



SALINE STRESS AND EXOGENOUS APPLICATION OF PROLINE IN CASHEW ROOTSTCK

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ABSTRACT

The scarcity of good quality water and the occurrence of long dry periods are limiting factors for irrigated agriculture, especially in semiarid regions, which induces the use of saline water and technologies that enable its use in agriculture. In this context, the objective of the present study was to evaluate the influence of exogenous application of proline on growth and phytomass of BRS 226 Planalto precocious dwarf cashew rootstock irrigated with water of different levels of salinity. The study was performed in a greenhouse, in the municipality of Pombal - PB, in a protected environment, using a randomized block design in a 5 x 4 factorial scheme. The treatments consisted of different levels of electrical conductivities of irrigation water – EC_w (0.3; 1.0; 1.7; 2.4 and 3.1 dS m⁻¹) associated with foliar application of proline - P (0, 4, 8 and 12 mM), with three repetitions and two plants per plot. The irrigation with saline water of up to 0.85 dS m⁻¹ can be used for the production of BRS 266 cashew rootstock. Proline concentration of 8 mM promoted largest dry phytomass of the BRS 226 Planalto cashew rootstock. The increase in proline concentration up to 12 mM applied via leaf does not attenuate the deleterious effects of salinity on the absolute growth rate of plant height and on the fresh and dry leaf phytomass of the BRS 226 Planalto cashew rootstock.

Palavras-chave:

Anacardium occidentale L.
osmolitos compatíveis
salinidade

ESTRESSE SALINO E APLICAÇÃO EXÓGENA DE PROLINA EM PORTA-ENXERTO DE CAJUEIRO

RESUMO

A escassez de água de boa qualidade e a ocorrência de longos períodos secos são fatores limitantes para a agricultura irrigada, principalmente em regiões semiáridas, o que induz a utilização de águas salinas e o uso de tecnologias que possibilitem seu uso na agricultura. Neste sentido, objetivou-se avaliar a influência de aplicação exógena de diferentes concentrações de prolina sobre crescimento e fitomassa de porta-enxertos de cajueiro anão precoce BRS 226 Planalto irrigados com águas de diferentes níveis de salinidade. O trabalho foi conduzido, em casa de vegetação, no município de Pombal – PB em ambiente protegido, utilizando-se delineamento de blocos casualizados, em esquema fatorial 5 x 4, cujos tratamentos consistiram em diferentes níveis de condutividades elétricas da água de irrigação - CE_a (0,3; 1,0; 1,7; 2,4 e 3,1 dS m⁻¹) associado as concentrações de prolina – P aplicadas via foliar (0; 4; 8 e 12 mM), com três repetições e duas plantas por parcela. A irrigação com águas de até 0,85 dS m⁻¹ podem ser utilizadas para a produção de porta-enxerto de cajueiro BRS 226 Planalto. Concentração de prolina de 8 mM proporcionaram maior fitomassa seca de raiz de porta-enxerto de cajueiro BRS 226 Planalto. O aumento das concentrações de prolina até 12 mM aplicada via foliar não atenuam os efeitos deletérios da salinidade sobre a taxa de crescimento absoluto de altura de planta e sobre a fitomassa fresca e seca de folha do porta-enxerto de cajueiro BRS 226 Planalto.

INTRODUCTION

The cashew tree (*Anacardium occidentale* L.) is one of the most prominent fruit species cultivated in the Brazilian Northeast, being responsible for generating employment, due to the industrialized products derived from its fruit and pseudofruit. The main producing states are Ceará, Piauí and Rio Grande do Norte, which have a planted area of 522,478 hectares, representing 92.60% of the cultivated area in the country (IBGE, 2018).

Despite the importance of this fruit tree, the great genetic variability of the cashew tree found in this region is considered a limiting factor in the expression of the productive potential of the crop. Therefore, in order to try to encourage the expansion of the orchards in these areas, the use of improved plants (early dwarf cashew) has been highlighted to replace the old orchards whose plants were propagated via seeds. These improved genotypes promote precocity and productivity superior to conventional plants, as well as a reduced size, which facilitates harvesting and cultural treatments (SOUZA *et al.*, 2020).

