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Original Article

Salicylic acid and irrigation with water of different cationic compositions on 'Paluma' guava seedlings[1]

Ácido salicílico e irrigação com água de diferentes composições catiônicas em mudas de goiabeira 'Paluma'

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HIGHLIGHTS:

Water salinity of 3.0 dS m^{-1} increases electrolyte leakage, succulence, and chlorophyll a fluorescence.
Salicylic acid does not influence chlorophyll total content at salinity above 2.4 dS m^{-1}, regardless of the cationic nature of the water.
Salicylic acid at concentrations above 3.2 mM reduces the total dry matter mass and quality of guava seedlings.

ABSTRACT: The occurrence of water sources with high concentrations of salts in the semi-arid region of Northeastern Brazil limits the expansion of irrigated agriculture. Therefore, it is extremely important to look for strategies capable of mitigating the effects of salt stress on plants. The objective of this study was to evaluate the effects of foliar application of salicylic acid in inducing tolerance of guava seedlings irrigated with water of distinct cationic composition. The experiment was carried out under greenhouse conditions using a randomized block design in a 5 × 5 factorial arrangement, with five cationic compositions of irrigation water (S1 - Control tap water, S2 - Na^++Ca^{2+}+Mg^{2+}, S3 - Na^++Ca^{2+}, S4 - Ca^{2+}+Mg^{2+}, and S5 - Na^++Mg^{2+}), so as to have equivalent ratios of 7:2:1 relative to Na^++Ca^{2+}+Mg^{2+} and 1:1 relative to other combinations, respectively maintaining electrical conductivity - ECw at 3.0 dS m^{-1}, and five concentrations of salicylic acid (0, 0.8, 1.6, 2.4, and 3.2 mM), with three replications and two plants per plot. Electrolyte leakage, succulence, and chlorophyll a fluorescence signals increased in guava seedlings irrigated with water up to 3.0 dS m^{-1}. Water with ECw of 3.0 dS m^{-1} can be used to produce guava seedlings with acceptable quality for transplanting to the field, regardless of its constitution. In the absence of salicylic acid, water salinity negatively affected the chlorophyll a and b contents of seedlings, regardless of the cationic nature of the water.

Key words: *Psidium guajava* L., salt stress, osmoregulation

RESUMO: A ocorrência de fontes hídricas com concentrações elevadas de sais no semiárido do Nordeste brasileiro limita a expansão da agricultura irrigada. Dessa forma, é de extrema importância a busca por estratégias capazes de amenizar os efeitos do estresse salino nas plantas. Objetivou-se com este trabalho avaliar os efeitos da aplicação foliar de ácido salicílico na indução de tolerância em mudas de goiabeira irrigadas com águas de distintas composições catiônicas da água. A pesquisa foi desenvolvida sob condições de casa de vegetação, utilizando o delineamento de blocos casualizados em arranjo fatorial 5 × 5, sendo cinco composições catiônicas da água de irrigação (S1 - Testemunha água de abastecimento; S2 - Na^++Ca^{2+}+Mg^{2+}; S3 - Na^++Ca^{2+}; S4 - Ca^{2+}+Mg^{2+} e S5 - Na^++Mg^{2+}), de modo a se ter uma proporção equivalente de 7:2:1, entre Na^++Ca^{2+}+Mg^{2+} e de 1:1 entre outras combinações, respectivamente, mantendo condutividade elétrica - CEa de 3.0 dS m^{-1} e cinco concentrações de ácido salicílico (0; 0,8; 1,6; 2,4 e 3,2 mM) com 3 repetições e duas plantas por parcelas. O extravasamento de eletrólitos, a suculência e os sinais de fluorescência da clorofila a aumentaram nas mudas de goiabeira irrigadas com água de 3,0 dS m^{-1}. Água com CEa de até 3,0 dS m^{-1} pode ser utilizada na formação de mudas de goiabeira com qualidade aceitável para o transplantio no campo, independente da sua constituição. Na ausência do ácido salicílico a salinidade da água afetou, de forma negativa, os teores de clorofila a e b das mudas, independente da natureza catiônica da água.

Palavras-chave: *Psidium guajava* L., estresse salino, osmorregulação

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Introduction

Guava (*Psidium guajava* L.) is a species that stands out for producing fruits with a pleasant flavor, high nutritional value and accessible on the market, as it can be consumed fresh or in various processed products, such as guava paste, jellies, pasta, fruit in syrup, purees, baby food, base for drinks, soft drinks, juices and syrups (Oliveira et al., 2015). It is a fruit crop classified as sensitive to salt stress, with water salinity threshold level of 0.3 dS m^{-1} (Ferreira et al., 2023).

Although the semi-arid region of Northeast Brazil has edaphic conditions for the cultivation of this fruit crop, the irregularity of rainfall and high rates of evapotranspiration hinder agricultural production (Lacerda et al., 2022). In addition, this region normally has sources of water with high levels of dissolved salts and variations in its composition. Water with high concentrations of salts alter the patterns of nutrient availability and transport, causing inhibition in plant growth, and Na^+ and Cl^- ions are the main competitors that restrict availability of nutrients such as Ca^{2+}, K^+ and N (Shahid et al., 2020).

A strategy that has been employed to reduce the negative effects of salt stress on plants is foliar application of tolerance inducers such as salicylic acid (SA) (Lacerda et al., 2022), due to its role as a signaling agent in stress mitigation. Salicylic acid is a phytohormone that participates in the activation of genes capable of acting on the plant's mechanism of defense against oxidative stress, in the photosynthetic process (Figueiredo et al., 2019) in the production of osmolytes and secondary metabolites (Khan et al., 2015), thus contributing to the maintenance of ionic and cellular homeostasis, reducing the intensity of the deleterious effects of salt stress. Several studies have reported beneficial effects of applying salicylic acid to guava plants under salt stress (Xavier et al., 2022a; Lacerda et al., 2022); however, their research was limited to evaluating the effect of irrigation with water of different salinity levels and foliar application of salicylic acid. Therefore, it is imperative to conduct new studies that explore the effects of the cationic composition of water on guava plants in the seedling formation phase, as a measure to identify the cation(s) that promotes the minimum impacts on the physiology, growth, and quality of guava seedlings under Brazilian semi-arid conditions.

The objective of this study was to evaluate the effects of foliar application of salicylic acid in inducing tolerance of guava seedlings irrigated with water of distinct cationic nature to salt stress.

Material and Methods

The experiment was carried out under protected environment conditions, in an arched greenhouse, with low-density polyethylene cover of 150 microns, belonging to the Centro de Ciências e Tecnologia Agroalimentar (CCTA) of the Universidade Federal de Campina Grande (UFCG), Campus of Pombal, PB, Brazil, whose local geographic coordinates of reference are 6° 48' 16" S, 37° 49'15" W and average altitude of 144 m. The mean maximum and minimum air temperatures and mean relative air humidity in the internal area of the greenhouse during the experimental period were 32.9 °C, 22.5 °C, and 66%, respectively.

A randomized block design was used in a 5 × 5 factorial arrangement, with five cationic compositions of irrigation water (S_1 - Control, S_2 - $Na^++Ca^{2+}+Mg^{2+}$, S_3 - Na^++Ca^{2+}, S_4 - $Ca^{2+}+Mg^{2+}$, and S_5 - Na^++Mg^{2+}), so as to have equivalent ratios of 7:2:1 relative to $Na^++Ca^{2+}+Mg^{2+}$, and 1:1 relative to $Ca^{2+}+Mg^{2+}$, Na^++Ca^{2+} and Na^++Mg^{2+}, respectively, and five concentrations of salicylic acid - SA (0, 0.8, 1.6, 2.4, and 3.2 mM), with three replicates and two plants in each plot. For the concentration of 0 mM, foliar application with distilled water was performed. Plants of the control treatment (S1) were irrigated with municipal-supply water (electrical conductivity - ECw of 0.3 dS m^{-1}), while the other types of water (S2, S3, S4, and S5) had ECw of 3.0 dS m^{-1}, prepared using compounds of different cations, in chloride form. The cationic compositions of the waters were in accordance with the study of Queiroga et al. (2023). Salicylic acid concentrations were based on research carried out by Xavier et al. (2022a).

The seedlings were propagated by seeds in polyethylene bags with dimensions of 15 × 30 cm, filled with a mixture in a ratio of 2:1:1 (volume basis) of a Neossolo Regolítico (Entisols - Psamment) of sandy loam texture, sand, and well-decomposed cattle manure. The soil was obtained from the rural area of the municipality of São Domingos, PB, at 0-20 cm depth.

