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Morphophysiology of 'Faga 11' Cashew Rootstock Under Saline Water Irrigation and Exogenous Proline Application

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Abstract

Due to the reduced availability of good-quality water in the semi-arid region of Northeast Brazil, the utilization of saline waters in irrigation became an alternative for the expansion of agriculture in this region. Thus, it is necessary to develop techniques which can make viable the use of these waters in agriculture. Given the above, this study aimed to evaluate the morphophysiology of 'FAGA 11' cashew rootstock subjected to different levels of irrigation water salinity and exogenous proline application through the leaves. The experiment was carried out in a greenhouse of the Federal University of Campina Grande, at the Center of Sciences and Agri-Food Technology, Campus of Pombal, PB, Brazil, in a randomized block design, in 5 × 4 factorial scheme, with three replicates and two plants per plot. Treatments consisted of different levels of irrigation water electrical conductivity—ECw (0.3; 1.0; 1.7; 2.4 and 3.1 dS m^{-1}) combined with proline concentrations applied through the leaves—PC (0; 4; 8 and 12 mM). Irrigations with water up to ECw of 1.37 dS m^{-1} may be used as it causes an acceptable 10% reduction in the variables of morphology of cashew FAGA 11 seedlings. The proline concentrations tested, with the exception of the Dickson's quality index of seedlings, did not attenuate the deleterious effects of irrigation water salinity on the cashew FAGA 11 rootstock seedlings.

Keywords: *Anacardium occidentale* L., saline stress, compatible osmolytes

1. Introduction

Cashew (*Anacardium occidentale* L.) is one of the most important fruit species cultivated in Northeast Brazil, responsible for the generation of jobs because of the industrialized products from its fruit and pseudofruit, especially for the states 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).

Most cashew plantations in Brazil are located in the semi-arid region, where there is negative water balance during the year. Thus, irrigation becomes essential to guarantee the agricultural production. However, the waters used for irrigation normally have high concentrations of salts, above 2 dS m^{-1}, which can hamper germination, initial growth, physiology and production of the plants through the toxicity caused by toxic ions (Na^+ and Cl^-) and nutritional imbalance (Silva et al., 2011, 2017).

A practice that can allow saline waters to be used in irrigation is the exogenous application of compatible solutes, especially proline, as observed in other crops such as melon (Lacerda et al., 2012), watermelon (Lacerda et al., 2014), pigeon pea (Monteiro et al., 2014) and bell pepper (Lima et al., 2016); however, there are no studies with the cashew crop.

Proline is an amino acid which accumulates in plants in situations of abiotic stress. Consequently, increased proline contents can activate various cell functions such as osmotic adjustment, without causing damages to plant tissues, reserves of carbon and nitrogen used for growth and reestablishment after stress, detoxification from excess ammonia, besides acting as stabilizer of proteins and membranes, and eliminator of free radicals. Thus, proline accumulation can increase plants' level of tolerance to salt stress (Paulus et al., 2010).

This fact makes the proline an attenuating potential of saline stress during the formation of cashew rootstocks promoting the quality of seedlings which is greatly affected by saline stress.

Given the above, this study aimed to evaluate the morphophysiology of 'FAGA 11' cashew rootstocks as a function of saline water irrigation and foliar application of proline.

2. Material and Methods

2.1 Experiment Localization and Treatments

The study was carried out from October to December 2017 in a protected environment (greenhouse) at the Center of Sciences and Agri-Food Technology of the Federal University of Campina Grande (CCTA/UFCG), in the municipality of Pombal - PB, Brazil (6º47′03″ S; 37º49′15″ W; ~193 m).

The experiment was set in a randomized block design in 5 × 4 factorial scheme, corresponding to five levels of electrical conductivity of water—ECw (0.3; 1.0; 1.7; 2.4 and 3.1 dS m^{-1}) and four proline concentrations (0; 4; 8 and 12 mM); together, these factors resulted in 20 treatments, with three replicates and two plants per plot, totaling 120 plants.

Proline concentrations were determined based on the work of Lima et al. (2016), who observed higher growth of bell pepper plants cv. 'All Big' subjected to saline stress when proline concentration of 12.17 mM was used. Salinity levels were based on the work of Sousa et al. (2011), who observed accentuated inhibition of cashew growth from the saline level of 1.58 dS m^{-1}.

