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Hydroponic cultivation of okra using saline nutrition solutions under application of salicylic acid

Cultivo hidropônico de quiabeiro utilizando soluções nutritivas salinas sob aplicação de ácido salicílico

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ABSTRACT - The limited availability of low-salinity water for irrigation in the Northeastern semi-arid region has restricted food production, making it necessary to use strategies to reduce the effects of salt stress on plants. Among the alternatives, the foliar application of salicylic acid stands out. In this context, the objective of this study was to evaluate the effects of foliar application of salicylic acid in mitigating salt stress on the gas exchange, chlorophyll *a* fluorescence, photosynthetic pigments, and growth of 'Canindé' okra in a hydroponic system. The experiment was carried out in a greenhouse, in Pombal - PB, using the Nutrient Film Technique - NFT hydroponic system. The experimental design used was completely randomized in a split-plot scheme, with four levels of electrical conductivity of the nutrient solution - ECns (2.1, 3.6, 5.1, and 6.6 dS m^{-1}) as the plots and four concentrations of salicylic acid - SA (0, 1.2, 2.4, and 3.6 mM) as the subplots, with four replicates and two plants per plot. SA concentration of 3.6 mM was able to minimize the effect of nutrient solution salinity on chlorophyll *a* fluorescence and increase the synthesis of chlorophyll *b* in okra plants, 34 days after transplanting. Nutrient solution salinity above 2.1 dS m^{-1} negatively affected gas exchange, relative water content, photosynthetic pigments, and growth and increased electrolyte leakage in the leaf blade of okra plants.

Keywords: *Abelmoschus esculentus* L. Salt stress. Phytohormone. NFT.

RESUMO - A limitada disponibilidade de água de baixa salinidade para irrigação no semiárido Nordestino tem restringido a produção de alimentos, fazendo necessário uso de estratégias para reduzir os efeitos do estresse salino sobre as plantas. Dentre as alternativas, destaca-se a aplicação foliar de ácido salicílico. Nesse contexto, objetivou-se com este trabalho avaliar os efeitos da aplicação foliar de ácido salicílico na mitigação do estresse salino nas trocas gasosas, fluorescência da clorofila *a*, pigmentos fotossintéticos e crescimento do quiabeiro 'Canindé' em sistema hidropônico. O trabalho foi conduzido em casa de vegetação, em Pombal – PB, utilizando-se o sistema de cultivo hidropônico tipo Técnica de Fluxo Laminar de Nutrientes - NFT. O delineamento experimental utilizado foi o inteiramente casualizado em esquema de parcelas subdivididas, sendo quatro níveis de condutividade elétrica da solução nutritiva - CEsn (2,1; 3,6; 5,1 e 6,6 dS m^{-1}) considerados as parcelas e quatro concentrações de ácido salicílico - AS (0; 1,2; 2,4 e 3,6 mM), as subparcelas, com quatro repetições e duas plantas por parcela. A concentração de 3,6 mM de AS foi capaz de minimizar o efeito da salinidade da solução nutritiva na fluorescência da clorofila *a* e promoveu aumento na síntese de clorofila *b* das plantas de quiabeiro, aos 34 dias após o transplantio. A salinidade da solução nutritiva acima de 2,1 dS m^{-1} afetou negativamente as trocas gasosas, o conteúdo relativo de água, os pigmentos fotossintéticos, o crescimento e elevou o extravasamento de eletrólitos no limbo foliar das plantas de quiabeiro.

Palavras-chave: *Abelmoschus esculentus* L. Estresse salino. Fitormônio. NFT.

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INTRODUCTION

Belonging to the Malvaceae family, okra (*Abelmoschus esculentus* L.) is a water-demanding vegetable crop with an annual cycle and adapted to the edaphoclimatic conditions of the Northeast region (GOES et al., 2019). In the semi-arid region of Northeast Brazil, to meet the water needs of this crop, it is necessary to evaluate the management of irrigation with alternative sources of water, such as surface and/or underground sources, which generally contain high levels of salts (SILVA JÚNIOR; GHEYI; MEDEIROS, 1999; SOARES et al., 2018), because the rains are irregular, with high temperature and evaporation, leading to quantitative and qualitative water restriction for irrigation.

Salt stress compromises the development of plants, as energy is redirected to signal the partial closure of the stomata, reducing water absorption, which can lead to a decrease in growth, followed by the change in ionic homeostasis due to the high accumulation of cations and anions in the cellular tissues, which hinders the absorption of water and nutrients by the roots, and triggering physiological changes and oxidative damage in cells (SILVA et al., 2020).

It should be considered that the use of water with high concentrations of salts in a conventional way causes the salinization of soils. In this context, hydroponic cultivation can be a promising alternative especially in semi-arid regions, as it allows greater efficiency in water use, reducing the effect of salt stress on plants since the matric potential, which is responsible for retaining water in the soil, is absent in the hydroponic system, and it offers other advantages such as the use of a smaller volume of water compared to conventional cultivation systems (SANTOS et al., 2018).

Another strategy that has been employed to mitigate salt stress effects on plants is the foliar application of salicylic acid (SA). SA is a phytohormone that acts as a defense mechanism of plants, inducing the production of osmolytes and secondary metabolism, acting on the physiological processes of plants under stress conditions (OLIVEIRA et al., 2022). SA has a positive effect on antioxidant activity in plants under salt stress, improving enzymatic and photosynthetic responses, with actions on stomatal opening and carbohydrate metabolism, imposing osmotic regulation and increasing the tolerance of plants (SILVA et al., 2021).

It is important to highlight that the use of SA as a mitigator of salt stress in plants can vary according to the species, cultivar, and concentration used, in addition to the environmental conditions under which the plant is being grown. Therefore, more research is needed to evaluate the potential of using SA in okra cultivation in a hydroponic system under salt stress conditions, to provide the basis for the development of sustainable practices in agricultural production. However, several studies have already identified the role of SA as a signaler and its effects on the tolerance of plants to salt stress, as observed in soursop (SILVA et al., 2021), guava (XAVIER et al., 2022), and okra (MENDONÇA et al., 2022).

In this context, the study aimed to evaluate the effects of foliar application of SA in mitigating salt stress on the gas exchange, chlorophyll *a* fluorescence, photosynthetic pigments, and growth of 'Canindé' okra in an NFT hydroponic system.

MATERIAL AND METHODS

The experiment was conducted from October 2021 to January 2022, in a greenhouse belonging to the Center of Sciences and Agri-Food Technology (CCTA) of the Federal University of Campina Grande (UFCG), in Pombal, Paraíba, Brazil, located by the geographical coordinates 6°46'13" South latitude and 37°48'6" West longitude, at an average altitude of 184 m. The data of temperature (maximum and minimum) and mean relative humidity of the air of the experimental site are shown in Figure 1.

