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ÍNDICES FISIOLÓGICOS E CRESCIMENTO DE PORTA-ENXERTOS DE CAJUEIRO SOB ESTRESSE SALINO E CONCENTRAÇÕES DE PROLINA

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1 RESUMO

Na região semiárida a baixa disponibilidade de água de boa qualidade tem se tornado um sério problema para expansão da agricultura, sendo necessário o uso de água com teores elevados de sais. Neste contexto, objetivou-se avaliar os índices fisiológicos e crescimento de clones de cajueiro submetidos à diferentes salinidades da água e aplicação exógena de prolina. O trabalho foi conduzido em condições de casa de vegetação em delineamento de blocos casualizados, distribuídos em fatorial 5 x 4 x 2, correspondente a cinco níveis de condutividades elétricas da água - CEa (0,3; 1,0; 1,7; 2,4 e 3,1 dS m⁻¹), quatro concentrações de prolina - CP (0; 4; 8 e 12 mM) e dois clones de cajueiro (CCP 76 e Faga 11) com três repetições e duas plantas por parcela. A irrigação com água salina limita os índices fisiológicos dos porta-enxertos de cajueiro, no entanto, a CEa de até 2,3 dS m⁻¹ promoveu reduções aceitáveis de menos de 10% nestas variáveis. O clone CCP 76 se destacou apresentando maior alocação de fitomassa seca de folha. A aplicação de prolina na concentração de 7,4 mM promoveu maior alocação de fitomassa seca de caule dos porta-enxertos de cajueiro irrigados com águas salinas.

Palavras-chave: Anacardium occidentale L., escassez hídrica, atenuante

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PHYSIOLOGICAL INDICES AND GROWTH OF CASHEW CLONES UNDER SALT STRESS AND PROLINE APPLICATION

2 ABSTRACT

In the semi-arid region, the low availability of good quality water has become a serious problem for the expansion of agriculture, requiring the use of water with high levels of salts. In this context, this study aimed to evaluate the physiological indices and growth of cashew

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clones subjected to different salinity of water and exogenous application of proline. The study was conducted under greenhouse conditions in a randomized block design, distributed in a factorial 5 x 4 x 2, corresponding to five levels of electrical conductivity of the water - ECw (0.3; 1.0; 1.7; 2.4 and 3.1 dS m⁻¹), four proline concentrations - PC (0; 4; 8 and 12 mM) and two cashew clones (CCP 76 and Faga 11) with three replicates and two plants per plot. Irrigation with saline water limits the physiological indices of cashew rootstocks; however, ECw up to 2.3 dS m⁻¹ promoted acceptable reductions of less than 10% in these variables. Clone CCP 76 stood out with greater allocation of phytomass. The application of proline at a concentration of 7.4 mM promoted a greater allocation of stem dry phytomass of the cashew rootstocks irrigated with saline water.

Keywords: Anacardium occidentale L., water scarcity, mitigating

3 INTRODUCTION

The cashew tree (Anacardium occidentale L.) plays a fundamental role in the economy of the semiarid region of Brazil, making this region the largest producer of nuts, with a planted area of approximately 616,189 hectares, accounting for approximately 98.7% of national production, with the states of Ceará (42,597 tons), Rio Grande do Norte (33,912 tons) and Piauí (28,292 tons) standing out as the main producers (SUASSUNA et al., 2017).

Cultivated areas in semiarid Northeast China are subject to water scarcity due to high temperatures and irregular rainfall, with water with high salt content being common (SERRANO et al., 2013), a situation that generally forces seedling producers to use low-quality water for irrigation.

Soil salinity caused by inadequate management of agricultural areas as well as the use of water rich in salts has progressively increased the levels of Na+ and Cl⁻ in the rhizosphere, leading to growth inhibition and directly affecting crop production (HOLANDA FILHO; SANTOS; AZEVEDO, 2011).

The use of these waters in crop irrigation promotes distinct responses in plants, depending on the genotype, plant development phase, cationic and/or anionic nature of the water and the time of exposure

to salinity, irrigation management, and edaphoclimatic conditions, among other factors (ALVARENGA et al., 2019).

