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Gas exchange, photosynthetic pigments, and growth of hydroponic okra under salt stress and salicylic acid[1]

Trocas gasosas, pigmentos fotossintéticos e crescimento de quiabeiro hidropônico sob estresse salino e ácido salicílico

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HIGHLIGHTS:

Salt stress limited gas exchange and inhibited the growth of okra in hydroponic system.
Saline nutrient solution up to 9.0 dS m^{-1} does not affect the relative water content and electrolyte leakage.
Salicylic acid at a concentration of 0.9 mM stimulates the synthesis of carotenoids under a saline solution of 9.0 dS m^{-1}.

ABSTRACT: The high concentrations of salts present in the water sources of the Brazilian Northeastern semi-arid region stand out as one of the limiting factors for agricultural production, contributing to soil salinization and/or sodification. Thus, it is extremely important to identify strategies to mitigate the effects of salt stress on plants, such as the foliar application of salicylic acid. In this context, the objective of the present study was to evaluate the effect of foliar application of salicylic acid as an attenuator of salt stress on leaf gas exchange, water relations, photosynthetic pigments, and growth of okra cv. Canindé grown in a hydroponic system. The experiment was conducted in a greenhouse, in Pombal, PB, Brazil. The treatments consisted of four levels of electrical conductivity of the nutrient solution - ECns (3.0, 5.0, 7.0, and 9.0 dS m^{-1}) and four concentrations of salicylic acid - SA (0, 1.2, 2.4, and 3.6 mM) distributed in a completely randomized design in a split-plot scheme, considering the ECns levels as the plots and SA concentrations as the subplots, with four replicates and two plants per plot. ECns from 3.0 dS m^{-1} inhibited the synthesis of photosynthetic pigments, leaf gas exchange, and growth of okra cv. Canindé. Foliar application of salicylic acid at concentrations of up to 3.6 mM does not alleviate the effects of salt stress on water relations, gas exchange and growth of okra. Salicylic acid at a concentration of 1.8 mM stimulates chlorophyll a and b biosynthesis in okra under ECns of 3.0 and 4.0 dS m^{-1}, respectively.

Key words: *Abelmoschus esculentus* L. Moench, salinity, soilless cultivation, phytohormone

RESUMO: As altas concentrações de sais presentes nas águas no semiárido do Nordeste brasileiro destacam-se como um dos fatores limitantes para produção agrícola, contribuindo para a salinização e/ou sodificação dos solos. Assim, é de extrema importância a identificação de estratégias para amenizar os efeitos do estresse salino sobre as plantas, como a aplicação foliar de ácido salicílico. Neste sentido, objetivou-se com este estudo avaliar o efeito da aplicação foliar de ácido salicílico como atenuante do estresse salino nas trocas gasosas, relações hídricas, pigmentos fotossintéticos e crescimento do quiabeiro cv. Canindé cultivado em sistema hidropônico. O experimento foi desenvolvido em casa de vegetação, em Pombal, PB. Os tratamentos foram constituídos de quatro níveis de condutividade elétrica da solução nutritiva - CEsn (3,0; 5,0; 7,0 e 9,0 dS m^{-1}) e quatro concentrações de ácido salicílico - AS (0; 1,2; 2,4 e 3,6 mM) distribuídos em delineamento inteiramente casualizado em esquema de parcelas subdivididas, sendo os níveis de CEsn considerados as parcelas e as concentrações de AS as subparcelas, com quatro repetições e duas plantas por parcela. CEsn a partir de 3,0 dS m^{-1} inibiu a síntese de pigmentos fotossintéticos, as trocas gasosas foliares e o crescimento do quiabeiro cv. Canindé. A aplicação foliar de ácido salicílico em concentração de até 3,6 mM não alivia os efeitos do estresse salino sobre as relações hídricas, trocas gasosas e crescimento do quiabeiro. Ácido salicílico com concentração de 1,8 mM estimula a biossíntese de clorofila a e b em quiabeiro sob CEsn de 3,0 e 4,0 dS m^{-1}, respectivamente.

Palavras-chave: *Abelmoschus esculentus* L. Moench, salinidade, cultivo sem solo, fitohormônio

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Introduction

Okra (*Abelmoschus esculentus* L. Moench), belonging to the Malvaceae family, is a vegetable crop, traditionally cultivated by small farmers in Brazil, due to its rusticity and adaptation to tropical and subtropical climate regions (Santos et al., 2019). The vegetable has a salinity threshold of 2.54 dS m^{-1} in saline nutrient solution (Mendonça et al., 2022). Due to the high rates of evapotranspiration and low rainfall, the semi-arid region of northeastern Brazil is characterized by qualitative and quantitative scarcity of water resources, limiting the expansion of conventionally irrigated agriculture due to the occurrence of water sources with high concentrations of dissolved salts (Lima et al., 2020a).

Hydroponic cultivation is a viable alternative in these regions where water sources have high concentrations of salts, allowing the rational use of water, saving up to 70% compared to conventional systems, control of pH and electrical conductivity, and efficient use of fertilizers (Sausen et al., 2020). This viability of using saline waters for growing vegetables, especially in the context of hydroponic cultivation, is related to the absence of matric potential (Lira et al., 2018), since cultivation is carried out without soil or any other substrate.

One strategy that has been employed to reduce salt stress on plants is the exogenous application of salicylic acid (Silva et al., 2020; Lacerda et al., 2022). Salicylic acid (SA) is a phytohormone that participates in the activation of genes capable of acting in the plant's defense mechanism against oxidative stress, in the photosynthetic process, reducing the intensity of the deleterious effects of salt stress (Figueredo et al., 2019).

