these lateral branches were cut when they reached 40 cm in length, as recommended by (Embrapa, 2010).

Before transplanting the seedlings, the soil moisture content was increased until reaching the maximum water retention capacity. After transplanting, irrigation was performed daily at 5 p.m., applying in each lysimeter a volume corresponding to that obtained by the water balance and a leaching fraction, estimated at 10% every 30 days. The volume of water to be applied to the plants was determined by Eq. 2:

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

Where:

VI - volume of water to be used in the irrigation event (mL);
Va - volume applied in the previous irrigation event (mL);
Vd - volume drained (mL); and
LF - leaching fraction of 0.10.

Fertilization with nitrogen, potassium and phosphorus was performed as recommended by (Cavalcante, 2008), applying 100, 100 and 60 g per plant of N, P_2O_5 and K_2O. Urea (45% N), monoammonium phosphate (50% P_2O_5) and potassium chloride (60% K_2O) were used as a source of N, P and K, respectively. Fertilization with N, P_2O_5 and K_2O started at 15 days after transplantation (DAT) and was performed in fortnightly applications.

Fertilization with micronutrients was performed fortnightly through the leaves, beginning at 30 DAT, on the adaxial and abaxial sides, considering the nutritional requirements of the crop, with concentration of 1 g L^{-1} of Dripsol Micro® (1.2% magnesium, 0.85% boron, 3.4% iron, 4.2% zinc, 3.2% manganese, 0.5% copper and 0.06% molybdenum).

Phytosanitary control was carried out preventively to control the possible emergence of pests: psyllid (*Triozoida limbata*), fruit fly (*Anastrepha* spp., *Ceratitis capitata*), bug (*Leptoglossus gonagra*) and cochineal (*Ceroplastes floridensis*), through selective chemicals based on Imidacloprid and Abamectin using 1 g for 10 L and 2.5 mL for 10 L, respectively, in the preparation of the mixture.

At 150 DAT, gas exchange was analyzed through the CO_2 assimilation rate - *A* (mol CO_2 m^{-2} s^{-1}), transpiration - *E* (mmol H_2O m^{-2} s^{-1}), stomatal conductance - *gs* (mol H_2O m^{-2} s^{-1}), internal CO_2 concentration - *Ci* (μmol CO_2 m^{-2} s^{-1}), instantaneous water use efficiency - *WUEi* [(μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$) and instantaneous carboxylation efficiency - *CEi* (μmol m^{-2} s^{-1}/μmol m^{-2} s^{-1}).

Gas exchange was determined using an infrared gas analyzer - IRGA (Infrared Gas Analyser, LCpro - SD model, from ADC Bioscientific, UK). The readings were performed between 7:00 and 9:00 a.m., on the third fully expanded leaf counted from the apical bud, under natural conditions of air temperature and CO_2 concentration, using an artificial radiation source of 1,200 μmol m^{-2} s^{-1}, established through the photosynthetic light response curve and determined the photosynthetic light saturation point (Fernandes et al., 2021).

At the same time, photosynthetic pigments, percentage of cell damage (%D) and relative water content (RWC) were also measured. Chlorophyll *a*, chlorophyll *b* and carotenoid contents were determined according to (Arnon, 1949), using a spectrophotometer at absorbance wavelengths (A) of 470, 646, and 663 nm, according to Eqs. 3, 4 and 5.

$$Chl\ a = 12.21A_{663} - 2.81A_{646} \quad (3)$$

$$Chl\ b = 20.13A_{646} - 5.03A_{663} \quad (4)$$

$$Car = (1000A_{470} - 1.82\,Chl\ a - 85.02\,Chlb)/198 \quad (5)$$

where:

Chl *a* - chlorophyll *a*;

Chl *b* - chlorophyll *b*; and

Car - carotenoids.