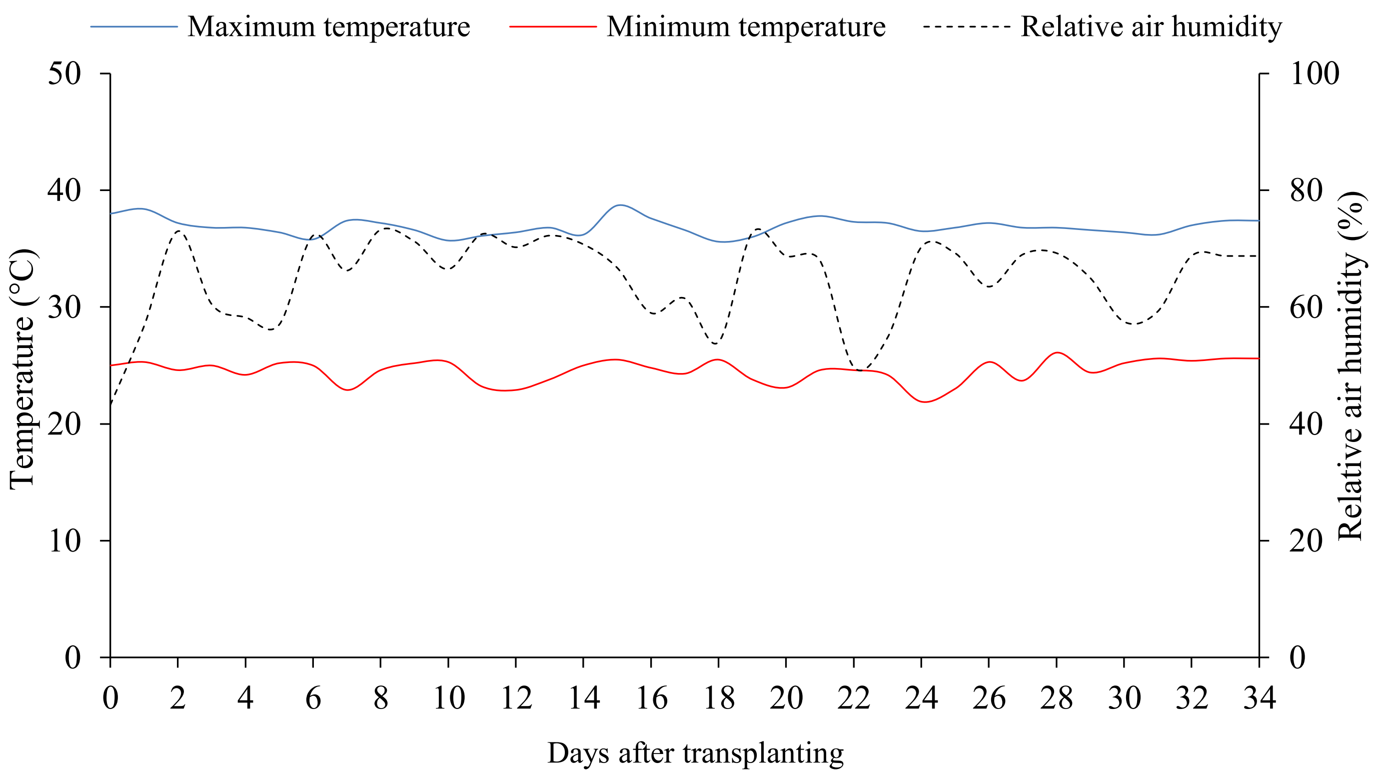


Figure 1. Maximum and minimum temperature and mean relative of air humidity observed in the internal area of the greenhouse during the experimental period.

Treatments consisted of four levels of electrical conductivity of the nutrient solution – ECns (2.1, 3.6, 5.1, and 6.6 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, with the ECns levels considered as the plots and SA concentrations as the subplots, with four replicates and two plants per plot. The SA concentrations used in this study were based on a study carried out with 'Gaúcho' melon (SOARES et al., 2022), while the salinity levels of the nutrient solution were adapted from the study conducted by Mendonça et al. (2022) with okra cv. 'Canindé'.

Seeds of 'Canindé' okra from ISLA® were used in the present study. This cultivar has a cycle of around 80 days, tall stature and highly productive plants, with excellent adaptability to different growing regions. The pods have five ridges and excellent post-harvest quality, with length between 10 and 15 cm and diameter ranging from 18 to 20 mm. In addition, 'Canindé' okra is resistant to the Yellow Vein Mosaic virus.

The hydroponic system used was of the Nutrient Film Technique (NFT) type, made with polyvinyl chloride (PVC) tubes of 100 mm in diameter and six meters in length, spaced 0.40 m apart. In the channels, the spacing was 0.50 m between plants and 1.0 m between treatments (subsystems), and the cells for planting on the upper part of the tube had diameter of 54.17 mm. The channels were supported on sawhorses with 0.60 m height and a 4% slope for the nutrient solution to flow. At the lowest point of each bench of the hydroponic system, a 150 L polyethylene recepient was placed to collect and conduct the nutrient solution back to the channels. The nutrient solution was injected into the cultivation channels by a pump of 35 W and a flow rate of 3 L min^{-1}. Nutrient solution circulation was programmed by a timer, with intermittent flow of 15 min every hour during the day and every 30 min at night.

The nutrient solution used in the experiment was prepared following the recommendation of Hoagland and Arnon (1950), using municipal-supply water with initial electrical conductivity of 0.3 dS m^{-1}. This electrical conductivity resulted in the lowest ECns level (2.1 dS m^{-1}). 150 L of nutrient solution with concentrations (mg L^{-1}) of 210, 31, 234, 200, 48, 64, 0.5, 0.5, 0.05, 0.02, 0.01, 5, 1.2, and 0.65 of N, P, K, Ca, Mg, S, B, Mn, Zn, Cu, Mo, Fe, Na, and Cl, respectively, were prepared using 136.09, 101.10, 236.15, and 246.49 g L^{-1} of KH_2PO_4, KNO_3, $Ca(NO_3)_2.4H_2O$ and $MgSO_4.7H_2O$, in addition to 3.10, 1.70, 0.22, 0.75, 1.25, 13.9, and 13.9 g L^{-1} of H_3BO_3, $MnSO_4.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $(NH_4)_6Mo_7O_2.4H_2O$, $FeSO_4$, and EDTA - Na.

Sowing was carried out in polyethylene containers with a capacity of 50 mL containing vegetable sponge, arranged on trays. Before sowing, the vegetable sponges were sanitized with sodium hypochlorite (2.5%), washed and dried in the open air. From seed germination to the appearance of the first true leaf (on average ten days after sowing), a half-strength nutrient solution was used (50% of the recommendation). After the appearance of the first true leaf, the vegetable sponge was removed and the plants were inserted into the hydroponic profiles and irrigated with full-strength nutrient solution (100% of the recommendation).

The saline solutions were obtained by adding sodium chloride (NaCl), calcium chloride ($CaCl_2.2H_2O$), and magnesium chloride ($MgCl_2.6H_2O$) salts to the nutrient solution prepared in water from the supply system of the municipality of Pombal, Paraíba, Brazil, incorporated in the equivalent ratio of 7:2:1, respectively. This is the proportion of Na, Ca, and Mg commonly found in sources of water used for irrigation in the semi-arid region of Northeast Brazil.

Complete replacement of the nutrient solution occurred every eight days, but the electrical conductivity and pH were monitored daily and, whenever necessary, the solution was adjusted by adding water with ECw of 0.3 dS m^{-1}, always maintaining the ECns according to the established treatments. The pH was kept between 5.5 and 6.5 by adding 0.1 M KOH or HCl. Okra plants were grown in the hydroponic profiles with vertical support using nylon twine.

The SA concentrations were obtained by dissolution in 30% ethyl alcohol, prepared in each application. The first application was performed 72 hours before the beginning of the application of the saline nutrient solution, between 5:00 and 6:00 p.m., applying an average of 18 mL of respective solution per plant, whereas the other applications were carried out at 10-day intervals, spraying the abaxial and adaxial sides of the leaves with a sprayer. The Wil fix adjuvant, at concentration of 0.5 mL L^{-1} of solution, was used to reduce the surface tension of the drops on the leaf. During the spraying of SA, a structure with plastic tarpaulin was used to prevent the solution from drifting onto neighboring plants.

Treatment effects were measured 34 days after inserting the plants in the hydroponic profiles. Gas exchange was measured by 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}), and internal CO_2 concentration - *Ci* (μmol CO_2 mol^{-1}) using the portable infrared carbon dioxide analyzer (IRGA), LCPro + Portable Photosynthesis System® model (ADC BioScientific Limited, UK), with irradiation of 1200 μmol photons m^{-2} s^{-1}, the airflow of 200 mL min^{-1}, and atmospheric CO_2 concentration. After data collection, instantaneous water use efficiency - *WUEi* - A/E [(μmol CO_2 m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$] and instantaneous carboxylation efficiency - *CEi* - *A/Ci* [(μmol CO_2 m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] were quantified.

