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Cell damage, gas exchange, and growth of *Annona squamosa* L. under saline water irrigation and potassium fertilization

Dano celular, trocas gasosas e crescimento da *Annona squamosa* L. irrigada com águas salinas e fertilização potássica

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Highlights

Irrigation water salinity negatively affects custard apple growth.
K_2O doses up to 22 g per plant attenuates salt stress effects on custard apple.
Cell damage and relative water content increases with the supply of K_2O.

Abstract

The semi-arid region of Northeastern Brazil has water limitations in terms of both quantity and quality, with salt stress as a limiting factor for increasing yield in most crops. In this context, the present study aimed to evaluate cell damage, gas exchange, and growth of custard apple under salt stress and potassium fertilization. The research was carried out at the Experimental Farm of CCTA/UFCG, in São Domingos-PB, Brazil. A randomized block design was arranged in a 2 × 5 factorial scheme, with two levels of electrical conductivity of irrigation water (ECw; 1.3 and 4.0 dS m^{-1}) and five potassium doses (10, 15, 20, 25, and 30 g of K_2O per plant per year). Water salinity of 4.0 dS m^{-1} negatively affected the stem diameter and number of leaves in custard apple at 179 and 210 days after transplanting (DAT). The highest relative growth in stem diameter in the period of 179-245 DAT was obtained in plants irrigated with 4.0 dS m^{-1} water and fertilized with

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20 g of K_2O per plant. Potassium doses of up to 30 g of K_2O resulted in a higher percentage of cell damage and relative water content in custard apple leaf tissue. Water saturation deficit decreased with the increase in K_2O doses in plants irrigated with water of 1.3 dS m^{-1}. Irrigation with 1.3 dS m^{-1} water and estimated K_2O doses ranging from 16 to 22 g per plant resulted in an increase in stomatal conductance, transpiration, CO_2 assimilation rate, and instantaneous carboxylation efficiency in custard apple plants at 210 DAT.

Key words: Semi-arid region. Salt stress. Attenuation.

Resumo

A região semiárida do Nordeste brasileiro apresenta limitações hídricas em termos quantitativos e qualitativos, tendo o estresse salino como um fator limitante para o aumento da produtividade na maioria das culturas. Neste contexto, o presente estudo teve como objetivo, avaliar o dano celular, as trocas gasosas e o crescimento da pinheira sob estresse salino e adubação potássica. A pesquisa foi realizada na Fazenda Experimental do CCTA/UFCG, em São Domingos-PB, Brasil. Foi utilizado o delineamento em blocos casualizados, arranjados em esquema fatorial 2 × 5 sendo dois níveis de condutividade elétrica da água de irrigação - CEa (1,3 e 4,0 dS m^{-1}) e cinco doses de potássio (10, 15, 20, 25 e 30 g de K_2O por planta por ano). A salinidade da água de 4,0 dS m^{-1} afetou de forma negativa o diâmetro de caule e o número de folhas da pinheira, aos 179 e 210 dias após o transplantio (DAT). O maior crescimento relativo em diâmetro de caule no período de 179-245 DAT foi obtido nas plantas irrigadas com água de 4,0 dS m^{-1} e adubação com 20 g de K_2O por planta. Doses de potássio de até 30 g de K_2O resultaram em maior percentual de dano celular e conteúdo relativo de água nos tecidos foliares da pinheira. O déficit de saturação hídrica diminuiu com o aumento nas doses de K_2O nas plantas irrigadas com água de 1,3 dS m^{-1}. Irrigação com água de 1,3 dS m^{-1} e doses estimadas de K_2O variando de 16 a 22 g por planta resultaram em incremento na condutância estomática, transpiração, taxa de assimilação de CO_2 e eficiência instantânea de carboxilação das plantas de pinheira, aos 210 DAT.

Palavras-chave: Semiárido. Estresse salino. Atenuação.

Introduction

Belonging to the Annonaceae family, custard apple (*Annona squamosa* L.) is a tropical fruit crop known in Brazil as 'ateira' or 'fruta-do-conde' (Lemos, 2014). It is a rustic plant with excellent adaptation to the climatic conditions of the semi-arid region of northeastern Brazil and good levels of yield under irrigated conditions (São José, Pires, Freitas, Ribeiro, & Perez, 2014).

However, in this region, the rainfall regime is characterized by prolonged periods of drought and annual irregularity of rainfall, causing water deficit in plants because evaporation rates exceed rainfall in most of the year, a situation that favors the occurrence of water sources with high concentrations of salt, mainly sodium, resulting in restrictions for use in agriculture (Lima et al., 2015; Lima, Fernandes, Soares, Gheyi, & Fernandes, 2020).

Given this scenario, the use of waters with high concentrations of salt has become increasingly necessary for the expansion of irrigated agriculture. However, excess salt in water is one of the abiotic stresses that limit the growth and yield of most crops due to the imbalance in the water and nutritional relationships of plants and the accumulation of ions considered toxic (Schossler, Machado, Zuffo, Andrade, & Piauilino, 2012). Plants that grow under saline conditions are exposed to various unfavorable conditions, which include low soil water potential and nutrient imbalance, with excessive accumulation of sodium and/or deficiency of Ca^{2+}, Mg^{2+}, and K^+ in the plants (Cuin & Shabala, 2007; Lima et al., 2020).

However, many plant species have developed several adaptation mechanisms that allow them to grow under saline conditions, including low absorption of Na+ and minimization of the concentration of salts in the cytosol (Arzani & Ashraf, 2016). Another important mechanism is the selectivity of K^+ over Na^+ to maintain a high K^+/Na^+ ratio in the cytosol, which is a determinant for salinity tolerance and has been suggested as one of the most important criteria for evaluating salinity tolerance in a large number of plant species (Hasanuzzaman et al., 2018).

In this context, among the techniques used to mitigate salt stress, the management of fertilization with potassium fertilizers stands out, since this element favors the formation and translocation of carbohydrates and efficient use of water by plants, balances nitrogen application, acting as an enzymatic activator and osmoregulation agent, controlling stomatal opening and closure, and its management may result in greater competition of this macro element with other cations, especially Na+ (Heidari & Jamshid, 2010; Araújo, Quadros, Cardoso, & Corrêa, 2012).

In this context, the present study aimed to evaluate the effects of irrigation with waters of different salinity levels and potassium fertilization on cell damage, gas exchange, and growth of custard apple plants.

