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Formation of guava seedlings under irrigation with water of different cationic natures and salicylic acid

Formação de mudas de goiabeira irrigadas com águas de diferentes natureza catiônica e ácido salicílico

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ABSTRACT - The objective of this study was to evaluate gas exchange, biomass, and quality of guava seedlings as a function of the cationic nature of the water used in irrigation and foliar application of salicylic acid. The experiment was carried out in a greenhouse in Pombal, PB, Brazil, using a randomized block design, in a 6 × 4 factorial scheme with six cationic compositions of irrigation water [S1 - Control (supply water); S2 - Na^+; S3 - Ca^{2+}; S4 - Na^++Ca^{2+}; S5 - Mg^{2+}, and S6 - $Na^++Ca^{2+}+Mg^{2+}$], associated with four concentrations of salicylic acid (0, 1.3, 2.6, and 3.9 mM), with 3 replicates. Plants in control (S1) were irrigated with water of electrical conductivity (ECw) of 0.3 dS m^{-1}, while in the other treatments were irrigated with different types of water and had an ECw of 4.3 dS m^{-1}, consisting of different cations, in the form of chloride. In the seedling formation phase, guava plants were sensitive to calcic water, which resulted in a marked decrease in their growth. Stomatal conductance, transpiration, and biomass accumulation of guava seedlings were more affected by variation in electrical conductivity than by cationic nature of the water. Salicylic acid at concentrations of 2.9 and 1.9 mM increased stomatal conductance and stem dry biomass, respectively, of guava seedlings. Water with ECw of 4.3 dS m^{-1} allowed the formation of guava seedlings with acceptable quality for transplanting to the field, regardless of the cationic nature of the water.

Keywords: Salt stress. Phytohormone. *Psidium guajava* L.

RESUMO - Objetivou-se com este trabalho avaliar as trocas gasosas, as fitomassas e a qualidade das mudas de goiabeira em função da natureza catiônica da água utilizada na irrigação e aplicação foliar de ácido salicílico. O experimento foi conduzido em casa-de-vegetação, Pombal, PB, utilizando-se o delineamento de blocos casualizados, em esquema fatorial 6 × 4, sendo seis composições catiônicas da água de irrigação [S1 – Testemunha (água de abastecimento); S2 - Na^+; S3 - Ca^{2+}; S4 - Na^++Ca^{2+}; S5 – Mg^{2+} e S6 - $Na^++Ca^{2+}+Mg^{2+}$], associados a quatro concentrações de ácido salicílico (0; 1,3; 2,6 e 3,9 mM), com 3 repetições. As plantas referentes à testemunha (S1) foram irrigadas com água de condutividade elétrica (CEa) de 0,3 dS m^{-1}, enquanto outros tratamentos diferentes tipos de águas foi utilizada com CEa de 4,3 dS m^{-1}, constituída de diferentes cátions, em forma de cloreto. Na fase de formação de mudas, a goiabeira foi sensível à água de natureza cálcica, obtendo-se diminuição acentuada no crescimento das plantas. A condutância estomática, a transpiração e o acúmulo de fitomassas das mudas de goiabeira foram mais afetados pela variação na condutividade elétrica em comparação com a natureza catiônica da água. Ácido salicílico nas concentrações de 2,9 e 1,9 mM aumentou a condutância estomática e a fitomassa seca do caule, respectivamente, em mudas de goiabeira. A água com CEa de 4,3 dS m^{-1} permitiu a formação de mudas de goiabeira com qualidade aceitável para o transplantio no campo, independente da natureza catiônica da água.

Palavras-chave: Estresse salino. Fitormônio. *Psidium guajava* L.

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INTRODUCTION

Belonging to the Myrtaceae family, guava (*Psidium guajava* L.) is a species native to tropical America that stands out for the high economic value, pleasant taste, and nutritional quality of the fruit, which guarantee the preference of various consumers of the domestic and foreign market and is a crop widely cultivated in Brazil (OLIVEIRA et al., 2015). The Brazilian guava production in 2021 was 552,393 tons, with an average yield of 24,953 kg ha^{-1} in 22,353 hectares of planted area. In the northeast, the states of Pernambuco, Bahia and Ceará stood out as the largest producers, obtaining production of 198,754, 46,836, and 22,062 tons with average yields of 36,032, 21,386, and 15,792 kg ha^{-1}, respectively (IBGE, 2022).

In regions with adverse climatic conditions, especially in semi-arid areas, low rainfall and intense evaporation are common in most months of the year, and water sources usually have high salt levels, especially in crystalline areas, with high amounts of chloride and sodium, low concentrations of sulfate, and varying concentrations of calcium, magnesium, carbonates, and bicarbonates (SILVA

JÚNIOR; GHEYI; MEDEIROS, 1999; PAIVA et al., 2021; PINHEIRO et al., 2022). On the other hand, in some parts, such as the Apodi Plateau (Rio Grande do Norte/Ceará), Gurgueia (Piauí) and Iracema (Bahia), there is a predominance of calcium/magnesium salts (HOLANDA et al., 2016).

However, salt concentrations in water that affect plant growth and development vary in total concentration and ionic composition of irrigation water (LIMA et al., 2019). Excess salts in water cause ionic imbalance and osmotic stress in plants, which causes changes in various physiological and metabolic processes, depending on the severity and duration of stress, and ultimately inhibit crop production (GUPTA; HUANG, 2014). Salt stress also causes an increase in the level of reactive oxygen species (ROS) that results in oxidative damage, which in turn affects plants at both cellular and metabolic levels (KUMAR et al., 2021).

The quantitative and qualitative reduction of water sources has led to the search for strategies aimed at more efficient use of water, as well as rational use of waters considered of inferior quality since using saline water in agriculture is a necessity in semi-arid regions. In this context, several authors have studied the effect of irrigation with water of different salinity levels on the cultivation of guava (SOUZA et al., 2016; BEZERRA et al., 2018; XAVIER et al., 2022; LACERDA et al., 2022a); however, these studies are restricted to the use of waters with different levels of electrical conductivity, making it imperative to conduct new studies, particularly to assess the use of waters with different cationic natures on the guava crop in the seedling formation phase.

Among the strategies that allow mitigating the effects of salt stress on plants, a prominent one is the use of elicitor substances, such as salicylic acid, an important signaling molecule that mitigates the adverse effects of salt stress on plants through the improvement in physiological and metabolic processes, resulting in their acclimatization to abiotic stresses, including salt stress (SILVA et al., 2020; LACERDA et al., 2022b; XAVIER et al., 2022).

Salicylic acid (SA) is a phytohormone that plays several physiological roles in plants, including the regulation of photosynthesis and an increase in the activity of enzymatic and non-enzymatic antioxidants. In addition, it promotes an increase in growth through the synthesis of photosynthetic pigments, maintains water relations, modulates mineral nutrients in the root system by restricting sodium (Na^+) influx, and induces the accumulation of osmotic regulators (KHAN; POOR; JANDA, 2022). In addition, SA can act in reducing the production of reactive oxygen species by promoting antioxidant reactions (ABDI et al., 2022). However, the effect of SA application depends on several factors, including concentration, plant species, stage of plant development, and mode of application (POÓR et al., 2019).

In light of the above, the objective of this study was to evaluate the gas exchange, biomass accumulation and quality of guava seedlings cultivated with waters of different cationic nature and foliar application of salicylic acid.