Saline waters were prepared by the addition, in water from the local supply system (ECw of 0.3 dS m^{-1}), of sodium chloride (NaCl), calcium chloride ($CaCl_2 \cdot 2H_2O$) and magnesium chloride ($MgCl_2 \cdot 6H_2O$), in equivalent ratio of 7:2:1, which prevails in the sources of water available for irrigation in Northeast Brazil (Medeiros, 1992), according to the relationship between ECw and the concentration of salts (mg L^{-1} = 640 × ECw) (Rhoades et al., 2000).

The cashew clone 'FAGA 11' was studied because it is recommended for rainfed cultivation in the semi-arid region of Northeast Brazil and due to characteristics such as high yield and large and heavy nuts (Almeida, 2002). Cashew seeds came from a commercial plantation area located in the municipality of Severiano Melo, RN.

2.2 Plant Material and Management of the Experiment

Rootstocks were produced in 12 × 20 cm polyethylene bags, perforated at the bottom to allow free drainage. The substrate used was composed of eutrophic Fluvic Neosol (95%) + decomposed bovine manure (5%). The soil was collected in the 0-20 cm layer in the Lot 14, Sector I, of the Irrigated Perimeter of Várzeas de Sousa – PB. The bags were arranged on metal benches at 0.8 m height from the soil to facilitate management and application of treatments.

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

Tabela 1. Chemical and physical characteristics of the substrate used in the experiment

Chemical characteristics						
pH (1:2.5)	ECse (dS m^{-1})	P (mg dm^{-3})	K	Na ($cmol_c$ dm^{-3})	Ca ($cmol_c$ dm^{-3})	Mg ($cmol_c$ dm^{-3})
7.22	1.28	0.30	0.14	3.50	1.70	0.14
Physical characteristics						
Size fraction (g kg^{-1})			Textural class	Total porosity (%)	AD (kg dm^{-3})	DP (kg dm^{-3})
Sand	Silt	Clay				
767.3	161.6	71.1	SL	47.63	1.44	2.75

Note. Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4 OAc at pH 7.0; ECse: Electrical conductivity of the saturation extract; SL: Sandy loam; AD: Apparent density; DP: particle density.

Sowing was carried out by planting one nut per bag in the substrate, maintained at field capacity by the addition of public-supply water (ECw of 0.3 dS m^{-1}). The nuts were planted in a vertical position with the base facing up (point of attachment to the cashew apple), at depth of approximately 1 cm, as recommended by EMBRAPA-CNPAT (Cavalcanti Júnior & Chaves, 2001).

Application of treatments or saline waters began at 25 days after sowing (DAS), with interval of one day, and irrigation was manually performed in the late afternoon (5:00 p.m.), using water depths based on the process of drainage lysimetry (using 20 bags with one collector in each), determined by the difference between the applied volume and drained volume in the previous irrigation (Bernardo et al., 2006). Every 10 days, the substrate received a leaching fraction of 0.15, based on the volume applied during this period, in order to reduce the accumulation of salts.

Likewise, proline solutions at concentrations according to treatment began at 25 DAS, every week. The different quantities of proline (0, 4, 8 and 12 mM) were separately diluted in water of the local supply system (electrical conductivity of 0.3 dS m^{-1}) and applied through foliar spraying, using a volume of 20 mL per plant, in a total of 600 mL per treatment.

2.3 Variables Measured

Manual weeding was performed always when necessary to control the incidence of invasive plants, which are harmful to the crop. The growth of the 'FAGA 11' cashew rootstock was evaluated at 65 days after sowing (DAS) based on stem diameter (SD), plant height (PH) and leaf area (LA). In addition, stomatal conductance (gs), transpiration (*E*) and CO_2 assimilation rate (*A*) were measured using an infrared gas analyzer—IRGA (LCI System, ADC, Hoddesdom). Phytomass accumulation was determined at 65 DAS, based on root dry phytomass (RDP) and total dry phytomass (TDP).

PH was measured as the distance from collar to apical meristem; SD was measured using a digital caliper in the collar region at 3 cm height from soil level. Leaf area was determined using the non-destructive method, by measuring with a millimetric ruler leaf length and width (cm) considering only leaves with minimum length of 1.5 cm and 50% of photosynthetically active area.

