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Scientific Article

Gas exchange and growth of colored cotton under salt stress and application of salicylic acid

Trocas gasosas e crescimento de algodoeiro colorido sob estresse salino e aplicação de ácido salicilico

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ABSTRACT - The occurrence of water sources with high salt content stands out as a limiting factor for the expansion of irrigated agriculture in the semi-arid region. In this context, it is essential to look for strategies to mitigate the effects of salt stress, and the application of salicylic acid stands out. The objective of this study was to evaluate gas exchange and growth of the naturally colored cotton cv. BRS Jade under saline water irrigation and foliar application of salicylic acid under semi-arid conditions. The experiment was carried out in containers adapted as drainage lysimeters, under open air conditions at the Center of Science and Agri-Food Technology of the Federal University of Campina Grande in Pombal, Paraíba, Brazil. A randomized block design was adopted, in a 5 × 5 factorial scheme, with treatments resulting from the combination of five levels of electrical conductivity of irrigation water - ECw (0.3, 1.8, 3.3, 4.8, and 6.3 dS m^{-1}) associated with five concentrations of salicylic acid - SA (0, 1.5, 3.0, 4.5, and 6.0 mM), with three replicates. Water salinity from 0.3 dS m^{-1} limited the photosynthetic efficiency and therefore the growth of cotton cv. BRS Jade. Foliar application of SA at concentration of 6.0 mM mitigated the effects of salt stress on the stomatal conductance and leaf transpiration of these plants. However, SA concentrations ranging from 0 to 6.0 mM reduce the internal CO_2 concentration, growth in stem diameter and plant height, and root and stem dry mass accumulation of cotton plants cv. BRS Jade.

Keywords: *Gossypium hirsutum* L.. Salinity. Growth regulator.

RESUMO - A ocorrência de fontes hidricas com elevados teores de sais se destaca como fator limitante para expansão da agricultura irrigada no semiárido. Neste contexto, é essencial a busca por estratégias de mitigação dos efeitos do estresse salino, destacando-se a aplicação de ácido salicilico. Objetivou-se com este trabalho avaliar as trocas gasosas e o crescimento do algodoeiro naturalmente colorido cv. BRS Jade irrigado com água salina e aplicação foliar com ácido salicílico em condições semiáridas. A pesquisa foi desenvolvida em vasos adaptados como lisímetros de drenagem, sob condições de céu aberto no Centro de Ciências e Tecnologia Agroalimentar da Universidade Federal de Campina Grande em Pombal, Paraíba. Foi adotado o delineamento em blocos casualizados, em esquema fatorial 5 × 5, sendo os tratamentos resultantes da combinação de cinco níveis de condutividade elétrica da água de irrigação - CEa (0,3; 1,8; 3,3; 4,8 e 6,3 dS m^{-1}) associados a cinco concentrações de ácido salicílico - AS (0; 1,5; 3,0, 4,5 e 6,0 mM) com três repetições. A salinidade da água a partir de 0,3 dS m^{-1} limitou a eficiência fotossintética e por conseguinte o crescimento do algodoeiro cv. BRS Jade. A aplicação foliar de AS na concentração de 6,0 mM amenizou os efeitos do estresse salino sobre a condutância estomática e transpiração foliar dessas plantas. Contudo, concentrações de ácido salicilico variando de 0 a 6,0 mM reduz a concentração interna de CO_2, o crescimento em diâmetro de caule e altura de plantas e o acúmulo de fitomassas seca de raiz e caule das plantas de algodoeiro cv. BRS Jade.

Palavras-chave: *Gossypium hirsutum* L.. Salinidade. Regulador de crescimento.

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INTRODUÇÃO

Colored fiber cotton (*Gossypium hirsutum* L.) belongs to the Malvaceae family and can be grown in tropical and subtropical regions. The Northeast region of Brazil has a favorable climate for the production of this crop, which generates essential income for family farming (SILVA et al., 2019). The cultivation of colored cotton has increased significantly, especially with the introduction of new varieties in the market, such as BRS Jade, which produces fiber with quality within the parameters required by the textile industry (VASQUES et al., 2020).

The naturally colored fiber, obtained from rustic cotton plants, provides farmers with a greater economic return, due to the high demand for this product by developed countries of the first world, with benefits in reducing the operating cost of artificial dyeing in industrialization and reducing the environmental impacts resulting from the use of dyes (BEZERRA et al., 2010).

Despite the advantages, some factors can reduce the yield of colored cotton in the Northeast region of Brazil, such as the use of saline water, which is an alternative to produce in periods of drought. The main sources, such as weirs and wells, have their capacity reduced by high evaporation and by human and agricultural consumption; in addition, the supply is not annually met due to low rainfall levels, which hinders cotton production and leads producers to resort to the use of water of low quality for irrigation (DIAS et al., 2020).

Excess salts in the root zone induce salt stress in plants, causing osmotic effect, evidenced by stomatal closure and reductions in water absorption, growth, and leaf expansion, which later with the accumulation of toxic ions such as Na^+ and Cl^- in the plant tissue advances to the ionic effect, impairing the photosynthetic process, biosynthesis of photosynthetic pigments and organic compounds, as well as crop development and production (SILVA et al., 2018). Salt stress can also cause changes in electron transport, modifying the activity of Photosystem II, which is responsible for the oxidation of water molecules to produce electrons (NAJAR et al., 2019).

In order to minimize the deleterious effect of salts from irrigation water on crops, salicylic acid (SA) has been exogenously applied, as it is considered a phenolic compound that acts as a stress signaler in plants, inducing the production of enzymatic and non-enzymatic compounds, regulators of metabolism as a defensive mechanism and detoxification of reactive oxygen species (ZRIG et al., 2021). SA also increases the activity of antioxidant enzymes, such as peroxidases, superoxide dismutase, and catalases, and improves osmotic and ionic homeostasis, contributing to efficient absorption of water and nutrients, consequently favoring photosynthesis and plant growth (NÓBREGA et al., 2018). The efficacy of this growth regulator has been observed in several crops, including sour passion fruit (GALVÃO SOBRINHO et al., 2023), soursop (SILVA et al., 2020) and West Indian cherry (DANTAS et al., 2021). However, in the literature, there are incipient studies with the application of SA in cotton crop under conditions of salt stress in semi-arid areas.

In view of this problem, it is relevant to use strategies capable of mitigating the effects of salt stress on the colored cotton cv. BRS Jade, since the crop has socioeconomic importance for the Northeastern semi-arid region, where producers depend on the use of saline water as an alternative in the period of water scarcity. In this context, the objective of this study was to evaluate the gas exchange and growth of naturally colored cotton cv. BRS Jade under saline water irrigation and foliar application of SA under semi-arid conditions.

