



DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v22n8p541-546>

Nitrogen fertilization to attenuate the damages caused by salinity on yellow passion fruit seedlings

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Key words:

Passiflora edulis f. *flavicarpa*
chlorophyll a
saline stress

ABSTRACT

The study aimed to evaluate the mitigating effect of nitrogen (N) on the damages caused by irrigation water salinity, in the production of yellow passion fruit seedlings. A randomized block design in split plots was used, with five levels of irrigation water salinity (plot) (0.3, 1.0, 1.7, 2.4 and 3.1 dS m⁻¹) and five doses of N fertilization (sub-plot) (180, 240, 300, 360 and 420 mg of N dm⁻³), with five replicates, totaling 125 experimental units, with one plant per plot. The seedlings were produced in 3.780 mL tubes, used as drainage lysimeter, which received a daily irrigation depth based on water balance. Growth and contents of chlorophyll and carotenoids were evaluated at 85 days after sowing. The increase in irrigation water salinity reduced stem diameter, plant height, number of leaves, chlorophyll a, chlorophyll b and total chlorophyll; increasing N doses also led to linear decline in stem diameter and plant height. Application of increasing doses of N did not attenuate the effect of salinity on growth and pigment contents.

Palavras-chave:

Passiflora edulis f. *flavicarpa*
clorofila a
estresse salino

Adubação nitrogenada como atenuante dos danos causados pela salinidade em mudas de maracujazeiro amarelo

RESUMO

Objetivou-se avaliar o efeito atenuante do nitrogênio nos danos causados pela salinidade da água de irrigação na produção de mudas de maracujazeiro amarelo. Utilizou-se o delineamento de blocos casualizados, com tratamentos formados a partir de parcelas subdivididas, compreendendo cinco níveis de salinidade da água de irrigação (parcela) (0,3; 1,0; 1,7; 2,4 e 3,1 dS m⁻¹) e cinco doses de adubação nitrogenada (subparcela) (180; 240; 300; 360 e 420 mg dm⁻³), com cinco repetições, perfazendo 125 unidades experimentais, com uma planta útil cada. As mudas foram formadas em tubetes de 3,780 mL, constituídos em lisímetros de drenagem, que recebiam uma lâmina de irrigação diária com base no balanço de água. Aos 85 dias após a semeadura, avaliaram-se os seguintes parâmetros de crescimento: diâmetro caulinar, altura de planta, número de folhas, taxa de crescimento relativo e os teores de pigmentos clorofila a, clorofila b, clorofila total e carotenoides. O incremento da salinidade da água de irrigação reduziu o diâmetro caulinar, altura de planta, número de folhas, clorofila a, clorofila b e clorofila total; as doses crescentes de nitrogênio também promoveram declínio linear no diâmetro caulinar e na altura de planta. A aplicação de doses crescentes de N não atenuou o efeito da salinidade sobre o crescimento e os teores de pigmentos.



INTRODUCTION

Passion fruit (*Passiflora edulis* Sims f. *Flavicarpa* Deg.) is a tropical climate plant, well adapted to the Brazilian Northeast, with great importance in the economy of the region, expressively contributing to socioeconomic aspects because its commercial exploitation generates jobs and income. In the 2016 season, with a harvested area of 49,889 ha and a production of 703.489 t, Brazil stood out as the largest producer and exporter of passion fruit. The state of Paraíba with 850 ha harvested obtained a production of 7893 t and the Northeast region stood out with harvested area of 36,778 ha and production of 489.898 t, corresponding to 69.64% of the national production (IBGE, 2016).

In this region, the scarce and irregular rains make it difficult to cultivate fruit crops under rainfed conditions (Silva et al., 2016), and the use of irrigation becomes necessary. Furthermore, according to Bezerra et al. (2016), despite the relevant expressiveness of the yellow passion fruit production in the Northeast region, the salinity of the soil and available water sources has been an obstacle for the production of seedlings and establishment of the crop under conventional management.

Salinity reduces the growth, development and production of crops, which is related to osmotic effects, decreasing the water or ionic potential due to the effect of specific ions or nutritional stress (Nobre et al., 2013), and the effect occurs on the growth and pigment contents of yellow passion fruit plants (Cavalcante et al., 2011; Bezerra et al., 2016).