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

The percentage of cell membrane damage was obtained according to (Scotti-Campos; Thu Phan Thi, A. 1997), using Eq. 6:

$$\%\,D = \frac{Ci}{Cf} \times 100 \quad (6)$$

Where:

% D - percentage of cell membrane damage;

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

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

The relative water content in the leaf blade (RWC) was determined according to the methodology of Weatherley (1950) using Eq. 7:

$$RWC = \frac{(FM - DM)}{(TM - DM)} \times 100 \quad (7)$$

Where:

RWC - relative water content (%);

FM - fresh mass of leaves (g);

TM - turgid mass (g); and DM - dry mass(g).

The growth in diameter of the rootstock and scion of guava plants was evaluated in the period from 90 to 150 DAT, through the absolute and relative growth rates in diameter of the rootstock (AGR_{DR} and RGR_{DR}) and scion (AGR_{DS} and RGR_{DS}), respectively, according to (Benincasa, 2003), using Eq. 8 and 9:

$$AGR = \frac{(SD_2 - SD_1)}{(t_2 - t_1)} \quad (8)$$

where:

AGR - absolute growth rate in diameter of rootstock and scion (mm d^{-1});

SD_1 - stem diameter (mm) at time t_1; and

SD_2 - stem diameter (mm) at time t_2.

$$RGR = \frac{(lnSD_2 - lnSD_1)}{(t_2 - t_1)} \quad (9)$$

where:

RGR - relative growth rate in diameter of rootstock and scion (mm mm^{-1} d^{-1}),

SD_1 - stem diameter (mm) at time t_1;

SD_2 - stem diameter (mm) at time t_2; and

ln - natural logarithm.

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

Results and Discussion

There was a significant effect of water salinity levels (SL) on relative water content (RWC), percentage of cell damage (% D), chlorophyll *a* and *b* contents (Chl *a* and Chl *b*) of guava cv. Paluma (**Table 2**). Salicylic acid (SA) concentrations and the interaction between factors (SL × SA) did not influence any of the variables analyzed at 150 days after transplantation.

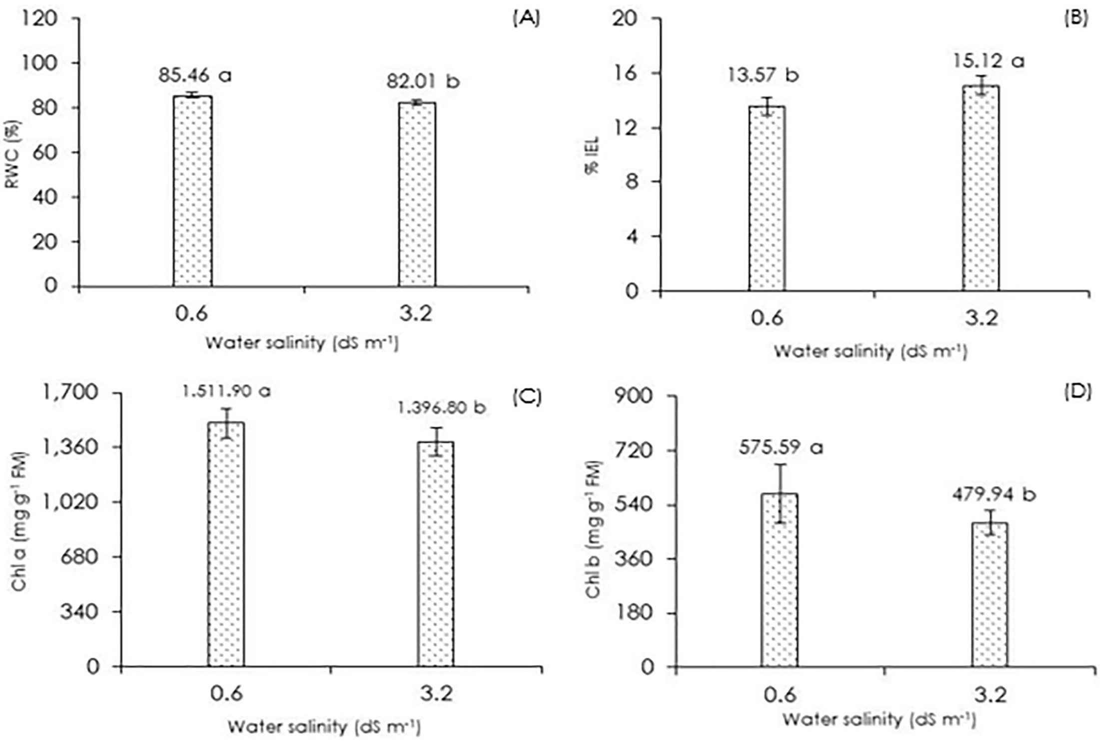
The relative water content of guava plants cv. Paluma decreased as a function of the increase in water salinity levels (**Figure 2**A). Plants irrigated using water with ECw of 0.6 dS m^{-1} obtained a RWC (4.03%) higher statistically than that of plants under the highest level of water salinity (ECw=3.2 dS m^{-1}). Reduction in the relative water content in leaf tissues is associated with the osmotic effect resulting from the salinity of irrigation water, which causes disturbances in the plant's water balance, due to the difficulty in water absorption (Barreiro Neto et al., 2017). (Silva Neta et al., 2020), working with passion fruit irrigated with water of different salinity levels (ECw from 0.3 to 3.58 dS m^{-1}) found a decrease in the relative water content of 2.91% per unit increment in ECw.