Material and Methods

This research was carried out in pots adapted as drainage lysimeters at the Rolando Enrique Rivas Castellon Experimental Farm, belonging to the Center for Science and Agri-Food Technology of the Federal University of Campina Grande (CCTA/UFCG), located in the municipality of São Domingos, Paraíba, Brazil, at the geographic coordinates 6°48′48.8″ S, 37°56′16.5″ W, elevation of 194 m.

The experiment was installed with a randomized block design, arranged in a 2 × 5 factorial scheme, corresponding to two levels of electrical conductivity of irrigation water (ECw; 1.3 and 4.0 dS m^{-1}) and five potassium doses (10, 15, 20, 25 and 30 g of K_2O per plant per year), with four replicates, totaling forty experimental units. Potassium doses were established according to the recommendations for Annonaceae species in the first year of production proposed by Silva and Silva (1997).

Custard apple seedlings were obtained from seeds by sowing two seeds in each polyethylene bag with a capacity of 1.5 dm^3, filled with substrate, consisting of a mixture of 75% soil, 25% sand, and 25% organic compost (bovine manure) in a proportion of 2:1:1 with mass of 1.7 kg. After the emergence of seedlings, thinning was performed when they were 10 cm tall, leaving only one plant per container. Notably, irrigation was carried out with the respective saline water since sowing.

At 190 days after sowing, seedlings were transplanted to pots adapted as drainage lysimeters with a capacity of 310 L (54 cm high, 105 cm upper diameter and 104 cm lower diameter), filled with a 0.5 kg layer of sand, followed by 310 kg of soil with a sandy loam texture (0-30 cm depth), properly pounded to break up clods, coming from the Rolando Enrique Rivas Castellón Experimental Farm of the municipality of São Domingos - PB, whose chemical and physical characteristics (Table 1) were obtained according to the methodologies proposed by Teixeira, Donagemma, Fontana and Teixeira (2017).

Table 1
Chemical and physical characteristics of the soil used in the experiment, before the application of treatments

Chemical characteristics								
pH (H_2O)	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+
(1:2.5)	dag kg^{-1}	(mg kg^{-1})	.... $cmol_c$ kg^{-1}					
6.95	0.96	13.75	0.39	0.83	3.74	2.30	0.00	0.00
.... Chemical characteristics				.... Physical characteristics				
EC_{se}	CEC	SAR	ESP	Size fraction (g kg^{-1})			Water content (dag kg^{-1})	
(dS m^{-1})	$cmol_c$ kg^{-1}	(mmol $L^{-1})^{0.5}$	%	Sand	Silt	Clay	33.42 kPa^1	1519.5 kPa^2
0.32	7.26	0.81	11.43	719.20	241.60	39.20	12.24	4.58

pH - Hydrogen potential, OM - Organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; $Al^{3+}+H^+$ extracted with 0.5 M CaOAc at pH 7.0; ECse - Electrical conductivity of saturation extract; CEC - Cation exchange capacity; SAR - Sodium adsorption ratio of the saturation extract; ESP - Exchangeable sodium percentage; ^{1,2} referring to field capacity and permanent wilting point, respectively.

Each lysimeter was perforated at the base to allow for drainage and coupled to a transparent drain with a diameter of 25 mm. The end of the drain inside the lysimeter was wrapped with a nonwoven geotextile (Bidim OP 30) to prevent clogging by soil material. A plastic container with a capacity of 3 L was placed below each drain to collect drained water to estimate water consumption by the plant. The water of lowest ECw, 1.3 dS m^{-1}, was obtained from an artesian well located in the experimental farm, while the highest salinity level (4.0 dS m^{-1}) was prepared by adding NaCl to the water of the lowest ECw, based on the relationship between ECw and the concentration of salts (mmol L^{-1} = 10*ECw dS m^{-1}) according to Richards (1954).

Irrigation using water with different levels of ECw were performed daily, applied in each lysimeter a volume of water corresponding to the water requirement of the plant. The volume applied in each irrigation event was estimated by means of the water balance, considering the volume of water applied to the plants and volume drained in the previous irrigation, as well as a leaching fraction of 20% every 20 days to reduce the salt accumulation in the root zone.

Fertilization with N_2-P_2O_5-K_2O was carried out as top-dressing via fertigation, based on the recommendation of fertilization for Annonaceae species proposed by Silva and Silva (1997), using urea, monoammonium phosphate, and potassium chloride as sources of nitrogen, phosphorus, and potassium, respectively. Fertilization started at 10 days after transplanting (DAT), split into 24 equal portions and applied at 15-day intervals throughout the first year of production. To meet the recommendations of N_2 and P_2O_5, 50 g of N and 40 g of P_2O_5 per plant were supplied every 15 days, diluted in the water of the lowest salinity level (1.3 dS m^{-1}). At fifteen-day intervals, plants received 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%)].

Throughout the experiment, formative pruning was performed to standardize and shape the crop, eliminating the apical bud with pruning shears when the main stem reached a 50 cm height from the soil level. Then, three shoots were selected according to the best vertical and horizontal distribution and when the main branches reached 50 cm in length, they were pruned to 30 cm, selecting three branches for each main branch, which were then pruned to 30 cm whenever they reached 50 cm in length, as recommended by Pereira et al. (2011). In field cultivation, a spacing of 2.0 m between rows and 2.0 m between plants was established.

Plant growth was evaluated based on the number of leaves and stem diameter at 179, 210, and 245 days after transplanting (DAT). Stem diameter data were used to determine the relative growth rate in stem diameter (RGR_{SD}) in the period from 179 to 245 DAT. At 210 DAT, the following parameters were determined: percentage of cell damage (%D), relative water content (RWC), water saturation deficit (WSD), stomatic conductance (*gs;* mol m^{-2} s^{-1}), transpiration (*E;* mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration (*Ci;* μmol mol^{-1}), and CO_2 assimilation rate (*A;* μmol m^{-2} s^{-1}). The ratio between A and *Ci* (*A/Ci*) was then used to determine the instantaneous carboxylation efficiency [*CEi;* (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$].