To alleviate the effect of saline stress, researchers have studied the use of nitrogen (N) as a stress reliever, based on which amino acids related to tolerance mechanisms, such as proline, can be produced more efficiently with the addition of this nutrient, since it participates in its composition (Taiz & Zeiger, 2013; Lima et al., 2016). Such effect can be evaluated using growth parameters and leaf contents of pigments.

According to Feijão et al. (2011), adequate N supply acts by increasing plant resistance due to the increase of organic compounds such as proline, free amino acids and glycine betaine, which act in cell osmotic adjustment, besides promoting the stabilization of subcellular structures, such as membranes and proteins, under saline stress.

This study aimed to evaluate the attenuating effect of N on the damages caused by irrigation water salinity in the production of passion fruit seedlings.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Center of Sciences and Agri-Food Technology of the Federal University of Campina Grande (CCTA-UFCG), Campus of Pombal, PB, at the coordinates 6° 48' 16" S and 37° 49' 15" W and 175 m of altitude, from February to April 2015.

Evaluated plants were arranged in a randomized block design, with treatments formed by split plots, corresponding to five levels of irrigation water salinity (plot) (0.3; 1.0; 1.7; 2.4 and 3.1 dS m⁻¹) and five doses of N fertilization (subplot) (180; 240; 300; 360 and 420 mg dm⁻³ of soil) according to Malavolta (1980), with five replicates, totaling 125 experimental units, with one plant each.

Yellow passion fruit seeds were planted on polyethylene trays of 166 cells containing commercial substrate. At 30 days after sowing (DAS), seedlings were transplanted to 3.780-mL citropote tubes containing substrate composed of a mixture of soil, well decomposed bovine manure and wood shavings at 2:1:0.5 proportion, respectively. Before filling the pots, the material was sieved through a 2-mm mesh. Samples were collected in the A horizon of a soil classified as Fluvic Neosol and bovine manure, and their physical and chemical characteristics (Table 1) were determined at the Laboratory of Soils and Plant Nutrition of the CCTA/UFCG, according to methodologies proposed by Claessen (1997).

For 10 days after transplanting, the seedlings received irrigation with non-saline water (0.3 dS m⁻¹), maintaining the soil at field capacity. After this period, treatments began to be applied, which lasted 30 days. Nitrogen fertilization was split into 4 portions, all applied at 5-day intervals through the irrigation water using urea as N source. Standardizing fertilization followed the recommendation of Lima (2002).

Irrigation waters were obtained by adding NaCl, CaCl₂(H₂O) and MgCl₂.6(H₂O) salts, at equivalent proportion of 7:2:1 g L⁻¹, respectively, in the public-supply water, based on the predominance of the ions in sources of water used for irrigation. After preparation, the solutions were stored in 60-L plastic containers, one for each EC_w level, and the electrical conductivity was daily measured with a portable conductivity meter adjusted to the temperature of 25 °C.

Irrigation was applied once a day, based on soil water balance, obtained by drainage lysimetry. For that, the lysimeters, represented by the citropote tubes, were installed on gutters to collect the drained volume, at each level of salinity, i.e., water management was different according to the salinity levels studied. The volume applied (V_a) in each citropote

Table 1. Physical and chemical characteristics of the soil and bovine manure used in the different treatments

Physical and chemical characteristics of the soil														
pH (H ₂ O)	EC dS m ⁻¹	P mg dm ⁻³	Cations						SB	t	T	V	N	OM g kg ⁻¹
			k ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Al ⁺	H + Al						
5.96	0.3	58	1.59	0.85	4.9	7.4	0	1.73	14.78	14.78	16.47	89.49	1.12	18
Density g cm ⁻³	Sand		Silt		Clay		Moisture - bar (% weight)							
	g kg ⁻¹		g kg ⁻¹		g kg ⁻¹		0.1	0.33	1	5	10	15		
1.48	800		140.6		50.49		20.33	17.11	7.91	3.97	3.57	3.43		
Chemical characteristics of the bovine manure														
pH (H ₂ O)	EC dS m ⁻¹	P mg dm ⁻³	Cations						SB	t	T	V	N	OM g kg ⁻¹
			k ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Al ⁺	H+Al						
7.62	1.23	80	2.15	4.4	3.2	10.7	0	0	20.45	20.45	20.45	100	2.49	41