Given the above, the objective of this study was to evaluate the effect of foliar application of salicylic acid as an attenuator of salt stress on leaf gas exchange, water relations, photosynthetic pigments, and growth of okra cv. Canindé grown in a Nutrient Film Technique (NFT) hydroponic system.

Material and Methods

The experiment was conducted from January to March 2022 under conditions of an arch-type greenhouse with 150-micron low-density plastic cover, at the Centro de Ciências e Tecnologia Agroalimentar (CCTA) pertaining to the Universidade Federal de Campina Grande (UFCG), in the municipality of Pombal, PB, Brazil, geographically located at 6° 46' 8" South and 37° 47' 45" West, with an altitude of 184 m, with an average annual precipitation of 700 mm. Air temperature (maximum and minimum) and relative air humidity data were collected daily using a digital thermo-hygrometer inside the greenhouse (Figure 1).

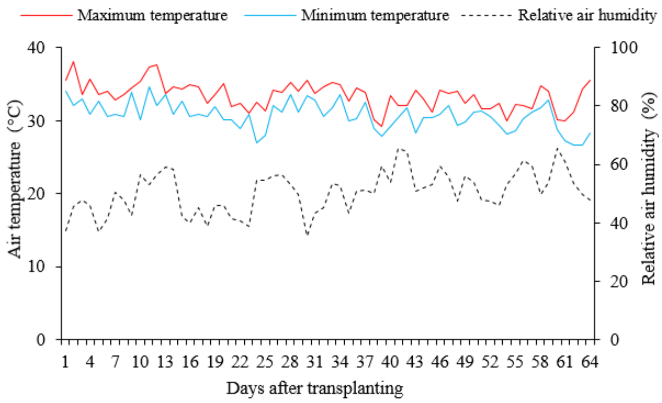


Figure 1. Daily data of air temperature (maximum and minimum) and mean relative air humidity during the experimental period

The treatments consisted of four levels of electrical conductivity of the nutrient solution - ECns (3.0, 5.0, 7.0, and 9.0 dS m^{-1}) and four concentrations of salicylic acid - SA (0, 1.2, 2.4, and 3.6 mM), distributed in a completely randomized design, in a split-plot scheme, considering ECns levels as the plots and SA concentrations as the subplots, with four replicates and two plants per plot. Salicylic acid concentrations were based on the study conducted by Silva et al. (2020). The levels of electrical conductivity of nutrient solution were defined based on the studies conducted by Soares et al. (2020) and Silva et al. (2023).

The nutrient solution used was that recommended by Hoagland & Arnon (1950), whose composition and nutrient concentrations are presented in Table 1. The solution was prepared in local-supply water (ECw = 0.3 dS m^{-1}), resulting in an ECns of 2.1 dS m^{-1}.

The saline nutrient solutions were prepared with the addition of non-iodized sodium chloride (NaCl), calcium chloride ($CaCl_2.2H_2O$), and magnesium chloride ($MgCl_2.6H_2O$) at a proportion of 7:2:1, respectively. The irrigation waters were prepared considering the relationship between ECw and salt concentration (Richards, 1954), according to Eq. 1:

$$Q \approx 10 \times ECw \tag{1}$$

where:

Q - sum of cations (mmol$_c$ L^{-1}); and,

ECw - desired electrical conductivity after discounting the ECw of the water from the municipal supply system (dS m^{-1}).

Seeds of okra cv. Canindé were sown in 50-mL disposable containers, containing coconut fiber substrate and one seed each. Prior to sowing, the coconut fiber was washed once to reduce salinity of substrate. A nutrient solution with 50% concentration was used from germination to the appearance of the second pair of true leaves; then, the coconut fiber

Table 1. Chemical composition relative to nutrients present in the nutrient solution of Hoagland & Arnon (1950), used in hydroponic cultivation of okra cv. Canindé

Nutrient	Fertilizer	Quantity (g 1000L^{-1})
N	KH_2PO_4	136.09
P	KNO_3	101.10
K	$Ca(NO_3)_2.4H_2O$	236.15
Ca	$MgSO_4.7H_2O$	246.49
Mg	H_3BO_3	3.10
S	$MnSO_4.4H_2O$	1.70
B	$ZnSO_4.7H_2O$	0.22
Mn	$CuSO_4.5H_2O$	0.75
Zn	$(NH_4)_6Mo_7O_{24}.4H_2O$	1.25
Cu	$CuSO_4$	0.75
Mo	EDTA – Na	13.9
Fe	$FeSO_4$	13.9

was removed, and the plants were inserted directly into the hydroponic profiles, using vertical staking in order to leave the stem upright, fixed with nylon strings, and a nutrient solution with 100% concentration.

The hydroponic system was an NFT type (Nutrient Film Technique), made with PVC pipe of 100 mm in diameter and 6 m in length, composed of four subsystems spaced 0.8 m apart, each of which with three channels spaced 0.4 m apart. In the channels, the spacing was 0.5 m between plants and 1.0 m between treatments.

The channels were supported on sawhorses with 0.6 m height and 4% slope for the nutrient solution to flow. At the lowest point of each bench of the hydroponic system, a 150-L polyethylene box was placed to collect and conduct the nutrient solution to the channels. The nutrient solution was driven to the head of the channels by a 35 W pump, with an average flow rate of 3 L min^{-1}. Nutrient solution circulation was programmed by a timer, with an intermittent flow of 15 min during the day and night.