At the same time, chlorophyll *a* fluorescence was evaluated in the third leaf, counted from the apex of the main branch of the plant, at 8:00 a.m., using an OS5p pulse-modulated fluorimeter from Opti Science, using the Fv/Fm protocol to determine the variables: initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum efficiency of photosystem II (Fv/Fm). This protocol was performed after adaptation of the leaves to the dark for 30 min.

Contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (CAR) were determined according to the methodology of Lichtenthaler (1987), using a disc of plant tissue collected from the third leaf counted from the apex. The disc was immersed in 80% acetone and stored in the dark for 48 hours in closed tubes. The extracts obtained were subjected to reading in a spectrophotometer with absorbance (ABS) wavelengths of 470, 646, and 663 nm, using Equations 1, 2 and 3.

$$\text{Chl } a = 12.21\ ABS_{663} - 2.81\ ABS_{646} \quad (1)$$

$$\text{Chl } b = 20.13\ ABS_{646} - 5.03\ ABS_{663} \quad (2)$$

$$CAR = (1000\ ABS_{470} - 1.82\ \text{Chl } a - 85.02\ \text{Chl } b) / 198 \quad (3)$$

Where:
Chl *a* - chlorophyll *a* (mg g^{-1} FM);
Chl *b* - chlorophyll *b* (mg g^{-1} FM); and
CAR - total carotenoids (mg g^{-1} FM)

The contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) were expressed in mg g^{-1} of fresh matter (FM).

In the same period, electrolyte leakage (EL) in the leaf blade was determined, by collecting leaves from the middle third and removing 8 leaf discs with an area of 113 mm², washed with distilled water to eliminate other electrolytes adhered to the leaves, and then placed in beakers, which contained 50 mL of bidistilled water and were closed with aluminum foil. The samples were kept at a temperature of 25 ºC for 24 hours, and the initial electrical conductivity (Ci) was determined. Subsequently, the beakers were taken to an oven with forced air circulation and subjected to temperature of 80 ºC for 150 minutes; after cooling, the final electrical conductivity (Cf) was determined. Thus, EL in the leaf blade was obtained according to Scotti-Campos et al. (2013), using Equation 4.

$$EL = \frac{Ci}{Cf} \times 100 \quad (4)$$

Where:
EL = Electrolyte leakage in the leaf blade (%);
Ci = initial electrical conductivity (dS m^{-1}); and
Cf = final electrical conductivity (dS m^{-1}).

To determine the relative water content (RWC) in the leaf blade, 3 fully expanded leaves located in the upper third of the plant were collected. After collection, the leaves were immediately weighed, avoiding moisture losses, to obtain fresh mass (FM); then, these samples were placed in plastic bags, immersed in 50 mL of distilled water and stored for 24 hours. After this period and after wiping excess water with paper towels, the turgid mass (TM) was obtained and the samples were dried in the oven (≈ 65 ºC ±3 ºC, until reaching constant weight) to obtain the dry mass (DM). RWC was determined according to Weatherley (1950), using Equation 5:

$$RWC = \frac{(FM - DM)}{(TM - DM)} \times 100 \quad (5)$$

Where:
RWC - Relative water content (%);
FM - Leaf fresh mass (g);
TM - Leaf turgid mass (g); and
DM - Leaf dry mass (g);

Plant growth was assessed based on measurements of plant height (PH), stem diameter (SD), and number of leaves (NL). PH (cm) was measured as the distance between the PVC tube and the insertion of the apical meristem, SD (mm) was measured three centimeters above the hydroponic profile, and NL was counted in each plant, considering only leaves with a minimum length of 3 cm.

The results were subjected to the data normality test (Shapiro-Wilk) and then to the F test at a 0.05 probability level. When significant, polynomial regression analysis (linear and quadratic) was performed to assess the relationships between the variables studied, using the statistical program SISVAR – ESAL version 5.6.

RESULTS AND DISCUSSION

The interaction between the salinity levels of nutrient solution (ECns) and salicylic acid (SA) had a significant effect on the instantaneous water use efficiency of okra plants (Table 1). However, individually, the salinity levels of the nutrient solution significantly affected all gas exchange variables evaluated, while salicylic acid did not have a significant effect on any of the variables analyzed. Unlike the results obtained here, Oliveira et al. (2022) observed in a study with hydroponic melon that the interaction between salinity levels of the nutrient solution and salicylic acid concentrations significantly influenced all gas exchange variables evaluated, except for instantaneous carboxylation efficiency.

Table 1. Summary of the analysis of variance for stomatal conductance (*gs*), transpiration (*E*), internal CO_2 concentration (*Ci*), CO_2 assimilation rate (*A*), instantaneous water use efficiency (*WUEi*), and instantaneous carboxylation efficiency (*CEi*) of 'Canindé' okra plants cultivated with saline nutrient solution (ECns) and foliar application of salicylic acid (SA) in a hydroponic system, 34 days after transplanting.

Source of variation	DF	Mean squares					
		gs	*E*	*Ci*	*A*	*WUEi*	*CEi*
Saline nutrient solution (ECns)	3	0.27^{**}	6.12^{**}	118222.53^{**}	1287.00^{**}	27.68^{**}	0.02^{**}
Linear Regression	1	0.06^{**}	17.99^{**}	32320.86^{*}	3684.05^{**}	76.76^{**}	0.05^{**}
Quadratic Regression	1	0.08^{*}	0.33^{ns}	3135.98^{ns}	102.98^{**}	0.00^{ns}	0.00^{ns}
Salicylic Acid (SA)	3	0.02^{ns}	0.65^{ns}	768.96^{ns}	1.00^{ns}	0.71^{ns}	0.00^{ns}
Linear Regression	1	0.01^{ns}	0.06^{ns}	1290.69^{ns}	0.47^{ns}	0.54^{ns}	0.00^{ns}
Quadratic Regression	1	0.01^{ns}	0.13^{ns}	802.78^{ns}	0.12^{ns}	1.07^{ns}	0.00^{ns}
Interaction (ECns × SA)	9	0.00^{ns}	1.94^{ns}	595.28^{ns}	13.58^{ns}	4.97^{*}	0.00^{ns}
Error 1	9	0.01	0.85	1321.87	4.32	1.54	0.00
Error 2	36	0.00	0.70	223.40	5.07	1.46	0.00
CV 1 (%)		24.63	21.37	24.75	9.10	23.04	28.58
CV 2 (%)		15.52	19.39	10.17	9.86	22.44	16.68

ns, *, **, respectively not significant and significant at a $p \leq 0.05$ and $p \leq 0.01$; CV - coefficient of variation; DF – Degrees of freedom.

Nutrient solution with electrical conductivity of 2.1 dS m^{-1} promoted higher stomatal conductance (0.59 mol H_2O m^{-2} s^{-1}) in okra plants, while the highest salinity level of the nutrient solution (6.6 dS m^{-1}) resulted in a minimum value of 0.31 mol H_2O m^{-2} s^{-1}, with a reduction of 47.70% in *gs* when comparing plants grown under ECns of 6.6 dS m^{-1} with those under 2.1 dS m^{-1} (Figure 2A). Under high salinity, plants close their stomata to reduce water loss to the atmosphere and decrease the absorption of salts, especially toxic ions such as Na^+ and Cl^-. This situation affects the production of photoassimilates (DIAS et al., 2018a). Similar to the result obtained in this study, Mendonça et al. (2023) found a reduction of *gs* in 'Canindé' okra plants with the increase in nutrient solution salinity from 2.1 to 9.0 dS m^{-1} in a hydroponic system, 63 days after transplantation.

The transpiration and internal CO_2 concentration of okra plants also decreased with the increase in the saline levels of the nutrient solution (Figures 2B and 2C), by 5.54 and 6.53%, respectively, per unit increase in ECns. Transpiration is a mechanism of plants to maintain water status, in which the difference in water potential between cell walls, intracellular space, and xylem drives the absorption of water and nutrients by the root system. Thus, plants close their stomata to reduce the absorption of toxic ions and the loss of water in the form of vapor to the atmosphere (SÁ et al., 2019). Partially closed stomata also compromise the entry of CO_2 into the substomatal chamber, because the smaller the opening of the stomata, the lower the CO_2 flux at the RuBisCO carboxylation site inside the chloroplasts, reducing water use and photosynthesis (DIAS et al., 2018b).

