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Photosynthetic pigments, photochemical efficiency and growth of custard-apple under salt stress and potassium fertilization[1]

Pigmentos fotossintéticos, eficiência fotoquímica e crescimento de pinha sob estresse salino e adubação com potássio

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HIGHLIGHTS:

Potassium with 98% of the recommendation increased the chlorophyll b in plants under water salinity of 1.3 dS m^{-1}.
The reduction in Fv/Fm indicates that photosynthetic performance was compromised by photoinhibitory damage.
The absolute and relative growth rates in stem diameter of the custard-apple decreased regardless of the potassium dose.

ABSTRACT: The salt stress caused by irrigation water with high concentration of salts stands out as one of the main limiting factors in agricultural production in the semiarid region of Northeastern Brazil. In this context, the objective of this study was to evaluate the photosynthetic pigments, the photochemical efficiency, and the growth of custard-apple irrigated with saline water and potassium doses. The research was carried out under field conditions in a randomized block design in a 2 × 5 factorial scheme, corresponding to two values of electrical conductivity of irrigation water - ECw (1.3 and 4.0 dS m^{-1}) and five potassium doses (50, 75, 100, 125 and 150% of the recommendation). The dose referring to 100% corresponded to the application of 20 g of K_2O per plant per year. ECw of 4.0 dS m^{-1} reduced the synthesis of chlorophyll a, total chlorophyll, and carotenoids in custard-apple, at 245 days after transplanting. Fertilization doses of 50 to 150% of the recommendation inhibited the synthesis of chlorophyll b and the absolute and relative growth rates in stem diameter of custard-apple plants irrigated with water of highest electrical conductivity. Reduction in the quantum efficiency of photosystem II in custard-apple cultivated under ECw of 4.0 dS m^{-1} is related to photoinhibitory damage to photosystem II. Potassium fertilization did not alleviate the stress caused by water salinity on the growth of custard-apple, during 151-245 days after transplantation.

Key words: *Annona squamosa* L., salinity, mitigation

RESUMO: O estresse salino ocasionado pela irrigação com águas de elevados teores de sais se destaca como um dos principais fatores limitantes na produção agrícola no semiárido do Nordeste brasileiro. Neste contexto, objetivou-se com este estudo avaliar os pigmentos fotossintéticos, a eficiência fotoquímica e o crescimento da pinheira irrigada com águas salinas e doses de potássio. A pesquisa foi realizada sob condições de campo no delineamento em blocos casualizados, arranjados em esquema fatorial 2 × 5, sendo dois valores de condutividade elétrica da água de irrigação - CEa (1,3 e 4,0 dS m^{-1}) e cinco doses de potássio (50, 75, 100, 125 e 150% da recomendação). A dose referente a 100% correspondeu à aplicação de 20 g de K_2O por planta por ano. Água de salinidade de 4,0 dS m^{-1} reduziu a síntese de clorofila a, clorofila total e carotenoides na pinheira, aos 245 dias após o transplantio. Doses de fertilização de 50 a 150% da recomendação inibiram a síntese de clorofila b, as taxas de crescimento absoluto e relativo em diâmetro de caule das plantas de pinha irrigadas com água de maior condutividade elétrica. A redução na eficiência quântica do fotossistema II na pinheira cultivadas sob salinidade da água de 4,0 dS m^{-1} está relacionada aos danos fotoinibitórios no fotossistema II. A adubação potássica não amenizou o estresse causado pela salinidade da água no crescimento da pinheira, no período de 151-245 dias após o transplantio.

Palavras-chave: *Annona squamosa* L., salinidade, mitigação

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Introduction

Belonging to the Annonaceae family, custard-apple (*Annona squamosa* L.) is a fruit appreciated fresh or as a sweet pulp and stands out as a source of vitamins A, B, C, E, and K1, antioxidant compounds, polyunsaturated fatty acids, and presence of essential minerals, preventing and fighting heart disease, diabetes, hyperthyroidism and cancer (Senthil & Silambarasan, 2015). The Northeast region of Brazil stands out with the largest national production, mainly in the states of Alagoas, Bahia, Ceará, and Pernambuco (Medeiros et al., 2014).

However, this region commonly has high temperatures, low rainfall levels, irregular distribution of rains, and intense evaporation in most months of the year, which makes the practice of irrigation indispensable to ensure security in agricultural production (Lima et al., 2019). However, in the custard-apple growing region in the semiarid region of Northeast Brazil, most waters contain relatively high contents of salts, mainly those of sodium and chloride, being one of the main obstacles to agricultural production (Andrade et al., 2019). Custard-apple is a fruit tree sensitive to water salinity, with a reduction in photosynthetic efficiency and production under an electrical conductivity of 3.0 dS m^{-1} (Ferreira et al., 2021).

In this context, the improvement of nutritional status with potassium fertilization in plants subjected to salt stress can be used as a tool to minimize oxidative cell damage, because K regulates growth and development through changes in physiological and biochemical attributes. Potassium also acts on the synthesis of osmolytes and increases antioxidant components, reducing the formation of reactive oxygen species (Ahanger et al., 2017). Several studies suggest that an adequate supply of potassium may attenuate the negative effects of salinity on different crops, such as strawberry (Khayyat et al., 2009), melon (Gurgel et al., 2010), and West Indian cherry (Lima et al., 2019).

