

Effect of water salinity and potassium doses on physiological traits and growth of 'Embrapa 51' precocious dwarf cashew (*Anacardium occidentale* L.) rootstock

Geovani Soares de Lima¹, Jailson Batista da Silva¹, Lauriane Almeida dos Anjos Soares, Reginaldo Gomes Nobre², Hans Raj Gheyi³, Genilson Lima Diniz¹, Pedro Dantas Fernandes⁴, Jussara Silva Dantas¹, Vera Lúcia Antunes de Lima⁴

¹Federal University of Campina Grande, Academic Unit of Agrarian Sciences, Pombal, 58.840-000, Paraíba, Brazil

²Federal Rural University of the Semi-arid Region, Department of Science and Technology, Caraúbas, 59.780-000, Rio Grande do Norte, Brazil

³Federal University of Recôncavo of Bahia, Nucleus of Soil and Water Engineering, Cruz das Almas, 44.380-000, Bahia, Brazil

⁴Federal University of Campina Grande, Academic Unit of Agricultural Engineering, Campina Grande, 58.109-970, Paraíba, Brazil

*Corresponding author: geovanisoareslima@gmail.com

Abstract

In the semiarid regions, plants are constantly exposed to different conditions of abiotic stresses due to the occurrence of excess salts in both soil and water. Thus, it is extremely important to identify an alternative capable of minimizing the effects of salt stress on plants as a way to ensure the expansion of irrigated areas. In this context, this study aimed to evaluate the chloroplast pigments, photochemical efficiency and growth of 'Embrapa 51' precocious dwarf cashew as a function of irrigation with saline water and potassium fertilization in the rootstock formation stage. The study was conducted under greenhouse conditions in the municipality of Pombal, PB, Brazil, using a randomized block design in a 5 x 2 factorial scheme, corresponding to five levels of irrigation water electrical conductivity - ECw (0.4; 1.2; 2.0; 2.8 and 3.6 dS m⁻¹) and two doses of potassium fertilization - KD (100 and 150% of the recommendation corresponding to 150 and 225 g K₂O kg⁻¹ soil), with two plants per plot and three replicates. Water salinity from 0.4 induced reductions in chlorophyll *a* and *b* synthesis, maximum and variable fluorescence and growth in sexually propagated precocious dwarf cashew seedlings and increases in carotenoid content and initial chlorophyll fluorescence. The quantum efficiency of photosystem II in cashew plants was decreased sharply with the increment in water salinity levels, standing out as indicative of damage to the photosystem II reaction centres. 'Embrapa 51' precocious dwarf cashew plants can be classified as sensitive to water salinity above 0.4 dS m⁻¹. Potassium doses of 100 and 150% of the recommendation did not alleviate the effects of salt stress during the precocious dwarf cashew rootstock production phase.

Keywords: *Anacardium occidentale* L.; salt stress; fertilization.

Abbreviations: Fo_initial fluorescence, Fv_variable fluorescence, Fm_maximum fluorescence, ΦPSII_quantum efficiency of photosystem II, Chl *a*_Chlorophyll *a*, Chl *b*_Chlorophyll *b*, ECw_irrigation water electrical conductivity, KD_potassium fertilization, PH_plant height, SD_stem diameter, LA_leaf area, DAS_days after sowing, FM_fresh matter.

Introduction

Cashew cultivation is one of the main socioeconomic activities conducted in the semiarid region of Northeastern Brazil, presenting itself as an important alternative to generate employment and income (Araújo et al., 2014). It is a fruit crop that can grow well in semiarid environments and stands out for the high potential both for the fresh consumption of its pseudofruit and in the nut industry. It can be used to obtain various products such as juices, soft drinks, candies, jams, nectars, flours and alcoholic beverages (Rabbani et al., 2012).

The precocious dwarf cashew plant differs from common cashew (or Giant cashew tree) due to its small size, height less than 4 m, homogeneous crown, smaller stem diameter and crown span compared to the common type, precocious cycle, beginning the flowering between 6 and 18 months

(Oliveira, 2008). Among the commonly used clones, the Embrapa 51 clone stands out for its small size, average height of 3.52 m at the sixth year of age, average crown diameter of 7.79 m, nut weight of 10.4 g, kernel/shell ratio of 24.5%, kernel weight of 2.6 g, resistance to anthracnose and moderate resistance to black mould (*Diplodinium anacardiacearum*) (Barros et al., 2000).

In this region, salinity is one of the abiotic stresses that most limit crop establishment (Amorim et al., 2010), affecting almost all aspects of plant development, including: emergence, vegetative growth and reproductive development. Excess salts impose ionic toxicity, osmotic stress, nutrient deficiency and oxidative stress on plants (Shrivastava et al., 2015) due to overproduction of reactive oxygen species (ROS), such as singlet oxygen (¹O₂), hydroxyl

radical ($\cdot\text{OH}$), superoxide anion ($\text{O}_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). ROS formation as a result of salt stress causes oxidative damage to lipids, proteins and other cellular components (Santos et al., 2018).