SB - sum of exchangeable bases; t - effective cation exchange capacity; T - cation exchange capacity at pH 7.0; V - base saturation (SB/T); OM - organic matter

tube was obtained by the difference between the total volume applied in the citropote tube on the previous day (V_{ta}) and the volume drained per citropote tube (V_d) on the next day, applying a leaching fraction of 0.20, according to Eq. 1.

$$V_a = \frac{V_{ta} - V_d}{1 - 0.2} \quad (1)$$

Treatment effects on seedling growth were evaluated at 55 and 85 days after sowing (DAS) by measuring stem diameter, plant height and number of leaves, and using these data to calculate the relative growth rate (RGR) of stem diameter, plant height and number of leaves, determined for the data obtained at 55 and 85 DAS, through Eq. 2 (Taiz & Zeiger, 2013).

$$RGR = \frac{(\ln A_2 - \ln A_1)}{t_2 - t_1} (\text{cm cm}^{-1} \text{ d}^{-1}) \quad (2)$$

where:

A_2 - stem diameter, plant height or number of leaves obtained at the end of the studied period;

A_1 - stem diameter, plant height or number of leaves obtained at the beginning of the studied period; and,

$t_2 - t_1$ - time difference between samplings.

The diagnosis of the contents of pigments was made with leaves collected at 85 DAS, using the methodologies presented in Arnon (1949) and Lichtenthaler (1987), at the Laboratory of Plant Physiology of the CCTA/UFCG, quantifying the contents of chlorophyll a, b and total and carotenoids through emission spectrophotometry, respectively, at wavelengths of 470, 645 and 663 nm, using Eqs. 3 and 6, according to the methodology of Lichtenthaler (1987).

$$\text{Chlorophyll a} = 12.21A_{663} - 2.81A_{647} \quad (3)$$

$$\text{Chlorophyll b} = 20.13A_{647} - 5.03A_{663} \quad (4)$$

$$\text{Chlorophyll total} = 17.3A_{647} + 7.18A_{663} \quad (5)$$

$$\text{Carotenoids} = \frac{(1000A_{470} - 1.82Ca - 85.02Cb)}{198} \quad (6)$$

where:

A - absorbance at the wavelength used, expressed in g m^{-2} , as a function of the area of the leaf discs;

Ca - chlorophyll a; and,

Cb - chlorophyll b.

The obtained data were subjected to analysis of variance by F test ($p < 0.05$) and, in cases of significance, linear and polynomial regression analyses were carried out using the statistical program Sisvar-ESAL, for the studied factors (Ferreira, 2014).

RESULTS AND DISCUSSION

According to the analysis of variance, there was no effect of the interaction between irrigation water salinity and N doses (Table 2) on the growth variables at 85 DAS, which is consistent with the results of Bezerra et al. (2016), who studied yellow passion fruit genotypes under water salinity. Irrigation water salinity affected all growth variables and the relative growth rate of the number of leaves, as observed by Mesquita et al. (2012), studying the growth of passion fruit seedlings under saline water.

Irrigation water salinity reduced stem diameter by 0.196 mm per unit increase in ECw (dS m^{-1}), at 85 days after sowing (Figure 1A). Comparing the data of plants subjected to irrigation with the highest salinity level (3.1 dS m^{-1}) with those cultivated at the lowest salinity level (0.3 dS m^{-1}), there was a reduction in stem diameter of 10.16% (0.55 mm). Similar results have been reported by Mesquita et al. (2010) and Bezerra et al. (2016) in yellow passion fruit seedlings under saline water irrigation, and such result can be attributed to the reduction in the osmotic potential (Ahmed et al., 2010), since no symptoms of toxicity were observed in the plants.

For the effect of N doses, there was a decline in stem diameter growth (Figure 1B) of approximately 8.39% (0.448 mm) comparing the N doses of 180 and 420 mg.

For Nobre et al. (2010), water salinity can compromise N absorption by plants, as a consequence of the ionic competition in the absorption sites. However, the present study did not find significant interaction, but a trend of reduction with the increase in the dose. Thus, there may have been a dose in excess, which may have caused damages to the plant.