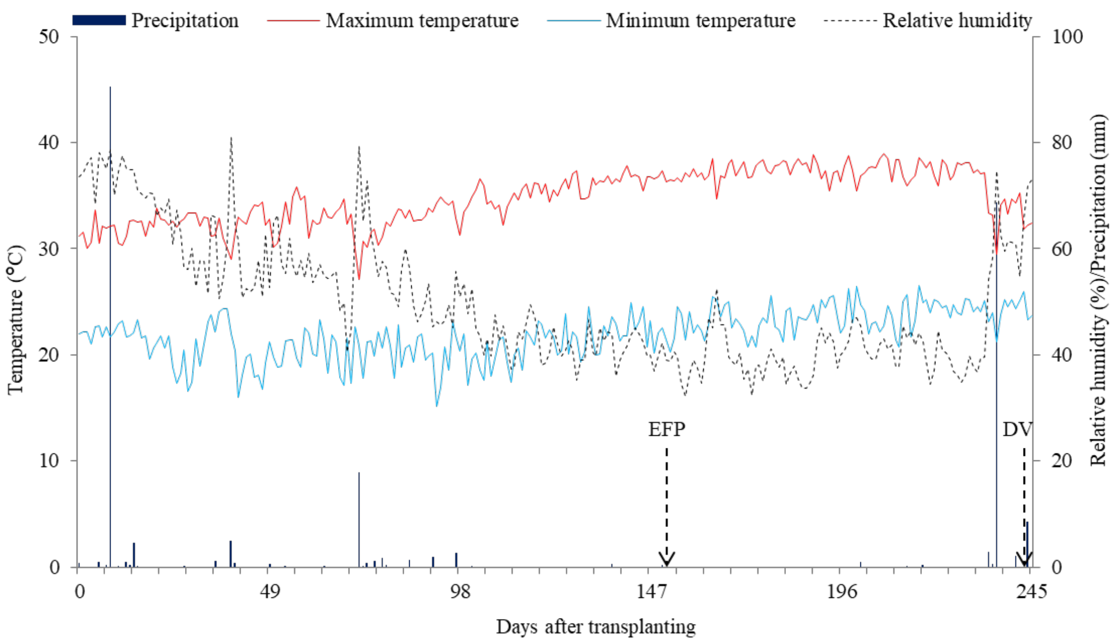
In this context, the objective of this study was to evaluate the photosynthetic pigments, photochemical efficiency, and growth of custard-apple under irrigated with waters of different salinity levels and potassium doses.

Material and Methods

The study was carried out in containers adapted as drainage lysimeters during the period from May 2019 to January 2020 under field conditions at the Rolando Enrique Rivas Castellón Experimental Farm belonging to the Center of Science and Agrifood Technology of the Universidade Federal de Campina Grande (CCTA/UFCG), located in the municipality of São Domingos, Paraíba, Brazil, at the geographic coordinates 6° 48’ 48.8” S latitude and 37° 56’ 16.5” W longitude, at an altitude of 194 m. The maximum and minimum temperature, relative humidity of the air, and precipitation data are presented in Figure 1.

The experiment was installed in a randomized block design, arranged in a 2 × 5 factorial scheme, corresponding to two values of electrical conductivity of irrigation water - ECw (1.3 and 4.0 dS m^{-1}) and five doses of potassium (50, 75, 100, 125, and 150% of the recommendation), with four replicates, totaling 40 experimental units, and each plot consisted of one plant. The dose referring to 100% corresponded to 20 g of K_2O per plant per year, according to the recommendation for Annonaceae plants for the first year of production proposed by Silva & Silva (1997). Potassium doses were established based on a study conducted by Dias et al. (2018), with the West Indian cherry crop.

Custard-apple seedlings were obtained from seeds, sowing two seeds per polyethylene bag with a capacity of 1.5 dm^3, filled with substrate, consisting of a mixture (volume basis) of 50% soil, 25% sand, and 25% bovine manure. After the seedlings emerged, thinning was performed, leaving only one plant per container when they were 10 cm tall. It is



EFP - End of formative pruning; DV- Determination of photosynthetic pigment variables, photochemical efficiency and growth

Figure 1. Maximum and minimum air temperature, relative air humidity, and precipitation data during the period of the experiment

emphasized that since sowing, irrigation was carried out with the respective waters.

At 190 days after sowing, the seedlings were transplanted to pots adapted as drainage lysimeters with a capacity of 310 L (54 cm height, 105 cm upper diameter, and 104 cm lower diameter). The lysimeters had a 0.5-kg layer of sand at the bottom, followed by 310 kg of a soil classified as Entisol with sandy loam texture (0-0.30 m depth), properly pounded to break up clods, from the Rolando Enrique Rivas Castellón Experimental Farm, whose chemical and physical characteristics (Table 1) were obtained according to the methodologies proposed by Teixeira et al. (2017).

Each lysimeter was drilled at the base to allow drainage and connected through a transparent tube of 25 mm in diameter. The tip of the drain inside the lysimeter was wrapped with a nonwoven geotextile (Bidim OP 30) to prevent clogging by soil material. Below each drain, a plastic container with a capacity of 3.0 L was placed to collect drained water to estimate the water consumption by the plant. The water with the lowest electrical conductivity (ECw), 1.3 dS m^{-1}, came from an artesian well located at the experimental farm, whereas the one with the highest electrical conductivity (4.0 dS m^{-1}) was prepared by adding iodine-free NaCl to the well water, based on the relationship between ECw and the concentration of salts (mg L^{-1} = 640 × ECw). ECw values were defined as a function of the quality of well water available at the CCTA/UFCG experimental area, which has the lowest electrical conductivity, 1.3 dS m^{-1}. The highest water salinity level (4.0 dS m^{-1}) was defined based on research results reported by Sá et al. (2015). The chemical characteristics of the water of low electrical conductivity are presented in Table 2.