MATERIAL AND METHODS

The assay was carried out from March to June 2021 in pots adapted as drainage lysimeters, under open air conditions at the Center of Science and Agri-food Technology - CCTA of the Federal University of Campina Grande - UFCG, located in the municipality of Pombal, Paraíba, Brazil, at the geographic coordinates 6º47’20” S latitude and 37º48’01” W longitude, at an altitude of 194 m. Meteorological data recorded during the experimental period, from March 30 to June 17, 2021, are presented in Figure 1.

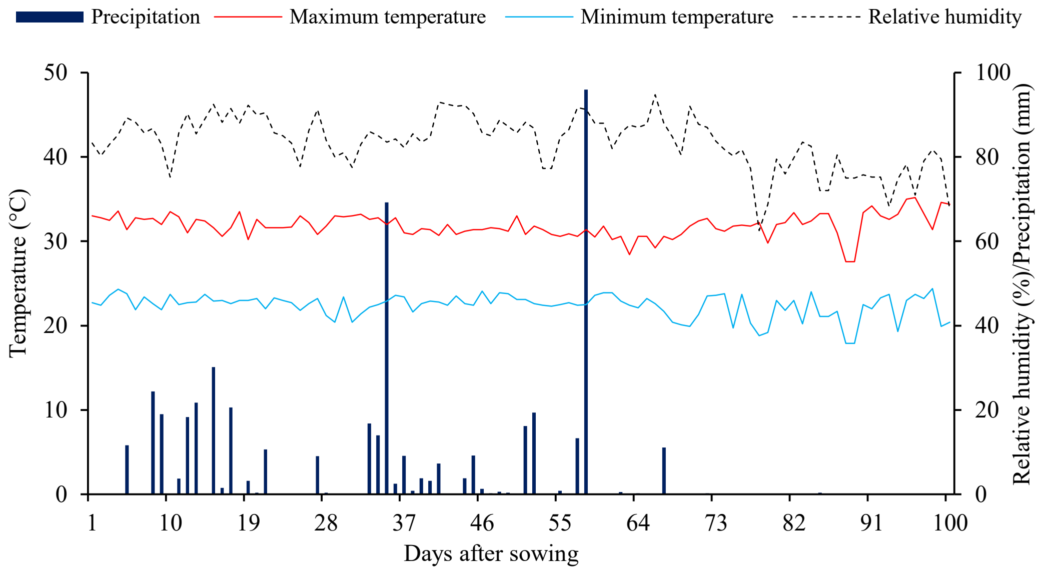


Figure 1. Climatic data of maximum and minimum temperature (ºC), precipitation (mm) and relative humidity of air (%) during the experimental period from March 30 to June 17, 2021.

A randomized block design was adopted in a 5 × 5 factorial scheme, and the treatments resulted from the combination of two factors: five levels of electrical conductivity of irrigation water - ECw (0.3, 1.8, 3.3, 4.8, and 6.3 dS m^{-1}), associated with five concentrations of salicylic acid - SA (0, 1.5, 3.0, 4.5, and 6.0 mM) with three replicates and one plant per plot, totaling 75 experimental units. The choice of electrical conductivity levels of irrigation water was based on a study conducted by Dias et al. (2020), while salicylic acid concentrations were determined based on previous studies conducted by Silva et al. (2020) and Veloso et al. (2021).

Cotton plants were grown in plastic pots adapted as drainage lysimeters with capacity of 20 L. The preparation of the lysimeters involved the perforation of two equidistant holes at the base of the containers, which were connected to two plastic tubes with diameter of 16 mm. These tubes were connected to plastic drainage bottles, each with volumetric capacity of 2 L. The purpose of this configuration was to determine the volume of water consumed by the plant.

A piece of geotextile was placed inside the lysimeter, followed by 0.5 kg of commercial crushed stone with size 01 (12.5 to 22 mm). Subsequently, the lysimeter was filled with 23.5 kg of soil, classified as *Neossolo Regolítico* (Entisol), with sandy loam texture (collected at 0-30 cm depth) from the rural area of the municipality of Pombal, PB, whose physical and chemical attributes (Table 1) were determined according to Teixeira et al. (2017).

Table 1. Chemical and physical characteristics of the soil (0-30 cm depth) used in the experiment, before application of the treatments.

Chemical Attributes								
pHse	ECse	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	$H + Al^{3+}$
$CaCl_2$ 1:2.5	(dS m^{-1})	mg dm^{-3}	cmol$_c$ dm^{-3}					
7.00	0.20	0.21	0.38	0.09	2.50	3.75	0	0

Physical Attributes							
Sand	Silt	Clay	BD	PD	Porosity	UD	Textural class
g kg^{-1}			kg dm^{-3}		%		
85.30	13.07	1.63	1.50	2.69	47.23	0.55	Loamy sand

pHse = pH of the substrate saturation extract; ECse = Electrical conductivity of saturation extract of the substrate, at 25 °C. Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; H^+ and Al^{3+} extracted with 0.5 M CaOAc at pH 7.0; BD = bulk density, PD = particle density. UD – Moisture (air dry soil).

Five seeds of colored cotton cv. BRS Jade were equidistantly distributed in each lysimeter, at 2 cm depth. At 15 days after sowing (DAS), thinning was performed, leaving one plant per container. Prior to sowing, the soil moisture content was raised to reach the field capacity, using water with electrical conductivity of 0.3 dS m^{-1}.

The water used in the irrigation of the treatment with lowest salinity (0.3 dS m^{-1}) was obtained from the public water supply system of Pombal, PB, while the other ECw levels were prepared by the dissolution of non-iodized sodium chloride (NaCl). Treatments were previously established taking into account the relationship between ECw and the concentration of salts (RICHARDS, 1954), according to Equation 1:

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

Where:
Q - Amount of salts to be added (mmol$_c$ L^{-1}); and
ECw - Electrical conductivity of water (dS m^{-1}).

After preparation, the waters were checked for ECw and stored individually in plastic containers with a capacity of 80 L each, in a shaded area and properly protected, covered to prevent loss due to evaporation and/or entry of rainwater.

