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# Salicylic acid attenuates the harmful effects of salt stress on the morphophysiology of early dwarf cashew

## Ácido salicílico atenua os efeitos do estresse salino sobre a morfofisiologia do cajueiro anão precoce

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#### ABSTRACT

Brazil is one of the largest cashew producers in the world, and its Northeast region stands out. However, cashew growth and development are negatively affected by irrigation with brackish water in its production. In this context, strategies have been employed to alleviate salt stress effects on plants. Among the strategies, the exogenous application of elicitor substances, such as salicylic acid, has stood out. Given the above, the objective of the present study was to evaluate the effect of foliar application of salicylic acid as an attenuator of salt stress on the morphophysiology of early dwarf cashew. The study was carried out in a greenhouse, in a randomized block design, in a 5 × 4 factorial arrangement, with five levels of electrical conductivity of the irrigation water – ECw (0.4, 1.2, 2.0, 2.8, and 3.6 dS m<sup>-1</sup>) and four concentrations of salicylic acid - SA (0, 1, 2, and 3 mM), with three replicates and one plant per plot. Irrigation with water of electrical conductivity above 0.4 dS m<sup>-1</sup> negatively affected the leaf relative water content, gas exchange, photosynthetic pigments, and growth of early dwarf cashew irrigated using water with ECw of up to 3.6 dS m<sup>-1</sup>, at 210 days after transplanting.

Index terms: Anacardium occidentale L.; abiotic stress; elicitor; salinity.

#### RESUMO

O Brasil é um dos maiores produtores de caju do mundo, com destaque para região Nordeste. Entretanto, o crescimento e desenvolvimento são reduzidos pela irrigação com águas salobras. Neste sentido, estratégias têm sido empregadas com intuito de amenizar o estresse salino sobre as plantas. Dentre as estratégias, a aplicação exógena de substância elicitoras, como o ácido salicílico têm se destacado. Diante do exposto, objetivou-se com o presente estudo, avaliar o efeito da aplicação foliar de ácido salicílico como atenuante do estresse salino sobre a morfofisiologia do cajueiro anão precoce. O estudo foi conduzido em casa de vegetação, no delineamento de blocos casualizados, em arranjo fatorial 5 × 4, com cinco níveis de condutividade elétrica da água de irrigação - CEa (0,4; 1,2; 2,0; 2,8 e 3,6 dS m<sup>-1</sup>) e quatro concentrações de ácido salicílico - AS (0, 1, 2 e 3 mM), com três repetições e uma planta por parcela. A irrigação com água de condutividade elétrica acima de 0.4 dS m<sup>-1</sup> afetou negativamente o conteúdo relativo de água nas folhas, as trocas gasosas, os pigmentos fotossintéticos e o crescimento das plantas. O ácido salicílico na concentração de 1 mM atenuou os efeitos do estresse salino sobre o extravasamento de eletrólitos, o conteúdo relativo de água, as trocas gasosas, a síntese de pigmentos fotossintéticos e o crescimento do cajueiro anão precoce irrigado com CEa de até 3,6 dS m<sup>-1</sup>, aos 210 dias após o transplantio.

Termos para indexação: Anacardium occidentale L.; estresse abiótico; elicitor; salinidade.

## INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a fruit crop widely cultivated in Brazil, especially in the semiarid region of the Northeast, having great socioeconomic importance in the generation of employment and income (Lima et al., 2020a; Sousa et al., 2023). The Northeast of Brazil has a planted area of approximately 616,189 ha and accounts for about 99% of the national production, having the states of Ceará (42,597 tons), Rio Grande do Norte (33,912 tons) and Piauí (28,292 tons) as main producers (Suassuna et al., 2017).

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The agricultural areas of the semi-arid region of northeastern Brazil are subject to the use of brackish water for irrigation as an alternative for the scarcity of low-salinity water and expansion of irrigated areas (Veloso et al., 2023). Due to the low rainfall and high evaporation rate in this region, there is a significant increase in the accumulation of soluble salts and/or exchangeable sodium in the soil, resulting in losses of vegetation cover and agricultural production (Castro; Santos, 2020).

Salt stress is one of the main abiotic factors that negatively affect crop growth, yield, and quality worldwide (Ahmadi; Souri, 2019; Rehman et al., 2022). Excess salts present in irrigation water reduce the osmotic potential of the soil, and hinder the absorption of water and nutrients, causing severe changes in plant metabolism (Silva et al., 2021). Salinity also induces secondary stress caused by the accumulation of reactive oxygen species (ROS) in plants, which negatively affects the structure of enzymes, nucleic acids, and lipids (Li et al., 2022), limits gas exchange, and damages cell integrity and chlorophyll structure, negatively affecting crop growth and development (Silva et al., 2022).

Phytohormone application can be used as a strategy to minimize salinity-induced yield losses and increase the tolerance of plants to salt stress (Quamruzzaman et al., 2021). Among the phytohormones, salicylic acid stands out, a phenolic compound that plays an important role in signaling stresses of biotic and abiotic origin (Lacerda et al., 2022).

In recent years, studies have reported that foliar application of salicylic acid can mitigate the harmful effects caused by salt stress on strawberries (Samadi; Habibi; Vaziri, 2019), tomato (Souri; Tohidloo, 2019), almonds (Mohammadi et al., 2020), grapes (Ekbic; Ozcan; Erdem, 2020), date (Jasim; Ati, 2020), orange (Mahmoud et al., 2021) and soursop (Silva et al., 2022).