Thus, research aimed at reducing the damage caused by salinity to plants under saline stress conditions has established important cultivation strategies and greater economic and social expression, since this information can be transmitted to rural producers (SILVA, 2016).

Under abiotic stress conditions, the amino acid proline accumulates in plants (LIMA et al., 2016) and activates several mechanisms, such as osmotic adjustment, without causing injury to plant tissues; the carbon and nitrogen reserves used in growth for recovery after stress; the detoxification of excess ammonia, proteins and membrane stabilizers; and the use of free radical scavengers (PAULUS et al., 2010). Thus, the accumulation of proline can increase the degree of tolerance of plants to salt stress.

Thus, the objective of this work was to evaluate the physiological indices and growth of cashew clones subjected to different saline levels of irrigation water and exogenous applications of proline.

4 MATERIALS AND METHODS

The research was conducted between October and December 2017 in an

agricultural greenhouse located at the Center for Agro-Food Science and Technology of the Federal University of Campina Grande (CCTA/UFCG), in the municipality of Pombal (PB), located at the geographic coordinates of 6°47'03" S, 37°49'15" W and an altitude of 144 m.

A randomized block design was adopted, in a $5 \times 4 \times 2$ factorial scheme, corresponding to five levels of water electrical conductivity (ECa (0.3; 1.0; 1.7; 2.4 and 3.1 dS m $^{-1}$)), four proline concentrations (0; 4; 8 and 12 mM), and two early dwarf cashew clones (CCP 76 and Faga 11) with three replicates and two plants per plot, totaling 240 experimental plots.

Owing to the lack of information on the application of proline in cashew or other fruit trees, the present study used a proline concentration (12 mM) that provided the greatest growth of 'All Big' pepper, as observed in the study by Lima et al. (2016).

The electrical conductivity levels of the water were based on the study developed by Sousa, Bezerra and Farias (2011), who reported marked inhibition of the growth of cashew seedlings BRS 274 and BSR 275 from a salinity level of 1.58 dS m⁻¹.

The saline waters were prepared by adding sodium chloride (NaCl), calcium (CaCl 2.2H 2 O) and magnesium (MgCl 2.6H 2 O) salts to the local water supply (ECw of 0.3 dS m⁻¹), maintaining an equivalent proportion of 7:2:1, a ratio that

predominates in the main water sources available for irrigation in Northeast Brazil (MEDEIROS, 1992), using the relationship between ECw and salt concentration (mg L $^{-1}$ = 640 × ECw) (RICHARDS, 1954; RHOADES et al., 2000).

The dwarf-early cashew clone used in the experiment was CCP 76, owing to its adaptability to different environments and the attractiveness and quality of the peduncle (VIDAL NETO et al., 2013), and Faga 11, because it is recommended for edaphoclimatic conditions of the semiarid Northeast, in addition to presenting characteristics such as high production and large and heavy nuts (ALMEIDA, 2002).

Seedlings were planted in 1250 mL polyethylene containers with a hole in the bottom for excess water drainage. The substrate was prepared from a Eutrophic Fluvic Neosol (95%) + aged cattle manure (5%); the soil was collected from the 0–0.20 m layer at Lot 14, Sector I, of the Várzeas de Sousa-PB Irrigated Perimeter. After filling, the containers were arranged on a metal bench (corner) at a height of 0.80 m above ground level to allow for better crop management.

The physical and chemical characteristics of the substrate used in the experiment (Table 1) were determined at the Irrigation and Salinity Laboratory of UFCG/Campina Grande, PB, according to the methodologies proposed by Teixeira (2017).

