Molecular Science

AIMS Molecular Science, 11(2): 206–220.
DOI: 10.3934/molsci.2024012
Received: 30 January 2024
Revised: 22 March 2024
Accepted: 26 March 2024
Published: 10 May 2024

https://www.aimspress.com/journal/Molecular

Research article

Differential metabolic rearrangements improve biomass and enhance the tolerance of two dwarf cashew (*Anacardium occidentale* L.) genotypes to salt stress

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Abstract: Salinity is one of the abiotic stresses that affect crop productivity and plant development the most. We aimed to analyze the physiological and biochemical responses of dwarf cashew (*A. occidentale* L.) genotypes subjected to salt stress. The experiment was carried out in a greenhouse with a completely randomized design in a 5 × 2 factorial scheme, with five salinity levels (0, 25, 50, 75, and 100 mM NaCl) and 2 dwarf cashew genotypes (Embrapa51 and CCP76). There was no significant effect of salinity on plant height, leaf number, and stem diameter; however, the dry biomass was significantly reduced. Chlorophylls, starch, and total free amino acids decreased with salt stress, mainly with 75 and 100 mM NaCl. The CCP76 genotype salt-stressed increased carotenoids, anthocyanins, total soluble carbohydrates, reducing sugars, sodium, and potassium ions compared to Embrapa51. Free proline was increased in response to salt stress in dwarf cashew genotypes. Interestingly, sucrose declined in Embrapa51 and increased in CCP76 in response to salinity. When submitted to 75 and 100 mM NaCl, i.e., under severe stress, CCP76 presented more sucrose than Embrapa51. Our results indicated that sucrose accumulation plays an important role in the acclimation of CCP76 to salinity. This disaccharide induces metabolic rearrangements, mostly in the levels of

soluble carbohydrates and amino acids, which contribute to rebalancing the osmotic potential and help to maintain favorable plant metabolism under salt stress. Overall, the dwarf cashew CCP76 was more tolerant to salinity than Embrapa51.

Keywords: cashew genotypes; chlorophylls; salinity; osmoprotectors

1. Introduction

Salinity is one of the most severe abiotic stresses affecting plant growth and crop productivity [1]. According to Dias et al. [2], salinity may be the result of anthropic action due to inadequate soil and water management in agricultural practices. In general, the water used for irrigation has a high salt content and therefore can be considered brackish water [3]. In arid and semiarid regions, irrigation with brackish waters together with high evapotranspiration in these environments results in the accumulation of soluble salts, with an emphasis on sodium ions (Na^+) [1]. The intracellular accumulation of Na^+ at toxic levels can compromise cellular ionic homeostasis [4] and limit the development of many plant species [5].

The responses of plant species to salinity are complex and involve a multitude of physiological and biochemical processes [6]. Salinity alters the osmotic potential of soils and therefore reduces the water availability to plants [7]. Plants more able to maintain a water potential below that of the soil are most competent to survive in dry or saline soils [8,9]. The decrease in soil osmotic potential caused by salinity affects important metabolic processes, mainly those related to the ability to maintain a favorable water balance and the assimilation and allocation of carbon [4,10]. Thus, salt stress promotes significant alterations in the dry weight of shoots and roots, leaf area and growth rate of plant species and therefore can lead to significant productivity losses [3]. According to Karimi et al. [9], plants grown under salt stress conditions induce osmotic adjustment to avoid structural damage due to changes in osmotic potential and cellular water potential.

Osmotic adjustment is a mechanism that involves the accumulation of inorganic (e.g., calcium (Ca^{2+}) and potassium (K^+) ions) solutes and/or compatible organic compounds [4,7]. Compatible organic solutes, also called osmoprotectants, are maintained in the cytoplasm to balance the vacuolar osmotic potential [6]. Among the osmoprotectants, soluble carbohydrates, polyalcohols, quaternary ammonium compounds, and amino acids, especially proline, are most commonly accumulated [5,11]. The accumulation of amino acids and soluble carbohydrates has been intensively studied in plants under conditions of salt stress, as they present a greater contribution to the osmotic potential [7]. According to Munns et al. [1], efficient osmotic adjustment results from the simultaneous accumulation of Na^+ and chloride (Cl^-) ions in vacuoles and K^+ and osmoprotectants in the cytoplasm.

Dwarf cashew (*Anacardium occidentale* L.) is one of the main perennial crops produced in Northeastern Brazil and is the source of several products of commercial importance, such as gum, peduncle, and nut. Cashew gum can be used in the pharmaceutical and food industry, while its peduncle is largely used in the production of juices [12]. Cashew nut is the main product of this crop and can be consumed in human food or used to extract cashew nut shell liquid [13]. Cashews display considerable adaptation to low fertility soils as well as to high temperatures and water stress [3]. According to Ferreira-Silva et al. [14], high temperature improves the oxidative protection of cashew plants under salinity, and these results can explain, at least in part, the rusticity of this culture.