To determine the percentage of damage to the cell membrane, 10 leaf discs with an area of 113 mm² were collected, washed with distilled water to remove other electrolytes adhered to the leaves, placed into beakers containing 50 mL of distilled water, and hermetically sealed with aluminum foil. The beakers were kept at a temperature of 25ºC for 90 minutes and evaluated for initial electrical conductivity (ECi); subsequently, the beakers were taken to a forced air ventilation oven and subjected to a temperature of 80ºC for 90 minutes, when the final electrical conductivity was measured (ECf). Thus, electrolyte leakage in the cell membrane was obtained according to Lima, Gheyi, Nobre, Soares and Santos (2019), as Eq.1:

$$\%\mathrm{D} = \frac{\mathrm{ECi}}{\mathrm{ECf}}\ x\ 100 \qquad (1)$$

Where:

%D = Percentage of membrane damage (%);

ECi = initial electrical conductivity (dS m^{-1});

ECf = final electrical conductivity (dS m^{-1});

Relative water content was determined according to the methodology of Weatherley (1950) using Eq. 2:

$$\mathrm{RWC} = \frac{\mathrm{FM-DM}}{\mathrm{TM-DM}} \times\ 100 \qquad (2)$$

Where: RWC = Relative water content (%);

FM = leaf fresh mass (g);

TM = turgid mass (g);

DM = Dry mass (g).

WSD was determined by following the methodology described by Taiz and Zeiger (2013), according to Eq. 3:

$$\text{WSD} = \frac{\text{TM}-\text{FM}}{\text{TM}-\text{DM}} \times 100 \qquad (3)$$

Where: WSD = water saturation deficit (%);

FM = leaf fresh mass (g);

TM = turgid mass (g);

DM = Dry mass (g).

Gas exchange determination was performed with an infrared gas analyzer (IRGA; Infra-Red Gas Analyser, LCpro - SD model, from ADC Bioscientific, UK). Readings were performed between 7:00 and 10:00 a.m. on the third fully expanded leaf counted from the apical bud, conducted under natural conditions of air temperature, CO_2 concentration, and using an artificial radiation source of 1,200 μmol m^{-2} s^{-1}, established through the photosynthetic light-response curve, and the photosynthetic light saturation point was determined.

The count of mature leaves was considered when leaves had a midrib length longer than 3 cm and characteristic color of the cultivar. Stem diameter was determined at 3 cm from the soil using a digital caliper, with readings in ‘mm’. RGR for stem diameter of s custard apple was determined according to Benincasa (2003), using Eq. 4.

$$\text{RGRSD} = \frac{(\text{InSD2}-\text{InSD1})}{(\text{t2}-\text{t1})} \qquad (4)$$

Where: RGRSD = Relative growth rate in stem diameter (mm mm^{-1} day^{-1});

SD_1 = Stem diameter (mm) at time t_1;

SD_2 = Stem diameter (mm) at time t_2;

In = natural logarithm.

The data were analyzed for normality and homoscedasticity and, subsequently, subjected to analysis of variance by an F test ($p<0.05$). When significant, a means comparison test (Tukey at 0.05 significance level) was performed for water salinity levels and regression analysis was performed for the potassium dose factor using the statistical program SISVAR version 5.1 (Ferreira, 2011).

Results and Discussion

There was a significant effect of salinity levels on water saturation deficit (WSD), internal CO_2 concentration (*Ci*), CO_2 assimilation rate (*A*), and instantaneous carboxylation efficiency (*CEi*) of custard apple plants (Table 2) at 210 days after transplanting. Potassium doses significantly influenced %D, RWC, and WSD. There was a significant interaction between the factors (SL x KD) on WSD, *gs, E, A,* and *CEi* in custard apple plants.

Table 2

Summary of the analysis of variance for the percentage of cell damage (%D), relative water content (RWC), water saturation deficit (WSD), stomatal conductance (*gs*), transpiration (*E*), internal CO_2 concentration (*Ci*), CO_2 assimilation rate (*A*), and instantaneous carboxylation efficiency (*CEi*) of custard apple cultivated with saline waters and potassium doses at 210 days after transplanting (DAT)

Source of variation	DF	Mean squares							
		%D	RWC	WSD	gs	E	Ci	A	CEi
Saline levels (SL)	1	6.65^{ns}	5.12^{ns}	93.20**	0.001^{ns}	0.24^{ns}	5405.62*	17.86*	0.012*
K dose (KD)	4	6.24*	436.73*	37.90**	0.0009^{ns}	0.10^{ns}	1453.71^{ns}	2.82^{ns}	0.0001^{ns}
Linear regression	1	9.38*	274.97*	55.71**	0.001^{ns}	0.04^{ns}	19.01^{ns}	8.77^{ns}	0.00006^{ns}
Quadratic regression	1	3.52^{ns}	55.46^{ns}	57.02**	0.00003^{ns}	0.01^{ns}	3924.72^{ns}	0.15^{ns}	0.0002^{ns}
Interaction (SL x KD)	4	3.29^{ns}	121.56^{ns}	41.33**	0.002*	2.73*	2121.43^{ns}	41.29**	0.001*
Blocks	3	0.84^{ns}	40.50^{ns}	14.21^{ns}	0.0006^{ns}	0.25^{ns}	2733.02^{ns}	1.00^{ns}	0.0003^{ns}
Experimental error	27	2.11	31.77	3.13	0.0008	0.30	1142.28^{ns}	2.99	0.0002
CV (%)		14.08	6.54	12.78	21.69	17.65	17.14	26.43	19.53

^{ns}, **, * not significant, significant at 1%, and 5% probability of error, respectively; DF -Degree of freedom; CV -Coefficient of variation.

Potassium doses caused a linear increase in the percentage of cell damage in leaf tissues of custard apple plants (Figure 1A), which increased by 0.479% for each 5 g increment in K_2O dose. There was a 20.47% increase in %D between plants fertilized with 30 g of K_2O and those that received the lowest dose (10 g of K_2O per plant). The increase in damage to the cell membrane led to a loss in integrity and destabilization in the cell membrane, and the amount of leakage was determined based on the leaked content from the cell; the higher it was, the greater the damage caused to the cell membrane (Ataíde, Flores, & Borges, 2012). However, the increase in the percentage of cell damage in leaf tissues of plants grown under a high concentration of salts may represent a mechanism to avoid tissue dehydration, due to the reduction in the osmotic component and water potential in the leaves (Fioreze, Rodrigues, Carneiro, Silva, & Lima, 2013). Lima et al. (2019) evaluated membrane damage, water status, and gas exchange in castor bean plants (*Ricinus communis* L.) cv. BRS Energia, in response to salinity and the cationic nature of irrigation water and verified that the high potassium concentration resulted in greater damage to the cell membrane.