MATERIAL AND METHODS

The experiment was conducted from October 2021 to April 2022 under greenhouse conditions at the Center of Sciences and Agri-Food Technology (CCTA) of the Federal University of Campina Grande (UFCG), Campus of Pombal-PB, whose local geographic coordinates are 6º48’16” S, 37º49’15” W and an average altitude of 144 m. The data of temperature (maximum and minimum) and relative humidity of the air during the experimental period were collected daily and are shown in Figure 1.

A randomized block design was used in a 6 × 4 factorial arrangement whose treatments resulted from the combination of two factors: six cationic nature of irrigation water (S1 - Control; S2 - Na^+; S3 - Ca^{2+}; S4 - Na^++Ca^{2+}; S5 - Mg^{2+}, and S_6 - Na^++Ca^{2+}+Mg^{2+}), to have equivalent ratios of 1:1 for Na^+:Ca^{2+} (S5) and 7:2:1 for Na^++Ca^{2+}+Mg^{2+} (S6), respectively, associated with four concentrations of salicylic acid - SA (0, 1.3, 2.6, and 3.9 mM) with three replicates and 2 plants in each plot. Salicylic acid concentrations were based on the study conducted by Silva et al. (2020) with the soursop crop, given the absence of studies with guava.

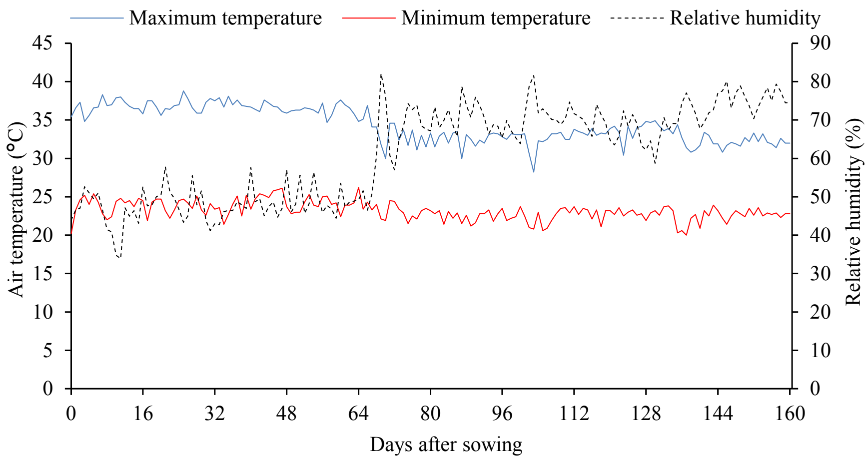


Figure 1. Data of maximum and minimum daily temperature and relative humidity of the air observed during the experimental period.

Plants in the control treatment (S1) were irrigated with municipal-supply water (ECw = 0.3 dS m^{-1}), whose chemical composition is presented in Table 1, while the other types of water (S2, S3, S4, S5, and S6) had ECw of 4.3 dS m^{-1}, prepared using compounds of different cations, in the form of chloride.

Table 1. Chemical characteristics of the water used in control treatment.

Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^{-}	CO_3^{2-}	Cl^{-}	EC	pH	SAR
(mmol$_C$ L^{-1})							dS m^{-1}		(mmol $L^{-1})^{0.5}$
0.39	0.78	1.45	0.33	0.96	0.00	1.75	0.30	7.04	1.34

EC - electrical conductivity. SAR - sodium adsorption ratio.

The guava cultivar studied was ‘Paluma’, derived from Rubi-Supreme guava, obtained from open-pollination seeds; its fruits are suitable for both table and industry, with a mass between 140 and 250 g, polar diameter from 8 to 10 cm and equatorial diameter from 7 to 9 cm, and pulp with intense red color (MEDINA et al., 1991).

Seedlings were produced in polyethylene bags with dimensions of 15 × 30 cm, filled with a mixture in a 2:1:1 ratio (volume basis) of soil classified as Entisol (Psamment) of sandy loam texture, sand, and organic matter (well-aged bovine manure). The soil came from the rural area of São Domingos, PB, 0-20 cm depth. The bags were distributed equidistantly, supported on benches at 0.80 m in height from the ground. The physical and chemical characteristics of the soil obtained according to the methodologies proposed by Teixeira et al. (2017) are shown in Table 2.

Table 2. Chemical and physical characteristics of the soil used in the experiment before the application of the treatments.

Chemical characteristics								
pH (H_2O)	OM	P	K^{+}	Na^{+}	Ca^{2+}	Mg^{2+}	Al^{3+}	H^{+}
(1:2.5)	g kg^{-1}	(mg kg^{-1})	...cmol$_c$ kg^{-1}...					
8.53	3.10	77.30	0.56	0.20	5.08	5.11	0	0
...Chemical characteristics...				...Physical characteristics...				
EC$_{se}$	CEC	SARse	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	33.42 kPa[1]	1519.5 kPa[2]
0.46	10.95	1.02	1.83	775.70	180.90	43.40	12.45	5.00

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

Fertilization with nitrogen, potassium, and phosphorus was performed based on the recommendation of Novais, Neves, and Barros (1991), applying 100, 150, and 300 mg kg^{-1} of N, K_2O, and P_2O_5, respectively, 1/3 supplied as basal dose and the remaining 2/3 as top-dressing via fertigation in 8 equal applications, at 10-day intervals, with the first application at 10 days after sowing (DAS). To meet the need for micronutrients, the plants were sprayed with a solution containing 2.5 g L^{-1} of Dripsol micro® (Mg^{2+} - 1.1%, Boron - 0.85%, Copper (Cu-EDTA) - 0.5%, Iron (Fe - EDTA) - 3.4%, Manganese (Mn-EDTA) - 3.2%, Molybdenum - 0.05%, and Zinc - 4.2% with 70% chelating agent EDTA), through the leaves (adaxial and abaxial sides), at 15-days intervals.

The solutions with desired concentrations of salicylic acid were prepared by dissolving salicylic acid (A.R.) in 30% ethyl alcohol. Salicylic acid applications started at 10 DAS and were subsequently performed weekly, spraying in such a way to completely wet the leaves (abaxial and adaxial sides), using a sprayer, with applications performed from 5 p.m., adapted from the study conducted by Silva et al. (2020). Plants were isolated with a structure using plastic tarpaulin during the applications of salicylic acid to prevent the solutions from drifting.

The irrigation waters with different cationic natures, with ECw = 4.3 dS m^{-1}, were obtained by the addition of Na^{+}, Ca^{2+}, and Mg^{2+} salts in chloride form, according to the pre-established treatments, based on the water from the local supply system (Pombal-PB), and their quantities were determined considering the relationship between ECw and the salt concentration (RICHARDS, 1954), according to Equation 1:

$$Q \approx 10 \times ECw \qquad (1)$$

where:
Q = Sum of cations ($mmol_c$ L^{-1}); and,
ECw = electrical conductivity of water (dS m^{-1})

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

Before sowing, the volume of water needed to raise the soil moisture content to field capacity was determined, applying water according to the established treatments. After transplanting, irrigation was performed daily at 5 p.m., applying in each bag the volume corresponding to that obtained by the water balance, and the volume of water to be applied was determined by Equation 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 in the previous irrigation event (mL); and,
LF - leaching fraction of 0.10.