The dwarf cashew has become a considerable source of income for the Brazilian Northeast, especially for the states of Ceará, Piauí, and Rio Grande do Norte, all in the Brazilian semiarid region where the use of irrigation with brackish water is common. Thus, adequate technical management and the correct choice of the genotype to be used in the cultivation areas are necessary. The dwarf cashew CCP76 and Embrapa51 genotypes are related to present resistance to drought; however, studies on the physiological behavior of these genotypes under salinity are scarce. We hypothesize that the salt-tolerant dwarf cashew genotype will be able to accumulate organic solutes, mostly total soluble carbohydrates, amino acids, and free proline, for osmotic adjustment to cope with salt stress. To test our hypothesis, the present study aimed to analyze the physiological and biochemical responses of two dwarf cashew genotypes (CCP76 and Embrapa51) grown under salt stress.

2. Materials and methods

2.1. Plant material

The two dwarf cashew genotypes, Embrapa51 and CCP76, were selected. The CCP76 genotype was one of the first early dwarf cashew genotypes to be made available to producers in 1983, while the Embrapa51 genotype was obtained by individual phenotypic selection in 1996. The Embrapa51 and CCP76 genotypes are resistant to anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.) and resinosis (*Lasiodiplodia theobromae* (Pat.) Griffon &. Maubl.), however only Embrapa51 is recognized as resistant to black mold (*Pilgeriella anacardii* Arx & Miller). Based on the dry biomass yield, the Embrapa51 and CCP76 genotypes were classified as sensitive and moderately tolerant, respectively, when grown with salinity of 3.6 dS/m [15].

2.2. Experimental conditions

The experiment was conducted in a greenhouse at the Department of Biology of the Center for Natural Sciences (Federal University of Piaui, Teresina, Piaui State, Brazil) at a temperature range of 28–39 ℃ with 50–70% relative humidity, a photoperiod of 12 hours, and an average maximum photosynthetic photon flux density of 1200 μmol/(m^2·s). A completely randomized design with a 2 × 5 factorial scheme was used, with two dwarf cashew genotypes (Embrapa51 and CCP76) and 5 doses of sodium chloride (NaCl). The dwarf cashew seedlings from Embrapa51 and CCP76 were obtained from the nuts (seeds) of these genotypes, which were purchased directly from cashew farmers at Fazenda Agropecuária Bom Futuro (Cocal de Telha, Piaui State, Brazil). The nuts were selected based on size, density, absence of injuries and then cleaned with distilled water. After cleaning, the nuts were planted in polypropylene pots containing 3.5 kg of medium vermiculite for the production of dwarf cashew seedlings.

Until thirty days after emergence (DAE), the seedlings of the dwarf cashew genotypes were irrigated, alternately, with distilled water and Hoagland & Arnon's nutrient solution [16]. At 30 DAE, the dwarf cashew seedlings were subjected to salt stress with the use of NaCl at the following doses: 0, 25, 50, 75, and 100 mM NaCl. All treatments consisted of five replications, and the experimental unit was composed of a pot with a plant. At 42 DAE, after 12 days under salt stress, all plants were collected, and stem diameter, plant height, and number of leaves were measured. The plants were divided into shoots and roots, and the plant material was packed separately in properly identified paper

bags and dried in a forced aeration oven at 65 ℃ to obtain the dry weight of the shoots and roots. Additionally, at the harvest (42 DAE), samples of fresh leaves of the dwarf cashew genotypes were collected and preserved at −20 ℃ to be used to determine the concentration of photosynthetic pigments, sodium and potassium ions, and organic solutes (total soluble carbohydrates, total free amino acids, free proline, sucrose, and starch).

2.3. Determination of the concentration of photosynthetic pigments

To measure the concentration of chlorophylls (*a* and *b*) and carotenoids [17], fresh samples of dwarf cashew leaves were macerated, in the dark, in the presence of 95% ethanol and centrifuged at 5,000 rpm for 5 min. The supernatant was collected and measured at 646, 664 and 470 nm. Based on readings, the concentrations of chlorophyll *a* and *b* and carotenoids were calculated, and the data were expressed in mg/g fresh weight (FW). To determine the concentration of anthocyanins [18], samples of fresh leaves were macerated in the presence of 0.1% methanol-HCl and centrifuged at 5000 rpm for 5 min. Subsequently, the supernatant was read at 530 nm. After calculating the concentration of anthocyanins, the data were expressed in mmol/g FW.

2.4. Determination of sodium (Na^+) and potassium (K^+) ion content

Samples of fresh dwarf cashew leaves (~ 100 mg), cut into small segments avoiding the vein, were mixed with distilled water (~ 10.0 mL) and taken to a water bath at 100 ℃ for one hour. Afterward, the supernatant (extract) was collected and aliquots of the extract were analyzed in a flame photometer (Micronal, model B462) to determine the concentration of Na^+ and K^+ ions based on standard curves obtained with sodium chloride and potassium chloride, respectively, according Viégas et al. [19]. Data were calculated and the results were expressed in mmol/kg DW (dry weight).

