

DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v27n2p92-100>

Salinity-tolerant dwarf cashew rootstock has better ionic homeostasis and morphophysiological performance of seedlings¹

Porta-enxerto de cajueiro anão tolerante à salinidade tem melhor homeostase iônica e desempenho morfofisiológico de mudas

Valéria F. de O. Sousa^{2*}, Gisele L. dos Santos³, Josemir M. Maia⁴, Sebastião de O. Maia Júnior⁵, João P. de O. Santos², José E. Costa², Anselmo F. da Silva², Thiago J. Dias², Sérgio L. Ferreira-Silva⁶ & Carlos A. K. Taniguchi⁷

¹ Research developed at Universidade Estadual da Paraíba/Centro de Ciências Humanas e Agrárias, Catolé do Rocha, PB, Brazil

² Universidade Federal da Paraíba/Departamento de Fitotecnia e Ciências Ambientais, Areia, PB, Brazil

³ Universidade Federal Rural do Semi-Árido/Departamento de Ciências Agrônômicas e Florestais, Mossoró, RN, Brazil

⁴ Universidade Estadual da Paraíba/Centro de Ciências Humanas e Agrárias, Catolé do Rocha, PB, Brazil

⁵ Universidade Estadual do Maranhão/Centro de Ciências Agrárias, São Luís, MA, Brazil

⁶ Universidade Federal Rural de Pernambuco/Unidade Acadêmica de Serra Talhada, Serra Talhada, PE, Brazil

⁷ Empresa Brasileira de Pesquisa Agropecuária/Embrapa Agroindústria Tropical, Fortaleza, CE, Brazil

HIGHLIGHTS:

The scion/rootstock combination CCP 76/09 kept the foliar K^+ concentration up to 100 mM L⁻¹ NaCl.

The self-graft CCP 76 and scion/rootstock combinations CCP 09 and CCP 76 were more sensitive to salt stress.

Salinity tolerance showed the following order: CCP 76/CCP 09 > CCP 09/CCP 09 > CCP 09/CCP 76 > CCP 76/CCP 76.

ABSTRACT: Considering the cashew tree's relevance and the limitations imposed by salinity stress in semi-arid regions, the use of alternatives capable of mitigating the harmful effects due to salinity is of great importance to the production sector. The use of grafted plants, especially with rootstock made of tolerant materials, influences the accumulation of toxic ions in leaves of grafted seedlings. Thus, the objective of this work was to evaluate morphophysiological characteristics and leaf concentrations of Na^+ , K^+ and Ca^{+2} of combinations of scion and rootstock of early dwarf cashew, contrasting in terms of salinity tolerance. The experiment was carried out in a completely randomized design with five replicates, in a 4×3 factorial arrangement, corresponding to four dwarf cashew scion/rootstock combinations (self-graft CCP 09, CCP 09/CCP 76, self-graft CCP 76, and CCP 76/CCP 09) and three NaCl concentrations (0, 50, and 100 mM L⁻¹). Height, number of leaves, leaf area, dry matter, tolerance index and leaf concentrations of Na^+ , K^+ and Ca^{+2} were evaluated after 30 days of application of NaCl concentrations. The scion/rootstock combination CCP 76/09 showed tolerance to 50 mM L⁻¹, due to the increase of leaf area and number of leaves. The scion/rootstock combination CCP 76/09 was more suitable, as it kept the leaf K^+ concentration and had the lowest Na^+ concentration.

Key words: *Anacardium occidentale* L., ion accumulation, grafting, salt stress

RESUMO: Considerando a relevância do cajueiro e a limitação imposta pelo estresse salino em regiões semiáridas, o uso de alternativas capazes de mitigar os efeitos deletérios em decorrência da salinidade são de grande importância ao setor produtivo. O uso de plantas enxertadas, principalmente com porta-enxertos de materiais tolerantes, influencia no acúmulo de íons tóxicos nas folhas das mudas enxertadas. Assim, o objetivo deste trabalho foi avaliar características morfofisiológicas e concentração foliar de Na^+ , K^+ , Ca^{+2} de combinações de copa e porta-enxerto de cajueiro anão precoce, contrastantes à salinidade. O experimento foi realizado em delineamento inteiramente casualizado, com cinco repetições, tendo os tratamentos dispostos em arranjo fatorial de 4×3 , correspondentes a quatro combinações de mudas enxertadas (auto-enxerto CCP 09/CCP 09, CCP 09/CCP 76, auto-enxerto CCP 76/CCP 76 e CCP 76/CCP 09) e a três concentrações de NaCl (0-controle, 50 e 100 mM L⁻¹). Foram avaliados altura, número de folhas, área foliar, massa seca, índice de tolerância e concentração foliar de Na^+ , K^+ e Ca^{+2} após 30 dias da aplicação das concentrações de NaCl. A combinação copa/porta-enxerto CCP 76/09 apresentou tolerância à salinidade de 50 mM L⁻¹, devido ao aumento da área foliar e número de folhas. A combinação copa/porta-enxerto CCP 76/09 foi mais adequada, pois manteve a concentração foliar de K^+ e o menor concentração de Na^+ .

Palavras-chave: *Anacardium occidentale* L., acumulação de íons, enxerto, estresse salino

• Ref. 263134 – Received 15 Apr, 2022

* Corresponding author - E-mail: valeriafernandesbds@gmail.com

• Accepted 02 Sept, 2022 • Published 19 Sept, 2022

Editors: Renér Luciano de Souza Ferraz & Walter Esfrain Pereira

This is an open-access article distributed under the Creative Commons Attribution 4.0 International License.



INTRODUCTION

Cashew (*Anacardium occidentale* L.) is widely cultivated in Brazil and, therefore, has great socioeconomic importance mainly in the semi-arid region of the Northeast, generating employment and income (Silva et al., 2018). However, cashew cultivation is carried out in areas irrigated with inferior quality water (30-50 mM L⁻¹ NaCl), due to the high salt concentration, which results in soil salinization, affecting the development of this crop (Alencar et al., 2021).

High concentrations of ions in the root region of plants, mainly Na⁺ and Cl⁻, trigger stress that causes ionic and osmotic effects, as it inhibits the absorption of K⁺ and Ca²⁺, because Na⁺ competes with K⁺ and Ca²⁺ for absorption sites on the cell membrane, causing nutritional disorders (Silva et al., 2022), inhibits water absorption, reduces leaf turgidity, plant growth and biomass production, and increases cell membrane degradation (Oliveira et al., 2018; Lima et al., 2020; Santana Júnior et al., 2020).