The irrigations with the respective water were carried out daily, applying in each lysimeter the volume of water corresponding to the water demand of the plant. The volume to be applied in each irrigation event was estimated through the water balance, considering the volume of water applied to the plants in the previous event and the volume drained, quantified in the morning of the following day, plus a leaching fraction of 20% every 20 days to avoid excessive accumulation of salts in the root zone (Lima et al., 2020).

Fertilization with NPK was based on the fertilizer recommendations for Annonaceae crops proposed by Silva & Silva (1997), using urea (45% N), monoammonium phosphate (61% P_2O_5, 12% N), and potassium chloride (60% K_2O) as sources of nitrogen, phosphorus, and potassium, respectively. Fertilization started at 10 days after transplanting (DAT), divided into 24 equal portions, applied at 15-day intervals throughout the first year of production. Potassium doses were supplied in the following quantities: 10, 15, 20, 25 and 30 g of K_2O per plant for doses of 50, 75, 100, 125, and 150%, respectively, in addition to 50 g of N and 40 g P_2O_5 per plant, respectively, dissolved in the well water (ECw = 1.3 dS m^{-1}). A micronutrient solution containing 1.5 g L^{-1} of Ubyfol® [(N (15%); P_2O_5 (15%); K_2O (15%); Ca (1%); Mg (1.4%); S (2.7%); Zn (0.5%); B (0.05%); Fe (0.5%); Mn (0.05%); Cu (0.5%); Mo (0.02%)] was applied at 15-day intervals through foliar spraying.

Throughout the experiment, formative pruning was performed aiming at standardizing and adapting the crop according to the methodology described by Fernandes et al. (2021). At 245 DAT, photosynthetic pigments were evaluated: chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl T), and carotenoids (Car). On the same date, photochemical efficiency was measured by evaluating initial fluorescence (F_0), variable fluorescence (Fv), maximum fluorescence (Fm), and quantum efficiency of photosystem II (Fv/Fm). Absolute (AGR_{SD}) and relative (RGR_{SD}) growth rates of stem diameter were determined in the period of 151-245 DAT. The period of 151-245 DAT corresponded to the end of crop formative pruning (151 DAT) and the beginning of the flowering stage (245 DAT).

To determine the photosynthetic pigments, leaf samples were collected from 06:00 to 07:00 in the morning. After collection, the samples were immediately sent to the laboratory for the removal of five discs (diameter 9 mm) with the help of a perforator, which were weighed and then immersed in acetone (80%) and stored in a refrigerator for 48 hours. After this period, the extracts obtained were evaluated with a spectrophotometer at the absorbance wavelength (A) of 470, 646, and 663 nm using Eqs. 1, 2, 3 and 4 to estimate the concentrations of chlorophyll

Table 1. Chemical and physical characteristics of the soil (0-0.30 m) used in the experiment, before the application of the treatments

Chemical characteristics									
pH (H_2O) (1:2.5)	OM dag kg^{-1}	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	$H^+ + Al^{3+}$	ESP (%)	EC_{se} (dS m^{-1})
			(cmol$_c$ kg^{-1})						
6.95	0.96	13.75	0.39	0.83	3.74	2.30	0	11.43	0.32
Physical characteristics									
Granulometric fraction (g kg^{-1})			Textural class	Water content (kPa)		AW	Total porosity (%)	AD	PD
Sand	Silt	Clay		33.42[1]	1519.5[2] (dag kg^{-1})			(kg dm^{-3})	
719.20	241.60	39.20	SL	12.24	4.58	7.66	47.76	1.40	2.68

OM - Organic matter: Walkley-Black Wet Digestion; P extracted with extractor of Mehlich 1; Ca^{2+} and Mg^{2+} extracted with 1 M KCl pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; $H^+ + Al^{3+}$ extracted with 0.5 M CaOAc at pH 7.0; ESP - Exchangeable sodium percentage; ECse - Electrical conductivity of soil saturation extract; SL - Sandy loam; AW - Available water; AD - Apparent density; PD - Particle density; [1,2] referring to field capacity and permanent wilting point

Table 2. Chemical characteristics of the water of low electrical conductivity used in the experiment

Ca^{+2}	Mg^{+2}	Na^+	K^+	HCO_3^-	CO_3^{-2}	Cl^-	EC	pH	SAR
			(mmol$_c$ L^{-1})				dS m^{-1}		(mmol L^{-1})$^{0.5}$
0.85	0.40	5.81	0.40	5.09	0.00	4.07	1.30	6.69	7.34

EC - Electrical conductivity; SAR - Sodium adsorption ratio a, b, carotenoids and total chlorophyll, respectively, according to the methodology of Arnon (1949). The results were expressed in mg g^{-1} of fresh matter (MF).

$$\text{Chl a} = 12.21A_{663} - 2.81A_{646} \quad (1)$$

$$\text{Chl b} = 20.13A_{646} - 5.03A_{663} \quad (2)$$

$$\text{Car} = \frac{(1000A_{470} - 1.82\text{Chl a} - 85.02\text{Chl b})}{198} \quad (3)$$

$$\text{Chl T} = 17.3A_{646} + 7.18A_{663} \quad (4)$$

where:

Chl a - Chlorophyll a;
Chl b - Chlorophyll b;
Car - Total carotenoids; and,
Chl T - Total chlorophyll.

Chlorophyll-a fluorescence was quantified considering fully expanded leaves, using the portable fluorometer Plant Efficiency Analyser - PEA II®. The leaves selected for analysis were pre-adapted to the dark, with appropriate clips, and the readings were taken after 30 min, between 7 and 9 a.m.