Table 2. Summary of analysis of variance for stem diameter (SD) (mm), plant height (PH) (cm), number of leaves (NL), chlorophyll a (CA) (g m^{-2}), chlorophyll b (CB) (g m^{-2}), total chlorophyll (TC) (g m^{-2}) and carotenoids (CRT) (g m^{-2}) at 85 days after sowing (DAS) and relative growth rate (RGR) in SD, PH and NL from 55 to 85 DAS, in yellow passion fruit seedlings under levels of irrigation water salinity and nitrogen doses

SV	DF	Mean square									
		SD	RGR-SD	PH	RGR-PH	NL	RGR-NL	CA	CB	TC	CRT
Block	4	0.9767	0.000015	358.45	0.000366 ^{ns}	6.5836	0.000091	0.129	0.026	0.258	0.008
Salinity (S)	4	1.4888*	0.000005 ^{ns}	5824.82*	0.000311 ^{ns}	38.822**	0.000167**	0.409**	0.042*	0.719**	0.014 ^{ns}
Error 1	16	0.3641	0.00002	1279.61	0.00014 ^{ns}	3.6907	0.000012	0.076	0.009	0.136	0.006
Nitrogen (N)	4	1.1919*	0.000015 ^{ns}	4969.02**	0.000076 ^{ns}	4.6734 ^{ns}	0.000034 ^{ns}	0.238 ^{ns}	0.025 ^{ns}	0.416 ^{ns}	0.008 ^{ns}
SXN	16	0.6077 ^{ns}	0.000020 ^{ns}	1135.00 ^{ns}	0.000162 ^{ns}	4.1342 ^{ns}	0.000019 ^{ns}	0.055 ^{ns}	0.004 ^{ns}	0.085 ^{ns}	0.004 ^{ns}
Error 2	80	0.388	0.000013	964.34	0.000130 ^{ns}	2.6198	0.000014	0.043	0.005	0.075	0.003
Mean		5.1236	0.0175	118.68	0.0704	16.818	0.0207	1.152	0.341	1.498	0.292
CV 1 (%)		11.78	25.61	20.14	16.87	11.42	16.57	24.04	24.71	24.63	26.56
CV 2 (%)		12.16	20.4	23.16	16.18	9.62	18.29	18.18	21.49	18.4	21.41

*, **Significant at 0.05 and 0.01 probability levels; ^{ns}Not significant; DF - Degrees of freedom; CV - Coefficient of variation; SV - Sources of variation

