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CROP SCIENCE

Differential responses of dwarf cashew clones to salinity are associated to osmotic adjustment mechanisms and enzymatic antioxidative defense

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Abstract: This study evaluate growth, gas exchange, solute accumulation and activity of antioxidant enzymes in dwarf cashew clones subjected to salinity. Shoot dry mass reduced 26.8% (CCP06) and 41.2% (BRS189) at 16 dS m^{-1}, concerning control. For net photosynthesis, CCP06 and BRS189 presented 69.8% and 34.7% of reduction, respectively. Na^+ and Cl^- contents increased in leaves and roots, in both clones, although CCP06 leaves presented Na^+ concentrations lower than those of BRS189, the first one was the clone that the most accumulated such toxic ion, whereas K^+ content remained almost unchanged for both clones. Soluble N-amino was the organic solute that more varied with salinity in cashew seedlings. Salt stress increased the activity of superoxide dismutase in both clones, mainly 16 dS m^{-1} treatment. Additionally, salinity promoted increases in ascorbate and guaiacol peroxidase activities, and the last enzyme was the main involved in H_2O_2 removal. Despite the reductions in growth and gas exchange, dwarf cashew seedlings of both clones presented an osmotic adjustment mechanism, and an efficient enzymatic antioxidant system that were able to attenuate the salt and oxidative stress, respectively. Our research suggested that BRS189 clone is more tolerant to salt stress than CCP06.

Key words: *Anacardium occidentale* L., osmoregulation, oxidative protection, photosynthesis, salinity.

INTRODUCTION

In natural and agricultural conditions, plants are exposed to many stressors originated from biotic and abiotic factors (Taiz et al. 2015). Among these factors, salinity is one of a major abiotic stress affecting plant growth and crop productivity, occurring specially in arid and semi-arid regions, where soil salt content is naturally high, and rainfall can be insufficient for leaching salt excess (Taiz et al. 2015). High salt levels in soil contribute to ion imbalance and hyperosmotic stress in plants, which affects water potential between soil and plant (Alencar et al. 2015, Praxedes et al. 2010). In addition to water deficit, ion toxic absorption, such as Na^+ and Cl^-, promoting ionic toxicity, following by nutritional and metabolic imbalance (Zhu 2003, Praxedes et al. 2010).

Salinity can affect plant in a multivariate way, primarily reaching plant growth due to osmotic and ionic components and mineral deficiency, and following with several metabolic processes as photosynthesis, protein synthesis and lipid metabolism (Praxedes et al. 2010, Silva et al. 2015, Mansour et al. 2016). The control of the absorption of toxic ions by the roots and their

transport and distribution through the plant, as well as the capacity to compartmentalize them in the vacuole, has been considered one of the main mechanisms of tolerance to salt stress (Ashraf & Harris 2004, Liang et al. 2018). Additionally, to this process, plant cells are also capable to develop adjustment osmotic, which are performed through organic solute accumulation (proline, soluble N-amino and soluble sugars) in cytosol, whereas to balance ion organic are accumulated in vacuole (Ashraf & Harris 2004, Liang et al. 2018).

In addition to effects on water status and plant cell ionic homeostasis, salinity can originate secondarily oxidative stress resulting from the increased concentration of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). (Miller et al. 2010, Gadelha et al. 2017). In general, plants use enzymatic and non-enzymatic antioxidative systems to cop the damage caused by ROS (Mittler 2002, Gondim et al. 2012). Among the components of antioxidant system, the ROS-scavenging enzymatic system stands out to cope the damages promoted by ROS, including activity of catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (G-POD), ascorbate peroxidase (APX) and glutathione reductase (GR) (Gondim et al. 2012, Gadelha et al. 2017). The antioxidant enzyme system is recognized as the main mechanism of plant tolerance to environmental stress (Miller et al. 2010). In this context, plants that own elevated levels of antioxidants can avoid more efficient the oxidative damages promoted by ROS (Miller et al. 2010).

The cashew (*Anacardium occidentale*), a member of the Anacardiaceae family, the Anacardiaceae family, which is composed of some 60 to 74 genera and 400 to 600 species (Bezerra et al. 2007). The cashew nut (*Anacardium occidentale* L.) cultivation is an important economic activity in Brazil Northeastern, which facing the limitations to plant growth and development due to high salinity in soil and low water available for plants (Bezerra et al. 2007). Salt negative effects on growth and seedling development have been studied in some aspects of plant development: on seed germination and reserve mobilization (Marques et al. 2013); ion homeostasis and H^+-ATPases, sterol and phospholipid contents (Alvarez-Pizarro et al. 2009), modulation of rootstock and scion on transpiration and photosynthetically aspects (Ferreira-Silva et al. 2010) and the identification of important protein involved in Cashew nuts responses to salt stress (Abreu et al. 2008).

Considering that the cashew (*Anacardium occidentale* L.) is an important crop for semiarid agriculture that contributes to social and economic development of several world regions, including the Brazil northeast. Moreover, considering that cashew culture in northeast Brazil is developed in irrigated area with inferior quality water, due to the high salt concentration, and in consequence promotes soil salinization. Despite its importance, very few studies were performed comparing different genotypes with salt tolerance mechanisms in different views. Therefore, more studies about physiological and biochemical parameters related to salinity tolerance mechanisms must be carried out to identify genotypes tolerant to such abiotic stress. Based on this, we hypothesized that salinity could affect negatively growth, gas exchange, water status, osmoregulation and antioxidative enzymes of two cashew genotypes, and we investigate if these genotypes show different tolerance responses to these parameters mentioned above.