2.5. Determination of organic solutes

Samples of dwarf cashew leaves (~ 100 mg), cut into small segments avoiding the vein, were mixed with distilled water (~ 10.0 mL) and taken to a water bath at 100 ℃ (60 min). Subsequently, the supernatant (extract) was collected and used to quantify the concentration of total soluble carbohydrates, total free amino acids, and free proline were measured as proposed by Dubois et al. [20], Yemm and Cocking [21], and Bates et al. [22], respectively. To measure the total soluble carbohydrates, aliquots of the extract were mixed with 5% phenol and concentrated sulfuric acid, and after vortexing the tubes, the mixture was read at 420 nm. The content of total soluble carbohydrates was calculated based on the standard glucose curve, and the data were expressed in mmol/kg DW. To measure the concentration of total free amino acids, aliquots of the extract were mixed with 0.2 M citrate buffer (pH 5.0) and the color developing reagent (5% ninhydrin in 0.2 mM KCN) and then taken to the water bath at 100 ℃ (15 min). Later, the tubes were cooled in an ice bath, and 60% ethanol was added. After reaching room temperature, the samples were read at 570 nm. The content of total free amino acids was calculated based on a standard glycine curve and expressed in mmol/kg DW. To determine the content of free proline, aliquots of the extract were mixed with glacial acetic acid and acidic ninhydrin (ninhydrin in 60% glacial acetic acid and 6 M phosphoric acid) and then taken to the water bath at 100 ℃ (60 min). Then, toluene was added for phase separation, and the chromophore was aspirated

and read at 520 nm. The concentration of free proline was calculated based on the standard proline curve and expressed in mmol/kg DW.

Sucrose content was measured as proposed by van Handel [23]. Samples of dwarf cashew leaves (~ 50 mg) were mixed with methanol:chloroform:water (MCW) solution (12:5:3), stirred for 25 min and then centrifuged at 10,000 rpm (10 min). The procedure was repeated, and the supernatants were combined to obtain the final extract. Aliquots of the final extract were mixed with 30% KOH (in methanol) and taken to the water bath (100 ℃) for 10 min. Then, 0.2% anthrone (in concentrated sulfuric acid) was added, and the mixture was taken to the water bath (40 ℃) for 20 min. The mixtures were read at 620 nm, and after calculation based on a standard sucrose curve, the data were expressed in mmol/kg DW. To measure the starch content [20,24], the pellet from sucrose extraction with MCW was mixed with 30% $HClO_4$, stirred for 25 min, and centrifuged at 10,000 rpm (10 min). Aliquots of the final extract were mixed with 5% phenol and concentrated sulfuric acid and read at 420 nm. The starch content was calculated based on the standard glucose curve and expressed in mmol/kg DW. Reducing sugars were determined by the difference between the concentration of total soluble carbohydrates and sucrose and expressed in mmol/kg DW.

2.6. Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) preceded by an F test at 5% probability. To analyze the effect of salinity (increasing doses of NaCl), the data were adjusted using regression analysis. The comparison between the average values of the dwarf cashew genotypes at each level of salt stress was performed using Tukey's test at 5% probability. Principal component analysis (PCA) was performed using all measured variables previously standardized. The correlation analysis was performed using Pearson's correlation (*r*). Univariate and multivariate statistical analyses were run using R 3.6.3 using RStudio (package ExpDes) and Past 4.03 statistics software, respectively.

3. Results

Differential responses of the dwarf cashew genotypes (Embrapa51 and CCP76) to salt stress were evaluated in terms of physiological and biochemical variables. There was a significant interaction between the 'genotype' and 'salinity' factors for all variables analyzed, except for plant height, number of leaves, and stem diameter (Table 1). The genotypes exhibited significant differences in physiological and biochemical variables, except in relation to plant height, total soluble carbohydrates, and total free amino acids. In addition, the ANOVA showed significant effects of salinity on all variables analyzed, except for plant height, number of leaves, and stem diameter. As shown in Table 2, Embrapa51 displays a number of leaves and stem diameter superior and inferior, respectively, compared to CCP76.

Plant biomass (shoot and root) of the dwarf cashew genotypes (Embrapa51 and CCP76) decreased as salt stress increased, with inferior biomass observed in Embrapa51 (Figure 1). When exposed to 100 mM NaCl, Embrapa51 displays a 36% decrease in root dry weight in relation to the control. In CCP76, a reduction of more than 30% in root dry weight was observed from 75 mM NaCl (Figure 1B). There was a reduction in the concentration of chlorophyll *a* in response to salt stress in both dwarf cashew genotypes (Figure 2A). The dwarf cashew genotypes showed chlorophyll-*a* values at approximately 1.0 mg/g FW when not exposed to salinity (control), and significant differences between the two genotypes for this variable were observed with the application of 50 and 75 mM NaCl

(Figure 2A). Embrapa51 showed chlorophyll-*a* concentrations 42% and 29% higher than those recorded for CCP76 when exposed to 50 and 75 mM NaCl, respectively.

Table 1. Analysis of variance (medium square) and normality test of the biometric and biochemical variables obtained after the exposure of the dwarf cashew genotypes to salt stress.

	Analysis of variance (medium square)				Shapiro-Wilk
Variables	**Genotypes (G)**	**Salt stress (S)**	**G x S**	**CV (%)**	
Plant height	0.41^{ns}	7.51^{ns}	5.26^{ns}	15.73	0.26
Number of leaves	50.7*	7.95^{ns}	8.45^{ns}	23.30	0.40
Stem diameter	6.63*	0.05^{ns}	0.43^{ns}	9.06	0.48
Shoot dry weight	8.64*	0.32*	0.28*	4.27	0.30
Root dry weight	1.82*	0.21*	0.02*	6.56	0.53
Chlorophyll *a*	0.08*	0.19*	0.02*	9.11	0.07
Chlorophyll *b*	0.15*	0.39*	0.08*	11.03	0.28
Carotenoids	0.03*	0.65*	0.00*	6.15	0.22
Anthocyanins	0.56*	2.61*	0.10*	9.04	0.18
Total soluble carbohydrates	1838^{ns}	850173*	57019*	5.99	0.12
Reduced sugars	2362^{ns}	847522*	57744*	6.09	0.11
Sucrose	32.4*	6.63*	31.6*	6.68	0.75
Starch	9781.3*	3143.8*	295.9*	5.41	0.31
Total free amino acids	723.2^{ns}	21745*	21244*	6.55	0.45
Free proline	5.04*	139.2*	8.39*	5.58	0.70
Sodium (Na^+)	313.6*	3092.2*	570.9*	4.94	0.79
Potassium (K^+)	241.4*	929.5*	78.3*	6.35	0.95
Degrees of freedom	1	4	4	-	

