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# Physiology of 'Paluma' guava under irrigation with saline water and nitrogen fertilization

## Fisiologia de goiabeira 'Paluma' sob irrigação com água salina e adubação nitrogenada

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### Abstract

The use of saline water in irrigation causes osmotic and toxic effects and nutritional imbalance in plants, leading to morphophysiological modifications in the leaves and compromising the production of photosynthetic pigments, which negatively reflects in the growth and development of the crops. Hence, this study aimed to evaluate the effect of irrigation water salinity on the content of photosynthetic pigments and leaf morphophysiology of guava seedlings cv. 'Paluma' under nitrogen (N) fertilization. A randomized block design was used, testing five levels of irrigation water electrical conductivity – EC<sub>w</sub> (0.3, 1.1, 1.9, 2.7, and 3.5 dS m<sup>-1</sup>) and four N doses (541.1, 773.0, 1,004.9, and 1,236.8 mg of N dm<sup>-3</sup> of soil) in a 5 x 4 factorial scheme with three replicates and five plants per plot. The contents of photosynthetic pigments in the leaves of the guava seedlings cv. 'Paluma' were inhibited by the increase in irrigation water salinity at 190 days after emergence, and the salt stress was lessened with the N dose of 1,004.9 mg dm<sup>-3</sup> up to an EC<sub>w</sub> level of 1.2 dS m<sup>-1</sup>. Leaf morphophysiology of guava seedlings was not compromised by irrigation water salinity up to 1.5 dS m<sup>-1</sup>, and the highest values were obtained in plants fertilized with 541.1 mg of N dm<sup>-3</sup>.

**Key words:** *Psidium guajava* L. Photosynthesis. Salinity and nitrogen.

### Resumo

O uso de água salina na irrigação promove efeitos osmóticos, tóxicos e desbalanço nutricional nas plantas, causando modificações morfofisiológicas nas folhas e comprometimento na produção dos pigmentos fotossintéticos, refletindo negativamente no crescimento e desenvolvimento das culturas. Nesse sentido, objetivou-se com o presente trabalho avaliar o efeito da salinidade da água de irrigação no teor de pigmentos fotossintéticos e na morfofisiologia foliar de mudas de goiabeira cv. Paluma sob adubação nitrogenada. Usou-se a aleatorização em blocos, testando cinco níveis de condutividade elétrica da água de irrigação – CE<sub>a</sub> (0,3; 1,1; 1,9; 2,7 e 3,5 dS m<sup>-1</sup>) e quatro doses de nitrogênio (541,1; 773,0; 1004,9 e 1236,8 mg de N dm<sup>-3</sup> de solo), em esquema fatorial 5 x 4, com três repetições e cinco plantas por parcela. Os teores de

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pigmentos fotossintéticos nas folhas das mudas de goiabeira cv. Paluma foram inibidos pelo aumento da salinidade na água de irrigação aos 190 dias após a emergência, sendo o estresse salino amenizado com adubação nitrogenada na dose de 1004,9 mg de N dm<sup>-3</sup> até a CEa de 1,2 dS m<sup>-1</sup>. A morfofisiológica foliar das mudas de goiabeira não foi comprometida pela salinidade da água de irrigação de até 1,5 dS m<sup>-1</sup>, e os maiores valores foram obtidos nas plantas adubadas com 541,1 mg de N dm<sup>-3</sup>.

**Palavras-Chave:** *Psidium guajava* L. Fotossíntese. Salinidade e nitrogênio.

## Introduction

In arid and semi-arid regions, irrigation emerges as one of the technologies that have most contributed to guaranteeing the production and exploitation of agricultural activities, because these areas are characterized by long drought periods. However, in these regions, water is not always of good quality, resulting in the use of irrigation water with different saline levels (HOLANDA et al., 2010).

The use of saline water in irrigation represents a serious issue in the agricultural production system, because of both the alterations in soil physical and chemical attributes (CAVALCANTE et al., 2010) and the negative impacts of the salts on the plants. These alterations are associated with osmotic and toxic effects, especially of Na<sup>+</sup> and Cl<sup>-</sup>, and with nutritional imbalance of K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>, induced by the competition with Na<sup>+</sup>, or even of NO<sub>3</sub><sup>-</sup> due to the high concentration of Cl<sup>-</sup> in the environment, which damages the functions of the enzymatic systems and impedes protein synthesis (APSE; BLUMWALD, 2007; DIAS et al., 2012b).

Plant species differently respond to salt stress and, among the fruit crops of economic interest, guava (*Psidium guajava* L.) is one of the species whose growth is affected by the use of saline irrigation water, especially at an early growth phase, being even classified as sensitive to salinity, with a salinity threshold of 1.2 dS m<sup>-1</sup> in the saturation extract, i.e., it suffers a decrease in its yield (TÁVORA et al., 2001). Some studies demonstrate that the increase in irrigation water salinity negatively affects leaf morphology of guava seedlings (CAVALCANTE et al., 2007, 2010) and, although not observed in this species, may decrease the concentration of photosynthetic pigments, which has been observed in leaves of passion fruit seedlings and tomato plants (CAVALCANTE et al., 2011; FREIRE et al., 2013; TATAGIBA et al., 2014).