Regarding the CO_2 assimilation rate of plants under the application of saline nutrient solution, the maximum and minimum values of 34.29 and 13.93 μmol CO_2 m^{-2} s^{-1} were obtained under ECns of 2.1 and 6.6 dS m^{-1}, respectively (Figure 2D). The reduction in CO_2 assimilation rate is a consequence of the lower stomatal conductance and CO_2 entry into the substomatal chamber with the increase in nutrient solution salinity, causing metabolic disorders in plants, such as phosphorylation and lower activity of RuBisCO and other enzymes involved in the Calvin cycle (DIAS et al., 2018b). Lima et al. (2020), in a study with okra plants cv. 'Valença' cultivated in pots under salt stress (ECw ranging from 0.3 to 3.1 dS m^{-1}), also observed reductions in transpiration, CO_2 assimilation rate, and instantaneous carboxylation efficiency with the increase in water salinity from 0.3 to 3.1 dS m^{-1}, 45 days after sowing.

Figure 2E shows that the instantaneous carboxylation efficiency decreased linearly with the increase in nutrient solution salinity, resulting in a decrease of 8.15% per unit increase in ECns. This decrease results from the low CO_2 concentration and CO_2 assimilation rate in the leaf mesophyll, negatively affecting the regeneration of the RuBisCO enzyme in the Calvin cycle and the photosynthetic efficiency (DIAS et al., 2019). Similar results were also obtained by Soares et al. (2022) with 'Gaúcho' melon under nutrient solution salinity (ECns from 2.1 to 5.4 dS m^{-1}) and salicylic acid application in hydroponic cultivation, whose reduction per unit increase in ECns was 1.96, 3.14, and 5.16% for transpiration, CO_2 assimilation rate, and instantaneous carboxylation efficiency, respectively.

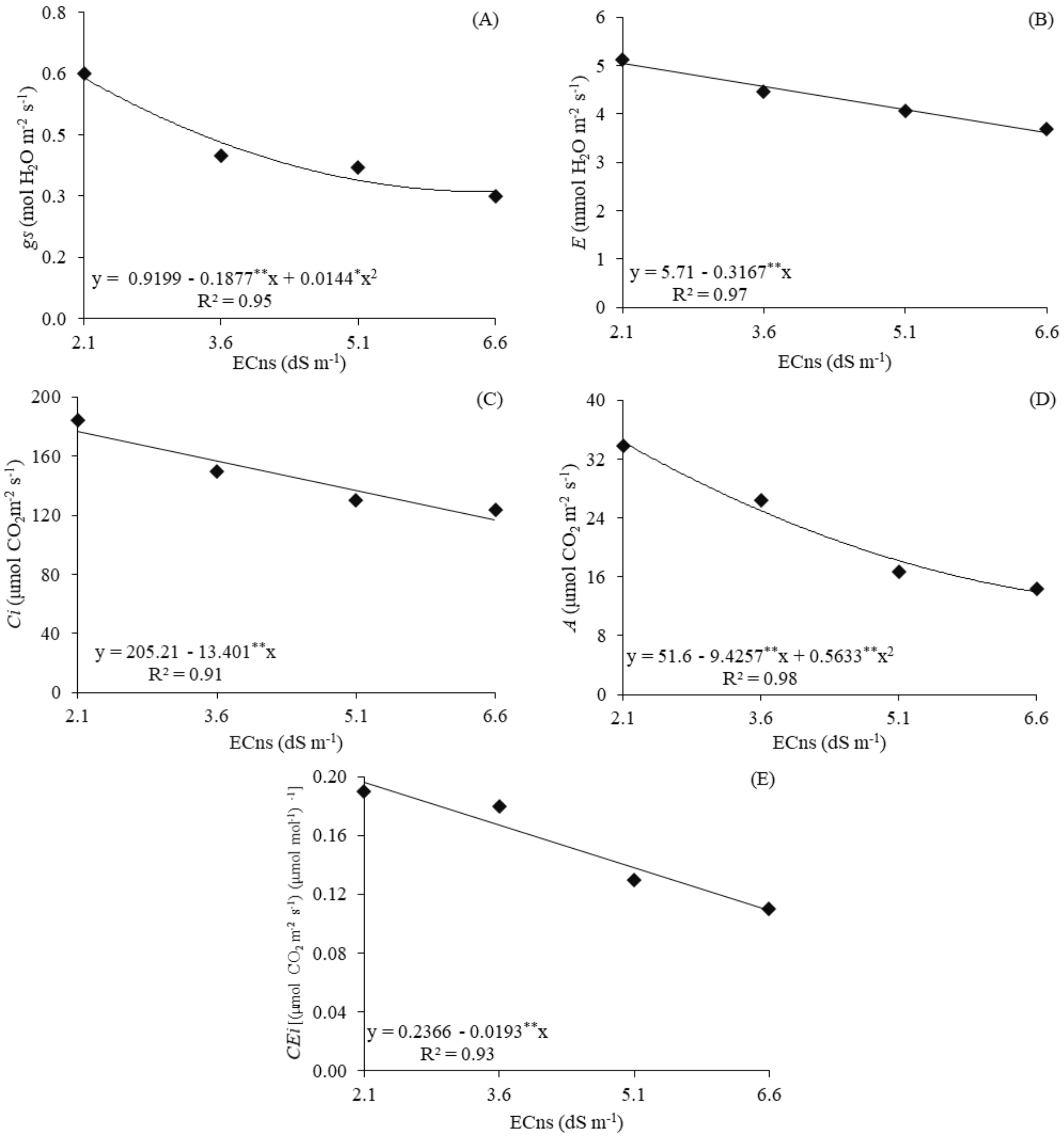
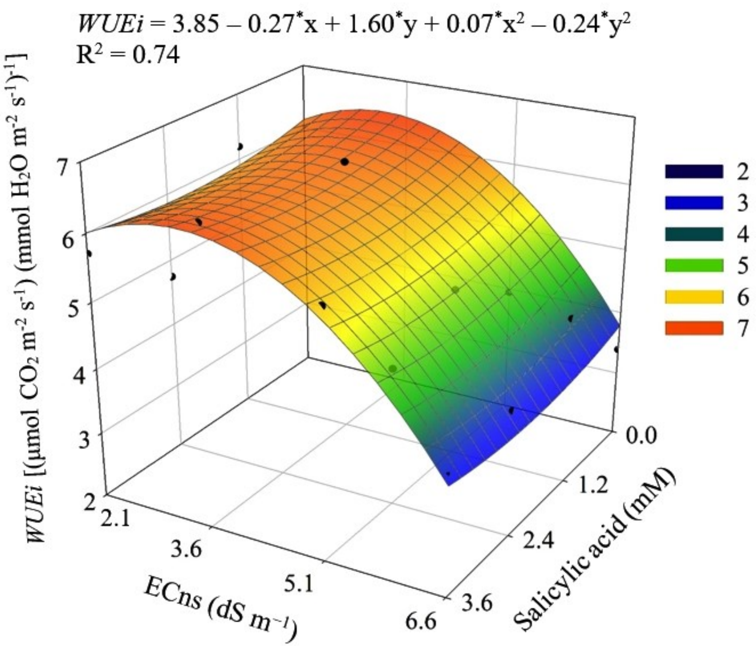


Figure 2. Stomatal conductance - *gs* (A), transpiration - *E* (B), internal CO_2 concentration - *Ci* (C), CO_2 assimilation rate - *A* (D), and instantaneous carboxylation efficiency - *CEi* (E) of 'Canindé' okra plants, as a function of the saline levels of the nutrient solution - ECns, in a hydroponic cultivation, 34 days after transplanting. ** significant at $p \leq 0.01$.