This study is based on the hypothesis that foliar application of salicylic acid acts in the regulation of physiological processes and induction of tolerance of early dwarf cashew to salt stress, by increasing chlorophyll biosynthesis and photosynthetic activity, in addition to reducing damage to the cell membrane. However, information on its use in early dwarf cashew irrigated with saline water under the semi-arid conditions of northeastern Brazil is incipient. Given the above, aimed to evaluate the effect of foliar application of salicylic acid as an attenuator of salt stress on the morphophysiology of early dwarf cashew.

## MATERIAL AND METHODS

The experiment was carried out from February to September 2022 in a protected environment (greenhouse), belonging to the Academic Unit of Agricultural Engineering - UAEA of the Federal University of Campina Grande - UFCG, in Campina Grande, Paraíba, Brazil, at the geographical coordinates 7°15'18" South latitude, 35°52'28" West longitude and an average altitude of 550 m. The greenhouse used was of the arch type, 30 m long and 21 m wide, with a 3.0 m ceiling height and low-density polyethylene cover (150 microns) with infrared treatment. The data of temperature (maximum and minimum) and average relative air humidity inside the greenhouse during the experimental period are shown in Figure 1.



**Figure 1**: Maximum and minimum temperature and average relative air humidity observed inside the greenhouse during the experiment.

In the experiment, grafted seedlings of early dwarf cashew were used as rootstock and the cultivars CCP 51 and BRS 226 Planalto were used as the scion, respectively, grafted by the full cleft method. The seedlings were acquired from a commercial nursery accredited by the National Registry of Seeds and Seedlings, located in the municipality of Pacajus - CE. In the transplanting period, the seedlings were 180 days old and showed a healed grafting point, a vigorous aspect, with at least 6 mature leaves and 25 cm height.

The early dwarf cashew clones were chosen based on their characteristics of adaptation to the edaphoclimatic conditions of the northeastern semi-arid region. The clone CCP 51 is recommended for both rainfed and irrigated cultivation and stands out due to the use of the peduncle for the table market, as it is one of the tastiest and has high production (Oliveira, 2002). BRS 226 has a high yield and good quality of the peduncle (cashew) and nut, being resistant to resinosis and black stem rot, diseases that have been causing significant losses to cashew growers (Serrano et al., 2013).

The treatments consisted of five levels of electrical conductivity of irrigation water - ECw (0.4, 1.2, 2.0, 2.8, and 3.6 dS m<sup>-1</sup>) and four concentrations of salicylic acid - SA (0, 1, 2, and 3 mM), in the  $5 \times 4$  factorial arrangement (Table 1), arranged in a randomized block design, with three replicates and one plant per plot, totaling 60 experimental units. Salicylic acid concentrations were applied by foliar spraying from 30 days after transplanting (DAT).

The concentrations of salicylic acid used in this study were adapted from the study conducted with 'Morada Nova' soursop (Silva et al., 2021), while the levels of electrical conductivity of water were based on the study conducted by Lima et al. (2020a) with the early dwarf cashew crop.

		,							
ECw (S) dS m <sup>-1</sup>	Concentration of salicylic acid (SA) - mM								
	0	1	2	3					
S1 0.4	S1SA1	S1SA2	S1SA3	S1SA4					
S2 1.2	S2SA1	S2SA2	S2SA3	S2SA4					
S3 2.0	S3SA1	S3SA2	S3SA3	S3SA4					
S4 2.8	S4SA1	S4SA2	S4SA3	S4SA4					
S5 3.6	S5SA1	S5SA2	S5SA3	S5SA4					

Table 1: Description of the analyzed treatments.

The experiment was conducted using plastic pots adapted as drainage lysimeters, with a capacity of 200 L, filled with a 1.0 kg layer of crushed stone followed by 250 kg of soil classified as Entisol (United States, 2014), collected at 0-30 cm depth, from the municipality of Lagoa Seca-PB, whose physical-chemical characteristics (Table 2) were determined according to Teixeira et al. (2017).

The irrigation waters with different levels of electrical conductivity were prepared by dissolving the salts of NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O, and MgCl<sub>2</sub>.6H<sub>2</sub>O, in the equivalent proportion of 7:2:1, respectively, in local-supply water (ECw =  $0.37 \text{ dS m}^{-1}$ ). This proportion is commonly found in water sources used for irrigation in small properties in the Northeast (Medeiros et al., 2003). The irrigation waters were prepared considering the relationship between ECw and the concentration of salts (Richards, 1954), according to Equation 1:

Chemical characteristics											
pH (H <sub>2</sub> O)	ОМ	Р	K <sup>+</sup> Na <sup>+</sup> C		Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup> + H <sup>+</sup>	ESP	ECse		
(1:2.5)	(dag kg <sup>-1</sup> )	(mg kg-1)			(%)	(dS m <sup>-1</sup> )					
6.12	1.36	6.80	0.22	0.22 0.16 2.60 3.66 1.93							
Physical-hydraulic characteristics											
Particle-size fraction (g kg <sup>-1</sup> )			Textural class	Moistu	re (kPa)	AW	Total porosity %	BD	PD		
Sand	Silt	Clay		33.42*	1519.5** dag kg <sup>-1</sup>						
760.9	164.5	74.6	SL	13.07	5.26	7.81	41.79	1.56	2.68		

**Table 2**: Chemical and physical attributes of the soil, in the 0-0.30 m layer, used in the experiment, before the application of the treatments.

OM - Organic Matter: Walkley-Black Wet Digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1 M KCl at pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted with 1 M NH<sub>4</sub>OAc at pH 7.0; Al<sup>3+</sup> and H<sup>+</sup> extracted with 0.5 M CaOAc at pH 7.0; ESP - Exchangeable sodium percentage; ECse - Electrical conductivity of saturation extract; SL - Sandy loam; AW - Available water; BD - Bulk density; PD - Particle density; \* - Field capacity; \*\* - Wilting point.