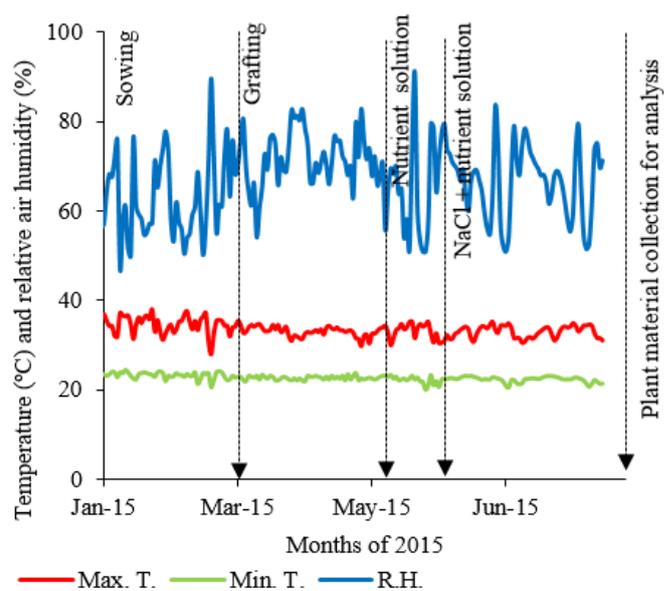
As an alternative to enable the production of dwarf cashew in regions with salinity problems, the use of grafted plants is recommended, especially with rootstock made of tolerant materials, since this influences the accumulation of toxic ions in leaves of grafted seedlings (Ferreira-Silva et al., 2009; Araújo et al., 2014). However, it is important to understand how contrasting materials in relation to salinity tolerance behave after grafting (Ferreira-Silva et al., 2010).

Thus, studies with grafted and contrasting materials in salinity tolerance can improve the understanding of morphophysiological mechanisms involved between scion and rootstock and interactions under salt stress (Ferreira-Silva et al., 2010), selecting more tolerant rootstocks, which results in more productive orchards (Lima et al., 2020). The hypothesis is that the use of tolerant rootstock promotes better morphophysiological performance of grafted seedlings. Thus, the objective of this work was to evaluate morphophysiological characteristics and leaf concentrations of Na⁺, K⁺ and Ca²⁺ of combinations of scion and rootstock of early dwarf cashew, contrasting in terms of salinity tolerance, based on plant initial growth traits.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse from January to June 2015, at the Centro de Ciências Humanas e Agrárias of the Universidade Estadual da Paraíba, located in the municipality of Catolé do Rocha, Paraíba, Brazil (6° 21' 08.7" S 37° 43' 32.0" W), at an altitude of 250 m. The climate of the region is BSw'h according to Köppen's classification, characterized by being semi-arid hot, with two distinct seasons, one rainy with irregular rainfall and the other without precipitation (Alvares et al., 2013). Air temperature and humidity data were monitored during the experimental period with a digital thermohygrometer (model 7666.02.0.00, Incoterm®, USA), as presented in Figure 1.

The experimental design was completely randomized, in a 4 × 3 factorial scheme, corresponding to four dwarf cashew scion/rootstock combinations (self-graft CCP 09, CCP 09/CCP



Min. T. - Minimum air temperature; Max. T. - Maximum air temperature; R.H. - Mean relative air humidity

Figure 1. Weather conditions during the cultivation of dwarf cashew seedlings

76, self-graft CCP 76 and CCP 76/CCP 09) and three NaCl concentrations (0, 50, and 100 mM L⁻¹), with five replicates, totaling 60 experimental plots, with each experimental plot composed of five seedlings.

Dwarf cashew seed-nuts from clones CCP 09 and CCP 76 came from Embrapa Agroindústria Tropical, Fortaleza, Ceará, Brazil. CCP 09 is widely cultivated in the commercial plantations of the Brazilian semiarid region and tolerant to salinity (Ferreira-Silva et al., 2010), and CCP 76 stands out for the quality of the nuts and the peduncle, but is moderately susceptible to salinity (Carneiro et al., 2007).

Before sowing, the nuts were selected according to size and health, eliminating those that floated after immersion in water. The nuts were disinfected in 5% (v/v) sodium hypochlorite solution for 10 min and germinated in polyethylene bags, with a capacity of 1,250 mL, filled with Plantmax® substrate. After the seedlings reached the stage of 10 leaves, at 60 days after sowing, grafting was performed by the side graft method, with scions from adult plants of the same clones, forming the four scion/rootstock combinations studied. The early dwarf cashew clones CCP 09 and CCP 06 used for scion were supplied by Embrapa Agroindústria Tropical, from the experimental field in Pacajus (4° 11' 07" S, 38° 30' 07" W, at 70 m altitude), in the state of Ceará, Brazil. The propagules came from the terminal, healthy and developed branches of 4-year-old mother plants, and were removed from the plants the day before the experiment was set up, transported in moistened paper bags and stored in a refrigerator at a temperature of 15 °C until grafting.

At 60 days after grafting, the most vigorous grafted seedlings were selected and transplanted to pots with capacity of 4 dm³ each, which were filled with a substrate composed of a mixture of vermiculite with sand in a 1:1 ratio. The grafted seedlings were standardized in terms of size in height so as not to influence the results. The grafted seedlings were kept under irrigation, containing nutrient solution with ¼ strength, according to Hoagland & Arnon (1950), for 15 days. After that

time, saline treatments were included in the nutrient solution with a pH ranging from 5.5 to 6.0, ideal for nutrient absorption by the crop (Silva et al., 2018). The irrigation depths were determined by a drainage lysimeter, using five seedlings that were subjected to 0 mM L⁻¹ NaCl and irrigated until drainage. The volume drained was subtracted from the volume of water applied, and the resultant amount was used for the remaining seedlings. The application of NaCl was carried out for 30 days, daily through irrigation in the late afternoon.

After 30 days of application of NaCl concentrations (DAP), the growth of dwarf cashew seedlings was evaluated based on their height, measured as the distance from the collar to the point of insertion of the apical meristem. The number of leaves, including those fully expanded with a minimum length of 2 cm, and leaf area (LA) per seedling, obtained from measurements of leaf width and length, were calculated using Eq. 1 (Murthy et al., 1985) and expressed in cm²:

$$LA = 0.21 + 0.69 \times W \times L \quad (1)$$

where:

- LA - leaf area;
- W - leaf width; and,
- L - leaf length.

The relative water content (RWC) was also measured at 30 DAP, being calculated according to the methodology described by Irigoyen et al. (1992). For that, 10 leaf discs (10 mm in diameter) were collected and their fresh mass was measured on a semi-analytical balance; then, the discs were submerged in distilled water for 24 hours to reach saturation. The turgid mass was then obtained, and the leaf discs were placed in an oven with air circulation at 65 °C for 24 hours, to determine the dry mass (DM). The relative water content was then calculated using Eq. 2:

$$RWC = \left(\frac{FM - DM}{TM - DM} \right) \times 100 \quad (2)$$

where:

- RWC - relative water content;
- FM - fresh mass;
- DM - dry mass; and,
- TM - turgid mass.

