

DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v25n7p446-452>

## Photosynthetic efficiency and production of *Annona squamosa* L. under salt stress and fertilization with NPK<sup>1</sup>

### Eficiência fotossintética e produção de *Annona squamosa* L. sob estresse salino e adubação com NPK

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#### HIGHLIGHTS:

*Electrical conductivity of water of 3.0 dS m<sup>-1</sup> reduces the chlorophyll synthesis and production of custard-apple.*

*NPK recommendation of 100-125-125, 125-125-100 and 125-125-125% cause more damage to the cell membrane.*

*Reduction in the quantum efficiency of custard-apple under salt stress is indicative of photoinhibitory damage.*

**ABSTRACT:** In the semi-arid region of Northeastern Brazil, the high concentration of salts found in the waters stands out as a limiting factor for irrigated agriculture. Thus, fertilization with nitrogen (N), phosphorus (P) and potassium (K) is a strategy capable of alleviating the effects of salt stress on plants. In this context, the objective of this study was to evaluate the concentrations of chloroplast pigments, chlorophyll fluorescence, cell membrane damage and the production of custard-apple irrigated using water with different electrical conductivities and fertilized with nitrogen, phosphorus and potassium. The treatments were distributed in randomized blocks, with three replicates, in a 2 × 8 factorial scheme, corresponding to two levels of electrical conductivity of irrigation water - EC<sub>w</sub> (0.8 and 3.0 dS m<sup>-1</sup>) and eight combinations of NPK fertilization (100-100-100; 100-100-125; 100-125-100; 100-125-125; 125-100-100; 125-100-125; 125-125-100 and 125-125-125% of the recommended doses of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O). EC<sub>w</sub> of 3.0 dS m<sup>-1</sup> reduced chlorophyll a and total chlorophyll concentrations, number of fruits per plant, photochemical efficiency, and increased carotenoid concentration and cell membrane damage in custard-apple. Fertilization with 100-125-125, 125-125-100 and 125-125-125% of the recommended doses of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O resulted in greater cell membrane damage in custard-apple.

**Key words:** water scarcity, salt stress, mineral nutrition, custard-apple

**RESUMO:** No semiárido do Nordeste brasileiro, a alta concentração de sais encontrada nas águas destaca-se como um fator limitante para agricultura irrigada. Assim, a adubação com nitrogênio (N), fósforo (P) e potássio (K) é uma estratégia capaz de amenizar o estresse salino sobre as plantas. Neste contexto, objetivou-se com esta pesquisa avaliar os teores de pigmentos cloroplastídicos, a fluorescência da clorofila, o dano na membrana celular e a produção de pinha irrigadas com água de diferentes condutividades elétricas e adubadas com nitrogênio, fósforo e potássio. Os tratamentos foram distribuídos em blocos ao acaso, em esquema fatorial 2 x 8, com três repetições, sendo dois níveis de condutividade elétrica da água de irrigação - CE<sub>a</sub> (0,8 e 3,0 dS m<sup>-1</sup>) e oito combinações de adubação NPK (100-100-100; 100-100-125; 100-125-100; 100-125-125; 125-100-100; 125-100-125; 125-125-100 e 125-125-125% das doses recomendadas de N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O). A CE<sub>a</sub> de 3,0 dS m<sup>-1</sup> diminuiu os teores de clorofila a e total, o número de frutos por planta, e eficiência fotoquímica, e aumentou os teores de carotenoides e o dano na membrana celular de pinha. A adubação com 100-125-125; 125-125-100 e 125-125-125% das dose recomendada de N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O resultou em maior dano na membrana celular de pinha.

**Palavras-chave:** escassez hídrica, estresse salino, nutrição mineral, pinheira

• Ref. 229631 – Received 06 Oct, 2019

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• Accepted 06 Mar, 2021 • Published 26 Mar, 2021

Edited by: Carlos Alberto Vieira de Azevedo

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## INTRODUCTION

Belonging to the Annonaceae family, custard-apple (*Annona squamosa* L.) has most of its production in the Northeast Region of Brazil, especially in Bahia, which accounts for 34% of the area, Pernambuco, with 17%, Rio Grande do Norte and Alagoas, with 11% (Guimarães et al., 2010).

In the Brazilian Northeast region, especially in semi-arid areas, irregular rainfall regime and high evapotranspiration, usually higher than the precipitated volume, are common, resulting in the scarcity of water resources, so the use of waters with high concentrations of salts becomes necessary (Santos & Brito, 2016). The excess of salts in water and/or soil alters the osmotic potential of the soil, reducing the absorption of water and nutrients by the plant, and promotes ion toxicity (commonly that of  $\text{Na}^+$  and  $\text{Cl}^-$ ) and nutritional imbalance, which causes changes in the physiological and biochemical functions of plants (Braz et al., 2019).