Total leaf area was obtained as sum of all leaves according to the methodology proposed by Carneiro et al. (2002), as shown in Equation 1:

$$LA = (L \times W) \times f \tag{1}$$

Where, LA = leaf area, L = length, W = width, and Factor "f" = 0.6544.

The dry weight of the cashew rootstocks was determined by collecting the plants, washing the roots to eliminate the adhered soil and dividing each plant into leaves, stem and roots. Then, this material was placed paper bags and dried in a forced-air oven at 65 ºC until constant weight, to determine root dry phytomass (RDP) and shoot dry phytomass (stem + leaves) (SDP). RDP and SDP values were summed to determine total dry phytomass (TDP).

Rootstock quality was determined at 65 DAS by the Dickson quality index (DQI) for seedlings, using the formula of Dickson et al. (1960), according to Equation 2:

$$DQI = \frac{TDW}{(PH/SD) + (SDW/RDW)} \tag{2}$$

Where, DQI = Dickson quality index, PH = plant height (cm), SD = stem diameter (mm), TDW = total dry weight (g), SDW = shoot dry weight (g) and RDW = root dry weight (g).

2.4 Statistical Analysis

The variables were subjected to analysis of variance by F test (0.01 and 0.05 probability levels) and, in cases of significant effects, regression analysis was carried out using the statistical program SISVAR (Ferreira, 2011).

3. Results and Discussion

Based on the analysis of variance summary (Table 2), the interaction between salinity levels and proline concentrations had significant effect on leaf area. However, stem diameter and plant height were only affected by irrigation water salinity, at 65 days after sowing (Table 2).

Table 2. Summary of analysis of variance for stem diameter (SD), plant height (PH) and leaf area (LA) of 'FAGA 11' cashew rootstock under saline water irrigation and exogenous application of different proline concentrations, at 65 days after sowing (DAS)

Source of variation	DF	Mean Square		
		SD	PH	LA
Saline Levels (S)	4	1.12^{**}	107.50^{**}	49743.15^{**}
Linear regression	1	3.02^{**}	410.70^{**}	189835.73^{**}
Quadratic regression	1	1.09^{**}	16.09^{ns}	3423.54^{ns}
Proline concentrations (PC)	3	0.46^{ns}	4.95^{ns}	19915.72^{**}
Interaction S*PC	12	0.80^{ns}	13.70^{ns}	145.46^{**}
Blocks	3	0.01^{ns}	26.66^{ns}	5697.48^{ns}
Residue	38	0.02	8.15	2585.78
CV (%)		2.72	7.43	13.72

Note. DF: Degree of freedom; CV: coefficient of variation; ns, **, * respectively not significant, significant at $p < 0.01$ and $p < 0.05$.

Increment in irrigation water salinity negatively affected stem diameter and plant height of 'FAGA 11' cashew and, according to the regression equations (Figures 1A and 1B), there was a negative linear effect, causing reductions of 3.93% in SD and 6.44% in PH per unit increase in ECw. Plants subjected to ECw of 3.1 dS m^{-1} had reduction of 0.63 mm (11.01%) in SD and 7.75 cm (18.04%) in PH when compared with those irrigated with 0.3 dS m^{-1} water. Such reduction in SD and PH under saline stress conditions is a response to the decrease in the osmotic potential of the soil solution, which causes stomatal closure and reduction in transpiration and, consequently, decrease in the absorption of water and nutrients by plants, resulting in lower growth (Lima et al., 2015).