Gas exchange, growth, and quality of the seedlings were evaluated at 160 DAS through stomatal conductance (*gs* - mol H_2O m^{-2} s^{-1}), CO_2 assimilation rate (*A*) (µmol CO_2 m^{-2} s^{-1}), transpiration (*E*) (mmol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration (*Ci*) (µmol CO_2 m^{-2} s^{-1}) with a portable infrared carbon dioxide analyzer (IRGA), "LCPro+" model from ADC BioScientific Ltda. Measurements were performed between 7:00 a.m. and 10:00 a.m. on the third fully expanded and photosynthetically active leaf, counted from the apical bud, under natural conditions of air temperature, CO_2 concentration and using an artificial radiation source of 1,200 µmol m^{-2} s^{-1}, established through the photosynthetic light response curve (FERNANDES et al., 2021).

Stem diameter (SD) was measured at 5 cm from the plant collar, with a digital caliper. Plant height (PH) was obtained as the distance from the plant collar to the insertion of the apical meristem. In the determination of leaf area (LA), only leaves that had a minimum length of 3 cm and at least 50% of their LA photosynthetically active were considered, according to the methodology of Lima et al. (2012), through Equation 3:

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

where:
LA = total leaf area (cm^2); and
L = leaf midrib length (cm).

Leaf succulence - SUC (g cm^{-2}) was determined according to the relationship proposed by Mantovani (1999), obtained through Equation 4:

$$SUC = \frac{(LFB - LDB)}{(LA)} \quad (4)$$

where:
SUC = leaf succulence (g cm^{-2});
LFB = leaf fresh biomass (g per plant);
LDB = leaf dry biomass (g per plant); and
LA = leaf area (cm^2).

To determine biomass accumulation the plants were cut at 160 DAS close to the soil surface and separated into leaves, stems, and roots. Subsequently, the different parts (leaves, stems, and roots) were placed in a paper bag and dried in a forced air ventilation oven at a temperature of 65 °C until reaching constant weight; then, the material was weighed to obtain the values expressed in gram (g), for the dry biomass of leaf (LDB), stem (StDB), and root (RDB), whose sum resulted in the total dry biomass (TDB) of the plant.

The quality of guava seedlings was determined using the Dickson Quality Index - DQI proposed by Dickson, Leaf and Hosner (1960), according to Equation 5:

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

where:
DQI = Dickson quality index;
PH = plant height (cm);
SD = stem diameter (mm);
TDB = total dry biomass (g per plant);
ShDB = shoot dry biomass (g per plant); and
RDB = root dry biomass (g per plant).

The obtained data were evaluated through analysis of variance by the F test after the normality test (Shapiro-Wilk). In the cases of significance, the Tukey test ($p \leq 0.05$) was performed for the cationic nature of irrigation water, and linear and quadratic polynomial regression analysis ($p \leq 0.05$) was performed for salicylic acid concentrations, using the statistical program SISVAR-ESAL version 5.6 (FERREIRA, 2019).

RESULTS AND DISCUSSION

There was a significant effect of the cationic nature of water (CNW) on all variables of gas exchange and growth of guava cv. 'Paluma', at 160 DAS (Table 3). Salicylic acid concentrations significantly affected ($p \leq 0.05$) only the stomatal conductance of guava plants cv. 'Paluma'. The interaction between the factors (CNW × SA) significantly influenced ($p \leq 0.05$) only the CO_2 assimilation rate of guava (Table 3).

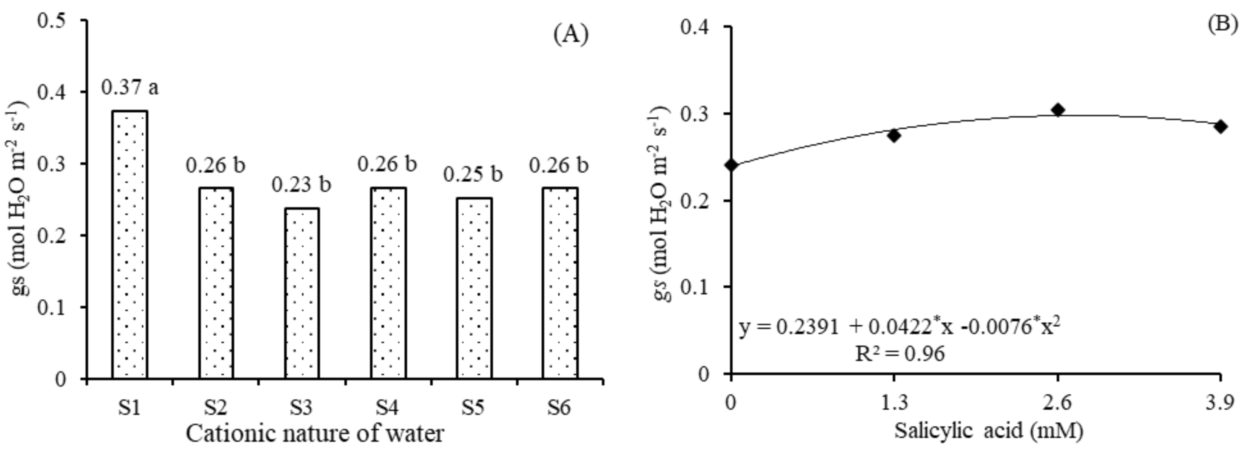
Table 3. Summary of the analysis of variance for stomatal conductance (*gs*), transpiration (*E*), internal CO_2 concentration (*Ci*), CO_2 assimilation rate (*A*), plant height (PH), stem diameter (SD), and leaf area (LA) of guava cv 'Paluma' under irrigation with water of different cationic nature (CNW) and exogenous application of salicylic acid (SA), 160 days after sowing.

Source of variation	DF	Mean squares						
		gs	*E*	*Ci*	*A*	PH	SD	LA
Cationic nature of water - CNW	5	0.028**	0.711**	2473.45*	84.056**	269.695*	4.797**	60766.64*
Salicylic acid (SA)	3	0.012*	0.142ns	875.94ns	23.667*	116.551ns	0.982ns	6092.13ns
Linear Regression	1	0.023*	-	-	-	-	-	-
Quadratic Regression	1	0.012*	-	-	-	-	-	-
Interaction (CNW × SA)	15	0.006ns	0.153ns	1185.12ns	18.118*	35.951ns	0.284ns	17388.39ns
Blocks	2	0.013*	0.506ns	6390.01*	1.593ns	72.844ns	1.585ns	6756.28ns
Residual	46	0.002	0.102	1005.27	9.054	62.430	0.490	17953.25
CV (%)		18.62	9.03	15.59	14.39	12.22	13.21	23.25

DF - Degrees of freedom; CV - Coefficient of variation; * significant at 0.05 probability level; ** significant at 0.01 probability level; ns not significant.