 $Q \approx 10 \times ECw$ 

#### Where:

 $Q - Sum of cations (mmol_ L<sup>-1</sup>); and$ 

ECw – Desired electrical conductivity of water (dS m<sup>-1</sup>).

(1)

After the addition of the salts in the water, the respective levels of ECw were checked with a benchtop conductivity meter and corrected, if necessary. At 45 days after transplanting (DAT), irrigation with respective saline water began, adopting a three-day interval in order to maintain soil moisture close to field capacity. The volume of water to be applied was determined according to the water requirements of the plants, estimated by the water balance, as presented in Equation 2:

$$VI = \frac{(Va - Vd)}{(1 - LF)}$$
(2)

Where:

VI - volume of water to be used in the next irrigation event (mL);

Va - volume applied in the previous irrigation event (mL); Vd - volume drained (mL);

LF - leaching fraction of 0.15, applied every 30 days to avoid excessive accumulation of salts in the soil.

The water consumption of cashew plants along the experiment is presented in Table 3.

**Table 3**: Water consumption of early dwarf cashew along the experiment (210 days after transplanting), for the different salinity levels of irrigation water (ECw).

ECw (dS m <sup>-1</sup> )	Water consumption (L per plant)	Water consumption* (mm per plant)
0.4	217.2	767.5
1.2	199.8	706.0
2.0	179.6	634.6
2.8	158.3	559.4
3.6	142.2	502.5

\* Water depth calculated considering the area of the lysimeter (0.283 m<sup>2</sup>).

Salicylic acid solutions of appropriate concentration were obtained by dissolution in 30% ethyl alcohol (95.5%), as it is a substance of low solubility in water at room temperature. To reduce the surface tension of the drops on the leaf surface, the adjuvant Wil fix was

used in the preparation of the solution at the concentration of 0.5 mL L<sup>-1</sup> solution.

Foliar applications of salicylic acid began 30 DAT, i.e., 15 days before irrigation with saline water, and spraying was performed on the abaxial and adaxial sides of the leaves (Souri; Tohidloo, 2019). Subsequent applications were performed at 30-day intervals using a backpack sprayer between 17:00 and 17:45 h. The sprayer used was the Jacto XP model from Jacto<sup>®</sup>, with capacity of 12 L, working pressure (maximum) of 88 psi (6 bar) and JD 12P nozzle and the average volume applied per plant was 300 mL.

The fertilization with nitrogen, phosphorus, and potassium were carried out according to the recommendation of Oliveira (2002) for the irrigated cultivation of early dwarf cashew, applying 60 g of nitrogen, 200 g  $P_2O_5$ , and 40 g  $K_2O$  per plant year, divided into 24 applications with intervals of 15 days. Calcium nitrate, monoammonium phosphate, and potassium sulfate were used as sources of nitrogen, phosphorus and potassium, respectively.

A Dripsol<sup>®</sup> micro solution was applied through the leaves, on the adaxial and abaxial sides, using a backpack sprayer, every two weeks to meet the micronutrient requirement at the concentration of 1.0 g L<sup>-1</sup> with the following composition: Mg (1.1%); Zn (4.2%); B (0.85%); Fe (3.4%); Mn (3.2%); Cu (0.5%); Mo (0.05%). During the experiment, cultural practices such as weeding, soil scarification, and phytosanitary control, recommended for the crop, were carried out using Deltamethrin at the concentration of 0.5 mL L<sup>-1</sup> solution and Azoxystrobin and Difenoconazole at the concentration of 0.3 mL L<sup>-1</sup>.

At 210 DAT, the following parameters were evaluated: relative water content (RWC), intercellular electrolyte leakage (% IEL), leaf gas exchange, photosynthetic pigments, and growth: plant height (PH), stem diameter below grafting (Dbe), at the grafting point (Dgp), and above grafting (Dab), crown diameter ( $D_{Crown}$ ) and crown volume ( $V_{Crown}$ ).

To determine the relative water content (RWC), two leaves were collected from the middle third of the main branch to obtain five discs (12 mm in diameter) of each leaf. The discs were weighed immediately after collection, avoiding moisture loss, to obtain the fresh mass (FM); then, these samples were placed in beaker, immersed in 50 mL of distilled water and stored for 24 hours. After this period, excess water was removed from the discs with paper towels and the turgid mass (TM) of the samples was obtained. Subsequently, they were dried in an oven at  $\approx 65 \pm 3$  °C until reaching constant weight to obtain the dry mass (DM) of the samples. RWC was determined according to Lima et al. (2015), by Equation 3:

$$RWC = \frac{FM - DM}{TM - DM}$$
(3)

Where: RWC = relative water content (%); FM = fresh mass of the leaf (g); TM = turgid mass (g); DM = dry mass (g).

To determine the intercellular electrolyte leakage (% IEL), a copper punch was used to obtain five leaf discs with an area of 1.54 cm<sup>2</sup> each, per experimental unit, which were washed and put into Erlenmeyer® flasks containing 50 mL of distilled water. After closing with aluminum foil, the Erlenmeyer® flasks were stored at a temperature of 25 °C for 24 hours and then the initial conductivity of the medium (Xi) was measured using a benchtop conductivity meter (MB11, MS Techonopon<sup>®</sup>). Subsequently, the Erlenmeyer® flasks were subjected to a temperature of 90 °C, for 120 minutes, in a drying oven (SL100/336, SOLAB®) and, after cooling their contents, the final conductivity (Xf) was measured. The percentage of intercellular electrolyte leakage in the leaf blade was expressed as the percentage of initial electrical conductivity relative to the electrical conductivity after treatment for 90 minutes at 90 °C, according to the methodology proposed by Scotti-Campos et al. (2013), considering Equation 4:

$$\% \text{IEL} = \frac{\text{Xi}}{\text{Xf}} \times 100 \tag{4}$$

Where:

% IEL - percentage of electrolyte leakage; Xi - initial electrical conductivity (dS m<sup>-1</sup>); Xf - final electrical conductivity (dS m<sup>-1</sup>).