The bags were distributed equidistantly, supported on benches at 0.80 m height. Physical and chemical attributes of the soil were obtained according to Teixeira et al. (2017): Ca^{2+}, Mg^{2+}, Na^+, K^+, H^++Al^{3+} = 5.08, 5.11, 0.20, 0.56, and 0 $cmol_c$ kg^{-1}, respectively; Exchangeable sodium percentage = 1.83%; Organic matter = 3.10 dag kg^{-1}; P = 77.30 mg kg^{-1}; electrical conductivity of the saturation extract = 0.46 dS m^{-1}; and pH in water (1:2.5) = 8.53; sand, silt, and clay = 775.70, 180.90, and 43.40 g kg^{-1}, respectively.

Top-dressing fertilization with nitrogen, potassium, and phosphorus was carried out based on the recommendation of Novais et al. (1991), with 100, 150, and 300 mg kg^{-1} soil of N, K_2O, and P_2O_5, respectively, applied via fertigation in 7 equal applications, at 12-day intervals, with the first application at 10 days after sowing (DAS). In order to meet the need for micronutrients, foliar applications were realized at 15 day intervals with 2.5 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).

Solutions with adequate concentrations of SA were prepared by dissolving salicylic acid (A.R.) in 30% ethyl alcohol (99.5%). Salicylic acid applications began at 10 DAS and were later carried out weekly (totaling 15 applications), spraying in such a way to fully wet the leaves (abaxial and adaxial sides), using a spray bottle and avoiding wetting of neighboring plants by means of a plastic curtain. Applications were carried out from 5:00 p.m.

Irrigation waters with the different cationic natures were obtained by the addition of Na^+, Ca^{2+} and Mg^{2+} salts in the form of chloride, according to the pre-established treatments, using the water from the local supply system (Pombal, PB). After preparing the waters, the ECw was checked and, if necessary, adjusted before use. The quantities of salts were determined considering the relationship between ECw and the concentration of salts (Richards, 1954), according to Eq. 1:

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

where:

Q - sum of cations ($mmol_c\ L^{-1}$); and,

ECw - electrical conductivity of water ($dS\ m^{-1}$).

Prior to sowing, the volume of water needed to raise the soil moisture content to field capacity was determined, applying water according to the treatments. After transplanting, irrigation was carried out daily at 5 p.m., applying to each bag the volume corresponding to that obtained by the water balance determined by Eq. 2:

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

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 after the previous irrigation event (mL); and,

LF - leaching fraction of 0.10.

At 129 DAS, moment when the seedlings were ready for grafting, the relative water content (RWC), electrolyte leakage (EL), leaf succulence (SUC), chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll total (Chl T), carotenoids (Car), initial fluorescence (F_0), variable fluorescence (Fv), maximum fluorescence (Fm), and quantum efficiency of photosystem II (Fv/Fm) were evaluated. At 129 DAS, the following growth variables were also measured: plant height (PH), stem diameter (SD), leaf area (LA), leaf (LDM), stem (SDM), root (RDM), and total (TDM) dry matter, and Dickson quality index (DQI) of guava seedlings.

Relative water content was determined according to Weatherley (1950). Electrolyte leakage in the leaf blade was obtained according to Scotti-Campos et al. (2013). Contents of photosynthetic pigments were quantified using the methodology of Arnon (1949). Photochemical efficiency (initial, maximum and variable fluorescence, and the quantum efficiency of photosystem II in leaves pre-adapted to dark with leaf clips for 30 minutes), was determined between 7:00 and 8:00 a.m., using a pulse-modulated fluorometer (Plant Efficiency Analyser - PEA II®).

Stem diameter was measured at 5 cm from the collar with a digital caliper. Plant height was obtained by taking as reference the distance from the collar to the insertion of the apical meristem. For leaf area determination, only leaves with minimum length of 3 cm and at least 50% of photosynthetically active area were considered, according to the methodology of Lima et al. (2012), as presented in Eq. 3:

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

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), using Eq. 4:

$$SUC = \frac{(LFM - LDM)}{(LA)} \qquad (4)$$

where:

SUC - leaf succulence ($g\ H_2O\ cm^{-2}$);

LFM - leaf fresh matter (g per plant);

LDM - leaf dry matter (g per plant); and,

LA - leaf area (cm^2).

To determine biomass accumulation, the plants were cut at 129 DAS close to the soil surface and separated into leaves, stems, and roots. Subsequently, the different parts were placed in paper bags and dried in an oven with forced air ventilation, at a temperature of 65 °C, until reaching constant weight; then, this material was weighed to obtain the values expressed in g for leaf (LDM), stem (SDM), and root (RDM) dry matter, whose sum resulted in the total dry matter (TDM) of the plant.

Quality of guava seedlings was determined using the Dickson quality index - DQI according to Dickson et al. (1960), as shown in Eq. 5:

$$DQI = \frac{(TDM)}{\left(\frac{PH}{SD}\right) + \left(\frac{SHDM}{RDM}\right)} \qquad (5)$$

where:

DQI - Dickson quality index of seedlings;

PH - plant height (cm);

SD - Stem diameter (mm);

TDM - total dry matter (g per plant);

SHDM - shoot (stem + leaves) dry matter (g per plant); and,

RDM - root dry matter (g per plant).

The obtained data were evaluated by analysis of variance by the F test after the normality test (Shapiro-Wilk). 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 software SISVAR-ESAL version 5.6.

Results and Discussion

There was significant effect of the different cationic nature of water (CNW) on the relative water content (RWC), electrolyte leakage (EL), and leaf succulence (SUC) of guava seedlings. Salicylic acid concentrations did not significantly influence any of the measured variables. However, the interaction between the factors (CNW × SA) significantly influenced the RWC of guava seedlings (Table 1).

For electrolyte leakage of guava (Figure 1A), the treatment with application of low- electrical conductivity water (control) was statistically inferior to the other treatments; however, when irrigated with water of cationic nature composed of

Table 1. Summary of the analysis of variance for the relative water content (RWC), electrolyte leakage (EL), and leaf succulence (SUC) of guava seedlings cv. Paluma under irrigation with water of different cationic compositions and application of salicylic acid 129 days after sowing

Source of variation	DF	Mean squares		
		RWC	EL	SUC
Cationic nature of water (CNW)	4	456.56^{**}	707.10^{**}	0.0034^{**}
Salicylic acid (SA)	4	20.29^{ns}	17.14^{ns}	0.0004^{ns}
Linear regression	1	3.14^{ns}	20.83^{ns}	0.0011^{ns}
Quadratic regression	1	56.08^{ns}	4.48^{ns}	0.0005^{ns}
Interaction (CNW × SA)	16	92.65^{**}	28.38^{ns}	0.0004^{ns}
Blocks	2	230.25^{**}	31.61^{ns}	0.0032^{*}
Residual	48	34.87	16.52	0.0006
CV (%)		7.86	17.79	2.50

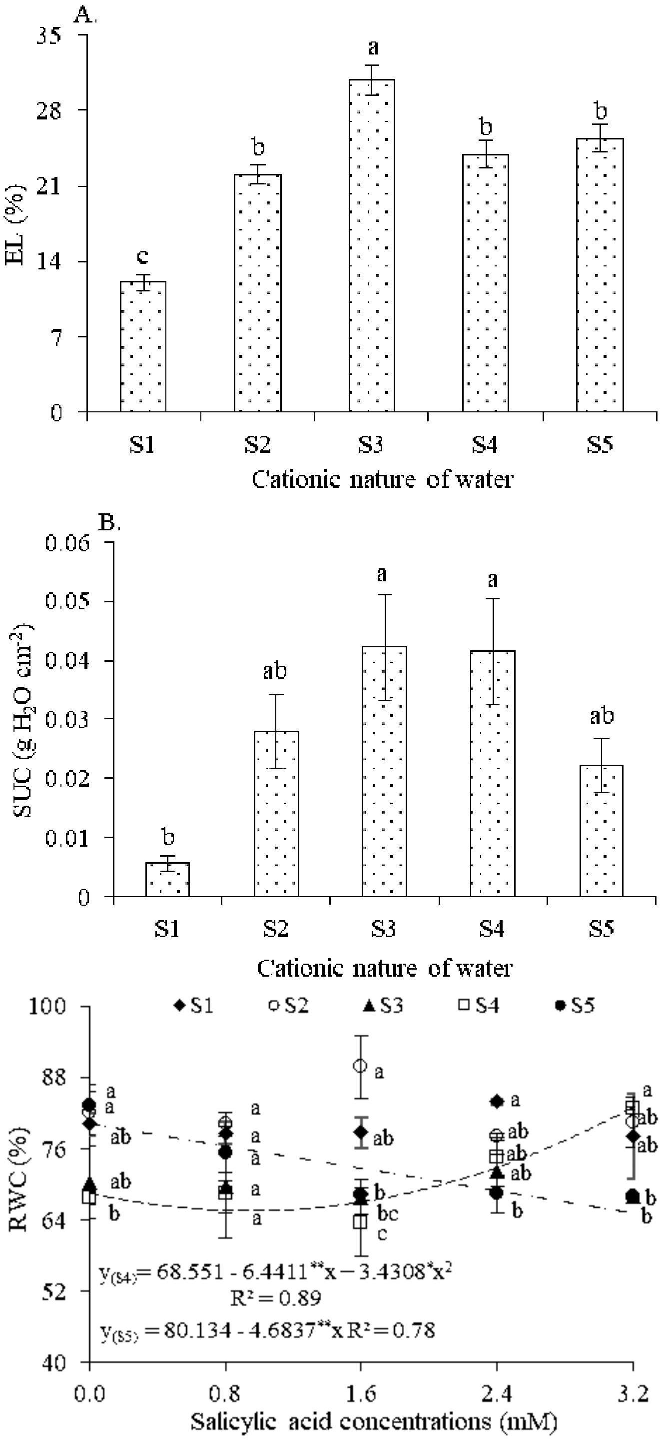
DF - Degrees of freedom; CV - Coefficient of variation; **, ^{ns} - Significant at $p \leq 0.01$, and not significant, respectively, by F test