Growth was evaluated based on the absolute (AGR_{SD}) and relative (RGR_{SD}) growth rates of stem diameter in the period of 151-245 DAT, according to Benincasa (2003), as shown in Eqs. 5 and 6.

$$AGR = \frac{(SD_2 - SD_1)}{(t_2 - t_1)} \quad (5)$$

where:

AGR - absolute growth rate in stem diameter (mm per day);
SD_1 - Stem diameter (mm) at time t_1; and,
SD_2 - Stem diameter (mm) at time t_2.

$$RGR = \frac{\ln(SD_2) - \ln(SD_1)}{(t_2 - t_1)} \quad (6)$$

where:

RGR - relative growth rate of stem diameter (mm mm^{-1} per day); and,
ln - natural logarithm.

The data obtained were evaluated by analysis of variance after the normality and homogeneity test (Shapiro-Wilk test). The F test ($p \leq 0.05$) was used for water electrical conductivity and linear and quadratic polynomial regression analysis ($p \leq 0.05$) for potassium doses, using the SISVAR statistical program (Ferreira, 2019).

Results and Discussion

According to the summary of the analysis of variance (Table 3), there were significant effects of water salinity levels on the concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids of custard-apple. Potassium doses did not significantly influence any of the variables analyzed, and the interaction between the factors salinity levels and potassium doses (SL × KD) significantly affected only chlorophyll b concentrations in custard-apple (Table 3).

Table 3. Summary of the analysis of variance for the concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl T), and carotenoids (Car) of custard-apple cultivated with saline waters and potassium doses, at 245 days after transplanting

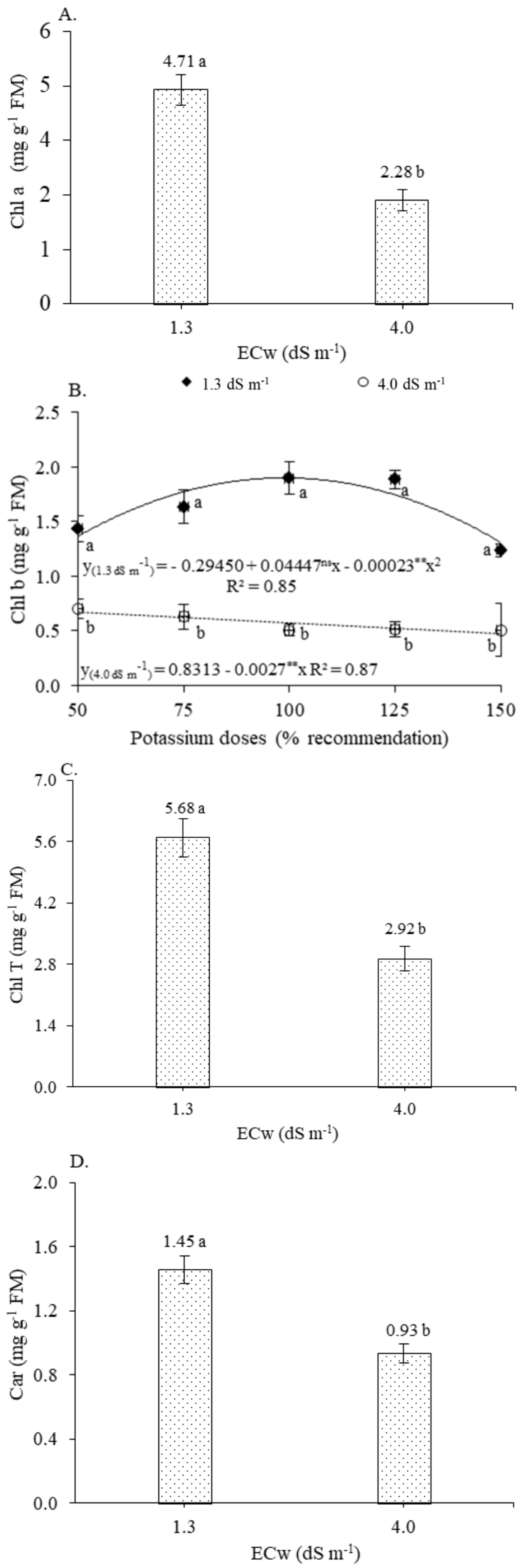
Source of variation	DF	Mean squares			
		Chl a	Chl b	Chl T	Car
Salinity levels (SL)	1	58.87**	10.70**	76.34**	2.73*
Potassium doses (KD)	4	0.69^{ns}	0.15^{ns}	1.94^{ns}	0.02^{ns}
Linear regression	1	0.10^{ns}	0.08^{ns}	0.39^{ns}	0.001^{ns}
Quadratic regression	1	0.32^{ns}	0.45^{ns}	0.0002^{ns}	0.0008^{ns}
SL × KD	4	2.92^{ns}	0.20*	3.89^{ns}	0.10^{ns}
Blocks	3	0.57^{ns}	0.01^{ns}	2.68^{ns}	0.03^{ns}
Residual	27	1.66	0.06	2.55	0.13
CV (%)		19.25	14.58	19.11	15.48

DF - Degree of freedom; CV - Coefficient of variation; ^{ns}, *,** - Respectively not significant, significant at $p \geq 0.05$ and $p \geq 0.01$

Chlorophyll a concentration of custard-apple plants was significantly reduced with the increase in water electrical conductivity (Figure 2A). Plants subjected to ECw of 1.3 dS m^{-1} obtained a Chl a concentration that was higher than those of plants irrigated with water of 4.0 dS m^{-1}. Comparing the plants grown under ECw of 4.0 dS m^{-1} to those irrigated with water salinity of 1.3 dS m^{-1}, there was a reduction of 51.59% (2.43 mg g^{-1} FM). Reduction in chlorophyll a synthesis in plants grown under salt stress has been considered a typical symptom of oxidative stress and may be attributed to the inhibition of chlorophyll synthesis, together with the activation of its degradation by the chlorophyllase enzyme, standing out as a mechanism of photoprotection through the reduction in light absorbance by decreasing chlorophyll concentration (Taibi et al., 2016).