The Northeast region, despite standing out as the largest cashew producer in Brazil, has irregular rainfall, high temperatures and high evapotranspiration rates, which limits water availability for irrigation. Thus, the use of saline water for irrigation appears as an alternative to supply the water demand of the crops, especially in the dry season, when the weirs in the region have reduced volumes of water and higher levels of salts in the water (SILVA *et al.*, 2015). Furthermore, in this region there is a large volume of low quality water.

Despite the possibility of using inferior quality water to irrigate crops, the excess of accumulated salts in the soil can reduce the availability of water for plants, due to the reduction of the osmotic potential in the soil solution (MEDEIROS *et al.*, 2016). Furthermore, it can promote specific toxicity and nutritional imbalance, inducing morphological, structural and metabolic changes in plants, negatively affecting seedling quality, growth and crop production (BEZERRA *et al.*, 2016).

In this context, several studies have a positive correlation between the accumulation of organic

solute or osmolytes (proline, glycine betaine, trehalose, sucrose, among others) and the tolerance of cultures to salt stress (ASHRAF *et al.*, 2011). The increase of these solutes increases the osmotic pressure of the cells, maintaining the water absorption and the cell turgor pressure, which contributes to the continuity of the physiological processes (MARIJUAN; BOSCH, 2013).

Ejaz *et al.* (2012) found an increase in proline in sugarcane plants submitted to 100 mM NaCl and a significant increase in the values of this osmolyte when, together with saline treatment, ascorbic acid was added. Lima *et al.* (2016) using application of proline via leaf observed that the highest values for fresh mass and number of sweet pepper fruits were obtained when using water with 0.6 dS m⁻¹ and doses of proline of 12.0 and 17.0 mM, respectively. According to Molazem and Azimi (2015), salt stress reduces growth and increases the content of proline in corn plants.

Thus, the objective of this study was to evaluate the influence of foliar applications of proline on growth and phytomass production of BRS 226 Planalto precocious dwarf cashew rootstocks irrigated with increasing salinity levels of irrigation water.

MATERIAL AND METHODS

The work was performed from October to December 2017, in a protected environment (greenhouse), at the Agri-Food Science and Technology Center at the Federal University of Campina Grande (CCTA / UFCG), in the municipality of Pombal - PB, located at 6°47'03" S, 37°49'15" W and altitude of 193 m.

A randomized block design was used, in a 5 x 4 factorial scheme, corresponding to five levels of water electrical conductivities - CEa (0.3; 1.0; 1.7; 2.4 and 3.1 dS m⁻¹) and four concentrations of proline (0; 4; 8 and 12 mM), with three replications and two plants per plot, totaling 120 plants. The sketch of the experimental area is shown in Figure 1.

Information on the application of proline in cashew or other fruit trees is scarce. Then, it was necessary to use the doses determined by Lima *et al.* (2016) who verified greater growth of pepper

B1S4N4	B1S3N1	B1S1N1	B1S4N3	B1S5N4	B1S2N2	B1S2N4	B1S5N1	B1S3N2	B1S4N1
B1S3N4	B1S2N3	B1S4N2	B1S5N3	B1S1N3	B1S5N2	B1S3N3	B1S1N2	B1S2N1	B1S1N4
B2S5N1	B2S4N3	B2S1N3	B2S3N2	B2S2N2	B2S1N1	B2S4N2	B2S5N4	B2S3N3	B2S5N2
B2S4N1	B2S1N4	B2S2N4	B2S5N3	B2S3N1	B2S4N4	B2S2N1	B2S3N4	B2S1N2	B2S2N3
B3S4N2	B3S1N1	B3S2N3	B3S1N3	B3S3N4	B3S5N1	B3S4N3	B3S4N4	B3S5N4	B3S2N2
B3S1N2	B3S3N3	B3S2N1	B3S5N2	B3S1N4	B3S3N2	B3S2N4	B3S4N1	B3S5N3	B3S3N1

Figure 1. Sketch of the experimental area S = salinity and N = doses of proline B = block

plants cv. All Big subjected to salt stress when 12.17 mM proline concentrations were used. The salt levels were based on studies by Sousa *et al.* (2011), who observed a marked inhibition in the growth of cashew seedlings BRS 274 and BSR 275 from the saline level of 1.58 dS m⁻¹.