Chemical characteristics									
	F		K^+	In the +	Ca	Mg	$H^{+} + Al^{3+}$		
рΗ	(mg k	(g -1)			2+	2+		-	Ees
F**			(cmol c kg ⁻¹					$(dS m^{-1})$	
)						
7.00	0.3	30	0.30	0.14	3.50	1.70	0.00	0.70	
Physical characteristics									
Granulometric fraction (g kg ⁻¹)			- Textural	Humidity (kPa) Total			Total	Ds	Dp
Sand Silt Clay		class	1519.5 dag kg ⁻¹		porosity %	(kg dm ⁻³)			
					•••••				
767.30	161.60	71.10	FAN	11.60	4.23		47.63	1.44	2.75

Table 1. Physical and chemical characteristics of the substrate used in the experiment.

pH = pH of the 1:2.5 substrate:water suspension; CEes = electrical conductivity of the substrate saturation extract at 25 °C; Ca $^{2+}$ and Mg $^{2+}$ extracted with 1 M KCl, pH 7.0; Na $^{+}$ and K $^{+}$ extracted with 1 M NH $_4$ OAc, pH 7.0; Al $^{3+}$ and H $^{+}$ extracted with 0.5 M CaOAc, pH 7.0; AD – available water; DS- soil bulk density; DP- particle density.

After the soil moisture content was increased to the level corresponding to the maximum water retention capacity, sowing was carried out by placing one seed per container. The seed was inserted in a vertical position with the base facing upward (the point where the nut was inserted into the peduncle) at a depth of approximately 1 cm of soil, according to the recommendations of Embrapa - CNPAT (CAVALCANTI JÚNIOR; CHAVES, 2001).

Saline water application began 25 days after sowing (DAS), with daily irrigation at 5:00 p.m., which was performed manually via a graduated cylinder. The applied depth was estimated via the drainage lysimetry principle. To reduce excessive salt accumulation in the soil, a leaching fraction of 0.15 was applied at 10-day intervals.

Proline concentrations were also applied at 25 DAS, weekly at 7-day intervals, totaling six applications according to the treatments. The different proline

concentrations (4, 8, and 12 mM) were prepared in water with a lower ECw (0.3 dS m⁻¹) and applied via foliar spray on the adaxial and abaxial surfaces, with an average volume of 20 mL per plant, totaling 600 mL per treatment. Plants that could serve as hosts for pests and diseases harmful to the crop were eliminated, in addition to periodic superficial scarification of the soil.

The effects of the different treatments were evaluated at 65 DAS, which was considered the average time required for the plants to be ready for grafting (SERRANO et al., 2013). The following parameters were measured: the plant height/stem diameter (AP/DC) ratio, leaf dry matter allocation (AFSF), stem dry matter allocation (AFSC) and root dry matter allocation (AFSR) of the cashew clones.

The allocation of dry matter to different organs (leaves, stems and roots) was determined according to Benincasa (2003), Equation 1.

(1)

where:

MSorgan - dry mass of the organ (leaf, stem or root)

MStotal - total dry mass

The shoot dry matter production index (IPFPA) was also evaluated via Equations 2 and 3 according to the methodology of Mantovani (1999) and Benincasa (2003), respectively.

$$IEF = (FSF)/(AF)$$
 (2)

where:

IEF - sclerophilia indices - gm⁻²; FSF - dry leaf phytomass; and AF - leaf area;

$$IPFPA = (FSPA)/(FST)$$
 (3)

where:

IPFPA - dry matter production index of the aerial part;

FSPA - dry aerial part phytomass; and FST - total dry matter.

Furthermore, the LMR obtained via Equation (4) was determined.

$$RMF = (FSF)/(FST) \tag{4}$$

where:

RMF - Leaf mass ratio, g g ⁻¹;

FSF - dry leaf phytomass; and FST - total dry matter.

The data were evaluated via analysis of variance and the F test (0.01 and 0.05 probability). In cases of significant effects, linear and quadratic polynomial regression analyses were performed for the water salinity factor and proline concentrations, and a mean test (Tukey) was performed for the cashew clones via Sisvar statistical software (FERREIRA, 2011).