For Cavalcante et al. (2010), the formation of seedlings and the cultivation of guava with saline irrigation water depends on the use of adequate techniques. In this case, the use of N fertilization emerges as a promising technology, because it reduces the Cl/N ratio in the plant, reestablishing the nutritional balance (BLANCO et al., 2008). However, there are not many studies testing this method in guava crops under salt stress to evaluate the effects of salinity on the contents of photosynthetic pigments and on leaf morphophysiology.

Mineral nutrition is an important factor, and N is the second macronutrient required in large amounts by guava seedlings (FRANCO et al., 2007). When applied at adequate doses, N increases the chlorophyll content in the leaves (DIAS et al., 2012a) due to its functions in plant metabolism, participating as a constituent of the chlorophyll molecule, nucleic acids, amino acids, and proteins (TAIZ; ZEIGER, 2013).

The quantification of chlorophyll contents and the evaluation of leaf morphophysiology are relevant in studies on fertilization practices, because they are related to the photosynthetic efficiency of the plants and, consequently, to their growth, production, and adaptability to different environmental conditions, such as salinity (TAIZ; ZEIGER, 2013). Therefore, this study aimed to evaluate the effect of irrigation water salinity on the contents of photosynthetic pigments and leaf morphophysiology of guava seedlings cv. 'Paluma' subjected to N doses.

## Material and Methods

The experiment was carried out from March to October 2014 in a greenhouse at the Center of Sciences and Agrifood Technology (CCTA) of the

Federal University of Campina Grande (UFCG), Pombal-PB, Brazil, 6°48'16" S, 37°49'15" W, at a mean altitude of 144 m asl.

The treatments consisted of the combination of five levels of irrigation water salinity (0.3, 1.1, 1.9, 2.7, and 3.5 dS m<sup>-1</sup>) and four N doses (70, 100, 130, and 160% of the N dose indicated for guava seedlings, i.e., 541.1, 773.0, 1,004.9, and 1,236.8 mg of N dm<sup>-3</sup> of soil), in randomized blocks with three replicates. Each experimental unit had five plants. The N doses were determined based on the dose of 773 mg of N dm<sup>-3</sup>, recommended by Dias et al. (2012a) for guava seedlings cv. 'Paluma' propagated from herbaceous cuttings, which corresponded to the N dose of 100%.

Saline irrigation waters were prepared using local supply water with electrical conductivity (ECw) of 0.3 dS m<sup>-1</sup> by adding the amounts of NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O, and MgCl<sub>2</sub>·6H<sub>2</sub>O salts, to obtain equivalent proportions of 7:2:1, which is the predominant salinity in the main water sources available in Northeast Brazil, following the

relationship between ECw and the concentration of salts (mmol L<sup>-1</sup> = EC x 10) (MEDEIROS, 1992).

The experiment was conducted using the guava cultivar 'Paluma', the most cultivated cultivar in Northeast Brazil (DIAS et al., 2012a). Three seeds per container were planted at a depth of 1.0 cm. The containers consisted of plastic tubes with a height of 19 cm, a diameter of 6.3 cm and a capacity of 288 cm<sup>3</sup>. Openings to allow drainage were located at the bottom. The containers were arranged on trays with a capacity for 54 tubes, supported by metal workbenches (angle brackets) at a height of 0.80 m from the soil.

The tubes were filled with substrate composed of Fluvic Neosol + sand + well decomposed bovine manure at the proportion of 82, 15, and 3%, respectively. The physical and chemical characteristics of the substrate (Table 1) were analyzed at the Laboratory of Soils and Plants of the CCTA/UFCG, according to the methodology of Claessen (1997).

**Table 1.** Physical and chemical characteristics of the substrate used in the experiment.

Textural class	Apparent density kg dm <sup>-3</sup>	Total porosity %	Organic matter g kg <sup>-1</sup>	P mg dm <sup>-3</sup>	Exchange complex					
					Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>		
					----- cmol <sub>c</sub> dm <sup>-3</sup> -----					
Sandy loam	1.38	47.00	32	17	5.4	4.1	2.21	0.28		
Saturation extract										
pH <sub>ps</sub>	EC <sub>se</sub> dS m <sup>-1</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Na <sup>+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	Saturation %
7.41	1.20	2.50	3.75	4.74	3.02	7.50	3.10	0.00	5.63	27.00

Contents of P, K, and Na in soil determined in Mehlich-1 extractor; Exchangeable Ca and Mg extraction by 1.0 mol L<sup>-1</sup> KCl; Organic matter: Walkley-Black wet digestion; pH<sub>ps</sub> = pH of the saturation paste; ECse = Electrical conductivity of the saturation extract of the substrate at 25°C.

When plants showed two pairs of true leaves, fully expanded (20 days after emergence), thinning was performed, leaving only the most vigorous plant per tube.

Phosphate fertilization was performed at the dose of 100 mg of P<sub>2</sub>O<sub>5</sub> dm<sup>-3</sup> in the form of single superphosphate, which was ground and mixed with the substrate before sowing (CORRÊA et al., 2003).