Salinity causes both osmotic and water stresses, reduction in leaf turgor, leading to stomatal closure and decrease in stomatal conductance, besides reduced content of photosynthetic pigments, inhibition of electron transport chain, disorganization of photosystem II complex (PSII), increase in non-photochemical quenching and alteration in the membrane structure (Hniličková et al., 2017). Thus, damage at any level caused by salt stress can reduce the photosynthetic capacity of the plants (Shraf and Harris, 2013).

An alternative that can reduce the effect of salt stress on cashew is the establishment of osmotic and ionic homeostasis through the supply of potassium, because the influx of Na^+ in plants occurs through the system of absorption of low- and high-affinity K^+ (Viégas et al., 2001). The positive regulation of K status in the plant reduces ROS production, due to the activity of enzymes involved in ROS detoxification (Cakmak, 2005). In addition, it performs vital regulatory functions in biochemical and physiological processes that contribute to plant growth and development (Hasanuzzaman et al., 2018). Potassium plays a fundamental role in cell osmoregulation: its ions are necessary to retain the pH gradient through the thylakoid membrane. In photosynthesis, potassium acts in the activation of numerous enzymes and in the synthesis of ATP (Kalaji et al., 2016).

Considering that the expansion in the cultivation of this fruit crop in the semi-arid region of Northeastern Brazil is conditioned on the use of salt stress-tolerant clones of precocious dwarf cashew, this study aimed to evaluate the chloroplast pigments, photochemical efficiency and growth of 'Embrapa 51' precocious dwarf cashew as a function of irrigation with saline water and potassium fertilization, in the rootstock formation stage.

Results and discussion

Effect of salt stress and potassium doses on chloroplast pigments of precocious dwarf cashew

The increasing levels of irrigation water salinity inhibited the chlorophyll *a* synthesis of precocious dwarf cashew plants, at 75 days after sowing. The regression equation (Fig 1A) shows that the increase in electrical conductivity of water resulted in a linear reduction of 17.10% in Chl *a* content per unit increase of ECw. There was a relative reduction in Chl *a* contents of $4.87 \text{ mg g}^{-1} \text{ FM}$ between plants cultivated under water salinity of 3.6 dS m^{-1} and those under the lowest ECw level (0.4 dS m^{-1}). The reduction in chlorophyll *a* content in response to salt stress occurs as a result of increased degradation through the enzyme chlorophyllase or inhibition of its synthesis. In addition, it may be associated with the decrease in the number of chloroplasts, which also affects the thylakoid membranes and plastids through their degradation (Mostajeran et al., 2014). According to Taibi et al. (2016), the reduction in chlorophyll *a* contents in plants under salt stress has been considered a typical symptom of oxidative stress. In a study with West Indian cherry under salt stress conditions (ECw of 0.8 and 3.8 dS m^{-1}), Lima et al. (2018) also found that the chlorophyll *a* content was markedly reduced with the increase in irrigation water salinity.

The chlorophyll *b* contents of the cashew plants were also linearly reduced by water salinity and, according to the regression equation (Fig 1B), there was a reduction of 17.60% per unit increase in ECw. It is observed that the stress caused by irrigation using water of highest salinity level (3.6 dS m^{-1}) caused a 60.60% reduction ($5.09 \text{ mg g}^{-1} \text{ FM}$) in the Chl *b* contents compared to plants irrigated under electrical conductivity of 0.4 dS m^{-1} . The reduction in chlorophyll *b* content in cashew is possibly associated with the inhibition in the synthesis of 5-aminolevulinic acid (chlorophyll precursor molecule) and with damage caused by salt stress, besides the formation of reactive oxygen species (ROS), which induce photo-oxidation of the pigments and degradation of chlorophyll molecules (Silva et al. 2016). A fact that may also be related to the sensitivity of the membranes to oxidative stress (Nedjimi, 2014).

The synthesis of carotenoids in precocious dwarf cashew plants (Fig 1C) was increased linearly in response to the increasing levels of water salinity, by 20.28% per unit increment in ECw. In relative terms, there was an increase of $3.78 \text{ mg g}^{-1} \text{ FM}$ (60.03%) in plants subjected to the highest ECw level (3.6 dS m^{-1}) compared to those receiving water of lowest salinity level (0.4 dS m^{-1}). The increase in the synthesis of carotenoids in plants under salt stress can be considered a tolerance mechanism. Because these are accessory pigments in the absorption and dissipation, in the form of heat and/or fluorescence and energy from the light photons captured by the pigments, which protect chlorophyll from photo-oxidation (Nascimento et al., 2015). In addition, carotenoids are pigments that can also act as antioxidants, protecting lipids of the plasmatic membrane from oxidative stress caused in plants exposed to salinity (Falk and Munné-Bosch, 2010). Unlike the data obtained in this study, Silva et al. (2017) evaluated the effects of water salinity (ECw ranging from 0.5 to 3.5 dS m^{-1}) in soursop plants at 110 days after sowing and observed reduction in the synthesis of carotenoids as a function of salt stress. The above-mentioned authors attributed the decrease in the synthesis of carotenoids to damage the photosynthetic apparatus resulting from salt stress.