Note: The means with the same letter are statistically equal by Tukey's test ($p < 0.05$).

Table 2. Biometric variables of the dwarf cashew genotypes subjected to salt stress.

Salt stress	**Plant height**		**Number of leaves**		**Stem diameter**	
(mM NaCl)	**EMBRAPA51**	**CCP76**	**EMBRAPA51**	**CCP76**	**EMBRAPA51**	**CCP76**
0	19.7	17.7	15.0	12.0	7.0	6.6
25	19.0	18.2	14.0	12.0	5.6	6.7
50	20.0	18.0	14.0	14.0	6.1	6.9
75	18.8	20.3	14.0	14.0	5.8	7.5
100	23.3	22.7	16.0	14.0	5.9	6.9
Mean	20.1 a	19.4 a	15.6 a	13.0 b	6.1 b	6.9 a

Note: The means with the same letter are statistically equal by Tukey's test ($p < 0.05$).

In relation to chlorophyll *b*, a significant reduction was observed in both dwarf cashew genotypes (Figure 2B). CCP76 had 98%, 138%, and 192% more chlorophyll *b* than Embrapa51 when submitted to 50, 75, and 100 mM NaCl, respectively. There was a significant increase in the concentration of

carotenoids (Figure 2C) in response to salinity for the two dwarf cashew genotypes. The carotenoid content differed significantly between the two genotypes, except when the plants were exposed to 100 mM NaCl. CCP76 displays carotenoid concentrations 54% higher than Embrapa51 in the control and approximately 20% higher in salinity. The salinity induced a significant increase in the anthocyanin content, and significant differences between genotypes were observed only when plants were exposed to 100 mM NaCl (Figure 2D). When exposed to 100 mM NaCl, CCP76 exhibited an anthocyanin content 34% higher than that registered for Embrapa51 (Figure 2D).

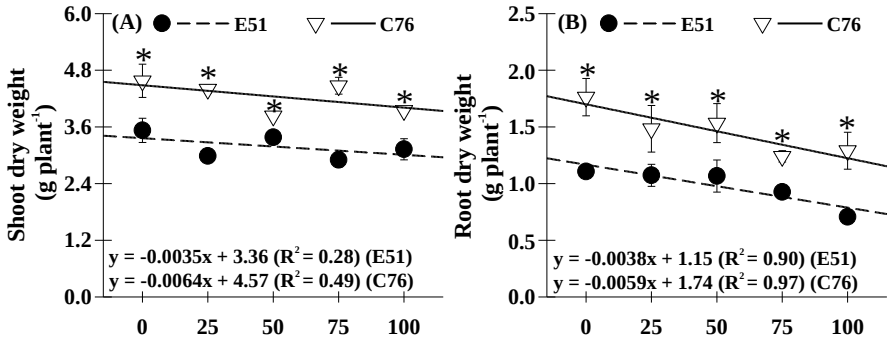

Figure 1. Dry weight of shoots (A) and roots (B) of the dwarf cashew genotypes subjected to salt stress (0–100 mM NaCl). The asterisk (*) indicates significant differences between the genotypes at each level of salt stress (Tukey's test at 5% probability). E51 = Embrapa51. C76 = CCP76.