MATERIALS AND METHODS

Plant material and growth condition

Dwarf cashew nuts of genotypes CCP06 and BRS 189, provided by Embrapa Agroindústria Tropical, Fortaleza, CE, Brazil, were surface disinfected in a 0.09% (w/v) thiophanate-methyl solution for 10 min and then thoroughly rinsed with distilled water. These nuts were sown in 5.5 L plastic pots containing vermiculite moistened, in a ratio of 2:1, with distilled water (0 dS m^{-1}) or NaCl solution with 8 and 16 dS m^{-1} of electric conductivity (CE). The trays were kept in a greenhouse under the following conditions: a midday photosynthetic photon flux density of approximately 1,200 µmol m^{-2} s^{-1}, a mean air temperature of 31.0° C (day) and 25.0° C (night), and a mean relative humidity of 73.9%. Five replicates (trays) of 10 nuts were used in each treatment. On the first experimental day, each tray was weighed so that the water lost by evapotranspiration could be replaced daily.

Growth parameters, SPAD index and gas exchange, organic and inorganic contents

Seedlings with four fully expanded primary leaves [27 days after sowing (DAS)], were subjected to SPAD index (chlorophyll relative content). These same seedlings were used to determination of net photosynthesis per unit leaf area (*A*), stomatal conductance to water vapor (g_s) and internal-to-ambient CO_2 concentration ratio (Ci/Ca) were measured at 08:00–09:00 h under artificial, saturating PPF (1200 μmol m^{-2} s^{-1}) with a portable open-system infrared gas analyzer (LCi, ADC, Hoddesdon, UK).

Seedlings at 28 DAS were harvested for determination of leaf area (LA) using a leaf area meter LI-3000 (LI-COR, Inc., Lincoln, NE, USA). In following, seedlings were separated into leaves, stem and roots, and immediately frozen in N_2 liquid. One portion of this material were frozen-dried and used for dry mass estimation (shoot and root dry mass) and oxidative enzyme stress.

During the seeding harvesting, some part of each tissue (leaf and root) were used to inorganic and organic estimation. For this, leaf and root fresh tissues were macerated in a mortar and pestle and filtered through nylon tissue using a 10 mL disposable syringe. Thereafter, leaf and root juices were centrifuged at 12.000×*g*, for 10 min, at environment temperature, and the supernatants were used for organic and inorganic solute determination.

Na^+ and K^+ concentrations were determined by flame photometry (Malavolta et al. 1989), whereas Cl^- concentration was determined spectrophotometrically following the $Hg(SCN)_2$-$Fe(NO_3)_3$ method described by Gaines et al. (1984). Proline, soluble N-amino and soluble carbohydrates (were also estimated using root and leaf juices). Soluble carbohydrate determination was based on the phenol-sulfuric acid method (Dubois et al. 1956). Soluble N-amino concentration was measured spectrophotometrically using the ninhydrin method performed by Yemm & Cocking (1955). Proline was quantified spectrophotometrically following the ninhydrin method described by Bates et al. (1973).

Enzymatic extraction and antioxidative metabolism estimation

For extract preparation, 100 mg of leaf and root lyophilized powder were homogenized in 5 mL of ice-cold extraction buffer [100 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA] using a mortar and pestle. For APX extracts, 2 mM ascorbic acid was added to the extraction buffer. After extraction, the homogenate was filtered through muslin cloth and centrifuged at 12,000 x g for 15 min at 4° C; the supernatant was separated and used as crude extract for enzyme

activity and lipid peroxidation determination by spectrophotometric.

Total SOD (EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT), as described previously by Beauchamp & Fridovich (1971). The reaction was conducted at 25°C in a chamber with two 20-W fluorescent tubes for 15 min (Giannopolitis & Ries 1977). One SOD activity unit (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT (nitroblue tetrazolium) photoreduction rate.

Total APX (EC 1.11.1.1) activity was performed through ascorbate oxidation by measuring absorbance at 290 nm and using the molar extinction coefficient (2.8 mM^{-1} cm^{-1}), according to Nakano & Asada (1981).

Total GPX (EC 1.11.1.7) activity was determined through guaiacol oxidation by measuring the absorbance at 470 nm and using the molar extinction coefficient (26.6 mM^{-1} cm^{-1}) as described by Kar & Mishra (1976).

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid reaction, using the extinction coefficient (155 mM^{-1} cm^{-1}), according to Cakmak & Horst 1991.

Experimental design and statistical analysis

The experimental design was completely randomized, following a factorial scheme composed of two cashew nut genotypes (CCP06 and BRS189) and three two salt treatments (CE of 8 and 16 dS m^{-1}) and 0 (distilled water-control). Analyses were performed using five replications containing 10 plants per treatment. The results were subjected to a two-way analysis of variance (ANOVA). When a difference was significant ($p \leq 0.05$), the values were compared through Tukey's test. Each replicate was measured twice, and the data were expressed as the means ± standard error.