Fifteen days after seedling emergence, irrigation was carried out daily, applying a specific volume of water to each lysimeter in order to keep soil moisture close to field capacity, and the applied volume was determined according to the water requirement of the plants, estimated by the water balance, calculated as expressed in Equation 2:

$$VC = \frac{VA - VD}{1 - LF} \quad (2)$$

Where:
VC - Volume consumed (L),
VA - Volume of water applied to the plants in the previous irrigation;
VD - Volume drained, quantified in the morning of the following day after irrigation; and
LF - Leaching fraction of 0.20, applied every 7 days.

Salicylic acid (SA) solutions of appropriate concentrations were prepared by dissolving salicylic acid (A.R.) in 30% ethyl alcohol (99.5%). Foliar applications with different SA concentrations started at 15 DAS. The second application occurred 15 days after the first, while the other applications were performed weekly, totaling seven SA applications throughout the experimental period. The applications were carried out by spraying, in such a way to fully wet the leaves, using a spray bottle, from 5 p.m.

Nitrogen, phosphorus, and potassium fertilization was performed as recommended by Novais, Neves, and Barros (1991) for pot experiments, using urea (45% N), monoammonium phosphate (12% N and 61% P_2O_5), and potassium chloride (60% K_2O). Basal fertilization was performed based on the total recommendation for phosphorus and 1/3 of the amount of nitrogen and potassium. The remaining two applications of nitrogen and potassium were topdressing, via irrigation water at 45 and 65 DAS. Micronutrient fertilization was carried out fortnightly, by foliar applications at a concentration of 0.5 g L^{-1} of the commercial product Dripsol Micro Rexene® Equilíbrio (1.2% - Mg; 0.85% - B; 3.4% - Fe; 4.2% - Zn; 3.2% - Mn; 0.5% - Cu; 0.06% - Mo) at 15 and 30 days after emergence.

Pest and disease management was performed with the chemical pesticide Battus® for the control of cotton aphid (*Aphis gossypii*) according to the manufacturer's recommendation. The application was carried out using a pre-compression manual knapsack sprayer, with a high molar mass polyethylene tank, with volumetric capacity of 12 L. For the control of weeds in the lysimeters, manual uprooting was periodically carried out to avoid interspecific competition for water and nutrients, favoring the development of the crop.

Gas exchange evaluations were performed at 50 DAS between 07:00 and 09:00 a.m., using an infrared gas analyzer - IRGA (InfraRed Gas Analyser, model LCpro - SD, from ADC Bioscientific, UK) to determine the CO_2 assimilation rate - *A* (μmol CO_2 m^{-2} s^{-1}), transpiration - *E* (mmol H_2O m^{-2} s^{-1}), stomatal conductance - *gs* (mol H_2O m^{-2} s^{-1}), and internal CO_2 concentration - *Ci* (μmol CO_2 m^{-2} s^{-1}). These data were then used to calculate water use efficiency - *WUEi* (*A*/*E*) [(μmol CO_2 m^{-2} s^{-1})(mol H_2O m^{-2} s^{-1})$^{-1}$] and instantaneous carboxylation efficiency - *CEi* (*A*/*Ci*) [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-2} s^{-1})$^{-1}$]. Readings were performed on the third fully expanded leaf, counted from the apical bud, under natural conditions of air temperature, CO_2 concentration and using an artificial radiation source of 1200 μmol m^{-2} s^{-1}.

In the same period (50 DAS), the following variables were obtained: number of leaves (NL), by directly counting leaves with length greater than 3 cm; plant height (PH), by measuring the length of the aerial part, from the plant collar to the apical bud of the main branch; and stem diameter (SD), measured 2 cm above the ground using a digital caliper. At the end of the crop cycle (100 DAS), the plants were collected, separated into leaves, stems, and roots, packed in paper bags and dried in an air circulation oven, maintained at 65 ºC, until they reached constant weight. Subsequently, the material was weighed on a precision scale, to obtain dry mass of leaf (LDM), stem (SDM), and root (RDM).

The data obtained were subjected to analysis of variance (F-Test) at 0.05 and 0.01 probability levels and, in cases of significance, linear and quadratic regression analysis was performed for the levels of electrical conductivity of irrigation water and salicylic acid concentrations, using the statistical software SISVAR-ESAL. In cases of significant interaction between the factors, TableCurve 3D software was used to construct the response surfaces.

RESULTS AND DISCUSSION

There was a significant effect of the interaction between factors (ECw × SA) for the variables of stomatal conductance (*gs*) and transpiration (*E*) (Table 2). The salinity levels of irrigation water significantly influenced the internal CO_2 concentration, CO_2 assimilation rate, instantaneous carboxylation efficiency, and water use efficiency in cotton cv. BRS Jade. On the other hand, SA concentrations significantly influenced only the internal CO_2 concentration (*Ci*) of cotton plants, at 50 DAS.

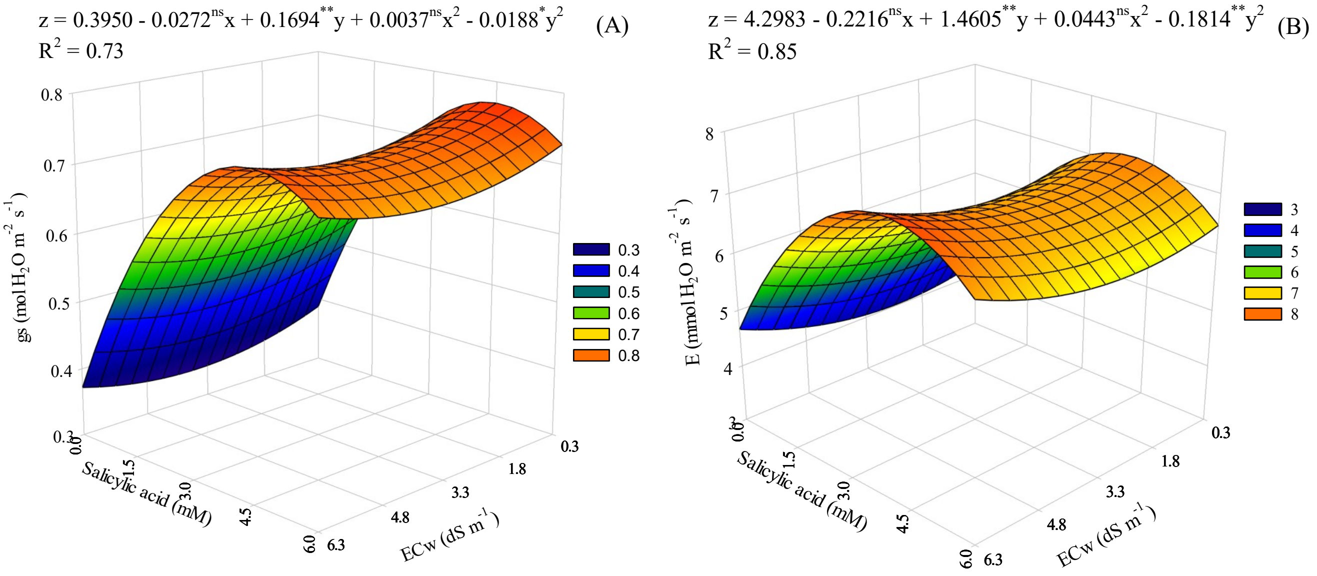
Table 2. Summary of the analysis of variance for the variables: stomatal conductance (*gs*), transpiration (*E*), internal CO_2 concentration (*Ci*), CO_2 assimilation rate (*A*), instantaneous carboxylation efficiency (*CEi*), and water use efficiency (*WUEi*) of cotton plants cv. BRS Jade irrigated with water of different electrical conductivities (ECw) and foliar application of salicylic acid (SA) at 50 days after sowing (DAS).