In research conducted by Ferreira et al. (2021) evaluating the effects of irrigation with saline water (ECw of 0.8 and 3.0 dS m^{-1}) and combinations of fertilization with NPK on the photosynthetic efficiency of custard-apple, verified that irrigation with ECw of 3.0 also inhibited the synthesis of photosynthetic pigments, such as total chlorophyll. It is noteworthy that in plants under irrigation with ECw of 3.0 dS m^{-1} the concentrations of photosynthetic pigments were lower than those observed in this study.

The chlorophyll b concentration of custard-apple was significantly influenced by the interaction between the two factors (Figure 2B). For custard-apple plants irrigated with water of 1.3 dS m^{-1}, the data were described by a quadratic model, with an estimated maximum value of 1.86 mg g^{-1} FM, obtained when potassium dose corresponding to 98% of the recommendation was used. When comparing plants fertilized with the dose of 150% to those that received the lowest dose (50%) of potassium, there was reduction in chlorophyll b concentration of 0.270 mg g^{-1} FM. This reduction may have been intensified by the fact that the nutrient source has a high salt index (116) associated with the higher value of



Means followed by different letters indicate a significant difference between treatments by F test (p ≥0.05); Vertical bars represent the standard error of the mean (n = 4)

Figure 2. Concentrations of chlorophyll a - Chl a (A), total chlorophyll - Chl T (C), and carotenoids - Car (D) of custard-apple irrigated with saline waters – ECw and chlorophyll b - Chl b (B) as a function of the interaction between ECw and potassium doses, at 245 days after transplanting

ECw prepared with NaCl. Another aspect that must also be considered is that both the irrigation water and the fertilizer had chloride, an ion considered toxic to plants.

In plants subjected to water salinity of 4.0 dS m^{-1}, Chl b concentration (Figura 2B) decreased linearly, by 8.12% with each 25% increment in potassium dose. Plants under water salinity of 4.0 dS m^{-1}, and fertilized with 150% K_2O reduced their chlorophyll b synthesis by 38.77% (0.270 mg g^{-1} FM) compared to those subjected to the lowest K_2O dose (50%) (Figure 2B). When analyzing the interaction between ECw and potassium doses, it was observed that the Chl b concentration of plants subjected to ECw of 1.3 dS m^{-1} differs significantly from those of plants irrigated using water with 4.0 dS m^{-1}. Plants grown under ECw of 1.3 dS m^{-1} stood out statistically with the highest concentration of Chl b, regardless of the K_2O dose applied.

Thus, it can be noted that the deleterious effects of salt stress on chlorophyll b synthesis (Figure 2B) were intensified with increasing doses of potassium in plants grown under ECw of 4.0 dS m^{-1} and possibly due to chloride toxicity. Corroborating the results obtained in this study, Lima et al. (2020), evaluating the photosynthetic pigments of 'BRS Rubi do Cerrado' passion fruit as a function of salt stress (ECw from 0.3 to 3.5 dS m^{-1}) and potassium fertilization (doses of 50 and 100% of the K_2O recommendation), found that fertilization with 100% K_2O decreased the Chl b concentration of plants subjected to the salinity of 3.5 dS m^{-1}.

Water salinity inhibited the synthesis of total pigments of custard-apple plants and, according to the means comparison test (Figure 2C), plants grown under ECw of 1.3 dS m^{-1} had Chl T concentration higher than those of plants subjected to the highest electrical conductivity (4.0 dS m^{-1}). Custard-apple plants under irrigation with ECw of 4.0 dS m^{-1} decreased Chl T synthesis by 47.71% (2.76 mg g^{-1} FM) compared to those subjected to water salinity of 1.3 dS m^{-1}. The decrease in total chlorophyll concentration is in line with the reductions in chlorophyll a and b concentrations (Figures 2A and B). In plants grown under water salinity, the excess of salts (especially the toxic ions Na^+ and Cl^-) causes an imbalance between the production and removal of reactive oxygen species (ROS), leading to imbalance and increase in the levels of ROS, triggering oxidative stress, which results in a series of changes in metabolic mechanisms, associated with the synthesis of photosynthetic pigments (Silva et al., 2016). The decrease in chlorophyll concentration is usually accompanied by the inactivation of photochemical reactions, especially those mediated by photosystem II (PSII) in plants exposed to salt stress (Zhao et al., 2019).

The carotenoid concentration (Car) of custard-apple decreased sharply with the increase in the electrical conductivity of the water and, according to the means comparison test (Figure 2D), the Car concentration of plants irrigated with water of 4.0 dS m^{-1} was lower than that observed in those subjected to ECw of 1.3 dS m^{-1}. Plants irrigated with ECw of 4.0 dS m^{-1} had reduction in Car concentration of 36.36% (0.52 mg g^{-1} FM) compared to those under lower water electrical conductivity (1.3 dS m^{-1}). Carotenoids function as light energy harvesters for photosynthesis and in the dissipation of excess energy through the xanthophyll cycle; in addition, they can act as stabilizers of the chloroplast membrane, acting between light-harvesting complexes and the lipid phase of thylakoid membranes, reducing membrane fluidity and susceptibility to lipid peroxidation. In addition, a decrease in carotenoid concentration is indicative that there was ß-carotene degradation as well as a reduction in zeaxanthin formation (Taibi et al., 2016).