The instantaneous water use efficiency (Figure 3) decreased as the nutrient solution salinity increased, and plants grown in the nutrient solution of 3.4 dS m^{-1} had the highest *WUEi*, with an estimated value of 6.52 [(µmol CO_2 m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]. On the other hand, plants grown in the nutrient solution of 6.6 dS m^{-1} and with the SA concentration of 1.6 mM showed the lowest *WUEi*, with a value of 3.70 [(µmol CO_2 m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]. This indicates that salinity can negatively affect instantaneous water use efficiency in plants and that the effects of the SA application depend on the concentration, plant development stage, and form of application. The results mentioned indicate that the water restriction imposed by the osmotic effects on the plants reduced their transpiration and CO_2 assimilation rate; however, up to the ECns of 3.4 dS m^{-1}, the plants maintained a certain capacity of efficient use of water. However, at high levels of 6.6 dS m^{-1}, instantaneous water use efficiency can be significantly reduced. In addition, the SA application did not improve the instantaneous water use efficiency of okra plants under high salinity conditions. Unlike the results obtained here, Oliveira et al. (2022) studying hydroponic melon under the salinity of the nutrient solution (ECns from 2.1 to 5.4 dS m^{-1}) found no significant effect of the sources of variation tested on *WUEi*.



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

Figure 3. Instantaneous water use efficiency - *WUEi* of 'Canindé' okra plants, as a function of the interaction between the salinity levels of the nutrient solution - ECns and exogenous application of salicylic acid - SA, in a hydroponic cultivation, 34 days after transplanting.

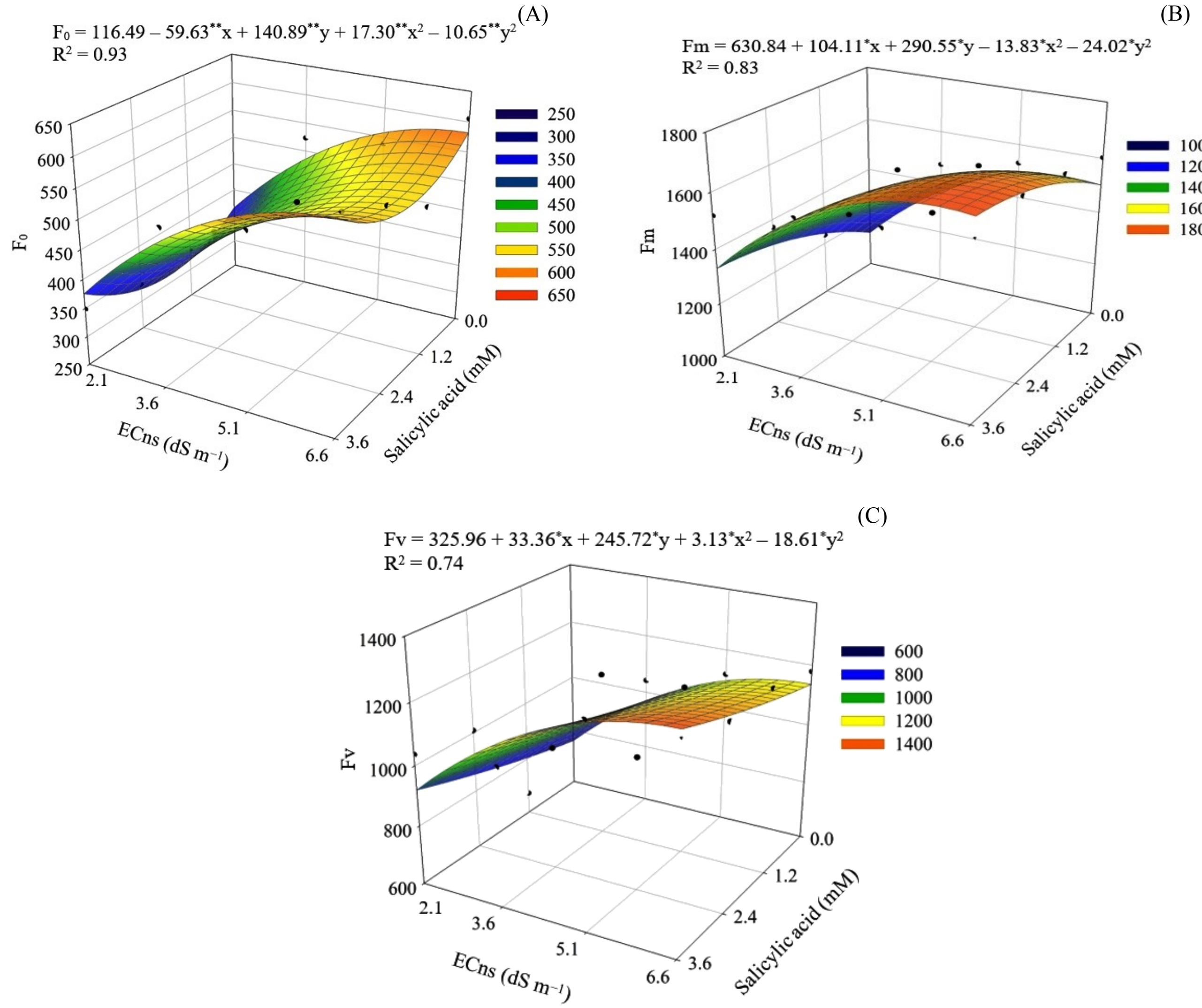
The interaction between the electrical conductivity of the nutrient solution and salicylic acid concentrations (ECns × SA) significantly influenced all chlorophyll *a* fluorescence variables of okra plants, except for the quantum efficiency of photosystem II (Table 2).

Table 2. Summary of the analysis of variance for initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum efficiency of photosystem II (Fv/Fm) of 'Canindé' okra plants cultivated with saline nutrient solution (ECns) and foliar application of salicylic acid (SA) in a hydroponic system, 34 days after transplanting.

Source of variation	DF	Mean squares			
		F_0	Fm	Fv	Fv/Fm
Saline nutrient solution (ECns)	3	162802^{**}	421126^{**}	276573^{**}	0.005^{ns}
Linear Regression	1	383550^{**}	1048168^{**}	765973^{**}	0.008^{ns}
Quadratic Regression	1	97170^{**}	161034^{**}	27225^{ns}	0.006^{ns}
Salicylic Acid (SA)	3	6891^{ns}	100303^{**}	80923^{*}	0.003^{ns}
Linear Regression	1	2840^{ns}	266325^{*}	231412^{**}	0.003^{ns}
Quadratic Regression	1	1175^{ns}	11944^{ns}	961^{ns}	0.007^{ns}
Interaction (ECns × SA)	9	11750^{*}	105222^{**}	121635^{**}	0.018^{ns}
Error 1		3528	13152	18821	0.012
Error 2		7376	23211	24290	0.009
CV 1 (%)		12	7.66	12.86	15.44
CV 2 (%)		17.79	10.18	14.61	13.51

ns, *, **, respectively not significant and significant at $p \leq 0.05$ and $p \leq 0.01$; CV - coefficient of variation; DF - Degrees of freedom.

For initial fluorescence (Figure 4A) and maximum fluorescence (Figure 4B), the maximum estimated values of 591.99 and 1704.97, respectively, were obtained in plants grown under ECns of 2.1 dS m^{-1} and salicylic concentration of 3.6 mM, whereas plants grown in the nutrient solution of 2.1 dS m^{-1} under SA application of 1.7 mM and 0 mM obtained minimum values of 314.02 and 1135.07 for F_0 and Fm, respectively. Although the increase in initial fluorescence indicates a reduction in the electron transfer capacity due to the dissociation of the reaction center of photosystem II (CINTRA et al., 2020), salicylic acid accumulated in plant tissues is synthesized under salt stress conditions and acts on plant resistance by regulating physiological processes through antioxidant enzymes, restoration of membrane potential, nitrogen metabolism, proline, glycine betaine production, stimulation of electron flow in photosystem II, and the electron transport activity associated with photosystem I (LOTFI; GHASSEMI-GOLEZANI; PESSARAKLI, 2020). Thus, the increase in maximum fluorescence may indicate an improvement in photosynthesis efficiency, despite the salt stress conditions. This is because the maximum fluorescence is related to the plant's capacity to transfer energy for the formation of NADPH, ATP and reduced ferredoxin, which are fundamental for CO_2 assimilation in the biochemical phase of photosynthesis (DIAS et al., 2019).