The preparation of saline waters was performed by adding the salts of sodium chloride (NaCl), calcium (CaCl₂.2H₂O) and magnesium (MgCl₂.6H₂O), in the local water supply (CEa of 0.3 dS m⁻¹), maintaining an equivalent ratio of 7: 2: 1. This ratio is predominant in the main sources of water available for irrigation in Northeast Brazil (MEDEIROS, 1992), using the ratio between CEa and the concentration of salts (mg L⁻¹ = 640 x CEa) (RHOADES *et al.*, 2000).

The BRS 226 Planalto cashew clone was used due to the characteristics of rusticity and adaptation to the climatic conditions of the semiarid (FERREIRA-SILVA *et al.*, 2009). The seeds came from a commercial exploration area located in the city of Severiano Melo - RN.

Polyethylene bags with a capacity of 1250 mL were used for the formation of rootstocks, drilled at the base, for free water drainage. The substrate used was composed of eutrophic fluvic neosol (95%) + tanned bovine manure (5%). The soil was collected in the 0-0.20 m layer in Lot 14, Sector

I, of the Irrigated Perimeter Várzeas de Sousa-PB. After filling, the bags were placed on a metal bench (angle bracket), at a height of 0.80 m from the ground to facilitate the handling and application of treatments.

The physicochemical characteristics of the substrate used in the experiment (Table 1) were determined according to the methodology proposed by Claessen (1997) at the Irrigation and Salinity Laboratory at UFCG / Campina Grande-PB.

Sowing was performed using one seed per bag. The substrate was in the field capacity using water supply (ECa of 0.3 dS m⁻¹). The seed was inserted in the vertical position with the base facing upwards (insertion point of the chestnut into the peduncle), at a depth of approximately 1 cm of soil, according to the recommendations of Embrapa - CNPAT (CAVALCANTI JÚNIOR; CHAVES. 2001).

The application of saline water started 25 days after sowing (DAS), with daily watering shift. The irrigation was performed in the late afternoon (5:00 p.m), manually. The applied blade was estimated by the drainage lysimetry principle: difference between the applied and the drained volume in the previous irrigation (Figure 2). In order to reduce the accumulation of salts in the soil, a leach fraction of 0.15 was applied every 10 days.

The application of proline concentrations

Table 1. Physicochemical characteristics of the substrate used in the experiment

		Chemical attributes						
pH _{es}	CE _{es}	P	K ⁺	Na ²⁺	Ca ²⁺	Mg ²⁺	Al ⁺	H + Al
1:2.5	dS m ⁻¹	mgdm ⁻³cmol _c dm ⁻³					
7.22	1.28	0.3	0.3	0.14	3.50	1.7	0.00	0.00
		Physical attributes						
		Sand	Silte	Clay	DS	DP	Porosity	Textural class
	g kg ⁻¹ g cm ⁻³		%	
		767.3	161.6	71.1	1.44	2.75	47.63	Sandy loam

pH_{es} = pH of the substrate saturation extract; CE_{es} = Electrical conductivity of the substrate saturation extract at 25 ° C; Ca²⁺ and Mg²⁺ extracted with 1 M KCl pH 7.0; Na⁺ and K⁺ extracted using 1 M NH₄OAc pH 7.0; Al³⁺e H⁺ extracted using 0.5 M CaOAc pH 7.0; DS- Density of the sun; DP- Particle density.

**Figure 2.** Collection of drained water

also started at 25 DAS and were performed weekly, totaling six applications depending on the treatments. The different concentrations of proline (0, 4.8 and 12 mM) were prepared in water of electrical conductivity of 0.3 dS m⁻¹ and applied via foliar spraying of the leaves on the adaxial and abaxial surfaces. An average volume of 20 mL was applied per plant, totaling 600 mL per treatment. Weeding was done whenever necessary to control the incidence of invasive plants, harmful to the culture of interest.

The effects of the treatments were evaluated by determining the absolute and relative growth rates for plant height (TCAap and TCRap) and stem diameter (TCAdc and TCRdc), in the period between 25 to 65 DAS. Fresh phytomass of stem (FFC) and leaves (FFF) and dry phytomass of

stem (FSC), leaves (FSF) and roots (FSR) from cashew at 65 DAS, as well as the ideal time for the production of cashew rootstocks were also determined.