Thus, due to the limitations of using saline water in irrigation, there is a need to develop alternatives to minimize the effects caused by excess salts on both plants and soil (Lima et al., 2020). Therefore, the supply of nutrients such as nitrogen (N), potassium ( $\text{K}^+$ ), and phosphorus (P) has stood out as an important tool to reduce the deleterious effects of the use of saline water in irrigation, due to the functions that these elements perform. Nitrogen is a constituent of compounds that are related to plant's tolerance mechanisms such as proline, betaine glycine and free amino acids (Wanderley et al., 2018).

Potassium participates in several biological processes such as enzymatic activation, respiration, photosynthesis and improvement in water balance, as well as competition with  $\text{Na}^+$  cation (Heidari & Jamshid, 2010), while phosphorus improves energy storage capacity, root development, water use efficiency and nutrient absorption and utilization (Diniz et al., 2018).

In this context, the objective of this study was to evaluate the concentrations of chloroplast pigments, chlorophyll fluorescence, cell membrane integrity and production of custard-apple irrigated with waters of different electrical conductivities and under different combinations of nitrogen, phosphorus and potassium fertilization.

## MATERIAL AND METHODS

The experiment was carried out in a protected environment of the Center for Technology and Natural Resources - CTRN of the Federal University of Campina Grande - UFCG, Campina Grande, PB, Brazil, at the geographic coordinates  $7^\circ 15' 18''$

latitude South,  $35^\circ 52' 28''$  longitude West and mean altitude of 550 m. The greenhouse used was of the arched type, 30 m long and 21 m wide, with ceiling height of 3.0 m and 150-micron, low-density polyethylene cover.

The experimental design was in randomized blocks, in a  $2 \times 8$  factorial scheme, with three replicates, whose treatments consisted of two values of electrical conductivity of irrigation water - ECw (0.8 and  $3.0 \text{ dS m}^{-1}$ ) and eight combinations of fertilization - C with nitrogen, phosphorus and potassium ( $C_1 = 100-100-100$ ;  $C_2 = 100-100-125$ ;  $C_3 = 100-125-100$ ;  $C_4 = 100-125-125$ ;  $C_5 = 125-100-100$ ;  $C_6 = 125-100-125$ ;  $C_7 = 125-125-100$  and  $C_8 = 125-125-125\%$  of the recommendation of  $\text{N-P}_2\text{O}_5\text{-K}_2\text{O}$ ) for Annonacea species proposed by Silva & Silva (1997). Each plot consisted of one plant. The combination of 100-100-100% corresponded to the application of 100, 60 and 60 g of N,  $\text{P}_2\text{O}_5$ ,  $\text{K}_2\text{O}$  per plant per year, respectively, referring to the second year of cultivation.

At the end of the first year of cultivation, the plants were subjected to a 15-day period of water stress and, subsequently, a pruning (cleaning) was performed. After pruning, the second year of cultivation began. The fruits formed were allowed to grow without any fruit thinning.

Waters of different electrical conductivities were prepared with the addition of  $\text{NaCl}$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  salts to the public-supply water ( $0.6 \text{ dS m}^{-1}$ ) of the municipality of Campina Grande, maintaining the equivalent proportion of 7:2:1, respectively, which represents the mean composition of the waters of the Northeastern semi-arid region. Saline water ( $\text{ECw} = 3.0 \text{ dS m}^{-1}$ ) was obtained considering the relationship between the electrical conductivity of irrigation water (ECw) and the concentration of salts [ $\text{mmol L}^{-1} = 10 \times \text{ECw} (\text{dS m}^{-1})$ ], according to Richards (1954).

To conduct the experiment, 250-L plastic pots adapted as drainage lysimeters, were filled with a 1.0 kg layer of crushed stone followed by 235 kg of soil classified as Psamments of sandy clay loam texture (0-30 cm layer) collected from the municipality of Esperança, PB, Brazil, whose physical and chemical attributes (Table 1) were determined according to methodologies proposed by Teixeira et al. (2017).

For the study, 'crioulas' custard-apple 180 days old seedlings produced from seeds were acquired from a certified commercial nursery in the state of Pernambuco. After transplanting, the seedlings were acclimatized for 105 days before the beginning of irrigation with saline waters. Manual weeding, soil surface scarification to avoid formation of compacted layers, and formative and corrective pruning were performed throughout the experiment. Pollination was

**Table 1.** Chemical and physico-hydric characteristics of the soil used in the experiment

Chemical characteristics									
pH ( $\text{H}_2\text{O}$ ) (1:2.5)	OM ( $\text{dag kg}^{-1}$ )	P ( $\text{mg kg}^{-1}$ )	$\text{K}^+$	$\text{Na}^+$	$\text{Ca}^{2+}$ ( $\text{cmol}_c \text{ kg}^{-1}$ )	$\text{Mg}^{2+}$	$\text{H}^+ + \text{Al}^{3+}$	ESP (%)	ECse ( $\text{dS m}^{-1}$ )
5.63	1.83	18.20	0.21	0.17	3.49	2.99	5.81	1.34	0.61
Physical characteristics									
Size fraction ( $\text{g kg}^{-1}$ )			Textural class	Water content (kPa)		AW	Total porosity ( $\text{m}^3 \text{ m}^{-3}$ )	Ds	Dp
Sand	Silt	Clay		33.42	1519.5 ( $\text{dag kg}^{-1}$ )			(kg $\text{dm}^{-3}$ )	
573	101	326	SCL	12.68	4.98	7.70	0.5735	1.13	2.65