5 RESULTS AND DISCUSSION

There was a significant effect of the interaction between the factors proline concentrations and cashew clones only for the allocation of root dry matter (AFSR) (Table 2). Water salinity significantly influenced the allocation of leaf dry matter (AFSF) and stem dry matter (AFSC), and proline concentration alone also influenced AFSF, AFSC, and AFSR. significantly whereas cashew clones affected all the variables (Table 2) at 65 days after sowing (DAS).

Table 2. Summary of analysis of variance for the plant height/stem diameter (AP/DC) ratio and allocation of leaf (AFSF), stem (AFSC) and root (AFSR) dry matter of cashew clones irrigated with saltwater and subjected to proline application at 65 days after sowing

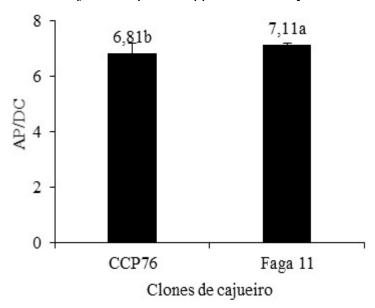
sowing			М	C			
Sources of variation	GL	Mean Square					
Sources of variation		AP/DC	AFSF	AFSC	AFSR		
Saline Levels (SL)	4	1.07 ns	73.73 **	24.64 *	81.67 ns		
Linear Reg.	1	1.53 ns	196.07 **	49.77 *	48.22 ns		
Quadratic Reg.	1	0.69 ns	19.07 ns	24:30 ns	86.31 ns		
Proline (P)	3	$0.09^{\rm ns}$	34.10 *	86.65 **	19.29 **		
Linear Reg.	1	0.23^{ns}	41.41 *	100.65 **	12.91 ^{ns}		
Quadratic Reg.	1	$0.01^{\rm ns}$	44.43 *	158.44 **	35.08 **		
Clones (C)	1	2.76 **	60,984 **	1193.41 **	97.07 **		
Interaction (NS x P)	12	2.27 ns	69.12 ns	100.04 ns	42.39 ns		
Interaction (NS x C)	4	$0.73^{\rm ns}$	17.68 ns	61.09 ns	24.57 ns		
Interaction (P x C)	3	0.58 ns	20.17 ns	79.89 ns	41.18 **		
Interaction (NS x P x	12	1.65 ns	69.88 ns	41.37 ns	45.47 ns		
C) .							
Blocks	2	0.39 ns	12.91 ns	4.52 ns	2.77 ns		
CV (%)		8.39	6.36	8.72	8.75		

us, $\overline{*, **}$ respectively, not significant, significant at p < 0.05 and p < 0.01;

Figure 1 shows that the cashew clone Faga 11 differed statistically from CCP 76, with the plant height/upper stem diameter ratio being 4.40% greater than that of the clone CCP 76. The lower AP/DC ratio of clone CCP76 is of utmost

importance in studies on rootstock formation, since DC is considered one of the most important characteristics of rootstocks at the time of grafting (SOUZA et al., 2015).

Figure 1. Plant height/stem diameter relationship - AP/DC of cashew clones irrigated with saline water and subjected to proline application 65 days after sowing.

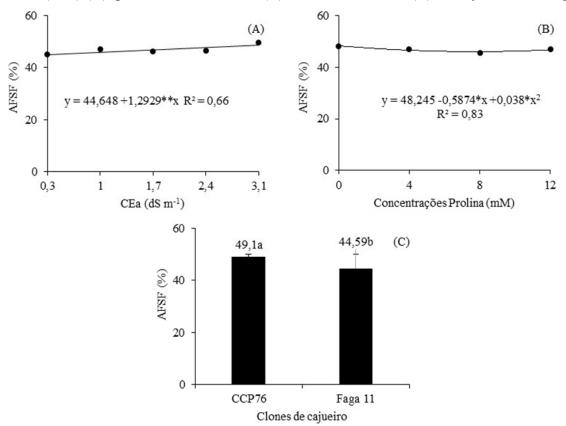


Means followed by different letters indicate significant differences between clones according to Tukey's test (p < 0.05).