The nutrient solution was completely replaced every eight days, with daily checking of electrical conductivity and pH, which were adjusted whenever necessary by adding local-supply water with ECw of 0.3 dS m^{-1}, always maintaining the ECns according to the treatments and the pH between 5.5 and 6.5 through the addition of 0.1 M KOH or HCl (Mendonça et al., 2022).

The solutions of salicylic acid were prepared by the dissolution of salicylic acid (A.R. grade) in 30% of ethyl alcohol (99.5%) and 0.05% of Haiten®, a non-ionic adhesive spreader used to break the surface tension and improve air absorption by the okra leaves.

The salicylic acid applications were performed 48 hours after inserting the plants into the hydroponic profiles and 72 hours before the beginning of the use of saline nutrient solutions. The applications were performed at 17:00 hours (time when temperature is mild to avoid evaporation of salicylic acid), manually with a sprayer, aiming to moisten the total area of the leaves (adaxial and abaxial sides) according to the treatments, applying on average 19 mL per plant, at 8-day intervals, totaling six applications. A cardboard structure was used to avoid the drift of treatments between plants. The plants were monitored and phytosanitary practices were performed whenever necessary.

At 63 days after transplanting (DAT), the following variables were evaluated: relative water content - RWC, intercellular electrolyte leakage in the leaf blade - % IEL, leaf gas exchange by determination of stomatal conductance - gs (mol H_2O m^{-2} s^{-1}), transpiration - E (mmol H_2O m^{-2} s^{-1}), CO_2 assimilation rate - A (μmol CO_2 m^{-2} s^{-1}), internal CO_2 concentration - Ci (μmol CO_2 m^{-2} s^{-1}), instantaneous water use efficiency - WUEi [(μmol CO_2 m^{-2} s^{-1}) (mmol H_2O^{-2} s^{-1})$^{-1}$], instantaneous carboxylation efficiency - CEi [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-2} s^{-1})$^{-1}$], contents of chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Car), and growth through plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA).

For the determination of the relative water content in the leaves (RWC), four leaf discs (113 mm^2) were collected and weighed (on an analytical scale) to obtain fresh mass (FM), turgid mass (TM) after submersion in distilled water for 24 hours and dry mass (DM) after drying in a forced air circulation oven at 60 °C for 48 hours. Relative water content was determined according to the methodology of Weatherley (1950), using Eq. 2:

$$\mathrm{RWC}(\%) = \frac{(\mathrm{FM}-\mathrm{DM})}{(\mathrm{TM}-\mathrm{DM})}\times 100 \tag{2}$$

where:

RWC - relative water content (%);

FM - leaf fresh mass (g);

TM - turgid mass (g); and,

DM - dry mass (g).

Intercellular electrolyte leakage (% IEL) was determined using four leaf discs (113 mm^2), which were placed in beakers with 50 mL of distilled water and kept at a temperature of 25 °C for 90 min. After this time, a benchtop conductivity meter was used to measure the initial electrical conductivity of the medium (IC). Then, the beaker was placed in a drying oven and kept at a temperature of 80 °C for 90 min, for subsequent measurement of the final electrical conductivity (FC). Electrolyte leakage in the cell membrane was expressed according to Scotti-Campos et al. (2013), as shown in Eq. 3:

$$\%\mathrm{IEL} = \frac{\mathrm{IC}}{\mathrm{FC}}\times 100 \tag{3}$$

where:

% IEL - inter cellular electrolyte leakage in the membrane (%);

IC - initial electrical conductivity (dS m^{-1}); and,

FC - final electrical conductivity (dS m^{-1}).

Leaf gas exchange was determined between 06:30 and 09:00 hours in the morning in leaves located in the middle third, using a portable infrared carbon dioxide analyzer (IRGA), LCPro+ Portable Photosynthesis System® (ADC BioScientific Limited, UK), with irradiation of 1200 μmol photons m^{-2} s^{-1}, airflow of 200 mL min^{-1}, and atmospheric CO_2 concentration.

The contents of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined according to Arnon (1949). By using 1 g of fresh matter homogenized in 4 mL of 80% acetone, after 72 hours in the dark in hermetically closed containers, chlorophyll and carotenoid concentrations were determined using a spectrophotometer (Thermo Scientific®, Genesys 20) at absorbance (ABS) wavelength (470, 646, and 663 nm), using Eqs. 4, 5 and 6.

$$\mathrm{Chl\ a} = 12.21\times \mathrm{ABS}_{663} - 2.81\times \mathrm{ABS}_{646} \tag{4}$$

$$\mathrm{Chl\ b} = 20.13\times \mathrm{ABS}_{646} - 5.03\times \mathrm{ABS}_{663} \tag{5}$$

$$\mathrm{Car} = \frac{(1000\times \mathrm{ABS}_{470} - 1.82\times \mathrm{Chl\ a} - 85.02\times \mathrm{Chl\ b})}{198} \tag{6}$$

The values obtained for chlorophyll a, chlorophyll b, and carotenoid contents were expressed in mg g^{-1} of fresh matter (mg g^{-1} FM).

Stem diameter was measured at 5 cm from the hydroponic profile, with a digital caliper. Plant height was obtained considering the distance from the hydroponic profile to the insertion of the apical meristem. In the quantification of NL, only leaves with a minimum length of 3 cm and at least 50% of photosynthetically active area were considered. Plant height and leaf area were determined with a graduated ruler. Leaf area of the plant was determined according to Fideles Filho et al. (2010), using Eq. 7:

$$Y = \sum 0.7254(x)^{2.08922} \tag{7}$$

where:

Y - leaf area of the plant (cm^2); and,

x - midrib length (cm).