OM - Organic matter: Walkley-Black Wet digestion;  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  extracted with 1 M KCl at pH 7.0;  $\text{Na}^+$  and  $\text{K}^+$  extracted with 1 M  $\text{NH}_4\text{OAc}$  at pH 7.0;  $\text{H}^+ + \text{Al}^{3+}$  extracted with 0.5 M  $\text{CaOAc}$  at pH 7.0; ECse - Electrical conductivity of saturated soil extract; SCL - Sandy clay loam; AW - Available water; Ds - soil density; Dp - Soil particle density

carried out manually with the aid of a brush, removing pollen from the flower in the male stage and deposited on the stigma of the flower in the female stage.

Fertilization with nitrogen, phosphorus and potassium was performed by fertigation twice a month, in the first and fourth week of each month. Urea (45% N), monoammonium phosphate (61% P<sub>2</sub>O<sub>5</sub>, 12% N) and potassium chloride (60% K<sub>2</sub>O), respectively, were used as sources of NPK. Micronutrient applications were performed weekly by foliar spraying with the foliar fertilizer Quimifol Nutri (0.5 g L<sup>-1</sup>), which contains 25% potassium (K<sub>2</sub>O), 2.5% magnesium, 6.0% sulfur, 2.0% boron, 0.5% copper, 0.3% molybdenum and 5.0% zinc.

After the seedlings were transplanted to the lysimeters, irrigation was performed manually at a three-day interval, applying the volume of water corresponding to that obtained by the water balance determined by Eq. 1:

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

where:

VI - volume of water to be applied in the irrigation event, mL;

Va and Vd - volume applied and drained in the previous irrigation event, mL; and,

LF - leaching fraction of 0.10.

Chlorophyll a fluorescence reading was performed during the flowering stage, at 695 days after transplanting, from 7:00 to 9:00 a.m. in mature leaves located in the upper third of each plant, after 30 min of adaptation to the dark, by determining the initial fluorescence (F<sub>0</sub>), maximum fluorescence (F<sub>m</sub>), variable fluorescence (F<sub>v</sub>) and the quantum efficiency of photosystem II (F<sub>v</sub>/F<sub>m</sub>). Immediately after fluorescence reading, leaf samples were collected for the quantification of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations, according to Arnon (1949).

To determine the percentage of cell membrane damage (%D), at 695 days after transplanting one leaf was collected from each plant, and 10 leaf discs were removed with an iron hole puncher, which were washed and placed in an Erlenmeyer flask containing distilled water. %D was expressed as the percentage of initial electrical conductivity in relation to the final electrical conductivity, as proposed by Scotti-Campos et al. (2013). Production was determined by counting the number of fruits per plant (NFr) harvested up to 760 days

after transplanting. The fruits were harvested as the segments changed color from light green to grayish-brownish green.

The collected data were subjected to analysis of variance by the F test and, when significant, subjected to the Tukey test (p ≤ 0.05) for salinity levels, while the N-P-K fertilization combinations factor was compared by the Scott-Knott test (p ≤ 0.05), using the statistical program Sisvar. Due to the heterogeneity of Chl a, Chl b, Chl total, Car and NFr data, verified through the tests of normality and homogeneity of variances, they were transformed to √x before analysis of variance.

## RESULTS AND DISCUSSION

According to the summary of the analysis of variance (Table 2), there was a significant effect of salinity levels on the chlorophyll a (Chl a), chlorophyll total (Chl total), carotenoids (Car) and percentage of cell damage (%D) of custard-apple at 695 days after transplanting. Except for %D, the combinations of N-P-K fertilization did not influence (p > 0.05) any of the variables analyzed. The interaction between the factors (ECw × C) also did not significantly affect (p > 0.05) any of the variables studied.

The chlorophyll a concentrations of custard-apple subjected to irrigation with water of electrical conductivity of 0.8 dS m<sup>-1</sup> differed statistically from those irrigated with 3.0 dS m<sup>-1</sup>, and there was an increase in Chl a of 1.04 mg g<sup>-1</sup> of FM (Figure 1A). The total chlorophyll concentration also showed behavior similar to that of chlorophyll a (Figure 1B). It was observed that the highest Chl total concentration (2.74 mg g<sup>-1</sup> FM) was obtained when plants were grown under the lowest electrical conductivity (0.8 dS m<sup>-1</sup>), resulting in an increase of 0.81 mg g<sup>-1</sup> of FM, compared to those observed in plants under ECw of 3.0 dS m<sup>-1</sup>.