At 30 DAP, the electrolyte leakage was measured according to Lutts et al. (1996). 10 leaf discs were collected, placed in a test tube with 10 mL deionized water, and incubated in a water bath at 25 °C for six hours. Then, conductivity meter readings were performed to determine the electrical conductivity of the solutions (L1). Subsequently, the tubes were placed again in a water bath for one hour at 100 °C, and new readings of the electrical conductivity of the solutions (L2) were made. Electrolyte leakage was estimated by Eq. 3:

$$\%EL = \left(\frac{L1}{L2} \right) \times 100 \quad (3)$$

where:

- %EL - electrolyte leakage;
- L1 - conductivity after six hours at 25 °C; and,
- L2 - conductivity after one hour at 100 °C.

Subsequently, the leaves and stems of the scion and rootstock were separated, and the vegetative parts were placed separately to dry in a forced air circulation oven at 65 °C for 72 hours to obtain a constant dry matter mass. Subsequently, the material was weighed on an analytical balance (0.001 g) to determine the dry matter mass of leaves, dry matter mass of the scion, dry matter mass of the rootstock (g).

These masses partitioned among vegetative organs were summed to obtain the total dry mass and calculate the salinity tolerance index, by comparing the data of saline treatments with those of the control (0 mM NaCl L⁻¹), using Eq. 4:

$$STI = \left(\frac{TDM \text{ salinity treatment}}{TDM \text{ control treatment}} \right) \times 100 \quad (4)$$

where:

- STI - salinity tolerance index; and,
- TDM - total dry mass.

Na⁺, K⁺, and Ca⁺² concentrations were determined using 50 mg of leaf dry mass, ground in a Wiley-type stainless steel knife mill with 20 mL of deionized water. The materials were placed in hermetically sealed test tubes and kept in a water bath at 100 °C for one hour. The extract was obtained by filtration, and content of the ions was measured with the help of readings in a flame photometer (Analyser 910M) (Malavolta et al., 1997).

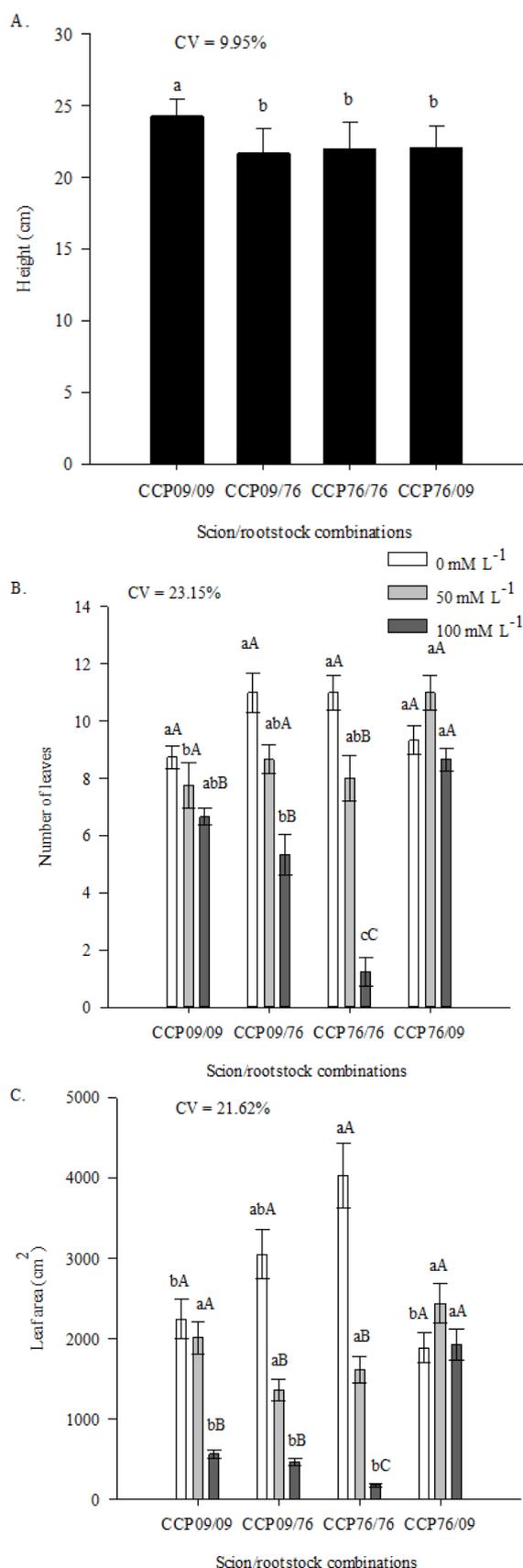
The data obtained were subjected to the F test at p ≤ 0.05, through analysis of variance, and the means were subjected to the Tukey test at the same probability level. Analyses were conducted using R 3.6.3 platform with ExpDes.pt computer package (Ferreira et al., 2018).

RESULTS AND DISCUSSION

Seedling height varied significantly only for scion/rootstock combinations, probably due to the short period of exposure to salt, which did not interfere with seedling height. It was observed that the self-graft CCP 09 seedlings showed greater height (24.29 cm), differing statistically from the others (Figure 2A). Similar results were reported by Serrano et al. (2013), who observed greater growth of clone CCP 09 compared to CCP 76; therefore, this fact may be related to the genetic factor of each material.

The number of leaves of the grafted seedlings was significantly affected by the scion/rootstock combinations and NaCl concentrations (Figure 2B). It was observed that the CCP 09/CCP 76 and self-graft CCP 76 reduced leaf production from 11 leaves to 5.33 and 1.25 leaves, respectively, between the lowest (0 mM L⁻¹) and highest (100 mM L⁻¹) concentration of NaCl.

