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Gas exchange and photochemical efficiency of guava under saline water irrigation and nitrogen-potassium fertilization¹

Trocas gasosas e eficiência fotoquímica de goiabeira irrigada com águas salinas e adubação nitrogenada-potássica

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HIGHLIGHTS:

Irrigation water electrical conductivity of less than 3.5 dS m⁻¹ does not damage the photosynthetic apparatus in guava. The maximum and variable fluorescence is reduced when electrical conductivity of irrigation water is above 0.3 dS m⁻¹. Excessive doses of N and K reduce the quantum efficiency of photosystem II.

ABSTRACT: The objective of this study was to evaluate the effects of different combinations of nitrogen and potassium fertilization on gas exchange and chlorophyll a fluorescence in seedlings of guava cv. Paluma irrigated with water of different salinity levels. The experiment was set up in a randomized block design and analyzed in 5 × 4 factorial scheme, with four replicates. The treatments were composed of the combination of the factor electrical conductivity of irrigation water - EC_w (0.3, 1.1, 1.9, 2.7, and 3.5 dS m⁻¹), with the factor combinations (C) formed from recommended doses of nitrogen (N) and potassium (K₂O), 70% N + 50% K₂O, 100% N + 75% K₂O, 130% N + 100% K₂O, and 160% N + 125% K₂O. The fertilization combination 130% N + 100% K₂O mitigated the effects of salt stress up to mean EC_w of 1.3 dS m⁻¹, resulting in increments in CO₂ assimilation rate, transpiration, instantaneous carboxylation efficiency, and electron transport rate. For maximum quantum efficiency of photosystem II, only treatment irrigated with EC_w 3.5 dS m⁻¹ under the combination 160% N + 125% K₂O showed damage to the photosynthetic apparatus.

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

RESUMO: Objetivou-se com o presente estudo avaliar os efeitos de diferentes combinações de adubação nitrogenada e potássica sobre as trocas gasosas e fluorescência da clorofila a em mudas de goiabeira cv. Paluma irrigadas com águas de distintas salinidades. O experimento foi instalado em delineamento em blocos casualizados e analisados no esquema fatorial 5 × 4, com quatro repetições. Os tratamentos foram compostos a partir da combinação do fator condutividade elétrica da água de irrigação - CE_a (0,3; 1,1; 1,9; 2,7 e 3,5 dS m⁻¹) com o fator combinações (C) formada a partir de doses recomendadas de nitrogênio (N) e potássio (K₂O): 70% N + 50% K₂O; 100% N + 75% K₂O; 130% N + 100% K₂O e 160% N + 125% K₂O. A combinação de adubação 130% N + 100% K₂O mitigou o estresse salino até a CE_a média de 1,3 dS m⁻¹, resultando em aumento na taxa de assimilação de CO₂, transpiração, eficiência instantânea de carboxilação e taxa de transporte de elétrons. Para a máxima eficiência quântica do fotossistema II, apenas os tratamentos irrigados com CE_a 3,5 dS m⁻¹ e combinação 160% N + 125% K₂O apresentaram danos no aparelho fotossintético.

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

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INTRODUCTION

Guava (*Psidium guajava* L.) is a plant native to Tropical America and exploited in several tropical and subtropical regions. Among the Brazilian regions with exploitation of this fruit crop, the Northeast stands out, but the irregularities of rainfall combined with high temperatures and evapotranspiration rates cause the sustainable exploitation of guava to be feasible only under the use of irrigation. Another limiting factor in the region is the quality of available water for irrigation, as it commonly contains high salt content, both in surface and underground sources (Lima et al., 2020).

Fertilizer management can improve availability and benefits to crops and induce competition of these with ions considered toxic in excess. The management of combined concentrations of N and K is important because N participates in several compounds that are essential for plants, such as amino acids, glycinebetaine, proline, chlorophyll, and nucleic acids (Taiz et al., 2017), while potassium, besides being a constituent of the structure of plants, also has a function of regulating biochemical and physiological processes, such as stomatal opening and photosynthesis (Mostofa et al., 2022), which can mitigate the deleterious effect of saline stress, since excess salts in irrigation water induce stomatal closure and changes in chlorophyll fluorescence signals, in addition to reducing plant photosynthesis and therefore growth and production (Lima et al., 2015; Silva et al., 2019).

N and K interact in several enzymatic processes between the root and the shoot (depending on the type of plant, nitrate supply and luminosity), where the increase in NO_3^- storage in the shoots is directly linked with the supply of K for the plant, and K deficiency can inhibit the translocation of NO_3^- and reduce the assimilation of N by the roots (Xu et al., 2020). The objective of this study was to evaluate the effects of different combinations of nitrogen and potassium fertilization on gas exchange and chlorophyll a fluorescence in seedlings of guava cv. Paluma irrigated with waters of different salinity levels.

MATERIAL AND METHODS

The study was conducted with a guava (*Psidium guajava*) cv. Paluma in a protected ambient from February to June 2020, at the Multidisciplinary Center of Caraúbas of UFRSA, located in the Oeste Potiguar mesoregion at the geographic coordinates $05^\circ 46' 23''$ S and $37^\circ 34' 12''$ W and an altitude of 144 m. Climatological data (Figure 1) were collected during the study period from an automatic weather station located at UFRSA, Caraúbas campus, in an area near the site where the experiment was set up.

A randomized block design was used, arranged in a 5×4 factorial scheme, referring to five salinity levels of irrigation water, with electrical conductivity (ECw) of 0.3, 1.1, 1.9, 2.7, and 3.5 dS m^{-1} and four combinations (C) of recommended dose of nitrogen (N) and potassium (K_2O) corresponding to C1 = 70% N + 50% K_2O , C2 = 100% N + 75% K_2O , C3 = 130% N + 100% K_2O , and C4 = 160% N + 125% K_2O . Four replicates were used, and each plot consisted of two plants.