SV	DF	Mean squares					
		gs	*E*	*Ci*	*A*	*CEi*	*WUEi*
ECw	4	0.4240^{**}	20.3527^{**}	12471.94^{**}	431.2490^{*}	0.6437^{*}	11.5876^{*}
Linear regression	1	1.1863^{**}	55.9370^{**}	83.62^{ns}	527.7938^{*}	0.0065^{ns}	40.9979^{**}
Quadratic regression	1	0.1558^{*}	24.2080^{**}	26387.21^{*}	998.6581^{*}	1.2389^{*}	2.0090^{ns}
SA	4	0.0136^{**}	1.0438^{ns}	4795.11^{*}	134.9810^{ns}	0.1145^{ns}	2.5609^{ns}
Linear regression	1	0.0022^{ns}	1.7002^{ns}	15504.16^{*}	149.3008^{ns}	0.3229^{ns}	0.2842^{ns}
Quadratic regression	1	0.0147^{ns}	2.0900^{ns}	177.37^{ns}	0.5941^{ns}	0.0066^{ns}	3.7306^{ns}
ECw × SA	16	0.0600**	1.9636^{**}	2881.24^{ns}	183.0457^{ns}	0.0990^{ns}	2.3195^{ns}
Block	2	0.0054^{ns}	0.0101^{ns}	982.49^{ns}	57.6306^{ns}	0.0290^{ns}	1.3481^{ns}
Residual	48	0.0206	0.5464	2046.64	117.55	0.1746	2.4136
CV (%)	-	23.27	11.96	39.09	23.00	73.40	20.04

SV - Source of variation; DF - Degrees of freedom; ECw – Electrical conductivity of water; SA – Salicylic acid; ECw x SA - Interaction between the electrical conductivity of water and salicylic acid; CV - Coefficient of variation; (*) significant at 0.05 probability level; (**) significant at 0.01 probability level; (^{ns}) not significant.

The stomatal conductance (*gs*) of cotton plants cv. BRS Jade was positively influenced by the application of salicylic acid at all salinity levels (Figure 2A), with the highest value of 0.769 mol H_2O m^{-2} s^{-1} obtained when the SA concentration of 4.5 mM was applied to plants under irrigation with water of 0.3 dS m^{-1}, a value that represents an increase of 98,56% compared to plants under irrigation with water of 0.3 dS m^{-1}, but without the application of SA (0.387 mol H_2O m^{-2} s^{-1}), which may be related to the stress signaling caused by the application of SA, which increases the production of secondary metabolites that reduce the osmotic potential of the roots to values lower than those caused by the accumulation of salts in the soil, improving the absorption of water and nutrients, perhaps increasing stomatal opening (SILVA et al., 2021).

A behavior similar to that of *gs* was observed in transpiration - *E* (Figure 2B), with the highest values recorded in plants under SA application, whose maximum value was reached when the SA concentration of 4.0 mM was associated with irrigation with water of 6.3 dS m^{-1} (7.60 μmol H_2O m^{-2} s^{-1}). On the other hand, the lowest transpiration was observed in plants without SA application (0 mM) and irrigated with water of 2.5 dS m^{-1} (4.02 μmol H_2O m^{-2} s^{-1}). Stomatal opening directly influences the loss of water by the plant (SADOK; LOPEZ; SMITH, 2021). This explains the high transpiration of cotton cv. BRS Jade when SA is applied, as the increase in stomatal opening promotes greater interaction with the environment, increasing xylem transpiration flow (NAEEM et al., 2020).



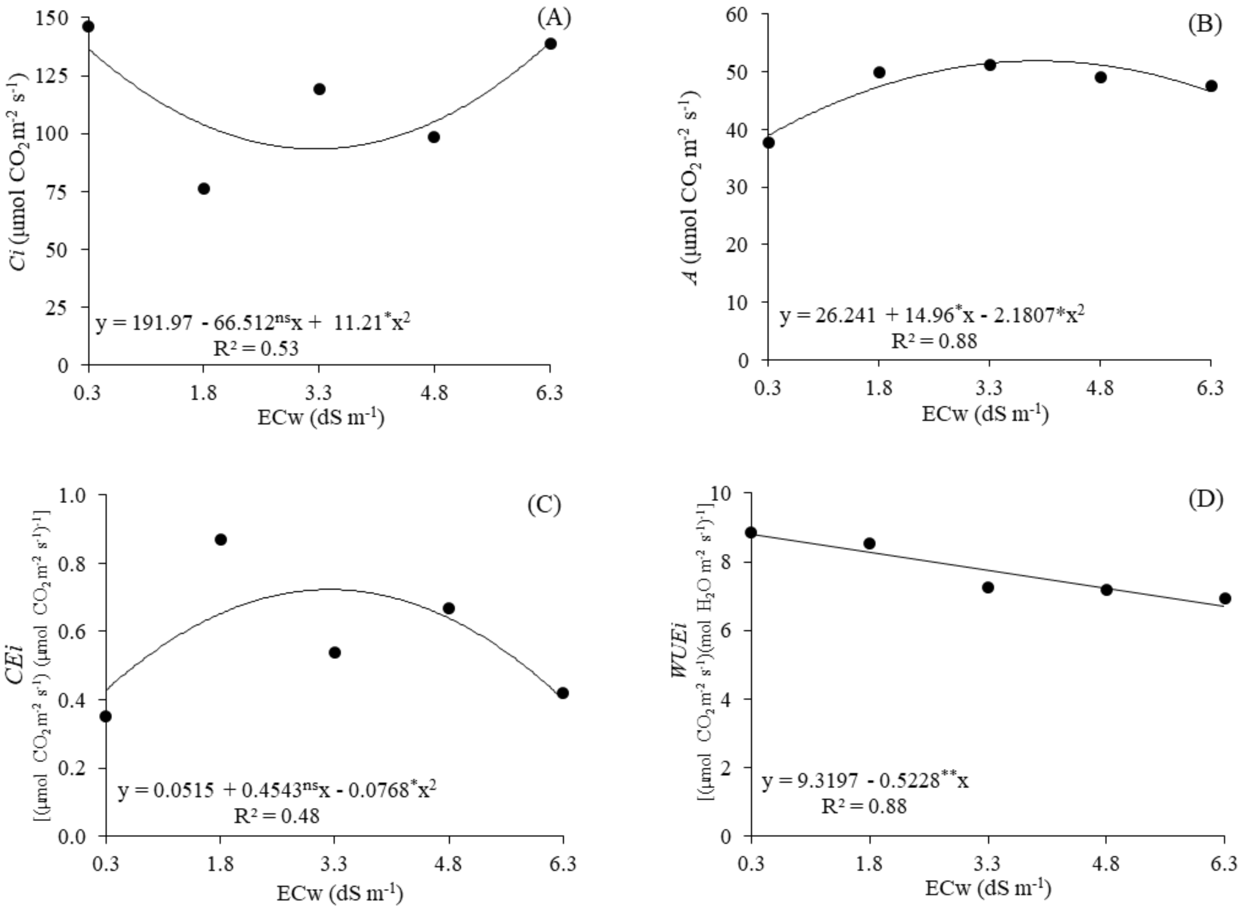
X and Y - Irrigation water salinity levels - ECw and concentrations of salicylic acid - SA, respectively; *, ** - Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively and ^{ns} - Not significant ($p > 0.05$) by the F test.