The determination of the absolute growth rate (TCA) was obtained using the methodology proposed by Benincasa (2003), as described in equation 1:

$$TCA = \frac{(A_2 - A_1)}{(t_2 - t_1)} \quad (1)$$

Where:

A₂ = plant growth (stem diameter or height) at time t₂;

A₁ = plant growth at time t₁; and

$t_2 - t_1$ = time difference between samples.

The relative growth rates (TCR) were obtained by equation 2. The growth is measured as a function of pre-existing matter, adapting to plant stem height or diameter according to Hunt *et al.* (2002).

$$TCR = \frac{(\ln A_2 - \ln A_1)}{(t_2 - t_1)} \quad (2)$$

Where:

A_2 = plant growth (stem diameter or height) at time t_2 ;

A_1 = plant growth at time t_1 ;

$t_2 - t_1$ = time difference between the samples; and

ln = natural logarithm

In order to determine the accumulation of phytomass, the stem of each plant was cut close to the soil. Then, the different parts (stem and leaf) were separated and weighed immediately on a precision scale (0.001 g) to determine the fresh phytomass. After determining fresh phytomass, the different parts of the plant (leaves, stem and root) were packed separately in paper bags properly identified and put to dry in a forced air circulation oven at 65 °C until dry phytomass of leaf (FSF), stem (FSC) and root (FSR) were obtained.

The variables were assessed using analysis of

variance by the F test (0.01 and 0.05 probability) and in cases of significant effect, linear and quadratic polynomial regression analysis was performed using the SISVAR statistical software (FERREIRA, 2011).

RESULTS AND DISCUSSION

Based on the results of the analysis of variance (Table 2) we can verify a significant effect of the interaction between the factors (irrigation water salinity x proline concentration) on the absolute growth rate of the height of the BRS 226 Planalto cashew rootstock. However, for relative growth rate of plant height and absolute and relative growth rate of stem diameter, there is a significant effect only for the irrigation water salinity factor.

Increasing levels of water salinity in interaction with different concentrations of proline inhibited the growth rate of plant height of BSR 226 cashew rootstocks (Figure 3). We can observe that the highest TCAap (0.69 cm d⁻¹) was obtained when the plants were irrigated with CEa of 0.3 dS m⁻¹ in the absence of proline. Despite the decrease at 65 DAS, the AP of the plants were within a standard suitable for grafting.

The effect of salinity on TCAdc is presented in Figure 4A. The best adjustment of the data occurs in a linear equation with a decrease in the rootstock

Table 2. Summary of analysis of variance of the absolute and relative growth rate of plant height (TCAap and TCRap) and stem diameter (TCAdc and TCRdc) of BSR 226 cashew rootstock in the period from 25 to 65 days after sowing. The cashew rootstock were irrigated with water of different levels of salinity under different concentrations of proline

Source of Variation	DF	Mean Square			
		TCAap	TCRap	TCAdc	TCRdc
Saline levels (S)	4	0.11**	0.00009**	0.001**	0.00006**
Linear regression	1	0.41**	0.0003**	0.005**	0.0001**
Quadratic regression	1	0.01ns	0.0000001ns	0.0007**	0.00002*
Proline (P)	3	0.04**	0.00004ns	0.0001ns	0.00001ns
Linear regression	1	0.01ns	0.00004ns	0.00002ns	0.00001ns
Quadratic regression	1	0.09**	0.00005ns	0.00007ns	0.00003ns
Interaction (S x P)	12	0.01*	0.00003ns	0.0004ns	0.00002ns
Block	2	0.005ns	2.16x10 ⁻⁷ ns	0.00008ns	0.000005ns
Error		0.005	0.000007	0.00008	0.000005
CV (%)		13.65	14.01	19.20	20.10

ns not significant; *significant to $p < 0.05$; ** significant to $p < 0.01$;