For the percentage of cell damage in guava plants (Figure 2B), there was an increase as a function of irrigation with water of high electrical conductivity, significantly differing from plants that received the lowest level of water salinity (0.6 dS m^{-1}). The increase in salinity and consequently higher concentrations of Na^+ and Cl^- ions in the leaves of the plants allowed an increase in the extrusion of electrolytes; as a result, these ions promoted structural changes of cell membranes and organelles, causing rupture of the cell membrane and loss of its

Table 2. Summary of the analysis of variance for relative water content (RWC), percentage of cell damage (% D), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (CAR) of guava plants cv. Paluma irrigated with saline water and subjected to exogenous application of salicylic acid, at 150 days after transplanting

Source of variation	DF	Mean squares				
		RWC	% D	Chl a	Chl b	CAR
Salinity levels (SL)	1	69.93*	14.53*	79511.08*	54885.88*	3718.56^{ns}
Salicylic acid (SA)	3	33.17^{ns}	8.52^{ns}	227296.63^{ns}	12006.53^{ns}	1809.34^{ns}
Linear regression	1	2.79^{ns}	0.69^{ns}	377804.34^{ns}	144339.54^{ns}	7488.34^{ns}
Quadratic regression	1	16.25^{ns}	0.72^{ns}	215082.66^{ns}	238833.42^{ns}	10366.72^{ns}
Interaction (SL x SA)	3	4.53^{ns}	3.33^{ns}	39084.65^{ns}	49332.27^{ns}	4416.40^{ns}
Blocks	2	9.39^{ns}	1.16^{ns}	30270.32^{ns}	19837.14^{ns}	8294.77^{ns}
Residue	14	4.56	1.88	14852.66	11329.50	61526.29
CV (%)		2.55	9.58	8.38	20.17	24.59
SMD		1.86	1.20	106.71	93.19	58.04

DF - degree of freedom; CV (%) - coefficient of variation; $^{ns, *}$ respectively not significant and significant at $p < 0.05$. SMD - significant minimum difference.



Means followed by different letters indicate significant difference between treatments by Tukey test ($p < 0.05$). Vertical bar represents the standard error of the mean (n=3).

Figure 2. Relative water content - RWC (A), percentage of cell damage - % D (B), chlorophyll *a* - Chl *a* (C) and chlorophyll *b* - Chl *b* (D) of guava plants cv. Paluma as a function of water salinity - ECw, at 150 days after transplanting.

internal content (Ferraz et al., 2015). Plants can synthesize reactive oxygen species (ROS) as a defense mechanism against salt stress, and ROS can cause the efflux of K^+, resulting in losses of this cell nutrient, stimulating proteases and endonucleases, and promoting programmed cell death, a mechanism that occurs when the plant is under severe stress (Demidchik et al., 2014).