Leaf gas exchange: stomatal conductance - gs (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), transpiration - E (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> assimilation rate - A (µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), internal CO<sub>2</sub> concentration - Ci (µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), instantaneous water use efficiency - WUEi ([(µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>)) (A/E), and instantaneous carboxylation efficiency - CEi ([(µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (µmol CO<sub>2</sub> m0<sup>-1</sup>)<sup>-1</sup>]) (A/Ci), were measured on the third leaf, counted from the apex of the main

branch of the plant, with irradiation of 1200  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> and airflow of 200 mL min<sup>-1</sup>, using the portable photosynthesis meter "LCPro+" from ADC BioScientific Ltda.

The contents of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were quantified according to Arnon (1949), with plant extracts from samples of discs from the blade of the third mature leaf counted from the apex. In each sample, 6.0 mL of 80% acetone (A.R.) was used. Through these extracts, the concentrations of chlorophyll and carotenoids were determined in the solutions using the spectrophotometer at absorbance wavelength (ABS) (470, 647, and 663 nm), according to Equation 5, 6, 7, and 8:

 $Chla = (12.25 \times ABS_{663}) - (2.79 \times ABS_{647})$  (5)

$$Chlb = (21.50 \times ABS_{647}) - (5.10 \times ABS_{647})$$
(6)

$$Chlt = (7.15 \times ABS_{663}) + (18.71 \times ABS_{647})$$
(7)

$$Car = \frac{|(1000 \times \text{ABS}_{470}) - (1.82 \times \text{Chl } a) - (85.02 \times \text{Chl } b)|}{198}$$
(8)

The values obtained for the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the leaves were expressed in  $\mu$ g mL<sup>-1</sup>.

PH was measured by taking as a reference the distance from the plant collar to the insertion of the apical meristem. Stem diameter was measured with a digital caliper below the grafting (Dbe), at the grafting point (Dgp), and above the grafting (Dab).  $D_{Crown}$  was obtained by means of the crown diameter observed in the row direction (DR) and interrow direction (DIR). Crown volume ( $V_{Crown}$ ) was calculated from the crown height (CH), DR, and DIR, using Equation 9, according to Portella et al. (2016).

$$V_{Crown} = \frac{\pi}{6} \times CH \times DR \times DIR$$
(9)

Where:

V<sub>Crown</sub> - crown volume (m<sup>3</sup>); CH - crown height (m); DR - crown diameter in the row direction (m); and DIR - crown diameter in the interrow direction (m).

The multivariate structure of the results was evaluated by means of principal component analysis (PCA), synthesizing the amount of relevant information contained in the original data set in a smaller number of dimensions, resulting from linear combinations of the original variables generated from the eigenvalues ( $\lambda \ge 1.0$ ) in the correlation matrix, explaining a percentage greater than 10% of the total variance (Govaerts et al., 2007).

From the reduction of dimensions, the original data of the variables of each component were subjected to multivariate analysis of variance (MANOVA) by the test of Hotelling (1947) at 0.05 probability level for the electrical conductivity of irrigation water and salicylic acid concentrations, as well as for the interaction between them. Only variables with a correlation coefficient greater than or equal to 0.6 were maintained in each principal component (PC) (Hair et al., 2009).

Data regarding gas exchange were removed from the principal component analysis because they showed autocorrelation. The data were subjected to the distribution normality test (Shapiro-Wilk test) at 0.05 probability level. Subsequently, analysis of variance was performed at 0.05 probability level and, in cases of significance, regression analysis was performed. In case of significance of the interaction between factors, SigmaPlot software v.12.5 was used to create the response surfaces. Statistica software v. 7.0 was used for statistical analysis (Statsoft, 2004).

#### **RESULTS AND DISCUSSION**

The multidimensional space of the original variables was reduced to two principal components (PC1 and PC2) with eigenvalues greater than  $\lambda \ge 1.0$ , according to Kaiser (1960). The eigenvalues and percentage of variance explained for each component (Table 4) represented together 88.21% of the total variation. PC1 explained 77.00% of the total variance, formed by most of the variables analyzed, except for the diameter at the grafting point (Dgp). PC2 represented 11.21% of the remaining variance, being formed by the Dgp variable.

There was a significant effect ( $p \le 0.01$ ) of the interaction between the levels of electrical conductivity of irrigation water (ECw) and concentrations of salicylic acid (SA) for the two principal components (Table 3). A significant effect ( $p \le 0.01$ ) of the factors was also observed when analyzed individually.

The two-dimensional projections of the effects of treatments and variables in the first and second principal components (PC1 and PC2) are presented in Figures 2A and 2B. In the first principal component (PC1), a process possibly characterized by the effect of the interaction between the levels of electrical conductivity of irrigation water and salicylic acid concentrations was identified. The correlation coefficients for RWC, % IEL, Chl *a*, Chl

*b*, Chl *t*, Car, Dbe, Dab, PH,  $D_{Crown}$ , and  $V_{Crown}$  were higher than 0.80 (Table 4).

Foliar application of salicylic acid at the concentration of 1 mM (SA2) stood out from the other concentrations with the highest values (Table 4), even in plants irrigated with ECw of 3.6 dS m<sup>-1</sup>. Excess salts present in irrigation water hinders osmotic and ionic homeostasis and causes accumulation of toxic ions, which induce lipid peroxidation and destabilize the production of lipids, proteins, and nucleic acids of the membrane, limiting the maintenance of cell turgor (Sharma et al., 2020; Soares et al., 2022). In addition, high *Ci* levels in the cell reflect the difficulty in assimilation due to the inhibition of RuBisCO activity (Prywes et al., 2022).