The doses of 100% N ($541.1 \text{ mg of N dm}^{-3}$ of soil) and 100% K_2O ($798.6 \text{ mg of K dm}^{-3}$ of soil) adopted were respectively based on indications of Souza et al. (2016) and Bonifácio et al. (2018); thus, the doses corresponding to the treatments with N and K combination were respectively equal to 378.8 and 399.3, 541.1 and 598.9, 703.4 and 798.6, and 865.8 and 998.25 mg of N and $\text{K}_2\text{O dm}^{-3}$ of soil. Urea (45% N) and potassium chloride (60% K_2O) were used as sources of nitrogen and potassium, respectively.

The water salinity levels were based on studies that indicate that guava is moderately sensitive to salinity of irrigation water, with reductions in growth, development, and production at water salinity above 1.5 dS m^{-1} (Bezerra et al., 2018).

The irrigation waters of the treatments were prepared by the addition of NaCl, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in the water from the municipal supply system, in an equivalent ratio of 7:2:1 (Medeiros, 1992), respectively, following the relationship between ECw and salt concentration ($\text{mmol}_e \text{ L}^{-1} \approx \text{EC} \times 10$) proposed by Richards (1954). This proportion of salts adopted was based on the predominant composition of the principal sources of water used for irrigation in the northeastern region.

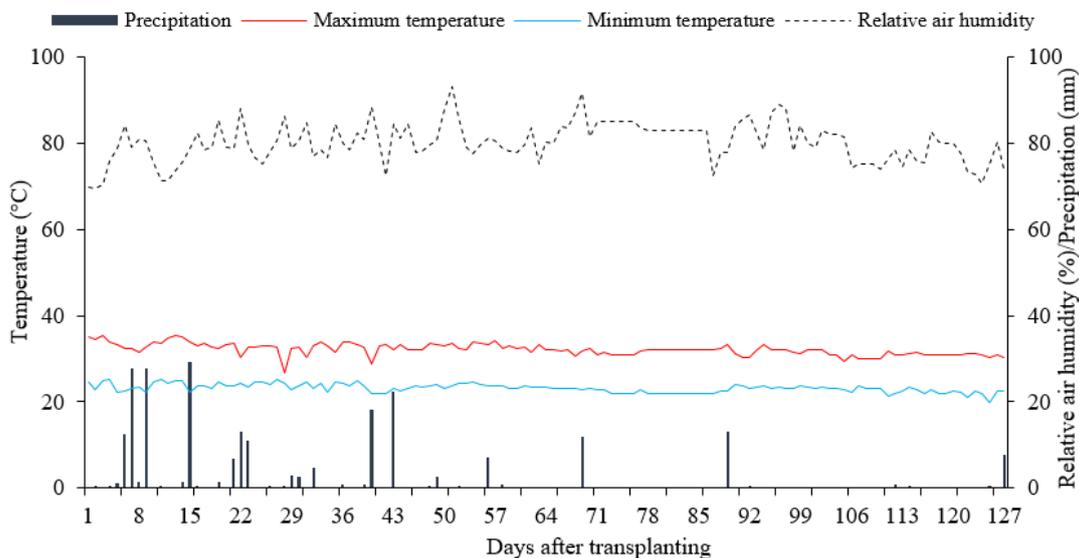


Figure 1. Daily maximum and minimum temperature, rainfall, and relative air humidity during the experimental period

Sowing was carried out in plastic bags with a capacity of 1150 mL, which were filled with a substrate formed by soil material collected from the 0-30 cm layer in the municipality of Caraúbas - RN, bovine manure, crushed carnauba straw, and coal powder, in a 2:2:2:1 ratio, and arranged on wooden pallets, at 0.20 m height from the ground. The bags had holes at the base to allow free drainage. Sowing was carried out by placing three seeds per bag at a depth of 1.0 cm. Thinning was performed after stabilization of emergence and when the plants had two pairs of true leaves, leaving only one plant in the bag, which had the best growth.

Before the experiment, the chemical and physical-hydraulic characteristics of the substrate (Table 1) were analyzed according to Teixeira et al. (2017).

During the experimental period, the substrate was maintained with moisture content close to field capacity. Until 30 days after sowing (DAS), the plants were irrigated with local supply water of 0.3 dS m⁻¹ and after this period, they were irrigated with water according to the respective treatment. The volume of water to be applied in each irrigation was determined by weighing a sample of bags of each treatment at weekly intervals according to the state of growth of the plants, thereby obtaining the mean evapotranspired volume of water for each treatment to raise soil moisture in the soil to the level of field capacity (Silva et al., 2019).

At 30 days after sowing, the combinations of N and K doses were applied at weekly intervals. Foliar fertilization (N - 10%, P₂O₅ - 52%, K₂O - 10%, Ca - 0.1%, Zn - 0.02%, B - 0.02%, Fe - 0.15%, Mn - 0.1%, Cu - 0.02%, and Mo - 0.005%) performed at 45 DAS and every 20 days in a proportion of 1 g of fertilizer for 1 L of water, applied with a backpack sprayer, distributing 5 L in the plants.

To mitigate the damage caused by pests and diseases, phytosanitary control, preventive and/or curative, was realized during the experimental period. Weeds that emerged in the bags were eliminated by manual uprooting.

The effects of the different treatments were analyzed at 125 DAS through physiological evaluations of leaf gas exchange [net CO₂ assimilation rate - A (μmol CO₂ m⁻² s⁻¹), stomatal conductance - gs (mol H₂O m⁻² s⁻¹), transpiration rate - E (mmol H₂O m⁻² s⁻¹), internal CO₂ concentration - Ci (μmol CO₂ m⁻² s⁻¹) were evaluated on the third leaf from the apex, and these data were used to calculate the instantaneous carboxylation efficiency - CEi [(μmol CO₂ m⁻² s⁻¹) (μmolCO₂ m⁻² s⁻¹)⁻¹] and instantaneous water use efficiency - WUEi (μmol CO₂ m⁻² s⁻¹) (μmol CO₂ m⁻² s⁻¹)⁻¹). Gas exchange measurements were performed at 08:00 a.m. under natural conditions of air temperature and CO₂ concentration using an artificial

radiation source of 1,200 μmol m⁻² s⁻¹, established through the curve of photosynthetic response to light and the point of photosynthetic saturation by light (Fernandes et al., 2021), using the portable photosynthesis meter "LCPro+" from ADC BioScientific Ltda.