Effect of salt stress and potassium doses on photochemical efficiency of precocious dwarf cashew

The initial chlorophyll fluorescence increased linearly as the water salinity levels increased. It is possible to observe by the regression equation (Fig 2A) that an increase of 8.88% in initial fluorescence (F_0) may occur per unit increment of ECw. There was an increment of 232.56 between cashew plants subjected to ECw of 3.6 dS m^{-1} and those that received the water of lowest salinity (0.4 dS m^{-1}). The increase of initial fluorescence (F_0) in cashew plants as a function of ECw levels is an indication of damage and inactivation of the PSII reaction centres (Mehta et al., 2010). Moreover, such an increase may be related to the reduction in the capacity to transfer the excitation energy from the antenna to the reaction centre (Baker, 2008). According to Yamane et al. (2008), the increase of F_0 in plants cultivated under abiotic stresses, such as salt stress, may be associated with increased leaf thickness due to lipid peroxidation caused by ROS. Dias et al. (2018), studied 'BRS 366 Jaburu' West Indian cherry cultivated under irrigation with saline waters (ECw: 0.8 and 3.8 dS m^{-1}) and concluded that salt stress causes reduction in F_0 , due to the damage caused by excess salts to chlorophyll *a*.

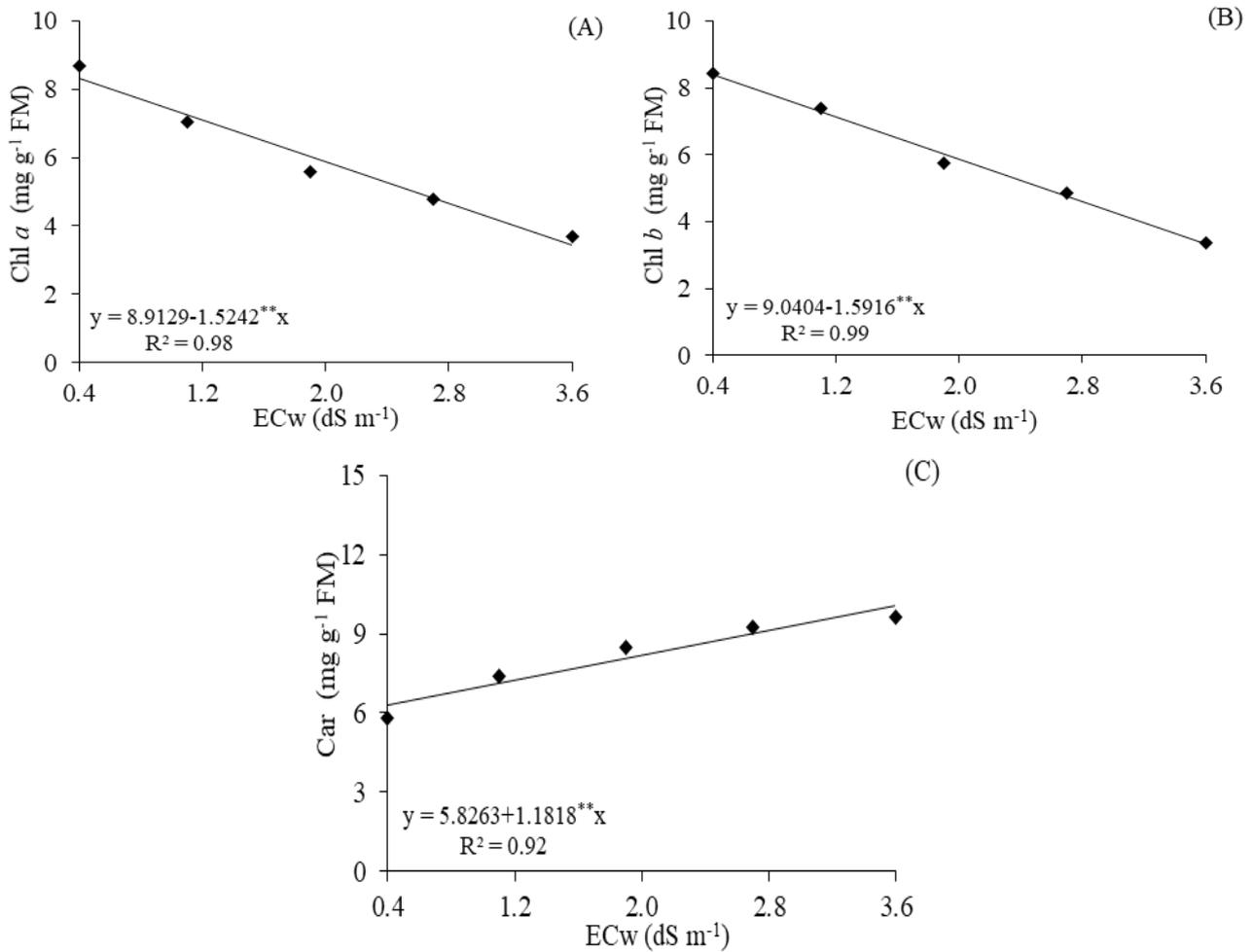


Fig 1. Contents of chlorophyll *a* - Chl *a* (A), chlorophyll *b* - Chl *b* (B) and carotenoids – Car (C) of ‘Embrapa 51’ precocious dwarf cashew plants, as a function of water salinity - ECw, at 75 days after sowing. **, significant at $p < 0.01$.