Na^++Ca^{2+}, guava plants showed the highest EL, indicating greater damage to the cell membrane, while plants subjected to the treatments Na^++Ca^{2+}+Mg^{2+}, Ca^{2+}+Mg^{2+}, and Na^++Mg^{2+} did not differ significantly. The increase in EL in the leaf blade is due to the ionic effects caused by excess salts in the irrigation water, especially ions such as Na^+, which, when absorbed and accumulated in the leaves, cause structural changes in cell membranes and consequent rupture, leading to increase in reactive oxygen species (ROS), which cause lipid peroxidation and oxidation of cell membranes (Sachdev et al., 2021).

Leaf succulence in plants irrigated with low-electrical conductivity water (S1) was statistically lower than that of plants irrigated with waters of S3 and S4 composition (Figure 1B); however, when analyzing only the cationic nature of the water, no significant differences were found among the treatments S2, S3, S4, and S5, an indication that the increase in leaf succulence is related to the variation in the electrical conductivity levels of the water. Increase in leaf succulence is indicative of the possible ability of plants to acclimatize to salt stress, facilitating the absorption of water by the roots, which results in higher water content in leaf tissues, promoting osmotic adjustment (Cruz et al., 2018).

Analysis of the interaction between the factors (CNW × SA) showed that foliar application of SA at a concentration of 0.8 mM did not significantly influence the RWC of guava seedlings, regardless of the CNW (Figure 1C). However, when comparing the RWC of plants that received application of 0, 1.6, 2.4, and 3.2 mM of SA, it was observed that the highest RWC values were obtained in plants subjected to S5, S2, S1, and S4, respectively. It is also observed that, in plants cultivated without SA application (0 mM) and in those treated with 1.6, 2.4, and 3.2, mM SA, the lowest RWC values were obtained under S4, S4, S5, and S3, respectively.

For plants cultivated under irrigation with low-electrical conductivity water (S1), with Na^++Ca^{2+}+Mg^{2+} (S2) and Na^++Ca^{2+} (S3) composition, the regression models adopted ($y_{(S1)} = 79.155 + 1.4134^{ns}x\ 0.4007^{ns}x^2$ $R^2 = 0.045$; $y_{(S2)} = 81.329 + 3.8336^{ns}x - 1.3996^{ns}x^2$ $R^2 = 0.16$; $y_{(S3)} = 69.845 + 0.0621^{ns}x - 0.096^{ns}x^2$ $R^2 = 0.03$) did not fit satisfactorily ($R^2 < 0.60$) to the data of relative water content in the leaf blade (Figure 1C). In plants subjected to irrigation with water of Ca^{2+}+Mg^{2+} (S4) composition, foliar application of salicylic acid at the estimated concentration of 3.2 mM promoted the maximum RWC value of 83.07%. On the other hand, irrigation with Na^++Mg^{2+} (S5) water resulted in a decrease in RWC with the application of salicylic acid, equal to 5.84% per unit increase in SA concentration.



At each salicylic acid level, means followed by the same letters do not differ significantly by Tukey test ($p \leq 0.05$); Vertical bars represent the standard error of the mean (n = 3)
S1 - Control, S2 - Na^++Ca^{2+}+Mg^{2+}, S3 - Na^++Ca^{2+}, S4 - Ca^{2+}+Mg^{2+}, and S5 - Na^++Mg^{2+}, Plants of control (S1) were irrigated with water of 0.3 dS m^{-1} electrical conductivity (ECw), while for S2, S3, S4, and S5, the ECw was 3.0 dS m^{-1}

Figure 1. Electrolyte leakage (A) and leaf succulence (B) of guava seedlings cv. Paluma as a function of the cationic nature of the water - CNW, and relative water content - RWC (C) as a function of interaction between CNW and salicylic acid concentration 129 days after sowing

The reduction in RWC is due to the disturbances caused by the excess of salts in the irrigation water on the water balance of the plants, due to the difficulty in the absorption of water imposed by the reduction in the osmotic potential of the soil solution (Lima et al., 2020). It is worth mentioning that the increase in RWC in guava seedlings subjected to salt stress is due to the action of SA in the regulation of several physiological and biochemical processes such as control of stomatal opening, transpiration, ionic absorption, and photosynthesis (Silva et al., 2020).

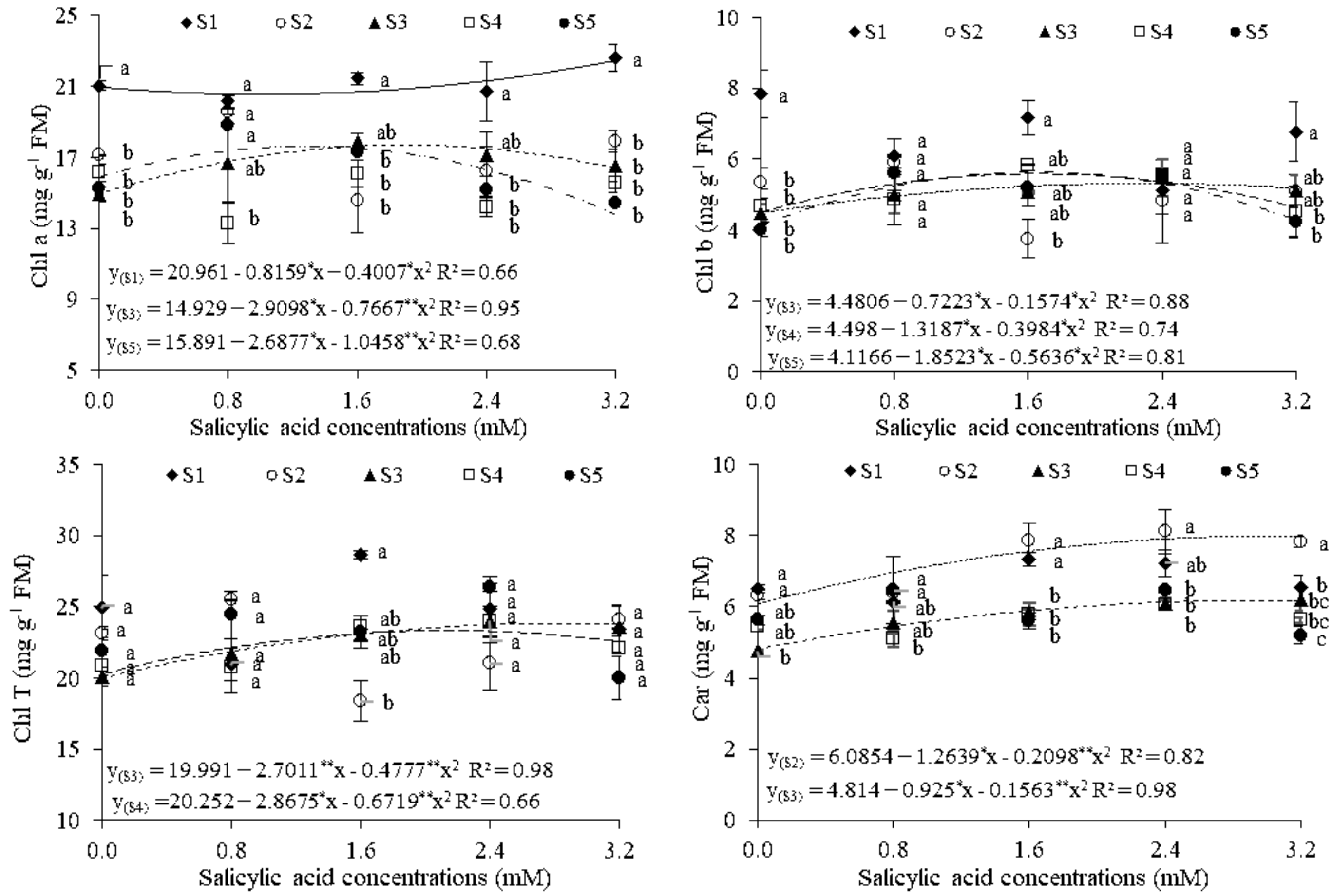
The summary of the analysis of variance (Table 2) showed a significant effect of the CNW on the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Car), initial fluorescence (F_0), variable fluorescence (Fv), and quantum efficiency of photosystem II (Fv/Fm) of guava cv. Paluma. Salicylic acid concentrations significantly influenced only carotenoid contents. On the other hand, the interaction between the factors (CNW × SA) significantly affected the contents of Chl a, Chl b, Chl T, and Car of guava seedlings at 129 days after sowing.