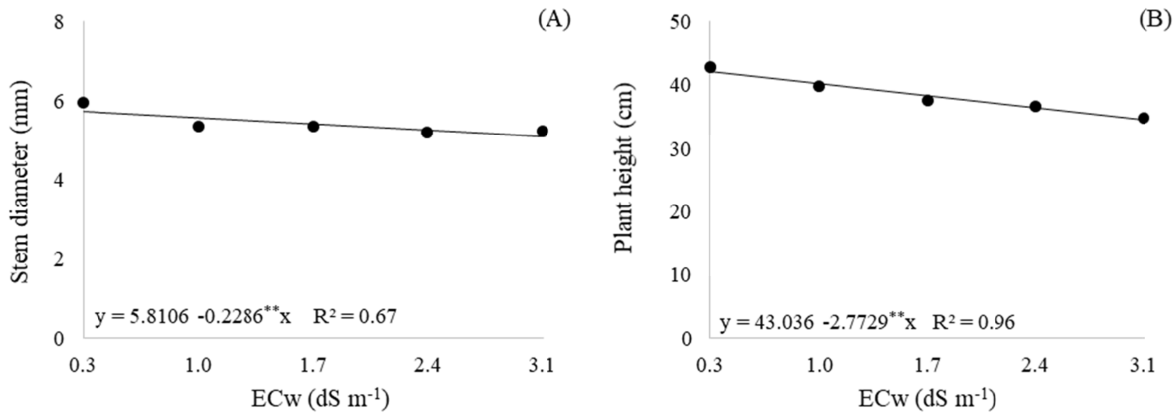

Figure 1. Stem diameter (A) and plant height (B) of 'FAGA 11' cashew rootstock, as a function of salinity of irrigation using water—ECw, at 65 days after sowing—DAS

Foliar application of proline at concentrations of 0 and 4 mM caused quadratic effect on leaf area and, according to the regression equation (Figure 2), the highest values of LA (401.37 and 479.35 cm^2) were found in plants irrigated using water with ECw levels of 1.6 and 0.3 dS m^{-1}, respectively. Based on the results, despite the reduction of LA due to salinity, the exogenous application of proline on the leaves at the concentration of 4 mM led to an increase in the leaf area in the saline levels of the irrigation water, when compared to the concentrations of proline tested. In addition, according to the regression equations (Figure 2), proline concentrations of 8 and 12 mM caused linear reduction in LA due to increase in irrigation water salinity, so that plants irrigated using water of highest salinity (3.1 dS m^{-1}) had reductions of 168.89 and 273.81 cm^2 compared with those subjected to ECw of 0.3 dS m^{-1} (Figure 2). Organic solutes such as proline are commonly found in plants under water/saline stress conditions, acting in the process of osmotic adjustment, but the excessive accumulation of proline under high concentrations of salts in the irrigation water intensifies the osmotic potentials, which negatively affect plant growth (Lima et al., 2016).

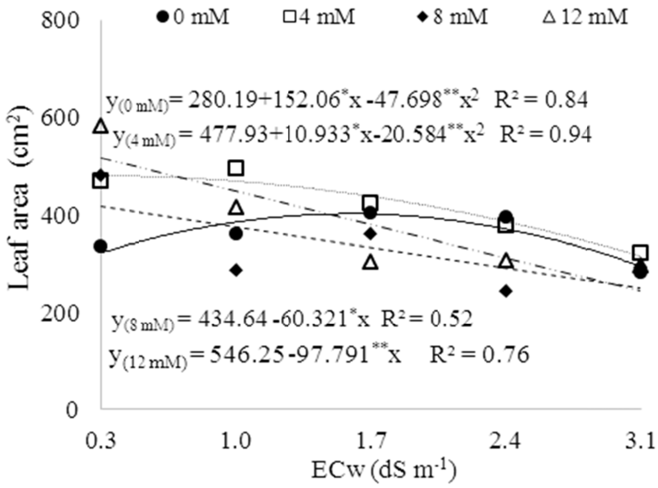

Figure 2. Leaf area of 'FAGA 11' cashew rootstock, as a function of the interaction between the levels of salinity of irrigation water—ECw and proline concentrations, at 65 days after sowing—DAS

Based on the analysis of variance summary (Table 3), there was significant interaction between the factors (irrigation water salinity and proline concentration), at 65 days after sowing, on the transpiration and total dry phytomass of 'FAGA 11' cashew rootstock. For stomatal conductance (*gs*), CO_2 assimilation rate (*A*), root dry phytomass (RDP) and Dickson quality index (DQI), significant isolated effects ($p < 0.01$) of factors (salinity levels and proline concentrations) were observed at 65 DAS.