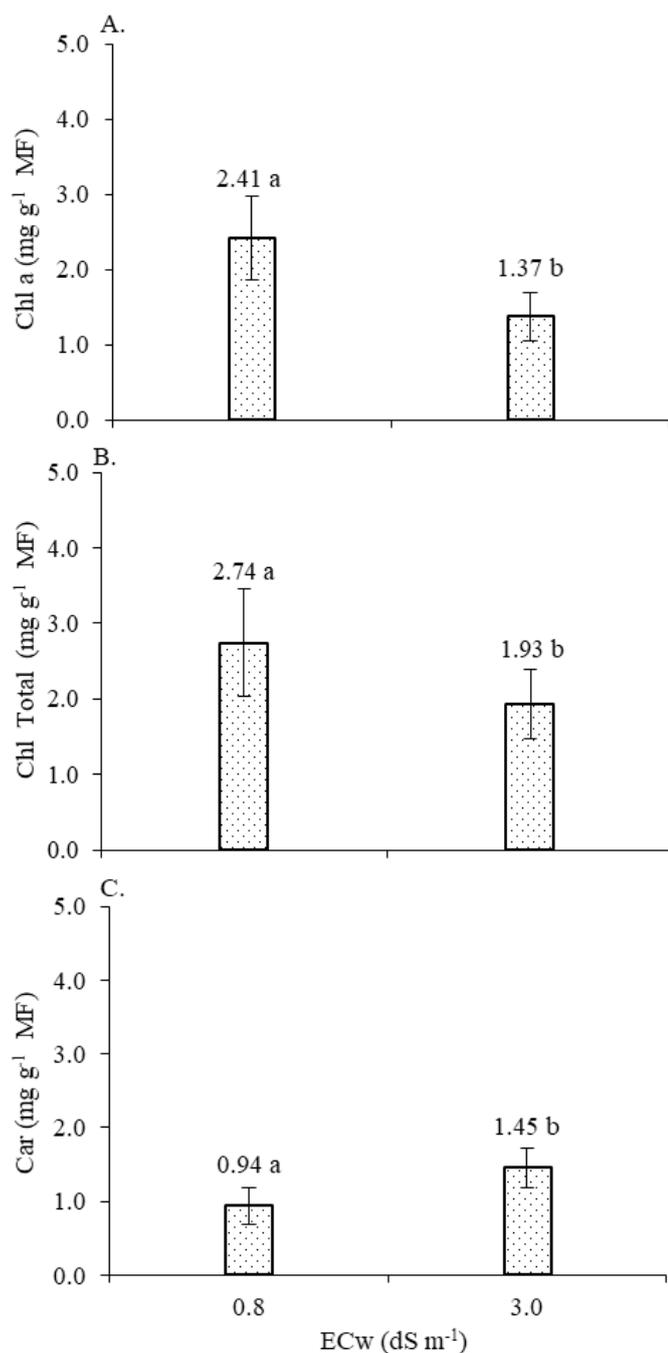
The reduction in chloroplast pigment concentrations occurs both as a result of the degradation of pigment molecules through the action of chlorophyll enzymes and due to the reduction in the chlorophyll synthesis process induced by high salinity conditions (Nunkaewa et al., 2014), standing out as an indication of oxidative stress, as a probable result of photooxidation of pigments (Silva et al., 2016). Corroborating these results, Oliveira et al. (2018) evaluated the concentration of chloroplast pigments in jackfruit plants under salt stress and found reductions of up to 10 and 27.43%, respectively, in Chl a and total Chl concentrations in the leaves of plants irrigated using waters with ECw of 4.0 dS m<sup>-1</sup>.

Regarding carotenoid concentrations, Figure 1C shows that plants grown under ECw of 0.8 dS m<sup>-1</sup> differed statistically from

**Table 2.** Summary of the analysis of variance for chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll total (Chl total), carotenoids (Car) and percentage of cell damage (%D) of custard-apple irrigated with saline waters under different combinations of N-P-K fertilization at 695 days after transplanting

Source of variation	DF	Mean squares				
		Chl a <sup>1</sup>	Chl b <sup>1</sup>	Chl total <sup>1</sup>	Car <sup>1</sup>	%D
Water salinity levels (ECw)	1	12.9800**	0.2299 <sup>ns</sup>	7.8926*	3.0552**	136.508**
Combinations of fertilization (C)	7	0.6810 <sup>ns</sup>	0.0597 <sup>ns</sup>	1.1565 <sup>ns</sup>	0.2883 <sup>ns</sup>	17.9130*
Interaction (ECw × C)	7	0.3037 <sup>ns</sup>	0.0696 <sup>ns</sup>	0.5699 <sup>ns</sup>	0.4434 <sup>ns</sup>	12.8866 <sup>ns</sup>
Blocks	2	0.4710 <sup>ns</sup>	0.00091 <sup>ns</sup>	0.7583 <sup>ns</sup>	0.1166 <sup>ns</sup>	0.22237 <sup>ns</sup>
Residual	30	1.0154	0.1008	1.8412	0.2199	6.9890
CV (%)		25.20	26.73	28.23	19.60	16.01

ns, \*, \*\* - Not significant, significant at p ≤ 0.05 and at p ≤ 0.01 by F test, respectively; DF - Degrees of freedom; CV - Coefficient of variation; <sup>1</sup>Statistical analysis performed after data transformation to √x