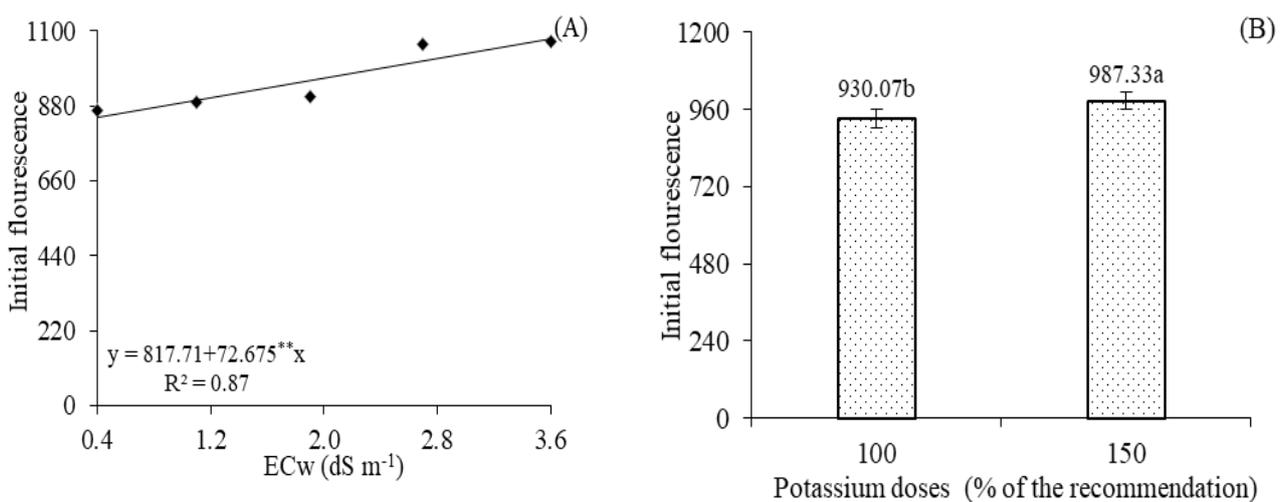


Fig 2. Initial fluorescence of ‘Embrapa 51’ precocious dwarf cashew plants as a function of water salinity - ECw (A) and potassium doses (B), at 75 days after sowing. Means followed by different letter indicate that the treatments differ by Tukey test, $p < 0.05$. **, significant at $p < 0.01$; Bars represent the standard error of the mean (n = 3).