Table 3. Summary of analysis of variance for stomatal conductance (gs), CO_2 assimilation rate (*A*), transpiration (*E*), root dry phytomass (RDP), total dry phytomass (TDP) and Dickson quality index (DQI) of cashew rootstock of 'FAGA 11' irrigated with saline water and subjected to different proline concentrations, at 65 days after sowing (DAS)

SV	DF	Mean Square					
		gs^1	*A*	*E*	RDP	TDP	DQI
Saline Levels (S)	4	0.001^{**}	20.83^{**}	2.36^{**}	0.44^{**}	7.65^{**}	0.06^{**}
Linear regression	1	0.006^{**}	72.77^{**}	8.89^{**}	1.57^{**}	27.95^{**}	0.21^{**}
Quadratic regression	1	0.0001^{ns}	6.78^{**}	0.14^{**}	1.57^{**}	2.59^{**}	0.02^{**}
Proline concentrations (PC)	3	0.0006^{*}	7.40^{**}	0.56^{**}	0.26^{**}	2.41^{**}	0.02^{**}
Interaction S*PC	12	0.0004^{ns}	3.90^{ns}	0.37^{**}	0.09^{ns}	1.76^{**}	0.01^{ns}
Blocks	3	0.000002^{**}	0.009^{ns}	0.004^{ns}	0.002^{ns}	0.04^{ns}	0.0008^{ns}
Residue	38	0.0001	0.14	0.003	0.002	0.02	0.0004
CV (%)		18.99	13.18	4.88	5.15	3.89	5.41

Note. ns, non-significant; ** significant at $p \leq 0.01$; SV = Sources of variation; DF: Degree of freedom; CV: coefficient of variation, [1] statistical analysis performed after data transformation to $\sqrt{X}$.

Stomatal conductance and CO_2 assimilation rate of 'FAGA 11' cashew rootstock (Figures 3A and 4A) were negatively affected by the increase in ECw and, according to the regression equations, there were linear reductions of 57.14 and 64.92%, respectively, in plants subjected to ECw of 3.1 dS m^{-1} in comparison to those irrigated with 0.3 dS m^{-1} water. The reduction in *gs* affects the exit of water vapor due to stomatal closure, causing an imbalance between the production and removal of reactive oxygen species produced during the photosynthetic process, which leads to metabolic alterations that culminate in oxidative damages to cell membranes and degradation of proteins, reducing net photosynthesis (Carvalho et al., 2011).

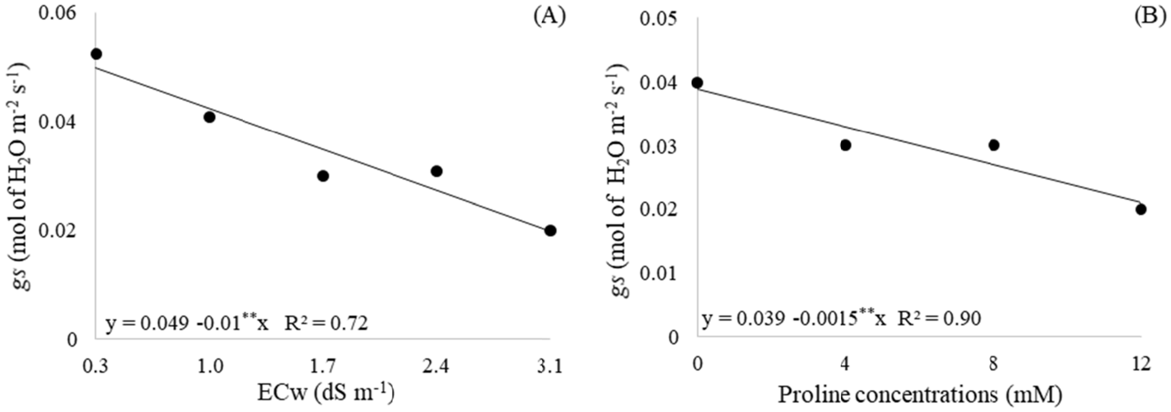

Figure 3. Stomatal conductance-*gs* of 'FAGA 11' cashew rootstock, as a function of different levels of salinity of irrigation water—ECw (A) and proline concentrations (B), at 65 days after sowing (DAS)

For stomatal conductance and CO_2 assimilation rate, the regression equations (Figures 3B and 4B) demonstrate that the 'FAGA 11' cashew rootstock when subjected to increasing proline concentrations had reductions in the physiological processes, 10.76% for *gs* and 9.91% for *A*, in plants which received the highest proline concentration (12 mM) through exogenous application. According to Lima et al. (2016), high proline concentrations applied through the leaves can cause deleterious effects on plants, because it possibly leads to alterations in cytosolic pH and redox status, resulting in irreversible damages to plant cell membranes.