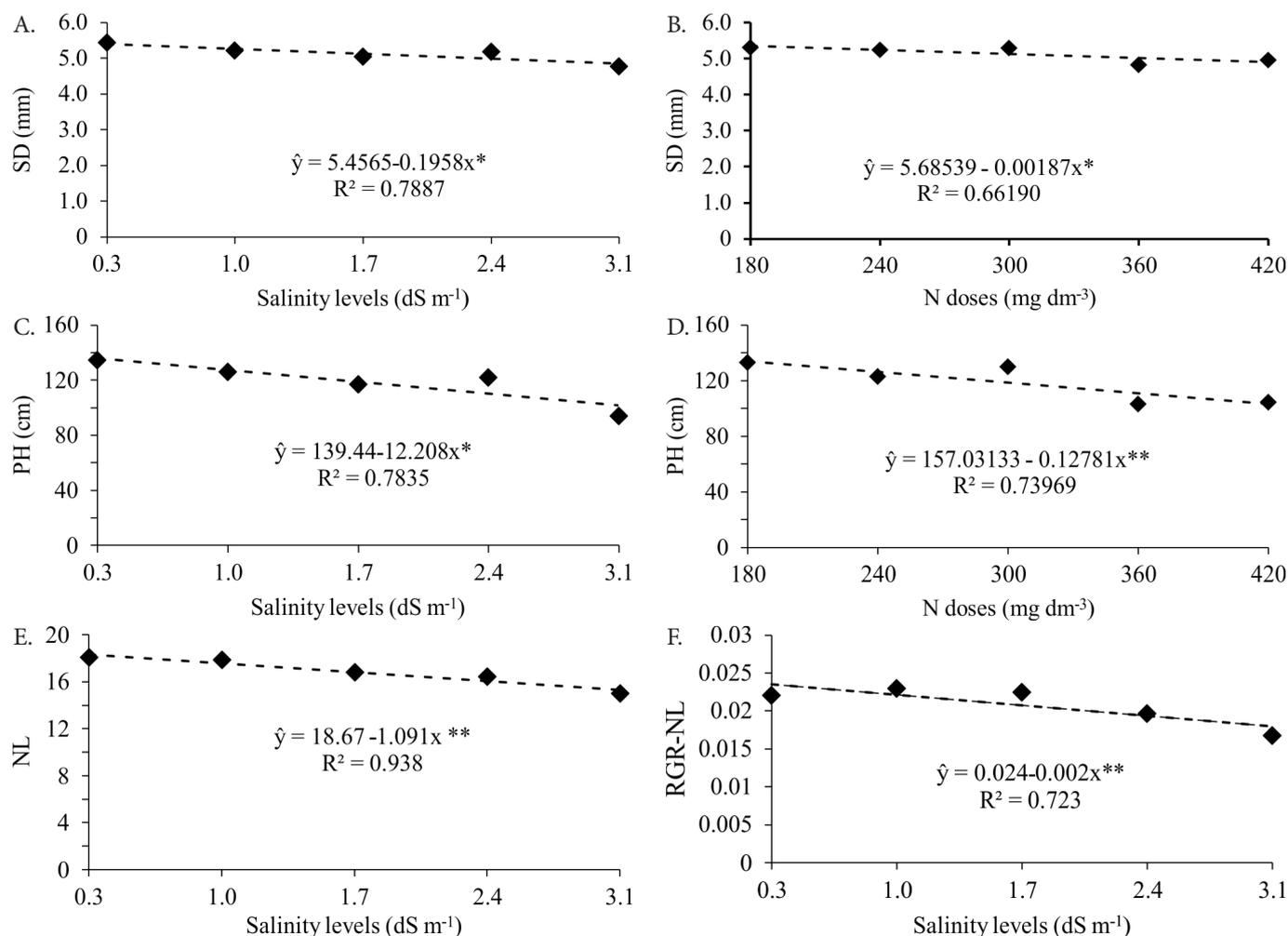


Figure 1. Stem diameter (SD) (A), plant height (PH) (C), number of leaves (NL) (E), relative growth rate of number of leaves (RGR-NL) (F) as a function of levels of irrigation water salinity; and stem diameter (SD) (B) and plant height (PH) (D) as a function of nitrogen doses, at 85 days after sowing in yellow passion fruit seedlings

The height of passion fruit seedlings was also reduced by the increase in irrigation water salinity (Figure 1C), at a rate of about 12.2 cm per unit increase in EC_w, resulting in a decline of 34.18 cm (25.18%) in the height of seedlings irrigated using 3.1 dS m⁻¹ water, in comparison to those subjected to 0.3 dS m⁻¹. It demonstrates the sensitivity to salinity of the growth in height, as observed by Mesquita et al. (2010), since the apical growth is more pronounced than longitudinal growth, corresponding to diameter.

Similarly to what occurred with stem diameter, the increase in N doses reduced the growth in height of the seedlings, with decline of approximately 22.89% (30.67 cm) between the highest dose (420 mg of N) and the lowest dose (180 mg of N) (Figure 1D), which increases the possibility of occurrence of dose above the recommended, since the soil had high CEC (Table 1). On the other hand, Caproni et al. (2013) mention a toxic effect of the ammonium, because the increasing N doses induced higher absorption of this nutrient in the ammoniacal form, which may also have occurred in the present study.

For the number of leaves (Figure 1E), the reduction caused by salinity was on the order of 1.09 leaves per unit increase in water salinity, which corresponds to a decrease of 16.65% (3.05 leaves) in the seedlings under higher salinity in comparison to those irrigated with 0.3 dS m⁻¹ water.

The reduction in the number of leaves by salinity can be attributed to the osmotic effect or is intensified by the fall of leaves due to the ionic effect of the salts, leading to burn and subsequent senescence of leaves, both aiming to reduce water loss (Oliveira et al., 2010). In the present study, it was observed that the effect of salinity can be attributed to the osmotic effect of such stress, because the increase in salinity caused an increment of about 23.93% in the relative growth rate of number of leaves (0.0056 RGR-NL) between the salinity levels of 0.3 and 3.1 dS m⁻¹. These results have also been observed by Sá et al. (2013) in papaya seedlings and by Silva et al. (2015) in guava plants under salinity.

According to the analysis of variance, there was no significant effect of the interaction between salinity and N doses on the contents of photosynthesizing pigments, and the contents of carotenoids were not significantly modified by the studied factors (Table 2), confirming the results of Furtado et al. (2014), who studied the pigment contents in cowpea irrigated with saline water and N doses and also found no effects of the interaction. Conversely, Nascimento et al. (2012) claim that N doses influenced the contents of pigments in cowpea leaves.