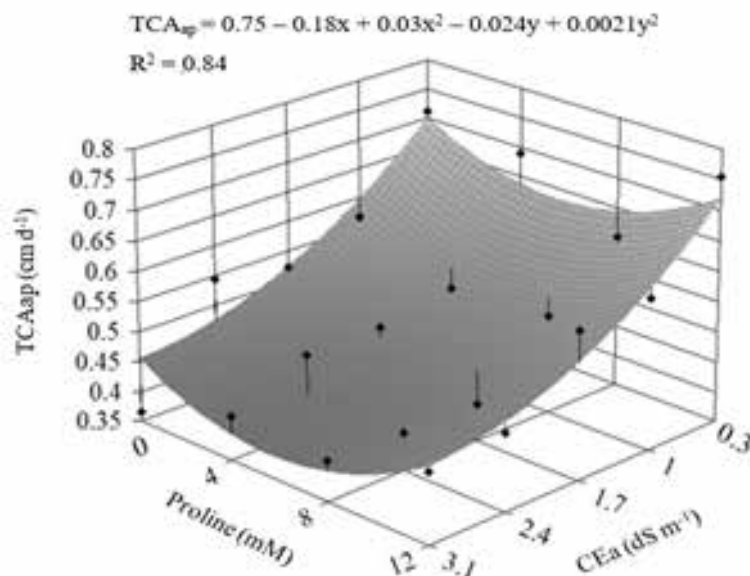


Figure 3. Absolute growth rate - (TCAap) of BRS 226 cashew rootstock as a function of the interaction between water salinity levels (CEa) and proline doses in the period from 25 to 65 days after sowing

submitted to greater CEa (3.1 dS m^{-1}) of 38.30% (0.024 mm d^{-1}). It is noted that this result can occur due to the effect of salinity on the turgor pressure in the cells. The decrease in the water content in the tissues results in a decline in cell wall expansion, causing less plant growth (FREIRE *et al.*, 2010; SOUZA *et al.*, 2017).

Similar behavior was observed for TCRdc (Figure 4B) by the increase in saline levels, with a linear reduction in the order of 12.23% due to a unit increase in CEa. There was a total decrease of 37.91% in the TCRdc of plants subjected to irrigation with CEa of 3.1 dS m^{-1} in relation to irrigated plants with the lowest salinity (0.3 dS m^{-1}). Irrigation with CEa water of 0.99 dS m^{-1} can be used in the production of cashew rootstocks due to the levels of DC and AP are within the standard cited by Serrano *et al.* (2013) which are 5.18 mm (DC) and 33.53 cm (AP). These values promote an acceptable average reduction of 10% over the TCA_{dc} and TCR_{dc} of the BRS 226 cashew rootstock.

TCR_{ap} was negatively and linearly affected by the salinity of the water (Figure 4C). According to the regression equation, there is a decrease of 10.66% per unit increase in CEa, which means that plants irrigated with CEa water of 3.1 dS m^{-1} suffered reductions in this variable of 0.0067 cm

$\text{cm}^{-1} \text{ d}^{-1}$ (29.86%) in relation to those irrigated with a level of 0.3 dS m^{-1} . It is noted that this reduction in plant growth was due to the lower consumption of water and / or nutrients, which can be explained by the osmotic effect since the amount of saline in the soil has been increasing over time (TORRES *et al.*, 2014).

Based on the summary of the analysis of variance (Table 3) we can observe that there was a significant effect of the interaction between the factors (salinity of the irrigation water and different concentrations of proline) for fresh and dry leaf phytomass. However, for fresh and dry stem phytomass there was a significant effect only of the irrigation water salinity factor. For dry root phytomass there was an effect in isolation of the factors salinity and concentration of proline at 65 days after sowing (Table 3).

FFF and FSF were significantly affected by the interaction between salinity of irrigation water and doses of proline. According to the regression equations (Figure 5A and B) we can verify that the plants submitted to 7.3 mM and 12 mM proline and irrigated with 0.3 dS m^{-1} CEa reached the largest fresh and dry phytomasses (9.97 g and $2.51 \text{ g plant}^{-1}$). However, the lowest production of FFF of 4.87 g and FSF 1.30 g were obtained in plants that received the 0 mM dose and irrigated with 3.1 dS m^{-1} CEa, corresponding to a reduction of 22.78 %