The relative water content of custard apple plants also increased linearly in response to potassium fertilization, and according to the regression equation (Figure 1B), there was an increase of 2.196% for each 5 g increment in K_2O dose. Plants subjected to fertilization with a higher potassium dose (30 g per plant) increased their RWC by 10.74% compared to those receiving the K_2O dose of 10 g. Potassium supply contributed to osmotic adjustment in plants under low water availability because excess salt in water reduces the water potential (A. F. L. de Sá et al., 2014). Osmotic adjustment is an important characteristic associated with the maintenance of high cell turgor potential and water retention in response to salt stress, being fundamental for the survival of plants under salt stress conditions, highlighting the role of K^+ in the regulation of turgor within the guard cells during stomatal movement (Wang, Zheng, & Guo, 2013).

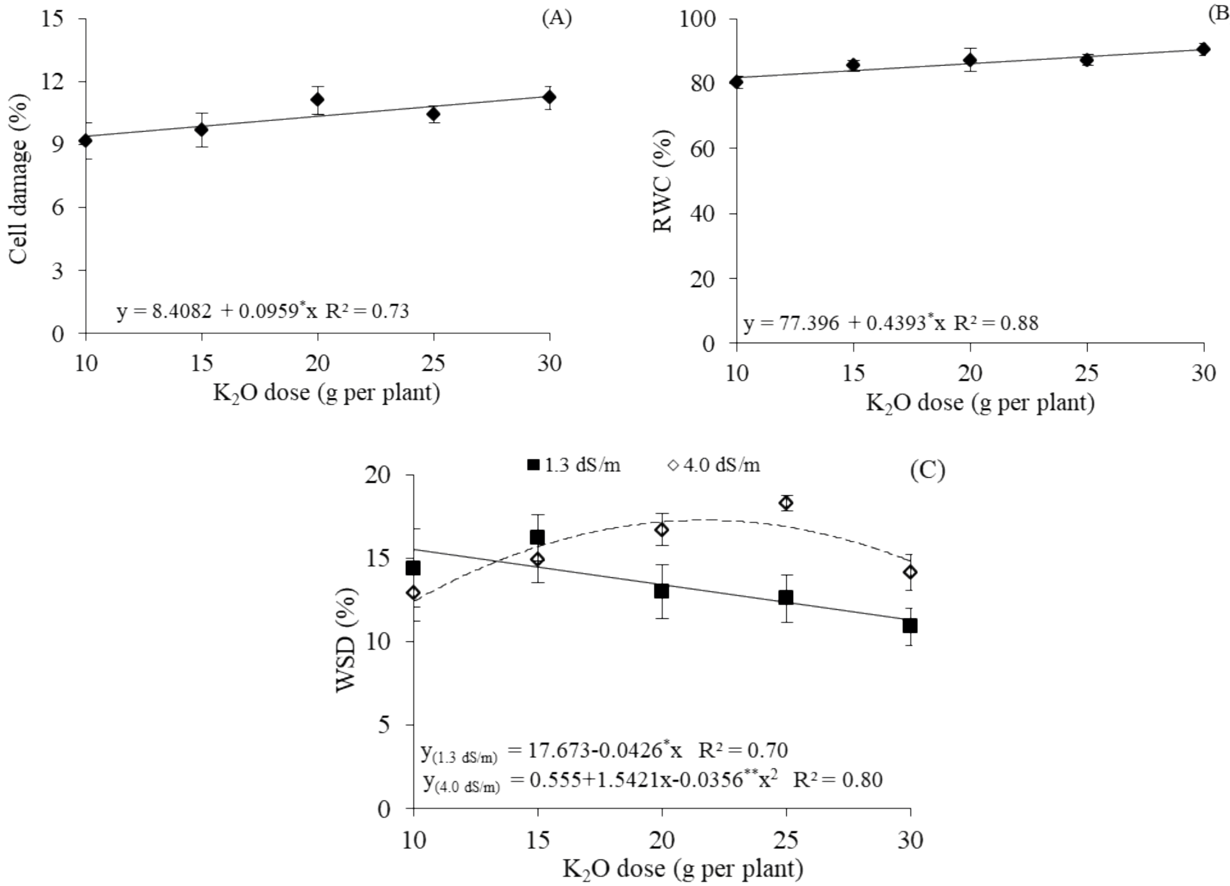

Figure 1. Cell damage (A) and relative water content - RWC (B) of custard apple plants as a function of potassium doses and water saturation deficit - WSD (C) as a function of the interaction between the factors ECw level and potassium dose at 210 days after transplanting. Vertical bars represent the standard error of the mean (n=4).

The interaction between factors (NL × KD) significantly influenced the water saturation deficit (Figure 1C). In plants grown under an ECw of 1.3 $dS\ m^{-1}$, WSD was reduced sharply with the increase in K_2O doses, by 0.213% for each 5 g increment in K_2O dose. There was a 4.93% reduction in WSD between plants fertilized with 30 g of K_2O and those subjected to the lowest dose (10 g K_2O per plant). The reduction in water saturation deficit was associated with a high relative water content and the functions performed by K in osmoregulation, stomatal movement, energy transfer, transport in the phloem, cation-anion balance, and stress tolerance (Wang et al., 2013).

The data of plants irrigated with an ECw of 4.0 dS m^{-1} were described by a quadratic model (Figure 1C), with an estimated maximum value of 17.25% at a K_2O dose of 22 g per plant. This situation may be related to the high salt index of the fertilizer and excess salt in water, which restricts the absorption of water and nutrients by plants. Pinheiro et al. (2019), in a study evaluating the water relations of West Indian cherry (Malpighia emarginata Sesse & Moc. ex DC.) cv. BRS 366 Jaburu as a function of irrigation with saline water (ECw of 0.8 and 3.8 dS m^{-1}) and potassium fertilization, observed that the highest dose of K_2O (125% of the recommendation) caused a reduction in the WSD of the plants.