RESULTS AND DISCUSSION

Growth, SPAD index and gas exchange

Growth and development of dwarf cashew seedlings were affected by salinity increment in the culture medium. Salt stress effects on shoot dry mass (SDM) and leaf area (LA) were similar in both clones, presenting evidenced damages only under higher salinity conditions (16 dS m^{-1}). Seedlings growing in such conditions had their SDM average and LA values reduced by 26.8% and 41.2%, for CCP 06 and BRS 189, respectively, when compared to the control treatment (0 dS m^{-1}) (Figure 1). The reduction of LA is highlighted as one of the first responses of the plants to saline stress, resulting in a smaller area for the capture of light energy, thus compromising photosynthesis and plant growth. (Taiz et al. 2015). Regarding root dry mass (RDM), only CCP06 clone seedlings were significantly affected by salinity, decreasing by 33.1% when compared to the control (Figure 1). Growth reduction is one of the main effects of salinity on plants. This behavior has been observed in both glycophytes and halophytes, but the first ones are affected even when submitted to low doses of salt (Srinivas et al. 2018). Additionally, Alvarez-Pizarro et al. (2009) studied physiological and biochemical responses to salt stress in dwarf-cashew during seedling establishment. Corroborating to our findings in the present study, these researchers observed that in terms of total dry mass, BRS189 genotype had a lower reduction of growth than CCP06, thus suggesting higher tolerance to salinity in the former.

Under control conditions (0 dS m^{-1}), the SPAD index of BRS189 clone seedlings was higher than CCP 06 clone seedlings, and there was a distinct effect of the salinity on each clone; we observed

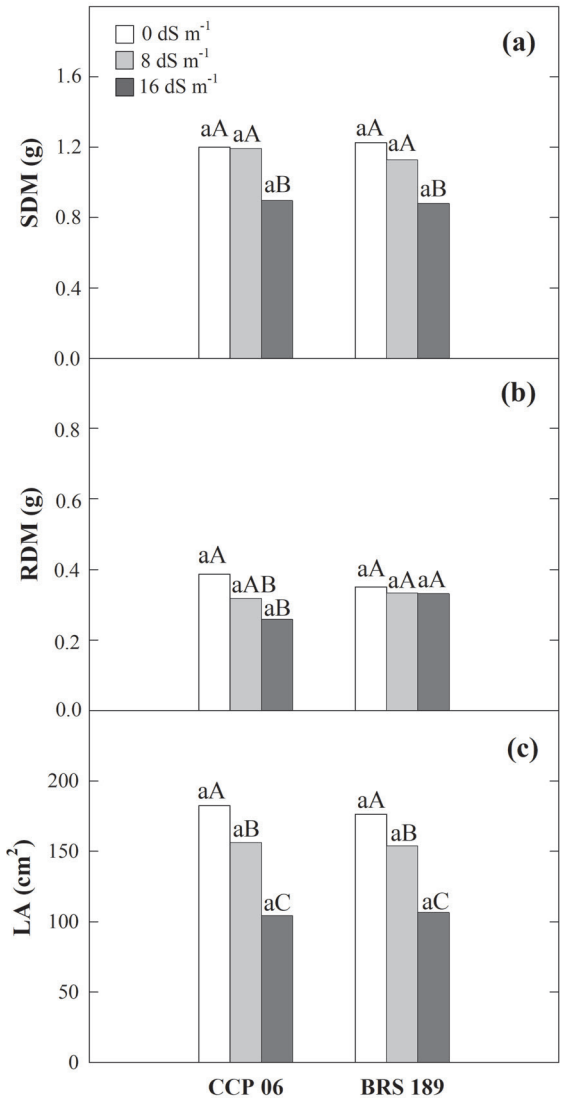

Figure 1. Shoot (SDM), root dry mass (RDM) and leaf area (LA) of seedlings of two cashew nut clones subjected to different salinity levels. The means followed by the same lowercase letter within cashew clones or by the same uppercase letter for salinity levels did not differ statistically (p>0.05).

CCP06 SPAD index remained unchanged as the EC of the medium increased, whereas for BRS189 this variable presented an average reduction of 12.8% compared to the control treatment (Figure 2). When it comes to gas exchanges parameters, we verified g_s was significantly reduced by salinity in both clones, but CCP06 clone was more affected than BRS189 clone. The first and latter one presented 87.4% and 60.9% of reduction under 16 dS m^{-1}, respectively (Figure 3).

A similar response to the SPAD index was observed for *A* variable under this same electrical conductivity, in which CCP06 clone was more sensitive to salinity than BRS189 clone, presenting 69.8% and 34.7% of reduction in comparison to control, respectively (Figure 3). Similarly, *E* mean values were also reduced as the EC was increased, mainly in clone CCP06 (Figure 3). On the other hand, BRS189 clone seedlings under 0 and 8 dS m^{-1} showed significantly higher *E* values than those of CCP06 clone, but under higher salt stress conditions (16 dS m^{-1}), these values did not differ significantly between both clones. Finally, C_i/C_e of both clones was also reduced due to salinity. Hence, corroborating to what we found for the previously mentioned gas exchange variables, CCP06 was more affected than BRS189 clone (Figure 3).

Agreeing to this study, Alvarez-Pizarro et al. (2009) report for these clones that net photosynthesis decreased in both genotypes due to salinity, but CCP06 was more affected than BRS189 clone. Also, Munns (2002) points out

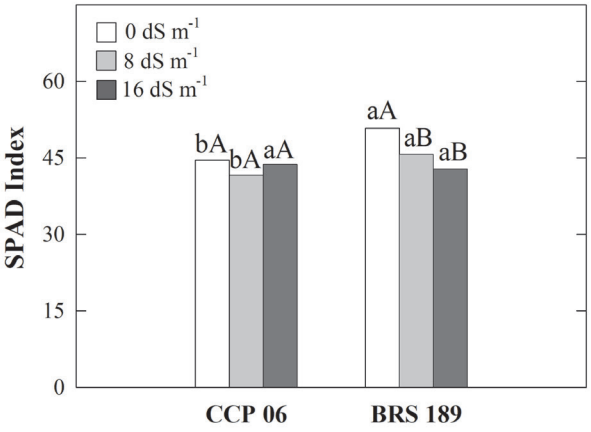

Figure 2. SPAD index of seedlings of two cashew nut clones subjected to different salinity levels. The means followed by the same lowercase letter within cashew clones or by the same uppercase letter for salinity levels did not differ statistically (p>0.05).