In the principal component 1, it is possible to notice that plants irrigated with ECw of 1.2 dS m<sup>-1</sup> and under foliar application of salicylic acid at the concentration of 1 mM (S2SA2) had the highest values (Table 4) of RWC (93.5%), Chl *a* (810.3  $\mu$ g mL<sup>-1</sup>), Chl b (358.4  $\mu$ g mL<sup>-1</sup>), Chl *t* (1168.7  $\mu$ g mL<sup>-1</sup>), Car (266.8  $\mu$ g mL<sup>-1</sup>), Dbe (29.23 mm), Dab (20.38 mm), PH (0.88 m), D<sub>Crown</sub> (0.82 m) and V<sub>Crown</sub> (0.33 m<sup>3</sup>).

When comparing the results obtained in plants of the S2SA2 treatment with those of plants grown under S2SA1, there were increments of 3.20% (RWC), 9.87% (Chl *a*), 12.88% (Chl *b*), 10.78% (Chl *t*), 3.13% (Car), 11.18% (Dbe), 7.83% (Dab), 7.32% (PH), 3.80% (D<sub>Crown</sub>) and 10.00% ( $V_{Crown}$ ), showing the beneficial effect of salicylic acid at the concentration of 1 mM. On the other hand, plants that did not receive salicylic acid application and were irrigated with ECw of 3.6 dS m<sup>-1</sup> (S5SA1) had the lowest mean values of RWC (69.2%), Chl *a* (492.6 µg mL<sup>-1</sup>), Chl b (244.3 µg mL<sup>-1</sup>), Chl t (736.9 µg mL<sup>-1</sup>), Car (190.6 µg mL<sup>-1</sup>), Dbe (17.45 mm), Dgp (17.28 mm), Dab (14.25 mm), PH (0.62 m), D<sub>Crown</sub> (0.54 m), and V<sub>Crown</sub> (0.22 m<sup>3</sup>).

In the principal component 1, it is possible to observe that the treatment S5SA4 (ECw =  $3.6 \text{ dS m}^{-1}$  and SA = 0 mM) resulted in the highest electrolyte leakage in the leaf blade - % IEL (37.9 %) of cashew plants.

For the principal component 2 (PC2), foliar application of salicylic acid at the concentration of 1.0 mM promoted an increase in diameter at the grafting point, even in plants irrigated with ECw of 3.6 dS m<sup>-1</sup> (Table 4), with the highest value of Dgp (28.99 mm) recorded in plants cultivated with ECw of 0.4 dS m<sup>-1</sup> (S1SA2). On the other hand, plants that did not receive treatment with SA (SA1) had a reduction in Dgp with the increase in electrical conductivity of irrigation water, and the lowest value of Dgp (17.28 mm) was found in plants irrigated with ECw of 3.6 dS m<sup>-1</sup> (S5SA1).

									Principal components			
								PC1		PC2		
Eigenvalues (λ)									9.24		1.35	
Percentage of total variance (S <sup>2</sup> %)									77.00		11.21	
Hotelling test (T <sup>2</sup> ) for levels of electrical conductivity of water (ECw)								0.01		0.01		
Hotelling test (T <sup>2</sup> ) for concentrations of salicylic acid (SA)								0.01		0.01		
		Hotelling	g test (T²)	for inte	raction (E	Cw × SA)			0.01		0.0	01
DCa					Coe	fficients	of correl	ation				
PCS	RWC	% IEL	Chl a	Chl b	Chl t	Car	Dbe	Dgp	Dab	PH	D <sub>Crown</sub>	V <sub>Crown</sub>
PC1	-0.94	0.93	-0.87	-0.89	-0.89	-0.92	-0.83	-0.53	-0.85	-0.93	-0.87	-0.93
PC2	-0.21	0.14	-0.42	-0.37	-0.42	-0.08	0.31	0.67	0.37	0.17	0.23	0.12
						Mean	values					
	RWC	% IEL	Chl a	Chl b	Chl t	Car	Dbe	Dgp	Dab	PH	D <sub>Crown</sub>	V <sub>Crown</sub>
S1SA1	85.2	18.0	640.7	288.6	929.4	247.7	24.04	26.59	17.4	0.77	0.75	0.30
S1SA2	86.1	16.4	701.0	325.8	1026.8	253.5	27.07	28.99	19.36	0.84	0.81	0.32
S1SA3	84.5	19.6	665.6	312.9	978.5	249.2	24.4	24.08	17.39	0.8	0.71	0.29
S1SA4	82.5	20.3	587.5	302.4	889.9	248.4	23.85	22.21	17.29	0.78	0.63	0.28
S2SA1	90.6	16.8	737.5	317.5	1055.0	258.7	26.29	23.74	18.90	0.82	0.79	0.30
S2SA2	93.5	15.4	810.3	358.4	1168.7	266.8	29.23	26.60	20.38	0.88	0.82	0.33
S2SA3	91.8	18.4	759.1	340.5	1099.6	262.3	26.64	20.98	18.56	0.86	0.68	0.28
S2SA4	89.7	19.0	711.0	332.6	1043.6	261.4	25.82	20.15	17.95	0.84	0.65	0.26
S3SA1	81.0	19.8	700.7	282.6	983.2	220.5	20.16	19.14	18.39	0.75	0.68	0.29
S3SA2	81.8	18.1	736.5	319.0	1055.5	225.6	25.11	19.93	16.53	0.81	0.7	0.29
S3SA3	80.3	21.6	721.2	306.3	1027.5	221.8	25.53	22.55	16.2	0.76	0.67	0.26
S3SA4	78.4	22.3	675.5	296.0	971.5	227.9	23.14	21.93	16.75	0.73	0.62	0.26
S4SA1	78.5	22.8	578.6	273.1	851.6	203.2	18.75	18.18	17.66	0.71	0.65	0.27
S4SA2	79.3	20.8	685.1	303.0	988.1	214.3	21.4	18.93	15.87	0.76	0.67	0.26
S4SA3	77.9	24.8	641.9	291.0	932.9	210.7	21.33	21.43	15.55	0.72	0.67	0.24
S4SA4	76.0	25.7	601.2	281.2	882.4	216.5	23.13	22.67	16.08	0.68	0.59	0.23
S5SA1	69.2	33.5	492.6	244.3	736.9	190.6	17.45	17.28	14.25	0.62	0.54	0.22
S5SA2	72.2	33.9	523.7	275.8	799.4	195.0	19.52	17.98	16.26	0.67	0.67	0.23
S5SA3	71.6	34.1	545.6	264.8	810.4	191.7	19.66	20.35	15.11	0.64	0.58	0.23
S5SA4	70.3	37.9	515.0	255.9	770.9	197.0	19.08	21.14	14.62	0.63	0.57	0.22