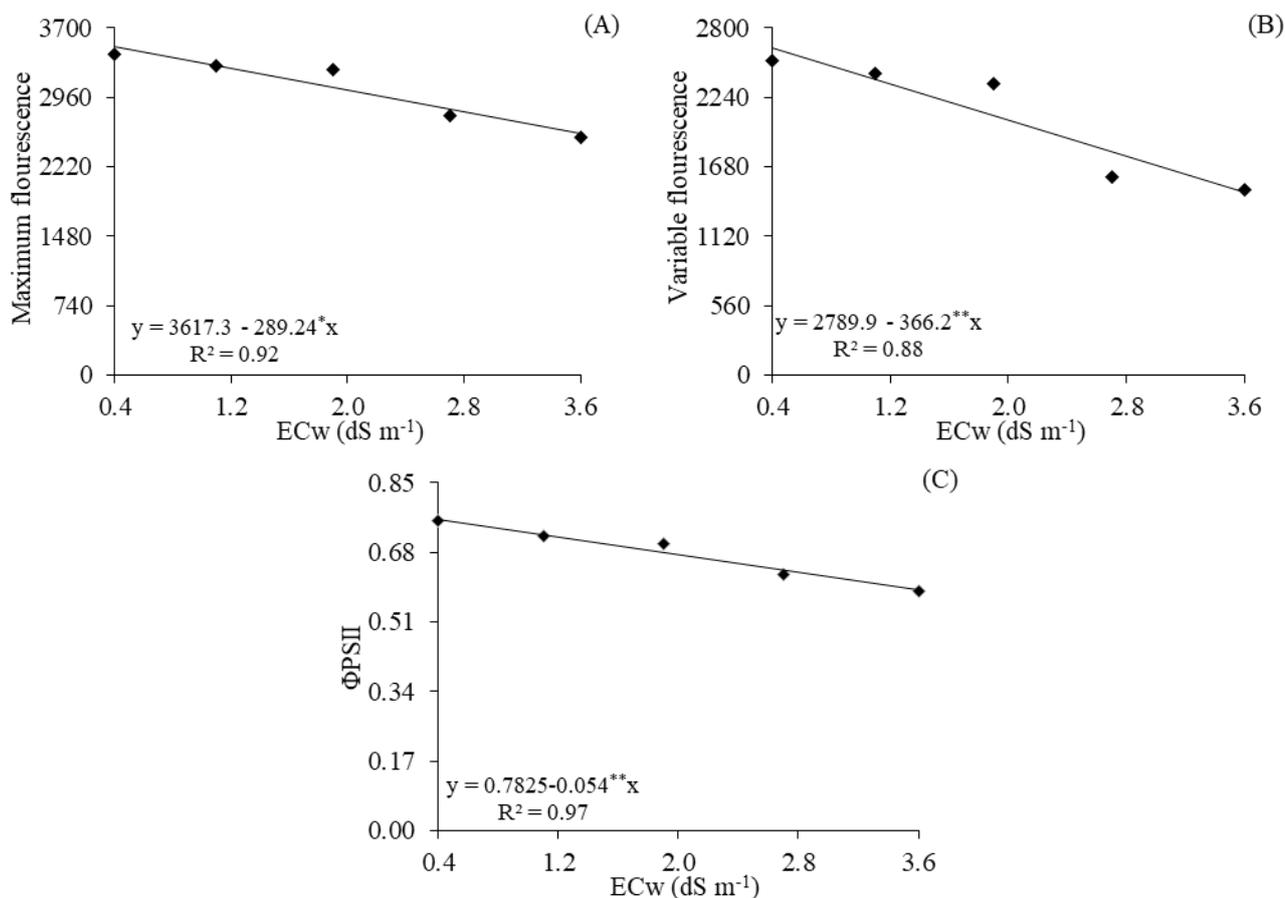


Fig 3. Maximum fluorescence (A), variable fluorescence (B) and quantum efficiency of photosystem II - ΦPSII (C) of 'Embrapa 51' precocious dwarf cashew plants as a function of water salinity - ECw (A) and potassium doses (B), at 75 days after the sowing. **, significant at $p < 0.01$.

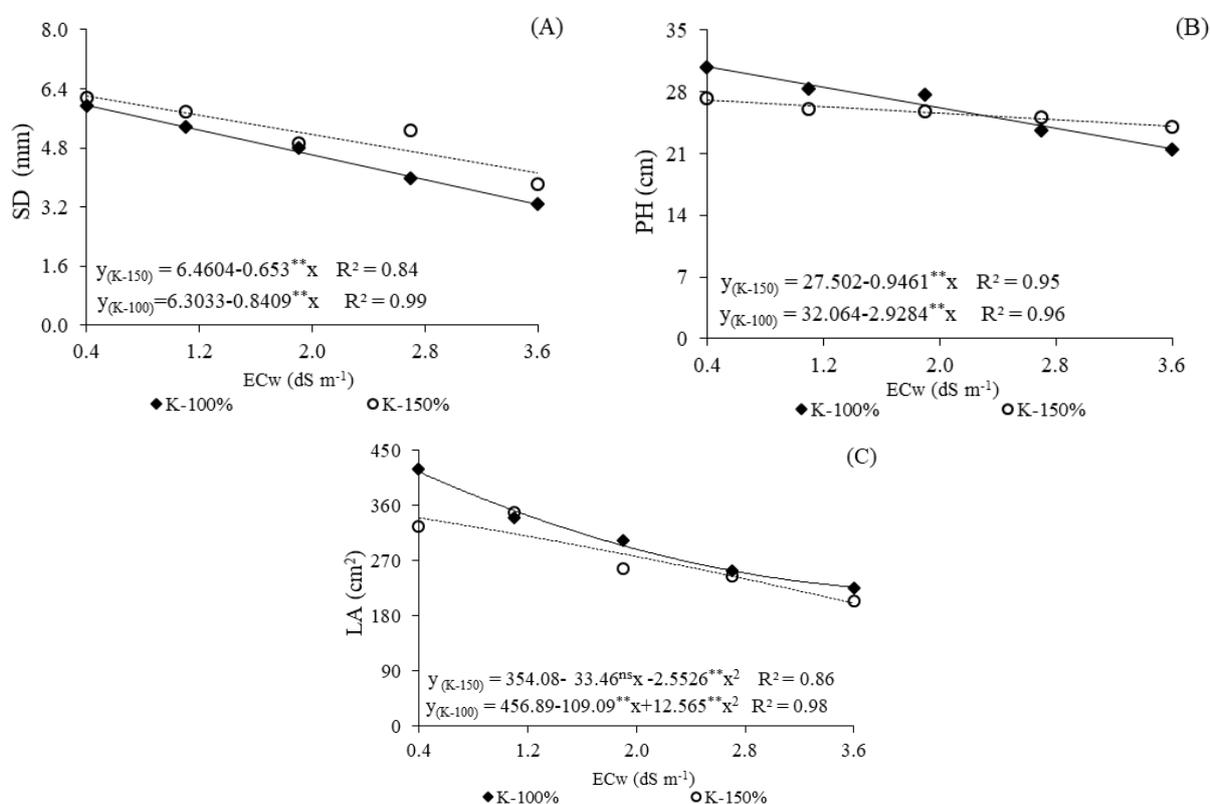


Fig 4. Stem diameter - SD (A), plant height - PH (B) and leaf area - LA (C) of 'Embrapa 51' precocious dwarf cashew plants as a function of the interaction between water salinity - ECw and potassium doses (A), at 25 days after sowing (DAS). ^{ns}, **, *Respectively, not significant, significant at $p < 0.01$ and significant at $p < 0.05$.