In addition, potassium fertilization was applied, using the dose of 726 mg of K dm<sup>-3</sup> of substrate (FRANCO et al., 2007), divided into four equal applications at 60, 90, 120, and 150 days after emergence (DAE) via fertigation with water of EC = 0.3 dS m<sup>-1</sup> for all treatments.

Application of saline water began at 25 DAE and irrigation of the different treatments was based

on crop water requirement, determined through drainage lysimetry, i.e., the volume retained in the tube was daily applied, determined by the difference between the applied volume and the volume drained in the previous irrigation. Irrigation was performed twice a day, in the early morning and late afternoon. Every 15 days, a leaching fraction of 0.15 was applied based on the volume applied in this period in order to avoid accumulation of salts in the substrate.

Nitrogen (N) fertilization began at 25 DAE, applied through fertigation and divided into 14 equal applications, every 10 days, using urea (45% of N) as N source.

The effect of the treatments on the plants was observed at 190 DAE through evaluation of the photosynthetic pigments: chlorophyll *a* (CLa), chlorophyll *b* (CLb), total chlorophyll (CLtotal), carotenoids (CAR), total chlorophyll/carotenoids ratio (CLtotal/CAR), and leaf morphophysiology: leaf area (LA), leaf dry mass (LDM), leaf mass ratio (LMR), and specific leaf area (SLA).

Determination of CLa, CLb, and CAR was performed using the third pair of fully expanded leaves, from the apical bud to the base of the plant, with a cork borer. For this, eight discs of leaf tissue were removed from the middle third of the leaves, corresponding to a total area of discs of 9.04 cm<sup>2</sup>. Then, the material was placed in small envelopes of aluminum paper and transported in thermal containers with ice to the refrigerator of the laboratory, where the samples remained totally in the dark for 48 h at a temperature of 8 °C. Subsequently, leaf discs were macerated in 90% acetone (5.0 mL) and 0.2 g of calcium carbonate (CaCO<sub>3</sub>). The obtained extracts were filtered using fast filter paper and collected in 25-mL volumetric flasks completing up to volume with 80% acetone; thus, the extract was diluted five times (ARNON, 1949).

Contents of chlorophyll *a*, *b*, and total carotenoids were quantified through spectrophotometry with readings in absorbance (A) at the wavelengths of 663, 646, and 470 nm, using 80% acetone as blank, through the equations 1, 2, 3, and 4, respectively, according to the methodology of Lichtenthaler

(1987):

$$\text{CLa} = 12.21 A_{663} - 2.81 A_{646} \quad \text{Eq. 1}$$

$$\text{CLb} = 20.13 \times A_{646} - 5.03 \times A_{663} \quad \text{Eq. 2}$$

$$\text{CLtotal} = 17.3 A_{646} + 7.18 A_{663} \quad \text{Eq. 3}$$

$$\text{CAR} = (1000 A_{470} - 1.82 \text{ CLa} - 85.02 \text{ CLb}) / 198 \quad \text{Eq. 4}$$

The data were obtained in mg L<sup>-1</sup> and, based on the area of the leaf discs (9.04 cm<sup>2</sup>) and on the dilution of the extract in 80% acetone (25 mL), the contents of CLa, CLb, and CAR were expressed as mass per m<sup>2</sup> (g m<sup>-2</sup>).

Leaf area (LA) was determined by measuring the leaves with fully opened leaf blades, according to Lima et al. (2012), considering the following equation:  $LA = 0.3205 \times L^{2.0412}$ , where L is the length of the leaf blade (cm).

The LDM was determined after collecting leaves and drying in forced-air ovens at 65 °C until constant weight, using a precision scale (0.001 g). The LMR was calculated by dividing leaf dry mass by the dry mass of the entire plant, and SLA was determined as the ratio between leaf area and leaf dry mass, according to Benincasa (2003).

The obtained data were evaluated through analysis of variance by F test at 0.05 and 0.01 probability levels and, in case of significance, regression analysis was performed using the statistical program SISVAR/UFLA (FERREIRA, 2011). The regression model was selected through the best fit based on the coefficient of determination (R<sup>2</sup>).

## Results and Discussion

Interaction between levels of irrigation water salinity and N doses had a significant effect ( $p < 0.01$  and  $p < 0.05$ ) on the contents of CLa, CL total, and CAR (Table 2). In addition, there were isolated significant effects ( $p < 0.01$ ) of water salinity on the contents of CLb and of N doses ( $p < 0.05$ ) on the total CLtotal/CAR ratio.