The stomatal conductance of plants grown under irrigation with water of 0.3 dS m^{-1} was statistically higher than that of those subjected to ECw of 4.3 dS m^{-1} with distinct cationic compositions (Na^+; Ca^{2+}; Na^++Ca^{2+}; Mg^{2+}, and Na^++Ca^{2+}+Mg^{2+}) (Figure 2A). When comparing the *gs* of plants grown with water containing Na^+ (S2), Ca^{2+} (S3), Na^++Ca^{2+} (S4), Mg^{2+} (S5), and Na^++Ca^{2+}+Mg^{2+} (S6), there was no significant difference among them, evidencing that the partial closure of stomata is related to the variation in the electrical conductivity of water. The reduction in stomatal conductance in plants subjected to salt stress occurs due to the decrease in leaf turgor and atmospheric vapor pressure, along with chemical signals generated by the root to limit water loss to the atmosphere (HNILIČKOVÁ et al., 2017). However, the decline in *gs* causes a decrease in CO_2 absorption, affecting the functioning of the photosynthetic apparatus and inhibiting plant growth (HANNACHI et al., 2022).

Lima et al. (2019), when evaluating gas exchange in castor bean plants as a function of the cationic nature of irrigation water (S1-Control; S2 - Na^+; S3 - Ca^{2+}; S4 - Na^++ Ca^{2+}; S5 - K^+, and S6 - Na^++ Ca^{2+}+ Mg^{2+}), also observed that, except for waters containing potassium and salinity of 4.5 dS m^{-1}, caused a decrease in stomatal conductance, regardless of the cationic nature of the water. In another study, Xavier et al. (2022) evaluated the gas exchange of 'Paluma' guava seedlings as a function of irrigation with saline waters (ECw: 0.6 to 4.2 dS m^{-1}) prepared with Na^++Ca^{2+}+Mg^{2+} in the equivalent proportion of 7:2:1 and found that stomatal conductance reduced quadratically with the increase in water salinity levels, at 180 days after sowing.



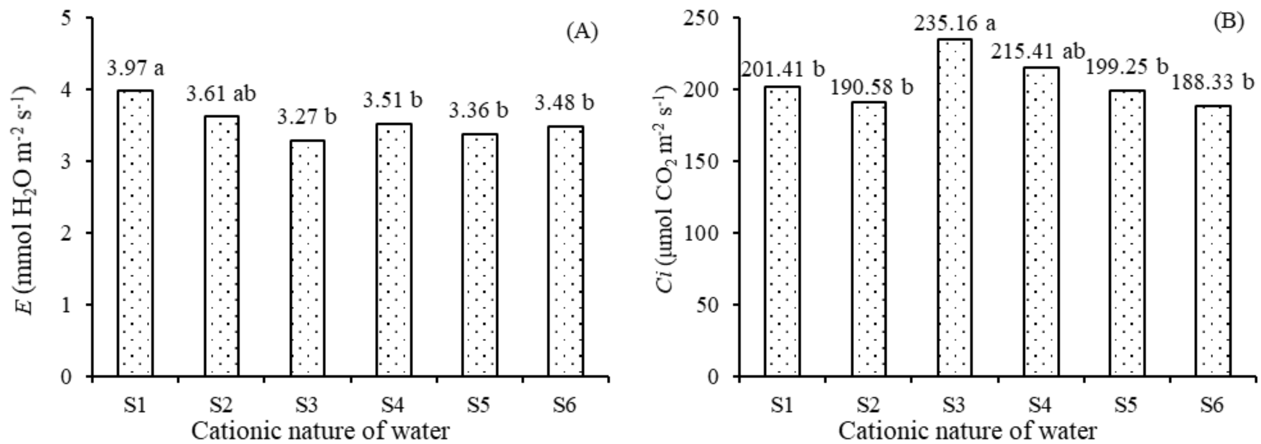
Means followed by different letters differ significantly from each other by Tukey test ($p \leq 0.05$).
S1 – Control, S2 - Na^+, S3 - Ca^{2+}, S4 - Na^++Ca^{2+}, S5 - Mg^{2+}, and S6 - Na^++Ca^{2+}+Mg^{2+}. In the control (S1), plants were irrigated with water of electrical conductivity (ECw) of 0.3 dS m^{-1}, while an ECw of 4.3 dS m^{-1} was used for S2, S3, S4, S5 and S6.

Figure 2. Stomatal conductance of guava cv 'Paluma' as a function of the cationic nature of water (A) and salicylic acid concentrations, 160 days after sowing.

Salicylic acid concentrations increased the stomatal conductance of guava quadratically (Figure 2B), with the maximum value of 0.298 mol H_2O m^{-2} s^{-1} obtained at the estimated concentration of 2.9 mM. On the other hand, the absence of SA application (0 mM) resulted in a minimum value of 0.239 mol H_2O m^{-2} s^{-1}. In relative terms, the application of 3.9 mM of salicylic acid promoted an increment of 0.049 mol H_2O m^{-2} s^{-1} compared to plants that did not receive the application (0 mM). The increase in stomatal conductance promoted by salicylic acid may be related to its function in the regulation of the antioxidant defense system, in the maintenance of chloroplast integrity, contributing to the control of stomatal opening and closure, and in the stability of the cell membrane (KHALVANDI et al., 2021).

The transpiration (Figure 3A) of guava plants irrigated with ECw of 0.3 dS m^{-1} (Control) differed significantly from that of plants that received water with Ca^{2+} (S3), Na^++Ca^{2+} (S4), Mg^{2+} (S5), and Na^++Ca^{2+}+Mg^{2+} (S6). However, when comparing plants in the control treatment with those subjected to the salinity of water of sodic nature (S2), no significant differences were found between them. It is important to highlight that the time scale at which specific damage by Na^+ occurs in plants depends on the rate of Na^+ accumulation in leaves and the effectiveness of Na^+ compartmentalization in leaf tissues and cells or interactions with other environmental factors (TESTER; DAVENPORT, 2003). Another factor that contributes as a mechanism of tolerance of plants to salt stress is the replacement of Na^+ with K^+, or the exclusion of Na^+ and the retention of intracellular K^+ (NAHAR et al., 2016).

On the other hand, the partial closure of the stomata observed in this study through stomatal conductance (Figure 2A) contributes to reducing water losses by transpiration to the atmosphere in plants subjected to irrigation with water containing Na^+ (S2) Ca^{2+} (S3), Na^++Ca^{2+} (S4), Mg^{2+} (S5) and Na^++Ca^{2+}+Mg^{2+} (S6), but does not compromise CO_2 diffusion in the leaf mesophyll (LIMA et al., 2020), as observed by the internal CO_2 concentration data (Figure 3B), and plays an important physiological function in the maintenance of osmotic and ionic homeostasis in the plant (MOHAMED et al., 2020). Paiva et al. (2021), in a study evaluating the effects of irrigation with waters of different cationic natures (S1 - Control; S2 - Na^+; S3 - Ca^{2+}; S4 - Mg^{2+}; S5 - Na^+ + Ca^{2+}; S6 - Na^+ + Mg^{2+}; S7 - Ca^{2+} + Mg^{2+} and S8 - Na^+ + Ca^{2+} + Mg^{2+}) on the gas exchange of passion fruit cv ‘BRS Rubi do Cerrado’, found the lowest values of leaf transpiration when plants were irrigated with water salinized by calcium (S3) and magnesium (S4) and observed no significant difference between plants that received the treatments S2, S5, S6, S7, and S8.