Figure 2. Stomatal conductance - *gs* (A) and transpiration - *E* (B) of cotton plants cv. BRS Jade, as a function of the interaction between irrigation water salinity levels - ECw and salicylic acid concentrations, at 50 days after sowing (DAS).

Regarding the effect of the electrical conductivity of irrigation water on the internal CO_2 concentration (Figure 3A), the highest value (138.80 μmol CO_2 m^{-2} s^{-1}) was observed in plants irrigated with water of 6.3 dS m^{-1}. However, the lowest value was established at the estimated ECw of 3.0 dS m^{-1} (93.32 μmol CO_2 m^{-2} s^{-1}). This result may be related to RuBisCO's carbon consumption in the Calvin cycle, which reduces the carbon present in substomatal chambers. Damage to this process reduces the consumption of carbon from the energy imbalance caused by the accumulation of salts in the plant, which intensifies the production of reactive oxygen species (SILVA et al., 2018; VELOSO et al., 2022).

The influence of saline water irrigation on the CO_2 assimilation rate (Figure 3B) and carboxylation efficiency (Figure 3C) was confirmed, showing significant increases up to the estimated electrical conductivity of 3.43 dS m^{-1} for *A* (51.89 μmol CO_2 m^{-2} s^{-1}) and up to 2.96 dS m^{-1} for *CEi* (0.72 (μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-2} s^{-1})$^{-1}$). Thus, the maintenance of the photosynthetic activity of cotton plants is evident. This may be related to the increased production of secondary metabolites that minimize the deleterious effects of salt accumulation, improving the activity of antioxidant enzymes, which fight free radicals produced in irregular energy dissipation (ACOSTA-MOTOS et al., 2017). These results corroborat with Zhang et al. (2014), who related the maintenance of photosynthetic activity in cotton plants (*Gossypium hirsutum* L.) to the increase in the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) under conditions of salt stress.

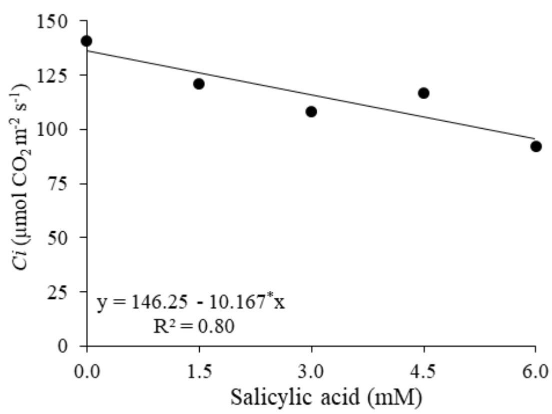
For water use efficiency (Figure 3D), the increase in irrigation water salinity resulted in a linear decrease of 5.60% per unit increase in ECw, from of 9.16 [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-2} s^{-1})$^{-1}$] in plants irrigated with water of 0.3 dS m^{-1} to 6.03 [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-2} s^{-1})$^{-1}$] in plants irrigated with water of 6.3 dS m^{-1}, resulting in a 34.23% reduction in *WUEi*. Water use efficiency is obtained through the relationship between the CO_2 assimilation rate and stomatal conductance, and the observed values refer to the amount of carbon fixed by the plant for a given stomatal conductance. Thus, it is likely that plants with the capacity to maintain reduced water use efficiency under saline conditions are those sensitive to salt stress (LIMA et al., 2020).



*, **, ns - significant at $p \leq 0.05$, $p \leq 0.01$ and not significant ($p > 0.05$) by the F test, respectively.

Figure 3. Internal CO_2 concentration - *Ci* (A), CO_2 assimilation rate - *A* (B), instantaneous carboxylation efficiency - *CEi* (C), and water use efficiency - *WUEi* (D) of cotton plants cv. BRS Jade, as a function of irrigation water salinity - ECw, at 50 days after sowing (DAS).

Salicylic acid application reduced the internal CO_2 concentration (Ci) of cotton cv. BRS Jade, at 50 DAS (Figure 4), showing a linear reduction of 10.42% for each 1.5 mM increase in SA concentration, i.e., there was a decrease of 41.71% in the *Ci* of plants under application of 6.0 mM of SA (85.25 μmol CO_2 m^{-2} s^{-1}) compared to those that were cultivated without SA application (146.25 μmol CO_2 m^{-2} s^{-1}). This behavior may be related to the high stomatal opening and transpiration observed in plants under SA application, leading to carbon losses to the external environment, in addition to that used in the carbon assimilation activity (VELOSO et al., 2021).



* - Significant at $p \leq 0.05$ by the F test.