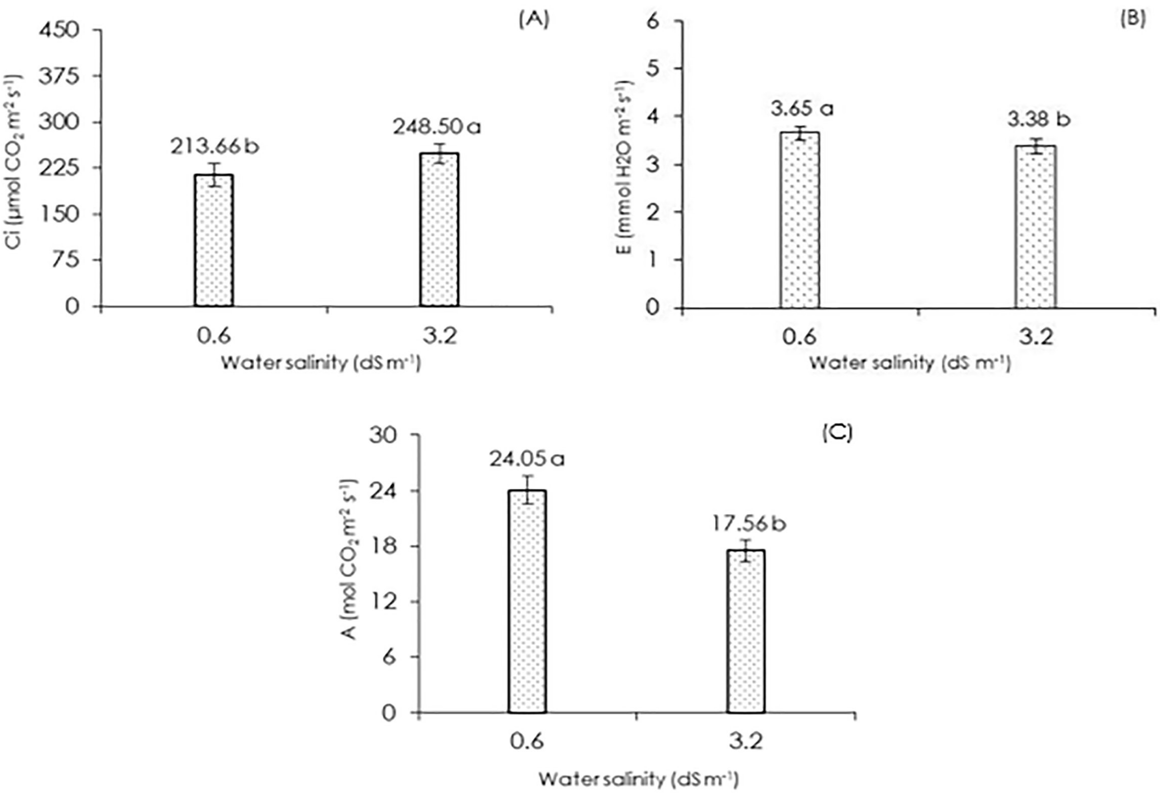
For the contents of chlorophyll *a* (Figure 2C) and chlorophyll *b* (Figure 2D), it was verified that the increase in water salinity led to reductions in plants subjected to salt stress (3.2 dS m^{-1}) when compared with those irrigated with ECw of 0.6 dS m^{-1}. Inhibition in chlorophyll synthesis may be associated with the reduction of the synthesis of 5-aminolevulinic acid, a molecule responsible for chlorophyll production and increase in the activity of the chlorophyllase enzyme, which degrades the molecules of these photosynthetic pigments under salt stress conditions (Silva et al., 2017; Sá et al., 2019). This behavior was also observed by (Cavalcante et al., 2011) when evaluating chlorophyll contents in yellow passion fruit irrigated with saline water (0.5, 1.5, 2.5, 3.5 and 4.5 dS m^{-1}); these authors noted that salinity levels higher than ECw=2.5 dS m^{-1} compromise the biosynthesis of these pigments.