The different cationic compositions of the irrigation water caused a significant effect on the chlorophyll a contents of guava seedlings subjected to different SA concentrations, but the highest contents of this pigment were observed in plants irrigated with low-electrical conductivity waters (0.3 dS m^{-1}), regardless of the SA concentration (Figure 2A). In plants subjected to concentrations of 0 and 3.2 mM of SA, irrigation

Table 2. Summary of the analysis of variance for the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll total (Chl T), carotenoids (Car), initial fluorescence (F_0), variable fluorescence (Fv), maximum fluorescence (Fm), and quantum efficiency of photosystem II (Fv/Fm) of guava cv. Paluma under irrigation with water of different cationic compositions and application of salicylic acid 129 days after sowing

Source of variation	DF	Mean squares							
		Chl a	Chl b	Chl T	Car	F_0	Fv	Fm	Fv/Fm
Cationic nature of water (CNW)	4	82.36**	7.72**	13.30^{ns}	8.30**	3591**	30467**	4375^{ns}	0.089**
Salicylic acid (SA)	4	2.72^{ns}	0.25^{ns}	7.98^{ns}	2.65**	1184^{ns}	8738^{ns}	3325^{ns}	0.012^{ns}
Linear regression	1	0.0004^{ns}	0.30^{ns}	7.95^{ns}	5.61**	1544^{ns}	23637^{ns}	10516^{ns}	0.011^{ns}
Quadratic regression	1	0.5738^{ns}	0.57^{ns}	15.46^{ns}	2.95**	101^{ns}	7680^{ns}	208^{ns}	0.033^{ns}
Interaction (CNW × SA)	16	7.18**	1.98*	17.70**	0.68**	778^{ns}	6718^{ns}	9430^{ns}	0.017^{ns}
Blocks	2	3.01^{ns}	0.69^{ns}	7.68^{ns}	0.08^{ns}	281^{ns}	25224*	33747*	0.001^{ns}
Residual	48	2.52	0.83	7.00	0.29	600	5560	6977	0.012
CV (%)		9.22	17.15	11.53	8.76	21.44	17.67	16.71	13.03

DF - Degrees of freedom; CV - Coefficient of variation; *,**, ^{ns} - Significant at p ≤ 0.05, at p ≤ 0.01, and not significant, respectively, by F test



Means followed by the same letters do not differ significantly by Tukey test (p ≤ 0.05); Vertical bars represent the standard error of the mean (n = 3). FM - fresh mass
S1 - Control, S2 - $Na^{+}+Ca^{2+}+Mg^{2+}$, S3 - $Na+Ca^{2+}$, S4 - $Ca^{2+}+Mg^{2+}$, S5 - $Na^{+}+Mg^{2+}$, Plants of control (S1) were irrigated with water of 0.3 dS m^{-1} electrical conductivity (ECw), while for S2, S3, S4, and S5, the ECw was 3.0 dS m^{-1}

Figure 2. Contents of chlorophyll a - Chl a (A), chlorophyll b - Chl b (B), chlorophyll total - Chl T (C), and carotenoids - Car (D) of guava seedlings cv. Paluma as a function of the interaction between cationic nature of the water and salicylic acid concentrations 129 days after sowing

with water of 0.3 dS m^{-1} (S1) resulted in the highest contents of Chl a, differing significantly from those cultivated under different cationic compositions (S2, S3, S4, and S5). The foliar application of 0.8 mM SA resulted in lower Chl a contents in plants irrigated with S4 (Ca^{2+}+Mg^{2+}) water, being statistically lower than the values obtained under S1, S2, and S5 (Figure 2A). On the other hand, foliar application of 1.6 and 2.4 mM SA led to low contents of Chl a in plants cultivated under S2, S4, and S5.

Regarding the effects of foliar application of salicylic acid on the synthesis of chlorophyll a for each cationic composition of the water (Figure 2A), it is observed that plants irrigated with water with low electrical conductivity (S1) and those consisting of Na^++Ca^{2+} (S3) and Na^++Mg^{2+} (S5) obtained maximum values of 22.45, 17.68, and 17.61 mg g^{-1} FM, respectively, under foliar application of SA at estimated concentrations of 3.2, 2.0, and 1.3 mM. On the other hand, data of plants grown under irrigation water with the composition Na^++Ca^{2+}+Mg^{2+} (S2) and Ca^{2+}+Mg^{2+} (S4) were not satisfactorily ($R^2 < 0.60$) described by the tested regression models ($y_{(S2)} = 18.157 - 2.0366^{ns}x + 0.5681^{ns}x^2$ $R^2 = 0.15$; $y_{(S4)} = 15.648 - 1.4205^{ns}x + 0.4342^{ns}x^2$ $R^2 = 0.16$). Reduction in chlorophyll contents in plants cultivated under salt stress occurs due to the inhibition of its synthesis as a consequence of the reduction in the synthesis of 5-aminolevulinic acid, the molecule responsible for the production of chlorophyll, and the increase in the activity of the enzyme chlorophyllase (Silva et al., 2017).

Regarding the chlorophyll b content, it was observed that, among the different concentrations of SA, there was statistical difference only in plants treated with 0, 1.6, and 3.2 mM of SA, and that at these concentrations the highest Chl b content was obtained in plants irrigated with low-salinity water - 0.3 dS m^{-1} (Figure 2B). Likewise, there was no significant difference in Chl b content in plants irrigated with saline water, regardless of its composition. From these results, it can be inferred that the use of water with 3.0 dS m^{-1} salinity causes a deleterious effect on Chl b content and that the foliar application of SA used in this study was not able to reverse the effects of salt stress caused by irrigation with water of different cationic compositions (S2 - Na^++Ca^{2+}+Mg^{2+}, S3 - Na^++Ca^{2+}, S4 - Ca^{2+}+Mg^{2+}, and S5 - Na^++Mg^{2+}). It is worth pointing out that the lowest Chl b content was obtained in plants subjected to 1.6 mM of SA and water with Na^++Ca^{2+}+Mg^{2+} (S2) composition compared to the control treatment; however, it did not differ from those cultivated under S3, S4, and S5.

When evaluating the effect of the interaction between the concentrations of salicylic acid in each cationic nature on the contents of chlorophyll b (Figure 2B), it was verified that plants subjected to treatments S3 (Na^++Ca^{2+}), S4 (Ca^{2+}+Mg^{2+}), and S5 (Na^++Mg^{2+}) reached maximum values (5.30, 5.58, and 5.63 mg g^{-1} FM), respectively, when they received estimated concentrations of 2.30, 1.70, and 1.60 mM. The data from plants subjected to S1 and S2 were not adequately ($R^2 < 0.60$) described by the regression models analyzed ($y_{(S1)} = 7.228 - 0.3912^{ns}x$ $R^2 = 0.22$; $y_{(S2)} = 5.6691 - 1.1379^{ns}x + 0.2946^{ns}x^2$ $R^2 = 0.29$).