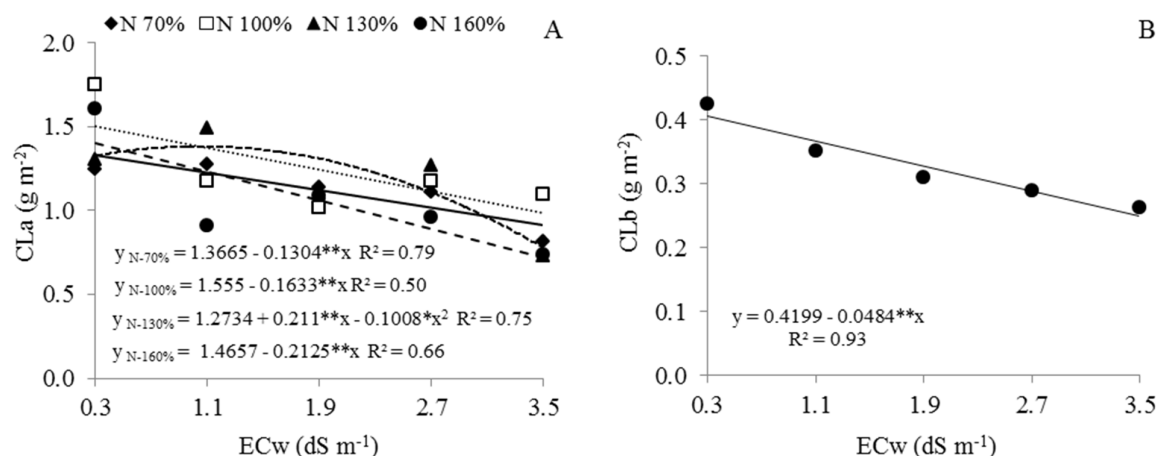
**Table 2.** Summary of the analysis of variance for chlorophyll *a* (CLa), *b* (CLb), and total chlorophyll (CLtotal), carotenoids (CAR) and total chlorophyll/carotenoid ratio (CLtotal/CAR) in leaves of guava seedlings cv. 'Paluma' irrigated with salinized water under nitrogen fertilization, at 190 days after emergence – DAE.

Source variation	Mean square				
	CLa	CLb	CLtotal	CAR	CLtotal/CAR
Salinity (S)	0.6314**	0.0485**	0.0116**	0.0857**	0.2686 <sup>ns</sup>
Linear regression	1.4608**	0.1801**	3.6436**	0.3131**	0.5644 <sup>ns</sup>
Quadratic regression	0.1248 <sup>ns</sup>	0.0121 <sup>ns</sup>	0.0511 <sup>ns</sup>	0.0139 <sup>ns</sup>	0.0351 <sup>ns</sup>
N doses (ND)	0.0933*	0.0078 <sup>ns</sup>	0.1492 <sup>ns</sup>	0.0010 <sup>ns</sup>	0.6144*
Linear regression	0.0037 <sup>ns</sup>	0.0088 <sup>ns</sup>	0.0884 <sup>ns</sup>	0.0020 <sup>ns</sup>	0.6293*
Quadratic regression	0.0778*	0.0138 <sup>ns</sup>	0.3511 <sup>ns</sup>	0.0009 <sup>ns</sup>	1.1426**
Interaction (S*ND)	0.1014**	0.0063 <sup>ns</sup>	0.1368*	0.0213*	0.1926 <sup>ns</sup>
Blocks	0.1067*	0.0035 <sup>ns</sup>	0.1467 <sup>ns</sup>	0.0259 <sup>ns</sup>	0.0396 <sup>ns</sup>
CV (%)	15.64	20.64	15.60	18.30	13.44

ns, \*\*, \*Respectively not significant and significant at  $p < 0.01$  and  $p < 0.05$ .

In the follow-up analysis of the factor water salinity level at different N doses (Figure 1A), CLa data fitted to a decreasing linear regression equation as a function of the increase in water salinity in plants that received fertilization at the doses of 70 (541.1), 100 (773), and 160% of N (1,236.8 mg dm<sup>-3</sup>), and the highest values of the variable (1.33,

1.50, and 1.40 g m<sup>-2</sup>) at the respective doses were obtained at the lowest EC<sub>w</sub> level (0.3 dS m<sup>-1</sup>), with decreases of 9.54, 10.50, and 14.50%, respectively, with per unit increase in EC<sub>w</sub>. However, at 130% of N (1,004.9 mg dm<sup>-3</sup>), the data best fitted to a quadratic model, with increasing effect on CLa content until the highest value (1.38 g m<sup>-2</sup>), at the EC<sub>w</sub> level of 1.2 dS m<sup>-1</sup>.

**Figure 1.** Follow-up analysis of the factor irrigation water salinity –EC<sub>w</sub> at the nitrogen doses for the contents of chlorophyll *a* – CLa (A) and chlorophyll *b* – CLb (B) in leaves of guava seedlings cv. 'Paluma' at 190 days after emergence – DAE.

According to Munns and Tester (2008), the reduction in chlorophyll content due to the increase in salinity occurs because the salt stress stimulates the enzymatic activity of chlorophyllase, which degrades the molecules of the photosynthesizing pigments and induces structural destruction of the

chloroplasts, besides causing loss and imbalance in the activity of pigmentation proteins. The harmful effects of irrigation water salinity on CLa contents have also been reported for passion fruit plants (CAVALCANTE et al., 2011; FREIRE et al., 2013).