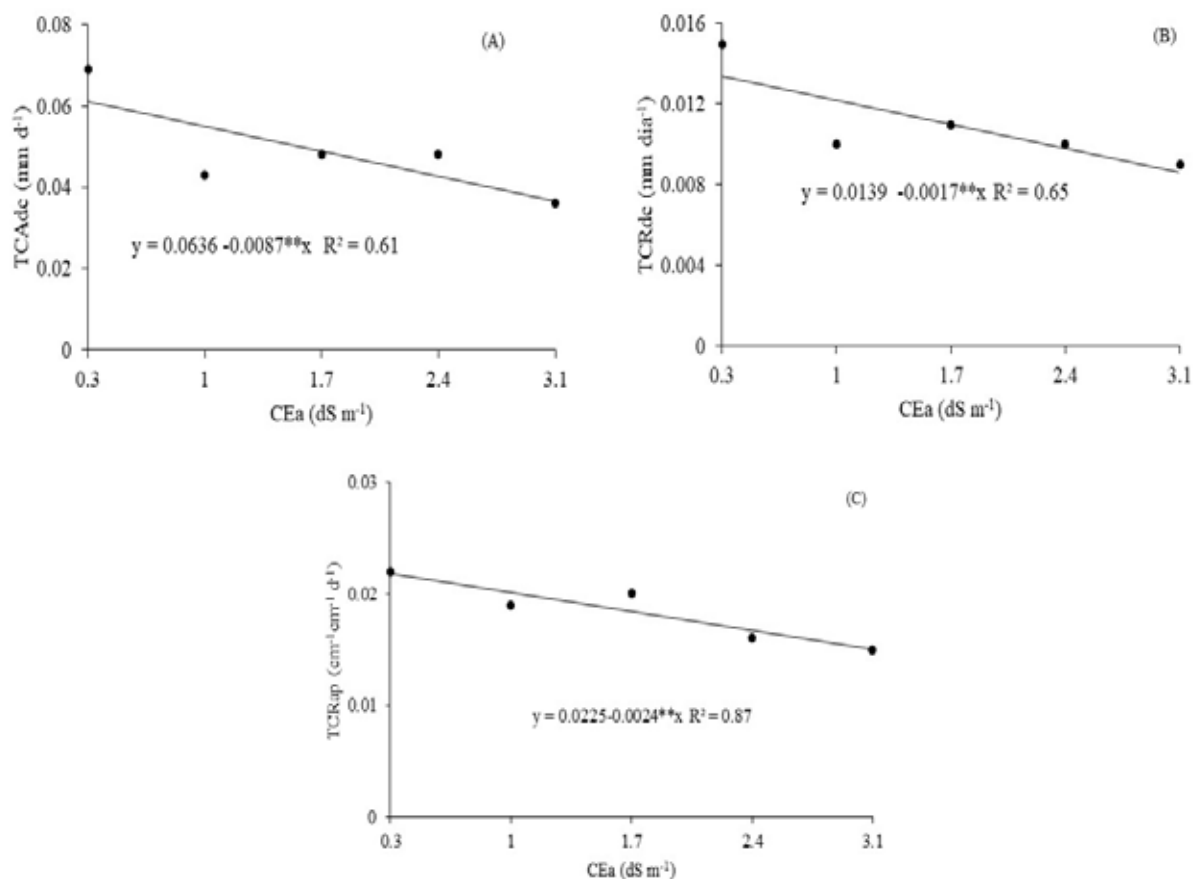


Figure 4. Absolute - TCAdc (A) and relative TCRdc (B) growth rate of stem diameter - and plant height relative growth rate - TCRap (C) as a function of CEA in the period from 25 to 65 days after sowing of the BRS 226 Planalto cashew tree

Table 3. Summary of analysis of variance for fresh phytomass from leaf (FFF), stem (FFC); and dry phytomass from leaf (FSF), stem (FSC) and roots (FSR) of BRS 226 cashew rootstock irrigated with different levels of salinity and different concentrations of proline at 65 days after sowing

Source of Variation	DF	Mean Square				
		FFF	FFC	FSF	FSC	FSR
Saline levels (S)	4	23.90**	11.42**	1.71**	1.33**	1.65**
Linear regression	1	72.18**	35.20**	6.63**	3.96**	3.80**
Quadratic regression	1	12.01**	4.71**	0.19ns	0.69**	1.79**
Proline (P)	3	7.55**	0.43ns	0.29**	0.10ns	0.10**
Linear regression	1	19.34**	0.98ns	0.74**	0.09ns	0.12*
Quadratic regression	1	3.21**	0.29ns	0.02ns	0.04ns	0.09*
Interaction (S x P)	12	5.30**	3.42ns	0.19*	0.26ns	0.13ns
Block	2	0.12ns	1.07ns	0.06ns	0.07ns	0.004ns
Error		0.12	0.22	0.06	0.02	0.02
CV (%)		4.82	9.64	13.71	12.55	14.60

ns not significant; *significant to $p < 0.05$; ** significant to $p < 0.01$.