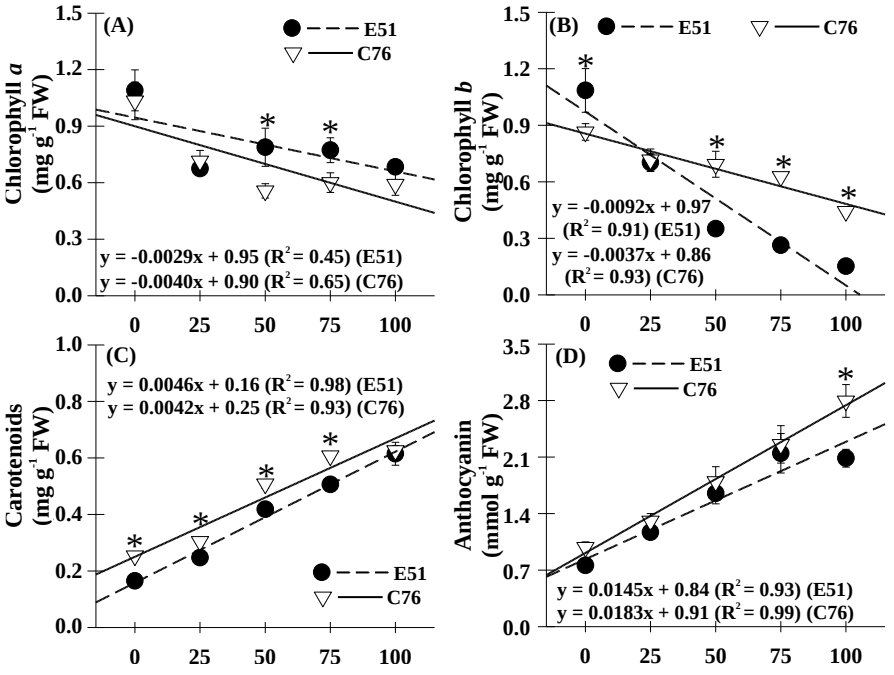

Figure 2. Photosynthetic pigments of the dwarf cashew genotypes subjected to salt stress (0–100 mM NaCl): (A) Chlorophyll *a*, (B) chlorophyll *b*, (C) carotenoids, and (D) anthocyanin. The asterisk (*) indicates significant differences between the genotypes at each level of salt stress (Tukey's test at 5% probability). E51 = Embrapa51. C76 = CCP76.

There was a linear increase in the concentration of total soluble carbohydrates (Figure 3A) and reduced sugars (Figure 3B) with the increase in salinity, and different responses between genotypes were observed when these plants were submitted to 50, 75, and 100 mM NaCl. CCP76 showed higher values of total soluble carbohydrate and reduced sugar than Embrapa51 when subjected to 50 and 100 mM NaCl, while Embrapa51 was superior to CCP76 when exposed to 75 mM NaCl. In relation to sucrose, the genotypes display an inverse response when exposed to salt stress (Figure 3C). While Embrapa51 displays a decrease in sucrose levels in response to salinity, CCP76 enhances the sucrose content as salt stress increases. Up to 50 mM NaCl, Embrapa51 showed higher sucrose values. This genotype was 92%, 68%, and 42% higher than CCP76 when subjected to control, 25 and 50 mM NaCl, respectively. In contrast, CCP76 showed a higher sucrose content when exposed to 75 and 100 mM NaCl (approximately 30% superior).

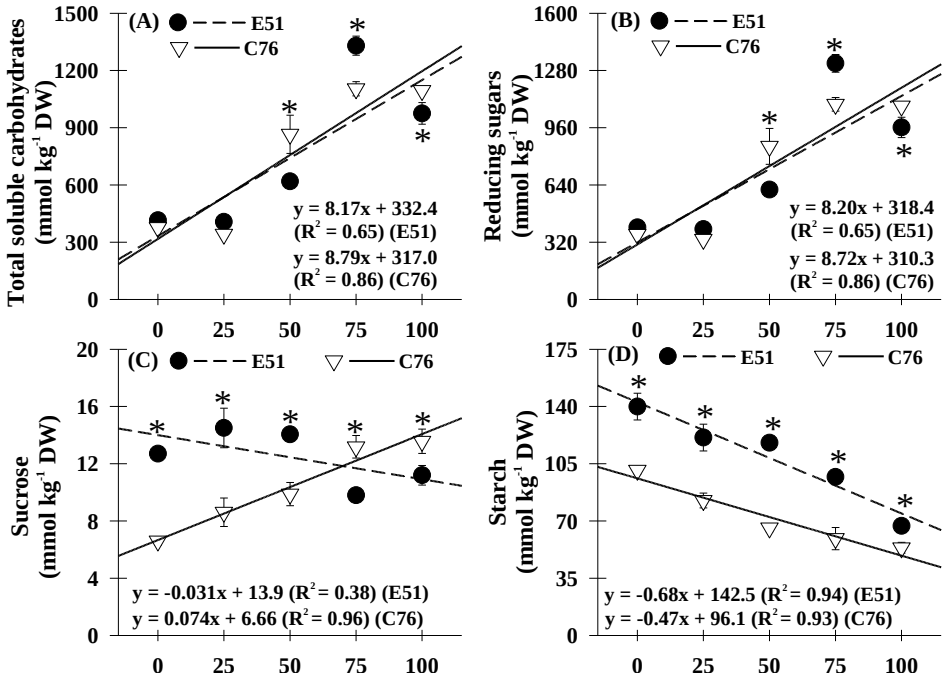

Figure 3. Total soluble carbohydrates (A), reducing sugars (B), sucrose (C), and starch (D) of the dwarf cashew genotypes subjected to salt stress (0-100 mM NaCl). The asterisk (*) indicates significant differences between the genotypes at each level of salt stress (Tukey's test at 5% probability). E51 = Embrapa51. C76 = CCP76.

The starch concentration was linear and significantly reduced in response to the increase in salt stress in the two dwarf cashew genotypes, and there was a significant difference between the two genotypes at each salinity level applied (Figure 3D). When exposed to 50 mM NaCl, the two dwarf cashew genotypes showed the greatest differences in starch content (approximately 80%). Different responses were observed between the dwarf cashew genotypes in relation to total free amino acids (Figure 4A). CCP76 showed a content of total free amino acids higher than Embrapa51 when subjected to the control and 25 mM NaCl, while Embrapa51 was superior to CCP76 when exposed to 50, 75, and 100 mM NaCl. The concentration of free proline was enhanced in both genotypes in response to salinity, and differences between the two genotypes were observed only in the control (Figure 4B).