Photochemical efficiency was also analyzed at 125 DAS, using a pulse-modulated fluorometer, OS5p model from Opti Science, Fv/Fm protocol, with determination of initial fluorescence (F₀), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum efficiency of photosystem II (Fv/Fm). To perform the measurements, it was necessary to adapt the leaves to the dark for 30 min using clips of the device to ensure that the reaction centers were open during the analysis period.

Following the evaluation of physiological parameters by Yield protocol, an actinic light source with a saturating multi-flash pulse was applied, coupled to a photosynthetically active radiation determination clip (PAR-Clip), to determine the following variables: initial fluorescence before the saturation pulse (Fs), maximum fluorescence after adaptation to saturating light (Fms), and electron transport rate (ETR).

The means of the variables were subjected to analysis of variance, with an F test (0.05 probability level) and regression studies for salinity levels. The means of the qualitative factor (combination of N and K fertilization) were compared by the Tukey test, using the statistical program SISVAR-ESAL (Ferreira, 2019).

RESULTS AND DISCUSSION

There was a significant interaction ($p \leq 0.01$) between the salinity levels of irrigation water and the combinations of N and K fertilization on stomatal conductance (gs), CO₂ assimilation rate (A), internal CO₂ concentration (Ci), transpiration (E), and instantaneous carboxylation efficiency (CEi). Instantaneous water use efficiency (WUEi) showed no significant response ($p > 0.05$) for the factors studied (Table 2).

For stomatal conductance (Figure 2A), the effect of salinity on plants fertilized with the fertilization combinations C1 and C2 caused a decreasing linear behavior, with reductions of 23.04% (C1) and 18.19% (C2) per unit increase in ECw. For the plants fertilized with the C3 combination, the gs data did not obtain a satisfactory adjustment to the tested models (0.0963 - 0.1146^{ns}x - 0.0402^{ns}x²). The fertilization combination C4 led to a decreasing quadratic behavior, with the highest gs (0.224 mol H₂O m⁻² s⁻¹) obtained when plants were subjected to ECw of 0.3 dS m⁻¹. When splitting the fertilization combinations at each water salinity level (Figure 2A), there were significant

Table 1. Chemical and physical characteristics of the substrate used for the sowing of guava cv. Paluma

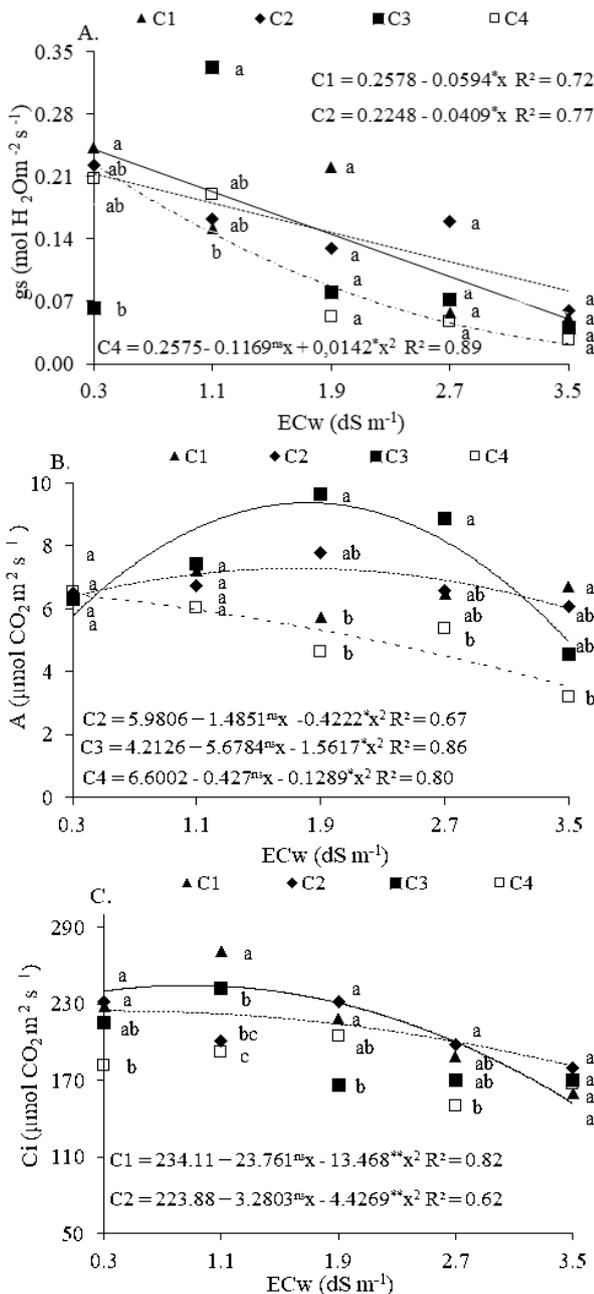
Sand		Silt (g kg ⁻¹)		Clay		Textural classification	ECse (dS m ⁻¹)	pHse H ₂ O	OM (g kg ⁻¹)			
447		411		143		Loam	0.68	6.03	37			
P (mg dm ⁻³)	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺	(H + Al)	SB	t	CEC	V	m	ESP
134.2	0.87	0.15	17.11	1.14	0	0.58	19.27	19.27	19.85	97	0	0.78
(cmol _c dm ⁻³)												
(%)												

Available phosphorus (P) - EMBAPA methodology; OM - Organic matter: Walkley-Black Wet Digestion; Ca²⁺ and Mg²⁺ - Extracted with 1 mol L⁻¹ KCl at pH 7.0; Na⁺ and K⁺ - Extracted with 1 mol L⁻¹ NH₄OAc at pH 7.0; Al³⁺ and (H⁺ + Al³⁺) - Extracted with 0.5 mol L⁻¹ CaOAc at pH 7.0; ECse - Electrical conductivity of the substrate saturation extract at 25 °C; pHse - pH of the substrate saturation extract; SB - Sum of bases; t - Effective CEC; CEC - Cation exchange capacity; V - Base saturation; m - Aluminum saturation; ESP - Exchangeable sodium percentage