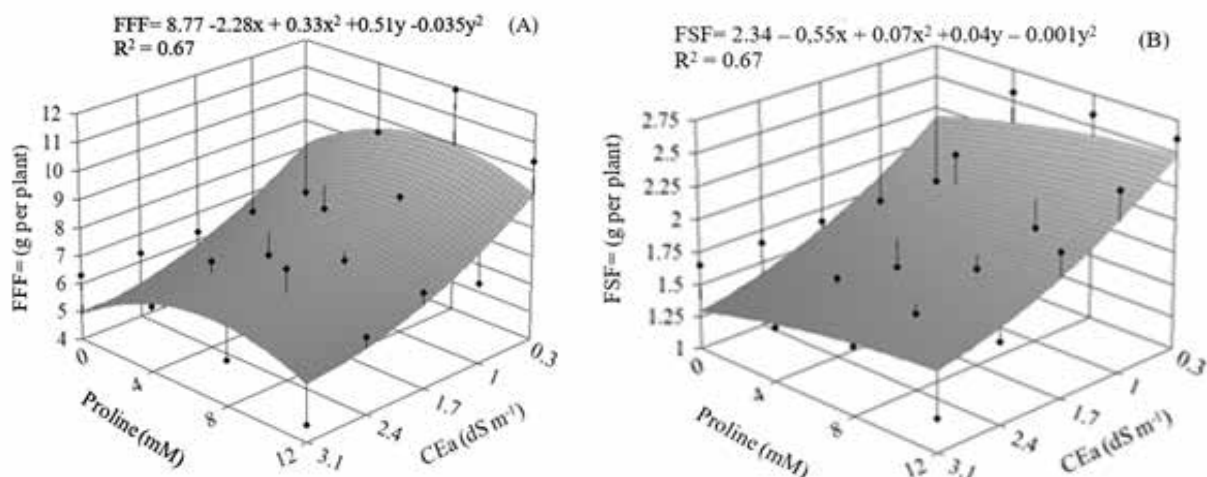


Figure 5. Fresh leaf phytomass - FFF (A) and dry leaf phytomass- FSF (B) of BRS 226 cashew rootstock as a function of the interaction between water salinity (CEa) and doses of proline at 65 days after sowing

(FFF) and 15.13 % (FSF).

This type of behavior demonstrates that the reduction of the photosynthetic process in saline treatment is not only due to the partial closure of the stomatal opening but also due to a damage to the cell structure responsible for CO₂ assimilation. This cell structure damage can be possibly caused by reduction in osmotic-water potential and accumulation of ions above the tolerated range. These changes can affect the accumulation of fresh and dry phytomass of plants grown under salt stress conditions (LACERDA *et al.*, 2012).

The behavior of the FFC and FSC variables as a function of the salinity levels of the irrigation water is shown in Figure 6. We can observe that variables values reduced with the increase of the CEa levels. Maximum values of 6.31 and 1.69 g per plant were found in the ECa of 0.3 dS m⁻¹ and the lowest values of 4.15 g per plant (FFC) and 0.97 g per plant (FSC) were found in the ECa of 3.1 dS m⁻¹. This decrease in the production of phytomass can be associated with the photosynthetic rate and the energy deviation intended for growth. This energy deviation may have been used for the activation and maintenance of metabolic activity associated with adaptation to salinity such as maintaining the integrity of membranes; for synthesis of organic solutes for osmoregulation and / or protection of macromolecules and regulation of ionic transport and distribution in various organs within cells

(SOUZA *et al.*, 2016).