Figure 4. Internal CO_2 concentration - *Ci* of colored cotton plants cv. BRS Jade, as a function of foliar application of salicylic acid, at 50 days after sowing (DAS).

There was a significant effect of the interaction between the factors (ECw × SA) on dry mass of leaf (LDM) and stem (SDM) of colored cotton plants at 100 DAS (Table 3). The electrical conductivity of irrigation water (ECw) significantly affected the stem diameter (SD), number of leaves (NL), and root dry mass (RDM) of cotton plants cv. BRS Jade. On the other hand, SA concentrations significantly affected plant height (PH), stem diameter (SD), and root dry mass (RDM) in cotton plants cv. BRS Jade.

Table 3. Summary of the analysis of variance for plant height (PH), stem diameter (SD), and number of leaves (NL) at 50 days after sowing (DAS), and dry mass of leaf (LDM), stem (SDM), and root (RDM) of cotton plants cv. BRS Jade irrigated with water of different electrical conductivities and under foliar application of salicylic acid, at 100 DAS.

SV	DF	Mean squares					
		PH	SD	NL	LDM	SDM	RDM
ECw	4	5.6966^{ns}	1.2117*	125.9466*	3.2411^{ns}	12.0930*	3.0936*
Linear regression	1	7.2600^{ns}	1.6875*	213.6066*	0.2713^{ns}	17.5719*	6.1448*
Quadratic regression	1	0.1714^{ns}	0.0044^{ns}	4.0047^{ns}	0.5411^{ns}	15.1796*	2.4710*
SA	4	47.4133*	2.1749**	96.3800^{ns}	29.0221**	40.8057**	5.6798*
Linear regression	1	182.6016*	6.5187**	291.2066*	45.4960**	103.8003**	19.7726*
Quadratic regression	1	0.7440^{ns}	0.3900^{ns}	7.2428^{ns}	28.0210**	25.0988*	2.6208*
ECw × SA	16	11.4216^{ns}	0.5827^{ns}	48.7383^{ns}	5.3382*	9.1479**	1.1357^{ns}
Block	2	46.9633*	1.2794*	97.6133^{ns}	1.1264^{ns}	2.8676^{ns}	0.8221^{ns}
Residual	48	1474.11	1.2794	52.3216	1.8406	2.4727	1.0854
CV (%)	-	5.63	5.31	16.21	8.27	8.57	11.51

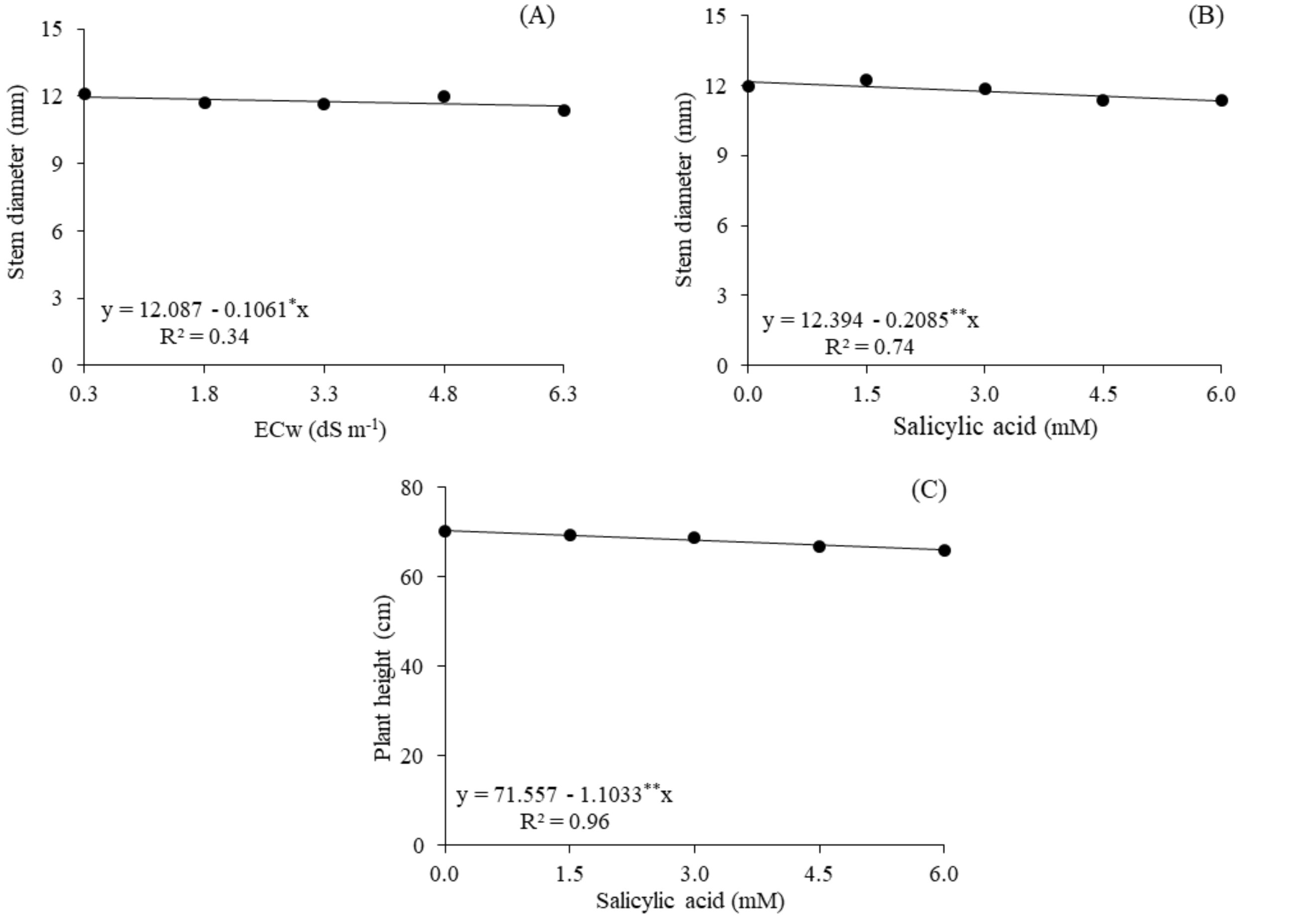
SV - Source of variation; DF - Degrees of freedom; ECw – Electrical conductivity of water; SA – Salicylic acid; ECw x SA - Interaction between the electrical conductivity of water and salicylic acid; CV - Coefficient of variation; (*) significant at 0.05 probability level; (**) significant at 0.01 probability level; (^{ns}) not significant.

