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Scientific Article

Physiological indices and growth of hydroponic cucumber under saline nutrient solutions and salicylic acid

Índices fisiológicos e crescimento de pepino hidropônico sob soluções nutritivas salinas e ácido salicílico

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ABSTRACT - The objective of this study was to evaluate the effect of foliar application of salicylic acid on the physiological indices and growth of Japanese cucumber cv. Hiroshi grown under saline nutrient solutions in a hydroponic system. The experiment was conducted in a greenhouse at the Center of Science and Agri-Food Technology of the Federal University of Campina Grande, Pombal, PB, Brazil, using the Nutrient Film Technique (NFT) hydroponic cultivation system. A completely randomized design was used in a split-plot scheme, with plots consisting of four levels of electrical conductivity of the nutrient solution - ECns (2.1, 3.6, 5.1, and 6.6 dS m^{-1}) and subplots consisting of concentrations of salicylic acid - SA (0, 1.8, 3.6, and 5.4 mM), with four replicates and two plants per plot. ECns of 4.8 dS m^{-1} associated with foliar application of 3.6 mM of SA resulted in higher relative water content. Growth, photosynthetic pigment synthesis, and biomass accumulation in Japanese cucumber plants are inhibited by nutrient solution from 2.1 dS m^{-1}. Salicylic acid at concentration of 2.0 mM promoted a higher relative growth rate of leaf area for plants under nutrient solution of 2.1 dS m^{-1}. SA concentration of 5.4 mM associated with saline nutrient solution of 6.6 dS m^{-1} resulted in a higher root/shoot ratio, but intensified the deleterious effects of salt stress on the biomass accumulation of cucumber plants.

Keywords: *Cucumis sativus L.* Salt stress. Gas exchange. Mitigator.

RESUMO - Objetivou-se com este estudo avaliar o efeito da aplicação foliar de ácido salicílico nos índices fisiológicos e crescimento de pepino japonês cv. Hiroshi cultivado sob soluções nutritivas salinas em sistema hidropônico. A pesquisa foi conduzida em casa de vegetação no Centro de Ciências e Tecnologia Agroalimentar da Universidade Federal de Campina Grande, Pombal - PB, utilizando-se o sistema de cultivo hidropônico tipo Técnica de Fluxo Laminar de Nutriente - NFT. Foi utilizado o delineamento inteiramente casualizado em esquema de parcelas subdivididas, sendo as parcelas constituídas de quatro níveis de condutividade elétrica da solução nutritiva - CEsn (2,1; 3,6; 5,1 e 6,6 dS m^{-1}) e as subparcelas de concentrações de ácido salicílico - AS (0, 1,8, 3,6 e 5,4 mM) com quatro repetições e duas plantas por parcelas. A CEsn de 4,8 dS m^{-1} associada a aplicação foliar de 3,6 mM de AS resultou em maior conteúdo relativo de água. O crescimento, a síntese de pigmentos fotossintéticos e o acúmulo de biomassa nas plantas de pepino japonês são inibidos pela solução nutritiva a partir de 2,1 dS m^{-1}. Ácido salicílico na concentração de 2,0 mM promoveu maior taxa de crescimento relativo em área foliar das plantas sob solução nutritiva de 2,1 dS m^{-1}. A concentração de 5,4 mM de AS associadas à solução nutritiva salina de 6,6 dS m^{-1} resultou em maior razão de raiz/parte aérea, entretanto, intensifica os efeitos deletérios do estresse salino no acúmulo de biomassas das plantas de pepino.

Palavras-chave: *Cucumis sativus L.* Estresse salino. Trocas gasosas. Mitigador.

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INTRODUCTION

Belonging to the Cucurbitaceae family, cucumber (*Cucumis sativus L.*) is a vegetable originally from India and cultivated in various parts of the world, standing out as a source of vitamins A, B, C, and K, as well as minerals, and can be consumed fresh in salads, used in preserves, stews, sandwiches, and as a raw material in the cosmetic and pharmaceutical industry (CARVALHO et al., 2013). In addition, it is an option of revenue for small and medium-sized producers, as it can reach high yields (PREVITAL et al., 2022). It is a vegetable crop classified as moderately sensitive to salinity, with threshold level of 2.5 dS m^{-1} in irrigation water and 3.5 dS m^{-1} in soil saturation extract (MAAS, 1984).

The semi-arid region of Northeast Brazil is characterized by spatial-temporal variability of rainfall and high evaporation demand, contributing to the qualitative and quantitative scarcity of water sources, which limits the growth and development of crops (MENDONÇA et al., 2022). In this region, water sources with moderate levels of dissolved salts are common. The excessive presence of soluble salts in water and/or soil restricts water and nutrient uptake by plants, causing limitations in physiology and growth, due to the reduction of osmotic potential and consequently water potential (LIMA et al., 2020).

Salt stress causes changes in plant metabolism, gas exchange, and growth, compromising photosynthetic efficiency and production components (DIAS et al., 2019). In this context, cultivation in a hydroponic system stands out as a viable option in areas that have water sources with high concentrations of salts, as it allows greater water use efficiency, resulting in savings of up to 70% compared to conventional systems (MENDONÇA et al., 2022; SOARES et al., 2023). In addition, this method provides greater control of the pH and electrical conductivity of the nutrient solution, as well as efficient use of fertilizers (SAUSEN et al., 2020).

Foliar application of salicylic acid (SA) is another strategy used to mitigate the effects of salt stress on plants (SILVA et al., 2020). SA is a phytohormone that plays an important role in the activation of genes responsible for the defense mechanism of plants against oxidative damage, contributing positively to the photosynthetic process, reducing the deleterious effects of salt stress (CHEN et al., 2020). The beneficial effects of SA on plants are associated with the increased synthesis of compatible osmolytes, such as glycine betaine, proline, sorbitol, trehalose, and sucrose, compounds that contribute to the maintenance of osmotic balance (SILVA et al., 2020).

Salicylic acid also improves enzymatic and photosynthetic activities and maintains the balance between the production and elimination of reactive oxygen species (SILVA et al., 2020). In this context, several studies have been conducted with horticultural species and have found positive effects of SA application via foliar, as observed in melon (SOARES et al., 2023) and okra (MENDONÇA et al., 2022). This study was based on the hypothesis that foliar application of SA increases the activity of antioxidant enzymes and the biosynthesis of compounds that play an important role in acclimatization, contributing to the reduction of the deleterious effects of salt stress on Japanese cucumber in hydroponic system.

In view of the above, the objective of this study was to evaluate the effect of foliar application of SA on the physiological indices and growth of Japanese cucumber cv. Hiroshi under salt stress in NFT-type hydroponic system.

MATERIAL AND METHODS

The experiment was conducted from May to June 2022 under conditions of greenhouse belonging to the Center of Sciences and Agri-Food Technology (CCTA), of the Federal University of Campina Grande (UFCG), in the municipality of Pombal, PB, Brazil, geographically located at 6° 46' 8" South and 37° 47' 45" West, with altitude of 184 m above sea level.

Temperature (maximum and minimum) and relative humidity data were collected daily using a digital thermo-hygrometer K29-5070H - KASVI® inside the greenhouse and are presented in Figure 1.