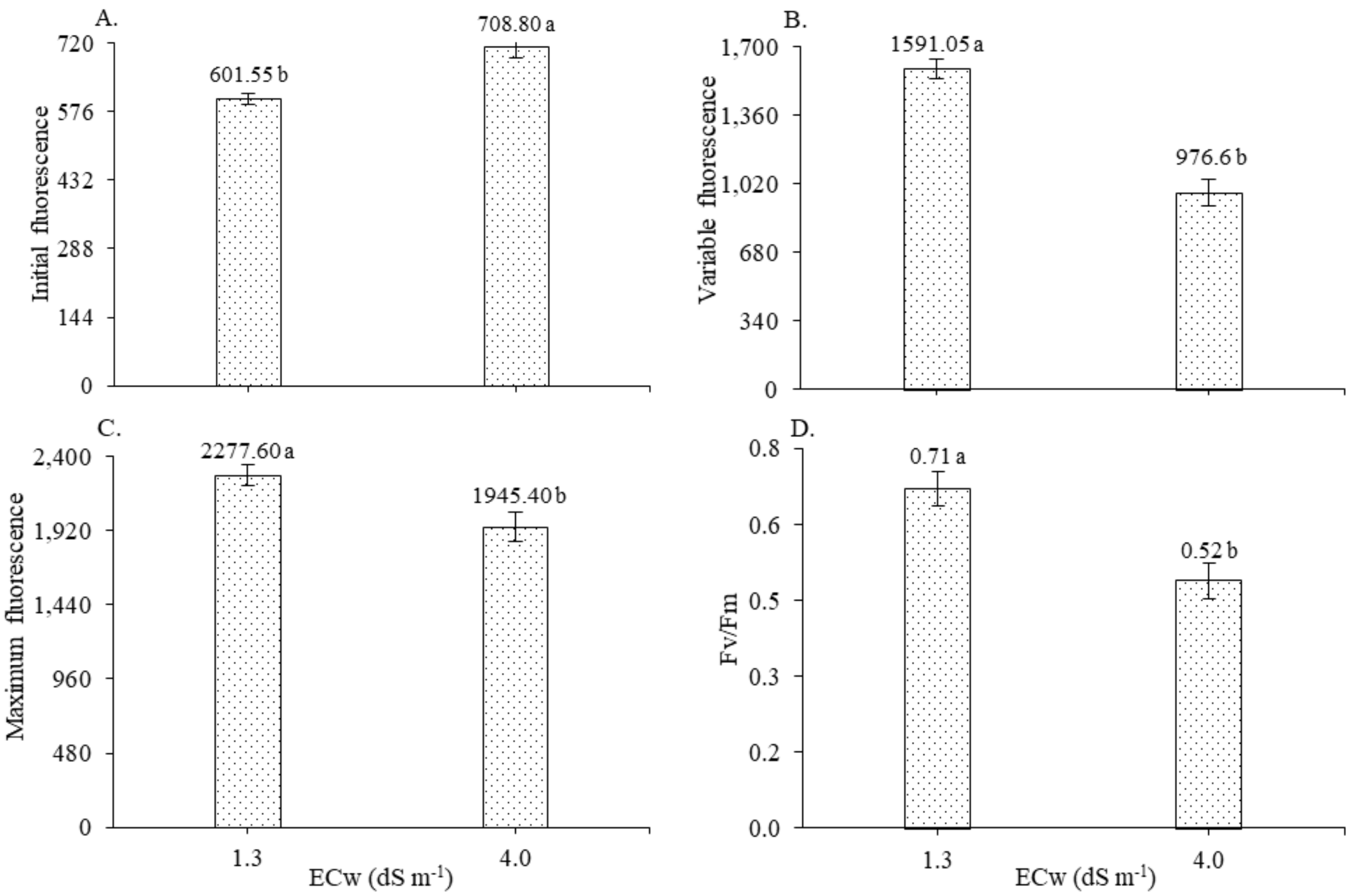
According to the summary of the analysis of variance (Table 4), there was significant effect of the water electrical conductivity on the initial fluorescence (F_0), variable fluorescence (Fv), maximum fluorescence (Fm), the quantum efficiency of photosystem II (Fv/Fm) of custard-apple, at 245 days after transplanting, and for the relative growth rate of stem diameter in the period of 151-245 DAT. Potassium doses did not significantly influence any of the variables analyzed; however, the interaction between the factors caused significant effect on the absolute (AGR_{SD}) and relative (RGR_{SD}) growth rate of stem diameter of custard-apple.

The initial fluorescence of custard-apple subjected to water salinity of 4.0 dS m^{-1} was significantly higher than that of plants that received ECw of 1.3 dS m^{-1} (Figure 3A). Plants irrigated with ECw of 4.0 dS m^{-1} had increase in F_0 of 17.82% (107.25) compared to those under the lowest ECw (1.3 dS m^{-1}). Initial fluorescence is the time when quinone A (PSII primary electron receptor) is completely oxidized and the

Table 4. Summary of the analysis of variance for initial fluorescence (F_0), variable fluorescence (Fv), maximum fluorescence (Fm), and quantum efficiency of photosystem II (Fv/Fm), at 245 days after transplanting (DAT) and the absolute (AGR_{SD}) and relative (RGR_{SD}) growth rates of stem diameter in the period of 151-245 DAT of custard-apple cultivated with saline waters and potassium doses