Table 2. Summary of the analysis of variance for stomatal conductance (gs), CO₂ assimilation rate (A), internal CO₂ concentration (Ci), transpiration (E), instantaneous carboxylation efficiency (CEi), and instantaneous water use efficiency (WUEi) in guava seedlings cultivated under different salinity levels of irrigation water and combinations of nitrogen and potassium fertilization, at 125 days after sowing (DAS)

Source of variation	DF	Mean squares					
		gs	A	Ci	E	CEi	WUEi
Salinity level (S)	4	0.074**	9.200*	9745.729**	0.256*	0.0003*	2.321 ^{ns}
Linear Regression	1	0.259**	10.790 ^{ns}	31621.784**	0.109 ^{ns}	0.0002 ^{ns}	6.557 ^{ns}
Quadratic Regression	1	0.007 ^{ns}	23.098**	2462.316*	0.914**	0.0007*	0.535 ^{ns}
NK Combination	3	0.008 ^{ns}	17.278**	4785.693**	0.353*	0.0004*	1.828 ^{ns}
Interaction (S x NK)	12	0.020*	5.718*	1981.178**	0.191*	0.0003*	3.104 ^{ns}
Blocks	3	0.004 ^{ns}	9.458*	2774.878**	0.821**	0.0008**	2.777 ^{ns}
CV (%)		32	26.42	12.13	24.25	33.88	27.18

ns, **, * - Respectively not significant, significant at $p \leq 0.01$ and significant at $p \leq 0.05$; CV - Coefficient of variation; DF - Degrees of freedom



^{ns}, *, ** - not significant, significant at $p \leq 0.05$ and $p \leq 0.01$ by the F test, respectively; C1 = 70% N + 50% K₂O, C2 = 100% N + 75% K₂O, C3 = 130% N + 100% K₂O, and C4 = 160% N + 125% K₂O

Figure 2. Stomatal conductance – gs (A), CO₂ assimilation rate – A (B), and internal CO₂ concentration – Ci (C) of guava seedlings as a function of salinity of irrigation water and combination of NK fertilization, at 125 days after sowing

differences in the gs of plants irrigated with an ECw of 0.3 dSm⁻¹ under C1 fertilization (70% N + 50% K₂O) and C4 (160% N + 125% K₂O). When using water salinity of 1.1 dS m⁻¹ there was a significant difference only between combinations C1 and C3. In plants grown under ECw of 1.9, 2.7, and 3.5 dS m⁻¹ there were no significant differences between the different fertilization combinations for gs.

The stomata are responsible for regulating gas exchange and, according to the results obtained in the present study (Figure 2A), gs was negatively affected by the increase in water salinity under the combinations C1, C2, and C4, directly influenced by the loss of water in leaf tissues because, according to Pereira et al. (2020), the closing of stomata reduces water loss through transpiration, resulting in a decrease in the photosynthetic rate, besides increasing the resistance to CO₂ diffusion.

According to Figure 2B, the CO₂ assimilation rate of plants fertilized with C1 was not influenced by the increase in irrigation water salinity, with a mean value of 6.511 μmol CO₂ m⁻² s⁻¹. Conversely, for the fertilization combinations C2 and C3, the regression equations showed a quadratic behavior, with the highest values of 7.285 and 9.374 μmol CO₂ m⁻² s⁻¹, respectively, obtained in plants under ECw of 1.8 dS m⁻¹. Plants irrigated with waters of 3.5 dS m⁻¹ had the lowest values of A. Fertilization with 160% N + 125% K₂O (C4) led to the highest value of A, 6.460 μmol CO₂ m⁻² s⁻¹, in plants irrigated with water of 0.3 dS m⁻¹.

When analyzing the effects of the fertilization combinations at each ECw level (Figure 2B), a superiority of the CO₂ assimilation rate of plants submitted to C3 is observed compared to those cultivated under C1 and C4 and irrigated with ECw of 1.9 dS m⁻¹. In the plants irrigated with ECw of 2.7 dS m⁻¹, there were differences in the A between the plants fertilized with the C3 and C4 combinations. Under irrigation with water of highest salinity (3.5 dS m⁻¹), there was superiority in the A of the plants fertilized with the combination C1 compared to C4. However, there were no significant differences between different fertilization combinations in plants irrigated with ECw of 0.3 and 1.1 dS m⁻¹. Results similar to the present study were observed by Lacerda et al. (2022) evaluating the effects of irrigation with saline water (ECw of 0.6 and 3.2 dS m⁻¹) on guava plants, observing a CO₂ assimilation rate of around 6 μmol CO₂ m⁻² s⁻¹ in plants cultivated under water salinity of 3.2 dS m⁻¹, i.e., a value close to that achieved under ECw of 3.5 dS m⁻¹ in the present study.

The decrease in CO₂ assimilation rate due to the increase in salinity, more markedly under fertilization management C4, may have occurred due to the decrease in the amount of carbon dioxide fixed, which is a response of the plant to salt stress (Zhao et al., 2020). The increase in CO₂ assimilation rate for ECw of up to 1.8 dS m⁻¹ and under fertilization combinations C2 and C3 is influenced by the increase in the synthesis of sugars by photosynthesis, stimulating the increase in sap flow to accelerate the compartmentalization of toxic ions in the vacuole.

In plants grown under water salinity of 1.9 dS m⁻¹, the fertilization combination C2 and C3 reached the highest estimated value for A (Figure 2B), reflecting the role of K in osmotic regulation and promoting maintenance of turgor in the guard cells by increasing the osmotic potential. This mechanism of acclimatization to water stress allows the translocation of photoassimilates through the phloem, which maintains ideal photosynthetic rates in the plant, preventing electrons from being used for the production of reactive forms of oxygen (Johnson et al., 2022).