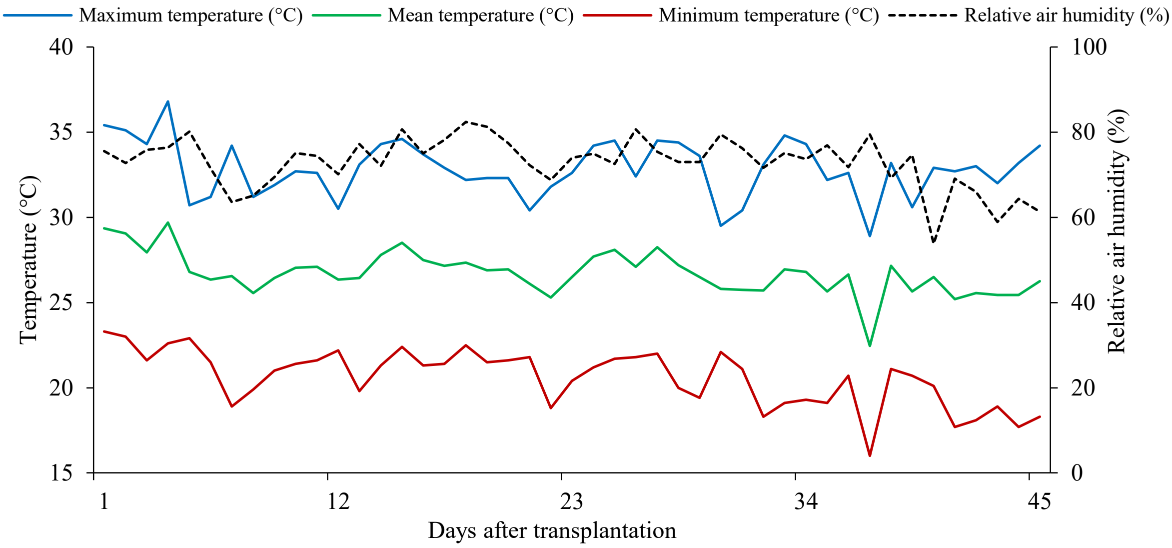


Figure 1. Daily values of maximum, mean, and minimum temperatures and mean relative humidity observed in the internal area of the greenhouse from May 01 to June 14, 2022.

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, 1.8, 3.6, and 5.4 mM) distributed in a completely randomized design, in a split-plot scheme, with four replicates and two plants per plot. ECns levels were considered the plots and the concentrations of SA were considered the subplots. The SA concentrations used in this study were based on a study conducted with melon (SOARES et al., 2023), while the salinity levels of the nutrient solution were based on the study conducted by Medeiros et al. (2010) with cucumber cv. Hokushin.

Japanese cucumber seeds cv. Hiroshi were used in the present study. The hydroponic system was NFT (Nutrient Film Technique) type, made with PVC pipes of 100 mm in diameter and six meters in length, spaced 0.40 m apart. In the hydroponic profile, the holes planted with the seedlings had diameter of 54.17 mm and were 0.50 m apart, with spacing between treatments (subsystems) of 1.0 m.

The hydroponic profiles were supported by 0.60-m-high sawhorses with a 4% inclination for the nutrient solution to flow. At the end of each subsystem, a 150 L polyethylene container was placed to collect return of nutrient solution and recirculate it in the system. The nutrient solution was injected into the hydroponic profile at the top of each channel with a 35 W pump at a flow rate of 3 L min^{-1}. A timer was used to program the circulation of the nutrient solution in the system, with intermittent flow of 15 min during the day and 30 min at night.

The nutrient solution recommended by Hoagland and Arnon (1950) was used in the study. The solution was prepared in local-supply water (0.3 dS m^{-1}), resulting in an electrical conductivity of 2.1 dS m^{-1}, and its pH was maintained between 5.5 and 6.5. A total of 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, was prepared using 101.10, 136.09, 236.15, 246.49, 3.10, 1.70, 0.22, 0.75, 1.25, 13.9, and 13.9 g L^{-1} of KNO_3, KH_2PO_4, $Ca(NO_3)_2.4H_2O$, $MgSO_4.7H_2O$, 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, respectively.

In the nutrient solution formulated by Hoagland and Arnon (1950), non-iodized sodium chloride (NaCl), calcium chloride ($CaCl_2.2H_2O$), and magnesium chloride ($MgCl_2.6H_2O$) were added in an equivalent ratio of 7:2:1, respectively. The different levels of electrical conductivity of the nutrient solution were obtained considering the relationship between ECw and salt concentration (RICHARDS, 1954), according to Equation 1.

$$Q \approx 10 \times ECw \quad (1)$$

where:
Q = Sum of cations ($mmol_c$ L^{-1}); and,
ECw = Desired electrical conductivity after discounting the ECw of the water of the municipal supply system (dS m^{-1}).

The saline nutrient solution was completely replaced every 8 days, and the electrical conductivity and pH were checked daily. Whenever necessary, the electrical conductivity was adjusted with the addition of local-supply water (0.3 dS m^{-1}), maintaining the ECns according to the treatments initially established, and the pH was maintained between 5.5 and 6.5 by adding 0.1 M potassium hydroxide (KOH) or hydrochloric acid (HCl).

Japanese cucumber cv. Hiroshi was sown in 50 mL polyethylene cups containing coconut fiber substrate, by placing one seed in each container, arranged in trays. The fiber was cleaned with sodium hypochlorite (2.5%) prior to sowing. Half-strength nutrient solution (50%) was used from germination to the emergence of the first true leaf. The fiber was removed after the emergence of the first true leaf, and the seedlings were inserted directly into the hydroponic channels, using a 100% nutrient solution. The plants were trained vertically so as to leave the stem erect with nylon twine.

Salicylic acid solutions were prepared by diluting salicylic acid (A.R.) in 30% ethyl alcohol (99.5%) and 0.05% Haiten®, a nonionic adhesive spreader used to break the surface tension and improve absorption by the cucumber leaves. Salicylic acid applications were carried out 48 h after inserting the plants in the hydroponic profiles and 72 h before using the saline nutrient solutions. Distilled water was applied to plants under the SA concentration of 0 mM.

Applications were carried out between 5:00 and 6:00 p.m. with a manual sprayer, so as to wet all the leaf area according to the treatments, applying an average of 80 mL per plant, at 10-day intervals. The applications stopped after the beginning of the fruiting stage, with a total of four applications. A plastic tarpaulin structure was used to avoid drift of the treatments between the plants. Plants were monitored and phytosanitary practices were carried out whenever necessary.

Physiological indices and growth of cucumber were evaluated at 30 days after transplanting (DAT). To determine the relative water content (RWC), two leaves were detached from the middle third of the main branch, and 10 discs with an area of 1.54 cm^2 each were collected. After collection, the discs were weighed to obtain the fresh mass (FM); then, they were transferred to beakers and immersed in 50 mL of distilled water for 24 hours. After this period, excess water was removed from the discs using paper towel, and the turgid mass (TM) was determined. Subsequently, the discs were dried at a temperature of ≈ 65 ± 3 °C in a forced air circulation oven, until reaching a constant weight, to obtain the dry mass (DM). RWC was determined according to the methodology of Weatherley (1950), using Equation 2.

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

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

Electrolyte leakage (EL%) was determined using five leaf discs with an area of 1.54 cm^2 each; the discs were placed in beakers with 50 mL of distilled water and kept at a temperature of 25 ºC for 24 hours. After this time, the initial electrical conductivity of the medium (Ci) was measured with a benchtop conductivity meter. The beakers were then placed in an oven at a temperature of 80 ºC and kept for 120 min, for subsequent measurement of final conductivity (Cf). Electrolyte leakage in the cell membrane was expressed according to Scotti-Campos et al. (2013), as shown in Equation 3.

$$\%\text{EL} = \frac{\text{Ci}}{\text{Cf}} \times 100 \quad (3)$$

where:
%EL = electrolyte leakage in the membrane;
Ci = initial electrical conductivity (dS m^{-1}); and,
Cf = final electrical conductivity (dS m^{-1}).

Gas exchange was determined 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}), internal CO_2 concentration - *Ci* (μmol CO_2 m^{-2} s^{-1}) on leaves located in the middle third using a portable infrared carbon dioxide analyzer (IRGA), model LCPro+ Portable Photosynthesis System® (ADC BioScientific Limited, UK), irradiation of 1200 μmol photons m^{-2} s^{-1} and air flow of 200 mL min^{-1}, and atmospheric CO_2 concentration and were performed between 7:00 and 9:00 a.m.