Stomatal conductance of custard apple plants was significantly affected by the interaction between water salinity levels and potassium doses, and the regression equations (Figure 2A) showed that the data obtained the best fit with the quadratic model for ECw levels of 1.3 and 4.0 dS m^{-1}. When irrigating with an ECw of 1.3 dS m^{-1}, the highest estimated value (0.0873 mol m^{-2} s^{-1}) was obtained in plants fertilized with 16 g K_2O per plant. Plants subjected to water salinity of 4.0 dS m^{-1} reached the highest gs (0.0750 mol m^{-2} s^{-1}) when they were fertilized with 18 g of K_2O. A decrease in stomatal activity was one of the main effects caused by salt stress, acting as a defense mechanism in the reduction of water loss by plants to the atmosphere, as well as an indication of the intensification of the osmotic effect (Sousa, Gheyi, Brito, Xavier, & Furtado, 2016). Dias, Lima, Pinheiro, Gheyi and Soares (2019), in a study with West Indian cherry (*Malpighia emarginata* Sesse & Moc. ex DC.) cv. BRS 366 Jaburu subjected to water salinity (ECw of 0.8 and 3.8 dS m^{-1}), also observed that the increase in ECw resulted in a reduction in the stomatal conductance of plants.

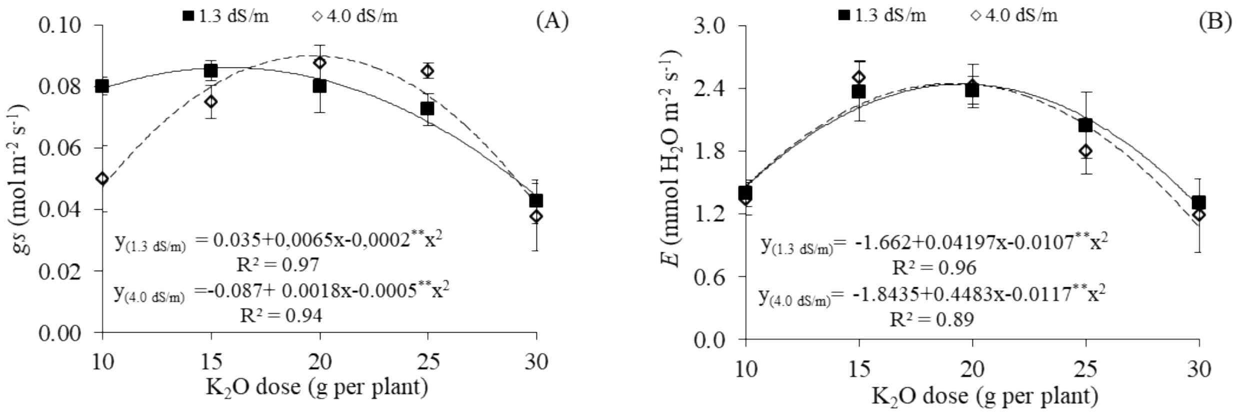

Figure 2. Stomatal conductance - *gs* (A) and transpiration - E (B) of custard apple plants as a function of the interaction between the factors water salinity levels (ECw) and potassium dose at 210 days after transplanting. Vertical bars represent the standard error of the mean (n=4).

The leaf transpiration of custard apple plants was also significantly influenced by the interaction between factors (SL x KD). Plants under irrigation with an ECw of 1.3 dS m^{-1} obtained the highest estimated value of 2.452 mmol of H_2O m^{-2} s^{-1} when they were fertilized with 20 g of K_2O, with reductions from this dose, reaching a value of 1.299 mmol of H_2O m^{-2} s^{-1} under fertilization with 30 g of K_2O. Similar behavior was observed in plants irrigated with the highest ECw level (4.0 dS m^{-1}), whose maximum estimated value (2.450 mmol of H_2O m^{-2} s^{-1}) was reached under a K_2O dose of 19 g. Transpiration played a significant physiological role in maintaining the osmotic concentration in the plant (Alam Juraimi, Rafii, & Hamid, 2015). The reduction of transpiration causes a decrease in the capacity of water absorption by the root system, as a response to excess salt and, therefore, a reduction in the absorption of toxic ions, especially Na^+ and Cl^-, decreasing toxicity by specific ions (Syvertsen & Garcia-Sanchez, 2014). Thus, a lower transpiration rate contributed to the lower accumulation of Na^+ and Cl^- in the plant. Lima et al. (2020), in an experiment conducted with sour passion fruit (*Passiflora edulis Sims*) cv. BRS Rubi do Cerrado irrigated with saline water (ECw from 0.3 to 3.5 dS m^{-1}) under greenhouse conditions, concluded that water salinity higher than 0.3 dS m^{-1} reduced plant transpiration.

The internal CO_2 concentration of custard apple decreased with the increase in water salinity levels, and the means comparison test (Figure 3A) showed that plants cultivated using water with an ECw of 4.0 dS m^{-1} had statistically lower *Ci* compared to those that were subjected to the lowest ECw level (1.3 dS m^{-1}). A reduction of 11.13% (23.25 μmol mol^{-1}) in *Ci* was observed in the comparison between plants cultivated with an ECw of 4.0 dSm^{-1} and those that received 1.3 dS m^{-1}. The reduction in the internal CO_2 concentration in plants grown under an ECw of 4.0 dS m^{-1} may be related to stomatal closure and, therefore, to the lower diffusion of CO_2 in the substomatal chamber (Oliveira, Souza, Cunha, Silva, & Veloso, 2017), observed in the present study through stomatal conductance (Figure 2A). In addition, stomatal closure may limit the CO_2 retention rate and, consequently, *Ci* decreases in intercellular spaces due to CO_2 consumption by photosynthetic activity (Dalastra, Echer, Guimarães, Hachmann, & Inagaki, 2014). A. A. R. da Silva et al. (2019a), in a study evaluating the gas exchange of passion fruit (*Passiflora edulis* f. *flavicarpa*) cultivar Guinezinho under irrigation with saline water (ECw from 0.7 to 2.8 dS m^{-1}), also observed that the increase in water salinity resulted in a decrease in internal CO_2 concentration.