The increments in CLa contents due to the use of N fertilization at the dose of 130% of N ( $1,004.9 \text{ mg dm}^{-3}$ ) under saline conditions (Figure 1A) is related to the stimulation of N absorption by the plant, in relation to chloride, because of the great affinity of the plants for nitrate. The increase in N fertilization up to a certain dose, under saline conditions where there is  $\text{Cl}/\text{NO}_3^-$  competition, can increase  $\text{NO}_3^-$  absorption, which reduces the  $\text{Cl}/\text{N}$  ratio in the leaves, reestablishing the nutritional balance and minimizing salt stress (BLANCO et al., 2008).

The content of CLb was negatively affected by the increment in irrigation water salinity (Figure 1B), with a linear loss of 11.53% ( $0.0484 \text{ g m}^{-2}$ ) per unit increase in ECw. Under these conditions, the light-capturing efficiency is reduced, since this pigment captures energy from other wavelengths and transfers it to CLa, which effectively acts in the photochemical reactions of photosynthesis, thus inhibiting photosynthetic activity and, consequently, plant growth (LIMA et al., 2011). Freire et al. (2013) also observed a deleterious effect of water salinity on CLb content in the leaves of passion fruit plants irrigated with water of  $\text{ECw} = 4.5 \text{ dS m}^{-1}$ .

In terms of the follow-up analysis of the water salinity levels with the N doses for the content of CL total (Figure 2A), highest CL total values of 1.76, 1.92, and  $1.84 \text{ g m}^{-2}$  were obtained for plants irrigated under water salinity of  $0.3 \text{ dS m}^{-1}$  and fertilized with N doses of 70, 100, and 160%, respectively. As water salinity increased, there were linear reductions of 10.68, 12.89, and 13.30% per unit increase in ECw in plants fertilized with the respective N doses, while at the N dose of 130% ( $1,004.9 \text{ mg dm}^{-3}$ ), there was a quadratic effect, with increase in CL total values up to the maximum value ( $1.78 \text{ g m}^{-2}$ ) at the water salinity level of  $1.2 \text{ dS m}^{-1}$ , which was justified by the same reasons that affected CLa under this dose. In guava seedlings cv. 'Paluma', Dias et al. (2012a) observed that leaf

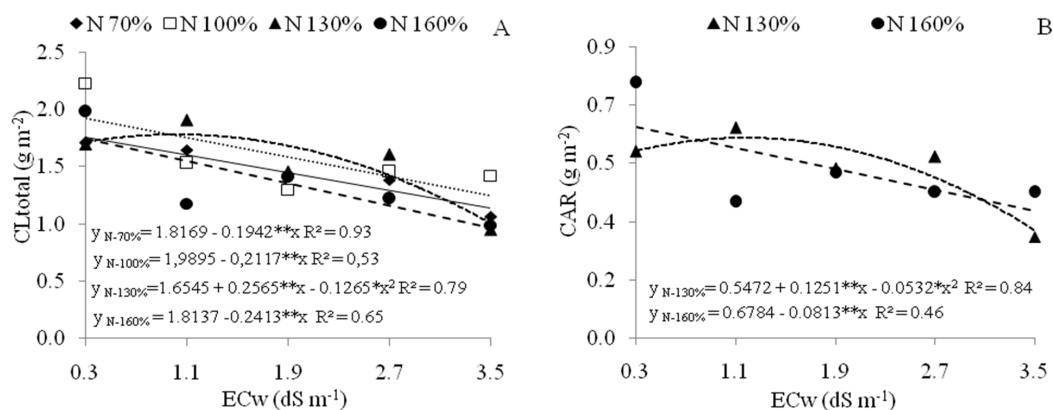
chlorophyll content under non-saline conditions was related to the increase in N fertilization up to a dose of  $793 \text{ mg of N dm}^{-3}$  of soil.

The results obtained in plants fertilized with N doses of 70, 100 and 160% (Figure 2A) are in agreement with those of Freire et al. (2013), who reported depletion of 19.2% in the content of CL total in passion fruit plants irrigated with ECw of  $4.5 \text{ dS m}^{-1}$ . For Apse and Blumwald (2007), the decreases in total CL due to the increase in salinity are related to osmotic and toxic effects of the ions and to nutritional imbalance.

For CAR contents, according to the follow-up analysis of salinity levels with N doses (Figure 2B), there was no significant difference of the water salinity levels in plants fertilized with the doses of 70 ( $541.1 \text{ mg of N dm}^{-3}$ ) and 100% ( $773 \text{ mg of N dm}^{-3}$ ) of N. In plants fertilized with the dose of 130% of N ( $1,004.9 \text{ mg dm}^{-3}$ ), there was a quadratic effect, and the highest value ( $0.62 \text{ g m}^{-2}$ ) was obtained at the ECw level of  $1.2 \text{ dS m}^{-1}$ , while at the dose of 160% of N ( $1,236.8 \text{ mg dm}^{-3}$ ), there was a decreasing linear effect as water salinity increased, with reductions of 11.98% in CAR content per unit increase in ECw.