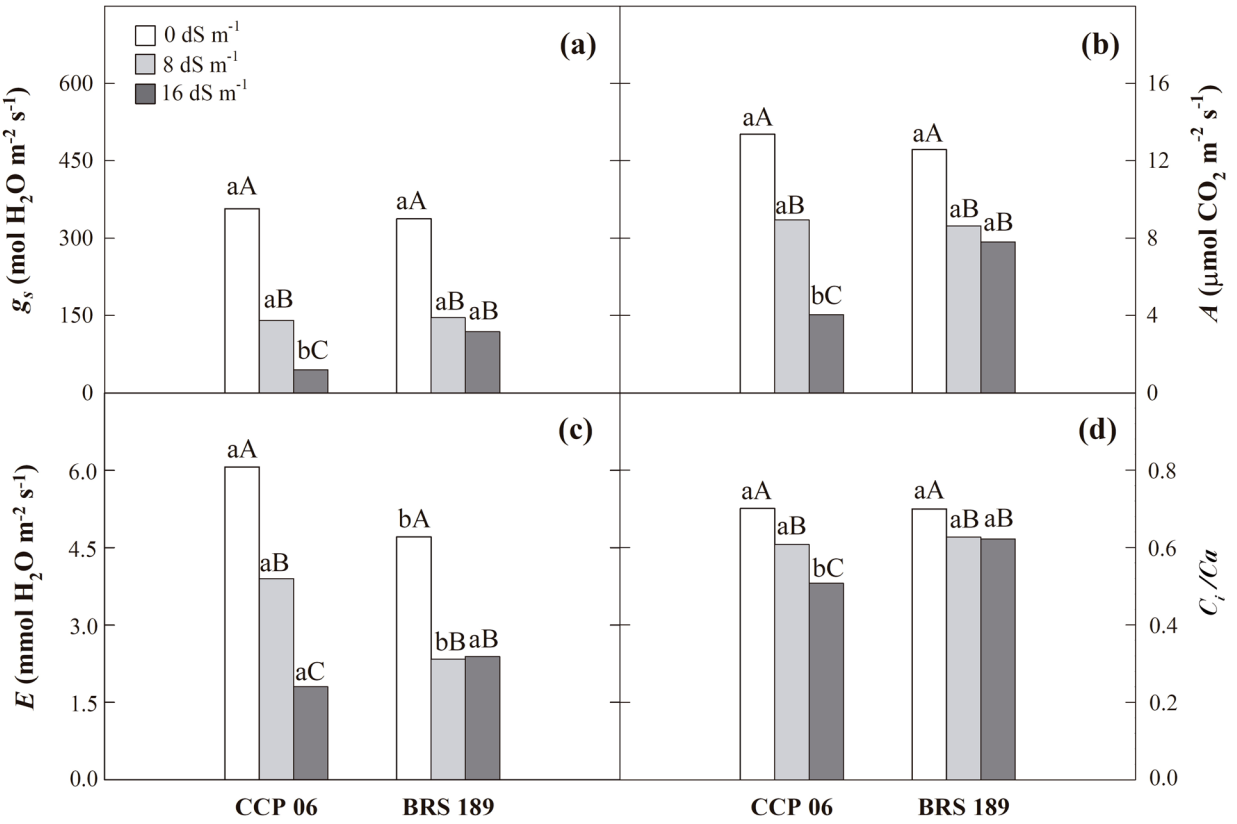

Figure 3. Stomatal conductance (g_s), Net photosynthesis (*A*), transpiration (*E*), and internal-to-ambient CO_2 concentration ratio (Ci/Ca) of seedlings of two cashew nut clones subjected to different salinity levels. The means followed by the same lowercase letter within cashew clones or by the same uppercase letter for salinity levels did not differ statistically (p>0.05).

that in addition to the physical injuries caused to foliar tissues, salinity can also accelerate the senescence of mature leaves because of chlorophylls degradation. Furthermore, Sharma et al. (2011) reported the main symptoms of salt stress in fruit plants are premature leaf drop, twig dieback, leaf scorching, blackening and necrosis. Nonetheless, in our study we observed that the strong reduction of *A* in CCP06 clone seedlings was not related to the leaves senescence or chlorophyll degradation. SPAD index, which corresponds to the relative chlorophyll content in leaf tissues, was not affected by salt stress in this research.

Osmoregulation

Foliar and root Na^+ concentrations were significantly increased due to salt stress increases in both clones. Although CCP06 leaves presented Na^+ concentrations lower than those of BRS189, the first one was the clone that accumulated such toxic ion the most. Thus, we estimated the average ion concentration was incremented by 3.9 and 2.8 times for both clones under salt stress, respectively. On the other hand, in the roots the highest accumulation of Na^+ was observed in BRS189, especially at 16 dS m^{-1} (Figure 4).