X and Y - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively;
*, ** significant at a p <0.05 and at a p<0.01, respectively

Figure 4. Initial fluorescence - F_0 (A), maximum fluorescence - Fm (B), and variable fluorescence - Fv (C) of 'Canindé' okra plants, as a function of the interaction between the salinity levels of the nutrient solution - ECns and concentrations of salicylic acid - SA, in a hydroponic cultivation, 34 days after transplanting.

Regarding variable fluorescence (Figure 4C), the maximum estimated value (1216.59) was obtained in plants subjected to ECns of 6.6 dS m^{-1} and foliar application of 3.6 mM of SA, while the minimum value of 759.90 was observed in plants that received the lowest ECns level (2.1 dS m^{-1}). Salicylic acid enabled better functioning of the photochemical activity of the leaves in plants subjected to the highest ECns level, as the increase in variable fluorescence resulted in the capacity to transfer the energy of electrons ejected from pigment molecules essential for the production of NADPH, ATP, and reduced ferredoxin (DIAS et al., 2018a). Soares et al. (2022), when evaluating the effects of foliar application of salicylic acid on 'Gaúcho' melon cultivated under saline nutrient solutions in a hydroponic system, also observed an increase in maximum and variable fluorescence in plants subjected to ECns of 4.3 and 4.2 dS m^{-1}.

The interaction between the salinity levels of the nutrient solution (ECns) and concentrations of salicylic acid (SA) did not significantly affect the variables studied (Table 3). However, the salinity levels of the nutrient solution significantly influenced the relative water content, electrolyte leakage in the leaf blade, the contents of photosynthetic pigments (Chl *a*, Chl *b* and CAR), and the growth of okra plants. The concentrations of salicylic acid significantly influenced only the chlorophyll *b* contents of okra plants, 34 days after transplantation.

Table 3. Summary of the analysis of variance for relative water content (RWC), electrolyte leakage in the leaf blade (EL), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (CAR), number of leaves (NL), stem diameter (SD), and plant height (PH) of 'Canindé' okra plants cultivated with saline nutrient solution (ECns) under foliar application of salicylic acid (SA) in a hydroponic system, 34 days after transplanting.

Sources of variation	DF	Mean squares							
		RWC	*EL*	*Chl* a	*Chl* b	CAR	NL	SD	PH
Saline nutrient solution (ECns)	3	438.60**	224.14**	470.75**	18.31**	0.93**	244.65**	6.18**	67.22*
Linear Regression	1	1259.28**	639.88**	1280.00**	70.14**	2.79**	689.82**	18.47**	277.77**
Quadratic Regression	1	5.11ns	3.49ns	1.37ns	11.67ns	0.01ns	25.20ns	0.00ns	0.34ns
Salicylic Acid (SA)	3	9.41ns	9.73ns	2.77ns	8.55*	0.06ns	12.06ns	0.13ns	5.73ns
Linear Regression	1	27.51ns	0.11ns	1.35ns	3.03*	0.04ns	7.55ns	0.00ns	16.95ns
Quadratic Regression	1	0.02ns	1.27ns	5.35ns	27.30na	0.00ns	2.84ns	0.34ns	0.03ns
Interaction (ECns × SA)	9	9.51ns	8.33ns	5.92ns	3.42ns	0.13ns	16.72ns	0.57ns	1.87ns
Error 1		10.32	6.74	2.72	1.64	0.18	9.00	0.62	6.44
Error 2		11.41	7.23	2.06	1.91	0.08	5.13	0.23	2.43
CV 1 (%)		4.49	19.00	8.64	15.77	6.36	28.58	16.78	20.58
CV 2 (%)		4.72	19.68	7.53	17.01	4.26	21.57	10.23	12.65

ns, *, **, respectively not significant and significant at a $p \leq 0.05$ and $p \leq 0.01$, CV= coefficient of variation.

The relative water content of okra plants decreased linearly with the increase in the electrical conductivity levels of the nutrient solution (Figure 5A), by 3.18% per unit increase in ECns. High concentrations of salts in the nutrient solution affect stomatal closure, which can lead to a reduction in water absorption by plants and consequently decreasing the relative water content in the leaf blade. In addition, increased transpiration can also aggravate the effects of salt stress (SILVA et al., 2021). Mendonça et al. (2022) also found a reduction, equal to 1.98% per unit increase in the electrical conductivity of the nutrient solution, in the relative water content in the leaf blade of okra plants grown in a hydroponic system with ECns of up to 6.6 dS m^{-1}, 60 days after transplantation.

The saline nutrient solution increased electrolyte leakage in the leaf blade of okra plants linearly (Figure 5B), which indicates that the increase in ECns resulted in an increase of 34.50% per unit increment in ECns. This response can be explained by the fact that, in general, plants subjected to salt stress tend to accumulate toxic ions such as Na^+ and Cl^-, which can lead to greater production of reactive oxygen species (ROS) and the imbalance between the production and elimination of ROS can cause lipid peroxidation, causing damage or even cell rupture, which can lead to internal leakage of the cell and oxidation of membranes (SACHDEV et al., 2021). However, for the electrolyte leakage in the leaf blade of okra plants subjected to saline nutrient solution, the maximum value obtained was lower than 50%. According to Sullivan (1972), the tissue is considered injured when more than 50% of the cells leak, which suggests that the okra plants in this study were not significantly affected by salt stress.

As for the chlorophyll *a* and *b* contents (Figure 6A and 6B) of okra plants, there were reductions of 8.68 and 5.17% for each unit increase in the electrical conductivity of the nutrient solution, respectively. When comparing plants grown under ECns of 6.6 dS m^{-1} with those subjected to the lowest salinity level (2.1 dS m^{-1}), there is a significant decline in the Chl *a* and Chl *b* contents of 47.82 and 26.06%, respectively. The effects of salt stress on plants are related to the increase in the activity of the chlorophyllase enzyme, breaking the thylakoids and the envelope layer of the chloroplasts, preventing the formation of new chlorophyll molecules in the presence of excess Na^+ and Cl^- ions, forming free radicals that reduce the action of the membranes involved in the photosynthetic process (OLIVEIRA et al., 2018). In a study conducted by Dantas et al. (2022) with zucchini under salt stress (ECns between 2.1 and 6.6 dS m^{-1}) and the application of hydrogen peroxide in a hydroponic system, these authors also found a reduction in the contents of chlorophyll *a*, chlorophyll *b,* and carotenoids with the increase in the electrical conductivity of the saline nutrient solution, 35 days after transplantation.

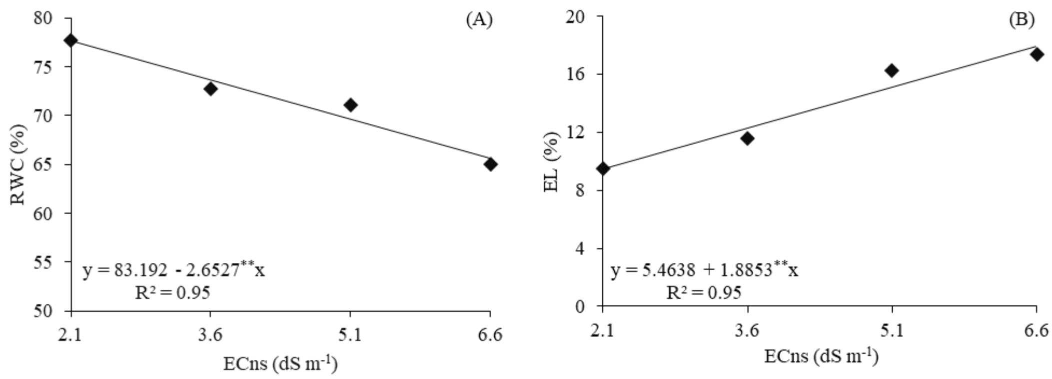


Figure 5. Relative water content – RWC (A) and electrolyte leakage – EL (B) in the leaf blade of 'Canindé' okra plants, as a function of the saline levels of the nutrient solution – ECns, in a hydroponic system, 34 days after transplanting. ** significant at $p \leq 0.01$.