The reduction in CAR content due to the increase in ECw can be attributed to the degradation of  $\beta$ -carotene under saline conditions and reduction in the synthesis of zeaxanthin, structural pigments of the carotenoids, which are apparently involved in the protection against photo-oxidative damages to chlorophyll (MUNNS; TESTER, 2008). However, this deleterious effect of salinity on CAR contents in the period of 190 DAE (Figure 2B) was lessened by N fertilization at the doses of 70 ( $541.1$ ) and 100% of N ( $773.0 \text{ mg of N dm}^{-3}$ ), because the plants that received these doses did not show significant difference when irrigated with water of different salinity levels up to  $1.2 \text{ dS m}^{-1}$  at the dose of 130% of N ( $1,004.9 \text{ mg dm}^{-3}$ ), as evidenced for the CLa and CL total contents.

**Figure 2.** Follow-up analysis of the factor irrigation water salinity -ECw at the nitrogen doses for the content of total chlorophyll – CLtotal (A) and carotenoids – CAR (B) in leaves of guava seedlings cv. 'Paluma' at 190 days after emergence – DAE.



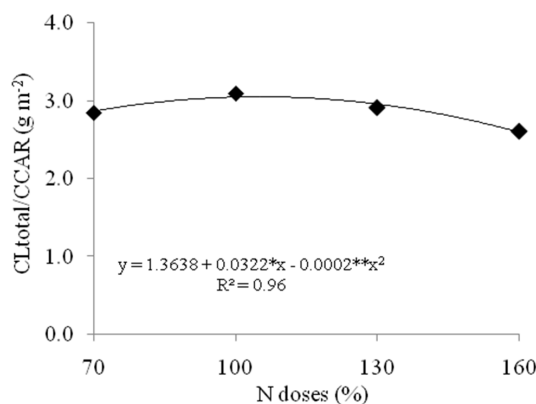
The production of CL total in relation to CAR (CLtotal/CAR) was positively influenced by the increase in N fertilization apparently (Figure 3), and the data best fitted to a quadratic regression equation, with the highest value (2.660 g m<sup>-2</sup>) at the dose of 80% of N (618.4 mg dm<sup>-3</sup>). Possibly, this is due to the high correlation between N contents and chlorophyll in the leaves, since this nutrient is a constituent of the chlorophyll molecule (TAIZ; ZAIGER, 2013). In this case, there is a higher gain in CLa content in relation to the CAR concentration.

In terms of leaf morphophysiology (Table 3), there was a significant effect ( $p < 0.01$  and  $0.05$ ) of irrigation water salinity on leaf area (LA), leaf dry mass (LDM), and leaf mass ratio (LMR), while

N doses significantly ( $p < 0.01$  and  $0.05$ ) affected LA, LDM, LMR, and SLA. However, there was no significant ( $p > 0.05$ ) interaction between the factors water salinity and N doses for these variables.

According to Figure 4, leaf morphophysiology of guava seedlings, represented by LA, LDM, and LMR, was not compromised by the increase in irrigation water salinity up to a mean ECw level of 1.5 dS m<sup>-1</sup>, and the data of LA, LDM, and LMR were best fitted to quadratic regression equations (Figures 4A, B, and C, respectively), with maximum values of 201.20 cm<sup>2</sup>, 1.57 g, and 0.442 g g<sup>-1</sup> obtained at water salinity levels of 1.3, 1.5, and 1.7 dS m<sup>-1</sup>, respectively, from which there were decreases until a ECw level of 3.5 dS m<sup>-1</sup>.

**Figure 3.** Total chlorophyll/carotenoid ratio – CLtotal/CAR in leaves of guava seedlings cv. 'Paluma' as a function of nitrogen doses at 190 days after emergence – DAE.





**Table 3.** Summary of the analysis of variance for leaf area (LA), leaf dry mass (LDM), leaf mass ratio (LMR), and specific leaf area (SLA) of guava seedlings cv. ‘Paluma’ irrigated with salinized water under nitrogen fertilization at 190 days after emergence – DAE.

Source variation	Mean square			
	LA <sup>1</sup>	LDM	LMR	SLA
Salinity (S)	23.637**	0.889**	0,008*	624,527 <sup>ns</sup>
Linear regression	59.408**	1.857**	0,005 <sup>ns</sup>	364,357 <sup>ns</sup>
Quadratic regression	29.812**	1.518**	0,017*	1828,464 <sup>ns</sup>
N doses (ND)	48.082**	0.723**	0,014**	2898,350*
Linear regression	135.455**	8.111**	0,039**	7053,646**
Quadratic regression	1.732 <sup>ns</sup>	0.018 <sup>ns</sup>	0,001 <sup>ns</sup>	353,614 <sup>ns</sup>
Interaction (S*ND)	4.380 <sup>ns</sup>	0.137 <sup>ns</sup>	0,001 <sup>ns</sup>	650,711 <sup>ns</sup>
Blocks	6.364 <sup>ns</sup>	0.076 <sup>ns</sup>	0,005 <sup>ns</sup>	5244,360*
CV (%)	15.59	21.75	12,23	19,88

ns, \*\*, \*Respectively not significant and significant at  $p < 0.01$  and  $p < 0.05$ ; <sup>1</sup> statistical analysis after data transformation in .