Silva et al. (2017), in a study evaluating the effects of irrigation with saline waters prepared with the addition of NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ salts in equivalent proportion of 7:2:1, found reduction in chlorophyll b content in 'Paluma' guava seedlings, equal to 11.53% per unit increase in ECw, compared to those subjected to 0.3 dS m^{-1}. For Cavalcante et al. (2011), the reduction in chlorophyll b contents is a consequence of oxidative stress and can be attributed to the inhibition of synthesis or degradation by the enzyme chlorophyllase in plants under salt stress.

For the chlorophyll total contents (Figure 2C), analysis of the interaction between the factors (CNW × SA) showed that there were statistical differences between the compositions of the water used in irrigation only when guava plants were subjected to 1.6 mM of SA. Under this concentration of SA, the S_2 composition (Na^++Ca^{2+}+Mg^{2+}) stood out for promoting the lowest content of chlorophyll total (18.3 mg g^{-1} FM), but it differed only from plants under low-salinity water (0.3 dS m^{-1}), which obtained the highest Chl T content (28.6 mg g^{-1} FM). The observed reductions in Chl a, Chl b, and Chl T contents may be related to changes in the structure of organelles, concentration of metabolites, and enzymatic activities involved in the photosynthetic process due to excess salts (Shahverdi et al., 2019).

Regarding the effects of salicylic acid concentrations in each cationic composition on chlorophyll total contents (Figure 2C), it can be seen that plants subjected to irrigation water with the composition Na^++Ca^{2+}(S3) and Ca^{2+}+Mg^{2+} (S4) obtained the estimated maximum values of 23.80 and 23.31 mg g^{-1} FM, respectively, under application of salicylic acid at concentrations of 2.70 and 2.10 mM. On the other hand, the minimum values (19.99 and 20.25 mg g^{-1} FM) were observed in plants that did not receive salicylic acid (0 mM). The Chl T data from plants under S1, S2, and S5 were not satisfactorily described by the regression models ($y_{(S1)} = 23.443 + 2.3816^{ns}x - 0.7087^{ns}x^2$ $R^2 = 0.09$; $y_{(S2)} = 24.517 - 4.2896^{ns}x + 1.2366^{ns}x^2$ $R^2 = 0.30$; $y_{(S5)} = 5.689 + 0.7782^{ns}x - 0.2768^{ns}x^2$ $R^2 = 0.40$).

Analysis of the interaction between the factors (CNW × SA) showed that the different irrigation water compositions had a significant effect on the carotenoid contents at all SA concentrations (0.0, 0.8, 1.6, 2.4, and 3.2 mM) (Figure 2D). It is worth pointing out that, at all concentrations of SA, irrigation with the composition S2 (Na^++Ca^{2+}+Mg^{2+}) resulted in higher Car contents, especially in plants subjected to 3.2 mM of SA, differing significantly from the other cationic compositions of the water (S1 - Control; S3 - Na^++Ca^{2+}, S4 - Ca^{2+}+Mg^{2+}, S5 - Na^++Mg^{2+}). The results obtained in the present study are superior to those obtained by Xavier et al. (2022b), in research evaluating the photosynthetic pigment content of 'Paluma' guava seedlings as a function of irrigation with waters of different salinities, in which case the maximum estimated value of 2.41 mg g^{-1} FM was obtained in plants irrigated with ECw of 0.6 dS m^{-1}.

The increase in the content of carotenoids can be considered an acclimatizing response to salinity, as these pigments act as antioxidant agents, protecting lipid membranes from oxidative stress generated in plants exposed to stress (Falk & Munné-Bosch, 2010). Thus, this increase may be related to the intensification of salt stress in plants, probably related to the degradation of β-carotene and integrated components of thylakoids, which act in the absorption and transfer of light to chlorophyll (Gomes et al., 2011).

The interaction between SA and CNW also significantly influenced the Car contents (Figure 2D), and plants subjected to irrigation with S2 and S3 reached maximum values (7.98 and 6.18 mg g^{-1} FM) when receiving the application of salicylic acid at concentrations of 3.2 and 2.9 mM, respectively. In these cationic compositions, the minimum values (6.08 and 4.81 mg g^{-1} FM) were observed in plants that did not receive foliar application of salicylic acid (0 mM). The data obtained from plants grown under S1 ($y_{(S1)} = 6.2337 + 0.8545^{ns}x - 0.2221^{ns}x^2$ $R^2 = 0.39$), S4 ($y_{(S4)} = 5.2769 + 0.3529^{ns}x - 0.0603^{ns}x^2$ $R^2 = 0.35$), and S5 ($y_{(S5)} = 5.6897 + 0.7782^{ns}x - 0.2768^{ns}x^2$ $R^2 = 0.40$) were not satisfactorily ($R^2 < 0.60$) described by the adopted regression models.

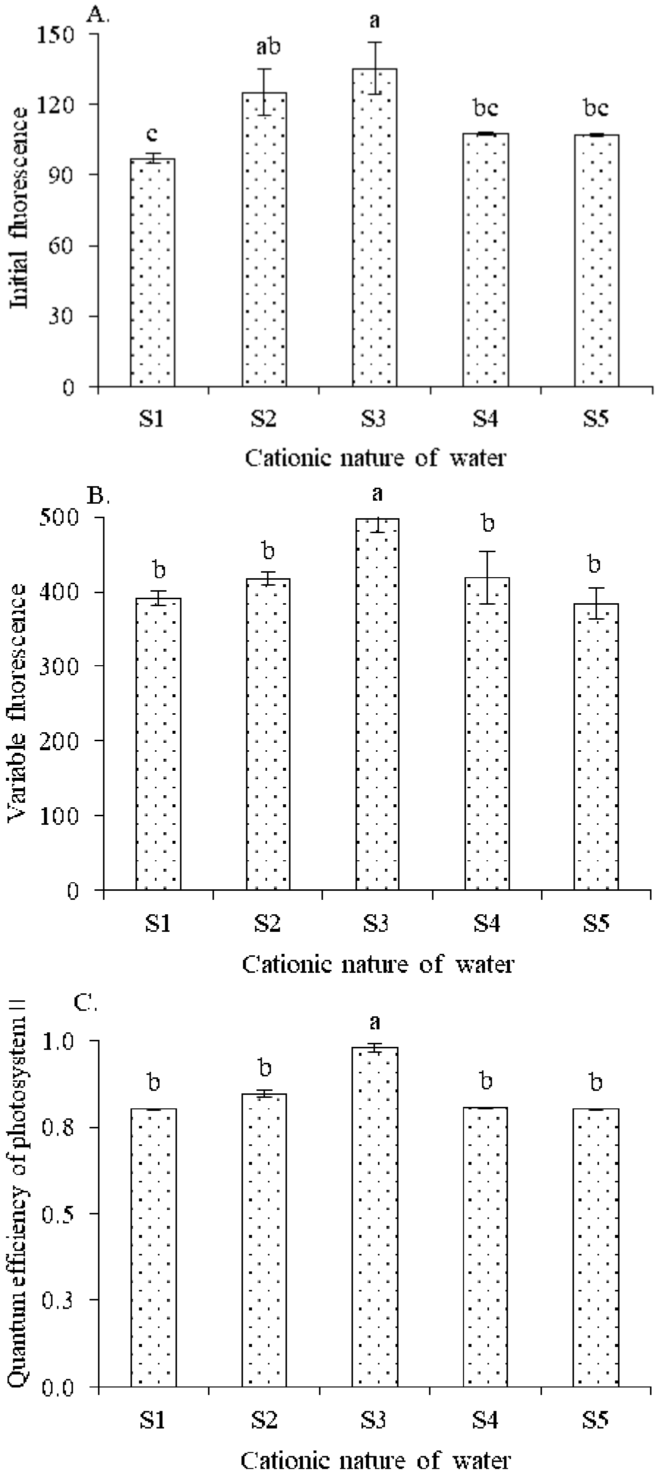
When evaluating the chlorophyll a fluorescence signals, it was observed that the initial fluorescence (Figure 3A) of plants grown with water with Na^++Ca^{2+} (S3) composition was statistically higher than that of plants under S1, S4, and S5. However, when comparing the different CNW (S2, S4, and S5), no significant differences were found among them. On the other hand, variable fluorescence (Figure 3B) and quantum efficiency of photosystem II (Figure 3C) showed similar trends when plants were subjected to different irrigation water compositions, with the highest values (497.53 and 0.98) observed in plants subjected to S_3 (Na^++Ca^{2+}). However, when comparing the Fv and Fv/Fm of plants subjected to water of cationic nature S1, S2, S4, and S5, no significant differences were observed among them.