The data obtained were subjected to the normality test (Shapiro-Wilk), followed by analysis of variance by the F test at $p \leq 0.05$. When significant, polynomial regression analysis (linear and quadratic) was performed for the saline nutrient solution and for salicylic acid concentrations, using the statistical program SISVAR – ESAL version 5.7 (Ferreira, 2019). SigmaPlot software was used to construct the response surface curves in cases in which the interaction between the factors was significant. Because of heterogeneity of gs, E, Ci, WUEi, and CEi data verified by the tests of normality and homogeneity of variance, the data were transformed in /x before the analysis of variance.

Results and Discussion

The interaction between the factors saline nutrient solution and concentrations of salicylic acid (ECns × SA) did not significantly influence any of the gas exchange variables of okra cv. Canindé, at 63 days after transplanting. However, there were significant effects of saline nutrient solution on stomatal conductance (gs), transpiration (E), CO_2 assimilation rate (A), and instantaneous water use efficiency (WUEi) of okra cv. Canindé (Table 2). The instantaneous carboxylation efficiency (CEi) and internal CO_2 concentration (Ci) were not affected significantly by the sources of variation tested in this study.

Stomatal conductance (gs) reached a maximum estimated value of 0.32 mol H_2O m^{-2} s^{-1} in plants cultivated under a saline nutrient solution of 3.0 dS m^{-1}, decreasing from this level and reaching the minimum value of 0.17 mol H_2O m^{-2} s^{-1} in plants subjected to ECns of 9.0 dS m^{-1}, which corresponded to a reduction of 45.67% compared to plants that had the highest gs (Figure 2A). Stomatal closure is a plant strategy to reduce water losses and maintain water balance, thus contributing to lower absorption of toxic ions (Dias et al., 2019). A negative effect of nutrient solution salinity was also verified by Dantas et al. (2022), who studied Italian zucchini cultivated with ECns (2.1 to 6.6 dS m^{-1}) in an NFT hydroponic system and observed a 26.84% reduction in stomatal conductance with the increase in nutrient solution electrical conductivity.

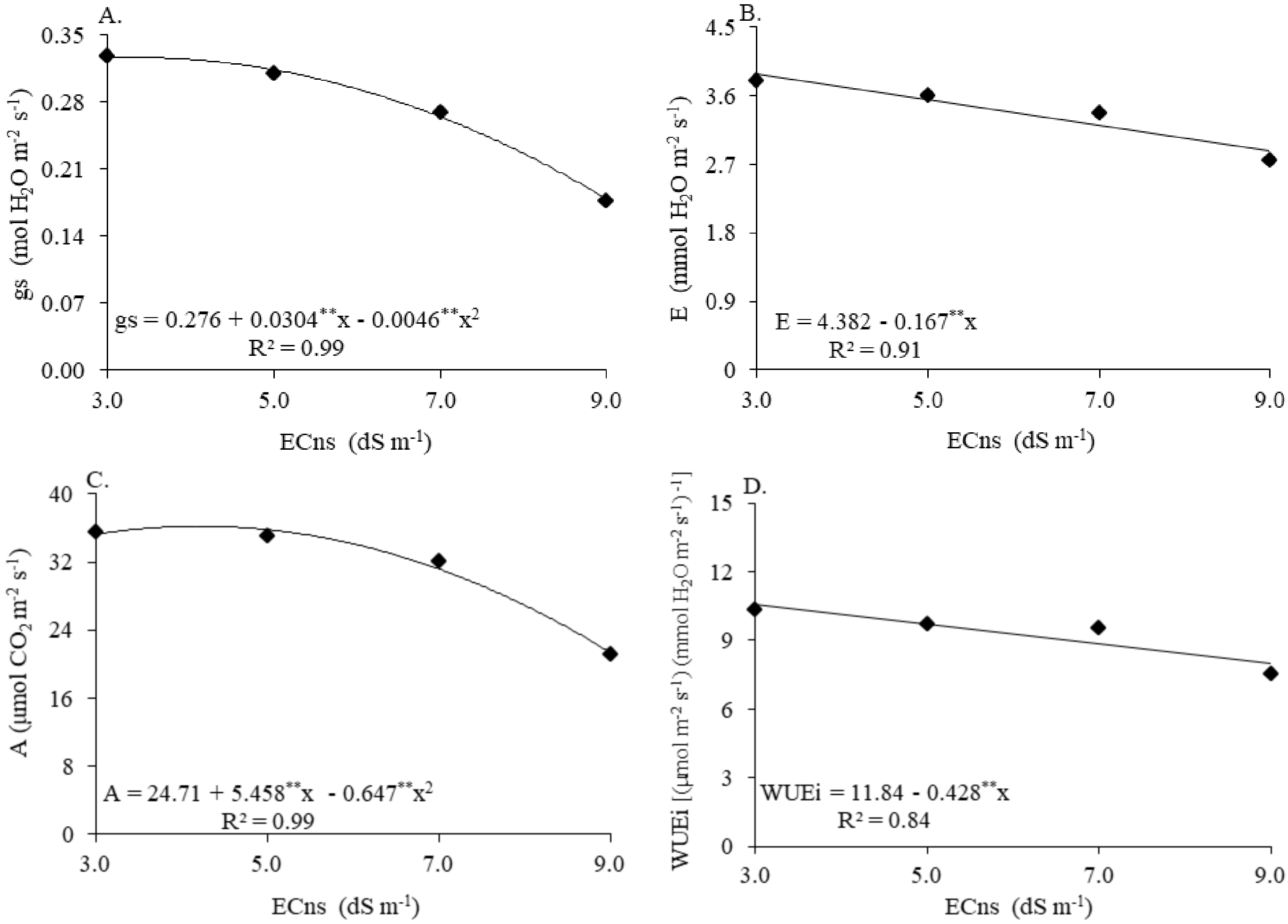
The transpiration (E) of okra plants decreased linearly with the increase in the electrical conductivity of the nutrient solution, with a reduction of 3.81% per unit increment in ECns (Figure 2B). When comparing plants subjected to ECns of 9.0 dS m^{-1} to those that received the lowest ECns (3.0 dS m^{-1}), a reduction of 25.81% was observed. The decrease in transpiration is directly related to the partial closure of the stomata (Figure 3A), restricting the loss of water from leaves to the atmosphere, and consequently promoting lower plant dehydration (Lima et al., 2017). Dantas et al. (2021), in a study with Italian zucchini cultivated with saline nutrient solutions (ECns ranging from 2.1 to 6.6 dS m^{-1}) in a NFT hydroponic system, also observed that the increase in the electrical conductivity of the nutrient solution from 2.1 dS m^{-1} reduced leaf transpiration.