This effect may have been caused by the exposure period, in addition to the high concentration of salts, as the leaves assimilated a large amount of toxic ions, such as Na⁺, causing



Means followed by the same lowercase letters in Figure 2A do not differ statistically by the Tukey test ($p \leq 0.05$). In the other figures, means followed by the same lowercase letters do not differ between the scion/rootstock combinations within each saline treatment, and means followed by the same uppercase letters do not differ between the saline treatments within each scion/rootstock combination (Tukey test, $p \leq 0.05$). Vertical bars - Standard error of the mean ($n = 5$); CV - Coefficient of variation

Figure 2. Height (A), number of leaves (B), and leaf area (C) of dwarf cashew seedlings subjected to 0, 50, and 100 mM L⁻¹ NaCl, observed at 30 days after beginning of the salt stress

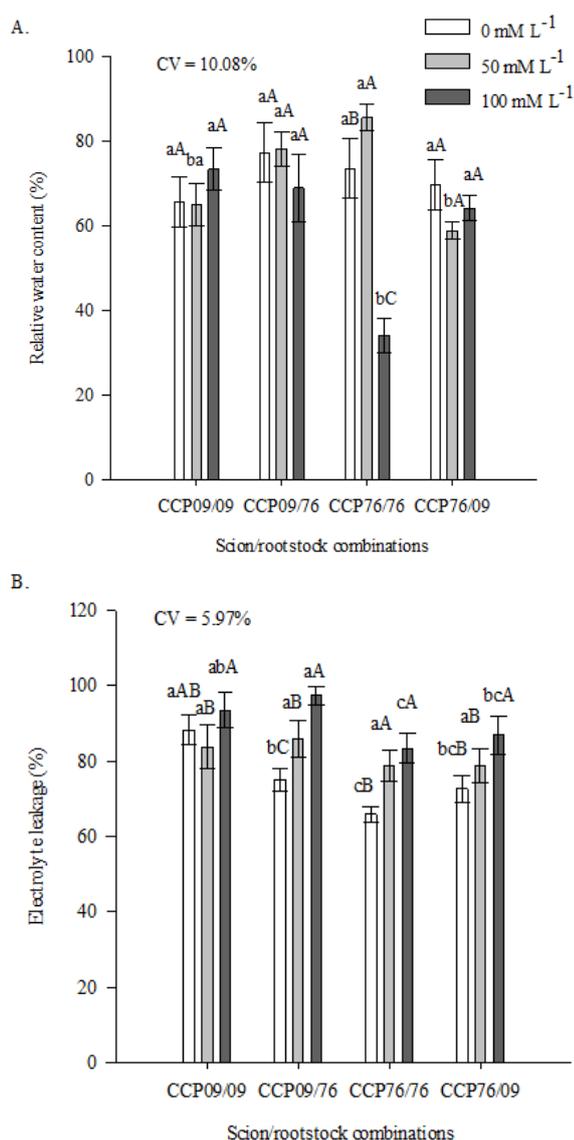
morphophysiological damage. Similarly, Araújo et al. (2014) found high Na⁺ accumulation in the leaves of cashew clone CCP 06. In contrast, the self-graft CCP 09 and the CCP 76/CCP 09 combination were not significantly influenced by increasing salt concentrations in terms of number of leaves. Rootstock CCP 09 may have been responsible for mitigating salt stress in the aerial part of cashew seedlings, since both combinations that contained it as rootstock did not suffer significant damage to the number of leaves, probably accumulating Na⁺ and Cl⁻ in the root system, from the perspective of reducing these ions in the shoot, as the damage by these ions in the leaves is the competition in ionic absorption between Na⁺ and K⁺ and between Cl⁻ and N, which causes nutritional disorders, reduction in the activation of several plant metabolism enzymes and consequently reduced growth, so the accumulation in the root system contributed to the lower Na⁺/K⁺ and Cl⁻/N ratios in the leaves, thus restoring ionic homeostasis (Gao et al., 2022).

Similar behavior to that for the number of leaves was observed in leaf area, with more pronounced decreases with the concentration of 100 mM L⁻¹ NaCl in self-graft CCP 09 (74.85%), CCP 09/CCP 76 (84.81%) combination, and self-graft CCP 76 (95.55%) seedlings, while CCP 76/CCP 09 was not affected by NaCl concentrations, with an average leaf area of 2,086.42 cm² (Figure 2C).

The behavior of CCP 76/CCP 09 seedlings, reaching a greater number of leaves and leaf area at a concentration of 50 mM L⁻¹, and self-graft CCP 09 with less reduction in leaf area at a concentration of 100 mM L⁻¹ compared to the other rootstocks, can be explained as a mechanism of adjustment to stress, accumulating in the vacuoles of stem or root cells toxic ions such as chlorine and sodium, decreasing the osmotic potential and increasing the turgor pressure in the leaves, thus accumulating water in the plant cells (Hessini et al., 2019), thus avoiding the concentration of toxic ions in the young leaves, where there is activity of leaf expansion and leaf production (Nawaz et al., 2020). Because the root system of the plant is responsible for the absorption of water and nutrients, the adjustment provided by the rootstock CCP 09 can be caused by reductions in the absorption of Na⁺ and Cl⁻ ions or their accumulation in the roots and stems, reflecting reductions in these ions in the leaves, causing the increase in leaf turgor and photosynthetic rates compared to the seedlings of the other studied rootstocks (Vila et al., 2020).

The relative water content (RWC) remained stable in self-graft CCP 09 and CCP 09/CCP 76 and CCP 76/CCP 09 combinations at all salt concentrations, while in the self-graft CCP 76, it increased 16.0% with the concentration of 50 mM L⁻¹ and decreased 53.56% with 100 mM L⁻¹ NaCl compared to the control, having the lowest RWC (34.12%) compared to the other scion/rootstock combinations with 100 mM L⁻¹ NaCl (Figure 3A).

Under saline conditions, the maintenance or increase of RWC indicated osmotic adjustment, in which salt stress did not affect the hydration of the leaves, and the excess of accumulated ions contributed to the retention of water in the tissues (Sanwal et al., 2022), increasing leaf succulence (Lima et al., 2020). Thus, higher RWC in seedlings under salinity conditions demonstrates greater adjustment to stress, suggesting that the self-graft CCP 76 combination had high



Means followed by the same lowercase letters do not differ between the scion/rootstock combinations within each saline treatment, and means followed by the same uppercase letters do not differ between the saline treatments within each scion/rootstock combination (Tukey, $p \leq 0.05$). Vertical bars - Standard error of the mean ($n = 5$); CV - Coefficient of variation

Figure 3. Relative water content (A) and electrolyte leakage (B) of dwarf cashew seedlings subjected to 0, 50, and 100 mM NaCl, at 30 days after beginning of the saline treatments

osmotic adjustment, only up to 50 mM NaCl, while the rest adjusted osmotically to 100 mM, mainly in the self-graft CCP 09 seedlings. Therefore, seedlings with CCP 09 rootstock adjusted better osmotically, as tolerant rootstocks control water transport to the scion maintaining high leaf turgor potential under stressful conditions (Sousa et al., 2022).

Grafted dwarf cashew increased the leakage of electrolytes with the addition of NaCl, except for self-graft CCP 09. These increases corresponded to 29.56% for CCP 09/CCP 76, 26.46% for self-graft CCP 76 and 19.66% for CCP 76/CCP 09, when comparing the 100 mM L⁻¹ with the 0 mM L⁻¹ NaCl concentration (Figure 3B). The highest electrolyte leakage at the concentration of 100 mM L⁻¹ was observed in CCP 09/CCP 76.