Irrigation with increasing levels of electrical conductivity of the water contributed to the reduction in stem diameter of 0.87% per unit increase in ECw (Figure 5A). A reduction of 0.63 mm (5.28%) was observed when comparing plants irrigated with 0.3 dS m^{-1} water to those cultivated with the lowest ECw level (6.3 dS m^{-1}). The reduction in stem diameter can be attributed to the decrease in the water potential of the soil solution, caused by the excess of soluble salts in the root zone, leading to decreases in cell turgor and expansion, reducing plant growth (DIAS et al., 2020). In a study with colored fiber cotton cv. BRS Rubi, Lima et al. (2018) observed a reduction in the growth in diameter of plants that were irrigated with saline water at increasing concentrations: EC from 5.1 to 9.1 dS m^{-1}.

Foliar application of SA reduced the stem diameter of cotton cv. BRS Jade linearly, by 2.52% for each 1.5 mM increase in SA concentrations (Figure 5B). This is because SA plays controversial roles in plant metabolism, depending on the concentration, growing conditions, and development stage of the plant. High concentrations of SA, which for some plant species are considered to be above 1.0 mM, can exert a negative regulation on plant growth and development (KOO; HEO; CHOI, 2020). Ribeiro et al. (2020), when studying the morphophysiological aspects of watermelon as a function of water salinity and exogenous application of SA, observed that SA did not attenuate the deleterious effects of salinity, but favored plant growth up to a concentration of 0.85 mM.

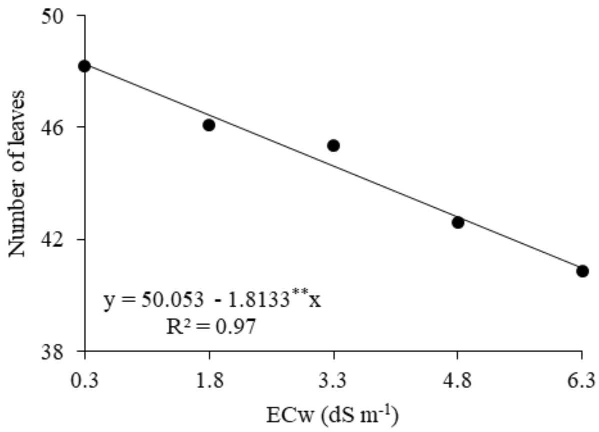
The height of cotton plants cv. BRS Jade was linearly reduced with the increase in SA concentrations (Figure 5C), decreasing by 2.31% per 1.5 mM increase in SA concentration, i.e., there was a decrease of 9.25% (6.61 cm) in cotton plants subjected to the application of 6.0 mM of SA compared to those that received the concentration of 0 mM. Thus, it can be inferred that the increasing application of SA concentrations without the influence of irrigation with saline water was not beneficial for the growth in height of cotton cv. BRS Jade, which may be related to the phytotoxic action of SA, since its application at low concentrations promotes the tolerance of the plant to salt stress. On the contrary, high concentrations do not play a beneficial role in the growth and production of plants, due to the low translocation of SA to the aerial part, which can unbalance their enzymatic activity, causing damage to the photosystem and growth (SOFY et al., 2020).

Irrigation with saline water compromised the number of leaves (NL) of cotton cv. BRS Jade, causing a linear reduction of 3.62% per unit increase in the electrical conductivity of the water. When comparing plants irrigated with ECw of 6.3 dS m^{-1} with those cultivated under the lowest level of water salinity (0.3 dS m^{-1}), a decrease of 21.97% was observed in NL (Figure 6). This reduction in NL can be explained as a mechanism of plant's survival under stress conditions, developing morphological and anatomical adaptations to reduce the loss of water to the environment and consequently the absorption of water and toxic ions, in an attempt to inhibit excessive accumulation in plant organs (LIMA et al., 2021). Dias et al. (2020) also observed a reduction in the number of leaves of white fiber cotton plants when irrigated with water of up to 6.7 dS m^{-1}.



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

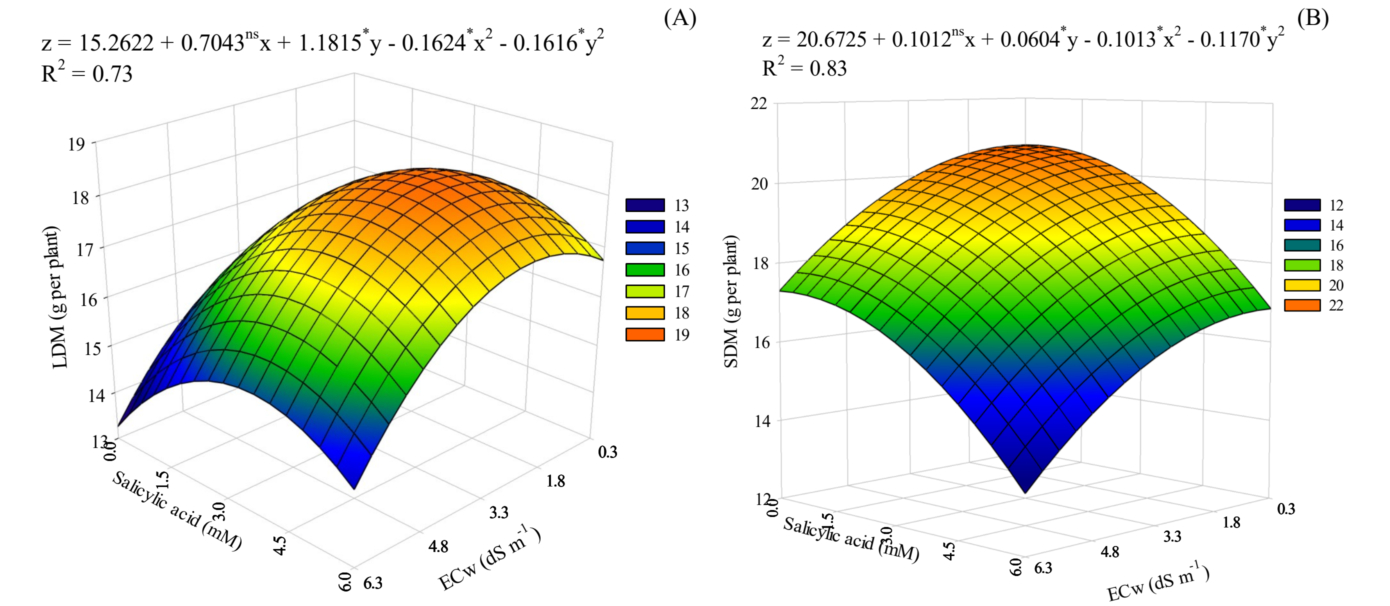
Figure 5. Stem diameter of cotton plants cv. BRS Jade, as a function of irrigation water salinity - ECw (A) and foliar application of salicylic acid - SA (B), and plant height as a function of SA concentrations, at 50 days after sowing (DAS).