For CO_2 assimilation rate (A) (Figure 2C), okra plants cv. Canindé cultivated under ECns of 4.3 dS m^{-1} obtained the maximum estimated value of 36.21 μmol CO_2 m^{-2} s^{-1}. On the other hand, the minimum value of A (21.42 μmol CO_2 m^{-2} s^{-1}) was found at ECns of 9.0 dS m^{-1}. A behavior similar to that of gs was observed, which reflects the restrictions on the entry of CO_2 in the substomatal chamber, probably due to phytotoxic damage resulting from the action of salts in the plant, leading to lower activity of RuBisCO and, consequently, the consumption of carbon in the Calvin cycle (Pan et al., 2021).

Table 2. Summary of the analysis of variance for relative water content (RWC), intercellular electrolyte leakage (% IEL), stomatal conductance (gs), transpiration (E), CO_2 assimilation rate (A), internal CO_2 concentration (Ci), instantaneous water use efficiency (WUEi), and instantaneous carboxylation efficiency (CEi) of okra plants cv. Canindé grown under saline nutrient solution (ECns) and application of salicylic acid (SA), 63 days after transplanting

Sources of variation	DF	Mean squares							
		RWC	% IEL	$gs^{(1)}$	$E^{(1)}$	A	$Ci^{(1)}$	$WUEi^{(1)}$	$CEi^{(2)}$
Saline nutrient solution (ECns)	3	25.88^{ns}	2.08^{ns}	0.05^{*}	2.57^{*}	540.84^{**}	3795.11^{ns}	17.51^{**}	0.10^{ns}
Linear regression	1	19.72^{ns}	0.03^{ns}	0.14^{**}	6.98^{**}	1282.88^{**}	3496.06^{ns}	43.98^{**}	0.21^{ns}
Quadratic regression	1	30.33^{ns}	4.95^{ns}	0.01^{**}	0.71^{ns}	321.57^{**}	7056.75^{ns}	5.86^{ns}	0.08^{ns}
Residual 1	6	557.51	19.14	0.02	8.70	275.03	15.25	49.07	0.06
Salicylic acid (SA)	3	52.82^{ns}	1.36^{ns}	0.0028^{ns}	0.19^{ns}	41.58^{ns}	3064.53^{ns}	4.04^{ns}	0.08^{ns}
Linear regression	1	97.74^{ns}	3.94^{ns}	0.0005^{ns}	0.02^{ns}	79.94^{ns}	6020.01^{ns}	9.16^{ns}	0.14^{ns}
Quadratic regression	1	26.28^{ns}	0.13^{ns}	0.004^{ns}	0.45^{ns}	39.24^{ns}	310.08^{ns}	0.33^{ns}	0.002^{ns}
Interaction (ECns × SA)	9	28.08^{ns}	0.68^{ns}	0.0024^{ns}	0.14^{ns}	36.96^{ns}	1640.42^{ns}	1.93^{ns}	0.05^{ns}
Residual 2	26	841.03	32.85	0.04	6.93	771.87	99.41	208.94	1.16
CV 1 (%)		12.62	17.44	13.35	18.81	21.89	15.40	13.83	6.34
CV 2 (%)		7.44	10.97	7.82	7.84	17.62	18.89	14.53	11.80

DF - Degrees of freedom; CV (%) - Coefficient of variation; **, *, ^{ns} - Significant at $p \leq 0.01$ and at $p \leq 0.05$ and not significant by F test, respectively; $^{(1)}$data transformed to $(x^{0.5})$; $^{(2)}$data transformed to $(x + 0.5)^{0.5}$



*, ** - Significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively

Figure 2. Stomatal conductance - gs (A), transpiration - E (B), CO_2 assimilation rate - A (C) and instantaneous water use efficiency - WUEi (D) of okra cv. Canindé in a hydroponic system, as a function of the saline nutrient solution - ECns, at 63 days after transplanting

The instantaneous water use efficiency (WUEi) of okra plants was also linearly reduced with the increase in ECns, by 3.61% per unit increment in ECns (Figure 2D). The decrease in WUEi may be associated with the fact that, in order to reduce osmotic stress, plants reduce the absorption of toxic ions through stomatal closure and transpiration, with the objective of increasing water use efficiency and maintaining the relative water content in the leaves (Sá et al., 2019).