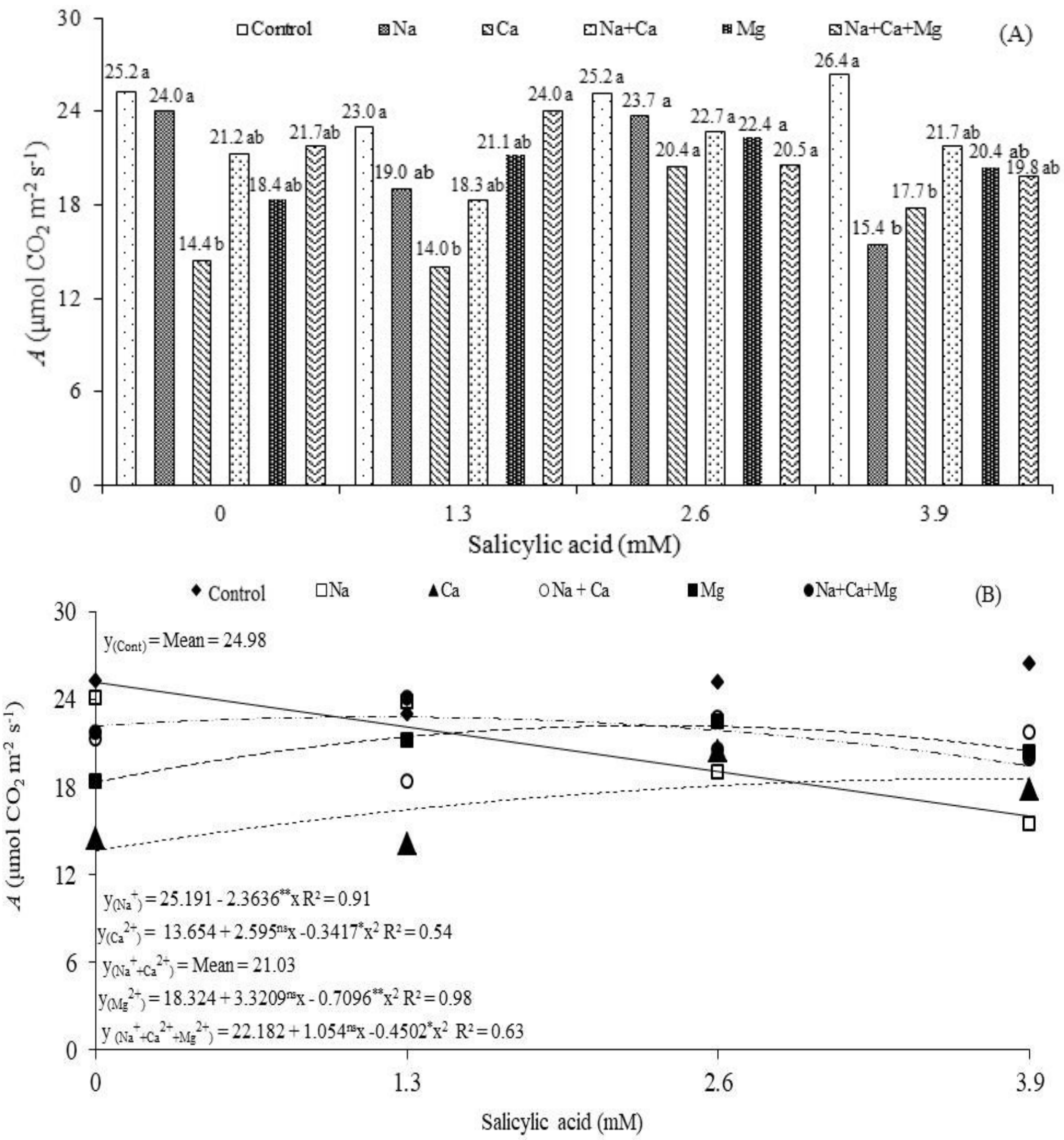


Means followed by different letters differ significantly from each other by Tukey test ($p \leq 0.05$).
S1 – Control, S2 - Na^+, S_3 - Ca^{2+}, S4 - Na^++Ca^{2+}, S5 - Mg^{2+}, and S6 - Na^++Ca^{2+}+Mg^{2+}. In the control (S1), plants were irrigated with water of electrical conductivity (ECw) of 0.3 dS m^{-1}, while an ECw of 4.3 dS m^{-1} was used for S2, S3, S4, S5 and S6.

Figure 3. Transpiration - *E* (A) and internal CO_2 concentration - *Ci* (B) of guava cv ‘Paluma’ irrigated with water of different cationic nature, 160 days after sowing.

The CO_2 assimilation rate of guava seedlings was significantly influenced by the interaction between the cationic nature of water and salicylic acid concentrations (Figure 4A). In the absence of foliar application of salicylic acid (0 mM), plants irrigated with ECw of 0.3 dS m^{-1} (Control) differed significantly only from those cultivated with water of calcic composition (S3). With the application of 1.3 mM of SA, there were significant differences in the *A* of plants subjected to low ECw (control) and water containing Na^++Ca^{2+}+Mg^{2+} when compared to those cultivated under salinity of water of calcic nature (S3). The application of 2.6 mM of SA did not significantly influence *A*, regardless of the cationic nature of the water. On the other hand, for plants that received 3.9 mM, irrigation with ECw of 0.3 dS m^{-1} (control) resulted in an *A* statistically higher than that of those cultivated with water containing sodium (S2) and calcium (S3).