There was a significant effect of water salinity levels

Table 3. Summary of the analysis of variance for internal CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*), CO_2 assimilation rate (*A*), instantaneous water use efficiency (*WUEi*) and instantaneous carboxylation efficiency (*CEi*) of guava cv. Paluma under irrigation with saline waters and exogenous application of salicylic acid at 150 days after transplanting

Source of variation	DF	Mean squares					
		Ci	*E*	*gs*	*A*	*WUEi*	*CEi*
Salinity levels (SL)	1	7280.161*	0.429*	0.001^{ns}	252.462**	11.333*	0.013*
Salicylic acid (SA)	3	309.617^{ns}	0.259^{ns}	0.001^{ns}	2.909^{ns}	0.544^{ns}	0.000^{ns}
Linear regression	1	1116.057^{ns}	0.084^{ns}	0.001^{ns}	2.127^{ns}	0.657^{ns}	0.000^{ns}
Quadratic regression	1	410.708^{ns}	0.470^{ns}	0.002^{ns}	0.098^{ns}	1.626^{ns}	0.000^{ns}
Interaction (SL x SA)	3	1116.055^{ns}	0.008^{ns}	0.001^{ns}	2.128^{ns}	0.657^{ns}	0.000^{ns}
Blocks	2	9285.795^{ns}	0.288^{ns}	0.031^{ns}	2.138^{ns}	0.895^{ns}	0.003^{ns}
Residue	14	1117.361	0.084	0.001	15.766	1.022	0.001
CV (%)		14.82	8.25	15.26	19.08	17.13	37.63
SMD		29.99	0.25	0.03	3.47	0.88	0.03

DF - degree of freedom; CV (%) - coefficient of variation; $^{ns, *}$ respectively not significant and significant at $p < 0.05$. SMD - significant minimum difference.

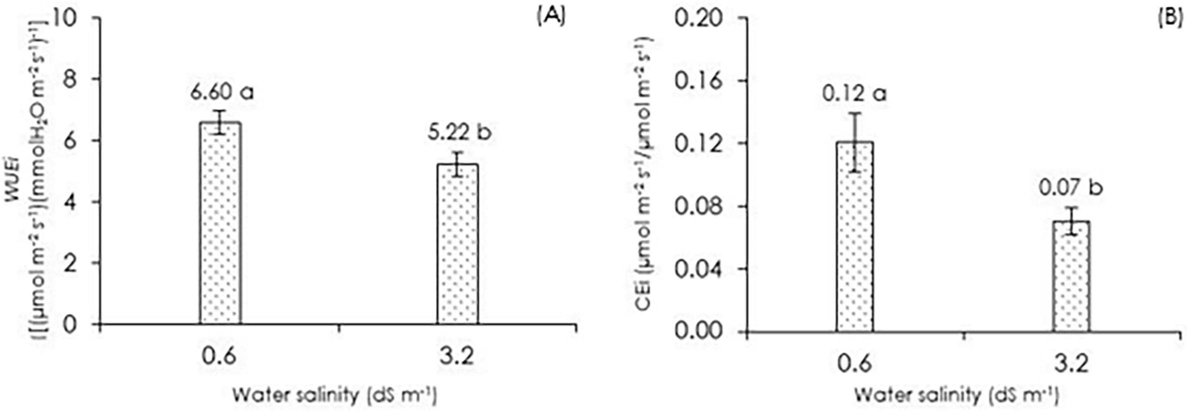


Means followed by different letters indicate significant difference between treatments by Tukey test ($p < 0.05$). Vertical bar represents the standard error of the mean (n=3).

Figure 3. Internal CO_2 concentration - *Ci* (A), transpiration - *E* (B), CO_2 assimilation rate - *A* (C) of guava plants cv. Paluma, as a function of the salinity of irrigation water - ECw, at 150 days after transplantation.

(SL) on the internal CO_2 concentration (*Ci*), transpiration (*E*), CO_2 assimilation rate (*A*), instantaneous efficiency water use (*WUEi*) and instantaneous carboxylation efficiency (*CEi*) of guava at 150 DAT (**Table 3**). Salicylic acid (SA) concentrations and the interaction between factors (SL × SA) did not influence any of the variables analyzed at 150 DAT.