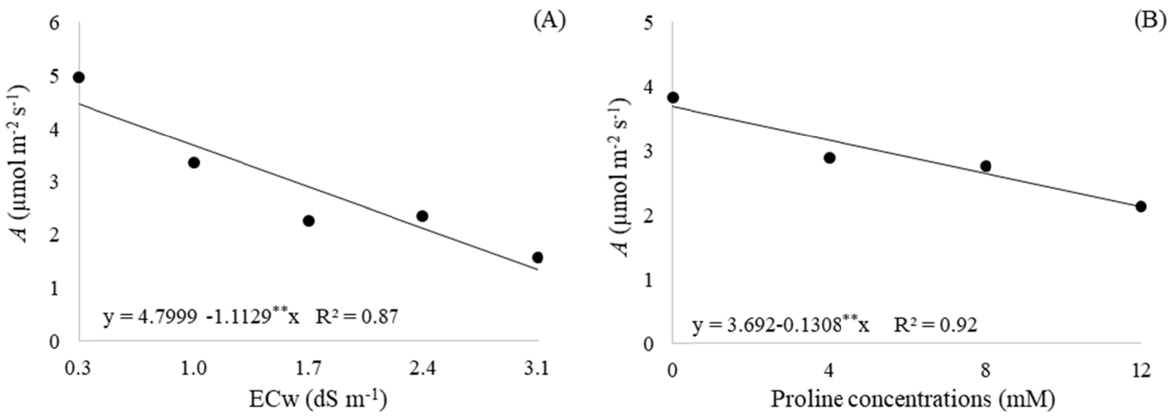

Figure 4. CO_2 assimilation rate-*A* of 'FAGA 11' cashew rootstock, as a function of different levels of salinity of irrigation water salinity—ECw (A) and proline concentrations (B), at 65 days after sowing—DAS

According to Figure 5, proline concentration of 8 mM led to the maximum estimated value of 1.67 mmol H_2O m^{-2} s^{-1} in the transpiration of plants irrigated with 0.6 dS m^{-1} water. However, proline concentrations of 0 and 4 mM resulted in reductions in this variable as the salinity levels increased, equivalent to 1.89 mmol H_2O m^{-2} s^{-1} (78.24%) and 1.13 mmol H_2O m^{-2} s^{-1} (66.37%) in plants subjected to ECw of 3.1 dS m^{-1} in comparison to those under the lowest salinity level (0.3 dS m^{-1}). Reduction in leaf transpiration in the 'FAGA 11' cashew rootstock can be a strategy to maintain a high leaf water potential and avoid excessive dehydration of the tissues. In addition, decrease in transpiration leads to decrease in the absorption of toxic ions, especially sodium and chloride.

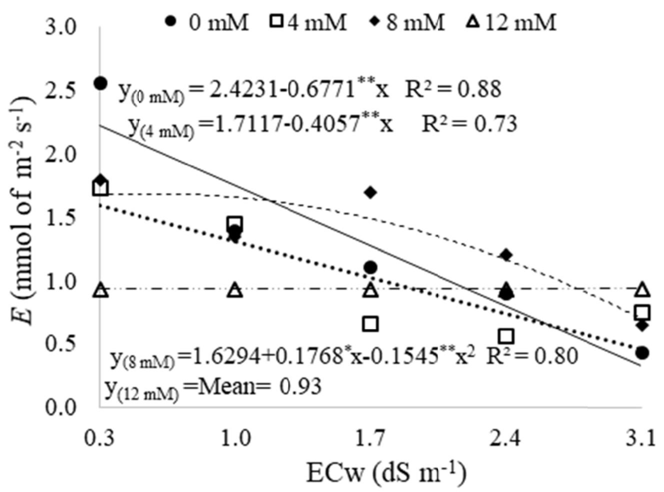

Figure 5. Transpiration-*E* of 'FAGA 11' cashew rootstock, as a function of the interaction between salinity of irrigation water—ECw and proline concentrations, at 65 days after sowing—DAS