Means followed by the same letters did not differ significantly by Tukey test ( $p \leq 0.05$ ); Vertical bars represent the standard error of the mean ( $n = 3$ )

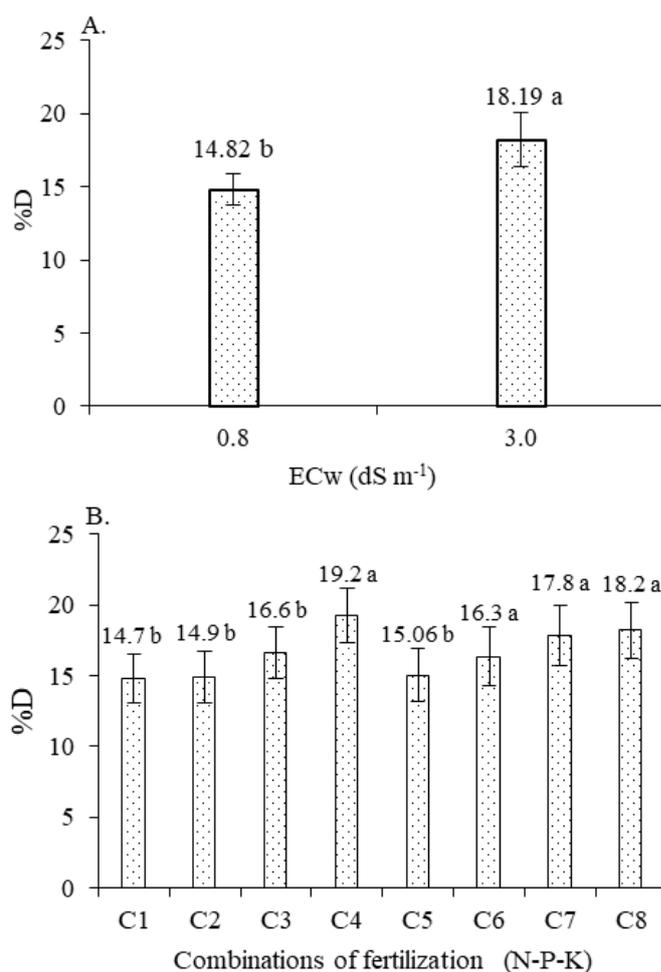
**Figure 1.** Mean concentrations of chlorophyll a - Chl a (A), chlorophyll total - Chl total (B) and carotenoids - Car (C) of custard-apple as a function of the electrical conductivity of irrigation water (ECw), at 695 days after transplanting

those grown under the highest salinity level ( $3.0 \text{ dS m}^{-1}$ ). When comparing the means of plants cultivated with  $3.0 \text{ dS m}^{-1}$  with those of plants subjected to  $0.8 \text{ dS m}^{-1}$ , there was an increase of 54.25% ( $0.51 \text{ mg g}^{-1}$  of FM) in carotenoid contents.

The increase in the biosynthesis of carotenoids in plants may be associated with mechanisms of protection of the antenna complex, as they act as reducing agents, protect pigments from oxidative reactions (Melo et al., 2017) and can regulate the activity of enzymes and endoproteases, protecting the lipid membranes of the chlorophyll molecule from oxidative stress caused by salt stress (Falk & Munné-Bosch, 2010).

For the percentage of cell membrane damage (%D) of custard-apple leaves, there was a difference between the levels of ECw, with the highest %D (18.19%) in plants grown under  $3.0 \text{ dS m}^{-1}$ , whereas those irrigated with water of lowest ECw ( $0.8 \text{ dS m}^{-1}$ ) showed mean cell damage of 14.82%, corresponding to a percentage increase of 22.73% in %D in comparison to those which received ECw of  $0.8 \text{ dS m}^{-1}$  (Figure 2A). The increase in %D in plants subjected to ECw of  $3.0 \text{ dS m}^{-1}$  is related to excessive absorption of toxic ions ( $\text{Cl}^-$  and or  $\text{Na}^+$ ), which affect ionic homeostasis in the cell when accumulated in leaf tissues, negatively interacting with the membrane walls. In line with the results obtained in this study, Sousa et al. (2017) evaluated the effects of irrigation with low-salinity water ( $0.6 \text{ dS m}^{-1}$ ) and high-salinity water ( $3.0 \text{ dS m}^{-1}$ ) and found an increase in the %D of citrus crop.