The concentrations of Na^+ and K^+ were significantly increased in response to salinity (Figure 4C and D). Overall, Embrapa51 maintained higher levels of K^+ than CCP76 at all salinity levels.

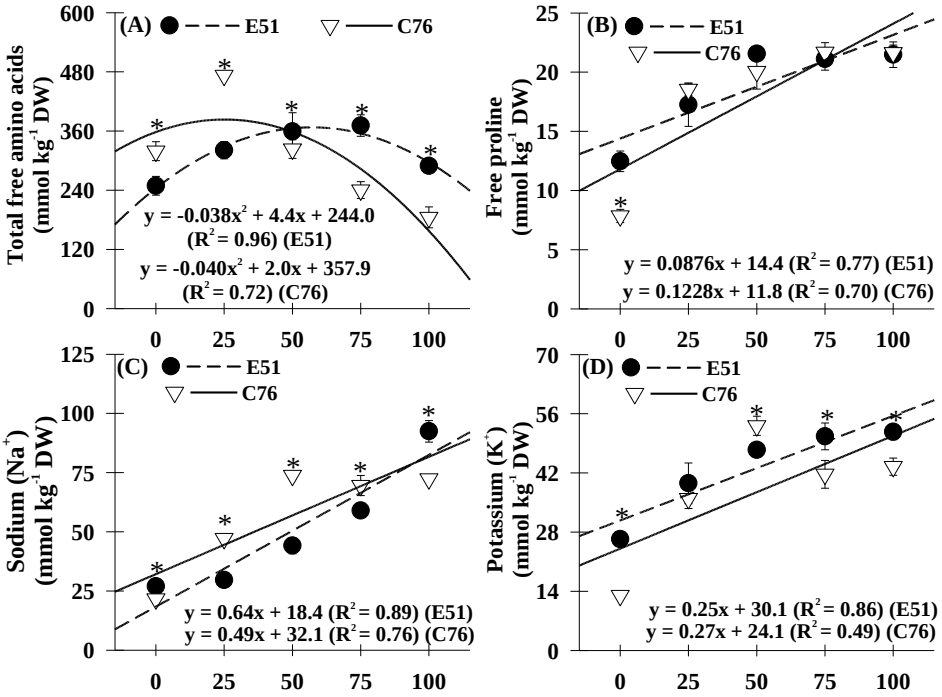

Figure 4. Total free amino acids (A), free proline (B), sodium (C), and potassium (D) of the dwarf cashew genotypes subjected to salt stress (0–100 mM NaCl). The asterisk (*) indicates significant differences between the genotypes at each level of salt stress (Tukey's test at 5% probability). E51 = Embrapa51. C76 = CCP76.

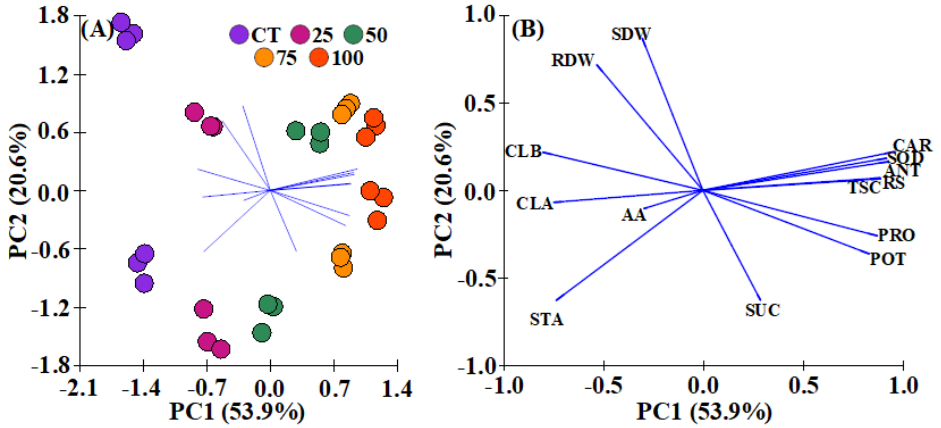

Figure 5. Principal component analysis (A and B) of the variables obtained in dwarf cashew genotypes subjected to salt stress (0-100 mM NaCl). CT = control. E51 = Embrapa51. C76 = CCP76. RDW = root dry weight. SDW = shoot dry weight. CAR = carotenoids. SOD = sodium. STA = starch. ANT = anthocyanin. RA = reduced sugar. TSC = total soluble carbohydrates. PRO = free proline. POT = potassium. CLA = chlorophyll *a*. CLB = chlorophyll *b*. SUC = sucrose. AA = total free amino acids.

The principal component (PC) analysis explained 58.7% of the total variation in all parameters measured when these genotypes were exposed to salinity. PC1 explained 53.9% of the total variance, while PC2 accounted for 20.6% of the variability (Figure 5C). PC1 was positively related to carotenoids, anthocyanin, Na^+, total soluble carbohydrates, reduced sugars, free proline, and K^+ (loadings ≥ 0.83) and negatively correlated with chlorophylls (*a* and *b*) and starch (loadings ≥ 0.73). PC2 is positively correlated with plant biomass (shoot and roots) and, to a lesser extent, negatively associated with sucrose (loading = −0.63).