Irrigation water salinity decreased chlorophyll contents (a, b and total) in the yellow passion fruit seedlings (Figure 2), causing reductions of about 8.22, 8.9 and 8.42% per unit increase in

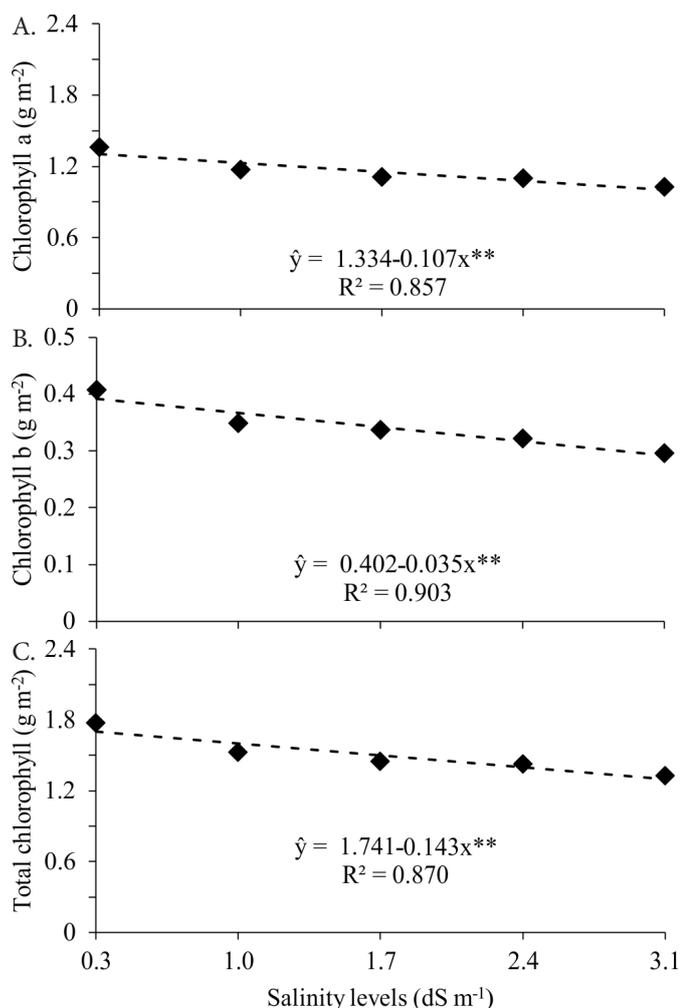


Figure 2. Chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) at 85 days after sowing in yellow passion fruit seedlings, as a function of the levels of irrigation water salinity

water salinity, i.e., as salinity increased to 3.1 dS m⁻¹ there were reductions of 23.01, 25.03 and 23.58%, respectively representing 0.3, 0.098 and 0.4 g m⁻² of chlorophylls a, b and total. Such response to salinity observed in chlorophyll contents confirm the limitation of photosynthesizing pigments, reducing the physiological potential and, consequently, the growth, as observed.

The results found in this study are similar to those observed by Freire et al. (2013) in yellow passion fruit and Furtado et al. (2014) in cowpea plants, all applying saline stress to the plants. For Melo et al. (2014), salinity can reduce the contents of photosynthesizing pigments in salt-sensitive plants and increase them in salt-tolerant plants.

In addition, this effect on the pigments confirms the predominance of the osmotic effect because there was no effect on the contents of carotenoids, which are components considered as protectants, and tend to be high when there is a more severe stress condition, as observed by Sá et al. (2017) in citrus plants. In this study, there were no effects of N doses on the contents of pigments; these results do not agree, partially, with those of Vieira et al. (2010), who found increment in chlorophyll a but not in chlorophyll b, studying the effect of four N concentrations, through the addition or not of ammonium sulfate, on 'Pérola' pineapple plants.

CONCLUSIONS

1. The increment in irrigation water salinity reduced the growth and contents of chlorophyll a, chlorophyll b and total chlorophyll.
2. Increasing doses of nitrogen fertilizer reduce stem diameter and plant height in yellow passion fruit seedlings.
3. Nitrogen fertilization did not mitigate the effect of salinity on the growth and contents of pigments in yellow passion fruit seedlings
4. The effect of salinity, in the stage of production of passion fruit seedlings, is predominantly evidenced by the osmotic effect.

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