Electrolyte leakage indicates the percentage of the integrity of cell membranes and, therefore, can be used as a selection criterion for the identification of seedlings tolerant to salt stress (Sharma et al., 2011). Thus, greater tolerance to salt

was observed for self-graft CCP 09 and CCP 76/CCP 09 combination, as they had lower electrolyte leakage with the increase in NaCl concentration. In grafted citrus seedlings, Sousa et al. (2017) observed less electrolyte leakage in the more salt-tolerant genotype, with less damage, due to greater stability in the plasma membrane.

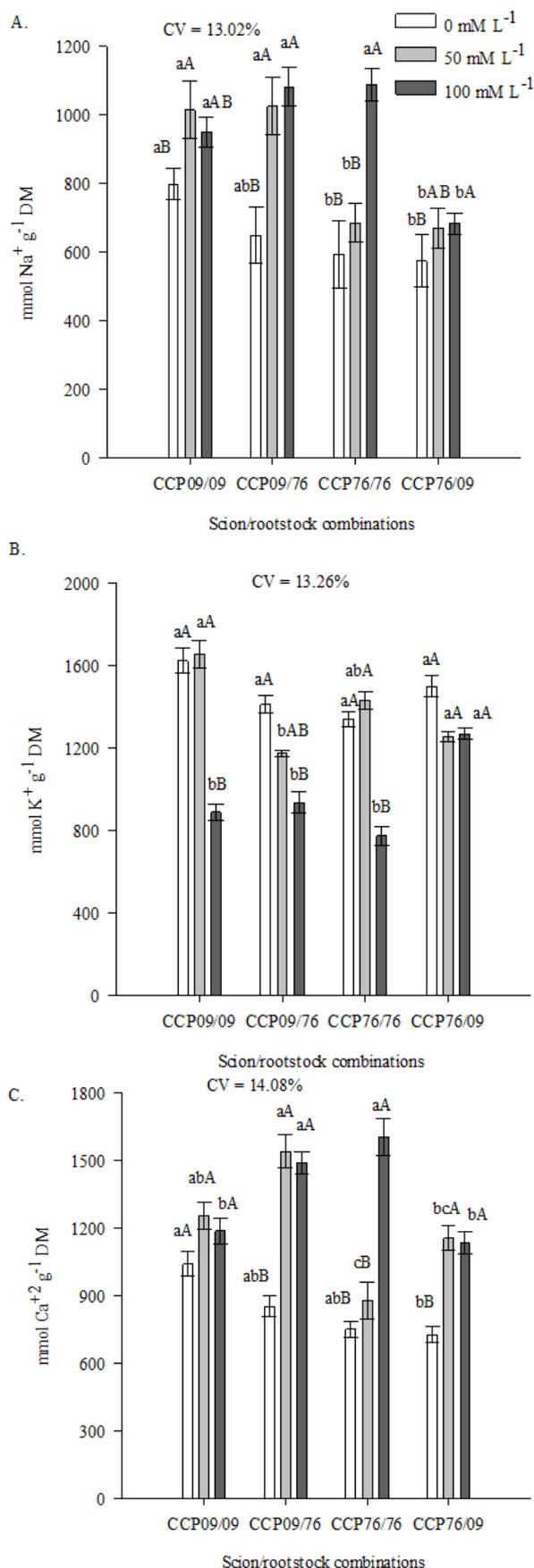
The Na⁺ concentration in the leaves of cashew seedlings was statistically lower in CCP 76/CCP 09 than in the other scion/rootstock combinations at the saline concentration of 100 mM L⁻¹ of NaCl (Figure 4A). In all scion/rootstock combinations there was an increase in Na⁺ content with increments of 21.34% for self-graft CCP 09, 67.36% for CCP 09/CCP 76, 83.41% for self-graft CCP 76, and 18.95% for CCP 76/CCP 09 compared to the 0 mM L⁻¹ NaCl concentration.

Regarding K⁺ concentration, there were 33.62 and 42.16% reductions in CCP 09/CCP 76 and self-graft CCP 76 seedlings, respectively, with the increase in NaCl from 0 to 100 mM L⁻¹ (Figure 4B). On the other hand, the CCP 76/CCP 09 combination kept the K⁺ concentration with the NaCl concentrations. Similarly, the self-graft CCP 09/CCP 09 seedlings kept it stable up to 50 mM L⁻¹, despite reducing it by 46.26% when irrigated with 100 mM L⁻¹ of NaCl.

The differences between cashew seedlings (CCP 76/CCP 09) that accumulated less Na⁺ and more K⁺ in the leaves indicated greater tolerance to NaCl, as a strategy of acclimatization to stress. This is similar to cashew rootstock BRS 226 (salt tolerant), which showed greater capacity for leaf exclusion of Na⁺ (Ferreira-Silva et al., 2009). Likewise, Alencar et al. (2021) observed that the cashew clone BRS 189 was more tolerant to salinity because it accumulates more Na⁺ in the roots and less Na⁺ and more K⁺ in the aerial part of the seedlings, being indicated for use as a rootstock in saline environments. One of the main responses of plants to salinity is the increase in Na⁺ input and decrease in K⁺ output in plant tissues, since sodium enters via the symplast into the same transport channels as potassium; nonetheless, to prevent the accumulation of high concentrations of Na⁺ in the cytosol, the cytoplasmic matrices limit the influx of Na⁺, increase the efflux of Na⁺ or compartmentalize Na⁺ in the vacuole. Therefore, the ability of plants to maintain low Na⁺ and high K⁺ concentrations is one of the mechanisms by which they tolerate salt stress (Sharma et al., 2011).

This is because high concentrations of Na⁺ ions are capable of triggering symptoms of salt toxicity in the leaves, such as chlorosis followed by necrotic areas (Silva et al., 2011), as observed in grafted cashew seedlings. In this way, plants can exclude toxic ions, compartmentalize them in the vacuole, and/or control their entry by the roots, avoiding harm to vital physiological processes, such as stomatal opening, photosynthesis, and cell expansion (Ferreira-Silva et al., 2009; Araújo et al., 2014).