Source of variation	DF	Mean squares					
		F_0	Fv	Fm	Fv/Fm	AGR_{SD}	RGR_{SD}
Saline levels (SL)	1	115025.62^{*}	3775488.02^{**}	1103568.40^{*}	0.37^{*}	0.001^{ns}	0.00009^{*}
Potassium doses (KD)	4	1813.22^{ns}	51040.16^{ns}	41279.50^{ns}	0.01^{ns}	0.0005^{ns}	0.000009^{ns}
Linear regression	1	500.00^{ns}	3498.01^{ns}	64468.01^{ns}	0.007^{ns}	0.001^{ns}	0.00001^{ns}
Quadratic regression	1	6032.89^{ns}	113220.72^{ns}	12495.43^{ns}	0.02^{ns}	0.0001^{ns}	0.000001^{ns}
SL × KD	4	4766.37^{ns}	89234.71^{ns}	207483.02^{ns}	0.02^{ns}	0.015^{**}	0.0001^{**}
Blocks	3	6656.62^{ns}	84194.29^{ns}	57236.73^{ns}	0.007^{ns}	0.001^{ns}	0.000007^{ns}
Residual	27	5644.06	63491.97	146672.86	0.031	0.0005	0.000009
CV (%)		11.47	19.63	18.14	28.74	19.19	22.97

DF - Degree of freedom; CV - Coefficient of variation; ^{ns}, *,** - Respectively not significant, significant at p ≥ 0.05 and p ≥ 0.01 by F test



Means followed by different letters indicate a significant difference between treatments by F test (p ≥ 0.05); Vertical bars represent the standard error of the mean (n = 4)

Figure 3. Initial fluorescence - F_0 (A), variable fluorescence - Fv (B), maximum fluorescence - Fm (C), and quantum efficiency of photosystem II - Fv/Fm (D) of custard-apple irrigated with saline water - ECw, at 245 days after transplanting

PSII reaction center (P_{680}) is open, an imminent situation to the activation of photochemical reactions (Azevedo Neto et al., 2011). Increase in F_0 is an indication of damage to the reaction center of photosystem II, or reduction of the capacity to transfer energy from the excitation of the light-harvesting system to the PSII reaction center (Melo et al., 2017), such as inactivation of partially reversible or even irreversible PSII reaction centers, when these plants were subjected to higher concentrations of salts (Lucena et al., 2012). Sousa et al. (2016), when evaluating the tolerance to salt stress (ECw of 0.6 and 3.0 dS m^{-1}) of 'Mimo do Céu' orange grafted on three rootstocks using as a physiological parameter the quantum efficiency of photosystem II, also observed an increase in F_0 with the increase in water salinity.

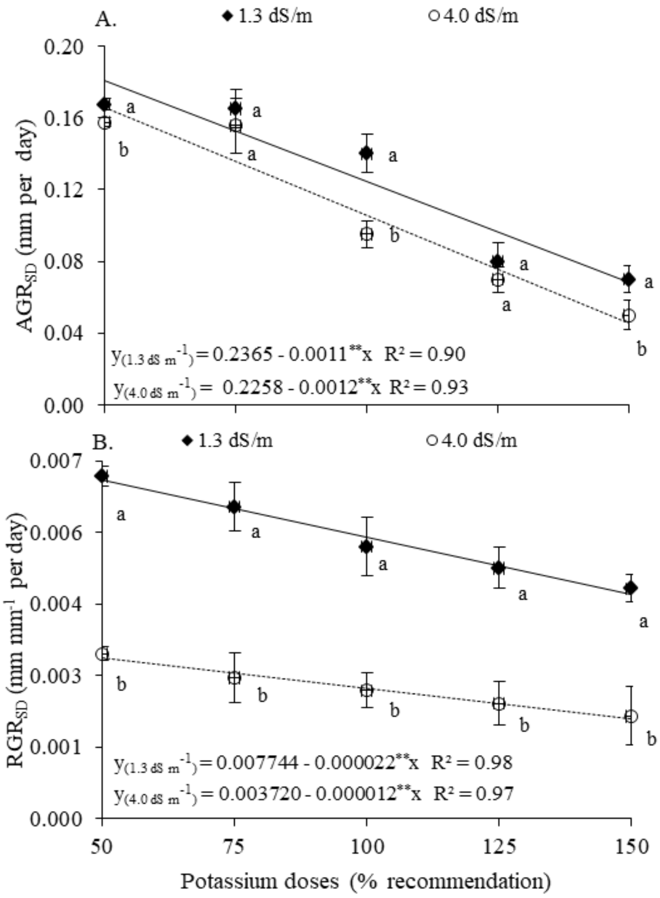
Variable fluorescence refers to the plant's ability to transfer energy from electrons ejected from pigment molecules to the formation of NADPH, ATP, and reduced ferredoxin (Freire et al., 2014). In this study, custard-apple was negatively affected by the increase of electrical conductivity in irrigation water and, according to the means comparison test (Figure 3B), plants grown under ECw of 1.3 dS m^{-1} obtained 62.91% higher Fv (614.45) compared to those subjected to ECw of 4.0 dS m^{-1}. Considering that chlorophyll is the main pigment responsible for capturing the light energy used in photosynthesis (Sacramento et al., 2014), the decrease in fluorescence is associated with the decline in the synthesis of Chl a. Dias et al. (2018), when evaluating the quantum yield of West Indian cherry cv. BRS 366 Jaburu under salt stress conditions (ECw of 0.8 and 3.8 dS m^{-1}), observed that as water salinity increased, there was reduction of 39.57% in variable fluorescence.