Water salinity caused an increase in the internal CO_2 concentration of guava cv. Paluma (**Figure 3**A) and, as the ECw level increased to 3.2 dS m^{-1}, there was an increase of 14.02% (34.84 μmol CO_2 m^{-2} s^{-1}) in *Ci* compared to plants subjected to ECw of 0.6 dS m^{-1}. The increase of *Ci* in plants under salt stress conditions is associated not only with stomatal factors, but also with the accumulation of salts in leaves, which causes deterioration of photosynthetic structures (Hussain et al., 2012). The increase of *Ci* in plants grown under ECw of 3.2 dS m^{-1} resulted in a lower CO_2 assimilation rate, which may possibly be related to low activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which demonstrates that carbon when entering the mesophilic cell was not being metabolized by the photosynthetic



Means followed by different letters indicate significant difference between treatments by Tukey test ($p < 0.05$). Vertical bar represents the standard error of the mean (n=3).

Figure 4. Instantaneous water use efficiency - *WUEi* (A) and instantaneous carboxylation efficiency - *CEi* (B) of guava plants cv. Paluma, as a function of the salinity of irrigation water - ECw, at 150 days after transplanting.

apparatus (Silva et al., 2019).

(Diniz et al., 2020), studying passion fruit crop cv. BRS GA1, observed that irrigation with water of 3.1 dS m^{-1} promoted an increase in the internal CO_2 concentration when compared to plants irrigated with water of 0.3 dS m^{-1}. (Lima et al., 2020) in a study with West Indian cherry also found an increase in the internal CO_2 concentration in plants irrigated using water with electrical conductivity of 4.5 dS m^{-1}.

Contrary to the situation observed for internal CO_2 concentration (Figure 2A), transpiration (Figure 3B) and CO_2 assimilation rate (Figure 2C) of plants decreased with increasing electrical conductivity of irrigation water. It is observed (Figure 2C) that guava plants subjected to ECw of 3.2 dS m^{-1} differed significantly from those grown with water of 0.6 dS m^{-1}. When comparing plants irrigated with water of 0.6 dS m^{-1} to those that received 3.2 dS m^{-1}, there were reductions in *E* and *A* of 7.4 (0.27 mmol CO_2 m^{-2} s^{-1}) and 26.99% (6.49 mol H_2O m^{-2} s^{-1}), respectively. The decrease in transpiration is a consequence of the reduction in the osmotic potential of the soil solution, which alters the rate of water absorption, a fact also observed in the RWC due to the reduction of the water content in the plant caused by the osmotic and ionic effects (Rodrigues et al., 2016).

The decrease in CO_2 assimilation rate as a function of salt stress is usually largely related to the reduction of stomatal opening, with restriction on the entry of CO_2 in the substomatal chamber (Lima et al., 2019; Sá et al., 2019). However, this situation was not observed in the present study, given that there was an increase in the internal CO_2 concentration with the increase in salinity, indicating that the decrease in CO_2 assimilation rate occurred because of the action of non-stomatal factors, such as the reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase, which performs CO_2 fixation (Silva, 2017).

The instantaneous water use efficiency (**Figure 4**A) and the instantaneous carboxylation efficiency (Figure 4B) of guava decreased significantly with the increase in the salinity level of irrigation water. When comparing the *WUEi* and *CEi* of plants subjected to ECw of 3.2 dS m^{-1} to those of plants that were subjected to the lowest salinity level (ECw=0.6 dS m^{-1}), there were reductions of 20.90 and 41.66%, respectively. This decrease in instantaneous water use efficiency is related to a natural mechanism of the plant when there is a reduction in the transpiration rate (Lima et al., 2019).