The increase in LA up to an ECw level of 1.3 dS m<sup>-1</sup> must be related to some adaptive mechanisms of this species to salinity, as a consequence of the exposure time to salinity during 180 days (Fig 4A). Under salt stress conditions, there is an accumulation of wax on the leaf surface and increase in the number of the palisade and spongy cells in the leaf tissue (PARIDA; DAS, 2005), which may have resulted in an accumulation of LDM up to a salinity level of 1.5 dS m<sup>-1</sup> (Figure 4B). This result is in agreement with the findings of Cavalcante et al. (2007), who observed that LDM levels of the guava cultivars ‘Paluma’, ‘Pentecoste’, and ‘Surubim’ increased with irrigation water salinity of up to 1.5 dS m<sup>-1</sup> at 180 DAS.

In relation to LMR, it is suggested that, up to an ECw level of 1.7 dS m<sup>-1</sup> (Figure 4C), the rate of photoassimilate exportation to the rest of the plant was lower, resulting in higher LMR, which may be related to adaptive mechanisms. Above this salinity level, the leaves tended to reach maturation early due to increased salt stress, resulting in the transport of photoassimilates to other plant parts, there by decreasing LMR. According to Rodrigues et al. (1993), LMR is a physiological component that expresses the fraction of dry mass not exported from the leaf to the rest of the plant; it tends to decrease with leaf maturation as the photoassimilated

compounds are directed to other regions of the plant (BENINCASA, 2003).

In general, leaf morphophysiology responds differently to salinity in relation to the contents of photosynthetic pigments, possibly due to mechanisms of adaptation to salinity adopted by the leaves.

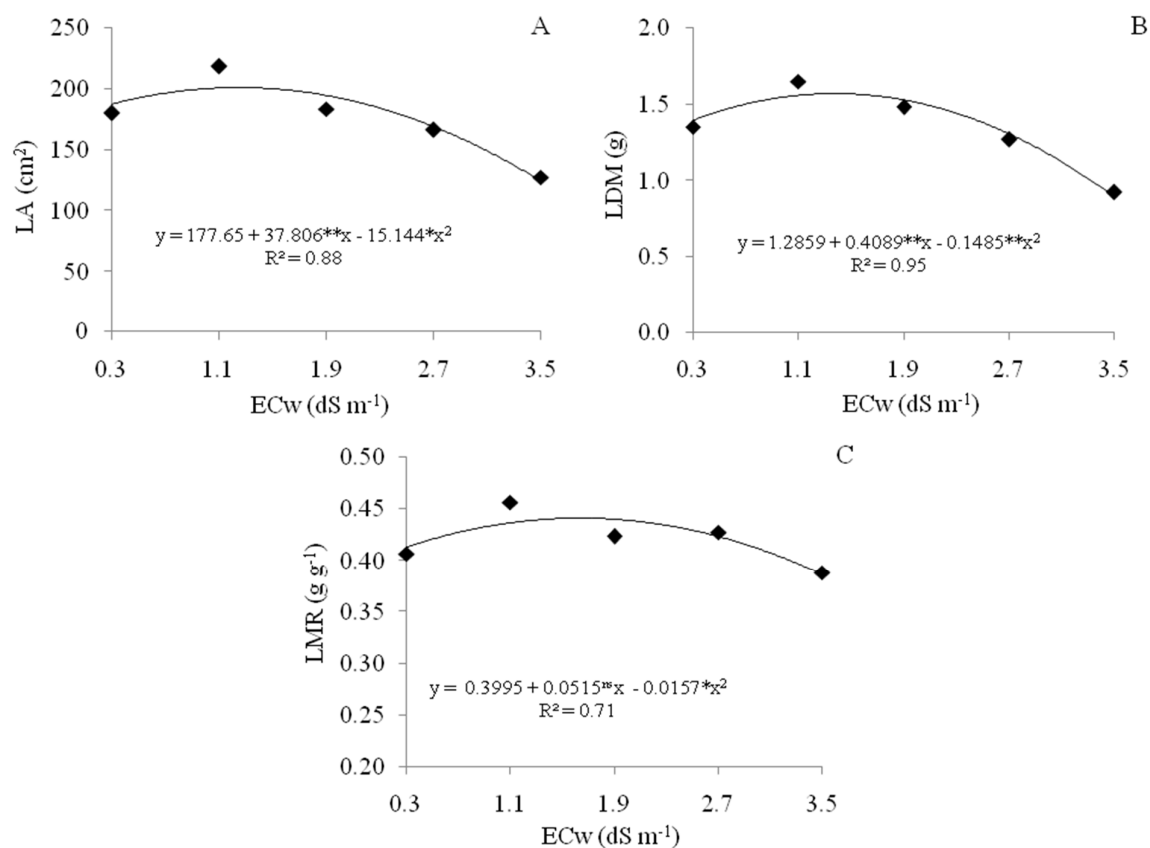
With regard to the isolated effect of N doses (Figures 5A, B, and C), the data of the variables LA, LDM, and LMR were fitted to decreasing linear regression equations, with reductions of 15.42, 18.06, and 5.31% in the respective variables. With the highest values obtained at the lowest dose, i.e., 70% of N (541.1 mg of N dm<sup>-3</sup>), this dose was close to the concentration of 552 mg of N plant<sup>-1</sup> accumulated in guava seedlings cv. ‘Paluma’ in a hydroponic cultivation system (FRANCO et al., 2007). It can be inferred that increasing N fertilization beyond a dose of 541.1 mg dm<sup>-3</sup>, using urea as N source, inhibits leaf morphophysiology by increasing soil ammonium levels, which may cause plant toxicity as observed by Silva et al. (2014) for jackfruit seedlings.