4. Discussion

In this study, the exposure of dwarf cashew genotypes to salinity did not significantly influence stem diameter, plant height or the number of leaves (Tables 1 and 2). It is likely that the number of leaves has not been significantly changed by the age of the dwarf cashew plants that were used, which were approximately 30 days (after emergence) and that were exposed to salinity for 12 days. In addition, dwarf cashew are plants with a long cycle and therefore slower leaf emission. Although the number of leaves was not altered, plants exposed to salinity showed an increase in chlorosis and leaf death, especially at the highest salinity levels. The results presented contrast with those of Souza et al. [25], who reported a reduction in the number of leaves of guava plants due to the increase in salinity. These authors relate the loss of leaves with a way to reduce transpiration, which is, therefore, a physiological process of adapting plants to salt stress.

Dwarf cashew genotypes submitted to salinity display decreases in shoot and root dry weight (Figure 1). The restriction of growth due to salinity is attributed to several factors ranging from changes in the absorption and use of nutrients and in photosynthesis to the very ionic toxicity caused by the excess Na^+ in the vicinity of the roots [6]. Similar results were recorded in trifoliate hybrids of citrus rootstocks submitted to salinity, which showed a 44% reduction in total dry weight [26]. Bader et al. [27] registered negative effects of salinity on the root dry weight of olive cultivars (*Olea europaea* L.). These researchers emphasize that salinity may have affected basic aspects of the development of these plants, such as cell division and elongation, due to the toxic effect of excess Na^+.

Salinity significantly altered the concentration of photosynthetic pigments in dwarf cashew genotypes, and chlorophyll (*a* and *b*) decreases in response to salt stress were registered (Figure 2A and B). These data are similar to those obtained in sorghum [28], bell pepper [29], and Indian cherry [30] irrigated with saline water. Changes in these pigments are related to tolerance or sensitivity to environmental stresses [31]. Overall, when exposed to salinity, Embrapa51 had the highest chlorophyll *a* content, and CCP76 had the highest chlorophyll *b* content. Chlorophyll *b*, present only in antenna complexes, protects the photosystem against photooxidative stress, and its content directly affects photosynthesis [32]. Thus, more chlorophyll *b* increases the transference of excess energy to carotenoids and thermal energy dissipation, resulting in the relief of the harmful effects of environmental stresses [31,33].

In this study, carotenoids and anthocyanins were increased in response to salinity, and this increase may be related to the function of these compounds. Carotenoids are accessory pigments with photoprotective action in the photochemical apparatus and prevent photooxidative damage to chlorophyll molecules [1,31,33]. Additionally, anthocyanins are secondary metabolites commonly described as antioxidants that protect plants against the negative effects of abiotic stresses, including salt and drought stress [7]. The increase in anthocyanins is possibly related to the increment in reducing

sugars and total soluble carbohydrates since the biosynthesis of this molecule is related to high levels of available sugars. In fact, the content of reducing sugars and total soluble carbohydrates in both genotypes increased (Figure 3A and B), and a positive correlation ($r = 0.85$) between anthocyanins and sugars (reducing sugars and total soluble carbohydrates) was observed.

Osmoregulation is among the mechanisms of tolerance to salt stress, and this process consists of increasing the concentration of solutes in the cells to maintain the cellular water potential at adequate levels [1]. In this study, total soluble carbohydrates and reducing sugars were significantly increased by salt stress (Figure 3). Soluble carbohydrates (monosaccharides and oligosaccharides, reducers, or not) have osmotic and protective effects and present themselves as primary sources of energy for plants in coping with stress [34]. It is possible that this increase in the sugar content was a strategy to mitigate the harmful effect of salt stress, considering that their accumulation can maintain plant growth under salinity due to osmotic adjustment [30]. Analogous to our results, soluble carbohydrates were improved in pistachio rootstocks [35] and bermudagrass [36] under salinity, while noni [34] and amaranth [8] exhibited a decline in soluble carbohydrates in response to salt stress.

Between the source and sink organs of a plant, carbohydrates are mostly transported as sucrose, non-reducing disaccharide composed of glucose, and fructose [30]. The sucrose provides energy for plant growth and development and contributes to osmotic adjustment of plant cells, as well as enhancing crop resistance to abiotic stresses [34,35]. Sucrose metabolism is important to cope the salt stress conditions in various plant species, like as soybean, sorghum, and Chinese rose [37]. In this study, under severe stress (up to 75 mM NaCl), the CCP76 genotype displays higher sucrose content and this response seems play an important role in the acclimation of this genotype to salt stress. The sucrose induces metabolic rearrangements, that contribute to osmotic adjustment and help to maintain favorable plant metabolism under salinity. According Kim et al. [38], salt tolerance is related to the high sucrose content.

In general, the reduction in the level of carbohydrates is associated with disorders in carbohydrate biosynthesis or translocation to other parts [37]. In our study, a reduction in starch concentration was recorded in the two dwarf cashew genotypes (Figure 3D). Nascimento et al. [10] reported similar results in bell pepper plants. The decline in starch content can be a way to mitigate the harmful effects of stress since this polysaccharide is a source of the simplest carbohydrates (mono- and oligosaccharides) commonly used by plants as compatible osmolytes in stressful conditions and then accumulates to promote osmotic adjustment [6,37]. According to Munns et al. [1], soluble carbohydrates and amino acids are among the organic solutes primarily involved in osmotic adjustment.