When plants are exposed to high salinity, changes occur in the functional status of chloroplast lamellae, causing changes in the characteristics of fluorescence signals, which may increase F_0 and Fv due to damage induced by reaction centers, limiting energy transfer between photosystems (Sá et al., 2018). In addition, the increase in Fv/Fm indicates that the reaction centers are potentially active, even under adverse conditions, inducing greater photochemical activity, which is characterized by an increase in fluorescence indices (Xavier et al., 2022b).

Increase in electrolyte leakage with ECw and S3 composition of the water (Na^++Ca^{2+}), as observed in this study (Figure 1A), is related to the ionic effect of salts, since their concentration in the root zone can alter the nutrient balance, promoting K^+ efflux in plant cells, favoring the intensification of lipid peroxidation caused by ROS, altering cellular homeostasis and resulting in membrane instability (Hnilicková et al., 2019).

Cationic nature of the water had significant effect on plant height (PH), stem diameter (SD), leaf area (LA), dry matter of leaf (LDM), root (RDM) and total (TDM), and Dickson quality index (DQI) of guava seedlings (Table 3). Salicylic acid concentrations significantly affected SDM, RDM, TDM and DQI, in addition, there was a significant effect of the interaction between the factors (CNW × SA) only on the RDM of guava seedlings at 129 days after sowing (Table 3).

For the growth in height, plants cultivated under electrical conductivity of 0.3 dS m^{-1} (S1 - Control) differed significantly from those subjected to the different cationic compositions of the water (Figure 4A). The reduction in plant growth induced by salt stress is a consequence of the osmotic effects



Means followed by the same letters do not differ significantly by Tukey test ($p \leq 0.05$); Vertical bars represent the standard error of the mean (n = 3)
S1 - Control, S2 - Na^++Ca^{2+}+Mg^{2+}, S3 - Na^++Ca^{2+}, S4 - Ca^{2+}+Mg^{2+}, S5 - Na^++Mg^{2+}, Plants of control (S1) were irrigated with water of 0.3 dS m^{-1} electrical conductivity (ECw), while for S2, S3, S4, and S5, the ECw was 3.0 dS m^{-1}

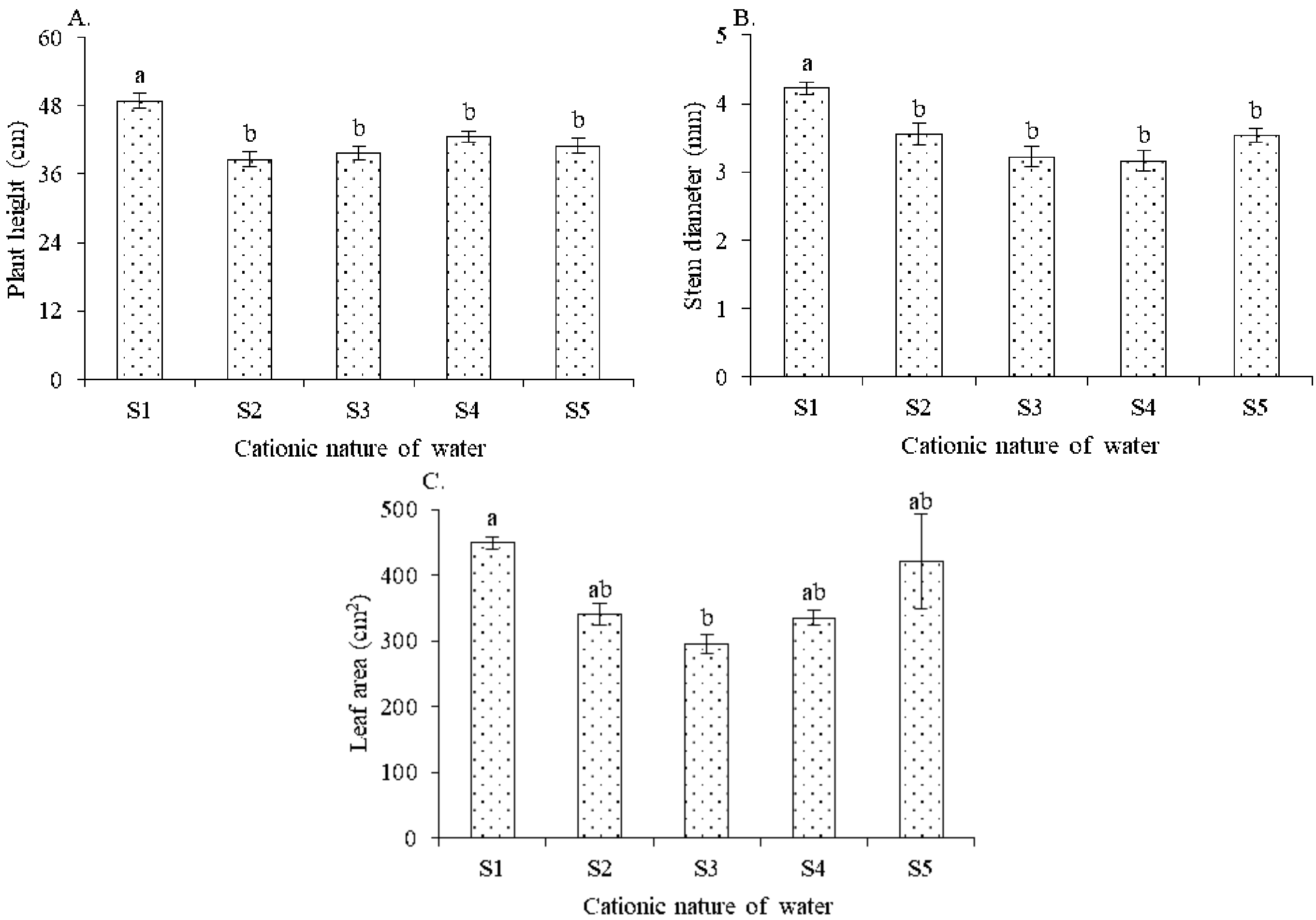
Figure 3. Initial fluorescence (A), variable fluorescence (B), and quantum efficiency of photosystem II (C) of guava seedlings cv. Paluma as a function of the cationic nature of the water 129 days after sowing

that interfere with the absorption of water and nutrients. Inhibition of growth in plant height is the result of changes in the soil water potential caused by excess salts, which restricts water absorption, reducing turgor pressure, and cell activity in plants, inhibiting cell expansion and elongation (Xavier et al., 2022b).

Table 3. Summary of the analysis of variance for plant height (PH), stem diameter (SD), leaf area (LA), dry matter of leaf (LDM), stem (SDM), root (RDM), and total (TDM), and Dickson quality index (DQI) of guava seedlings cv. Paluma under irrigation with water of different cationic compositions and application of salicylic acid 129 days after sowing

Source of variation	DF	Mean squares							
		PH	SD	LA	LDM	SDM	RDM	TDM	DQI
Cationic nature of water (CNW)	4	242.7**	2.66**	61690*	1.03*	0.23^{ns}	1.04**	8.64**	0.071**
Salicylic acid (SA)	4	18.27^{ns}	0.10^{ns}	11076^{ns}	0.35^{ns}	0.89*	0.36**	1.99*	0.015*
Linear regression	1	10.93^{ns}	0.14^{ns}	1645^{ns}	1.15^{ns}	2.83**	0.60**	5.04**	0.0435**
Quadratic regression	1	47.14^{ns}	0.09^{ns}	888^{ns}	0.11^{ns}	0.14^{ns}	0.04^{ns}	0.52^{ns}	0.0002^{ns}
Interaction (CNW × SA)	16	20.14^{ns}	0.12^{ns}	10660^{ns}	0.50^{ns}	0.50^{ns}	0.18**	0.82^{ns}	0.002^{ns}
Blocks	2	34.41^{ns}	1.64**	57044^{ns}	0.16^{ns}	0.38^{ns}	0.34**	0.28^{ns}	0.028**
Residual	48	21.95	0.24	18711	0.38	0.33	0.06	0.58	0.004
CV (%)		11.13	13.88	15.23	12.88	13.24	21.67	17.68	21.81

DF - Degrees of freedom; CV - Coefficient of variation; *,**, ^{ns} - Significant at $p \le 0.05$, at $p \le 0.01$, and not significant, respect



Means followed by the same letters do not differ significantly by Tukey test ($p \le 0.05$); Vertical bars represent the standard error of the mean (n = 3)
S1 - Control, S2 - Na^{+}+Ca^{2+}+Mg^{2+}, S3 - Na^{+}+Ca^{2+}, S4 - Ca^{2+}+Mg^{2+}, S5 - Na^{+}+Mg^{2+}, Plants of control (S1) were irrigated with water of 0.3 dS m^{-1} electrical conductivity (ECw), while for S2, S3, S4, and S5, the ECw was 3.0 dS m^{-1}