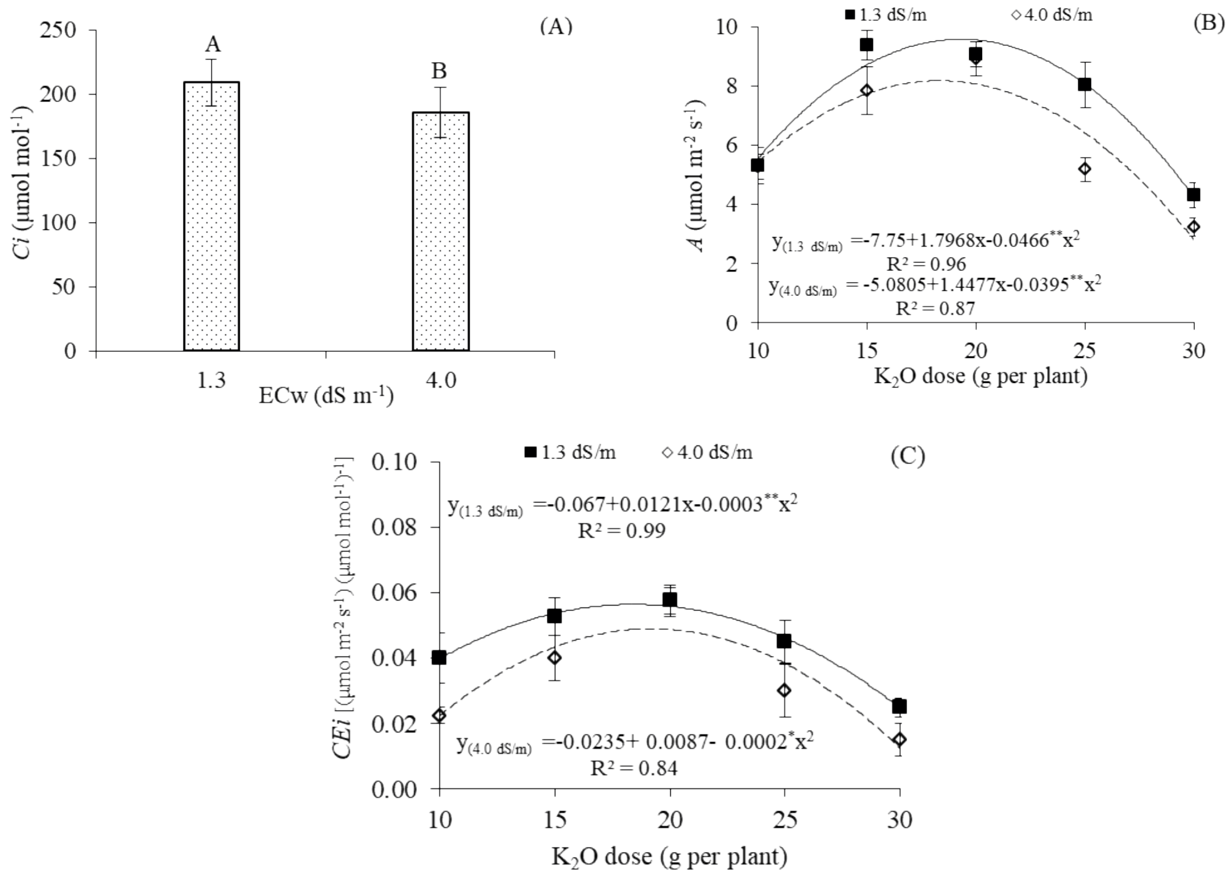

Figure 3. Internal CO_2 concentration - *Ci* (A) of custard apple plants as a function of water salinity levels - ECw and CO_2 assimilation rate - *A* (B) and instantaneous carboxylation efficiency - *CEi* (C) as a function of the interaction between the factors ECw level and potassium dose at 210 days after transplanting. Means followed by different letters indicate significant differences between treatments by a Tukey test ($p<0.05$). Vertical bars represent the standard error of the mean (n=4).

The CO_2 assimilation rate of custard apple plants was significantly influenced by the interaction between factors (SL x KD), and the regression equations (Figure 3B) showed that, for custard apple plants irrigated using water with an ECw of 1.3 and 4.0 dS m^{-1}, the data were described by a quadratic model, with estimated highest A values of 9.556 and 8.180 μmol m^{-2} s^{-1} obtained at K_2O doses of 19 and 18 g per plant, respectively. From these doses, there was a marked reduction in A, regardless of the ECw level, with the lowest estimated values obtained in plants fertilized with 30 g of K_2O per plant (4.214 and 2.800 μmol m^{-2} s^{-1}).

Despite the functions performed by potassium in maintaining ionic balance and turgor of cells, through the control of stomatal opening and closure (Gurgel, Gheyi, & Oliveira, 2010), in the present study, the highest K_2O dose resulted in a reduction in the CO_2 assimilation rate of custard apple plants irrigated with high-salinity water (4.0 dS m^{-1}). This situation may be related to the source of potassium used in this study (KCl) because this fertilizer has a high salt index in its composition (116.3) and when associated with the use of water with a high salt concentration, may have induced a reduction in the osmotic potential of the soil solution, hampering the absorption of water and nutrients by plants (Lima et al., 2020). A decrease in the CO_2 assimilation rate of plants grown under salinity has also been observed in West Indian cherry (Malpighia emarginata Sesse & Moc. ex DC.) (Dias et al., 2019) and sour passion fruit (Passiflora edulis Sims) (Lima et al., 2020).

For instantaneous carboxylation efficiency (Figure 3C), it was verified that plants subjected to a water salinity of 1.3 and 4.0 dS m^{-1} showed quadratic behavior, with the highest estimated values of 0.0550 and 0.0711 (μmol m^{-2} s^{-1})/(μmol mol^{-1})$^{-1}$ obtained in plants fertilized with 20 and 22 g of K_2O per plant, respectively. As observed for the CO_2 assimilation rate (Figure 3B), it was verified that despite the reduction in *CEi,* the highest value was obtained at the lowest water salinity level. The decrease in *CEi* may be associated with metabolic restrictions in the Calvin cycle and stands out as an indication that non-stomatal factors have acted in photosynthetic activity, such as low activity of the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), probably due to the low availability of reducing potential (ATP and NADPH) for the activation and regeneration of the enzyme (F. V. da S. Sá et al., 2017). In addition, due to the low intercellular concentration of CO_2 there was possibly an elevation in the photorespiration of the plants. A. A. R. da Silva et al. (2019b), in a study evaluating the gas exchange of soursop seedlings (*Annona muricata* L.) cv. Morada Nova irrigated with saline water (ECw from 0.7 to 3.5 dS m^{-1}), verified a reduction in CEi of 18.49% per unit increase in ECw.