According to our findings, BRS189 clone seedlings accumulated less Na^+ in shoots and more Na^+ in roots. Such behavior observed in this research is likely to be related to the existence

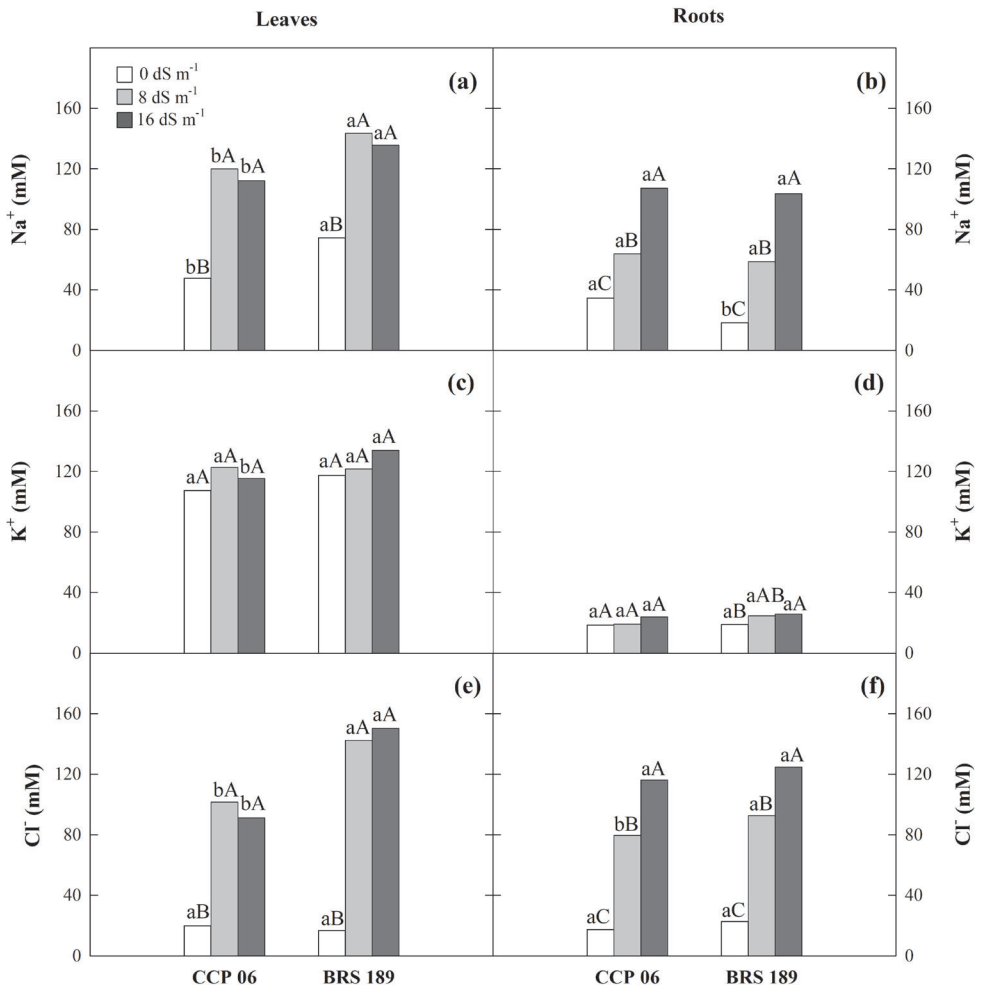

Figure 4. Na^+, K^+ and Cl^- concentrations in leaves and roots of two cashew nut clones subjected to different salinity levels. The means followed by the same lowercase letter within cashew clones or by the same uppercase letter for salinity levels did not differ statistically ($p>0.05$).

of mechanisms to control the absorption and translocation of Na^+ to the shoots, which is considered an important tolerance trait for acclimation to salt stress (Munns 2002, Oliveira et al. 2011, Taiz et al. 2015). Similarly, Silva et al. (2015) reported that *Jatropha curcas* L. seedlings presented changes in key physiological processes that allow this species to adjust to salinity. According to these authors, such responses are related to accumulation of Na^+ and Cl^- in the leaves and roots, K^+ / Na^+ homeostasis, transport of K^+ and selectivity (K^+-Na^+) for roots and accumulation of organic solutes contributing to the osmotic adjustment of the species.

Although salinity significantly increased Na^+ concentration in dwarf-cashew seedlings, there was slight variation due to this abiotic stress on leaf and root K^+ concentrations, which generally did not differ among the clones studied (Figure 4). Therefore, in this study the maintenance of K^+ levels in salt-stressed plants like the control treatment played an essential role as an adaptive response to salt stress, considering that K^+ is essential for the activity of many enzymes and for the regulation of cell turgescence and ion transport (Munns 2002, Rodrigues et al. 2016).