Water salinity caused a linear increase of 2.89% per unit increase in salinity in the allocation of dry phytomass to cashew rootstock leaves (Figure 2A). In other words, plants subjected to a higher CEa (3.1 dS m ⁻¹) increased the CEa by 8.10% compared with the control (CEa = 0.3 dS m ⁻¹), demonstrating that there was

less export of phytomass to the leaf (considered the production center) compared with other parts of the plant. As the salinity of the irrigation water increased, there was greater export of assimilates from the leaf to other plant organs (XAVIER et al., 2014).

Figure 2. Allocation of leaf dry matter (AFSF) as a function of irrigation with saline water (CEa) (A), proline concentrations (B) and cashew clones (C) 65 days after sowing.



Means followed by different letters indicate significant differences between clones according to Tukey's test (p < 0.05).

Foliar application with different concentrations of proline had a quadratic effect on AFSF (Figure 2B) and AFSC (Figure 3B) at 65 DAS. According to the regression equations, the maximum values of 48.24% (AFSF) and 32.74% (AFSC) were reached in plants subjected to

concentrations of 0 and 7.4 mM, respectively. The decrease in AFSCs (Figure 3A) may be a reflection of the decline in CO2 assimilation due to the increase in the concentration of proline supplied via foliar application (MONTEIRO et al., 2014).

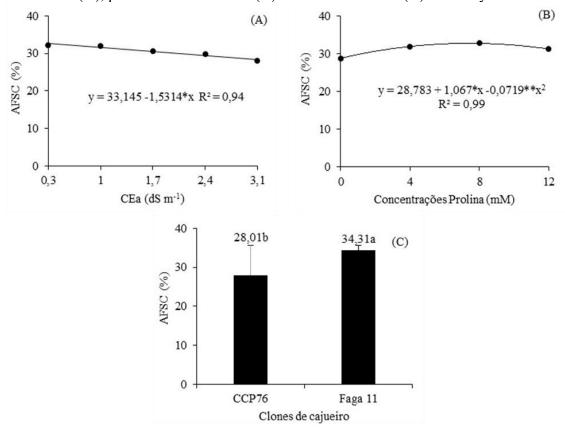


Figure 3. Allocation of stem dry matter—AFSCs as a function of saline water irrigation—CEa (A), proline concentrations (B) and cashew clones (C) at 65 days after sowing

Means followed by different letters indicate significant differences between clones according to Tukey's test (p < 5%).

For AFSF (Figure 2C), clone CCP 76 was 9.18% superior to clone Faga 11. However, for AFSCs (Figure 3C), the opposite behavior was observed, with clone Faga 11 being superior to CCP 76 by 22.49%. Thus, clone CCP 76 stood out in terms of growth, perhaps because it provided greater CO2 entry into the substomatal chambers (WEHR et al., 2017).

The allocation of stem dry matter decreased linearly in response to increasing salinity levels in the irrigation water, with a 4.62% reduction per unit increase in salinity and a total loss of 12.93% in plants irrigated with water with a salinity of 3.1 dS m⁻¹ compared with those grown under the lowest ECw level (Figure 3A). This corresponds to a 10% reduction when water with an ECw of 2.81 dS m⁻¹ was used. The

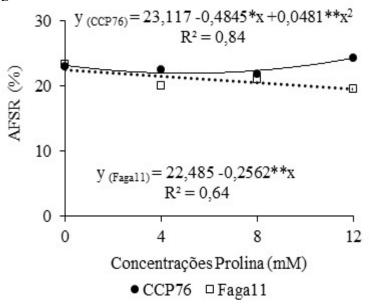
reduction in phytomass allocation is a consequence of the reduced photosynthetic rate and the diversion of energy allocated to growth toward the activation and metabolic maintenance of activities associated with adaptation to salinity, such maintaining membrane integrity, solutes synthesizing organic for osmoregulation and/or protection of macromolecules, and regulating ionic transport and distribution in various organs and within cells (XAVIER et al., 2014).