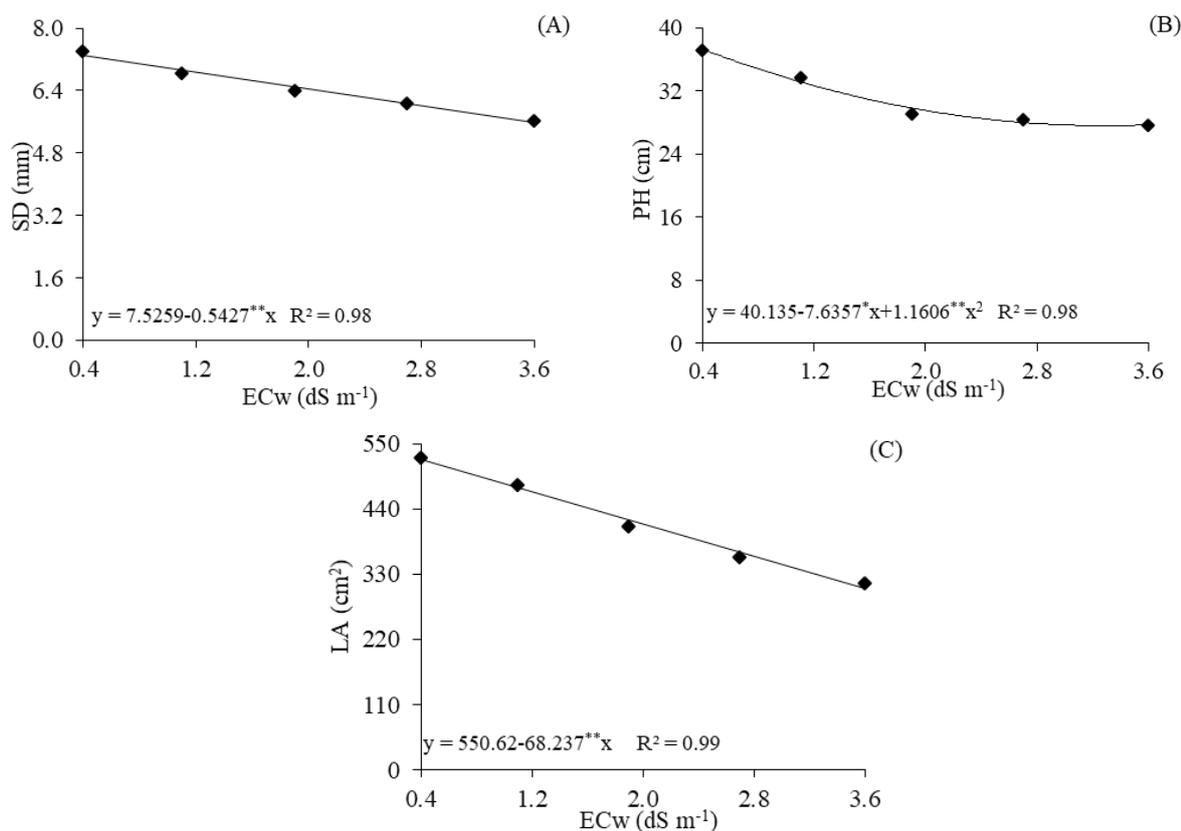


Fig 5. Stem diameter - SD (A), plant height - PH (B) and leaf area - LA (C) of 'Embrapa 51' precocious dwarf cashew plants as a function of water salinity - ECw, at 75 days after sowing (DAS). **, * Respectively, significant at $p < 0.01$ and $p < 0.05$.

The initial fluorescence of precocious dwarf cashew was significantly influenced by potassium fertilization and, according to the means comparison test (Fig 2B), plants subjected to fertilization with 150% of the K recommendation had higher initial fluorescence (57.26), compared to plants that received 100%. Dias et al. (2018), evaluated the effects of salt stress on the photochemical efficiency of West Indian cherry and did not observe significant effect of potassium doses (KD ranging from 50 to 125% of the recommendation) on the initial fluorescence of chlorophyll.

Maximum fluorescence represents the maximum intensity of fluorescence, when virtually all the quinone is reduced and the reaction centres reach their maximum capacity of photochemical reactions (Monteiro et al., 2018). According to the regression equation (Fig 3A), Fm decreased linearly by 7.99% per unit increment in water electrical conductivity, i.e., plants under irrigation with ECw of 3.6 dS m⁻¹ had Fm reduced by 925.56, compared to those cultivated under the lowest salinity (0.4 dS m⁻¹). Thus, the lower values of Fm obtained in plants under irrigation with saline waters is indication of a deficiency in the reduction of quinone A in thylakoid membranes and in the electron flux between the photosystems (Tatagiba et al., 2014).

For Fv (Fig 3B), it was observed that the use of water with electrical conductivity of 3.6 dS m⁻¹ caused a reduction of 13.12% per unit increment of ECw. By comparing the Fv of plants irrigated with ECw of 3.6 dS m⁻¹ with that of plants cultivated under 0.4 dS m⁻¹ water, it was possible to note a reduction of 44.33%. The reduction of variable fluorescence is due to the decrease in chlorophyll contents (Melo et al.,

2017), which results in the loss of plant capacity to transfer the energy of the ejected electrons from the pigment molecules to the formation of the reducing agent NADPH, ATP and Fd_r, leading to lower CO₂ assimilation capacity in the biochemical phase of photosynthesis, hence causing reduction of growth (Baker, 2008; Silva et al., 2017). Reduction in Fv due to the use of saline water has been observed in studies conducted by Silva et al. (2017) with soursop and by Monteiro et al. (2018) with sweet sorghum. The quantum efficiency of photosystem II in cashew plants decreased linearly under salt stress. According to the regression equation (Fig 3C), there were reductions of 6.90% per unit increase in ECw. There was a reduction of 22.70% in ΦPSII between plants cultivated under ECw of 3.6 dS m⁻¹ and those subjected to the lowest level of electrical conductivity (0.4 dS m⁻¹). Thus, the low values of ΦPSII indicates that part of the PSII reaction centres is damaged or photochemically inactive, thus reducing the electron transport capacity in PSII, a phenomenon commonly observed in plants under stress (Hniličková et al., 2017). Decrease in the quantum efficiency of photosystem II in plants due to the imposition of salt stress has also been observed in other crops, such as soursop (Silva et al., 2017) and West Indian cherry (Dias et al., 2018).