**Significant at $p \leq 0.01$, by the F test

Figure 6. Number of leaves of cotton plants cv. BRS Jade, as a function of irrigation water salinity – ECw, at 50 days after sowing (DAS).

Cotton plants cv. BRS Jade irrigated with 2.2 dS m^{-1} water and subjected to SA concentration of 3.6 mM attained the highest LDM value (18.18 g per plant) (Figure 7A), equivalent to an increase of 13.47% (2.16 g per plant) compared to plants under control treatment (0 mM) and irrigated using water with the same salinity level (2.2 dS m^{-1}). This result is due to the regulatory function that SA plays in several physiological processes, contributing to plant growth and development, in addition to reducing the osmotic potential of the root, facilitating the absorption of water and nutrients, and, consequently, cellular turgor, which facilitates the expansion and gain of biomass (SILVA et al., 2021). In a study conducted with soursop plants under salt stress, Silva et al. (2021) also observed that foliar application of 1.3 and 1.6 mM of SA favored plant growth parameters.



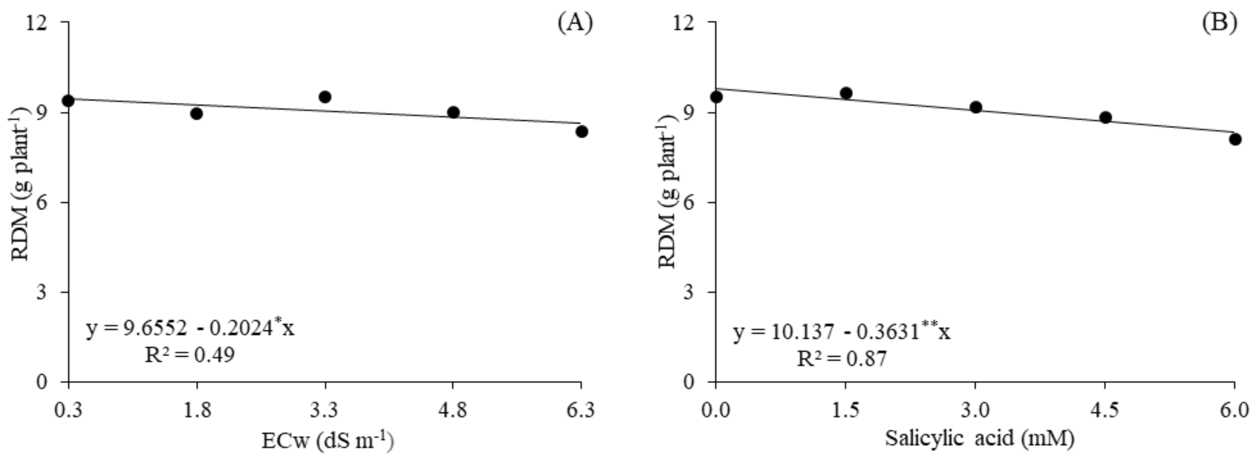
X and Y - Irrigation water salinity levels - ECw and concentrations of salicylic acid - SA, respectively; *, ns - significant at $p \leq 0.05$ and not significant ($p > 0.05$) by the F test, respectively

Figure 7. Dry mass of leaf - LDM (A) and stem - SDM (B) of colored cotton plants cv. BRS Jade, as a function of the interaction between the electrical conductivity of irrigation water and foliar application of salicylic acid at 100 days after sowing.

In relation to the stem dry mass (Figure 7B) of cotton plants cv. BRS Jade, the maximum estimated value of 20.69 g per plant was obtained under irrigation with 0.3 dS m^{-1} water and without foliar application of SA (0 mM). However, there was a decrease in SDM when SA concentrations above 1.5 mM were used, regardless of the electrical conductivity of the irrigation water. The lowest SDM (12.73 g per plant) was observed in plants irrigated with 6.3 dS m^{-1} water and subjected to SA concentration of 6.0 mM, corresponding to a reduction of 38.47% (7.96 g per plant), compared to plants with the highest SDM. Reduction in biomass accumulation in plants under salt stress conditions is a consequence of changes in the photosynthetic rate caused by the excess of salts, which restricts the absorption of water and nutrients by plants.

For the root dry mass of cotton plants (Figure 8A), there was a linear decrease of 2.09% per unit increment of ECw, i.e., plants subjected to the highest level of water salinity (6.3 dS m^{-1}) had a reduction in RDM accumulation of 12.65% (1.21 g per plant) compared to those subjected to ECw of 0.3 dS m^{-1}. This can be attributed to the inhibition of root growth under high salt concentrations, due to the increased metabolic cost of energy involved in the synthesis of osmotically active organic compounds, necessary in the processes of compartmentalization and in the regulation of ion transport, associated with adaptation to salt stress (LIMA et al., 2017).

The root dry mass of cotton also decreased linearly with the increase in SA concentrations, by 5.37% per 1.5 mM increase in SA concentration (Figure 8B). When comparing the RDM of plants cultivated under SA concentration of 6.0 mM to that of plants that did not receive foliar application (0 mM), a reduction of 21.49% was observed. The decrease in biomass accumulation due to the increase in SA concentration can be explained by the fact that this growth regulator influences the structure of the root meristem and its architecture, resulting in changes in the synthesis of auxins (PASTERNAK et al., 2019), because SA also interacts with other hormones involved in the regulation of cell division and expansion (LI; SUN; LIU, 2022).



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

Figure 8. Root dry mass (RDM) of colored cotton plants cv. BRS Jade, as a function of irrigation water salinity – ECw (A) and foliar application of salicylic acid (B), at 100 days after sowing (DAS).

CONCLUSIONS

Water salinity from 0.3 dS m^{-1} limits the photosynthetic efficiency and growth of cv. BRS Jade. Foliar application of salicylic acid at concentration of 4.5 mM mitigates the effects of salt stress on the stomatal conductance and leaf transpiration of these plants. However, salicylic acid concentrations ranging from 0 to 6.0 mM reduce the internal CO_2 concentration, growth in stem diameter, and plant height, and root and stem dry mass accumulation of cotton cv. BRS Jade.

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