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids) were quantified according to Arnon (1949), with extracts from samples of discs of the third fully matured leaf from the apex. 6.0 mL of 80% acetone (A.R.) was added to each sample, and then the samples were kept in the dark under refrigerated conditions for 48 hours. In these extracts, the chlorophyll and carotenoid contents were determined using a spectrophotometer at the absorbance wavelengths of 470, 647, and 663 nm, using Equations 4, 5, 6, and 7, with results expressed in mg g^{-1} FM.

$$\text{Chl } a = 12.21 \text{ ABS}_{663} - 2.81 \text{ ABS}_{646} \quad (4)$$

$$\text{Chl } b = 20.13 \text{ ABS}_{646} - 5.03 \text{ ABS}_{663} \quad (5)$$

$$\text{Chl } T = 7.15 \text{ ABS}_{646} + 1.82\text{ABS}_{663} \quad (6)$$

$$\text{Car} = (1000 \text{ ABS}_{470} - 1.82 \text{ Chl } a - 85.02 \text{ Chl b})/198 \quad (7)$$

At 17 and 30 days after transplanting, growth in stem diameter (SD), plant height (PH) and leaf area (LA) was evaluated. SD was measured 5 cm above the hydroponic profiles. PH was determined from the hole of the hydroponic system to the insertion of the apical meristem. LA was determined based on leaf width (> 5 cm) and was estimated by Equation 8, according to the methodology of Yang et al. (1990):

$$\text{LA} = \sum 0.739 \times \text{L} \times \text{W}^{0.00104} \quad (8)$$

where:
LA – leaf area per plant (cm^2);
L – leaf length (cm); and
W – leaf width (cm).

From the PH, SD, and LA data, the relative growth rates (RGR) in plant height (RGR_{PH}), stem diameter (RGR_{SD}), and leaf area (RGR_{LA}) were determined for the interval between 17 and 30 DAT, according to the methodology of Benincasa (2003), using Equation 9:

$$\text{RGR} = \frac{(\ln \text{A2} - \ln \text{A1})}{(\text{T2} - \text{T1})} \quad (9)$$

where:
RGR = relative growth rate;
A1 – variable at time t1;
A2 – variable in time t2;
T1 – time 1 in days;
T2 – time 2 in days; and
ln = natural logarithm.

To determine the biomass accumulation, plants were collected at 42 DAT, separated into leaves, stem, and root. After separation, the material was placed in previously identified paper bags and dried in an air circulation oven at 65 ºC for 72 hours. After obtaining constant weight, leaf dry biomass (LDB), stem dry biomass (StDB), root dry biomass (RDB), shoot dry biomass (ShDB) (sum of leaf and stem dry biomass), and total dry biomass - TDB (ShDB + RDB) were determined on a balance with precision of 0.01 g.

According to Benincasa (2003), the root/shoot ratio (R/S) was obtained by the ratio between root dry biomass and shoot dry biomass, using Equation 10:

$$\text{R/S} = \frac{\text{RDB}}{\text{ShDB}} \quad (10)$$

where:
R/S – root/shoot ratio (g g^{-1})
RDB - root dry biomass (g per plant); and
ShDB - shoot dry biomass (g per plant).

Root volume (RV) (cm^3) was obtained following the methodology described by Basso (1999). Roots were placed in a graduated cylinder containing a known volume of water, and the difference directly represented the root volume per unit of equivalence (1 mL = 1 cm^3).

The collected data were subjected to the distribution normality test (Shapiro-Wilk) at 0.05 probability level. Then, analysis of variance was performed at 0.05 probability level, and in cases of significance of an individual factor, regression analysis was performed using the statistical software SISVAR -ESAL. The choice of the model was based on the values of the coefficients of determination (R^2) and considering a probable biological explanation. Due to the heterogeneity of *gs*, *E,* and *Ci* data (Table 1), it was necessary to perform exploratory data analysis, with data transformation into $\sqrt{x}$. In cases of significant interaction between factors, SigmaPlot v.12.5 software was used to construct the response surfaces.

RESULTS AND DISCUSSION

There was a significant effect of the interaction between the factors electrical conductivity of the nutrient solution and SA concentrations on the relative water content and internal CO_2 concentration of Japanese cucumber plants at 30 days after transplanting (Table 1). The salinity levels of the nutrient solution affected the stomatal conductance of Japanese cucumber plants.

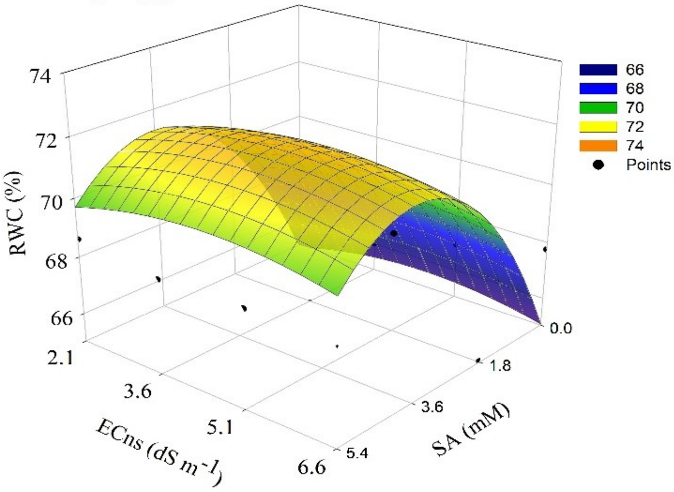
SA concentrations of up to 3.6 mM promoted an increase in RWC, even when the plants were cultivated under the highest level of electrical conductivity of 6.6 dS m^{-1}, with the highest estimated value of 72.23% obtained in plants subjected to ECns of 4.8 dS m^{-1} and SA concentration of 3.7 mM (Figure 2). However, the lowest value of 64.92% was observed in plants subjected to ECns of 2.1 dS m^{-1} and without SA application. RWC is a variable indicative of the plant's water status, and its reduction as a function of salinity may occur due to osmotic effects, which restrict water absorption by plants (LIMA et al., 2023). The increase in RWC in plants subjected to SA concentration of 3.6 mM may be associated with the physiological functions of SA on the accumulation of osmolytes, contributing to the absorption and increase of RWC in the tissues (SILVA et al., 2020).

Table 1. Summary of the analysis of variance for the relative water content (RWC) and electrolyte leakage (%EL) in the leaf blade, stomatal conductance (*gs*), transpiration (*E*), internal CO_2 concentration (*Ci*), and CO_2 assimilation rate (*A*) of Japanese cucumber plants cultivated with saline nutrient solution (ECns) and salicylic acid (SA) concentrations in hydroponic system, at 30 days after transplantation.

Sources of variation	DF	Mean squares					
		RWC	%EL	$gs^{(1)}$	$E^{(1)}$	$Ci^{(1)}$	*A*
Saline nutrient solution (ECns)	3	341.624^{ns}	153.29^{ns}	0.09^{**}	2.62^{ns}	7839.14^{*}	97.21^{ns}
Linear regression	1	514.64^{*}	40.16^{ns}	0.15^{**}	1.20^{ns}	16230.75^{*}	37.79^{ns}
Quadratic regression	1	4.461^{ns}	2.01^{ns}	0.000^{ns}	0.00^{ns}	1816.89^{ns}	74.34^{ns}
Residual 1	9	93.06	52.70	0.009	0.61	1819.16	23.89
Salicylic acid (SA)	3	178.36^{ns}	197.45^{ns}	0.01^{ns}	0.52^{ns}	8884.47^{**}	13.51^{ns}
Linear regression	1	9.50^{ns}	360.28^{ns}	0.000^{ns}	0.15^{ns}	538.20^{ns}	2.88^{ns}
Quadratic regression	1	39.64^{ns}	107.15^{ns}	0.037^{ns}	0.98^{ns}	13427.01^{**}	0.24^{ns}
Interaction (ECns × SA)	9	224.11^{*}	279.75^{ns}	0.01^{ns}	0.46^{ns}	4668.76^{**}	47.34^{ns}
Residual 2	36	102.27	253.03	0.01	0.55	1601.09	22.94
CV 1 (%)		13.42	17.64	31.51	24.91	23.18	19.34
CV 2 (%)		14.07	38.65	38.10	23.57	21.74	18.96

ns, * and ** respectively, not significant, significant at $p \leq 0.05$, and significant at $p \leq 0.01$; CV: coefficient of variation, DF: degrees of freedom; (1) Data transformed into $\sqrt{x}$.