**Table 4:** Eigenvalues, percentage of total variance explained, in the multivariate analysis of variance (MANOVA) and correlation coefficients (r) between original variables and the principal components.

S – ECw, S1 (0.4 dS m<sup>-1</sup>); S2 (1.2 dS m<sup>-1</sup>); S3 (2.0 dS m<sup>-1</sup>); S4 (2.8 dS m<sup>-1</sup>); S5 (3.6 dS m<sup>-1</sup>); SA – Salicylic acid, SA1 (0 mM); SA2 (1.0 mM); SA3 (2.0 mM); SA4 (3.0 mM); RWC (relative water content - %); % IEL (percentage of intercellular electrolyte leakage); Chl *a* (chlorophyll *a* -  $\mu$ g mL<sup>-1</sup>); Chl *b* (chlorophyll *b* -  $\mu$ g mL<sup>-1</sup>); Car (carotenoids -  $\mu$ g mL<sup>-1</sup>); Dbe (stem diameter below the grafting); Dgp (stem diameter at the grafting point); Dab (Stem diameter above the grafting); PH (Plant height - m); D<sub>Crown</sub> (Crown diameter - m); V<sub>Crown</sub> (Crown volume - m<sup>3</sup>).



**Figure 2**: Two-dimensional projections of the scores of the principal components PC1 and PC2 for the factors S (electrical conductivity of irrigation water - ECw) and SA (concentrations of salicylic acid) (A) and the analyzed variables (B).

The leaf relative water content is an important indicator of salt stress (Ahmad et al., 2018). The results obtained in this study indicate a reduction in the RWC of early dwarf cashew due to exposure to salt stress. However, foliar application of salicylic acid at the concentration of 1 mM was able to raise RWC in plants irrigated with ECw of 3.6 dS m<sup>-1</sup>. According to Sousa et al. (2023), under conditions of salt stress, the maintenance or increase of RWC indicates a possible osmotic adjustment, allowing the turgor of the leaves.

Salt stress caused an increase in % IEL in the leaf blade of early dwarf cashew in this case, membrane rupture may be associated with the accumulation of toxic ions and the induction of lipid peroxidation, which destabilizes the production of lipids and proteins of the membrane, limiting the maintenance of cell turgor (Samadi; Habibi; Vaziri, 2019). This result corroborates the reduction observed in the relative water content of the leaves of cashew plants that did not receive treatment with SA.

Increased electrolyte leakage due to salt stress was also verified by Sousa et al. (2023) when evaluating the morphophysiology of early dwarf cashew rootstocks under salt stress (ECw ranging from 0 to 100 mM  $L^{-1}$ ). These authors found an increase of 25.23% when comparing plants irrigated with the highest salinity level (100 mM  $L^{-1}$ ) to those of the control (0 mM  $L^{-1}$ ), with the % IEL 29.56% obtained in the CCP 09/CCP 76 rootstock.

In the present study, it was also found that the increase in the electrical conductivity of irrigation water reduced the contents of photosynthetic pigments and growth of early dwarf cashew. Degradation in the synthesis of photosynthetic pigments is one of the most noticeable effects in plants under salt stress (Lotfi; Ghassemi-Golezani; Pessarakli, 2020). Salinity increases the activity of the chlorophyllase enzyme, making the pigment-protein complex unstable, while the ROS induced by salt stress degrades the photosynthetic pigments (Soares et al., 2021). Reductions in the synthesis of photosynthetic pigments were also observed by Lima et al. (2020b) in early dwarf cashew rootstocks (Faga 11, Embrapa 51, and CCP 76) under salt stress (ECw ranging from 0.4 to 3.6 dS m<sup>-1</sup>) during seedling development.

Despite the harmful effects caused by the increase in ECw observed in the present study, foliar application of salicylic acid at the concentration of 1.0 mM mitigated the effects of salt stress, promoting a reduction in intercellular electrolyte leakage, increments in the relative water content and synthesis of photosynthetic pigments, and improvement growth of early dwarf cashew.

Lamnai et al. (2022) evaluated the effects of the application of salicylic acid in strawberry plants under salt stress (80 mM of NaCl). They found that the application of salicylic acid at the concentration of 0.25 mM attenuated the damage caused by salinity and maintained high levels

of RWC in treated plants. Soares et al. (2022) found that the application of salicylic acid up to a concentration of 4.5 mM stimulated the synthesis of total chlorophyll and increased the  $CO_2$  assimilation rate and instantaneous water use efficiency of melon cultivated in a hydroponic system.