There was a significant effect of salinity levels on the stem diameter (SD) and number of leaves (NL) of custard apple plants at 179, 210, and 245 days after transplanting (Table 3). Potassium doses did not significantly influence (*p*>0.05) any of the variables analyzed, regardless of the time of evaluation. The interaction between factors (SL × KD) caused a significant effect on SD at 245 DAT and on RGRSD in custard apple plants in the period of 179-245 DAT.

The stem diameter of custard apple plants was negatively affected by the increase in ECw levels at 179 and 210 DAT (Figure 4A). Plants grown under an ECw of 1.3 dSm^{-1} showed statistically higher SD compared to those under irrigation with the highest salinity level (4.0 dS m^{-1}) in both evaluation times. A comparison of the SD of plants subjected to the highest level of ECw (4.0 dS m^{-1}) to the value of plants irrigated with water of 1.3 dS m^{-1} showed reductions of 1.594 and 1.893 mm at 179 and 210 DAT, respectively. Inhibition of growth in plants subjected to salt stress was a consequence of changes in leaf gas exchange (gs, E, Ci, and A). In addition, another factor that contributed to the reduction in SD was the decrease in the osmotic potential of the soil solution caused by excess soluble salts in the root zone, which limits the absorption of water by the roots, causing the plant to reduce stomatal opening as a mechanism to reduce water loss, which leads to a reduction in cell turgor and, as a consequence, a decrease in cell expansion and plant growth (Souza, Lima, Gheyi, Nobre, & Soares, 2018). Corroborating the results obtained in this study, F. V. da S. Sá et al. (2015) evaluated the initial growth of custard apple (Annona squamosa L.) seedlings as a function of water salinity (ECw from 0.3 to 4.8 dS m^{-1}) and observed that an ECw higher than 0.3 dS m^{-1} caused negative effects on the growth in stem diameter.

Table 3

Summary of the analysis of variance for stem diameter (SD) and number of leaves (NL) at 179, 210, and 245 days after transplanting (DAT) and relative growth rate in stem diameter (RGR_{SD}) in the period from 179 to 245 DAT of custard apple cultivated with saline water and potassium doses

Days after transplanting	Mean squares					
	Saline levels (SL)	K dose (KD)	Interaction (NL × KD)	Blocks	Experimental error	CV (%)
			Stem diameter			
179	25.40*	1.02^{ns}	9.39^{ns}	5.01^{ns}	4.24^{ns}	8.21
210	35.85*	6.60^{ns}	5.49^{ns}	1.94^{ns}	5.17	8.46
245	19.95*	6.83^{ns}	167.53**	1.83^{ns}	3.54	6.19
			Number of leaves			
179	13727.02*	1267.85^{ns}	7714.15^{ns}	19058.42^{ns}	2784.16	21.57
210	910530.62**	15378.08^{ns}	18408.18^{ns}	25011.55^{ns}	6733.28	26.62
245	72590.40*	14648.65^{ns}	12876.27^{ns}	8026.30^{ns}	8978.81	21.34
			RGR_{SD}			
179-245	0.00000001^{ns}	0.000005^{ns}	0.0001**	0.000005^{ns}	0.000004^{ns}	23.64
DF	1	4	4	3	27	-

^{ns}, **, * not significant, significant at 1%, and 5% probability of error, respectively; DF -Degree of freedom; CV -Coefficient of variation.

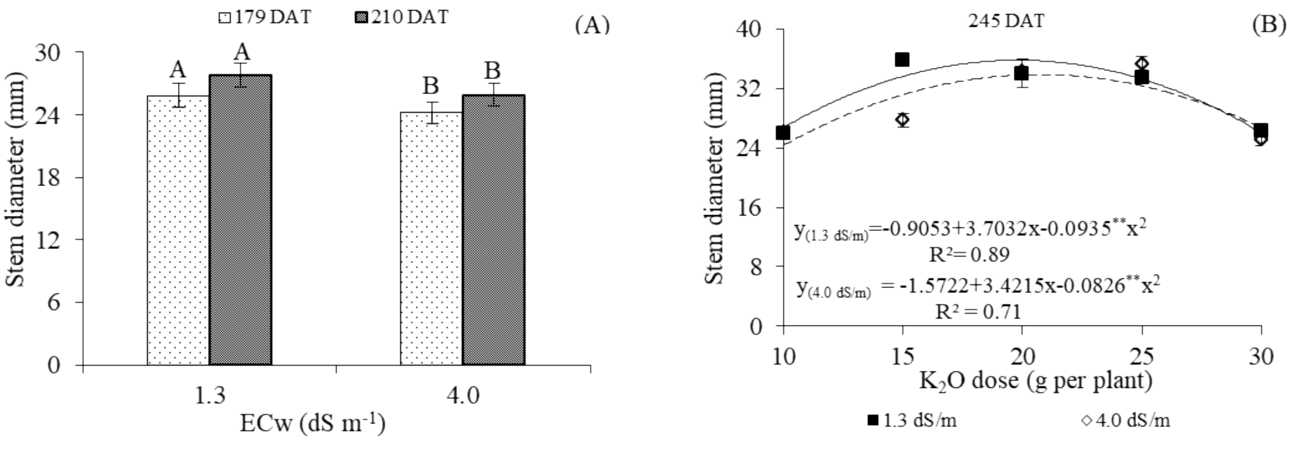

Figure 4. Stem diameter (A) at 179 and 210 days after transplanting (DAT) as a function of water salinity levels (ECw) and stem diameter (B) of custard apple plants at 245 DAT, as a function the interaction between the factors ECw level and potassium dose. Means followed by different letters indicate significant differences between treatments by a Tukey test (*p*<0.05). Vertical bars represent the standard error of the mean (n=4).

Stem diameter of custard apple was significantly affected by the interaction between factors (NS × DK) at 245 DAT (Figure 4B). Plants subjected to water salinity of 1.3 and 4.0 dS m^{-1} had the highest growth in SD (35.758 and 33.852 mm) when they were fertilized with estimated K_2O doses of 20 and 22 g per plant, respectively. Notably, from these K_2O doses, there was a decrease in growth, reaching SD values of 26.040 and 26.732 mm in plants fertilized with 30 g of K_2O per plant. The reduction in growth of the stem diameter in custard apple plants was a consequence of lower water potential in the soil, due to the high concentrations of salt in the external environment and may also be related to energy expenditure to maintain metabolic activities. Plants grown under high doses of potassium may have reduced Ca^{2+} concentrations through competitive inhibition between these ions (Inthichack, Nishimura, & Fukumoto, 2012) and, considering that Ca^{2+} acts on the integrity of the plasma membrane and composes the binding sites of the cell wall pectin molecules, low concentrations of this element can affect the rates of cell resistance, extensibility, elongation, and division, with negative effects on growth (Proseus & Boyer, 2012).