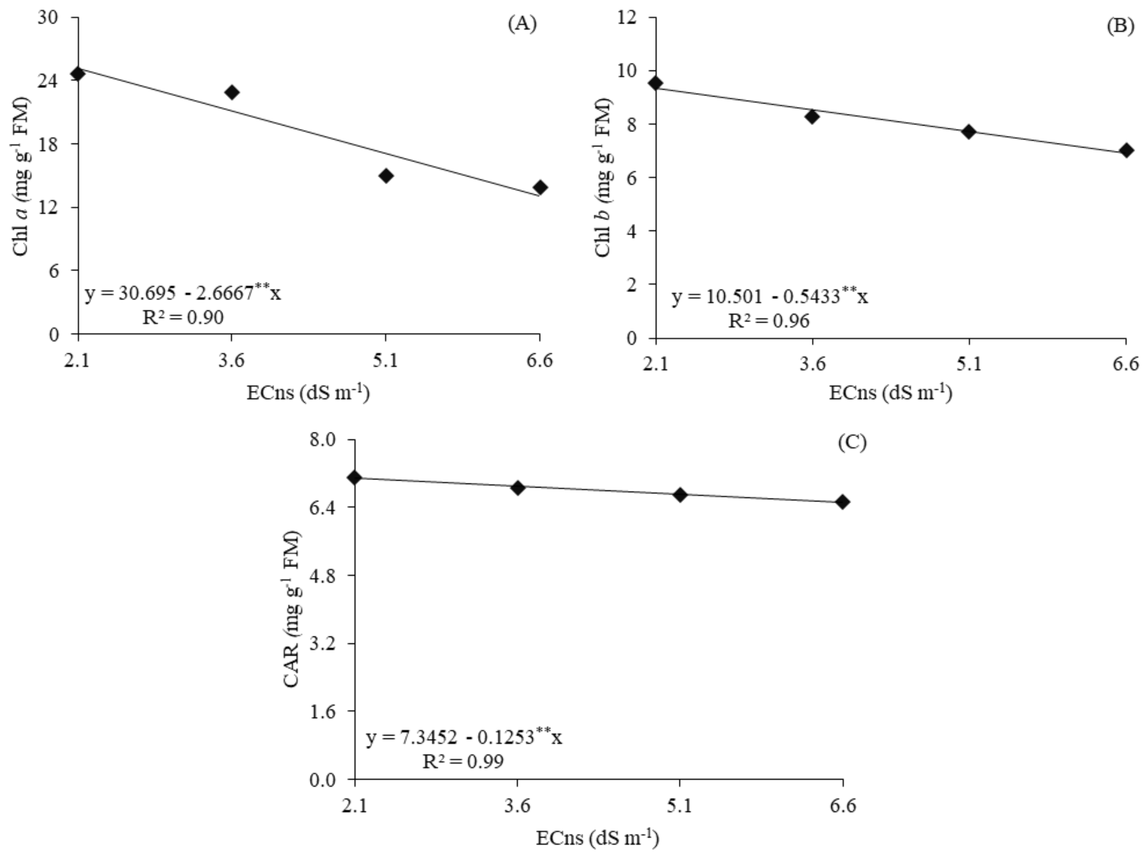


Figure 6. Chlorophyll *a* – Chl *a* (A), chlorophyll *b* – Chl *b* (B), and carotenoids – CAR (C) of 'Canindé' okra plants, as a function of the salinity levels of the nutrient solution – ECns, in a hydroponic system, 34 days after transplanting. ** significant at a $p \leq 0.01$.

The carotenoid contents of okra plants were also reduced linearly with the increase in the salinity levels of the nutrient solution (Figure 6C), with decreases of 1.70% per unit increment in ECns. The reduction in the contents of carotenoids in plants subjected to a high salinity of the nutrient solution is due to the photo-oxidation of β-carotene, negatively affecting their function of absorption and transfer of light to chlorophyll, reducing photosynthesis and plant growth (SILVA et al., 2016).

Salicylic acid increased the chlorophyll *b* contents of okra plants (Figure 7), by 4.95% per unit increase in SA concentration. According to Ram, Verma, and Gadi (2014), SA induces plants to synthesize cytokinin, which influences the increase of photosynthetic pigments by the mechanism of chlorophyll biosynthesis. A positive effect of salicylic acid was also reported by Hamani et al. (2020), who evaluated the levels of photosynthetic pigments in cotton plants under salt stress (0 and 150 mM) and foliar application of salicylic acid and observed that the SA concentration of 1.0 mM stimulated the biosynthesis of chlorophyll *b* in cotton plants.

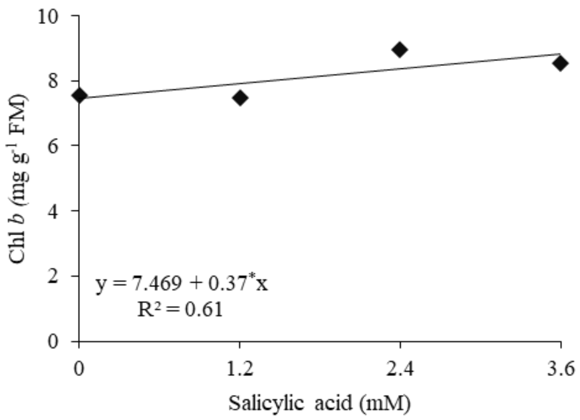


Figure 7. Chlorophyll *b* – Chl *b* of 'Canindé' okra plants, as a function of exogenous application of salicylic acid – SA, in a hydroponic system, 34 days after transplanting. * significant at a $p \leq 0.05$.

The growth of okra plants was reduced linearly with the increase in ECns (Figures 8A, B, and C), with decreases of 10.30, 5.25, and 6.23% per unit increment in ECns in the number of leaves, stem diameter, and plant height, respectively. When comparing plants grown under ECns of 6.6 dS m^{-1} with those subjected to the lowest ECns level (2.1 dS m^{-1}), reductions of 8.82 leaves, 1.44 mm, and 4.74 cm were observed in NL, SD, and PH, respectively. This negative effect is a consequence of the stomatal closure, which reduces water and nutrient absorption, destabilizing osmotic and ionic homeostasis, oxidizing proteins, nucleic acids, and lipids, and preventing cell expansion and elongation, resulting in lower growth and photosynthetic limitation with the reduction in the number of leaves (SILVA et al., 2018).