The maximum fluorescence of custard-apple was also reduced sharply with salt stress. It was verified through the means comparison test (Figure 3C) that the Fm of plants cultivated under water salinity of 1.3 dS m^{-1} differed statistically from that of plants irrigated with ECw of 4.0 dS m^{-1}. When comparing the Fm of plants subjected to ECw of 4.0 dS m^{-1} to that of plants under the lowest ECw (1.3 dS m^{-1}), there was a reduction of 14.59% (332.2). The decrease in the Fm of plants subjected to salt stress is indicative that there was deficiency in the photoreduction of quinone A (Q_A) in the thylakoid membranes and inflow of electrons between the photosystems (Tatagiba et al., 2014). Reduction in maximum fluorescence as a function of salt stress has also been observed in other crops such as West Indian cherry (Dias et al., 2018), cashew (Lima et al., 2019), and in 'Mimo do Céu' orange (Sousa et al., 2016).

The quantum efficiency of photosystem II (PSII) of custard-apple, determined through the ratio between Fv and Fm (Fv/Fm) decreased with the increase in water electrical conductivity (Figure 3D). According to the means comparison test (Figure 2D), the PSII of plants irrigated with water of 4.0 dS m^{-1} was significantly reduced compared to those subjected to ECw of 1.3 dS m^{-1}. Plants grown under ECw of 4.0 dS m^{-1} had reduction in PSII of 26.76% compared to those irrigated using water with 1.3 dS m^{-1}. Thus, it can be inferred that, in plants subjected to ECw of 1.3 and 4.0 dS m^{-1}, there was photoinhibition in the reaction centers of PSII or it was photochemically inactive because, when the photosynthetic apparatus is intact, the values of Fv/Fm vary between 0.75 and 0.85 (Reis & Campostrini, 2011). According to Carvalho et al. (2011), changes in the photosynthetic process in plants under salt stress result in excessive production of ROS and, in the absence of efficient protection mechanisms (enzymatic or not), there may be metabolic changes that result in oxidative damage.

The absolute growth rate - AGR_{SD} of custard-apple (Figure 4A) was significantly affected by the interaction between the factors. When irrigation was carried out using water with ECw of 1.3 and 4.0 dS m^{-1}, there was linear decrease in AGR_{SD}, with reductions of 11.62 and 13.28% per 25% increment in potassium doses, respectively. The AGR_{SD} of plants fertilized with 150% of the recommended potassium dose decreased by 60.60 and 72.37% (0.110 and 0.120 mm per day) compared to those that received 50% of the recommendation, respectively, at the ECw values of 1.3 and 4.0 dS m^{-1}.

When analyzing the effects of water electrical conductivity at each potassium dose (Figure 4A), it was observed that AGR_{SD} was negatively influenced by the salinity of irrigation water, with the highest values obtained at ECw (1.3 dS m^{-1}). As observed with the chlorophyll b concentration (Figure 2B), the increase in potassium doses intensified the effects of salt stress on AGR_{SD}, with greater magnitude in plants grown under the highest ECw (4.0 dS m^{-1}). The reduction in the growth of



Means followed by different letters indicate significant difference between water electrical conductivities by F test ($p \geq 0.05$); Vertical bars represent the standard error of the mean (n = 4)

Figure 4. Absolute - AGR_{SD} (A) and relative - RGR_{SD} (B) growth rates of stem diameter of custard-apple as a function of the interaction between water electrical conductivity - ECw and potassium doses, during 151-245 days after transplanting

custard-apple plants with the increase in potassium doses may be related to the saline effect of the fertilizer, because KCl has a high salt index (116). In addition, the excess of soluble salts in the root zone promotes metabolic alterations, mainly due to the restriction in the absorption of water and nutrients, causing a reduction in cell turgor, with a consequent reduction in cell expansion (Lima et al., 2019).

The relative growth rate of stem diameter - RGR_{SD} (Figure 4A) of custard-apple was also significantly affected by the interaction between the factors. According to the regression equations (Figure 4B), there was decreasing linear behavior for plants irrigated with ECw of 1.3 and 4.0 dS m^{-1}, whose reductions were 7.10 and 8.06% per 25% increment in K_2O dose, respectively. When comparing the RGR_{SD} of plants irrigated with ECw of 1.3 and 4.0 dS m^{-1}, there were reductions of 33.11 and 38.46% between those that were fertilized with doses of 50 and 150% of potassium recommendation, respectively. As observed for AGR_{SD} (Figure 3A), the increase in potassium dose intensified the effects of salt stress on custard-apple.

Analysis of the interaction (Figure 4B) showed that the RGR_{SD} of plants irrigated using water with electrical conductivity of 1.3 dS m^{-1} was statistically higher than that of plants cultivated under ECw of 4.0 dS m^{-1} at all potassium doses. However, as potassium doses increased, there was a reduction in RGR_{SD}, regardless of the water ECw. The inhibition in custard-apple growth due to the increase in potassium doses observed through RGR_{SD} (Figure 4B) may be related to the competitive absorption relative to the total sum of cations inside the plant, because the high potassium concentration induces calcium and magnesium deficiencies, reducing the growth of the entire plant (Dias et al., 2019).

Conclusions

1. Irrigation with water of 4.0 dS m^{-1} reduces the synthesis of chlorophyll a, total chlorophyll, and carotenoids in custard-apple, at 245 days after transplanting.

2. Increase in potassium dose from 50 to 150% of the recommendation intensifies the effect of salt stress on the chlorophyll b synthesis and the absolute and relative growth rates in stem diameter of custard-apple plants irrigated with water of electrical conductivity of 4.0 dS m^{-1}.

3. Reduction in the quantum efficiency of photosystem II in custard-apple irrigated with water of electrical conductivity of 4.0 dS m^{-1} is related to photoinhibitory damage to photosystem II.

4. Potassium does not mitigate the deleterious effects of salt stress on photosynthetic pigment biosynthesis and growth of custard-apple.

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