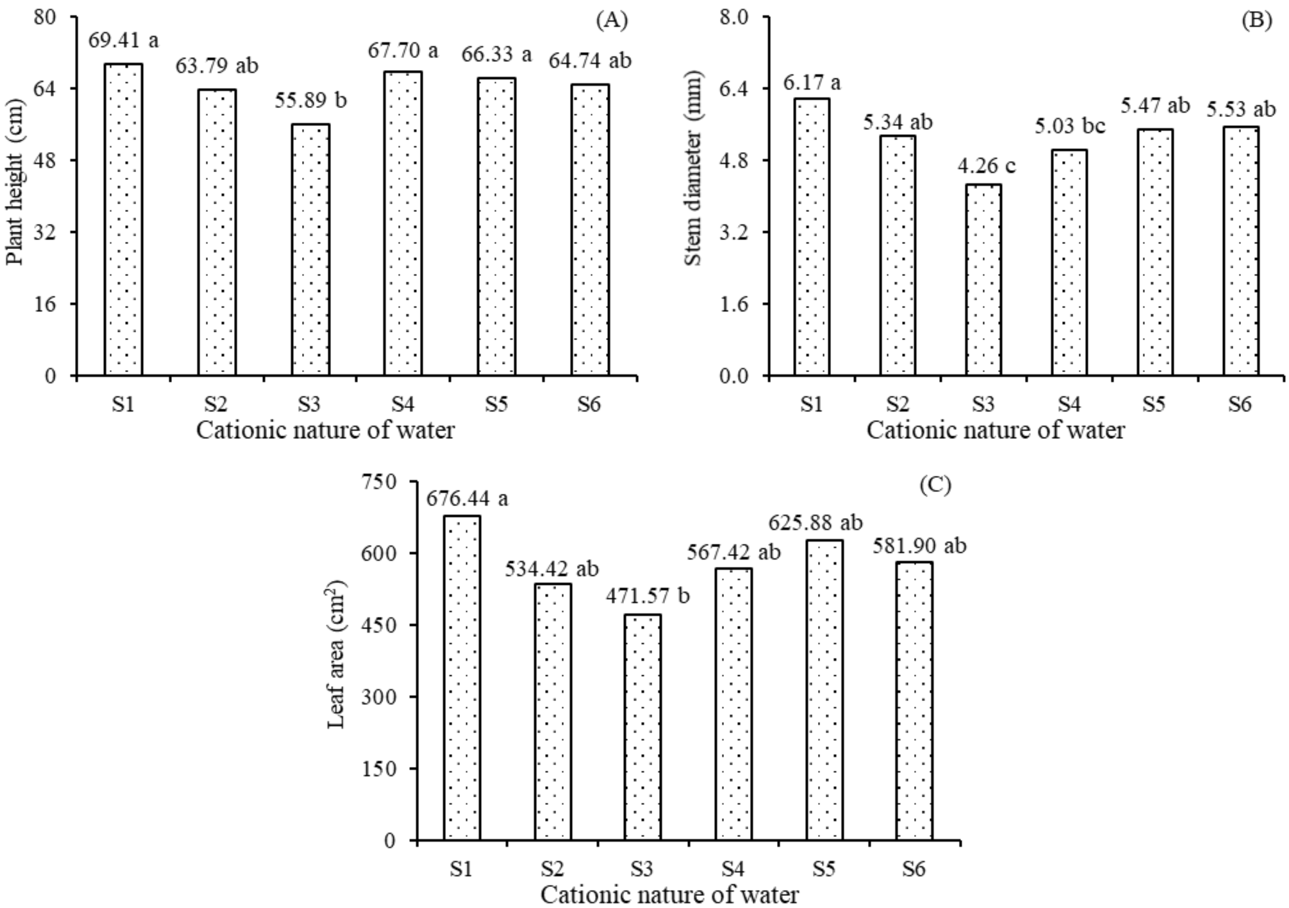
When analyzing the effects of salicylic acid concentrations considering each cationic nature of water (Figure 4B), it was observed that plants cultivated with water containing sodium (S2) showed a linear reduction in the CO_2 assimilation rate of 9.38% per unit increase in salicylic acid concentration. Plants subjected to the treatments S3, S5 and S6 showed a quadratic behavior (Figure 4B), with the maximum values (18.58, 22.20, and 22.79 µmol CO_2 m^{-2} s^{-1}) estimated at the SA concentrations of 3.8, 2.3, and 1.2 mM, respectively. However, there was no satisfactory fit of the regression models to the data of the CO_2 assimilation rate of plants subjected to the control treatment (S1) and S4, and the mean values were 24.98 and 21.03 µmol CO_2 m^{-2} s^{-1}, respectively. The photosynthetic capacity of the plants is reduced due to osmotic stress and partial closure of the stomata (MOHAMED et al., 2020). In addition, the salts absorbed by plants and accumulated in the photosynthetically active mesophyll tissues can inhibit CO_2 assimilation, mainly affecting photosynthesis processes in chloroplasts, standing out as a non-stomatal limitation. The alterations include inhibition of the activity of photosynthetic enzymes of the Calvin cycle, disturbance in chlorophyll biosynthesis, and reduction in operational efficiency and structural integrity of the photosynthetic apparatus and thylakoid membranes (PAN et al., 2021).



Means followed by different letters differ significantly from each other by Tukey test ($p \leq 0.05$).
S1 – Control, S2 - Na^{+}, S_3 - Ca^{2+}, S4 - Na^{+}+Ca^{2+}, S5 - Mg^{2+} and S6 - Na^{+}+Ca^{2+}+Mg^{2+}. In the control (S1), plants were irrigated with water of electrical conductivity (ECw) of 0.3 dS m^{-1}, while an ECw of 4.3 dS m^{-1} was used for S2, S3, S4, S5 and S6.

Figure 4. CO_2 assimilation rate - *A* of guava seedlings cv. 'Paluma' as a function of the interaction between cationic nature of the water and salicylic acid concentrations, 160 days after sowing.

Irrigation with water of low ECw (control) and containing $Na^{+}+Ca^{2+}$ (S4) and Mg^{2+} (S5) promoted plant height statistically higher than that of plants grown under salinity and water of calcic nature (S3) (Figure 5A). When comparing the PH of plants subjected to the treatments S1, S2, S4, S5 and S6, it was observed that there were no significant differences among them. The reduction in the growth in height in plants irrigated with water containing calcium may be associated with increased permeability of the cell membrane induced by the ionic and osmotic effect, leading to the generation of reactive oxygen species (ROS). These ROS can affect the integrity of cell membranes, enzyme activities, and the plant's photosynthetic apparatus (XU et al., 2017).



Means followed by different letters differ significantly from each other by Tukey test ($p \leq 0.05$).
S1 – Control, S2 - Na^{+}, S_3 - Ca^{2+}, S4 - $Na^{+}+Ca^{2+}$, S5 - Mg^{2+} and S6 - $Na^{+}+Ca^{2+}+Mg^{2+}$. In the control (S1), plants were irrigated with water of electrical conductivity (ECw) of 0.3 dS m^{-1}, while an ECw of 4.3 dS m^{-1} was used for S2, S3, S4, S5 and S6.

Figure 5. Plant height (A), stem diameter (B), and leaf area (C) of guava cv. 'Paluma' irrigated with water of different cationic natures, 160 days after sowing.

The SD of guava was also influenced by the cationic nature of the water (Figure 5B), and plants subjected to irrigation with water containing calcium obtained the lowest value for this variable, differing significantly from those cultivated under S1, S2, S5, and S6. However, when comparing the SD of plants grown under the treatments S3 and S4, no significant difference was observed. Excessive absorption of calcium ions, due to their high concentration in irrigation water, may have induced partial closure of stomata, reducing photosynthesis and consequently plant growth (BLATT, 2000). High concentrations of free Ca^{2+} in the cytoplasm can also precipitate PO_4^{3-} ions, interfering with physiological processes related to phosphorus metabolism, inhibiting respiration and affecting plant growth (WENG et al., 2022). Xavier et al. (2022), in a study with 'Paluma' guava seedlings in the rootstock formation phase irrigated with saline waters (ECw: 0.6 to 4.3 dS m^{-1}) prepared with $Na^{+}+Ca^{2+}+Mg^{2+}$ in the equivalent proportion of 7:2:1, found that the growth rate was linearly reduced with the increase in ECw levels.

As observed for PH and SD (Figures 5A and 5B), irrigation with water containing calcium (S3) reduced leaf area, with significant differences only compared to plants grown under ECw of 0.3 dS m^{-1} (control). However, no significant effect was observed on the LA of plants subjected to different cationic compositions of water. The decrease observed in leaf area can be considered a mechanism of tolerance of plants to maintain cell turgor, reducing water losses by transpiration and, consequently, reducing the transport of toxic ions such as Na^+ and Cl^- through the xylem (LIMA et al., 2020). Bezerra et al. (2018) evaluated the growth of grafted guava cv 'Paluma' under different salinities of irrigation water with sodic composition (ECw: 0.3 to 3.5 dS m^{-1}) and observed that water salinity from 0.3 dS m^{-1} negatively affected the number of leaves and leaf area, at 90, 120, and 150 DAT.

There was a significant effect of the cationic nature of water on the dry biomass of leaf (LDB) ($p \leq 0.05$), stem (StDB), root (RDB), total (TDB), and Dickson's quality index (DQI) of guava cv 'Paluma' ($p \leq 0.01$) (Table 4). Salicylic acid concentration significantly influenced only stem dry biomass. There was no effect of the interaction ($p > 0.05$) between the factors (CNW × SA) for any of the variables measured.