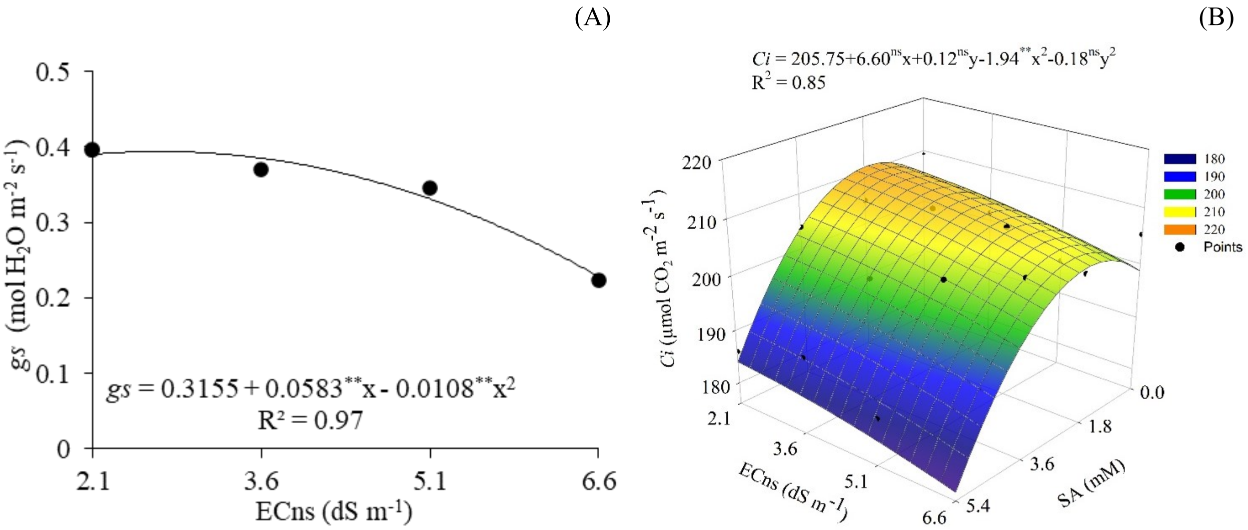
$$RWC = 63.16+3.56^{ns}x+1.07^{ns}y-0.49^{ns}x^2-0.11^{*}y^2$$
$$R^2 = 0.51$$



Y and X - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively; * - Significant at $p \leq 0.05$ and ns – Not significant ($p > 0.05$) by the F test, respectively.

Figure 2. Relative water content (RWC) in the leaf blade of Japanese cucumber plants, as a function of the interaction between the levels of electrical conductivity of the nutrient solution (ECns) and the concentrations of salicylic acid (SA), cultivated in a hydroponic system, at 30 days after transplanting.

The stomatal conductance (*gs*) of cucumber plants decreased with the increase in ECns levels (Figure 3A), with the maximum estimated value of 0.394 mol $H_2O\ m^{-2}\ s^{-1}$ obtained in plants cultivated under ECns of 2.7 dS m^{-1}, and the minimum value of 0.230 mol $H_2O\ m^{-2}\ s^{-1}$ in plants subjected to ECns of 6.6 dS m^{-1}, resulting in a decrease of 41.62% compared to the highest value found. Stomatal closure is a strategy to reduce water losses and maintain water balance, contributing to a lower absorption of toxic ions (DIAS et al., 2019). Under conditions of salt stress, plants can partially close their stomata as a strategy to increase stomatal resistance to the flow of water vapor from leaves to the atmosphere in order to maintain the water potential in the leaves and avoid the dehydration of guard cells; consequently, there is a reduction in the absorption of salts (LIMA et al., 2020). Negative effect of nutrient solution salinity was also observed by Dantas et al. (2022) in a study with zucchini (*Cucurbita pepo* L.) cultivated with ECns ranging from 2.1 to 6.6 dS m^{-1} in an NFT-type hydroponic system, with a reduction of 26.84% in *gs* per unit increment in the electrical conductivity of the nutrient solution.



** - Significant at $p \leq 0.01$ and ns – Not significant ($p > 0.05$) by the F test, respectively. In Fig. B, Y and X - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively.

Figure 3. Stomatal conductance - *gs* (A) of Japanese cucumber plants as a function of levels of electrical conductivity of the nutrient solution (ECns) and internal CO_2 concentration - *Ci* (B), as a function of the interaction between ECns levels and concentrations of salicylic acid (SA), at 30 days after transplanting.

For the internal CO_2 concentration (*Ci*) of cucumber plants (Figure 3B), the maximum estimated value of 210.82 µmol CO_2 m^{-2} s^{-1} was recorded in plants subjected to ECns of 2.1 dS m^{-1} and SA concentration of 1.7 mM. The increase in *Ci* observed in plants grown under the lowest ECns level (2.1 dS m^{-1}) is related to the greater stomatal opening (Figure 3A) and the absence of restriction of normal CO_2 flux to the substomatal chamber. On the other hand, cucumber plants subjected to the highest ECns level and SA concentration of 5.4 mM had the lowest value of internal CO_2 concentration (177.77 µmol CO_2 m^{-2} s^{-1}). Such reduction in the *Ci* of plants subjected to ECns of 6.6 dS m^{-1} and SA concentration of 5.4 mM may be related to the lower diffusion of CO_2 in the intercellular space of the leaf mesophyll, due to the partial closure of the stomata, and may be an indication that this phytohormone did not interact with the signaling pathways of reactive oxygen species (ROS) and consequently with oxidative stress (SILVA et al., 2020). In addition, the restriction of CO_2 to RuBisCO (ribulose-1,5-biphosphate carboxylase oxygenase) predisposes the photosynthetic apparatus to increased energy dissipation and downregulation of photosynthesis (VELOSO et al., 2022).

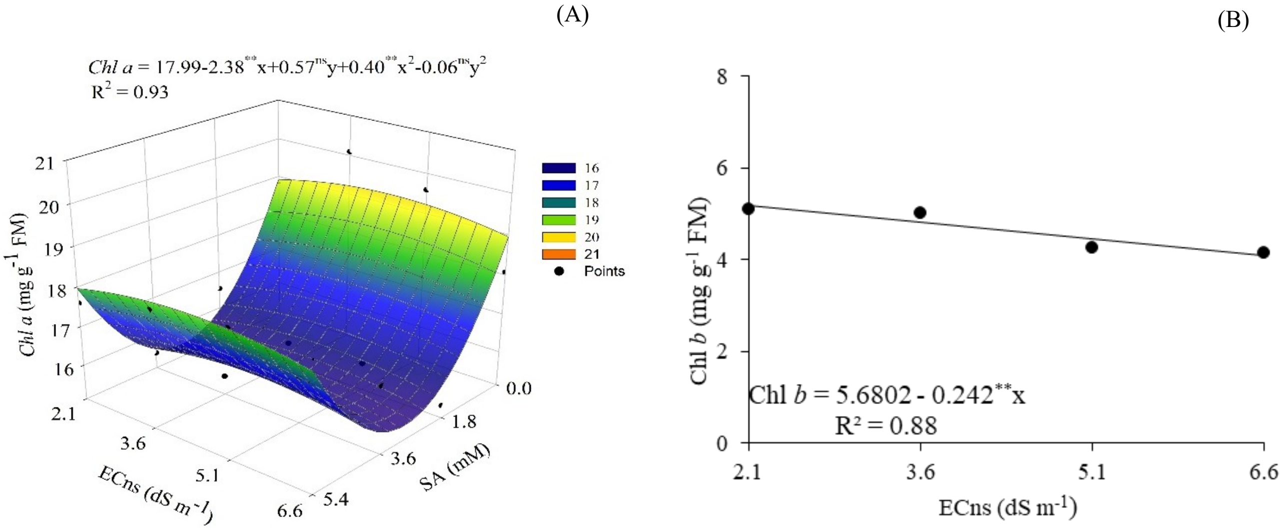
The interaction between the levels of electrical conductivity of the nutrient solution and SA significantly influenced the contents of chlorophyll *a*, total chlorophyll, and carotenoids (Table 2). The salinity levels of the nutrient solution significantly influenced the chlorophyll *b* content of Japanese cucumber plants at 30 days after transplanting.