The different combinations of fertilization with  $\text{N-P}_2\text{O}_5\text{-K}_2\text{O}$  also significantly influenced %D (Figure 2B). The highest percentage of cell membrane damage (19.3%) was obtained when plants were subjected to the combination  $\text{C}_4$ , but it



$\text{C}_1 = 100\text{-}100\text{-}100$ ;  $\text{C}_2 = 100\text{-}100\text{-}125$ ;  $\text{C}_3 = 100\text{-}125\text{-}100$ ;  $\text{C}_4 = 100\text{-}125\text{-}125$ ;  $\text{C}_5 = 125\text{-}100\text{-}100$ ;  $\text{C}_6 = 125\text{-}100\text{-}125$ ;  $\text{C}_7 = 125\text{-}125\text{-}100$  and  $\text{C}_8 = 125\text{-}125\text{-}125\%$  of the recommended dose of  $\text{N-P}_2\text{O}_5\text{-K}_2\text{O}$ ; Means followed by the same letter do not differ significantly for irrigation water salinity and different combinations of fertilization with  $\text{N-P}_2\text{O}_5\text{-K}_2\text{O}$ , respectively, by Tukey test and Scott-Knott test at  $p \leq 0.05$ , respectively; Vertical bars represent the standard error of the mean ( $n = 3$ )

**Figure 2.** Percentage of cell membrane damage - %D of custard-apple as a function of the electrical conductivity of irrigation water - ECw (A) and combinations of fertilization with nitrogen, phosphorus and potassium - N-P-K (B), at 695 days after transplanting

did not differ statistically from the combinations  $C_8$  and  $C_7$ . On the other hand, the combinations  $C_4$ ,  $C_7$  and  $C_8$  differed significantly from the others, while these did not show significant differences from one another. It is worth pointing out that the lowest value of %D was obtained in plants fertilized with the combination  $C_1$ .

According to the analysis of variance (Table 3), there were significant effects of water salinity levels on maximum fluorescence (Fm), variable fluorescence (Fv), quantum efficiency of photosystem II (Fv/Fm) and number of fruits per plant (NFr). The combinations of fertilization with N-P-K did not significantly influence any of the variables analyzed. The interaction between the two was significant only for the maximum fluorescence (Fm) of custard-apple, at 695 DAT.

For the maximum fluorescence of custard-apple (Table 4), it can be observed that when plants were subjected to ECw of 0.8 dS m<sup>-1</sup> there was no significant difference between the different fertilization combinations. Thus, it can be inferred that the percentage increase from 100 to 125% in the mineral supply for plants, be it N or P or K either separately or together, does not affect the Fm of custard-apple when irrigated with low-salinity water. This makes it possible to deduce that, depending on the cost of acquisition of the inputs, the supply of  $C_1$ , which corresponds to 100-100-100% of the recommendation of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O for this crop under salinity of 0.8 dS m<sup>-1</sup>, is an alternative of fertilization for the crop. On the other hand, in plants irrigated with water of 3.0 dS m<sup>-1</sup> (Table 4), the highest values of Fm (3348.66, 3463.00, 3538.33, 3486.66 and 3282.66) were achieved in those that received the combinations  $C_3$ ,  $C_4$ ,  $C_5$ ,  $C_6$  and  $C_7$ , differing significantly from the other combinations.

When analyzing the follow up of the interaction between salinity levels at each combination of N-P-K (Table 4), it can be verified that under ECw of 0.8 dS m<sup>-1</sup>, the highest values of Fm (3718.66, 3184.33, 3382.00, 3392.00, 3691.33, 3632.00 and 3617.33) were obtained in plants that received the combinations of 100-100-100, 100-100-125, 100-125-100, 125-100-100, 125-100-125, 125-125-100 and 125-125-125% of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, respectively. On the other hand, when using water with electrical conductivity of 3.0 dS m<sup>-1</sup>, the highest values of Fm (3011.66, 3348.66, 3463.00, 3538.33, 3486.66 and 3282.66) were observed in plants fertilized with the combinations  $C_2$ ,  $C_3$ ,  $C_4$ ,  $C_5$ ,  $C_6$  and  $C_7$ , respectively, and plants under these combinations had a statistically higher Fm than those that received  $C_1$  and  $C_8$ .

The increase in Fm may indicate an attempt at photosynthetic compensation, increasing its activity to obtain greater

**Table 4.** Interaction between the electrical conductivity of irrigation water (ECw) and the combinations of fertilization N-P-K for maximum fluorescence (Fm) of custard-apple, at 695 days after transplanting

Combinations of fertilization N-P-K (%)	ECw (dS m <sup>-1</sup> )	
	0.8	3.0
$C_1$ - 100-100-100	3718.66 aA ± 63.67	2665.66 bB ± 174.76
$C_2$ - 100-100-125	3184.33 aA ± 97.17	3011.66 bA ± 182.30
$C_3$ - 100-125-100	3382.00 aA ± 163.74	3348.66 aA ± 128.86
$C_4$ - 100-125-125	2899.00 aB ± 393.58	3463.00 aA ± 130.81
$C_5$ - 125-100-100	3392.00 aA ± 113.01	3538.33 aA ± 216.04
$C_6$ - 125-100-125	3691.33 aA ± 78.90	3486.66 aA ± 164.44
$C_7$ - 125-125-100	3632.00 aA ± 104.61	3282.66 aA ± 122.66
$C_8$ - 125-125-125	3617.33 aA ± 104.25	2589.00 bB ± 125.87

Means followed by the same lowercase and uppercase letters do not differ significantly for irrigation water salinity and different combinations of fertilization with N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, respectively, by Tukey test and Scott-Knott test at  $p \leq 0.05$ , respectively; number ± standard error (n = 3)

assimilation of CO<sub>2</sub>, while its reduction indicates a slowdown in photosynthetic activity in order to minimize the toxic effects of salinity (Flowers & Flowers, 2005). This occurs because the maximum fluorescence represents the maximum intensity of fluorescence, when virtually all the quinone is reduced and the reaction centers reach their maximum capacity of photochemical reactions (Suassuna et al., 2010; Silva et al., 2015).