According to the regression equations (Figure 2C), the salinity of the irrigation water caused a quadratic effect on the Ci in the plants in the combinations C1 (70% N + 50% K₂O), C2 (100% N + 75% K₂O). There was no satisfactory fit of the quadratic model in treatments C3 (130% N + 100% K₂O) and 160% N + 125% K₂O (C4), as demonstrated by the equations: C3 = 238.77 - 32.01^{ns}x + 3.05^{ns}x²; R² = 0.57, and C4 = 182.65 + 13.42^{ns}x - 5.8781^{ns}x²; R² = 0.39, with respective mean values of 192.867 μmol CO₂ m⁻² s⁻¹ and 179.401 μmol CO₂ m⁻² s⁻¹. The highest values of internal CO₂ concentration (C1 - 244.545 μmol CO₂ m⁻² s⁻¹) and (C2 - 224.483 μmol CO₂ m⁻² s⁻¹) were obtained, respectively, in plants under ECw of 0.9 dS m⁻¹ and 0.4 dS m⁻¹ and for both treatments, the lowest values were observed in plants under ECw of 3.5 dS m⁻¹.

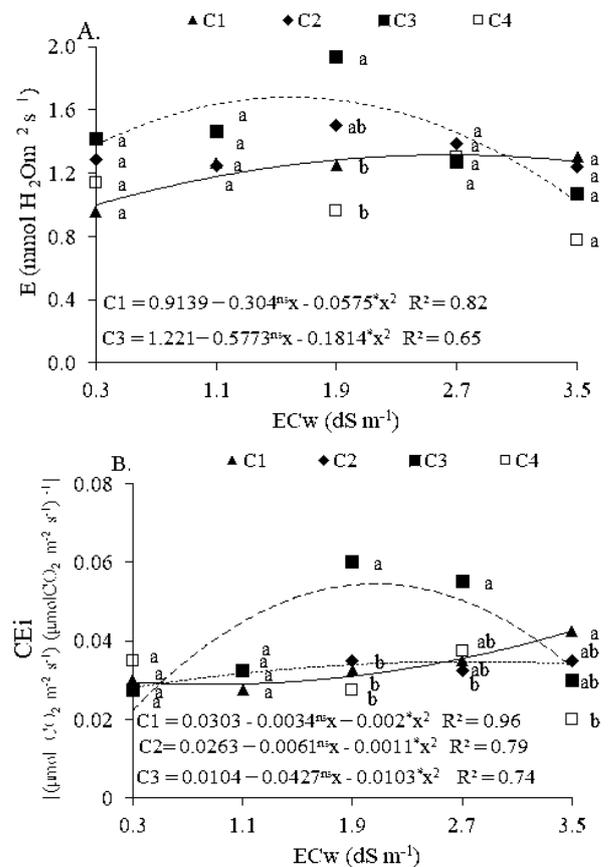
Regarding the splitting of the fertilization combinations at each water salinity level (Figure 2C), it is observed that the internal concentration of CO₂ of the plants cultivated under ECw of 0.3 dS m⁻¹ and submitted to combinations C1 and C2 was statistically superior to that of C4. In plants submitted to ECw of 1.1 dS m⁻¹, there were significant differences in Ci between combinations C1, C2 in relation to those that received C4. On the other hand, the plants cultivated with water of 1.9 dS m⁻¹, the highest Ci values were obtained in the C1 and C2 fertilization combinations, differing significantly only compared to the C3 combination. Under water salinity of 2.7 dS m⁻¹ there were significant differences in Ci only between C2 and C4 combinations. On the other hand, under highest ECw (3.5 dS m⁻¹) there were no significant difference among the different combinations of NK fertilization.

The decrease in Ci may have occurred due to the consumption of CO₂ by the RuBP carboxylase-oxygenase enzyme (RuBisCO) during the Calvin cycle, and under this condition it may also capture O₂, giving rise to photorespiration, an activity in which there is a waste of energy and decreased sugar synthesis, a condition that occurs more often when the stomata are closed (Dias et al., 2019), proven by the data presented in Figure 2A for stomatal conductance, which is a response of plants to the condition of

salt stress. Xavier et al. (2022a) also observed in guava plants that the increase in ECw from 0.6 to 4.2 dS m⁻¹ markedly reduced the internal concentration of CO₂ at 180 DAS.

Regarding transpiration, the regression equations (Figure 3A) point to a quadratic effect of water salinity for combinations C1 and C3; under the C1 fertilization management, the plants had the lowest E (0.999 mmol H₂O m⁻² s⁻¹) for a salinity of 0.3 dS m⁻¹ and maximum E (1.315 mmol H₂O m⁻² s⁻¹) under ECw of 2.6 dS m⁻¹. For C3, maximum transpiration values were verified under ECw levels of 1.6 dS m⁻¹ equal to 1.680 mmol H₂O m⁻² s⁻¹, with a least estimated value of 1.019 mmol H₂O m⁻² s⁻¹, obtained in ECw level of 3.5 dS m⁻¹. For treatments C2 (1.1647 + 0.26^{ns}x - 0.0667^{ns}x²; R² = 0.52) and C4 (1.1213 + 0.2456^{ns}x - 0.0937^{ns}x²; R² = 0.44) data did not show satisfactory adjustments to the tested models (Figure 3A). When verifying the effects of the fertilization combinations in each level of ECw (Figure 3A), significant differences were observed between the combinations of fertilization for foliar transpiration only in the plants irrigated with ECw 1.9 dS m⁻¹, highlighting superiority in the ones submitted to combination C3 compared to those under C1 and C4.