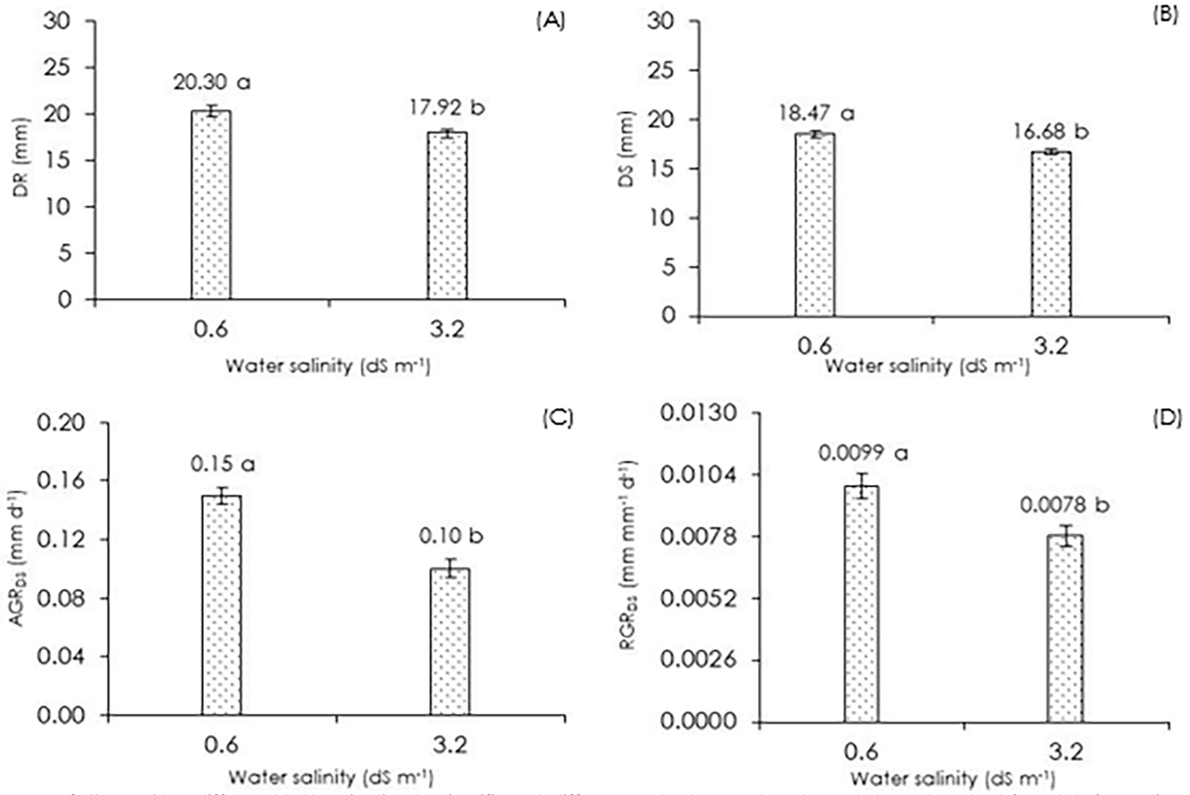
Reduction in instantaneous carboxylation efficiency is associated with the action of other environmental factors, thus promoting oxygenation of RuBisCO and increase in the photorespiratory pathway, resulting in the reduction of carbon compounds (Voss et al. 2013). This fact may also be associated with metabolic restrictions in the Calvin cycle, where the carbon received was not being fixed in the carboxylation phase in mesophilic cells (Souza et al. 2016). The result obtained reinforces those found for CO_2 assimilation rate, as there was a reduction in RuBisCO activity, thus also promoting a decrease in instantaneous carboxylation efficiency (Silva et al., 2019).