Figure 4. Plant height (A), stem diameter (B), and leaf area (C) of guava seedlings cv. Paluma, as a function of the cationic nature of the water 129 days after sowing

As observed for plant height (Figure 4A), the growth in stem diameter (Figure 4B) of plants grown under ECw of 0.3 dS m^{-1} (S1) was statistically higher than that of plants grown under water salinity of 3.0 dS m^{-1} (S2, S3, S4, and S5). It was also observed that there were no significant differences in the SD of plants cultivated under waters of Na^{+}+Ca^{2+}+Mg^{2+}, Na^{+}+Ca^{2+}, Ca^{2+}+Mg^{2+}, and Na^{+}+Mg^{2+} composition. The restriction in water uptake imposed by the excess of salts in the water limits turgor pressure in the cells and, consequently, plant growth (Lacerda et al., 2022). Queiroga et al. (2023), when evaluating the effects of salt stress caused by the water of different cationic natures and electrical conductivity of 4.3 dS m^{-1} (Na^{+}, Ca^{2+}, Na^{+}+Ca^{2+}, Mg^{2+}, and Na^{+}+Ca^{2+}+Mg^{2+}) prepared with chloride salts, also found that the greatest reduction in the growth in stem diameter of the plants occurred due to water salinity, regardless of the cationic nature of the water.

It should be noted that the intensity of the effects of salt stress on plants depends on the crop, cultivar, development stage, cationic composition of the water, irrigation, and fertilization management. According to Ferreira et al. (2023), the guava cv. Paluma is classified as sensitive to salt stress, with a water salinity threshold level of 0.3 dS m^{-1}, with a reduction of 11.48% per unit increase in ECw.

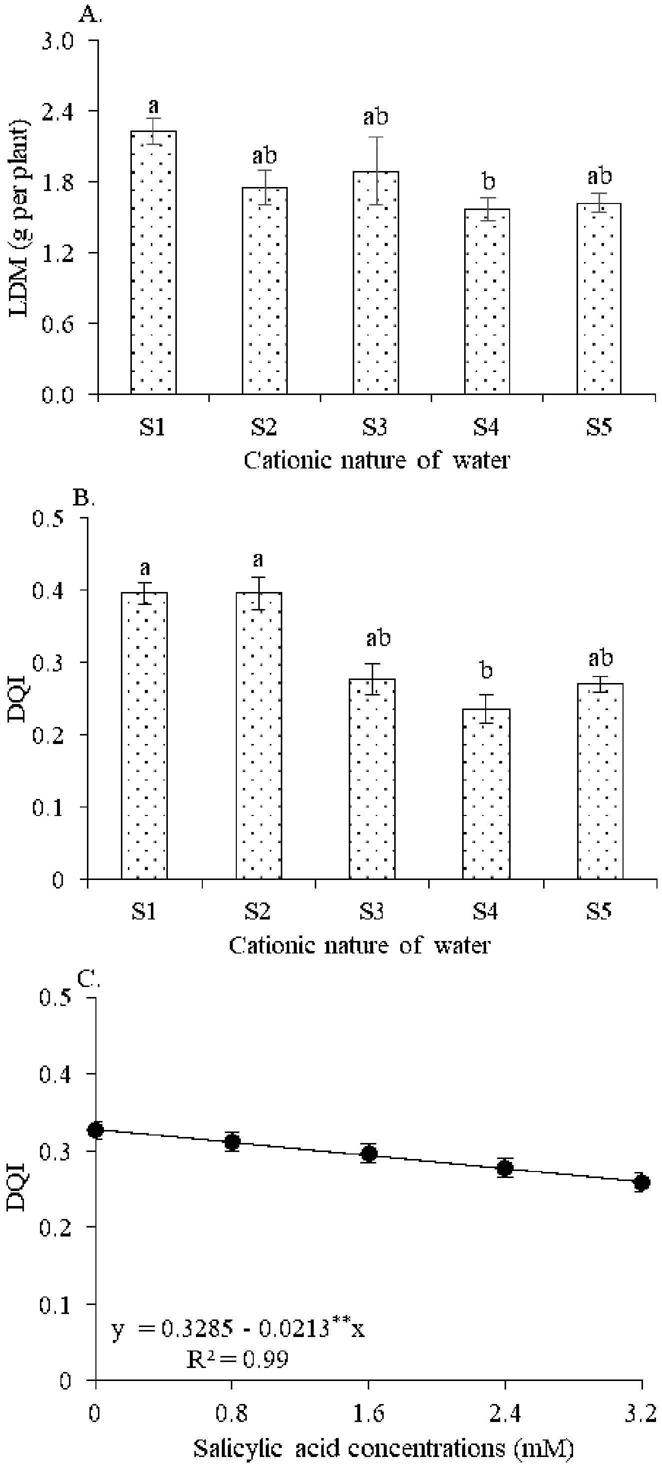
For leaf area, guava seedlings irrigated with water of 0.3 dS m^{-1} (S1) differed significantly from those cultivated under water composed of sodium and calcium (S3) (Figure 4C). On the other hand, when comparing the different cationic compositions of water, no significant differences were observed among them. The reduction in the growth in leaf area observed in plants irrigated with water consisting of sodium + calcium (S3) compared to the control treatment (S1) is possibly related to the increase in cell membrane permeability caused by the ionic and osmotic effects, leading to the generation of ROS. In addition, high concentrations of Ca^{2+} in the cytoplasm can also precipitate PO_4^{3-} ions and cause changes in physiological processes related to phosphorus metabolism, inhibiting respiration and affecting plant growth (Weng et al., 2022).

Leaf dry matter in guava seedlings cultivated under ECw of 0.3 dS m^{-1} (S1) was statistically higher than that of those irrigated with water with $Ca^{2+}+Mg^{2+}$ (S4) composition (Figure 5A). There were no significant differences among plants subjected to water salinity of 0.3 dS m^{-1} (S1 - Control) and water with $Na^{+}+Ca^{2+}+Mg^{2+}$, $Na^{+}+ Ca^{2+}$, and $Na^{+}+Mg^{2+}$ (S2, S3, and S5) composition. The reduction in biomass accumulation in plants cultivated under salt stress conditions may be related to changes in the partitioning of photoassimilates caused by the limitation in the absorption of water and nutrients (Xavier et al., 2022a).

For the Dickson quality index (Figure 5B), plants grown under water salinity of 0.3 (S1) and irrigated using water with $Na^{+}+Ca^{2+}+Mg^{2+}$ composition (S2) had a statically higher DQI compared to those subjected to S4. There was no significant effect among plants subjected to treatments S1, S2, S3, and S5. In the seedling formation phase, DQI is a morphological variable considered to be an indicator of the quality of seedlings to be used in the field, as it relates sturdiness (plant height and stem diameter) to the balance in biomass distribution. It is important to highlight that, regardless of the cationic nature of the water, the guava seedlings were of acceptable quality for transplanting to the field, as they attained DQI higher than 0.2 (Dickson et al., 1960). Queiroga et al. (2023), in a study with guava plants in the seedling formation phase under irrigation with waters of different cationic compositions, concluded that the use of 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.

Regarding the effect of SA on Dickson quality index, a linear reduction of 6.48% per unit increase in SA concentration was observed (Figure 5C). When comparing the DQI of plants grown under 0 and 3.2 mM of SA, a reduction of 20.74% was observed. Despite the decrease in DQI, seedlings formed using SA concentrations of up to 3.2 mM have acceptable quality for transplanting aiming at field cultivation, because according to Dickson et al. (1960) this value should be higher than 0.20. The beneficial effect of foliar application of SA is related to the concentration, mode of application, stage of development, and crop (Lacerda et al., 2022).