From an ECw of 2.6 dS m⁻¹ for combination C1 and 1.6 dS m⁻¹ for C3, there was a reduction in transpiration (Figure 3A), i.e., activating a plant defense mechanism associated with stomatal closure (Figure 2A). Transpiration is a process in which energy in the form of latent heat is transferred from the



ns, * - not significant, significant at p ≤ 0.05 by the F test, respectively; C1 = 70% N + 50% K₂O, C2 = 100% N + 75% K₂O, C3 = 130% N + 100% K₂O, and C4 = 160% N + 125% K₂O
Figure 3. Transpiration - E (A) and instantaneous carboxylation efficiency - CEi (B) of guava seedlings as a function of salinity of irrigation water and combination of NK fertilization, at 125 days after sowing

leaf to the air, being a mechanism of acclimatization of the plant to stress (Savvides et al., 2022). Pinheiro et al. (2022) mention that there is a close relationship between *g_s* and *E* because the flow of water vapor to the atmosphere decreases when the stomata are closed, thus triggering a process of reduction in transpiration due to the decrease in stomatal conductance.

Regarding the instantaneous efficiency of carboxylation (Figure 3B), with the management of C1 fertilization the maximum value 0.043 [(μmol CO₂ m⁻² s⁻¹) (μmol CO₂ m⁻² s⁻¹)⁻¹] was observed in the EC_w of 3.5 dS m⁻¹. Treatment C2 promoted maximum and minimum estimated results of 0.035 and 0.028 [(μmol CO₂ m⁻² s⁻¹) (μmol CO₂ m⁻² s⁻¹)⁻¹] for EC_w of 2.8 and 0.3 dS m⁻¹, respectively. For the C3 combination, the highest CE_i value 0.0546 [(μmol CO₂ m⁻² s⁻¹) (μmol CO₂ m⁻² s⁻¹)⁻¹] was obtained with an EC_w of 2.1 dS m⁻¹ and the lowest value (0.022) estimated with an EC_w of 0.3 dS m⁻¹. In treatment C4, there was no adequate adjustment to the tested regression models (0.0325 + 0.0032^{ns}x - 0.0017^{ns}x²; R² = 0.41) (Figure 3B).

When analyzing the effects of the fertilization combinations at each EC_w level (Figure 3B), it is observed that under water salinity of 0.3 and 1.1 dS m⁻¹ there were no significant differences between the fertilization combinations for CE_i. The CE_i of plants irrigated with EC_w of 1.9 dS m⁻¹ and fertilized with C3 differed significantly from those that received C1, C2, and C4. In case of water salinity of 2.7 dS m⁻¹, there were significant differences only in the CE_i of plants submitted to C2 and C3 combinations. At the highest water salinity level (3.5 dS m⁻¹), there is a significant effect for CE_i only in plants cultivated under combinations C1 and C4.

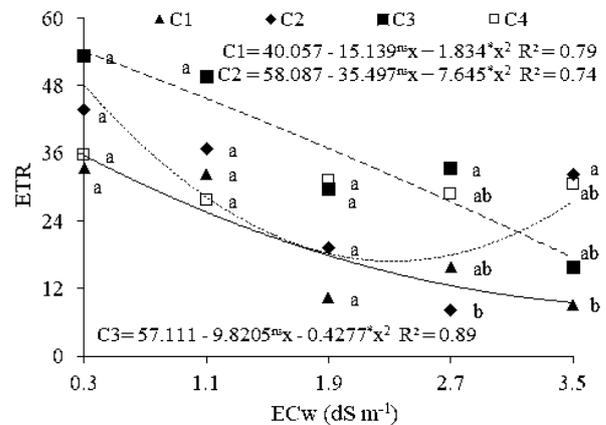
The observed increase in CE_i is indicative that guava seedlings showed greater availability of CO₂ in the mesophyll to be carboxylated or higher CO₂ assimilation rate (Pereira et al., 2020). The increase of CE_i in plants under salt stress is caused by the non-fixation of CO₂ in the carboxylation phase, reaching the mesophyll cells due to metabolic restrictions in the Calvin cycle (Hameed et al., 2021). Xavier et al. (2022a) in a study with guava cv. Paluma under irrigation with saline water (EC_w from 0.6 to 4.2 dS m⁻¹), observed that the highest CE_i was obtained in plants grown under water salinity of 0.6 dS m⁻¹. On the other hand, the lowest CE_i was verified in plants submitted to EC_w of 4.2 dS m⁻¹, 180 days after sowing. According to Silva et al. (2022), reductions in *A* allow CE_i to decrease, reducing the availability of ATP, NADPH and substrate for RuBisCO.

CE_i indicates that non-stomatal factors are interfering in the photosynthesis process and, under a condition of salt stress, the decrease in CE_i is indicative of a reduction in the activity of

ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) due to the accumulation of salts in leaf tissues, mainly Na⁺ and Cl⁻ associated with other environmental factors, which favor the oxygenation of RuBisCO and an increase in the photorespiratory pathway (Lima et al., 2019).

The summary of the analysis of variance (Table 3) shows a significant effect for the interaction between the factors (salinity levels of irrigation water and combinations of nitrogen and potassium fertilization - NK) on electron transport rate (ETR) and quantum efficiency of photosystem II (Fv/Fm). There were also significant simple effects of EC_w on variable fluorescence (Fv) and maximum fluorescence (Fm). There was no significant effect of any of the sources of variation studied on initial fluorescence (F₀).

For the interaction between irrigation water salinity and NK fertilizer combination on the electron transport rate at 125 DAS, the regression equations (Figure 4) showed a better fit of the quadratic model to the data, and the highest ETR values (C1 - 35.680; C2 - 48.126; C3 - 54.126) were obtained at EC_w of 0.3 dS m⁻¹, with decreases in ETR with increasing salinity. Regarding the C4 treatment (Figura 4), the data did not obtain adjust satisfactorily to the tested regression models (36.612 - 6.9695^{ns}x + 1.5212^{ns}x²; R² = 0.58). The decrease in ETR shows that the studied cultivar, under saline stress conditions, cannot maintain the normal flow of electron transport in chloroplasts, i.e., stress can affect the process, causing inactivation of photosynthetic electron transport,



^{ns}, *, ** - not significant, significant at p ≤ 0.05 and p ≤ 0.01 by the F test, respectively; C1 = 70% N + 50% K₂O, C2 = 100% N + 75% K₂O, C3 = 130% N + 100% K₂O, and C4 = 160% N + 125% K₂O