Table 2. Summary of the analysis of variance for the contents of chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), total chlorophyll (Chl *t*), and carotenoids (Car) of Japanese cucumber plants cultivated with saline nutrient solution (ECns) and concentrations of salicylic acid (SA) in a hydroponic system, at 30 days after transplanting.

Sources of variation	DF	Mean squares			
		Chl *a*	Chl *b*	Chl *t*	Car
Saline nutrient solution (ECns)	3	43.43**	8.77**	90.20**	3.88**
Linear regression	1	114.65**	23.68**	242.26**	6.83**
Quadratic regression	1	0.18ns	1.28	2.43ns	0.09ns
Residual 1	9	3.29	0.84	6.66	0.12
Salicylic acid (SA)	3	7.11ns	2.09ns	16.88ns	0.13ns
Linear regression	1	11.72ns	3.84ns	28.97ns	0.36ns
Quadratic regression	1	4.76ns	0.92ns	9.86ns	0.03ns
Interaction (ECns × SA)	9	8.26*	1.39ns	16.12*	0.46*
Residual 2	36	2.96	1.12	7.04	0.19
CV 1 (%)		9.52	15.47	10.33	5.67
CV 2 (%)		9.03	17.90	10.62	7.24

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

For chlorophyll *a* contents (Figure 4A), plants cultivated under ECns of 4.7 dS m^{-1} obtained the highest estimated value (19.33 mg g^{-1} FM) when subjected to SA concentration of 0 mM. On the other hand, the lowest Chl *a* content (15.38 mg g^{-1} FM) was achieved at SA concentration of 2.9 mM and ECns of 2.1 dS m^{-1}. Reduction in chlorophyll synthesis is a result of oxidative stress, besides being attributed to the inhibition of chlorophyllase synthesis or degradation caused by the chlorophyllase enzyme in plants under salt stress (LIMA et al., 2020).



* - Significant at $p \leq 0.01$ and ns – Not significant ($p > 0.05$) by the F test, respectively; In Fig. A, Y and X - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively.

Figure 4. Chlorophyll *a* - Chl *a* (A) contents in Japanese cucumber plants, as a function of the interaction between levels of electrical conductivity of the nutrient solution (ECns) and concentrations of salicylic acid (SA), and chlorophyll *b* - Chl *b* (B) contents as a function of ECns levels, at 30 days after transplanting.

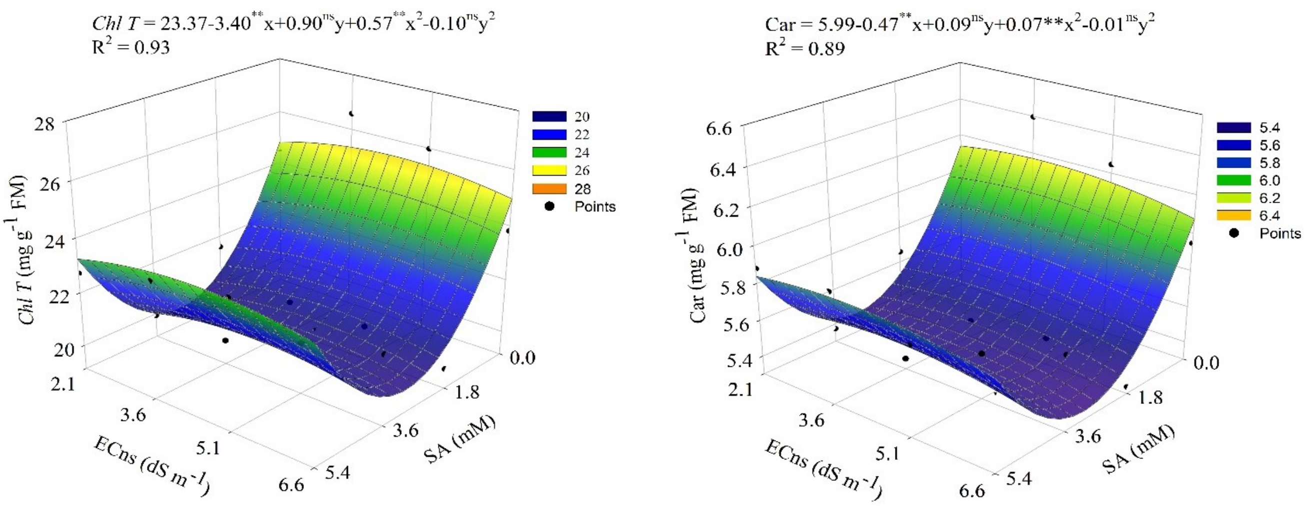
The increase in the levels of electrical conductivity of the nutrient solution reduced chlorophyll *b* contents in Japanese cucumber plants, with losses of 4.26% per unit increase in ECns (Figure 4B). When comparing the Chl *b* contents of plants subjected to ECns of 6.6 dS m^{-1} to those of plants that received the lowest salinity level of the nutrient solution, a decrease of 21.06% was observed. Inhibition in chlorophyll synthesis has been considered a typical symptom of oxidative stress and can be attributed to the inhibition of synthesis or degradation by the enzyme chlorophyllase (SOARES et al., 2021). In addition, the reduction may occur due to slow synthesis or rapid degradation, which is an indication that there was a mechanism of photoprotection through the reduction of light absorbance, reducing chlorophyll contents (TAIBI et al., 2016). Mendonça et al. (2023) in a study with okra (*Abelmoschus esculentus* L. Moench) cv. Canindé cultivated with saline nutrient solutions with EC ranging from 2.1 to 9.0 dS m^{-1} in an NFT-type hydroponic system, observed that the highest synthesis of chlorophyll *b* was obtained under ECns of 4.0 dS m^{-1} and foliar application of SA at concentration of 1.8 mM.

For the total chlorophyll contents (Figure 5A), plants cultivated under ECns of 4.5 dS m^{-1} obtained the highest estimated value (25.40 mg g^{-1} FM). On the other hand, the lowest contents of Chl *t* (19.749 mg g^{-1} FM) were achieved in plants subjected to SA concentration of 3.0 mM and ECns of 2.1 dS m^{-1}. Foliar application of SA at high concentrations can cause high levels of oxidative stress in plants, due to the excessive formation of ROS, resulting in reduced tolerance to stress. However, the mechanism of action of SA depends on the species, concentration, method of application, and environmental conditions (MENDONÇA et al., 2022).

Regarding carotenoid contents (Figure 5B), plants cultivated under ECns of 4.5 dS m^{-1} and without SA application obtained the highest estimated value (6.19 mg g^{-1} FM). On the other hand, the lowest CAR contents (5.35 mg g^{-1} FM) were achieved in plants subjected to ECns of 2.1 dS m^{-1} and SA concentration of 3.0 mM. Carotenoids are integrated components of thylakoids responsible for absorbing and transferring light to chlorophyll, acting as accessory pigments and with photoprotective action of the photochemical apparatus (CAVALCANTE et al., 2011). Thus, the increase in carotenoid synthesis observed in cucumber plants cultivated under ECns of 4.5 dS m^{-1} and foliar application of 0 mM possibly occurred as a mechanism to prevent photo-oxidative damage to chlorophyll molecules. In addition, this photosynthetic pigment acts as an antioxidant agent, protecting lipid membranes from oxidative reactions (MENDONÇA et al., 2023).

According to the summary of the analysis of variance (Table 3), there was a significant effect of the interaction between the factors electrical conductivity of the nutrient solution and SA concentrations on the relative growth rates of stem diameter, plant height, and leaf area of Japanese cucumber plants, in the period from 17 to 30 days after transplanting.

(A) (B)



Y and X - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively; ** - Significant at $p \leq 0.01$ and ns - Not significant ($p > 0.05$) by the F test, respectively.