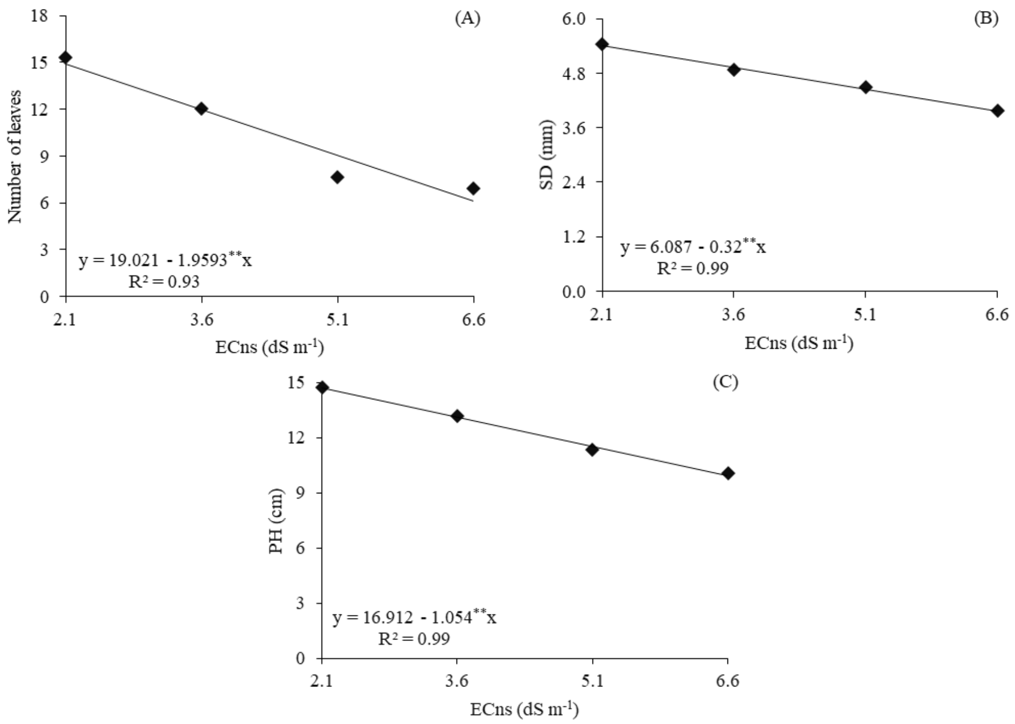


Figure 8. Number of leaves – NL (A), stem diameter – SD (B), and plant height – PH (C), as a function of the salinity levels of the nutrient solution – ECns of 'Canindé' okra plants, in a hydroponic system, 34 days after transplanting. ** significant at a $p \leq 0.01$.

CONCLUSIONS

Nutrient solution salinity above 2.1 dS m^{-1} negatively affected gas exchange, photosynthetic pigments, relative water content, electrolyte leakage in the leaf blade and growth in stem diameter, plant height, and number of leaves of 'Canindé' okra in hydroponic cultivation.

Salicylic acid at concentration of 3.6 mM reduces the negative effects on the initial, maximum, and variable fluorescence of okra plants grown under nutrient solution salinity of 6.6 dS m^{-1}, but it does not mitigate the effect of salt stress on instantaneous water use efficiency.

Foliar application of 3.6 mM of salicylic acid promotes an increase in the chlorophyll *b* contents of hydroponic okra plants.

REFERENCES

CINTRA, P. H. N. et al. Análise de fluorescência da clorofila a em mudas de cafeeiro sob estresse hídrico. **Brazilian Journal of Development**, 6: 28006-28014, 2020.

DANTAS, M. V. et al. Hydrogen peroxide and saline nutrient solution in hydroponic zucchini culture. **Semina: Ciências Agrárias**, 43: 1167-1186, 2022.

DIAS, A. S. et al. Trocas gasosas e eficiência fotoquímica do gergelim sob estresse salino e adubação com nitrato-amônio. **Irriga**, 23: 220-234, 2018a.

DIAS, A. S. et al. Gas exchanges and photochemical efficiency of West Indian cherry cultivated with saline water and potassium fertilization. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 22: 628-633, 2018b.

DIAS, A. S. et al. Gas exchanges, quantum yield and photosynthetic pigments of West Indian cherry under salt stress and potassium fertilization. **Revista Caatinga**, 32: 429-439, 2019.

GOES, G. F. et al. Ambiência agrícola e estresse salino em mudas de quiabo. **Revista Brasileira de Agricultura Irrigada**, 13: 3646-3655, 2019.

HAMANI, A. K. M. et al. Responses of leaf gas exchange attributes, photosynthetic pigments and antioxidant enzymes in NaCl-stressed cotton seedlings to exogenous glycine betaine and salicylic acid. **BMC Plant Biology**, 20: 1-14, 2020.

HOAGLAND, D. R.; ARNON, D. I. **The waterculture method for growing plants without soil**. 2. ed. Circular. Berkeley: California Agricultural Experiment Station, 1950. n. 347, 32 p.

LICHTENTHALER, H. K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. **Plant Cell Membranes**, 148: 350-382, 1987.

LIMA, G. S. et al. Gas exchanges, growth and production of okra cultivated with saline water and silicon fertilization. **Semina: Ciências Agrárias**, 41: 1937-1950, 2020.

LOTFI, R.; GHASSEMI-GOLEZANI, K.; PESSARAKLI, M. Salicylic acid regulates photosynthetic electron transfer and stomatal conductance of mung bean (*Vigna radi*ata L.) under salinity stress. **Biocatalysis and Agricultural Biotechnology**, 20: 1-14, 2020.

MENDONÇA, A. J. T. et al. Gas exchange, photosynthetic pigments, and growth of hydroponic okra under salt stress and salicylic acid. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 27: 673-681, 2023.

MENDONÇA, A. J. T. et al. Salicylic acid modulates okra tolerance to salt stress in hydroponic system. **Agriculture**, 12: 1-24, 2022.

OLIVEIRA, V. K. N. et al. Salicylic acid does not mitigate salt stress on the morphophysiology and production of hydroponic melon. **Brazilian Journal of Biology**, 82: e262664, 2022.

OLIVEIRA, W. J. et al. Fluorescência da clorofila como indicador de estresse salino em feijão caupi. **Revista Brasileira de Agricultura Irrigada**, 12: 2592-2603, 2018.

RAM, A.; VERMA, P.; GADI, B. R. Effect of fluoride and salicylic acid on seedling growth and biochemical parameters of watermelon (*Citrullus lanatus*). **Fluoride**, 47: 49-55. 2014.

SÁ, F. V. S. et al. Ecophysiology of West Indian cherry irrigated with saline water under phosphorus and nitrogen doses. **Bioscience Journal**, 35: 211-221, 2019.

SACHDEV, S. et al. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. **Antioxidantes**, 10: 1-37, 2021.

SANTOS, R. S. S. et al. Cultivo da rúcula em substrato de fibra de coco sob solução nutritiva salina. **Cultura Agronômica**, 27: 12-21, 2018,

SCOTTI-CAMPOS, P. et al. Physiological responses and membrane integrity in three Vigna genotypes with contrasting drought tolerance. **Emirates Journal of Food and Agriculture**, 25: 1002-1013, 2013.

SILVA, A. A. R. et al. Salicylic acid as an attenuator of salt stress in soursop. **Revista Caatinga**, 33: 1092-1101, 2020.

SILVA, A. A. R. et al. Salicylic acid relieves the effect of saline stress on soursop morphysiology. **Ciência e Agrotecnologia,** 45: e007021, 2021.

SILVA, A. R. A. et al. Pigmentos fotossintéticos e potencial hídrico foliar em plantas jovens de coqueiro sob estresse hídrico e salino, **Revista Agro@mbiente**, 10: 317-325, 2016.

SILVA, E. M. et al. Growth and gas exchanges in soursop under irrigation with saline water and nitrogen sources. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 22: 776-781, 2018.

SILVA JÚNIOR, L. G. A.; GHEYI, H. R.; MEDEIROS, J. F. Composição química de águas do cristalino do Nordeste Brasileiro. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 3: 11-17, 1999.

SOARES, L. A. A. et al. Growth and fiber quality of colored cotton under salinity management strategies. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 22: 332-337, 2018.

SOARES, M. D. M. et al. Physiology and yield of 'Gaúcho' melon under brackish water and salicylic acid in hydroponic cultivation. **Arid Land Research and Management**, 37: 1-20, 2022.

SULLIVAN, C. Y. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. In: RAO, N. G. P.; HOUSE, L. R. (Eds.). **Sorghum in the seventies**. New Delhi, NCP: Oxford & IBH Publishing Co, 1972. v. 1, cap. 1, p. 247-264.

WEATHERLEY, P. E. Studies in the water relations of the cotton plant. I - The field measurements of water deficits in leaves. **New Phytologist**, 49: 81-97, 1950.

XAVIER, A. V. O. et al. Gas exchange, growth and quality of guava seedlings under salt stress and salicylic acid. **Revista Ambiente & Água**, 17: e2816, 2022.