As for the Ca⁺² concentration, there was no variation between salt concentrations for the self-graft CCP 09 seedlings, but it increased, especially when the seedlings were irrigated with 100 mM L⁻¹ NaCl. The increases were 74.29% for CCP 09/CCP 76, 113.34% for self-graft CCP 76, and 55.99% for CCP 76/CCP 09, compared to the control (Figure 4C). Calcium plays a vital role in the formation of cell walls and in the relationship between cells, also acting as a regulator in the cation-anion



Means followed by the same lowercase letters do not differ between the scion/rootstock combinations within each saline treatment, and means followed by uppercase letters do not differ between the saline treatments within each scion/rootstock combination (Tukey, $p \leq 0.05$). Vertical bars - Standard error of the mean ($n = 5$); CV - Coefficient of variation

Figure 4. Na⁺ (A), K⁺ (B), and Ca²⁺ (C) concentrations of dwarf cashew seedlings subjected to 0, 50, and 100 mM L⁻¹ NaCl, at 30 days after beginning of the saline treatments

balance and as a catalyst for some enzymes. Therefore, the increase in calcium levels under saline conditions shows regulation of enzyme metabolism (Nawaz et al., 2020).

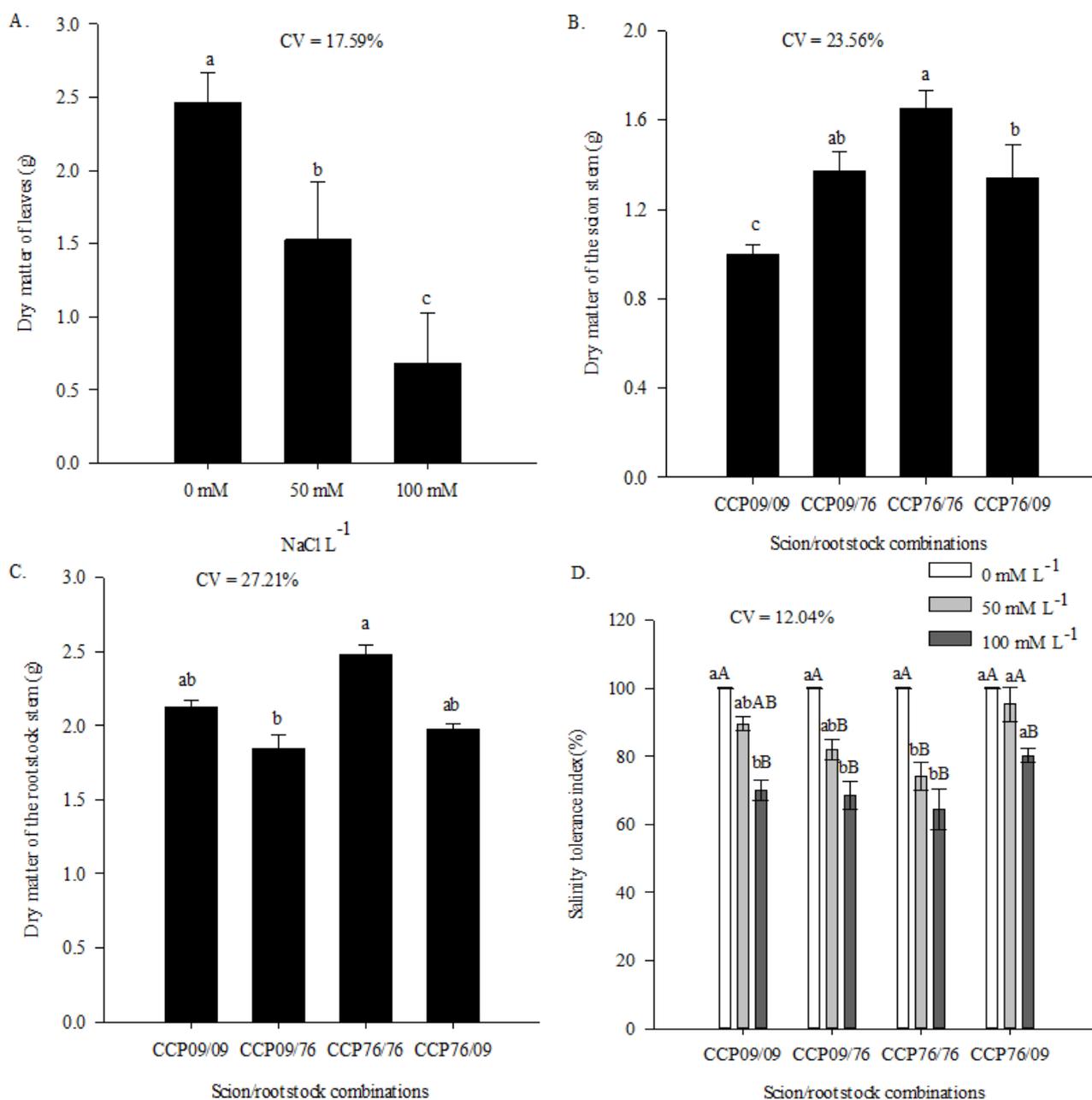
According to Imran et al. (2021), salinity tolerance by translocation of toxic ions to the vacuole in order to avoid harmful effects on the metabolic processes of the cytoplasm is dependent on Ca²⁺, as it is the main component for the exclusion of Na⁺ in leaves. Citrus seedlings with lower Ca²⁺ output and Na⁺ input from leaf tissues show greater tolerance to salt stress (Sharma et al., 2011), as observed in self-graft CCP 09 in this study. According to Ferreira-Silva et al. (2009), physiological changes in grafted early dwarf cashew seedlings under salinity depend on the compatibility between scion and rootstock, indicating that the CCP 09 rootstock was the one that best adjusted physiologically to salt stress.

Several studies reported that combinations of scion and rootstock were more efficient in the absorption and transport of macro and micronutrients (Alencar et al., 2021; Parthasarathi et al., 2021), due to alterations in the absorption, accumulation, or exclusion of salts, as a strategy of adjustment to the harmful effects of salt stress, triggering less damage when compared with other combinations of scions or non-grafted seedlings (Al-Juthery et al., 2019).

The dry matter accumulation of the leaves (Figure 5A) decreased with the addition of NaCl in the saline solution, from 2.46 g (0 mM NaCl L⁻¹) to 0.68 g (100 mM NaCl L⁻¹). The decrease in the dry matter production of cashew seedlings under salinity probably occurs due to the reduction in soil water availability and/or the excessive accumulation of toxic ions in plant tissues. Under these conditions, the loss of plant leaves under salt stress is due to early senescence caused by the toxic effects of the salts (Silva et al., 2011), as also observed for early dwarf cashew (Silva et al., 2018, Lima et al., 2020, Alencar et al., 2021), in addition to other fruit seedlings treated with saline water (Oliveira et al., 2018).