The different levels of water salinity also negatively interfered with the number of leaves of custard apple plants, at 179, 210, and 245 DAT (Figure 5A). Plants irrigated using water with an electrical conductivity of 1.3 dS m^{-1} had increments of 37.05, 219.35, and 187.60 in the number of leaves compared to those subjected to ECw of 4.0 dS m^{-1}. The reduction in leaf production in plants under salt stress stands out as a strategy to decrease transpiration and maintain a lower need for the absorption of saline water and, consequently, toxic ions (especially Na^+ and Cl^-), besides maintaining a higher water potential in plants in the face of the osmotic effect of salts (Bonifácio et al., 2018). However, a reduction in the number of leaves may result in negative effects on net photosynthesis rate, hampering the physiological and biochemical processes of plants (G. G. de Sousa et al., 2014). A reduction in the number of leaves in plants cultivated under salt stress has also been observed in custard apple (*Annona squamosa* L.) (Nunes, Bernardes, Glória, & Oliveira, 2012) and other crops, such as tamarind (*Tamarindus indica* L.) (Alves, Véras, Melo, Irineu, & Dias, 2019), soursop (*Annona muricata* L.) (A. A. R. da Silva et al., 2019a), and passion fruit (*Passiflora edulis* Sims) (Lima et al., 2020).

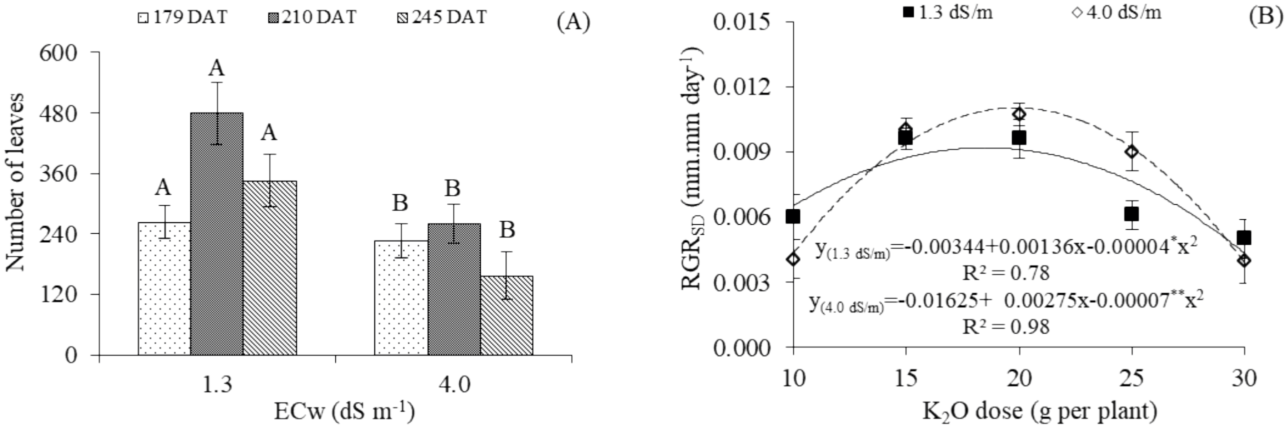

Figure 5. Number of leaves (A) at 179, 210, and 245 DAT as a function of water salinity levels (ECw) and relative growth rate in stem diameter - RGR_{SD} (B) of custard apple plants in the period from 179 to 245 DAT, as a function of the interaction between the factors ECw level and potassium dose. Means followed by different letters indicate significant differences between treatments by a Tukey test ($p<0.05$). Vertical bars represent the standard error of the mean (n=4).

The relative growth rate in stem diameter (RGRSD) of custard apple plants (Figure 5B) in the period of 179-245 DAT was significantly affected by the interaction between factors (SL x KD). When water of 1.3 and 4.0 dS m^{-1} was used in irrigation, the data were described by a quadratic model, with highest estimated values of 0.00812 and 0.01075 mm day^{-1} under fertilization with 17 and 20 g of K_2O per plant. When comparing custard apple plants irrigated with the lowest ECw (1.3 dS m^{-1}) and those subjected to the highest water salinity level (4.0 dS m^{-1}), a greater reduction in RGRSD was observed in plants cultivated with the highest water salinity. Such an increase in plant growth under stress may be related to the functions of K^+ in plant metabolism, acting on the translocation of photoassimilates for growth. In addition, K^+ plays a crucial role in regulating turgor within the guard cells during stomatal movement, contributing to the maintenance of a high water potential in the plant (Wang et al., 2013). Corroborating this study, Gurgel et al. (2010) evaluated the effects of using low-salinity water (ECw 0.52 dS m^{-1}), high-salinity water (ECw 2.41 dS m^{-1}), and potassium fertilization (218, 273, 328, 383 and 438 kg ha^{-1}) on the growth of melon (Cucumis melo L) cv. Goldex and found that the highest values in relative growth were obtained in melon irrigated using water with a high ECw (2.41 dS m^{-1}).

Conclusion

Water salinity of 4.0 dS m^{-1} negatively affected the growth in stem diameter and number of leaves in custard apple at 179 and 210 days after transplanting.

Potassium doses up to 30 g of K_2O resulted in a higher percentage of cell damage and relative water content in the leaf tissues of custard apple at 210 days after transplanting. A water saturation deficit decreased with the increase in K_2O dose in plants irrigated with water of 1.3 dS m^{-1}.

Custard apple plants subjected to water salinity of 1.3 dS m^{-1} and estimated K_2O doses ranging from 16 to 22 g per plant showed an increase in stomatal conductance, transpiration, CO_2 assimilation rate, and instantaneous carboxylation efficiency at 210 days after transplanting.

The highest relative growth in stem diameter of custard apple in the period of 179-245 days after transplanting was obtained in plants irrigated with water of 4.0 dS m^{-1} and fertilized with 20 g of K_2O per plant.

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