Increasing levels of water salinity linearly inhibited root dry phytomass production (Figure 6A), which decreased by 14.23% per unit increase in ECw, corresponding to a reduction of 39.86% in plants subjected to irrigation with ECw of 3.1 dS m^{-1} in comparison to those cultivated under the lowest salinity level (0.3 dS m^{-1}). Reduction in root dry phytomass can be related to the fact that this organ is highly susceptible to saline stress, especially because the deleterious action of the salts results in considerable decrease in its dry biomass, since root growth is determined by a high metabolic activity. In addition, root is the plant organ directly exposed to the excess of salts in the soil; in this case, as ECw levels increased, there was an increase in the stress and thus the metabolic activities decrease and plants tend to produce less phytomass (Guimarães et al., 2013; Souza et al., 2017).

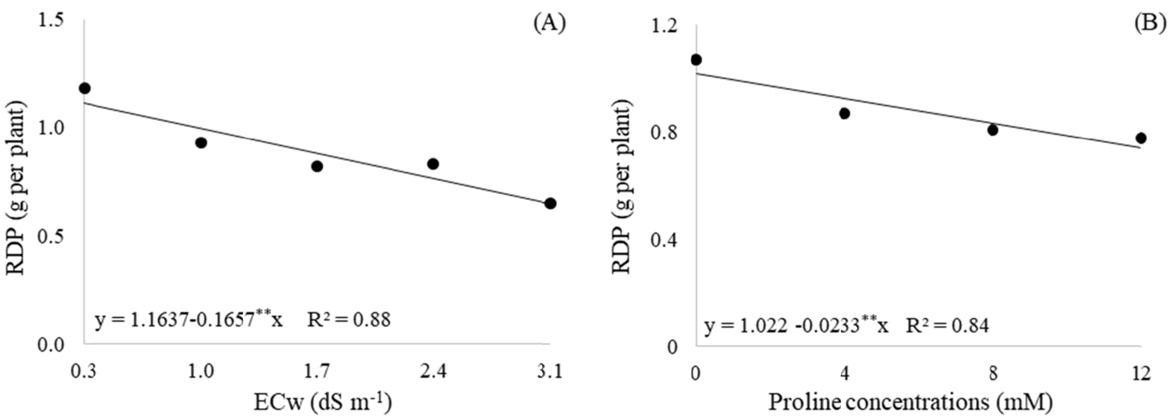

Figure 6. Root dry phytomass—RDP of 'FAGA 11' cashew rootstock, as a function of different levels salinity of irrigation water—ECw (A) and proline concentrations (B), at 65 days after sowing—DAS

Analyzing the effect of proline on the root dry phytomass of 'FAGA 11' cashew rootstock (Figure 6B), it is observed that RDP decreased by 2.27% per unit increase in proline concentration, *i.e.*, losses of 6.38% in plants that received the highest concentration (12 mM) in comparison to those that did not receive proline application. The decline in root dry phytomass production can be attributed to the increments in water salinity and proline concentration, which intensified the deleterious effect of saline stress on the root system of 'FAGA 11' cashew plants.

The total dry phytomass of 'FAGA 11' cashew rootstock was significantly affected by the interaction between saline levels and proline concentrations. According to the regression equations (Figure 7), plants that received exogenous proline at concentrations of 4, 8 and 12 mM fitted to the linear model, with reductions of 12.25, 17.77 and 17.25% per unit increase in ECw, *i.e.*, reductions of 34.32, 49.77 and 48.30%, respectively, in plants subjected to ECw of 3.1 dS m^{-1} in comparison to those cultivated under 0.3 dS m^{-1}. Reduction in total dry phytomass is a consequence of the decrease in CO_2 assimilation due to excessive accumulation of proline supplied through the leaves, which possibly led to reduction in cell osmotic potential and, along with saline levels, caused severe damages to the photosynthetic apparatus, compromising total dry phytomass production (Monteiro et al., 2014).