Increased N doses stimulated SLA, and the data best fitted to an increasing linear regression equation, with increment of 7.95% for every 30% increase in N dose (231.9 mg of N dm<sup>-3</sup>) (Figure

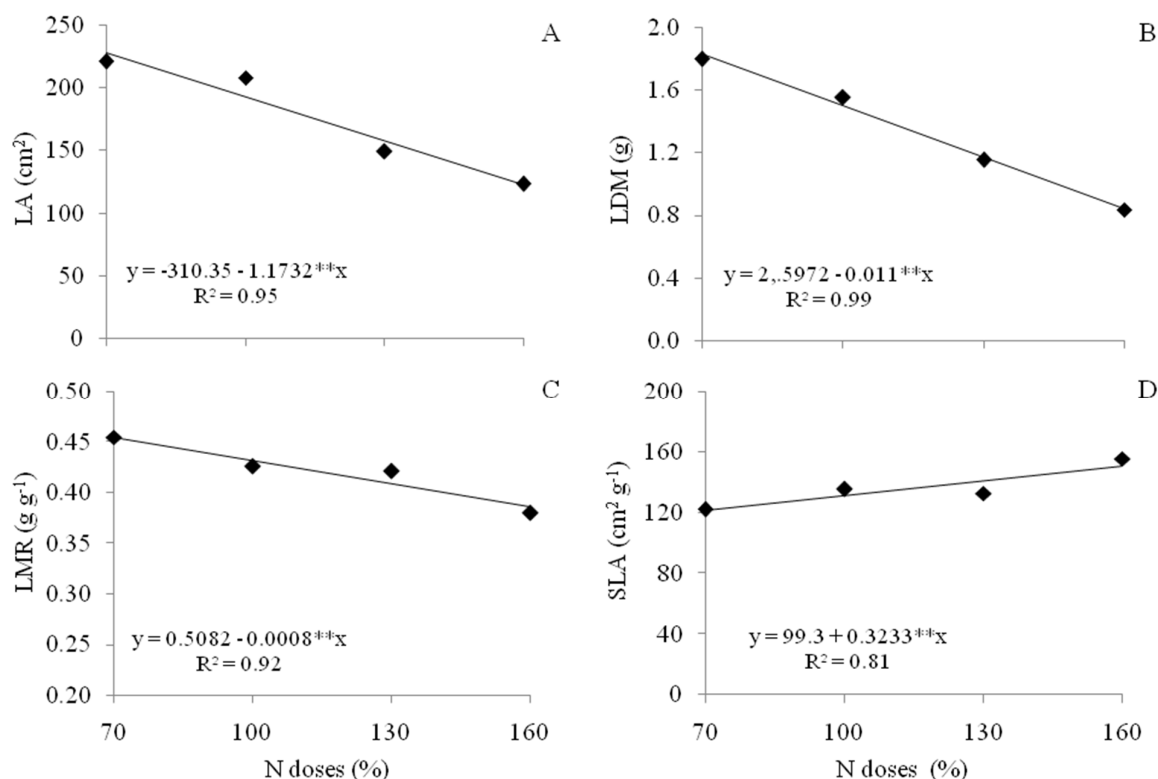
5 D). According to Benincasa (2003), SLA is a physiological component that relates leaf surface to the dry mass of the leaf itself, and the increase in this variable with the increment in N fertilization

is related to the lesser thickness of the leaf blade and to the reduction in leaf lignification, resulting in lighter leaves, contributing to increasing leaf area per unit of mass (PEREIRA et al., 2012).

**Figure 4.** Leaf area – LA, leaf dry mass – LDM and leaf mass ratio – LMR of guava seedlings cv. 'Paluma' as a function of irrigation water salinity at 190 days after emergence – DAE.



**Figure 5.** Leaf area – LA (A), leaf dry mass – LDM (B), leaf mass ratio – LMR (C), and specific leaf area – SLA (D) of guava seedlings cv. ‘Paluma’ as a function of nitrogen doses at 190 days after emergence – DAE.



## Conclusions

The contents of photosynthetic pigments in the leaves of guava seedlings cv. ‘Paluma’ are inhibited by the increase in irrigation water salinity, while leaf morphophysiology is not compromised by irrigation with mean water salinity of up to 1.5 dS m<sup>-1</sup> at 190 days after emergence.

Salt stress on the contents of chlorophyll *a*, CL total, and carotenoids is lessened with nitrogen fertilization at the dose of 1,004.9 mg of N dm<sup>-3</sup> of soil up to an irrigation water salinity level of 1.2 dS m<sup>-1</sup>.

Nitrogen fertilization higher than 541.1 mg of N dm<sup>-3</sup> of soil, regardless of the salinity level, is deleterious for leaf morphophysiological variables.

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