The improvements observed in the physiological variables of early dwarf cashew due to the foliar application of salicylic acid at the concentration of 1 mM were reflected in the growth variables, especially in plants irrigated with ECw of 0.4 and 1.2 dS m<sup>-1</sup>. Similar results were obtained in a study conducted by Silva et al. (2021), who evaluated the morphophysiology of soursop (*Annona muricata* L.) under salt stress (ECw ranging from 0.8 to 4.0 dS m<sup>-1</sup>) and foliar application of salicylic acid (SA ranging from 0 to 3.6 mM) and found that the foliar application of SA at the concentration of 1.6 mM attenuated the deleterious effects of salinity, promoting increments in stem diameter, plant height, and crown diameter.

Gas exchange variables, for being autocorrelated, as described above, were presented through univariate analysis. According to the analysis of variance summary (Table 5), the interaction between water electrical conductivity levels and salicylic acid concentrations (ECw × SA) significantly influenced (p≤0.01) the internal CO<sub>2</sub> concentration (*Ci*), stomatal conductance (gs) and instantaneous carboxylation efficiency (*CEi*). The ECw levels significantly affected (p≤0.01) all gas exchange variables, whereas salicylic acid concentrations did not significantly influence instantaneous water use efficiency (*WUEi*). The increase in the electrical conductivity of irrigation water increased *Ci*, regardless of the concentration of salicylic acid (Figure 3A). The highest *Ci* value (288.23  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) was obtained in plants irrigated with ECw of 3.6 dS m<sup>-1</sup> and subjected to SA concentration of 3.0 mM. On the other hand, the lowest *Ci* value (137.49  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) was recorded in plants irrigated with ECw of 0.4 dS m<sup>-1</sup> and without application of SA (0 mM). Stomatal conductance (Figure 3B) was favored by the application of salicylic acid at the concentration of 1.0 mM, with the highest *gs* value (0.255 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) recorded in cashew plants irrigated with ECw of 0.4 dS m<sup>-1</sup>, corresponding to an increase of 7.16% (0.017 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> compared to plants irrigated with the same ECw level (0.4 dS m<sup>-1</sup>) and without application of SA (0 mM).

The increase in the electrical conductivity of irrigation water negatively affected the transpiration of early dwarf cashew (Figure 3C), with reductions of 7.28% per unit increment in ECw. When comparing the transpiration of plants irrigated with ECw of 3.6 dS m<sup>-1</sup> with that of those subjected to water salinity of 0.4 dS m<sup>-1</sup>, a reduction of 24.0% (0.572 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) was observed. On the other hand, foliar application of salicylic acid up to the concentration of 1.0 mM promoted increase in transpiration (Figure 3D), with the highest *E* value (2.20 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) obtained at the concentration of 1.0 mM, corresponding to an increase of 2.12% compared to the control (0 mM).

<b>Table 5</b> : Summary of the analysis of variance for the internal CO <sub>2</sub> concentration ( <i>Ci</i> ), stomatal conductance (gs),
transpiration (E), CO, assimilation rate (A), instantaneous carboxylation efficiency (CEi) and instantaneous water
use efficiency (WUEi) of early dwarf cashew irrigated with saline waters and under foliar application of salicylic
acid, at 210 days after transplanting.

Source of variation		Mean squares							
		Ci	gs	Ε	A	CEi	WUEi		
Electrical conductivity of water (ECw)		41714.44**	0.016**	0.54**	50.86**	0.01**	0.67**		
Linear regression	1	164724.30**	0.062**	1.95**	199.97**	0.05**	1.72**		
Quadratic regression		1755.05*	8.60×10 <sup>-4ns</sup>	0.23 <sup>ns</sup>	3.05 <sup>ns</sup>	0.01*	0.75 <sup>ns</sup>		
Salicylic acid (SA)		3724.68**	0.011**	0.61**	35.26**	0.02**	3.75 <sup>ns</sup>		
Linear regression	1	11156.90**	0.001*	0.05 <sup>ns</sup>	35.23 <sup>ns</sup>	0.04**	-		
Quadratic regression		16.54 <sup>ns</sup>	0.014**	0.15**	46.61**	0.01*	-		
Interaction (ECw × SA)		202.48**	0.001**	0.04 <sup>ns</sup>	4.13 <sup>ns</sup>	0.03**	0.68 <sup>ns</sup>		
Blocks		294,18 <sup>ns</sup>	3.08×10 <sup>-4ns</sup>	0.02 <sup>ns</sup>	3.85 <sup>ns</sup>	0.002 <sup>ns</sup>	0.72 <sup>ns</sup>		
Residual		13.62	1.47×10-4	0.01	2.74	7.40×10 <sup>-5</sup>	0.68		
CV (%)		7.86	6.32	2.98	9.77	10.35	10.17		

<sup>ns, \* and \*\*</sup> respectively not significant, significant at  $p \le 0.05$  and  $p \le 0.01$ . CV: Coefficient of variation DF: degrees of freedom.



**Figure 3**: Response surface for internal CO<sub>2</sub> concentration - *Ci* (A) and stomatal conductance - *gs* (B) of early dwarf cashew as a function of the interaction between the levels of electrical conductivity of irrigation water (ECw) and salicylic acid concentrations, and transpiration - E as a function of ECw levels (C) and salicylic acid concentrations (D), at 210 days after transplanting. X and Y – water electrical conductivity levels and salicylic acid concentrations, respectively; <sup>ns, \* and \*\*</sup> respectively not significant, significant at  $p \le 0.05$  and  $p \le 0.01$ . Vertical lines represent standard error of mean (n=3).