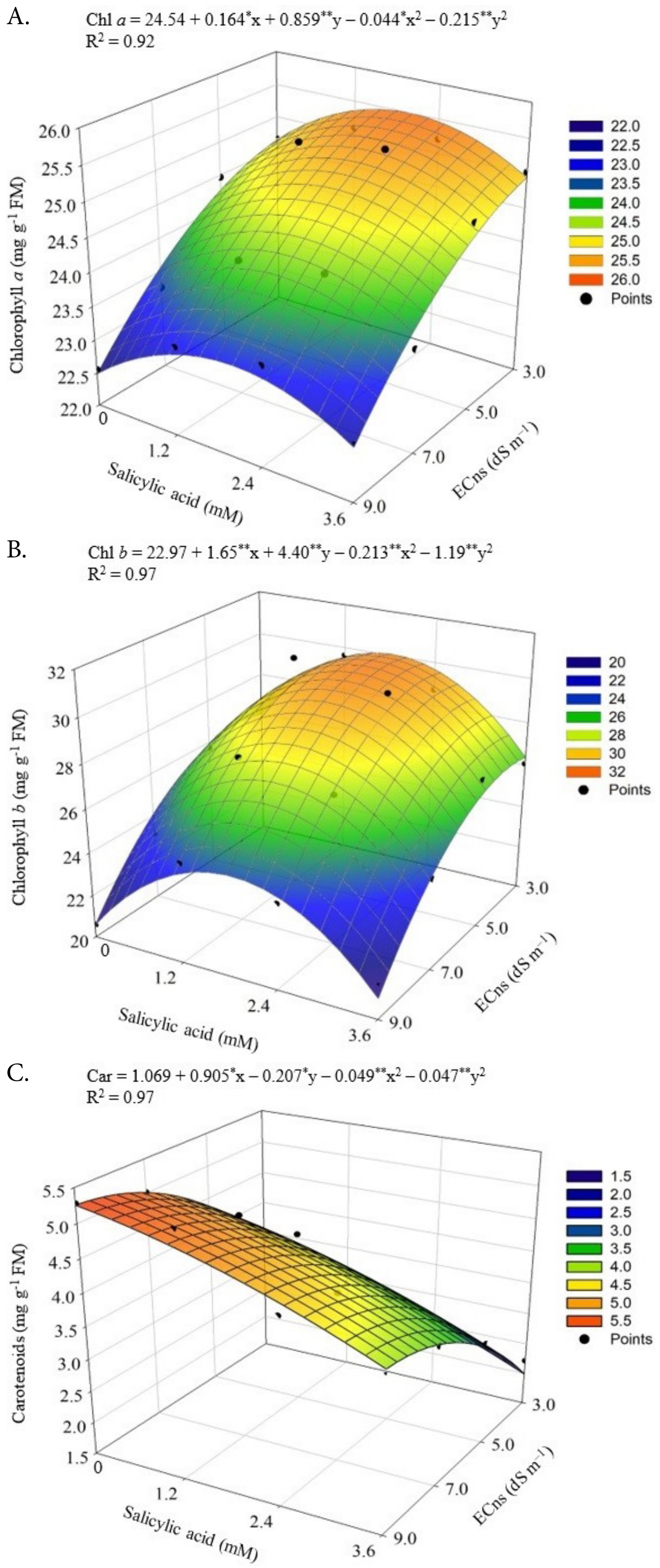
There was a significant effect of ECns and of the interaction between saline nutrient solution and salicylic acid concentrations (ECns × SA) for chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Car) contents of okra plants cv. Canindé, at 63 days after transplanting, while salicylic acid concentrations as a single factor affected Chl a and Car (Table 3).

For chlorophyll a content (Chl a), it was observed that plants subjected to ECns of 3.0 dS m^{-1} and salicylic acid at a concentration of 1.8 mM obtained the highest estimated value of 25.49 mg g^{-1} FM (Figure 3A). On the other hand, the lowest content of Chl a (22.50 mg g^{-1} FM) was obtained in plants grown under salicylic acid concentration of 0 mM and ECns of 9.0 dS m^{-1}. For chlorophyll b (Chl b), nutrient solution salinity of 4.0 dS m^{-1} promoted the highest value (30.23 mg g^{-1} FM) when plants were subjected to a salicylic acid concentration of 1.8 mM, whereas the lowest content of Chl b (20.57 mg g^{-1} FM) was recorded in plants grown under the concentration of 0 mM and nutrient solution salinity of 9.0 dS m^{-1} (Figure 3B). The reduction in chlorophyll content results from oxidative stress and can be attributed to inhibition of synthesis or degradation by the chlorophyllase enzyme in plants under salt stress (Lima et al., 2020b).