Figure 4. Electron transport rate - ETR of guava seedlings as a function of salinity of irrigation water and combination of NK fertilization, at 125 days after sowing

Table 3. Summary of the analysis of variance for electron transport rate (ETR), initial fluorescence (F₀), variable fluorescence (Fv), maximum fluorescence (Fm), and quantum efficiency of photosystem II (Fv/Fm), at 125 days after sowing, in guava seedlings under different salinity levels of irrigation water and combinations of nitrogen and potassium fertilization

Source of variation	DF	Mean squares				
		ETR	F ₀	Fv	Fm	Fv/Fm
Salinity level (S)	4	1457.437**	3246.117 ^{ns}	38372.881*	65394.278*	0.009**
Linear Reg.	1	4752.291**	5475.717 ^{ns}	113565.255*	183059.547**	0.019**
Quadratic Reg.	1	641.018*	218.692 ^{ns}	5015.692 ^{ns}	9972.718 ^{ns}	0.008*
NK combination	3	898.055**	3786.558 ^{ns}	54135.408*	33982.300 ^{ns}	0.011**
Interaction (S × NK)	12	301.815*	2261.648 ^{ns}	18221.933 ^{ns}	22042.289 ^{ns}	0.005**
Blocks	3	171.985 ^{ns}	7316.144**	10362.544 ^{ns}	24630.811 ^{ns}	0.001 ^{ns}
CV (%)		41.71	10.67	9.45	8.49	5.62

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

which may cause inactivation and irreversible disruption of the ETR pathway by proteins (Tatagiba et al., 2014).

In the breakdown of the fertilization combinations in each level of electrical conductivity of the water (Figure 4), it was observed that there were no significant differences in the ETR of the plants irrigated with EC_w of 0.3; 1.1, and 1.9 dS m⁻¹ under the different fertilization combinations. Under EC_w of 2.7 dS m⁻¹, there were significant differences only between the C3 and C2 fertilization combinations. On the other hand, for the plants cultivated under EC_w of 3.5 dS m⁻¹, the ETR of those that received the combination C2 was statistically superior to those fertilized with C1.

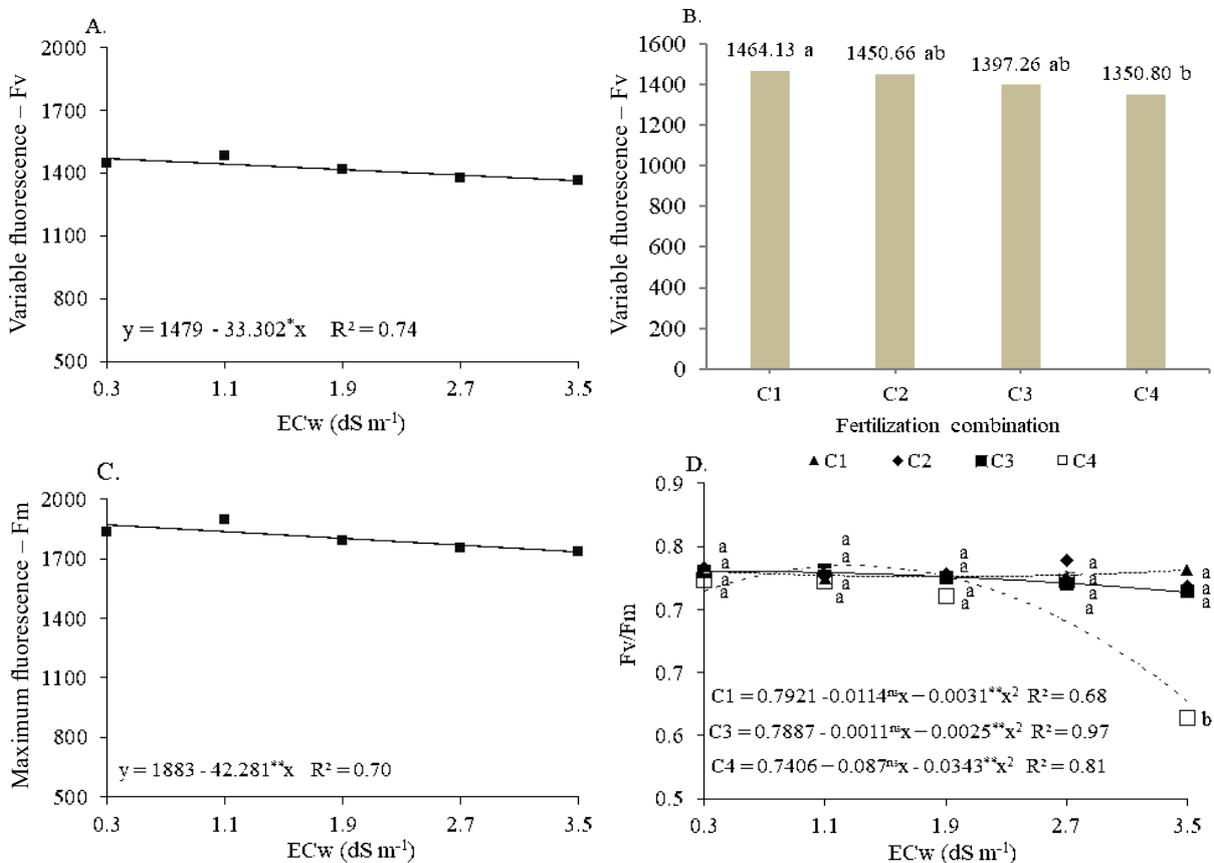
Despite the decrease in ETR due to the increase in water salinity (Figure 4), the fertilizer combination of C2 and C3 promoted higher ETR in comparison to C1 at an EC_w of 3.5 dS m⁻¹, indicating that adequate doses of these fertilizers favor the best development of plants and attenuate the effect of salt stress, because, according to Tighe-Neira et al. (2018), K is involved in chlorophyll fluorescence, an important variable in the photosynthetic processes. On the other hand, N can favor the increase of the ETR of the PSII reaction center, promoting a better direction of the flow of electrons so that the electrons participate in the carboxylation of RuBisCO, increasing the photosynthetic rate.