While the concentration of total free amino acids was reduced in response to salt stress, the concentration of free proline was elevated linearly with an increase in salinity levels (Figure 4). Proline is a multifunctional amino acid, and its accumulation is a common response when plants are subjected to abiotic stresses, with an emphasis on salt stress [11]. The accumulation of free proline contributes to the reduction of cellular water potential and, therefore, promotes increased water retention in cells under stress conditions. In addition to acting on osmotic adjustment, proline has several functions that range from the stabilization of membranes, subcellular structures and important proteins to the capture of reactive oxygen species [1]. As recorded in this study for the two dwarf cashew genotypes, noni [34], amaranth [8], and cassava [39] showed an increase in proline levels in response to salt stress.

The exposure of plants to salt stress also affects the levels of inorganic solutes, with an emphasis on K^+ [40]. K^+ is an important macronutrient and a limiting factor in the crop yield rate and is considered the main cationic inorganic nutrient in glycophyte plants [34,40]. In addition, K^+ plays an

important role in the response of plants to abiotic stresses, such as drought, salinity and flooding [1,7]. In our study, the concentration of K^+ was increased in response to salinity, and this increase was positively correlated (r = 0.76) with the increase in Na^+ levels in both genotypes (Figure 6B and D). In some plants, especially those tolerant to salinity, the compartmentalization of ions (K^+, Na^+ and Cl^-) in the vacuole can be a useful strategy in the search for osmotic balance since they can be balanced with the synthesized organic solutes in the cytosol of plant cells [37].

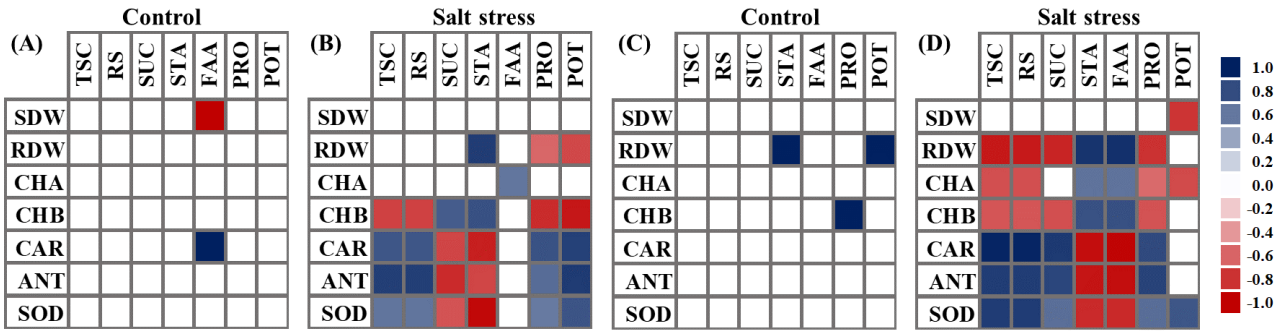

Figure 6. Pearson's correlation analysis of growth, photosynthetic pigments and sodium content and organic solutes and potassium content in in dwarf cashew Embrapa51 (A, B) and CCP76 (C, D) genotypes subjected to control and salt stress (25–100 mM NaCl). In horizontal lines: SDW, shoot dry weight; RDW, root dry weight; CHA, chlorophyll-*a*; CLB, chlorophyll-*b*; CAR, carotenoids; ANT, anthocyanin; SOD, sodium. In vertical columns: TSC, total soluble carbohydrates; RS, reduced sugar; SUC, sucrose; STA, starch; FAA, total free amino acids; PRO, free proline; POT, potassium. Blue or red color represents the significant positive or negative correlation, respectively ($p < 0.05$).

Overall, according the principal component analysis (PCA), the salinity levels are separated in ascending order in PC1 (53.9%). The negative effects of salinity are mostly on plant biomass and chlorophyll. Furthermore, accessory pigments (carotenoids and anthocyanin), Na^+, K^+, monosaccharides (reduced or not), and free proline are part of the biochemical arsenal against the negative effects of salinity. The results suggest that the difference between these genotypes is established in the ability to maintain higher levels of chlorophyll *b*, carotenoids, anthocyanin, and sucrose, jointly with greater remobilization of starch and amino acids. These features were registered in CCP76, and therefore, this genotype seems more tolerant to salinity than the Embrapa51.

5. Conclusions

Salinity significantly affected the growth of dwarf cashew genotypes, mostly in the deposition of biomass in the shoot and roots. It is possible that changes in the shoot and root dry weight are the result of the toxic effect of excessive Na^+ ions on primary metabolism, especially in the photosynthetic process. The reduction in the levels of chlorophylls due to salinity reinforces this idea. We concluded that dwarf cashew genotypes altered their biochemical metabolism in the search to re-equilibrate their osmotic potential and maintain their development under salt stress. Regarding the genotypes, CCP76 showed better results and was considered more tolerant to salinity than Embrapa51.

Use of AI tools declaration

The authors declare that they have not used Artificial Intelligence (AI) tools in the creation of this article.

Acknowledgments

The authors are grateful to the National Council for Scientific and Technological Development (CNPq) and National Council for the Improvement of Higher Education (CAPES), Brazilian research-funding agencies, for financial support (CNPq-Universal n° 426655/2018-4).

Conflict of interest

The authors declare no conflicts of interest.

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