Table 3. Summary of the analysis of variance for chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Car) contents of okra plants cv. Canindé cultivated under saline nutrient solution (ECns) and concentrations of salicylic acid (SA) in a hydroponic system, 63 days after transplanting

Sources of variation	DF	Mean squares		
		Chl a	Chl b	Car
Saline nutrient solution (ECns)	3	11.21^{**}	449.49^{**}	17.59^{**}
Linear regression	1	19.04^{ns}	1126.25^{**}	30.70^{**}
Quadratic regression	1	12.38^{ns}	215.30^{**}	18.70^{**}
Residual 1	6	2.82	39.60	2.12
Salicylic acid (SA)	3	3.22^{**}	15.34^{ns}	2.71^{**}
Linear regression	1	0.53^{ns}	0.07^{ns}	1.63^{ns}
Quadratic regression	1	2.97^{*}	0.50^{ns}	1.09^{ns}
Interaction (ECns × SA)	9	4.94^{**}	27.81^{*}	4.10^{**}
Residual 2	26	6.25	269.58	15.92
CV 1 (%)		2.79	10.92	19.39
CV 2 (%)		2.00	13.69	25.50

DF - Degrees of freedom; CV (%) - Coefficient of variation; **, *, ^{ns} - Significant at $p \leq 0.01$ and at $p \leq 0.05$ and not significant by F test, respectively



*, ** - Significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively; X and Y - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively

Figure 3. Response surface for the contents of chlorophyll a - Chl a (A), chlorophyll b - Chl b (B) and carotenoids - Car (C) of okra plants cv. Canindé in a hydroponic cultivation, as a function of saline nutrient solution - ECns and concentrations of salicylic acid (SA), 63 days after transplanting

Regarding carotenoid content (Figure 3C), it was observed that plants grown under ECns of 9.0 dS m^{-1} obtained the highest estimated value (5.20 mg g^{-1} FM) when subjected to the SA concentration of 0.9 mM. On the other hand, the lowest Car content (1.06 mg g^{-1} FM) was observed in plants subjected to the SA concentration of 3.0 mM and ECns of 4.6 dS m^{-1}. Carotenoids can act as antioxidant agents, protecting lipid membranes from oxidative stress generated in plants exposed to salinity (Falk & Munné-Bosch, 2010). Thus, this increase in the synthesis of carotenoids may be related to the intensification of salt stress on okra plants, probably related to the degradation of β-carotene and integrated components of thylakoids, which act in the absorption and transfer of light to chlorophyll (Gomes et al., 2011).

There were significant effects of saline nutrient solution on plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA) of okra plants cv. Canindé. The interaction between the factors (ECns × SA) and the concentrations of salicylic acid did not significantly influence any of growth variables of okra cv. Canindé, 63 days after transplanting (Table 4).

The height of okra plants decreased linearly with the increase in the electrical conductivity of the nutrient solution (Figure 4A), with a reduction of 7.23% per unit increment in ECns. When comparing in relative terms the growth in PH of plants cultivated under ECns of 9.0 dS m^{-1} to that of plants subjected to the lowest salinity level (3.0 dS m^{-1}), a reduction of 55.42% was observed. Plant growth inhibition is a consequence of changes in the total water potential caused by osmotic effects that restrict the absorption of water and nutrients (Lima et al., 2020b). The high levels of soluble salts present in the waters may also have caused nutritional imbalance and toxicity by specific ions, interfering in plant growth.

The stem diameter of okra plants cv. Canindé (Figure 4B) also decreased linearly with the increase in ECns, by 6.12% per unit increment in ECns. This reduction may be related to the increase of reactive oxygen species that cause several biochemical disorders and physiological changes, such as the decrease in stomatal opening, leading to limitations in their growth (Dantas et al., 2021). Similar results were observed by Modesto et al. (2019), who studied the growth of okra cv. Speedy as a function of water salinity (ECsol 2.8 to 18.61 dS m^{-1}) and observed that there was a linear decrease in stem diameter as water salinity increased. According to these authors, salt stress induces restriction in physiological variables, thus affecting its growth.