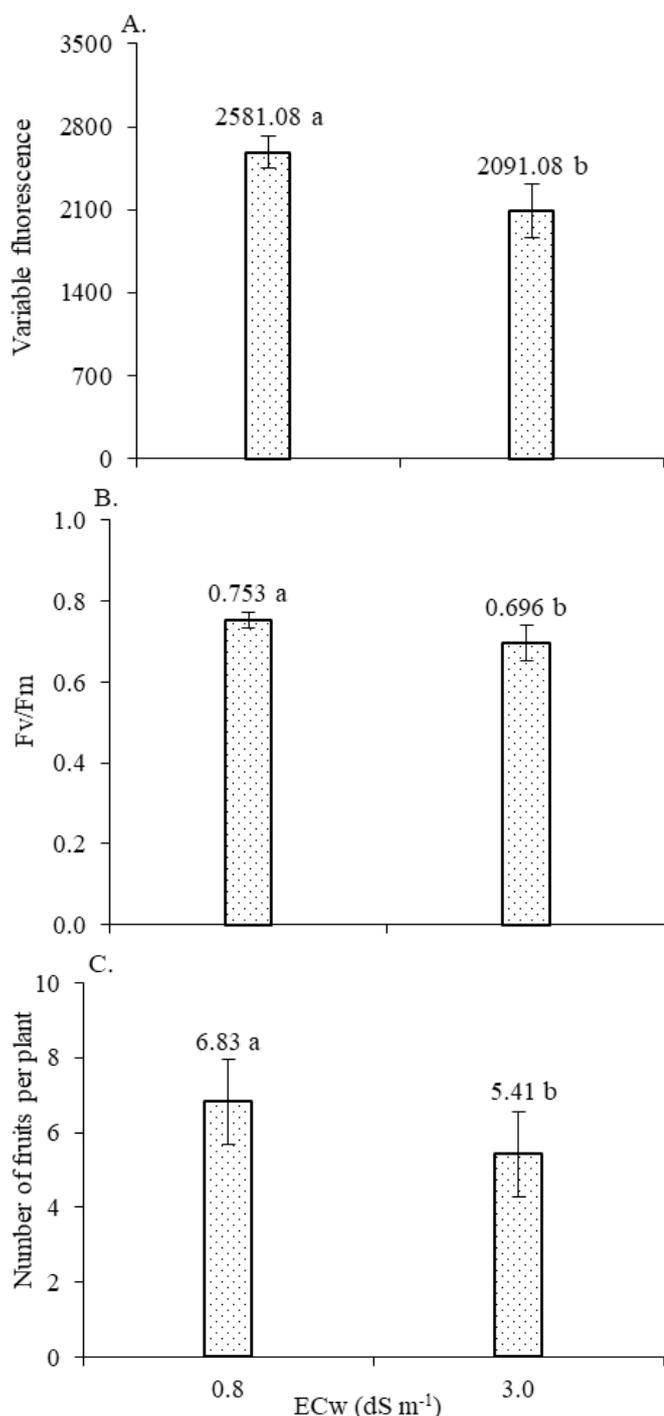
The variable fluorescence of custard-apple plants differed statistically between the ECw values (Figure 3A). It was observed that plants subjected to irrigation with water of 0.8 dS m<sup>-1</sup> had Fv of 2581.1, while the lowest value (2091.1) was verified in plants irrigated with ECw of 3.0 dS m<sup>-1</sup>, that is, custard-apple plants showed a reduction in Fv of 490 (18.98%) under the highest salinity level (3.0 dS m<sup>-1</sup>). The decrease in Fv is indicative that the photosynthetic apparatus was damaged by salt stress, given the decreases obtained in chlorophyll a and chlorophyll total contents (Figures 1A and B), compromising photosystem II, with negative effects on the photosynthetic process. In addition, Fv reflects the plant's ability to transfer the energy of electrons ejected from pigment molecules to the formation of the reducing agent NADPH, ATP and reduced ferredoxin (Fdr) and, consequently, greater capacity for CO<sub>2</sub> assimilation in the biochemical phase of photosynthesis (Baker, 2008; Dias et al., 2018).