Like Na^+ behavior, Cl^- concentration in both organs studied increased drastically due to salinity. We verified that leaves presented the significantly increase in this variable due to saline treatments, in which BRS189 clone presented higher increases (1650%) approximately ten

times higher than CCP06 concerning control. On the other hand, in roots we observed this increase resulted from salinity was similar among the clones, resulting in Cl^- concentration five times greater than control treatment under 16 dS m^{-1} (Figure 4). Corroborating to our findings, Bader et al. (2015) observed olive tree (*Olea europaea* L.) presented greater sodium accumulation in leaves and roots with increased external NaCl concentration, but differently according to cultivars. These authors reported the salinity-tolerant cultivar ('Picholine') accumulated less Na^+ and Cl^- in leaves and was able to maintain higher K^+/Na^+ ratios compared to others. Similarly, Zarei et al. (2016) reported the ability to sequester Na^+ and Cl^- ions in roots differs among the four fig genotypes they used in their study. Overall, their results indicated that salinity tolerance in fig tree is strongly associated with Na^+ and Cl^- ions exclusion mechanism from shoots. Therefore, the regulation of Cl^- transport from roots to shoots seems to be an essential process for salinity tolerance, mainly in fruit trees, that are generally more sensitive to Cl^- and Na^+ ions (Munns 2002, Taiz et al. 2015, Bader et al. 2015, Zarei et al. 2016). Moreover, high Cl^- and Na^+ concentrations in leaves, mainly the latter ion, may have been responsible for such strong reduction in CCP06 clone photosynthesis (Figure 3), which was the most affected by salt stress.

Proline is the most common compatible osmolyte in plants and it has therefore been extensively studied. In this research we noticed that proline concentration in leaves did not differ statistically among the clones analyzed. Nevertheless, this organic solute concentration increased progressively with salinity, presenting mean values 128.0% higher under 16 dS m^{-1} in comparison to control treatment (Figure 5). In roots, proline concentration was just slightly altered by salinity in CCP06 clone, whereas in BRS189 it increased significantly, specially under higher salinity (16 dS m^{-1}), reaching mean values 267% greater than the control treatment (Figure 5). Although we observed the salt stress effects on this solute concentration in roots were significantly more evident than shoots, leaves proline accumulation was considerably higher than in roots. It has been suggested that leaves accumulate more proline to maintain chlorophyll level and cell turgor to protect photosynthetic activity under salt stress (Silva-Ortega et al. 2008, Huang et al. 2013, Mansour et al. 2016). This is consistent with our results indicating that proline accumulation was greatest in the leaves of stressed plants. Therefore, the accumulation of this amino acid is an important regulatory mechanism under osmotic stress (Oliveira et al. 2011, Huang et al. 2013, Mansour et al. 2016).

Soluble carbohydrates concentration in leaves did not present significant changes due to either salinity or the clone studied (Figure 5). Although similar results were observed in BRS189 clone roots, salinity induced a slight increase in these solutes concentration in CCP06 clone (Figure 5). The role of carbohydrates as osmoregulators has been studied in many plant species, showing contrasting results that vary according to the genotype, organ studied, severity and duration of stress (Oliveira et al. 2011, Mansour et al. 2016, Ramteke & Sachin 2016). In this way, Oliveira et al. (2013) highlight that exposure to salt stress affects plant metabolism through complexes mechanisms, and as a result, several compounds that can function as osmolytes or compatible solutes, including carbohydrates and free proline, will be accumulated. In species of the genus Citrus, Arbona et al. (2003) observed that leaf and root contents of soluble carbohydrates were proportionally reduced due to the increasement of salt concentration in the soil solution, indicating that carbohydrates were not essential in maintaining these plants water potential.