The increase in the electrical conductivity of irrigation water also negatively affected the CO<sub>2</sub> assimilation rate (Figure 4A) and instantaneous water use efficiency (Figure 4C) of early dwarf cashew, with reductions of 8.01% in the CO<sub>2</sub> assimilation rate and 10.23% in the instantaneous water use efficiency, per unit increment in ECw. When comparing the *A and WUEi* of plants cultivated with ECw of 3.6 dS m<sup>-1</sup> to those of plants subjected to ECw of 0.4 dS m<sup>-1</sup>, reductions of 26.49% (5.17  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and 34.11% (2.93 ( $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>) were observed in *A* and *WUEi*, respectively.

Foliar application of salicylic acid at the concentration of 1.0 mM promoted an increase in  $CO_2$  assimilation rate (Figure 4B), which was equal to

4.78% (0.83 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) compared to plants that did not receive treatment with SA (0 mM). However, the observed increase in the CO<sub>2</sub> assimilation rate at the SA concentration of 1.0 mM did not lead to gains in instantaneous carboxylation efficiency (Figure 4C), considering that the highest value of *CEi* (0.148 [(µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>)(µmol CO<sub>2</sub> mol<sup>-1</sup>]<sup>-1</sup>) was obtained in plants irrigated with ECw of 0.4 dS m<sup>-1</sup> and without application of salicylic acid (0 mM)

Excess of salts in irrigation water, especially Na<sup>+</sup> and Cl<sup>-</sup>, restricts the capacity of roots to absorb water from soils (Soni et al., 2021). Later, salt stress causes osmotic stress and ionic imbalance (Farouk; Elhindi; Alotaibi, 2020), which results in stomatal closure and reductions in transpiration,  $CO_2$  assimilation rate, and instantaneous carboxylation and water use efficiency (Chrysargyris et al., 2019; Silva et al., 2020).

Stomata are the structures responsible for controlling the gas exchange of plants (Fernandes et al., 2022). The regulation of stomata under salt stress may also be related to the synthesis of abscisic acid (ABA), a plant hormone that acts on the accumulation of compatible osmolytes and protein synthesis, which help maintain leaf turgor (Verslues et al., 2006; Jones, 2016).

Reductions in gs, E, A, CEi, and WUEi with the increase in the electrical conductivity of irrigation water have also been observed in other studies with fruit crops, such as West Indian cherry (Dias et al., 2021; Dantas et al., 2021), passion fruit (Silva Neta et al., 2021), sugar apple (Sá et al., 2021), soursop (Silva et al., 2021) and guava (Lacerda et al., 2022).

Beneficial effects of salicylic acid were also observed by Mohammadi et al. (2020), who evaluated the effect of foliar spraying of salicylic acid (0 to 2 mM) on almond plants (*Prunus amygdalus* L.) under salt stress (8 dS m<sup>-1</sup>) and observed that the application of SA at the concentration of 1 mM mitigated the deleterious effects of salinity on stomatal conductance, transpiration, and  $CO_2$ assimilation rate of the plants.

Salicylic acid is a phenolic plant hormone that plays an important role in the signaling of biotic and abiotic stresses, such as salinity, and has emerged as an inducer of stress-responsive pathways (Ma et al., 2017; Moustafa-Farag et al., 2020). However, the induction of salt stress tolerance mediated by SA depends on several aspects such as concentration, application method, salt stress intensity, type of species, and stage of development (Nazar et al., 2011).



**Figure 4:** CO<sub>2</sub> assimilation rate – *A* (A) and instantaneous water use efficiency - *WUEi* (D) of early dwarf cashew, as a function of the levels of electrical conductivity of irrigation water (ECw), *WUEi* as a function of salicylic acid concentrations (B) and response surface for instantaneous carboxylation efficiency - *CEi* (C) as a function of the interaction between ECw and salicylic acid concentrations at 210 days after transplanting. X and Y – water electrical conductivity levels and salicylic acid concentrations, respectively; <sup>ns, \* and \*\*</sup> respectively not significant, significant at p ≤ 0.05 and p ≤ 0.01. Vertical lines represent standard error of mean (n=3).

The beneficial effect of salicylic acid may be related to its capacity to reduce the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions and increase K<sup>+</sup> absorption in plants, resulting in a better K<sup>+</sup>/Na<sup>+</sup> ratio (Hundare; Joshi; Joshi, 2022). In addition, SA acts in the intensification of the antioxidant capacity of the plant and in the protection of membranes against deterioration (Antonic et al., 2016; Esan et al., 2017), thus promoting greater tolerance to salt stress.

Many strategies have been employed by scientists to mitigate the harmful effects of salt stress and make plants tolerant to these conditions. The findings of this study show that foliar application of salicylic acid at the concentration of 1 mM can be used to promote greater tolerance of early dwarf cashew to salt stress and thus contribute to the development of irrigated agriculture in semi-arid regions, where waters with high salt concentrations are commonly found.

## CONCLUSIONS

Early dwarf cashew physiology and growth are negatively affected by the increase in the electrical conductivity of irrigation water above 0.4 dS m<sup>-1</sup>. However, foliar application of salicylic acid at the concentration of 1 mM attenuates the effects of salt stress on the percentage of electrolyte leakage, leaf relative water content, synthesis of photosynthetic pigments and growth of early dwarf cashew irrigated with ECw of up to 3.6 dS m<sup>-1</sup>.

## **AUTHOR CONTRIBUTIONS**

Conceptual idea: Silva, A. A. R. da; Lima, G. S. de; Data collection: Silva, A. A. R. da; Arruda, T. F. L.; Data analysis and interpretation: Silva, A. A. R. da; Soares, L. A. dos A.; Writing and editing: Silva, A. A. R. da; Lima, G. S. de; Reviewer: Azevedo, C. A. V. de; Gheyi, H. R.

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