As observed for Fv, the Fv/Fm of custard-apple was also significantly influenced by the salinity of irrigation water (Figure 3B). The quantum efficiency of photosystem II of plants subjected to 3.0 dS m<sup>-1</sup> was negatively affected, being 0.057 lower compared to the values of plants under ECw of 0.8 dS m<sup>-1</sup>. In addition, it can be observed (Figure 3B) that the highest

**Table 3.** Summary of the analysis of variance for initial fluorescence (F<sub>0</sub>), maximum fluorescence (Fm), variable fluorescence (Fv), quantum efficiency of photosystem II (Fv/Fm), at 695 days after transplantation (DAT), and number of fruits per plant (NFr) of custard-apple irrigated with saline water under different combinations of fertilization with N-P-K, at 760 DAT

Source of variation	DF	Mean squares				
		F <sub>0</sub>	Fm	Fv	Fv/Fm	NFr <sup>1</sup>
Water salinity levels (ECw)	1	28372.68 <sup>ns</sup>	851467.68*	2881200.00**	0.0380**	24.08*
Combinations of fertilization (C)	7	8504.87 <sup>ns</sup>	209856.33 <sup>ns</sup>	893556.47 <sup>ns</sup>	0.00712 <sup>ns</sup>	2.94 <sup>ns</sup>
Interaction (ECw × C)	7	10714.25 <sup>ns</sup>	457069.25**	156036.80 <sup>ns</sup>	0.00392 <sup>ns</sup>	6.27 <sup>ns</sup>
Blocks	2	718.520 <sup>ns</sup>	63828.64 <sup>ns</sup>	99348.58 <sup>ns</sup>	0.00634 <sup>ns</sup>	13.93 <sup>ns</sup>
Residual	30	9750.80	112580.17	154880.58	0.00427	4.82
CV (%)		11.29	10.15	16.85	9.02	19.14

ns, \*, \*\* - Not significant, significant at  $p \leq 0.05$  and at  $p \leq 0.01$  by F test, respectively; DF - Degrees of freedom; CV - Coefficient of variation; <sup>1</sup>Statistical analysis performed after data transformation to  $\sqrt{x}$



Means followed by the same letter do not present significant differences between each other by the Tukey test ( $p \leq 0.05$ ); Vertical bars represent the standard error of the mean ( $n = 3$ )

**Figure 3.** Variable fluorescence - Fv (A), quantum efficiency of the photosystem - Fv/Fm (B), at 695 days after transplantation (DAT), and number of fruits per plant (C) of custard-apple, as a function of the electrical conductivity of irrigation water (ECw), at 760 days after transplantation

value of Fv/Fm (0.753) was obtained in plants grown under the lowest salinity ( $0.8 \text{ dS m}^{-1}$ ) while those under the highest ECw had the lowest value (0.696). It is worthwhile to mention that Fv/Fm values between 0.75 and 0.85 are indicative that the photosynthetic apparatus is intact (Reis & Campostrini, 2011; Silva et al., 2015).

The inhibition in the quantum efficiency of PSII detected in plants cultivated with saline water indicates the occurrence of photoinhibitory damage in PSII reaction centers, which

promotes the formation of reactive oxygen species (Gonçalves et al., 2010; Lima et al., 2019). Corroborating this study, Freire et al. (2014) evaluated the quantum yield and gas exchange of yellow passion fruit irrigated with low-salinity water ( $0.50 \text{ dS m}^{-1}$ ) and high-salinity water ( $4.50 \text{ dS m}^{-1}$ ) and also observed deleterious effect of high ECw on both Fv and Fv/Fm.

For the number of fruits per plant (Figure 3C), it was verified that plants subjected to irrigation using water with ECw of  $0.8 \text{ dS m}^{-1}$  showed statistically higher NFr compared to those cultivated with water of high salinity level ( $3.0 \text{ dS m}^{-1}$ ). In a comparison of the number of fruits per plant - NFr (Figure 3C) under ECw of  $3.0 \text{ dS m}^{-1}$  with that of plants subjected to salinity level of  $0.8 \text{ dS m}^{-1}$ , there was a reduction of 20.79% (1.42 fruits per plant). The reduction in number of fruits per plant - NFr is related to lower absorption of water and nutrients by plants, resulting from the increase in soil salinity levels, due to irrigation using water with high ECw, causing damage to the cell membrane (Figure 2A) and reduction in water potential due to oxidative stress in the plant, which leads to a reduction in agricultural production (Lima et al., 2015).

Bezerra et al. (2019), when analyzing guava production as a function of irrigation water salinity and nitrogen fertilization, found that the increase in ECw from 0.3 to  $3.5 \text{ dS m}^{-1}$  reduced by 15.46 and 28.13% the number of fruits harvested in the first and second cycles of the crop, respectively.

## CONCLUSIONS

1. Irrigation water electrical conductivity of  $3.0 \text{ dS m}^{-1}$  reduces chlorophyll a and chlorophyll total concentrations, number of fruits per plant, photochemical efficiency, and increases the carotenoid concentrations and damage to the cell membrane of custard-apple, at 695 days after transplantation.
2. Fertilization with combinations of 100-125-125, 125-125-100 and 125-125-125% of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O results in greater damage to the cell membrane of custard-apple.
3. The highest maximum fluorescence of custard-apple is obtained under irrigation water electrical conductivity of  $3.0 \text{ dS m}^{-1}$  and fertilization with 100-100-100 and 125-125-125% of the recommended dose of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O.

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