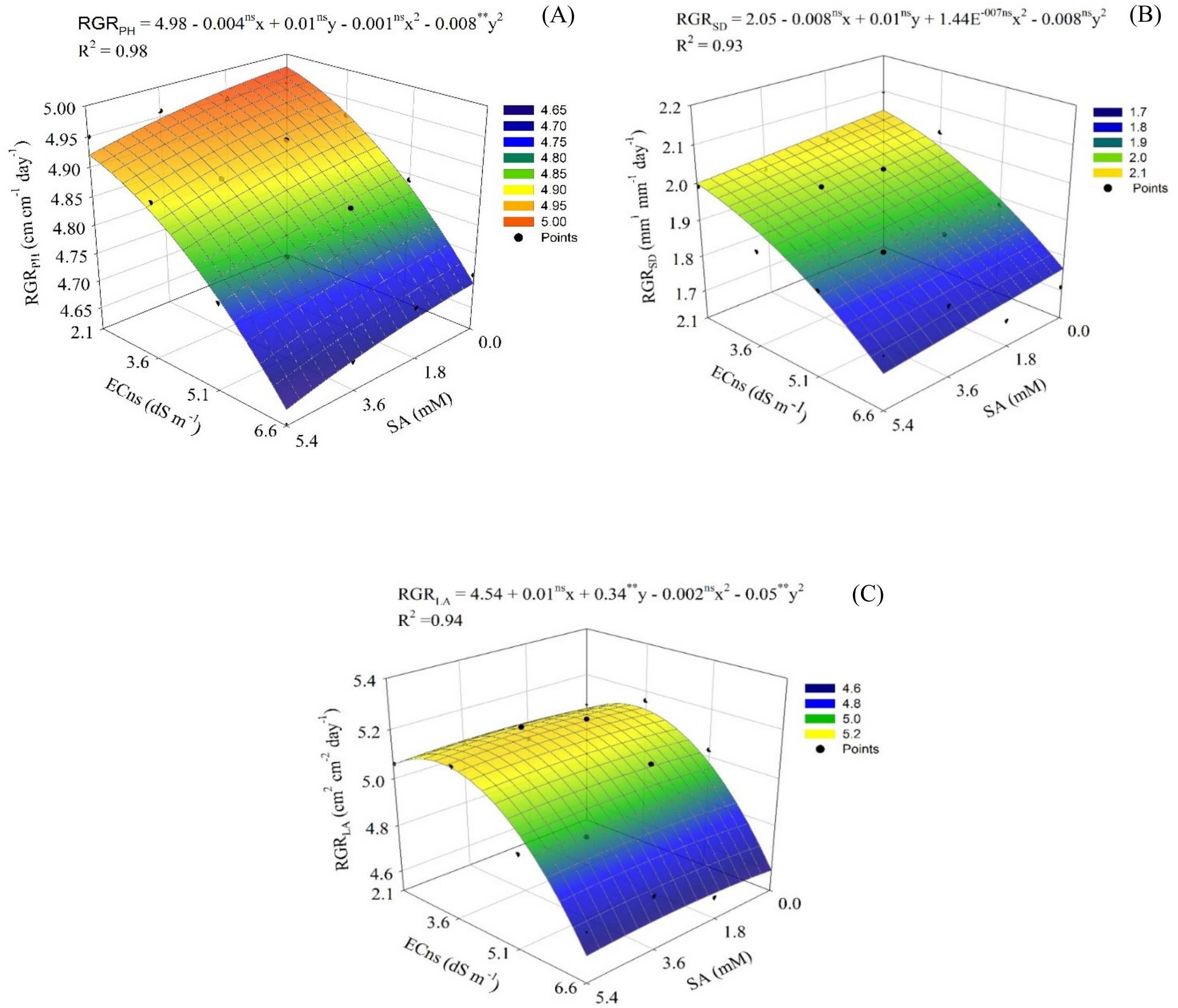
Figure 5. Total chlorophyll - Chl *t* (A) and carotenoids - Car (B) contents of Japanese cucumber plants, as a function of the interaction between the electrical conductivity of the nutrient solution (ECns) and concentrations of salicylic acid (SA), cultivated in a hydroponic system, at 30 days after transplanting.

Table 3. Summary of the analysis of variance for the relative growth rates of plant height (RGR_{PH}), stem diameter (RGR_{SD}), and leaf area (RGR_{LA}) of Japanese cucumber plants cultivated with saline nutrient solution (ECns) and concentrations of salicylic acid (SA) in a hydroponic system, in the interval from 17 to 30 days after transplanting.

Sources of variation	DF	Mean squares		
		RGR_{PH}	RGR_{SD}	RGR_{LA}
Saline nutrient solution (ECns)	3	0.23^{**}	0.23^{**}	0.84^{**}
Linear regression	1	0.69^{**}	0.68^{**}	1.68^{**}
Quadratic regression	1	0.027^{**}	0.020^{*}	0.82^{**}
Residual 1	9	0.0003	0.0023	0.001
Salicylic acid (SA)	3	0.009^{**}	0.01^{**}	0.002^{ns}
Linear regression	1	0.02^{**}	0.01^{**}	0.003^{ns}
Quadratic regression	1	0.001^{*}	0.000^{ns}	0.002^{ns}
Interaction (ECns × SA)	9	0.001^{**}	0.009^{**}	0.03^{**}
Residual 2	36	0.0001	0.001	0.001
CV 1(%)		0.37	2.53	0.81
CV 2(%)		0.26	1.73	0.67

$^{ns, *}$, and **, respectively, not significant, significant at $p \leq 0.05$ and significant at $p \leq 0.01$; CV - Coefficient of Variation, DF - Degrees of freedom.

The relative growth rates of plant height (Figure 6A), stem diameter (Figure 6B), and leaf area (Figure 6C) of Japanese cucumber plants were reduced with the increase in the levels of electrical conductivity of the nutrient solution. For the relative growth rates of plant height (Figure 6A) and stem diameter (Figure 6B), plants cultivated under ECns of 2.1 dS m^{-1} obtained the highest estimated values (4.97 cm cm^{-1} day^{-1} and 2.13 mm mm^{-1} day^{-1}, respectively) without SA application. However, foliar application of SA at a concentration of 2.0 mM reduced the deleterious effects of stress on the RGR_{LA}, promoting the maximum estimated value of 5.13 cm^2 cm^{-2} day^{-1} in plants grown at ECns of 3.5 dS m^{-1} (Figure 6C).



Y and X - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively; ** - Significant at $p \leq 0.01$ and ^{ns} - Not significant ($p > 0.05$) by the F test, respectively.

Figure 6. Relative growth rates of plant height - RGR_{PH} (A), stem diameter - RGR_{SD} (B), and leaf area - RGR_{LA} (C) of Japanese cucumber plants, as a function of the interaction between the levels of electrical conductivity of the nutrient solution - ECns and concentrations of salicylic acid (SA), cultivated in a hydroponic system, in the interval from 17 to 30 days after transplanting.

The increase in the relative growth rate of leaf area observed in plants subjected to SA concentrations of up to 2.0 mM (Figure 6C) may reflect the ability of SA to prevent the reduction of cytokinin and auxin, which stimulates cell division and consequently plant growth (ESTAJI; NIKNAM, 2020). Salt stress can cause inhibition of plant growth due to restrictions in water and nutrient uptake (MENDONÇA et al., 2023). In addition, the partial closure of the stomata resulting from osmotic and ionic effects causes changes in the photosynthetic rate and metabolism of plants, reducing turgor pressure, cell elongation, and cell wall elasticity, thus inhibiting their growth (LIMA et al., 2020).