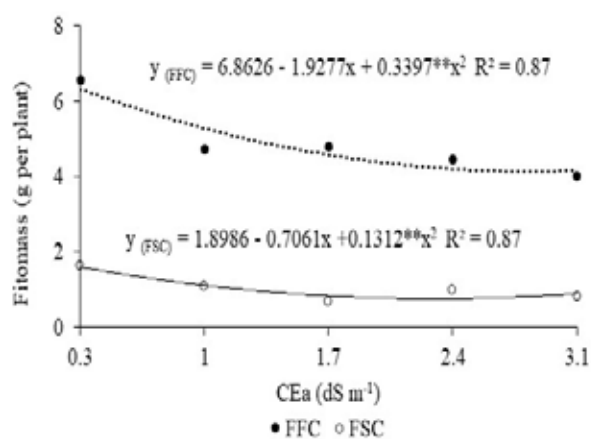


Figure 6. Fresh stem phytomass (FFC) and dry stem phytomass (FSC) of BRS 226 cashew rootstock as a function of water salinity (CEa) at 65 days after sowing

Regarding the effects of salinity on the dry root phytomass of the BRS 226 cashew rootstock (Figure 7A), we can verify a linear reduction with the increase in the electrical conductivity of irrigation water. There was a decrease in this variable of 17.27% due to a unit increase in CEa. This result is equivalent to a reduction of 48.35% (0.70 g per plant) of plants subjected to 3.1 dS m⁻¹ when compared to those that were under the lowest salinity level of (0.3 dS m⁻¹).

The reduction in the biomass accumulation may be the result of mechanisms for adjusting to salt stress conditions. These mechanisms include

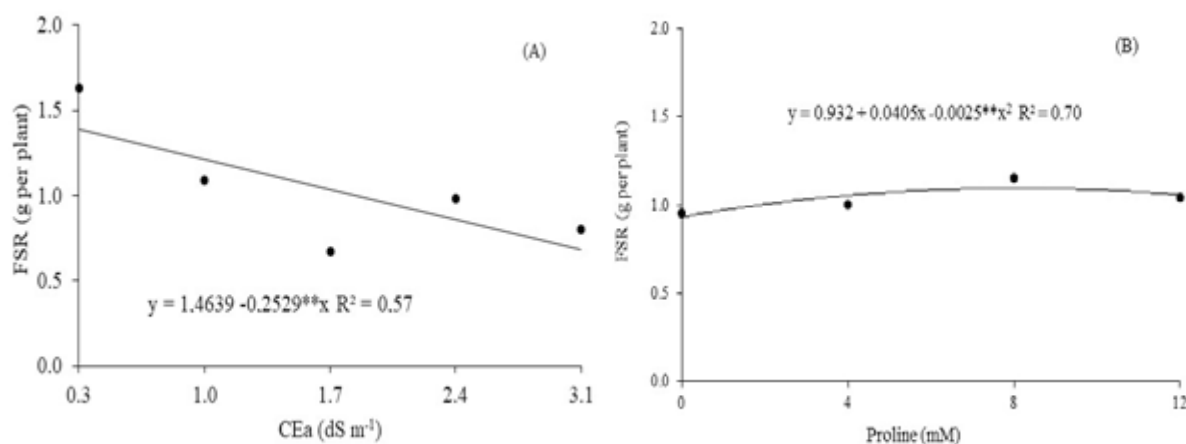


Figure 7. Dry root phytomass (FSR) of BRS 226 cashew rootstock as a function of CEa water salinity (A) and proline concentrations (B) at 65 days after sowing

changes in the ion balance, water potential, mineral nutrition, stomatal closure, photosynthetic efficiency and carbon allocation (SILVA *et al.*, 2008).

According to Figure 7B for FSR at 65 DAS, we can observe a better adjustment of the data in quadratic regression due to the increase in proline concentrations, whose highest FSR value of 1.09 g per plant was obtained when the plants were under the concentration of 8 mM proline. This result may be associated with the ability of a given dose of proline to provide osmotic adjustment without causing tissue damage. Therefore, a protective role was developed through the accumulation of nitrogenous organic compounds, reflecting an osmoregulatory mechanism (LARCEDA *et al.*, 2014).

CONCLUSION

- Irrigation with slightly saline water up to 0.85 dS m⁻¹ can be used for the production of BRS 226 Planalto cashew rootstock.
- 8 mM proline concentration provides greater value for dry roots phytomass of BRS 226 Planalto cashew rootstock.
- The increase in concentrations of proline applied via leaf does not attenuate the deleterious effects of salinity on height growth rate and leaf phytomass of the BRS 226 cashew rootstock.

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