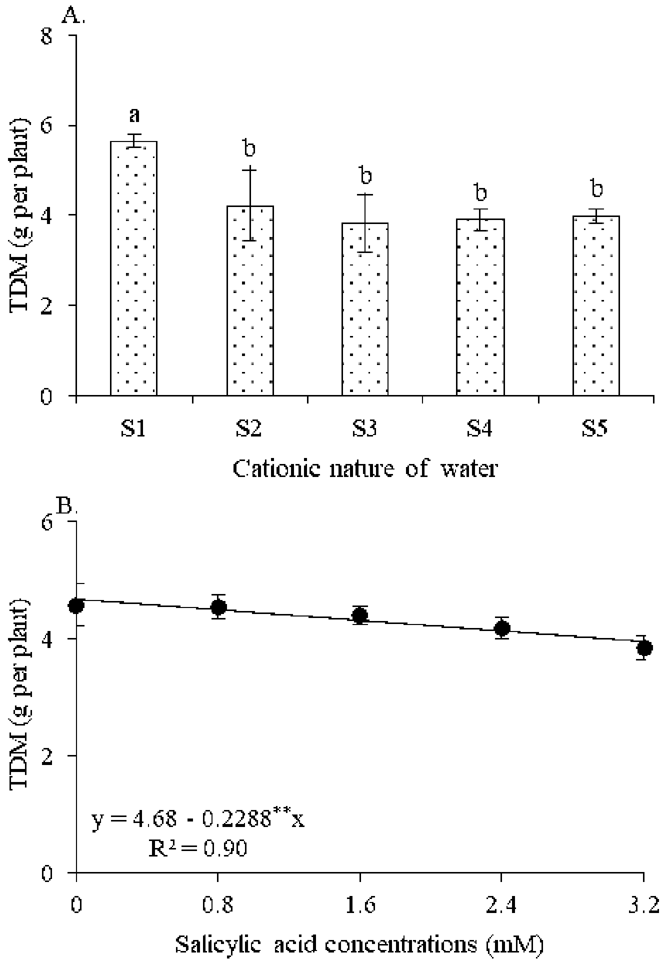
Regarding total dry matter of plant (Figure 6A), guava seedlings irrigated with water of 0.3 dS m^{-1} were on average 42% superior to those cultivated under ECw of 3.0 dS m^{-1}, and there were no significant differences in the TDM of seedlings



Means followed by the same letters do not differ significantly by Tukey test ($p \leq 0.05$); Vertical bars represent the standard error of the mean (n = 3)
S1 - Control, S2 - $Na^{+}+Ca^{2+}+Mg^{2+}$, S3 - $Na^{+}+Ca^{2+}$, S4 - $Ca^{2+}+Mg^{2+}$, S5 - $Na^{+}+Mg^{2+}$, Plants of control (S1) were irrigated with water of 0.3 dS m^{-1} electrical conductivity (ECw), while for S2, S3, S4, and S5, the ECw was 3.0 dS m^{-1}

Figure 5. Leaf dry matter - LDM (A) and Dickson quality index - DQI (B) of guava seedlings cv. Paluma, as a function of the cationic nature of the water and DQI as a function of salicylic acid concentrations (C) 129 days after sowing

cultivated under different cationic natures of water (S2, S3, S4, and S5). The greatest reduction in TDM is possibly associated with the energy expenditure due to several metabolic changes such as lipid peroxidation, reduction in chlorophyll content, regulation of ion transport and distribution in various organs inside the cells (Lima et al., 2020).

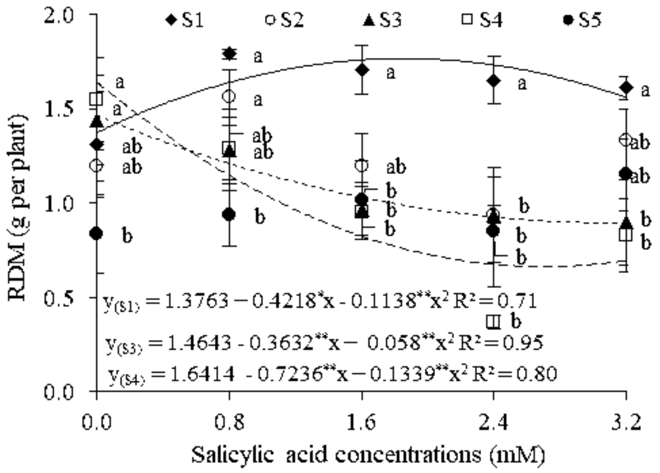


Means followed by the same letters do not differ significantly by Tukey test ($p \leq 0.05$); Vertical bars represent the standard error of the mean (n = 3)
S1 - Control, S2 - $Na^{+}+Ca^{2+}+Mg^{2+}$, S3 - $Na^{+}+Ca^{2+}$, S4 - $Ca^{2+}+Mg^{2+}$, S5 - $Na^{+}+Mg^{2+}$, Plants of control (S1) were irrigated with water of 0.3 dS m^{-1} electrical conductivity (ECw), while for S2, S3, S4, and S5, the ECw was 3.0 dS m^{-1}

Figure 6. Total dry matter of guava seedlings cv. Paluma, as a function of the cationic nature of the water (A) and salicylic acid concentrations (B) 129 days after sowing

The total dry matter decreased linearly with the increase in SA concentrations, by 4.89% per unit increase (Figure 6B). When comparing the TDM of plants grown under foliar application of 3.2 mM to the value of those receiving 0 mM, a decrease of 15.64% (0.73 g per plant) was observed. Reduction in biomass accumulation as a function of foliar application of SA was also observed by Xavier et al. (2022a), who evaluated guava seedlings subjected to irrigation with saline water in the proportion of 7:2:1 of Na^{+}, Ca^{2+}, and Mg^{2+} and found a 66.13% decrease in total dry matter in plants grown under the highest SA concentration (3.2 mM) compared to those in the control treatment (0 mM).

Root dry matter was significantly affected by the interaction CNW × SA (Figure 7). It was observed that under foliar application of 0 mM SA, there were significant differences in RDM only between plants grown under S4 ($Ca^{2+}+Mg^{2}$) and S5 ($Na^{+}+Mg^{2+}$). In plants subjected to SA concentration of 0.8 mM, the RDM of plants under cationic natures S1 and S2 was significantly higher than those that received water consisting of $Na^{+}+Mg^{2+}$ (S5). When using 1.6 mM of SA, there were significant differences between plants cultivated under S1 and those irrigated with S3, S4, and S5.



Means followed by the same letters do not differ significantly by Tukey test ($p \leq 0.05$); Vertical bars represent the standard error of the mean (n = 3)
S1 - Control, S2 - $Na^{+}+Ca^{2+}+Mg^{2+}$, S3 - $Na^{+}+Ca^{2+}$, S4 - $Ca^{2+}+Mg^{2+}$, S5 - $Na^{+}+Mg^{2+}$, Plants of control (S1) were irrigated with water of 0.3 dS m^{-1} electrical conductivity (ECw), while for S2, S3, S4, and S5, the ECw was 3.0 dS m^{-1}

Figure 7. Root dry matter - RDM of guava seedlings cv. Paluma, as a function of the interaction between the cationic nature of the water and salicylic acid concentrations 129 days after sowing

On the other hand, foliar application of 2.4 mM promoted the highest RDM value in plants cultivated under ECw of 0.3 dS m^{-1} (S1), statistically differing from those under the other cationic compositions of the water (S2, S3, S4, and S5). In plants grown under 3.2 mM, there were significant differences in RDM between plants under the cationic composition S1 and those under S3 and S4. Foliar application of 3.2 mM of SA increased the RDM of plants grown under S1, S2, and S5. From the results obtained for RDM (Figure 7), it is evident that the beneficial effect of foliar application of SA is related to the concentration of this phytohormone and to the cationic nature and electrical conductivity level of the water used in irrigation.

Regarding the effects of salicylic acid concentrations in each cationic nature of water on root dry matter (Figure 7), plants irrigated with low-electrical conductivity water (S1) and consisting of $Na^{+}+Ca^{2+}$ (S3), and $Ca^{2+}+Mg^{2+}$ (S4) reached maximum values of 1.76, 1.46, and 1.64 g per plant under salicylic acid at concentrations of 1.90, 0, and 0 mM, respectively. For plants that received water with the composition $Na^{+}+Ca^{2+}+Mg^{2+}$ ($y_{(S2)} = 1.3409 - 0.1021^{ns}x + 0.0179^{ns}x^2$, $R^2 = 0.07$) and $Na^{+}+Mg^{2+}$ ($y_{(S5)} = 0.854 + 0.0662^{ns}x$, $R^2 = 0.42$), the data were not satisfactorily described by the regression models ($R^2 < 0.60$).

Conclusions

1. Electrolyte leakage, succulence and chlorophyll a fluorescence signals increase in guava seedlings irrigated with 3.0 dS m^{-1} water, regardless of its cationic nature.

2. Despite the inhibition in growth, water with electrical conductivity of 3.0 dS m^{-1} can be used in the formation of guava seedlings with acceptable quality for transplantation to the field, regardless of cationic composition.

3. In the absence of foliar application of salicylic acid, water salinity negatively affects the chlorophyll a and b contents of guava seedlings, regardless of the cationic nature of the water.

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