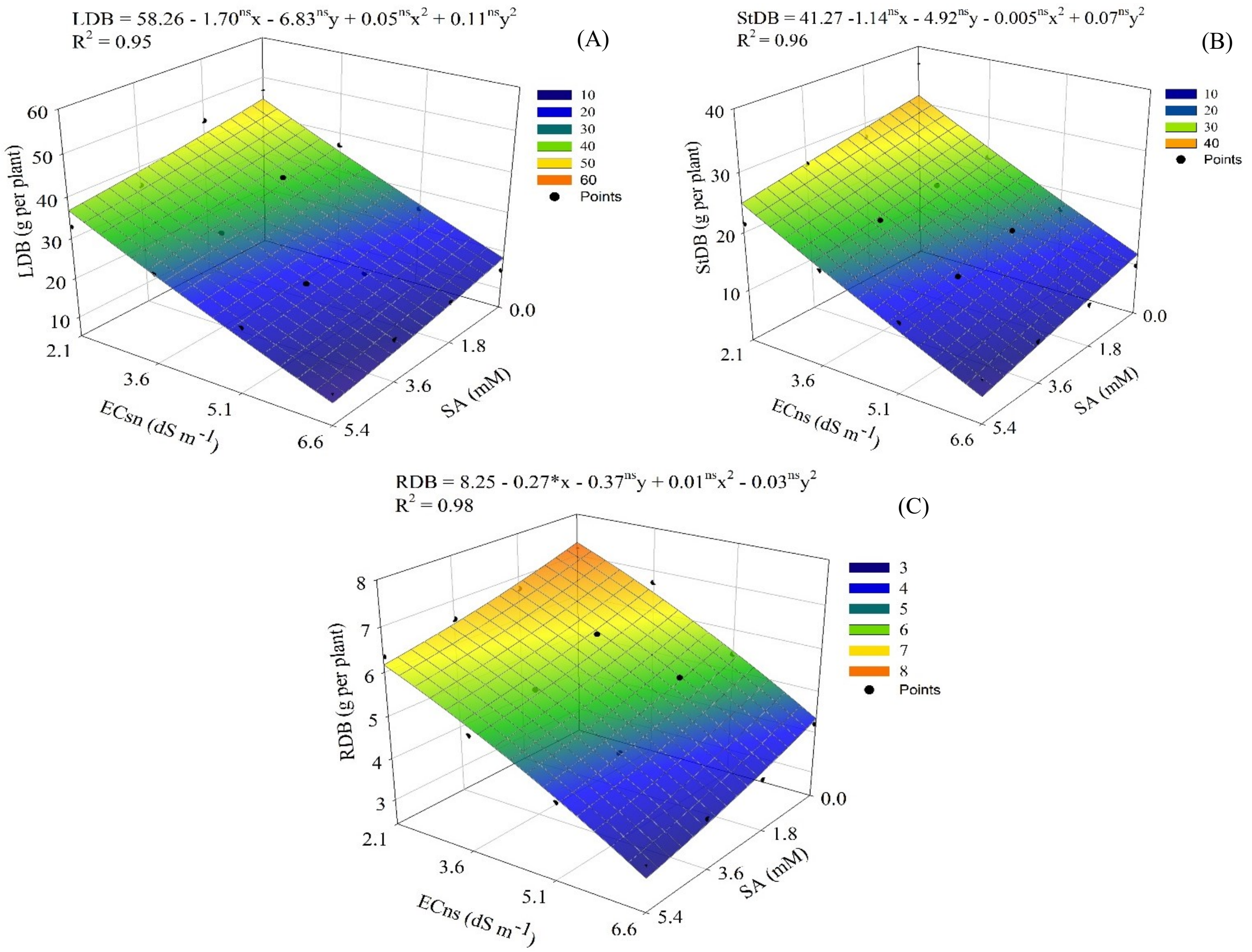
The summary of the analysis of variance (Table 4) shows a significant effect of the interaction between saline nutrient solution and SA concentrations on the leaf, stem, and root dry biomass of Japanese cucumber plants at 42 days after transplanting.

Regarding leaf dry biomass (Figure 7A), plants subjected to ECns of 2.1 dS m^{-1} and without SA application obtained the maximum estimated value of 44.4 g per plant. On the other hand, the minimum value (10.25 g per plant) was observed in plants cultivated under ECns of 6.6 dS m^{-1} and SA concentration of 5.4 mM.

Table 4. Summary of the analysis of variance for leaf dry biomass (LDB), stem dry biomass (StDB) and root dry biomass (RDB) of Japanese cucumber plants cultivated with saline nutrient solution (ECns) and concentrations of salicylic acid (SA) in a hydroponic system, at 42 days after transplanting.

Sources of variation	DF	Mean squares		
		LDB	StDB	RDB
Saline nutrient solution (ECns)	3	1923.03^{**}	1112.51^{**}	26.44^{**}
Linear regression	1	5760.38^{**}	3325.10^{**}	78.94^{**}
Quadratic regression	1	8.44^{ns}	1.31^{ns}	0.35^{ns}
Residual 1	9	4.86	4.27	0.18
Salicylic acid (SA)	3	226.57^{**}	118.70^{**}	3.94^{**}
Linear regression	1	628.68^{**}	348.86^{**}	11.69^{**}
Quadratic regression	1	12.95^{ns}	0.001^{ns}	0.09^{ns}
Interaction (ECns × SA)	9	30.70^{**}	31.21^{**}	0.28^{**}
Residual 2	36	6.53	2.45	0.08
CV 1(%)		7.97	11.30	8.16
CV 2(%)		9.13	8.05	5.41

^{ns} and ** respectively, not significant and significant at $p \leq 0.01$; CV - Coefficient of Variation; DF - Degrees of freedom.



Y and X - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively; * - Significant at $p \leq 0.05$ and ^{ns} - Not significant ($p > 0.05$) by the F test, respectively.

Figure 7. Leaf dry biomass - LDB (A), stem dry biomass - StDB (B), and root dry biomass – RDB (C) of Japanese cucumber plants, as a function of the interaction between the levels of electrical conductivity of the nutrient solution (ECns) and concentrations of salicylic acid – SA, cultivated in a hydroponic system, at 42 days after transplanting.

For stem dry biomass (Figure 7B), it was observed that nutrient solution salinity of 2.1 dS m^{-1} without application of SA promoted the maximum value of 31.25 g per plant, while the lowest value of StDB (5.55 per plant) was observed in plants cultivated under ECns of 6.6 dS m^{-1} and SA concentration of 5.4 mM. The root dry biomass of Japanese cucumber plants was also significantly affected by the interaction between the factors (Figure 7C), with the highest value (7.34 g per plant) obtained in plants cultivated under ECns of 2.1 dS m^{-1} without SA application.

Nutrient solution salinity of 6.6 dS m^{-1} and foliar application of 5.4 mM of SA resulted in the lowest value (3.33 per plant). It is important to highlight that the increase in nutrient solution salinity (6.6 dS m^{-1}) associated with a higher SA concentration (5.4 mM) in this study can be considered excessive and intensified the deleterious effects of salt stress on Japanese cucumber cv. Hiroshi in hydroponic system.

The inhibition of biomass accumulation in plants subjected to salt stress occurs due to the decrease in water and nutrient absorption and the ionic effects responsible for nutritional and metabolic imbalances (GUEDES et al., 2024). Salt stress also causes changes in photosynthetic processes and nutritional balance that result in reduced plant growth (LIMA et al., 2021), which was observed in this study in biomass accumulation.

There were significant effects of the interaction between saline nutrient solution and SA concentrations on shoot dry biomass, total dry biomass, root/shoot ratio, and root volume of cucumber plants under salinity levels of the nutrient solution and SA concentrations, at 42 days after transplanting (Table 5).

Table 5. Summary of the analysis of variance for shoot dry biomass (ShDB), total dry biomass (TDB), root/shoot ratio (R/S), and root volume (RV) of Japanese cucumber plants grown with saline nutrient solution (ECns) and concentrations of salicylic acid (SA) in hydroponic system, at 42 days after transplanting.