In terms of AFSR as a function of the interaction between proline concentration and cashew clone (Figure 4), the regression equation revealed that the highest AFSR value (24.22%) in clone CCP 76 was obtained when a proline concentration of 12 mM was applied at 65

DAS. For clone Faga 11 (Figure 4), a linear reduction in AFSR was detected, where plants that received the highest concentration of proline (12 mM) presented a decrease of 13.67% compared with that of the plants in the control treatment (0 mM). According to Araújo et al. (2017), decreases in phytomass allocation are caused by a

decrease in photosynthesis, as well as the redirection of energy that would be used for growth to other metabolic activities related to acclimation to the stress suffered by the cashew clone, such as the maintenance of membrane stability, the production of organic solutes and control of the transport and distribution of ions.

Figure 4. Allocation of root dry matter (AFSR) as a function of the interaction between proline concentrations and cashew clones irrigated with saltwater at 65 days after sowing



A significant interaction effect on the shoot dry matter production index was observed between the proline concentration and cashew clone (Table 3). The factors of salinity level, proline concentration, and cashew clone individually affected the L/M ratio. The sclerophylly index was significantly influenced by the saline concentration and proline concentration individually.

Table 3. Summary of analysis of variance for the shoot dry matter production index (APFI), leaf mass ratio (LMR) and sclerophylly index (SSI) of cashew clones irrigated with saltwater and proline concentrations at 65 days after sowing.

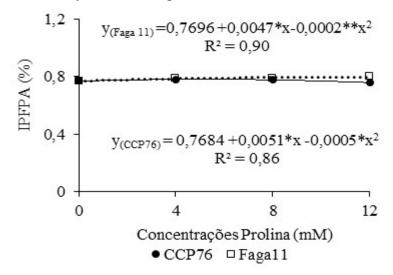
Courses of evenieties	CI	Mean Squar	e	
Sources of variation	GL	IPFPA	RMF	IEF
Saline Levels (SL)	4	0.007 ns	0.007 **	0.00002 *
Linear Reg.	1	0.004 ns	0.02 **	0.00007 st
Quadratic Reg.	1	0.009 ns	0.001 ns	0.000007^{ns}
Proline (P)	3	0.001 *	0.003 *	0.00002 *
Linear Reg.	1	0.001 *	0.003 *	0.00004 st
Quadratic Reg.	1	0.003 ns	0.004 ns	$0.000008\ ^*$
Clones (C)	1	0.01 **	0.05 **	$0.00002~^{\mathrm{ns}}$
Interaction (NS x P)	12	0.004 ns	0.006 ns	0.00004 ns
Interaction (NS x C)	4	0.002_{ns}	0.001 ns	$0.00002^{\text{ ns}}$
Interaction (P x C)	3	0.004 **	0.002 ns	$0.00001 ^{\mathrm{ns}}$
Interaction (NS x P x C)	12	0.004 ns	0.006 ns	$0.00002^{\text{ ns}}$
Blocks	2	0.0002 ns	0.001 ns	8x10 ^{-7ns}
CV (%)		2.44	6.48	22.09

us, *, *** respectively, not significant, significant at p < 0.05 and p < 0.01;

The proline concentration had a quadratic effect on the dry matter production index of the aerial part (Figure 5) of the cashew clones Faga 11 and CCP 76, with an increase as a function of the proline concentration applied via foliar application, with the maximum estimated value (0.79%) obtained in plants that

received 12 mM (Faga 11) and 4.8 mM (CCP 76) proline corresponding to 0.78% IPFPA. This increase may be related to the fact that the leaves at the highest levels are thicker and less flexible, a form of adaptation to resist the negative effects caused by the application of proline (SANTOS et al., 2016).