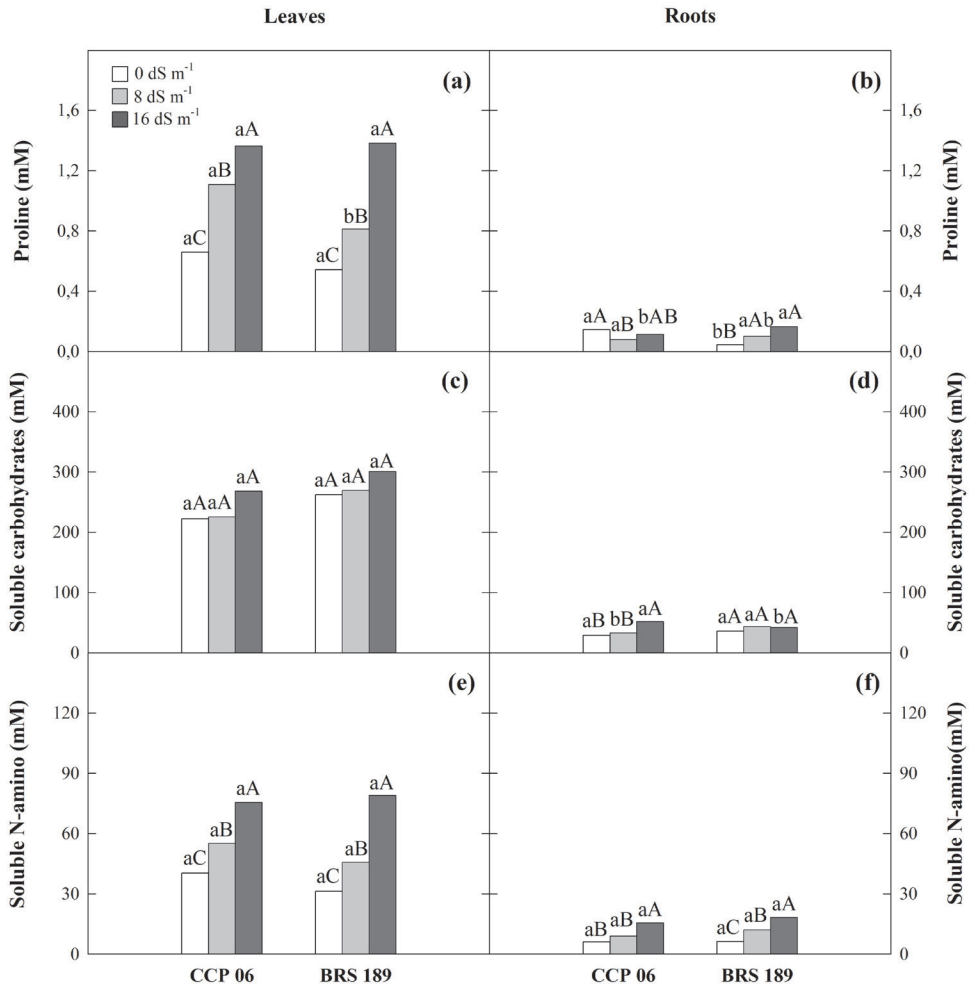

Figure 5. Proline, soluble carbohydrates and soluble N-amino in leaves and roots of two cashew nut clones subjected to different salinity levels. The means followed by the same lowercase letter within cashew clones or by the same uppercase letter for salinity levels did not differ statistically (p>0.05).

Such results contrast with those obtained in the present study, because although the carbohydrate contents were only slightly altered by salinity, their elevated levels in dwarf-cashew tissues indicate that these solutes played an important role in the osmotic adjustment of this plant.

Leaf and root soluble N-amino concentrations did not differ statistically among the clones but increased progressively as a function of salinity. Thus, CCP06 and BRS189 clones growing under 16 dS m^{-1} reached main values 113% and 170% higher than the control, respectively (Figure 5). The results suggest that soluble N-amino, together with carbohydrates, were the solutes that contributed the most to the maintenance of the osmotic adjustment of the dwarf cashew seedlings under salt stress. Soluble N-amino are considered important for osmotic adjustment, protection of macromolecules, protection against ROS, maintenance of intracellular pH, and serve as nitrogen reserves (Arbona et al. 2003, Ramteke & Sachin 2016). Furthermore, our findings suggest that dwarf-cashew BRS189 clone is more tolerant to salt stress than CCP06 clone, synthesizing carbohydrates and soluble N-amino as major compatible solutes to adjust the osmotic pressure when the Na^+ is accumulated in its cells and sustain the cell against homeostasis.

Antioxidative metabolism estimation

The enzymatic ROS scavenging mechanisms in plant includes production of SOD, GPX, APX, and other enzymes. The metalloenzyme SOD converts O_2 to H_2O_2, catalase and a variety of peroxidases catalyze (Rajput et al. 2016). SOD activity did not differ significantly between the clones in both organs studied. This enzyme's activity in leaves of salt-stressed CCP06 clone seedlings (16 dS m^{-1}) slightly increased in comparison to the control, while in roots both clones showed similar increases in SOD activity (about 50%) (Figure 6). Several authors have pointed out that salinity changes the activity of SOD as a response to increased production of ROS, especially superoxide (Oliveira et al. 2012, Wang et al. 2016, Liang et al. 2018). The results showed that SOD activity was higher in leaves than in roots, either under control conditions or salt stress, and this behavior is in agreement