Sources of variation	DF	Mean squares			
		ShDB	TDB	R/S	RV
Saline nutrient solution (ECns)	3	5952.13**	6770.67**	0.0111**	51728.95**
Linear regression	1	17838.36**	20289.88**	0.03**	153006.89**
Quadratic regression	1	3.08^{ns}	5.55^{ns}	0.002**	540.09*
Residual 1	9	7.06	7.95	0.0001	39.26
Salicylic acid (SA)	3	646.45**	749.85**	0.0005*	2396.21**
Linear regression	1	1914.53**	2225.15**	0.0005^{ns}	7177.50**
Quadratic regression	1	12.70^{ns}	10.63^{ns}	0.0009*	5.38^{ns}
Interaction (ECns × SA)	9	103.94**	105.14**	0.0003*	344.24**
Residual 2	36	10.31	10.82	0.0001	36.01
CV 1(%)		5.78	5.50	8.70	5.28
CV 2(%)		6.98	6.41	9.22	5.06

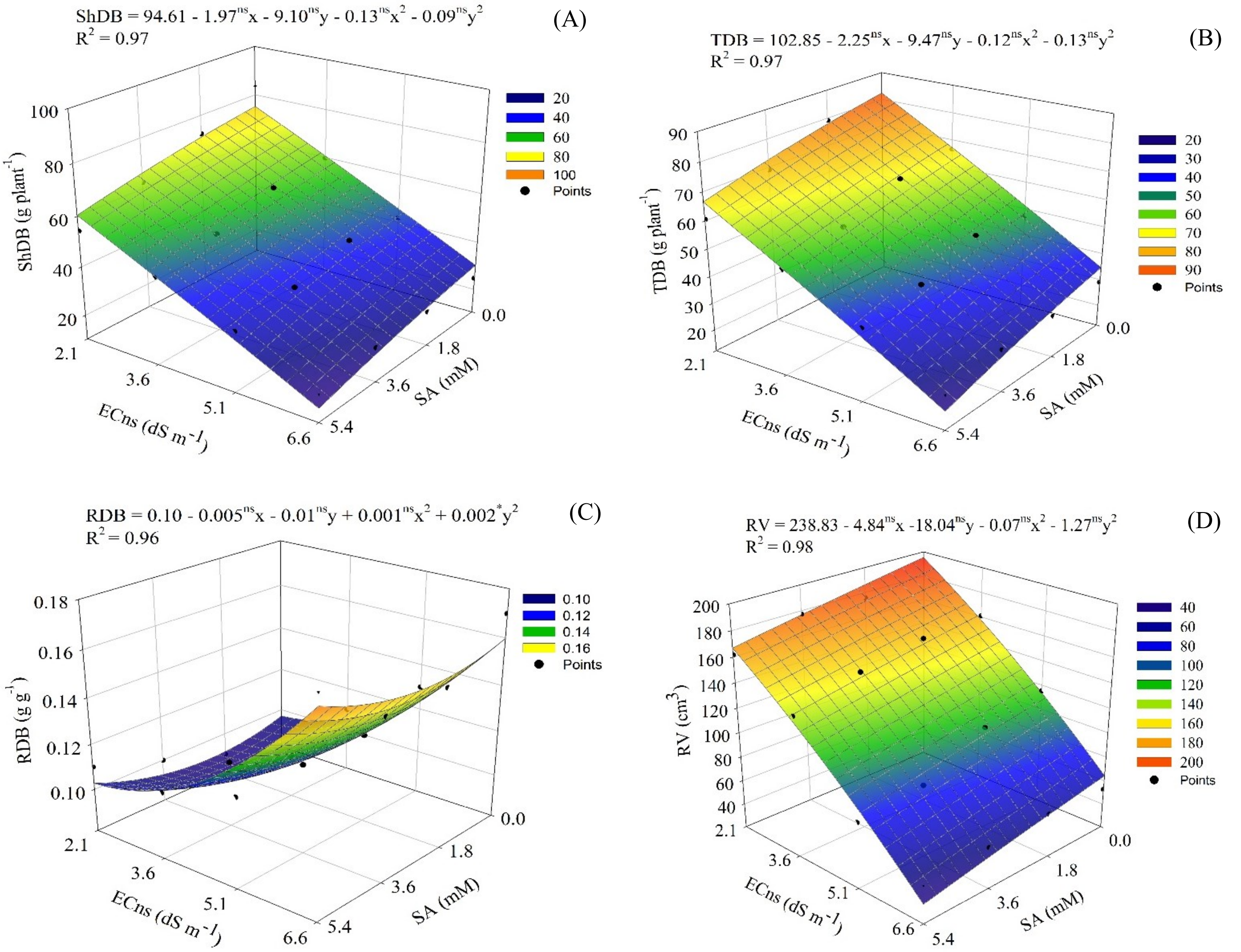
$^{ns, *\ and\ **}$ respectively not significant, significant at $p \leq 0.05$, and $p \leq 0.01$; CV - Coefficient of variation; DF - Degrees of Freedom.

Regarding shoot dry biomass (Figure 8A), plants subjected to ECns of 2.1 dS m^{-1} and SA concentration of 0 mM attained the highest estimated value (75.10 g per plant). On the other hand, the lowest value was obtained in plants cultivated with ECns of 6.6 dS m^{-1} when subjected to SA concentration of 5.4 mM. The total dry biomass of Japanese cucumber plants also decreased markedly with the increase in the salinity levels of the nutrient solution, with maximum value of 82.39 g per plant obtained under ECns of 2.1 dS m^{-1} and SA concentration of 0 mM (Figure 8B). The lowest value of TDB (19.04 g per plant) was achieved in plants subjected to nutrient solution salinity of 6.6 dS m^{-1} and SA concentration of 5.4 mM.

The inhibition in the accumulation of total dry biomass reflects the decline in LDB, StDB, RDB, and ShDB obtained in plants cultivated under the highest level of ECns (6.6 dS m^{-1}) and under foliar application of 5.4 mM of SA. Thus, it was clear that SA at a concentration of 5.4 mM intensifies the effects of salt stress on Japanese cucumber cv. Hiroshi. In addition, under conditions of salt stress, cell expansion is compromised, leading to leaf senescence, reducing the photosynthetic area and consequently the production of photoassimilates (LIMA et al., 2021).

For the root/shoot ratio of Japanese cucumber plants, the highest estimated value (0.1233) was obtained in plants subjected to ECns of 6.6 dS m^{-1} and SA concentration of 5.4 mM (Figure 8C). On the other hand, the lowest value of 0.0816 was observed in plants subjected to ECns of 2.1 dS m^{-1} and SA concentration of 2.3 mM. R/S indicates the level of contribution of the reserves stored in the root system in favoring the growth of the shoots. Increasing R/S ratio presents itself as a tolerance strategy for plants, since when they are subjected to stress, they seek to increase their roots in search of water and nutrients (DINIZ et al., 2020).

The root volume of Japanese cucumber plants subjected to ECns of 2.1 dS m^{-1} without SA application stood out with the highest estimated value (195.34 cm^3), as shown in Figure 8D. The lowest value of 36.26 cm^3 was observed in plants cultivated under ECns of 6.6 dS m^{-1} and SA concentration of 5.4 mM. High concentrations of salts present in irrigation water can cause ionic, osmotic, hormonal, and nutritional changes, restricting water absorption and negatively affecting crop growth and development (DINIZ et al., 2020).



Y and X - Electrical conductivity of the nutrient solution - ECns and concentration of salicylic acid - SA, respectively; * - Significant at $p \leq 0.05$ and ^{ns} - Not significant at $p > 0.05$ by the F test, respectively.

Figure 8. Shoot dry biomass - ShDB (A), total dry biomass - TDB (B), root/shoot ratio - R/S (C), and root volume - RV (D) of Japanese cucumber plants, as a function of the interaction between the levels of electrical conductivity of the nutrient solution (ECns) and concentrations of salicylic acid (SA), cultivated in a hydroponic system, at 42 days after transplanting.

CONCLUSIONS

Nutrient solution salinity of 4.8 dS m^{-1} associated with foliar application of 3.6 mM of salicylic acid results in higher relative water content in the leaf blade of Japanese cucumber plants. Growth, photosynthetic pigment synthesis, and biomass accumulation in Japanese cucumber plants are inhibited by nutrient solution salinity above 2.1 dS m^{-1} at 42 days after transplanting. Salicylic acid at a concentration of 2.0 mM promotes higher relative growth rate of leaf area of Japanese cucumber plants under nutrient solution salinity of 2.1 dS m^{-1}. Salicylic acid concentration of 5.4 mM associated with nutrient solution salinity of 6.6 dS m^{-1} results in a higher root/shoot ratio, but intensifies the deleterious effects of salt stress on the biomass accumulation of cucumber plants.

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