Effect of salt stress and potassium doses on growth of precocious dwarf cashew

The growth in stem diameter, plant height and leaf area of 'Embrapa 51' cashew, at 25 days after sowing, was significantly influenced by the interaction between factors

(SL x KD). SD and PH were decreased linearly, regardless of the K dose applied. According to the regression equations (Fig 4A and 4B), there were decreases per unit increase in ECw of 10.10 and 13.34% in SD and of 3.44 and 9.13% in PH of plants fertilized with 100 and 150% of the K recommendation, respectively. In relation to leaf area (Fig 4C), a quadratic model fitted to the data of plants fertilized with 100 and 150% of the K recommendation, with maximum values of 415.56 and 340.28 cm² obtained at the lowest level of ECw (0.4 dS m⁻¹) and the minimum values of 223.77 and 190.07 cm² obtained in plants under the highest level of water salinity (3.6 dS m⁻¹). The reduction of stem diameter, plant height and leaf area in cashew plants is probably related to competition of K in the absorption of Ca²⁺ and Mg²⁺ because, during the absorption process by roots, these nutrients use the same transport sites (Malavolta et al., 1997).

Andriolo et al. (2010), studied the effect of potassium applied via fertigation and in the aerial part (leaves) of strawberry, also found that high K doses resulted in a reduction of plant growth. These authors attributed the negative effect of high K concentration on plant growth to the competitive absorption in relation to the total sum of cations within the plant, inducing calcium and magnesium deficiencies.

At 75 days after sowing, the growth in stem diameter, plant height and leaf area was also negatively affected by the increasing levels of water salinity. According to the regression equations (Fig 5), stem diameter and leaf area were decreased linearly by 7.21 and 12.39%, respectively, per unit increase of water salinity. By comparing the SD and PH of plants cultivated under ECw of 3.6 dS m⁻¹ to those of plants subjected to the lowest level of salinity (0.4 dS m⁻¹), we observed reductions of 1.73 mm and 218.35 cm², respectively. Regarding to plant height (Fig 5B), a quadratic model fitted to the data, and a maximum value of 37.26 cm was obtained in plants subjected to ECw level of 0.4 dS m⁻¹, decreasing from this level and reaching the lowest PH value of 27.87 cm at the highest level of salinity (ECw = 3.6 dS m⁻¹). The reduction in the growth of cashew plants can be attributed to the inhibition of the synthesis of photosynthetic pigments imposed by the increasing levels of salinity, since chlorophyll is part of the light-harvesting antenna complex for the photosynthetic process (Tatagiba et al., 2014). According to Pak et al. (2009), the reduction in Φ PSII is one of the main factors responsible for the drastic decrease in the photosynthetic rate, and consequently, in the growth of plants cultivated under salt stress. Shah et al. (2018), evaluated the effects of irrigation with saline water (ECw from 0.3 to 3.1 dS m⁻¹) on the growth of 'Faga 11' precocious dwarf cashew. They found that stem diameter, plant height and leaf area were markedly reduced by the increase in water salinity.

Materials and methods

Experiment location, procedure and treatments

The study was conducted from August to October 2018 in plastic containers under greenhouse conditions, at the Centre of Science and Agri-Food Technology of the Federal University of Campina Grande (CCTA/UFCG), in the municipality of Pombal, PB, Brazil, located at the geographic coordinates 6° 48' 16" S latitude, 37° 49' 15" W longitude and mean altitude of 175 m.

The treatments resulted from the combination of five levels of irrigation water electrical conductivity (0.4; 1.2; 2.0; 2.8 and 3.6 dS m⁻¹) associated with two doses of potassium fertilization - KD [100 and 150% of the recommendation, according to Novais et al. (1991)]. The experimental design was randomized blocks in a 5 x 2 factorial arrangement, with two plants per plot and three replicates. The dose is referring to 100% corresponded to 150 mg of K₂O kg⁻¹ of the soil.

The irrigation waters with the respective levels of electrical conductivity were prepared by dissolving NaCl in public-supply water (ECw = 0.30 dS m⁻¹) from the municipality of Pombal-PB, based on the relationship between ECw and the concentration of salts (mmol_c L⁻¹ = 10*ECw - dS m⁻¹) according to Richards (1954).

Fertilization with phosphorus and nitrogen was performed according to the recommendation of Novais et al. (1991), applying equivalent to 300 mg of P₂O₅ and 100 mg of N kg⁻¹ of the soil, respectively. Monoammonium phosphate, urea and potassium chloride were used as sources of phosphorus, nitrogen and potassium, respectively. The fertilizations were split into 9 portions, which were applied weekly. Micronutrient requirements by cashew were met by biweekly applications of a solution (1.0 g L⁻¹) of Ubyfol [(N (15%); P₂O₅ (15%); K₂O (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%)], sprayed on the adaxial and abaxial sides of the leaves.