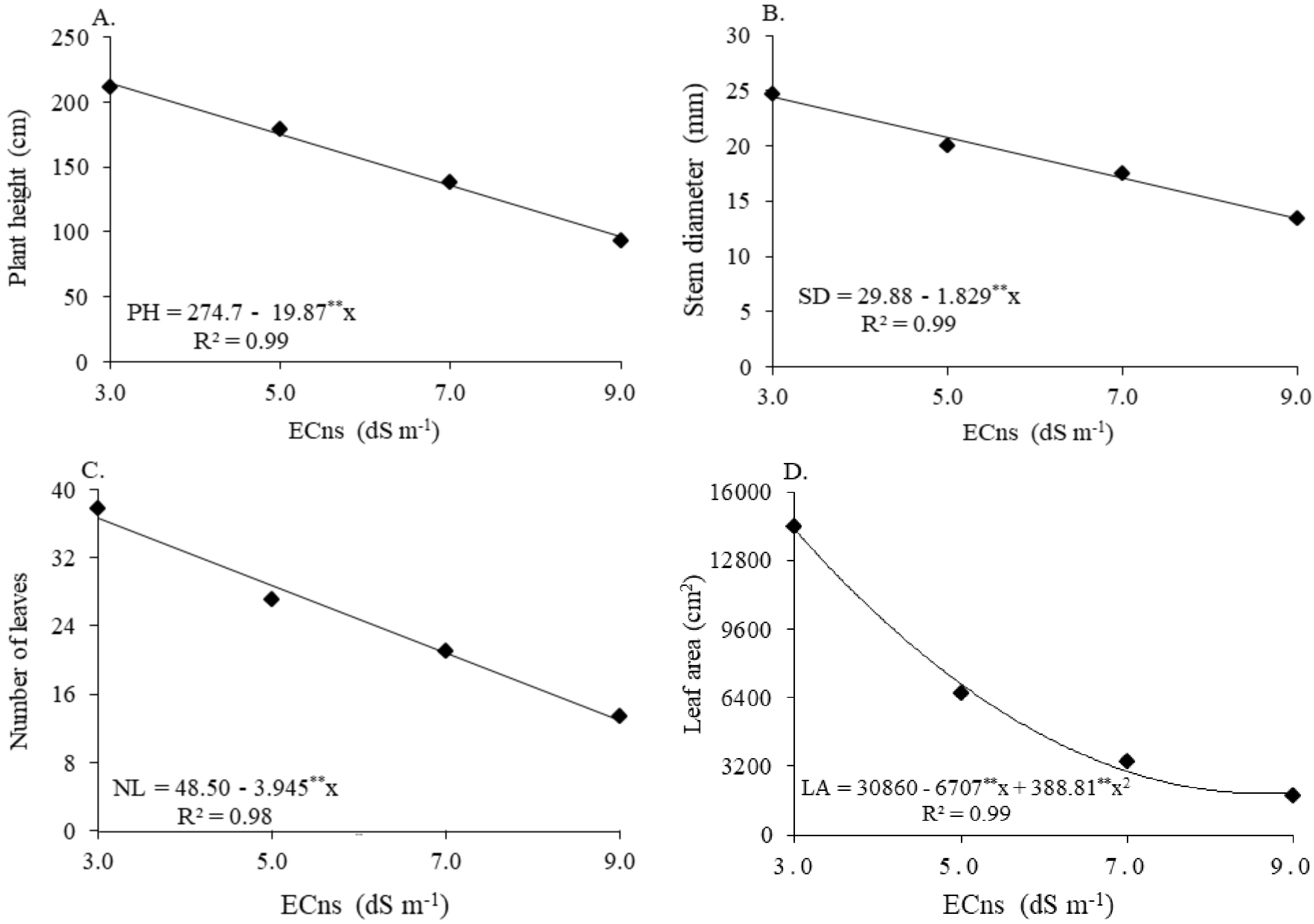
The number of leaves was reduced linearly with the increase in ECns, by 8.13% per unit increment in ECns (Figure 4C). There was a reduction of 64.55% between the ECns levels of 9.0 and 3.0 dS m^{-1}. The reduction in the number of leaves in plants in saline treatments is considered a strategy of protection and/or acclimatization to high salt concentrations, as a way to reduce water losses through transpiration (Lima et al., 2020c). Modesto et al. (2019) also reported a reduction in the number of leaves in okra cv. Speedy with increasing water salinity from 1.8 dS m^{-1}, 101 days after sowing.

For leaf area (Figure 4D), plants under the saline nutrient solution of 3.0 dS m^{-1} reached the maximum estimated value of 14238.29 cm^2, while plants subjected to ECns of 9.0 dS m^{-1} showed leaf area of 1990.61 cm^2. One of the signs of stress

Table 4. Summary of the analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA) of okra plants cv. Canindé cultivated under saline nutrient solution (ECns) and concentrations of salicylic acid (SA) in a hydroponic system, 63 days after transplanting

Sources of variation	DF	Mean squares			
		PH	SD	NL	LA
Saline nutrient solution (ECns)	3	31761.29**	270.67**	1262.18**	373931359.02**
Linear regression	1	94803.75**	802.96**	3736.70**	999947179.26**
Quadratic regression	1	462.52ns	1.27ns	27.00ns	116102865.15**
Residual 1	6	2092.25	32.26	145.67	2063154.31
Salicylic acid (SA)	3	208.43ns	1.88ns	23.90ns	2637518.49ns
Linear regression	1	279.50ns	1.11ns	63.03ns	5832858.19ns
Quadratic regression	1	200.08ns	4.44ns	8.33ns	336837.49ns
Interaction (ECns × SA)	9	358.80ns	4.18ns	19.39ns	1687857.99ns
Residual 2	26	12208.75	183.10	366.65	2795605.17
CV 1 (%)		12.01	12.26	19.84	21.90
CV 2 (%)		13.94	14.03	15.12	25.49

DF - Degrees of freedom; CV (%) - Coefficient of variation; **, *, ns - Significant at $p \leq 0.01$ and at $p \leq 0.05$ and not significant by F test, respectively



** - Significant at $p \leq 0.01$ by F test

Figure 4. Plant height - PH (A), stem diameter - SD (B), number of leaves - NL (C), and leaf area - LA (D) of okra cv. Canindé in a hydroponic cultivation, as a function of the saline nutrient solution – ECns, 63 days after transplanting

caused by excess salts is the rapid decrease in growth rate, especially in leaf area, a response caused by the alteration of physiological variables such as transpiration and CO_2 assimilation (Lima et al., 2020c). Despite the reduction in growth, the intensity of the effects of salt stress on plants depends on other factors such as species, cultivar, stage of the development cycle, cultivation system employed, and cultural practices (Pinheiro et al., 2022).

The absence of single significant effect of salicylic acid on the measured growth variables may be associated with the interaction of some factors such as tested concentrations, mode of application, crop, and the development stage of the plants (Poor et al., 2019).

Conclusions

1. Increase in nutrient solution salinity levels from 3.0 dS m^{-1} inhibits the synthesis of photosynthetic pigments, leaf gas exchange, and growth of okra cv. Canindé in hydroponic cultivation, 63 days after transplanting.

2. Foliar application of salicylic acid up to 3.6 mM did not attenuate effects of salt stress on water relations, gas exchange, and growth of okra cv. Canindé, 63 days after transplanting.

3. Salicylic acid at estimated concentration of 1.8 mM stimulates biosynthesis of chlorophyll a and b in okra plants cv. Canindé under nutrient solution salinity of 3.0 and 4.0 dS m^{-1}, respectively.

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