The increase in irrigation water salinity caused a decreasing linear effect of 2.25% per unit increase in EC_w on variable

fluorescence (Fv) at 125 DAS (Figure 5A), when seedlings irrigated with water of 3.5 dS m⁻¹ showed a reduction in Fv of 7.20% (78.667) compared to those irrigated with water of 0.3 dS m⁻¹. Variable fluorescence indicates the capacity to transfer the energy of electrons ejected from pigment molecules for the formation of NADPH, ATP, and reduced ferredoxin (Fdr), causing an increase in CO₂ assimilation capacity in the biochemical phase of photosynthesis (Tatagiba et al., 2014; Veloso et al., 2020). Xavier et al. (2022b) when evaluating the quantum yield of the photosystem II of guava plants under irrigation with saline water (EC_w ranging from 0.6 to 4.2 dS m⁻¹), 180 days after sowing, observed that there was no damage to the photosynthetic apparatus of the plants since the values of Fv/Fm were between 0.75 and 0.85.

An analysis of the effect of NK fertilization combinations on Fv (Figure 5B) showed that the highest values of Fv in guava seedlings occurred in plants fertilized with the combination C1 (70% N + 50% K₂O), followed by plants that received C2 (100% N + 75% K₂O) and C3 (125% N + 100% K₂O), whose means did not differ statistically. Plants fertilized with the combination C4 (160% N + 125% K₂O) had significantly lower values of Fv. Therefore, the plants fertilized with the combinations C1, C2, and C3 have a higher capacity to produce NADPH, ATP, and Fdr (Tatagiba et al., 2014).

Possibly there may have been greater nutritional imbalance and intensification of osmotic effect for the combinations C3



^{ns}, *, ** - not significant, significant at $p \leq 0.05$ and $p \leq 0.01$ by the F test, respectively; C1 = 70% N + 50% K₂O, C2 = 100% N + 75% K₂O, C3 = 130% N + 100% K₂O, and C4 = 160% N + 125% K₂O. Means followed by different letters indicate significant difference between treatments by Tukey test ($p \leq 0.05$)

Figure 5. Variable fluorescence – Fv (A) and maximum fluorescence – Fm (C) as a function of salinity of irrigation water - EC_w; variable fluorescence – Fv as a function of NK fertilization combination (B); and maximum quantum efficiency of photosystem II – Fv/Fm (D) of guava seedlings as a function of EC_w and NK fertilization combination, at 125 days after sowing

and C4, reducing water absorption due to the high doses of urea and KCl fertilizers that have a high salt index. Similar results have been reported by Dias et al. (2019), who observed that the increase in K fertilization intensified salt stress in the production of West Indian cherry with damage to variable fluorescence as the K dose increased.

The increase in water salinity caused a linear reduction in the maximum fluorescence of guava seedlings (Figure 5C), which showed a decrease of 2.25% per unit increase in ECw, and the difference between plants irrigated with water of the highest ECw (3.5 dS m⁻¹) and lowest ECw (0.3 dS m⁻¹) was 7.18% (98.583). This reduction in Fm caused by salt stress indicates that there was a decrease in chlorophyll a activity, and this condition can also reduce energy production and reducing power, negatively affecting the functioning of RuBisCO in the CO₂ carboxylation process (Sá et al., 2019). Maximum fluorescence is a variable that indicates the state in which the PSII reaction centers reach their maximum capacity, indicating that quinone (QA) was reduced by electrons transferred from P680 with the increase in salinity (Sá et al., 2019).

When evaluating the maximum quantum efficiency of photosystem II (Figure 5D) under the effect of irrigation water salinity for each combination of NK fertilization, it was possible to observe based on the regression equations a quadratic behavior for the combinations C1, C3, and C4; the C2 combination led to an average result of 0.786. The C1 combination resulted in an estimated maximum value of 0.789 at a salinity level of 0.3 dS m⁻¹ and a minimum value of 0.781 for ECw of 1.9 dS m⁻¹. The combinations C3 and C4 led to maximum values of 0.789 and 0.796 for the salinity levels of 0.3 and 1.30 dS m⁻¹, respectively, and lower estimated values of 0.762 and 0.625 for the same salinity level of 3.5 dS m⁻¹.

By analyzing the unfolding of the combinations at each water salinity level of the water (Figure 5D), it is verified that the quantum efficiency of the photosystem II of the plants submitted to ECw of 0.3, 1.1, 1.9, and 2.7 dS m⁻¹ did not differ significantly between the different NK fertilization combinations. However, under water salinity of 3.5 dS m⁻¹, the Fv/Fm of the plants cultivated under fertilization with the C1, C2, and C3 combinations was statistically superior to that of C4. According to Veloso et al. (2020), the photosynthetic apparatus is intact when the Fv/Fm ratio is between 0.75 and 0.85; thus, the Fv/Fm values found in the present study indicate that there was no damage to the photosynthetic apparatus, regardless of the salinity of the irrigation water applied, except for the combination C4 (160% N + 125% K₂O) at the salinity level of 3.5 dS m⁻¹, which led to a value below the recommended range.

The variation found in the efficiency of photosystem II is due to the changes observed in the initial fluorescence and maximum fluorescence, and its decrease with the increase in salinity is related to the reduction in the internal CO₂ concentration, closure of the stomata and increase in lipid peroxidation (Dias et al., 2019).

CONCLUSIONS

1. Irrigation water with ECw above 0.3 dS m⁻¹ reduces variable fluorescence and maximum fluorescence in guava seedlings cv Paluma.

2. The fertilization combination 130% N + 100% K₂O promotes higher estimated values for electron transport rate, CO₂ assimilation rate, transpiration, and instantaneous carboxylation efficiency of guava seedlings cv. Paluma, attenuating salt stress up to the mean value of 1.3 dS m⁻¹.

3. For maximum quantum efficiency of photosystem II, only treatments irrigated with ECw of 3.5 dS m⁻¹ and under the combination 160% N + 125% K₂O showed damage to the photosynthetic apparatus.

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