Table 4. Summary of the analysis of variance for the dry biomass of leaf (LDB), stem (StDB), root (RDB), total (TDB), leaf succulence (SUC), and Dickson's quality index (DQI) of guava cv 'Paluma' under irrigation with water of different cationic nature (CNW) and exogenous application of salicylic acid (SA), 160 days after sowing.

Source of variation	DF	Mean squares					
		LDB[1]	StDB	RDB[1]	TDB	SUC[1]	DQI
Cationic nature of water - CNW	5	5.644^{*}	7.714^{**}	14.709^{**}	76.137^{**}	0.00002^{ns}	1.228^{**}
Salicylic acid (SA)	3	1.565^{ns}	1.385^{*}	0.320^{ns}	5.586^{ns}	0.00004^{ns}	0.029^{ns}
Linear Regression	1	-	0.296^{*}	-	-	-	-
Quadratic Regression	1	-	0.203^{*}	-	-	-	-
Interaction (CNW × SA)	15	2.374^{ns}	0.561^{ns}	0.445^{ns}	4.494^{ns}	0.0001^{ns}	0.039^{ns}
Blocks	2	0.376^{ns}	0.419^{ns}	0.141^{ns}	1.570^{ns}	0.00001^{ns}	0.020^{ns}
Residual	46	1.613	0.500	0.555	3.647	0.00002	0.039
CV (%)		16.95	24.43	19.22	22.48	18.78	24.88

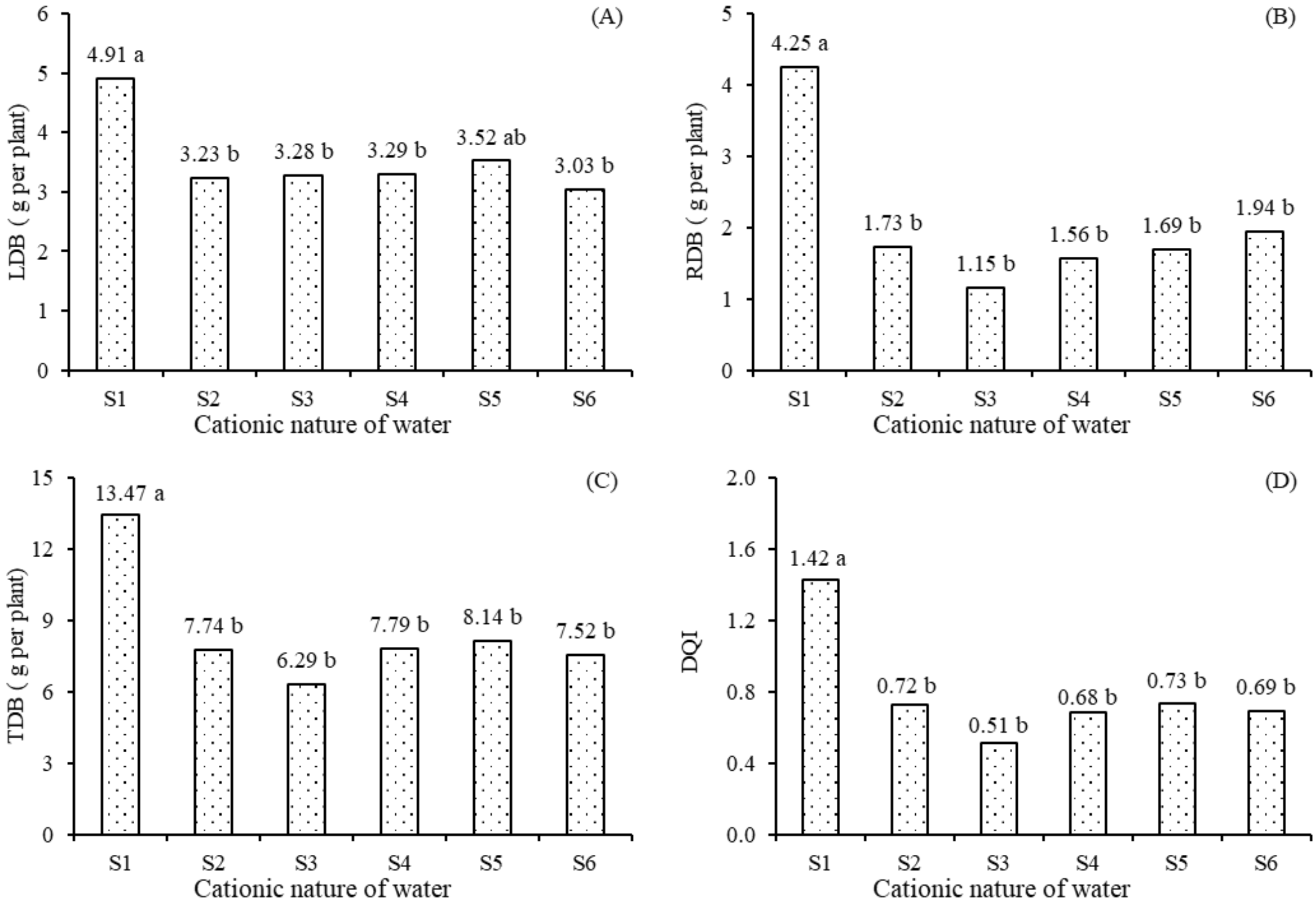
DF - Degrees of freedom; CV - Coefficient of variation; * significant at 0.05 probability level; ** significant at 0.01 probability level; ns not significant; [1]Data transformed into √x

The LDB of guava plants (Figure 6A) subjected to irrigation with water containing Na^+, Ca^{2+}, Na^++Ca^{2+}, and Na^++Ca^{2+}+Mg^{2+} was significantly reduced compared to those that received ECw of 0.3 dS m^{-1}. However, when comparing plants cultivated under irrigation with water containing Mg^{2+} (S5) to those subjected to the different cationic nature, no significant difference was observed. The reduction in biomass accumulation in plants subjected to ECw of 4.3 dS m^{-1} (S2, S3, S4, and S6) may be associated with changes in the partition of photoassimilates, with the displacement of energy, which is directed to growth for the activation and maintenance of metabolic activities associated with salinity tolerance mechanisms, such as the maintenance of membrane integrity and the regulation of transport and ionic distribution in various organs inside cells (DINIZ et al., 2020).

The decrease in biomass accumulation in plants subjected to salt stress also reflects the action of osmotic and ionic effects, which interferes with photosynthetic efficiency through energy expenditure to maintain membrane integrity, synthesis of organic solutes for osmoregulation and/or protection of macromolecules and regulation of ion transport and distribution, and, consequently, with plant growth and development, as observed in different crops, such as yellow passion fruit (DINIZ et al., 2020; LIMA et al., 2020), pomegranate (SOARES et al., 2021), and cherry tomatoes (ROQUE et al., 2022).

The RDB (Figure 6B) and TDB (Figure 6C) of guava irrigated with water of 0.3 dS m^{-1} were statistically higher than those of plants cultivated under different cationic compositions (Na^+, Ca^{2+}, Na^++Ca^{2+}, Mg^{2+}, and Na^++Ca^{2+}+Mg^{2+}). Thus, it is clear that the variation in electrical conductivity levels from 0.3 to 4.3 dS m^{-1} is more harmful to the biomass accumulation of guava, regardless of the cationic nature in irrigation water.