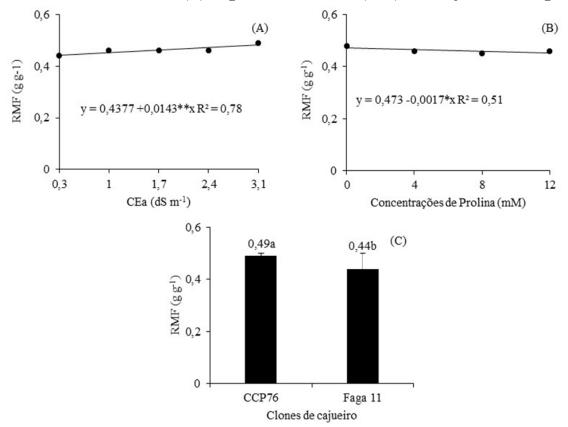
Figure 5. Dry matter production index of the aerial part – IPFPA as a function of the interaction between proline concentrations and cashew clones irrigated with saltwater at 65 days after sowing



The leaf mass ratio (LMR) increased as the salinity of the irrigation water increased (Figure 6A), with an increase of 3.27% per unit increase in ECw; that is, seedlings irrigated with an electrical conductivity of 3.1 dS m⁻¹ presented an increase of 9.14% compared with those subjected to an ECw of 0.3 dS m⁻¹. The

behavior observed in this research may be related to greater energy expenditure by the plant to increase the concentration of ions and secondary metabolites within it and thus reduce its water potential to values lower than those found in the soil, which caused an increase in the amount of water within it (SHELDON et al., 2017).

Figure 6. Leaf mass ratio (RMF) as a function of salinity level (A), proline concentration (B) and cashew clone (C) irrigated with saltwater (CEa) at 65 days after sowing



Means followed by different letters indicate significant differences between clones according to Tukey's test (p < 0.05).

For RMF (Figure 6B), a decrease of 0.35% was observed per unit increase in proline at 65 DAS. The RMF was affected by the increase in proline concentration, reaching a decrease of 4.31% at the highest proline concentration (12 mM) in relation to the control treatment. According to Lima et al. (2016), high concentrations of proline applied via foliar application can have deleterious effects on plants, as they can

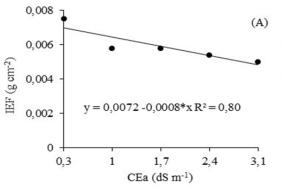
probably cause changes in the cytosolic pH and redox state.

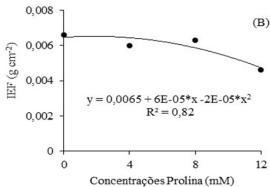
Cashew clones differed significantly from each other, with clone CCP 76 being statistically superior to Faga 11 in terms of RMF at 65 DAS (Figure 6C). Engineer et al. (2016) reported that a high leaf mass ratio may correlate with better crop yield; thus, low adaptation may result in suboptimal yields.

The sclerophylly index (SII) decreased linearly with increasing water salinity, with an index of 0.0047 g cm⁻² when the plants were subjected to an ECw of 3.1 dS m⁻¹, that is, a reduction of 31.11% (0.0022 g cm⁻²) compared with that of the plants subjected to the lowest salinity level (Figure 7A). When irrigating

with an ECw of 1.17 dS m⁻¹, a 10% reduction in the SII was observed. The sclerophylly index indicates the amount of dry mass per unit area; thus, the reduction in this index demonstrates that irrigation with saltwater reduces the thickness of the leaf blade (FURTADO et al., 2013).

Figure 7. Sclerophylly index – IEF as a function of salt level – CEa (A) and proline concentration (B) at 65 days after sowing





The sclerophylly index (SFI) fit the quadratic regression model best (Figure 7B), with the maximum estimated value (0.0065 g cm⁻²) reached in plants sprayed with 1.6 mM proline, which decreased from this concentration and reached the lowest value under 12 mM. The decline in the SFI can be attributed to the increase in proline due to the intensification of its deleterious effect on the sclerophylly index.

6 CONCLUSION

Irrigation with saline water limits the physiological indices of cashew rootstocks; however, water with an ECw of up to 2.3 dS m⁻¹ can be used, as it promotes acceptable reductions of 10% in these variables. Clone CCP 76 stood out for its greater phytomass allocation. The application of proline at a concentration of 7.4 mM promoted greater allocation of stem dry matter in cashew rootstocks irrigated with saltwater.

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