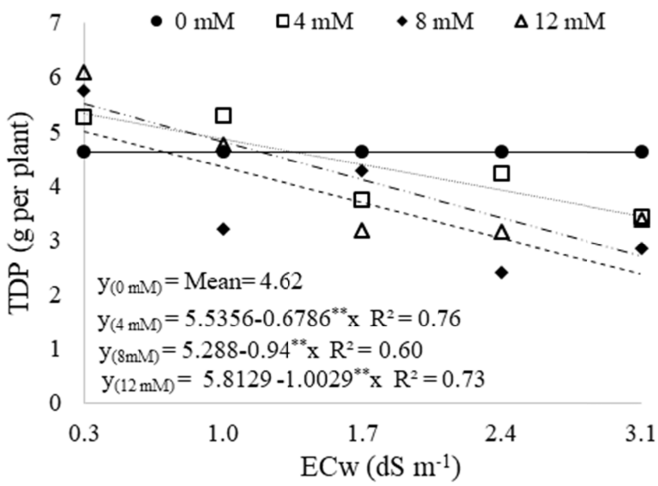

Figure 7. Total dry phytomass—TDP of 'FAGA 11' cashew rootstock, as a function of the interaction between salinity of irrigation water—ECw and proline concentrations, at 65 days after sowing—DAS

The different water salinity levels also negatively interfered with the Dickson quality index of the cashew rootstock seedlings and, based on the regression equation (Figure 8 A), there was a linear effect, with reduction of the order of 7.33% per unit increase in ECw. In other words, plants irrigated with ECw of 3.1 dS m^{-1} had DQI of 0.25 with reductions of 20.53% (0.06) compared with those irrigated with 0.3 dS m^{-1} water. From the agronomic point of view, it is an interesting situation because, under saline conditions, 'FAGA 11' cashew rootstocks showed DQI higher than 0.2, considered as of good quality for establishment in the field, since it relates robustness and balance in biomass distribution (Oliveira et al., 2013; Souza et al., 2017).

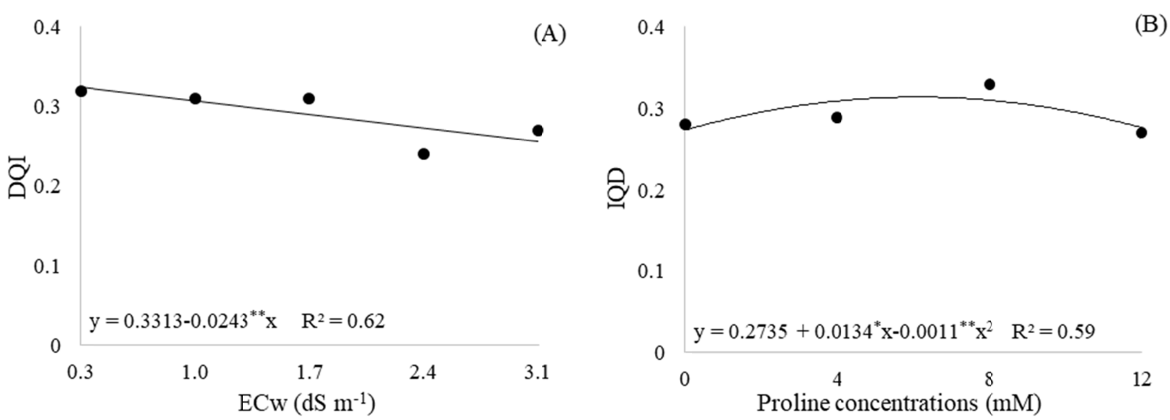

Figure 8. Dickson quality index—DQI of 'FAGA 11' cashew rootstock seedlings, as a function of different levels of salinity of irrigation water—ECw (A) and proline concentrations (B), at 65 days after sowing—DAS

Regarding the effect of proline concentrations on DQI (Figure 8B), the data fitted best to the quadratic model, and the highest estimated value for DQI (0.31) was obtained when plants were subjected to the concentration of 6.1 mM. According to Lacerda et al. (2014), the increment in DQI can be an indication of cellular ion homeostasis in response to the osmotic adjustment promoted by proline.

4. Conclusion

Irrigations with water of electrical conductivity up to 1.37 dS m^{-1} can be used in irrigation with acceptable reduction of 10% in the morphology of cashew FAGA 11 rootstock seedlings.

The proline concentrations tested, with the exception of the Dickson's quality index of seedlings, did not attenuate the deleterious effects of irrigation water salinity on the cashew FAGA 11 rootstock seedlings.

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