The DQI (Figure 6D) also followed the same trend observed for RDB and TDB (Figures 6B and 6C), in which plants subjected to ECw of 0.3 dS m^{-1} (control) obtained a higher DQI when compared to those that received water containing Na^+ (S2), Ca^{2+} (S3), Na^++Ca^{2+} (S4), Mg^{2+} (S5) and Na^++Ca^{2+}+Mg^{2+} (S6). When comparing plants grown with waters prepared with Na^+, Ca^{2+}, Na^++Ca^{2+}, Mg^{2+}, and Na^++Ca^{2+}+Mg^{2+}, no significant differences were observed among them. It is important to highlight that, despite the reduction in DQI, plants subjected to ECw of 4.3 dS m^{-1} (Na^+; Ca^{2+}; Na^++Ca^{2+}; Mg^{2+}, and Na^++Ca^{2+}+Mg^{2+}) were suitable to be transplanted to the field, because they had DQI higher than 0.20, being considered seedlings of acceptable quality for transplanting to the field (DICKSON; LEAF; HOSNER, 1960).



Means followed by different letters differ significantly from each other by Tukey test ($p \leq 0.05$).
S1 – Control, S2 - Na^+, S3 - Ca^{2+}, S4 - Na^++Ca^{2+}, S5 - Mg^{2+}, and S6 - Na^++Ca^{2+}+Mg^{2+}. In the control (S1), plants were irrigated with water of electrical conductivity (ECw) of 0.3 dS m^{-1}, while an ECw of 4.3 dS m^{-1} was used for S2, S3, S4, S5, and S6.

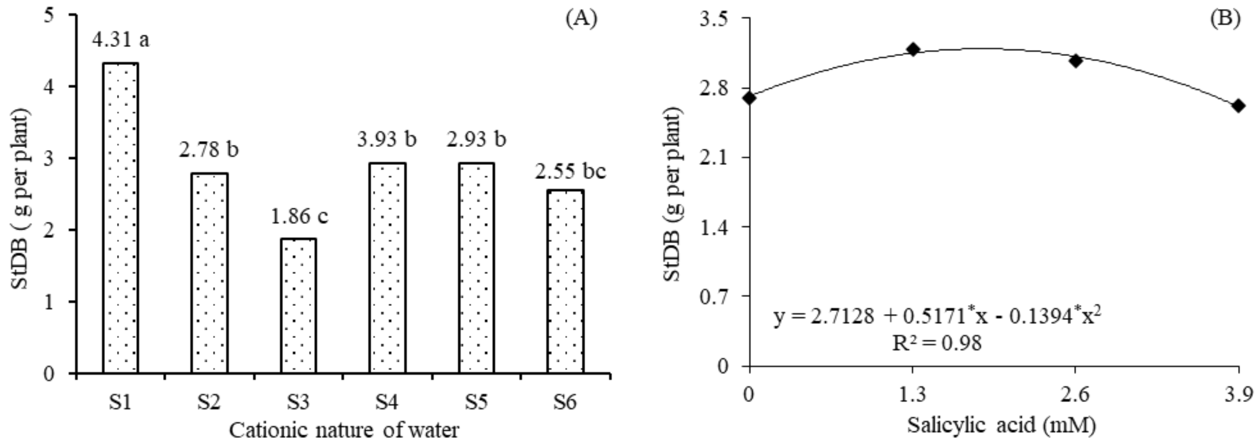
Figure 6. Leaf dry biomass - LDB (A), root dry biomass - RDB (B), total dry biomass - TDB (C), and Dickson's quality index - DQI (D) of guava cv 'Paluma' irrigated with water of different cationic nature, at 160 days after sowing.

Lima et al. (2021a) evaluated the quality of passion fruit seedlings, cv 'BRS Rubi do Cerrado', as a function of the cationic nature of irrigation water and observed that, although there was a reduction in the DQI, regardless of the cationic nature, the use of water with electrical conductivity of 3.0 dS m^{-1} led to the production of seedlings with Dickson quality index higher than 0.2, considered acceptable.

For the dry biomass of the stem of guava (Figure 7A), irrigation with water of different cationic compositions caused a negative effect, regardless of the type of cation, compared to those cultivated under ECw of 0.3 dS m^{-1} (control). The StDB of plants irrigated with water prepared with Na^+ (S2), Na^++Ca^{2+} (S4), and Mg^{2+} (S5) differed significantly only from the values of those subjected to the lowest level of ECw (S1) and calcic composition (S3). However, there were no significant differences in the StDB of plants grown with water containing calcium (S3) and Na^++Ca^{2+}+Mg^{2+} (S6).

The water of calcic nature caused a greater reduction in StDB, which was lower than that of plants subjected to S1, S2, S4, and S5. However, plants grown under ECw of 4.3 dS m^{-1} reduced their StDB accumulation compared to those that received the lowest salinity level (0.3 dS m^{-1}), regardless of the cationic nature of the water. The reduction in StDB accumulation is a consequence of the decrease in water availability due to the osmotic effect, which in turn interferes with the absorption of water and nutrients by plants (LIMA et al., 2021b).

Regarding the effects of salicylic acid on StDB (Figure 7B), the maximum estimated value (3.192 g per plant) was obtained under application of 1.90 mM, decreasing from this concentration and reaching the lowest value (2.609 g per plant) in plants that received 3.90 mM. At high concentrations, SA inhibits catalase activity and increases the concentration of H_2O_2 in the cytoplasm of the guard cells (CHEN; SILVA; KLESSIG, 1993). H_2O_2 oxidizes the plasma membrane and increases its permeability to K^+. K^+ efflux induces the loss of turgor pressure and causes partial closure of stomata (JOON-SANG, 1998), limiting the photosynthetic rate and therefore, the biomass accumulation of plants. However, the intensity of the effects of salicylic acid depends on other factors such as mode of application, concentration, crop and plant development stage (POÓR et al., 2019).



Means followed by different letters differ significantly from each other by Tukey test ($p \leq 0.05$).
S1 - Control; S2 - Na^+; S3 - Ca^{2+}; S4 - Na^++Ca^{2+}; S5 - Mg^{2+}, and S6 - Na^++Ca^{2+}+Mg^{2+}; In the control (S1), plants were irrigated with water of electrical conductivity (ECw) of 0.3 dS m^{-1}, while an ECw of 4.3 dS m^{-1} was used for S2, S3, S4, S5 and S6.

Figure 7. Stem dry biomass (StDB) of guava cv 'Paluma' as a function of the cationic nature of water (A) and salicylic acid concentrations (B), at 160 days after sowing.

CONCLUSIONS

In the seedling formation phase, guava is sensitive to water salinity of calcic nature, with a marked decrease in plant growth, 160 days after sowing.

Stomatal conductance, transpiration, and biomass accumulation of guava seedlings cv 'Paluma' are more affected by the variation in electrical conductivity than by the cationic nature of water.

Application of salicylic acid at concentrations of 2.9 and 1.9 mM increases stomatal conductance and stem dry biomass accumulation, respectively, in guava seedlings.

Water with electrical conductivity of up to 4.3 dS m^{-1} allows the formation of guava seedlings cv 'Paluma' with acceptable quality for transplanting to the field, regardless of the cationic nature of the water.

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