There was a significant effect of water salinity levels (SL) on the diameters of the rootstock (DR) and scion (DS), absolute and relative growth rates of stem diameter of the scion (AGR_{DS} and RGR_{DS}) of guava plants cv. Paluma at 150 DAT (**Table 4**). Salicylic acid (SA)

Table 4. Summary of the analysis of variance for diameter of rootstock (DR) and diameter of scion (DS) at 150 days after transplantation (DAT) and absolute growth rate (AGR_{DR}) and relative growth rate of diameter of rootstock (RGR_{DR}) and absolute growth rate (AGR_{DS}) of diameter of scion (RGR_{DS}) of guava plants cv. Paluma under irrigation with saline waters and exogenous application of salicylic acid in the period of 90-150 DAT

Source of variation	DF	Mean squares					
		DR	DS	AGR_{DR}	RGR_{DR}	AGR_{DS}	RGR_{DS}
Salinity levels (SL)	1	32.201^{**}	19.206^{**}	0.00026^{ns}	0.000006^{ns}	0.01653^{**}	0.000027^{**}
Salicylic acid (SA)	3	0.424^{ns}	0.173^{ns}	0.00035^{ns}	0.000002^{ns}	0.00083^{ns}	0.000005^{ns}
Linear regression	1	0.672^{ns}	0.287^{ns}	0.00021^{ns}	0.000001^{ns}	0.00074^{ns}	0.000007^{ns}
Quadratic regression	1	0.370^{ns}	0.001^{ns}	0.00002^{ns}	0.000003^{ns}	0.00022^{ns}	0.000002^{ns}
Interaction (SL x SA)	3	1.393^{ns}	0.360^{ns}	0.00028^{ns}	0.000001^{ns}	0.00002^{ns}	0.000007^{ns}
Blocks	2	0.196^{ns}	0.908^{ns}	0.00002^{ns}	0.000001^{ns}	0.00013^{ns}	0.000021^{ns}
Residue	14	2.034	0.855	0.00024	0.000007	0.00012	0.000072
CV (%)		7.48	5.25	14.11	12.08	10.33	12.06
SMD		1.24	0.80	0.01	0.01	0.01	0.03

DF - degree of freedom; CV (%) - coefficient of variation; $^{ns, *}$ respectively not significant and significant at p < 0.05. SMD - significant minimum difference.



Means followed by different letters indicate significant difference between treatments by Tukey test (p < 0.05). Vertical bar represents the standard error of the mean (n=3).

Figure 5. Diameter of rootstock - DR (A), diameter of scion - DS (B) at 150 days after transplanting – DAT, absolute growth rate - AGR_{DS} (C) and relative growth rate - RGR_{DS} (D) of diameter of the scion of guava plants cv. Paluma as a function of the salinity of irrigation water – ECw, in the period of 90 -150 DAT.

concentrations and the interaction between factors (SL × SA) did not significantly interfere in any of the variables analyzed at 150 days after transplantation.

The increase in irrigation water salinity also inhibited the growth in stem diameter of guava plants cv. Paluma (**Figure 5**). Plants irrigated with water of 3.2 dS m^{-1} reduced their DR (Figure 5A), DS (Figure 5B), AGR_{DS} (Figure 5C) and RGR_{DS} (Figure 5D) by 11.72, 9.69, 33.33 and 21.21%, respectively, in comparison to those that received ECw of 0.6 dS m^{-1}. The free energy state of water in the soil may decrease due to excess ions and can also promote modifications in genes that are responsible for the transition of the synthesis of suberin, lignin and polysaccharides of the cell wall, where such characteristics negatively affect the rate of cell elongation and division of the tissues (Byrt et al., 2018). The decrease in growth may also be associated with the osmotic and toxic effects of ions (Na^+ and Cl^-), which can concentrate in stem tissues during plant growth (Lima et al., 2019b). This reduction in growth variables may also reflect the decrease in the CO_2 assimilation rate observed in plants irrigated using water with electrical conductivity of 3.2 dS m^{-1}, as tits reduction limited the growth of the plants.

Conclusions

Irrigation with water of 3.2 dS m^{-1} promotes reductions in gas exchange, chlorophyll *a* and *b* contents, relative water content and growth of guava plants cv. Paluma.

Water salinity of 3.2 dS m^{-1} results in an increase in the percentage of cell damage in guava plants at 150 days after transplanting.

The interaction between water salinity levels and salicylic acid concentrations do not influence the physiological indices and growth of guava in the post-grafting phase.

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