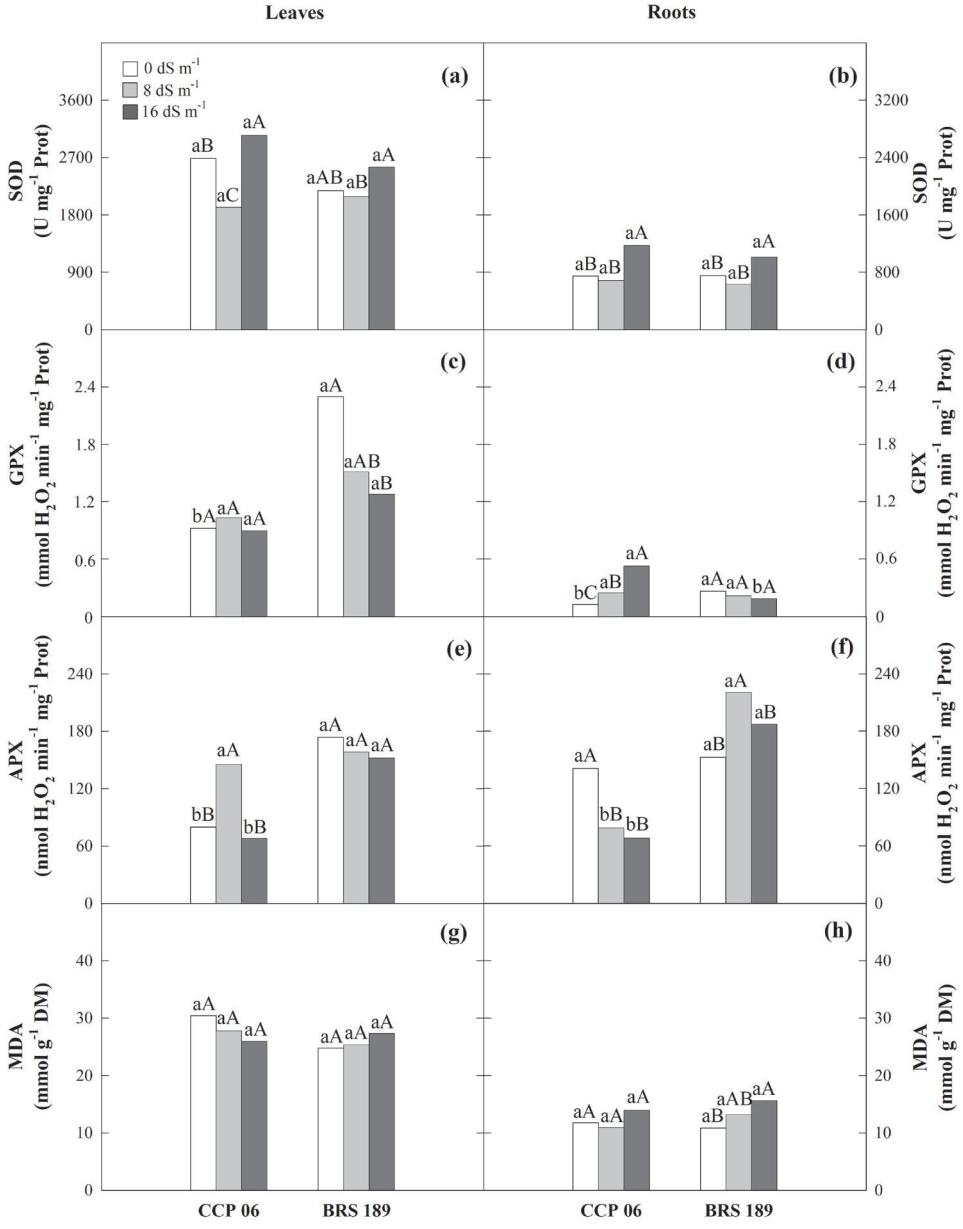

Figure 6. Activities of Superoxide dismutase (SOD), guaiacol peroxidase (GPX) ascorbate peroxidase (APX) and malondialdehyde contents (MDA) in leaves and roots of two cashew nut clones subjected to different salinity levels. The means followed by the same lowercase letter within cashew clones or by the same uppercase letter for salinity levels did not differ statistically (p>0.05).

with the fact that leaves are the main organs producing ROS. Additionally, Rajput et al. (2016) emphasized the balance and stability of SOD synthesis is crucial for suppressing lethal level of ROS within the cells.

Among various antioxidant defense mechanisms observed in salt-stressed plants to protect them against the potentially cytotoxic species of activated oxygen, increases in GPX activity have been pointed out as a relevant strategy for this purpose in some species. In this research, GPX activity in leaves was significantly reduced by salinity in BRS189 clone, whereas in roots of salt-stressed (16 dS m^{-1}), CCP06 clone seedlings the GPX activity increased significantly (Figure 6). The altered GPX activity has been used as an indicator of stress in plants submitted to abiotic stresses, such as salinity (Oliveira et al. 2012, Liang et al. 2018). This enzyme plays an important role in the protection of cells against H_2O_2, also participating in the removal of this ROS, produced by the enzymatic dismutation of superoxide by SOD (Arbona et al. 2003). Our results, therefore, corroborate with the findings of Kartashov et al. (2008), who reported that in both control and experimental plants, the highest SOD activity was found in the roots of the glycophytes whereas highest activity of guaiacol-dependent peroxidase was detected in the roots of both control and experimental plants of the halophyte.

As the EC in the growth medium increased, APX activity in leaves increased transiently in CCP06 clone, but remained unchanged in BRS189 clone seedlings. On the other hand, this enzyme activity in CCP06 clone roots was significantly reduced (Figure 6). APX plays an important role in the regulation of intracellular H_2O_2 levels, along with catalase and GPX. According to Parida et al. (2004), the increase in its activity may be related to the activation of preexisting forms, salt-induced increase effects on this enzyme synthesis, or the increased production of H_2O_2 in the cytosol. Although the values of APX activity were much lower than those of GPX, the maintenance or increase of its activity in saline treatments suggests this enzyme also played an essential role for H_2O_2 elimination, especially in leaves and roots of the BRS189 clone.

Among the two peroxidases studied, the GPX enzyme was the one that most contributed to the H_2O_2 removal since it presented values with order of magnitude much higher than those of the APX (Figure 6). Our findings showed the enzymatic antioxidant system is crucial to improve salt-tolerance in dwarf-cashew seedlings. Similarly, in a recent study, Lima et al. (2018) reported that cashew plants growing under multiple abiotic stresses did not suffered photoinhibition and oxidative stress, and these responses were associated with increases in ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities, glutathione (GSH) oxidation and ascorbate (ASC) synthesis.

MDA levels varied little in leaves and roots, either as a function of salinity or in the comparison of the clones studied (Figure 6). MDA is a byproduct of lipid peroxidation caused by high amount of ROS, and its quantification is used to evaluate oxidative damage in membranes (Rajput et al. 2016, Gadelha et al. 2017). The absence of variation in MDA contents suggests that the antioxidative system of dwarf-cashew seedlings was efficient to avoid the damages caused by ROS. A few changes in MDA levels due to salt stress have also been described by other authors, because of increased activity of antioxidative enzymes (Oliveira et al. 2012, Rajput et al. 2016, Gadelha et al. 2017, Liang et al. 2018).

CONCLUSIONS

In this research, we observed significative reductions in dwarf cashew seedlings' growth, photosynthesis and gas exchange due to the excessive accumulation of Na^+ and Cl^- ions in their tissues confirming that this crop cultivation can be severally affected by salinity.

Despite the reductions in growth and gas exchange, dwarf cashew seedlings of both clones exhibited mechanisms of osmotic adjustment, plus an enzymatic antioxidant system that was able to attenuate the additional oxidative stress.

Although we reported similar responses to salinity for some parameters evaluated in this study, we would recommend the use BRS 189 in salt-affected soils rather than CCP 06, since the fact that the first clone is more tolerant to salinity than the latter.

Acknowledgments

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