Establishment and management of the experiment

The containers were filled by placing a 2:1:1 proportion of a sandy-textured Entisol, sand and organic matter (well-decomposed bovine manure was used as source), from the rural area of the municipality of São Domingos, PB, whose chemical and physical characteristics were obtained according to the methodology of Donagema et al. (2011): Ca²⁺ = 2.42 cmol_c kg⁻¹; Mg²⁺ = 5.84 cmol_c kg⁻¹; Na⁺ = 0.09 cmol_c kg⁻¹; K⁺ = 0.21 cmol_c kg⁻¹; H⁺ + Al³⁺ = 0.00 cmol_c kg⁻¹; CEC = 8.56 cmol_c kg⁻¹; organic matter = 3.80 dag kg⁻¹; P = 11.99 mg kg⁻¹; pH in water (1:2.5) = 7.00; electrical conductivity of the saturation extract = 0.20 dS m⁻¹; sand = 846.3 g kg⁻¹; silt = 137.0 g kg⁻¹; clay = 16.3 g kg⁻¹; moisture at 33.42 kPa = 11.16 dag kg⁻¹; moisture at 1519.5 kPa = 4.23 dag kg⁻¹.

Prior to sowing, the soil moisture content was raised to field capacity using the respective water of each treatment. After transplanting, irrigation was performed daily by applying a volume of water sufficient to maintain soil moisture close to field capacity in each container, and the applied volume was determined according to the water requirement of the plants, estimated by water balance: volume applied minus volume drained in the previous irrigation, plus a leaching fraction of 0.10.

This study used seeds of precocious dwarf cashew, clone 'Embrapa 51', which were planted at 3 cm depth, by placing one seed per container in the vertical position, i.e. with the thinner end facing down and the peduncle attachment scar facing up.

Traits measured

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b* [Chl *a*, Chl *b*] and carotenoids [Car]) and photochemical efficiency were determined at 75 DAS, and growth was determined at 25 and 75 DAS. Photochemical efficiency was measured by

the initial fluorescence (Fo), variable fluorescence (Fv) and maximum fluorescence (Fm) and quantum efficiency of photosystem II (ΦPSII). Chlorophylls *a* and *b* contents (mg g⁻¹ of fresh matter - FM) were determined according to the methodology of Arnon (1949), through samples of 5 discs of the blade of the third mature leaf from the apex.

Chlorophyll and carotenoid concentrations were quantified in the solutions using a spectrophotometer at the absorbance (ABS) wavelengths 470, 646, and 663 nm, according to the following equations: Chlorophyll *a* (Chl *a*) = 12.21 ABS₆₆₃ - 2.81 ABS₆₄₆; Chlorophyll *b* (Chl *b*) = 20.13 A₆₄₆ - 5.03 ABS₆₆₃; Total carotenoids (Car) = (1000 ABS₄₇₀ - 1.82 Chl *a* - 85.02 Chl *b*)/198. Chlorophyll *a* fluorescence was quantified considering fully expanded leaves, using the portable fluorometer Plant Efficiency Analyser - PEA II®. The leaves selected for the analysis were pre-adapted to the dark using appropriate clips, and after a period of 30 minutes, the readings were taken.

Plant height was obtained considering as reference the distance from the plant collar to the insertion point of the apical meristem. Stem diameter was measured at 5 cm from the plant collar, using a digital calliper. Leaf area was obtained by measuring the length and width of all leaves of the plants according to the methodology described by Carneiro et al. (2002), as shown in Eq 1:

$$LA = (L \times W) \times f \quad (1)$$

LA = Leaf area of the plant (cm²), L = leaf length, W = leaf width, and f = equivalent factor = 0.6544.

Statistical analysis

After verifying the homogeneity of variances, the obtained data were subjected to analysis of variance by F-test at 0.05 and 0.01 probability levels and in cases of significance, linear and quadratic polynomial regression analysis was performed for the water salinity levels, as well as a means comparison test (Tukey) for potassium doses. When the interaction between factors (SL x KD) was significant, the factor salinity levels was further analysed for each potassium dose, using the statistical program SISVAR-ESAL.

Conclusions

Water salinity from 0.4 induced reductions in chlorophyll *a* and *b* synthesis, maximum and variable fluorescence and growth in sexually propagated precocious dwarf cashew seedlings and increases in carotenoid content and initial chlorophyll fluorescence. However, there was an increase in carotenoid content and chlorophyll initial fluorescence, at 75 days after sowing. Quantum efficiency of photosystem II in cashew plants were decreased sharply with the increment in irrigation water salinity, standing out as indicative of damage to the PSII reaction centres. 'Embrapa 51' precocious dwarf cashew plants can be classified as sensitive to water salinity from 0.4 dS m⁻¹. Potassium doses of 150 and 225 g kg⁻¹ soil, corresponding to 100 and 150% of the recommendation, do not alleviate the effects of salt stress during the precocious dwarf cashew rootstock production phase.

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