The stem dry matter, of both the scion and the rootstock, differed statistically only for the scion/rootstock combinations, indicating that the period of exposure to salts did not interfere in these structures (Figure 5B). A greater accumulation of dry matter of the scion stem (1.65 g) and rootstock stem (2.48 g) was observed in the self-graft CCP 76 (Figures 5B and C), while a smaller scion matter (1.0 g) was observed for self-graft CCP 09 and a smaller rootstock matter (1.84 g) was observed for CCP 09/CCP 76. Despite the high accumulation of dry matter of the scion and rootstock in self-graft CCP 76, its grafting success rate was 76.9%, which was low when compared to that of the CCP 09/CCP 76 combination (93.1%) (Serrano et al., 2013). According to the authors, it is important for producers of dwarf cashew seedlings to choose combinations that have high rates of grafting success, which will maximize the productive efficiency of the nursery.

The salinity tolerance index was higher with the scion/rootstock combination CCP 76/CCP 09 (95.37%), differing from the other combinations at the concentration of 50 mM L⁻¹ of NaCl (Figure 5D). With 100 mM L⁻¹ NaCl, the tolerance index was 80.17% (CCP 76/CCP 09), 70.11% (self-graft CCP 09), 68.54% (CCP 09/CCP 76) and 64.5% for self-graft CCP76. According to the tolerance index, the scion/rootstock combination CCP 76/CCP 09 was more tolerant, followed



Means followed by the same lowercase letters do not differ statistically by the Tukey test ($p \leq 0.05$). In Figure 5D means followed by the same lowercase letters do not differ between the scion/rootstock combinations within each saline treatment, and means followed by the same uppercase letters do not differ between the saline treatments within each scion/rootstock combination (Tukey, $p \leq 0.05$). Vertical bars - Standard error of the mean ($n = 5$); CV - Coefficient of variation

Figure 5. Dry matter of leaves (A), dry matter of the scion stem (B), dry matter of the rootstock (C) and salinity tolerance index of dwarf cashew seedlings subjected to 0, 50, and 100 mM L⁻¹ NaCl, at 30 days after beginning of the saline treatments

by self-graft CCP 09, CCP 09/CCP 76 and self-graft CCP 76. These results indicate that under saline conditions, the low accumulation of Na⁺ in the leaves contributes to maintaining the number of leaves and leaf area, which was more evident in the scion/rootstock combination CCP 76/CCP 09, conferring greater tolerance to salt stress.

CONCLUSIONS

1. The scion/rootstock combination CCP 76/CCP 09 showed tolerance to 50 mM L⁻¹ NaCl, due to the increase of leaf area and number of leaves.

2. The scion/rootstock combination CCP 76/CCP 09 kept the leaf K⁺ concentration and had the lowest Na⁺ concentrations under the NaCl concentrations.

ACKNOWLEDGEMENTS

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financing the project (485799/2013-8) and to Embrapa Agroindústria Tropical for granting the plant material.

LITERATURE CITED

Alencar, M. L. M.; Oliveira, A. B. de; Alvarez-Pizarro, J. C.; Marques, E. C.; Prisco, J. T.; Gomes-Filho, E. Differential responses of dwarf cashew clones to salinity are associated to osmotic adjustment mechanisms and enzymatic antioxidative defense. *Anais da Academia Brasileira de Ciências*, v.93, p.1-14, 2021. <https://doi.org/10.1590/0001-3765202120180534>

- Al-Juthery, H. W.; Al-Swedi, F. G.; Al-Tae R. A.; AL-Taey, D. K. Grafting of vegetable crops improve diseases control, salt and drought stress tolerance and nutrients, water efficiency (article review). *International Journal of Botany*, v.4, p.108-114, 2019.
- Alvares, C. A.; Stape, J. L.; Sentelhas, P. C.; Gonçalves, J. L. de M.; Sparovek, G. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, v.22, p.711-728, 2013. <https://doi.org/10.1127/0941-2948/2013/0507>
- Araújo, L. F. de; Lima, R. E. M.; Costa, L. de O. da; Silveira, E. M. de C.; Bezerra, M. A. Alocação de íons e crescimento de plantas de cajueiro anão-precoce irrigadas com água salina no campo. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.18, p.34-38, 2014. <https://doi.org/10.1590/1807-1929/agriambi.v18nsuppS34-S38>
- Carneiro, P. T.; Cavalcanti, M. L.; Brito, M. E.; Gomes, A. H.; Fernandes, P. D.; Gheyi, H. R. Sensibilidade do cajueiro anão precoce ao estresse salino na pré-floração. *Revista Brasileira de Ciências Agrárias*, v.2, p.150-155, 2007. <https://doi.org/10.5039/agraria.v2i2a1894>
- Ferreira, E. B.; Cavalcanti, P. P.; Nogueira, D. A. ExpDes.pt: Pacote experimental designs (Portuguese). R package version 1.2.0, 2018. Available on: <<https://CRAN.R-project.org/package=ExpDes.pt>>. Accessed on: Dec. 2021.
- Ferreira-Silva, S. L.; Silva, E. N.; Carvalho, F. E. L.; Lima, C. S. de; Alves, F. A. L.; Silveira, J. A. G. Physiological alterations modulated by rootstock and scion combination in cashew under salinity. *Scientia Horticulturae*, v.127, p.39-45, 2010. <https://doi.org/10.1016/j.scienta.2010.09.010>
- Ferreira-Silva, S. L.; Voigt, E. L.; Viégas, R. A.; Paiva, J. R. de; Silveira, J. A. G. Influência de porta-enxertos na resistência de mudas de cajueiro ao estresse salino. *Pesquisa Agropecuária Brasileira*, v.44, p.361-367, 2009. <https://doi.org/10.1590/S0100-204X2009000400005>
- Gao, Z.; Zhang, J.; Zhang, J.; Zhang, W.; Zheng, L.; Borjigin, T.; Wang, Y. Nitric oxide alleviates salt-induced stress damage by regulating the ascorbate–glutathione cycle and Na⁺/K⁺ homeostasis in *Nitraria tangutorum* Bobr. *Plant Physiology and Biochemistry*, v.173, p.46-58, 2022. <https://doi.org/10.1016/j.plaphy.2022.01.017>
- Hessini, K.; Issaoui, K.; Ferchichi, S.; Saif, T.; Abdelly, C.; Siddique K. H. M.; Cruz, C. Interactive effects of salinity and nitrogen forms on plant growth, photosynthesis and osmotic adjustment in maize. *Plant Physiology and Biochemistry*, v.139, p.171-178, 2019. <https://doi.org/10.1016/j.plaphy.2019.03.005>
- Hoagland, D. R.; Arnon, D. I. The water culture method for growing plants without soils. Berkeley: California Agricultural Experimental Station, 1950.
- Imran, Q. M.; Falak, N.; Hussain, A.; Mun, B. G.; Yun, B. W. Abiotic stress in plants; stress perception to molecular response and role of biotechnological tools in stress resistance. *Agronomy*, v.11, p.1-20, 2021. <https://doi.org/10.3390/agronomia11081579>
- Irigoyen, J. J.; Emerich, D. W.; Sánchez-Díaz, M. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum*, v.84, p.67-72, 1992. <https://doi.org/10.1111/j.1399-3054.1992.tb08764.x>
- Lima, G. S. de; Silva, J. B. da; Souza, L. de P.; Nobre, R. G.; Soares, L. A. dos A.; Gheyi, H. R. Tolerance of precocious dwarf cashew clones to salt stress during rootstock formation stage. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.24, p.474-481, 2020. <https://doi.org/10.1590/1807-1929/agriambi.v24n7p474-481>
- Lutts, S.; Kinet, J.; Bouharmont, J. NaCl induced senescence in leaves of rice (*Oryza sativa*) cultivars differing in salinity resistance. *Annals of Botany*, v.78, p.389-398, 1996. <https://doi.org/10.1006/anbo.1996.0134>
- Malavolta, E.; Vitti, G. C.; Oliveira, S. A. de. Avaliação do estado nutricional das plantas: princípios e aplicações. 2.ed. Piracicaba: POTAFOS, 1997.
- Murthy, K. N.; Kumar, K. V.; Bhagavan, S.; Subbaiah, C. C.; Kumaran, P. M. A rapid non-destructive method of estimating leaf area in cashew. *Acta Horticulturae*, v.108, p.46-48, 1985. <https://doi.org/10.17660/ActaHortic.1985.108.8>
- Nawaz, F.; Shehzad, M. A.; Majeed, S.; Ahmad, K. S.; Aqib, M.; Usmani, M. M.; Shabbir, R. N. Role of Mineral Nutrition in Improving Drought and Salinity Tolerance in Field Crops. In: Hasanuzzaman M. (eds). *Agronomic Crops*. Singapore: Springer, 2020. p.129-147. https://doi.org/10.1007/978-981-15-0025-1_8
- Oliveira, F. I. F. de; Souto, A. G. de L.; Cavalcante, L. F.; Medeiros, W. J. F. de; Medeiros, S. A. da S.; Oliveira, F. F. de. Biomass and chloroplast pigments in jackfruit seedlings under saline stress and nitrogen fertilization. *Revista Caatinga*, v.31, p.622-631, 2018. <https://doi.org/10.1590/1983-21252018v31n310rc>
- Parthasarathi, T.; Ephrath, J. E.; Lazarovitch, N. Grafting of tomato (*Solanum lycopersicum* L.) onto potato (*Solanum tuberosum* L.) to improve salinity tolerance. *Scientia Horticulturae*, v.212, p.1-9, 2021. <https://doi.org/10.1016/j.scienta.2021.110050>
- Santana Júnior, E. B.; Coelho, E. F.; Gonçalves, K. S.; Cruz, J. L. Physiological and vegetative behavior of banana cultivars under irrigation water salinity. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.24, p.82-88, 2020. <https://doi.org/10.1590/1807-1929/agriambi.v24n2p82-88>
- Sanwal, S. K.; Mann, A.; Kumar, A.; Kesh, H.; Kaur, G.; Rai, A. K.; Kumar, R.; Sharma, P. C.; Kumar, A.; Bahadur, A.; Singh, B.; Kumar, P. Salt Tolerant Eggplant Rootstocks Modulate Sodium Partitioning in Tomato Scion and Improve Performance under Saline Conditions. *Agriculture*, v.12, e183, 2022. <https://doi.org/10.3390/agriculture12020183>
- Serrano, L. A. L.; Melo, D. S.; Taniguchi, C. A. K.; Vidal Neto, F. das C.; Cavalcante Júnior, L. F. Porta-enxertos para a produção de mudas de cajueiro. *Pesquisa Agropecuária Brasileira*, v.48, p.1237-1245, 2013. <https://doi.org/10.1590/S0100-204X2013000900007>
- Sharma, D. K.; Dubey, A. K.; Srivastav, M.; Singh, A. K.; Sairam, R. K.; Pandey, R. N.; Dahuja, A.; Kaur, C. Effect of putrescine and paclobutrazol on growth, physicochemical parameters, and nutrient acquisition of salt-sensitive citrus rootstock *Karna khatta* (*Citrus karna* Raf.) under NaCl Stress. *Journal of Plant Growth Regulation*, v.30, p.301-311, 2011. <https://doi.org/10.1007/s00344-011-9192-1>
- Silva, A. F. da; Sousa, V. F. de O.; Santos, G. L. dos; Araújo Júnior, E. S.; Silva, S. L. F. da; Macedo, C. E. C. de; Melo, A. S. de; Maia, J. M. Antioxidant protection of photosynthesis in two cashew progenies under salt stress. *Journal of Agriculture Science*, v.10, p.388-404, 2018. <https://doi.org/10.5539/jas.v10n10p388>
- Silva, E. B. da; Viana, T. V. de A.; Sousa, G. G. de; Sousa, J. T. M. de; Santos, M. F. dos; Azevedo, B. M. de. Growth and nutrition of peanut crop subjected to saline stress and organomineral fertilization. *Revista Brasileira de Engenharia Agrícola*, v.26, p.495-501, 2022. <https://doi.org/10.1590/1807-1929/agriambi.v26n7p495-501>

- Silva, E. N. da; Ribeiro, R. V.; Ferreira-Silva, S. L.; Viégas, R. A.; Silveira, J. A. G. Salt stress induced damages on the photosynthesis of physic nut young plants. *Scientia Agrícola*, v.68, p.62-68, 2011. <https://doi.org/10.1590/S0103-90162011000100010>
- Sousa, A. R. de O.; Silva, E. M. de A.; Coelho Filho, M. A.; Costa, M. G. C.; Soares Filho, W. dos S.; Micheli, F.; Maserti, B.; Gesteira, A. da S. Metabolic responses to drought stress and rehydration in leaves and roots of three Citrus scion/rootstock combinations. *Scientia Horticulturae*, v. 292, e110490, 2022. <https://doi.org/10.1016/j.scienta.2021.110490>
- Sousa, J. R. M. de; Gheyi, H. R.; Brito, M. E. B.; Silva, F. de A. F. D. da; Lima, G. S. de. Dano na membrana celular e pigmentos clorofilianos de citros sob águas salinas e adubação nitrogenada. *Irriga*, v.22, p.353-368, 2017. <https://doi.org/10.15809/irriga.2017v22n2p353-368>
- Vila, H. F.; Di Filippo, M. L.; Venier, M.; Hugalde, I. P.; Filippini, M. F. A dynamic model for sodium intoxication unravels salt tolerance in grapevine (*Vitis vinifera* L.) rootstocks. *Revista de la Facultad de Ciencias Agrarias*, v.52, p.88-101, 2020.