

# Induction of salt stress tolerance in cherry tomatoes under different salicylic acid application methods

## Indução a tolerância ao estresse salino em tomate cereja sob diferentes métodos de aplicação de ácido salicílico

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### Highlights

Salt stress reduces plant photosynthetic pigment levels and photochemical efficiency.

Water salinity drastically affects gas exchange.

Foliar spray with salicylic acid minimizes the deleterious effects of salinity.

### Abstract

Salinity is among the biggest challenges of irrigated agriculture, as it induces several limitations to the growth and physiology of plants; therefore, strategies should be sought that minimize its impacts on plants. In this scenario, the present study was developed to examine the effects of different salicylic acid (SA) application methods on photosynthetic pigments, chlorophyll *a* fluorescence, gas exchange, and biomass accumulation of cherry tomato under salt stress. The study was carried out in a greenhouse, using a Regosol soil (Psamments) with a sandy-loam texture. The treatments were distributed in a completely randomized design, in a 2 × 4 factorial arrangement consisting of two levels of electrical conductivity in the irrigation water (0.6 or 2.6 dS m<sup>-1</sup>) and four salicylic acid application methods (M1 = without SA [control] application; M2 = foliar spray; M3 = irrigation; or M4 = spray and irrigation), with five replicates. Irrigation with 2.6 dS m<sup>-1</sup> salinity water negatively affected chlorophyll *a* fluorescence and the total chlorophyll, chlorophyll *a*, and

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carotenoid contents, in addition to inhibiting stem dry biomass production and root/shoot ratio. Foliar spray with salicylic acid minimized the deleterious effects of salt stress on gas exchange and chlorophyll content and increased leaf and root dry biomass accumulation and the root/shoot ratio of cherry tomatoes at 120 days after sowing.

**Key words:** Acclimatization. Irrigation. Mitigation. *Solanum lycopersicum* L.. Salinity.

## Resumo

A salinidade está entre os maiores desafios da agricultura irrigada, induzindo várias limitações no crescimento e na fisiologia das plantas, fazendo necessária a busca por estratégias que visem minimizar seus impactos sobre as plantas. Neste contexto, objetivou-se avaliar os efeitos de diferentes métodos de aplicação de ácido salicílico sobre os pigmentos fotossintéticos, a fluorescência da clorofila *a*, as trocas gasosas e o acúmulo de fitomassa de tomate cereja sob estresse salino. O estudo foi conduzido em casa de vegetação, utilizando-se um Neossolo Regolítico Psamítico de textura franco-arenosa. Os tratamentos foram distribuídos em delineamento inteiramente casualizados, em arranjo fatorial  $2 \times 4$ , sendo dois níveis de condutividade elétrica da água de irrigação ( $0,6$  e  $2,6 \text{ dS m}^{-1}$ ) e quatro métodos de aplicação de ácido salicílico (M1= Testemunha - sem aplicação de AS, M2= via pulverização, M3= via irrigação e M4= pulverização e irrigação), com cinco repetições. A irrigação com água de  $2,6 \text{ dS m}^{-1}$  afetou de forma negativa a fluorescência da clorofila *a*, os teores de clorofila *a*, total e carotenóides, além de inibir a produção de fitomassa seca de caule e a relação raiz/parte aérea. O método de aplicação de ácido salicílico via pulverização foliar minimizou os efeitos deletérios do estresse salino sobre as trocas gasosas e teores de clorofila *b* e proporcionou maior acúmulo de fitomassa seca de folha e raiz, aumentando também a relação raiz/parte aérea de tomate cereja, aos 120 dias após a semeadura.

**Palavras-chave:** *Solanum lycopersicum* L. Salinidade. Irrigação. Mitigação. Aclimação.

## Introduction

Tomato (*Solanum lycopersicum* L.) is a vegetable widely consumed and produced worldwide. In 2020, Brazil produced about 3,956,559 t of the fruit, ranking among the ten largest tomato producers (Instituto Brasileiro de Geografia e Estatística [IBGE], 2020). In northeastern Brazil, tomato production was 496,721 t, of which 241,200 t came from the main producing state, Bahia, from an area of 5,340 ha (IBGE, 2020).

As one of the major tomato groups sold in Brazil (Santa Cruz, Carmen, Italian, cherry, and industrial), cherry tomatoes are appreciated for their high antioxidant activity,

in addition to having high levels of nutrients and soluble solids, rendering them attractive and healthy appetizers in gastronomy (Londoño-Giraldo et al., 2020).

Despite the production potential of the Brazilian northeast, tomato growing is limited due to the use of saline water for irrigation. Its production cycle is drastically affected because the plant is sensitive to the effects of salts in water with electrical conductivity above  $2.5 \text{ dS m}^{-1}$  (P. R. F. Medeiros, Duarte, Uyeda, Silva, & Medeiros, 2012). Some studies have shown a reduction in the yield of tomato when the crop was subjected to salt stress; e.g., in conventional cultivation, Parvin et al. (2015) reported a 57.2% reduction in yield

when the crop was irrigated with water with 8.0 dS m<sup>-1</sup> salinity. Batista et al. (2021) evaluated the production of tomato cv. Caroline in a hydroponic system under salt stress (2.5 to 8.5 dS m<sup>-1</sup>) and found a 62% decrease in the yield of plants grown under higher salinity.

The salinity of irrigation water and the soil is one of the main abiotic stresses that lowers the productivity of arable areas to uneconomical levels. This phenomenon is particularly intense in irrigated soils in semi-arid regions, where water with high levels of salts is used to meet the water needs of crops as a way to address the scarcity of quality water (Machado & Serralheiro, 2017; Zörb, Geilfus, & Dietz, 2019).

In recent years, there has been an expansion in vegetable growing in protected environments. However, the accumulation of salts in the soil, resulting from the use of fertilizers and the lack of leaching, has become a limiting factor for production in this cropping system (Azevedo, Oliveira, Martins, Silva, & Ribeiro, 2018). Lima et al. (2016) investigated irrigation with saline water (0.6 and 3.0 dS m<sup>-1</sup>) in the cultivation of 'All Big' peppers in a protected environment and found that increasing water salinity negatively affected the growth and production of the plants.

Exogenous application of salicylic acid (SA) to plants is an alternative to induce tolerance to salt stress in an attempt to ensure agricultural activities in regions with quantitative and qualitative shortages of water resources. This organic acid is a secondary metabolite produced by the plant organism that is responsible for numerous regulatory functions such as floral induction, growth, enzyme biosynthesis, stomatal movements, membrane protection, and cellular respiration.

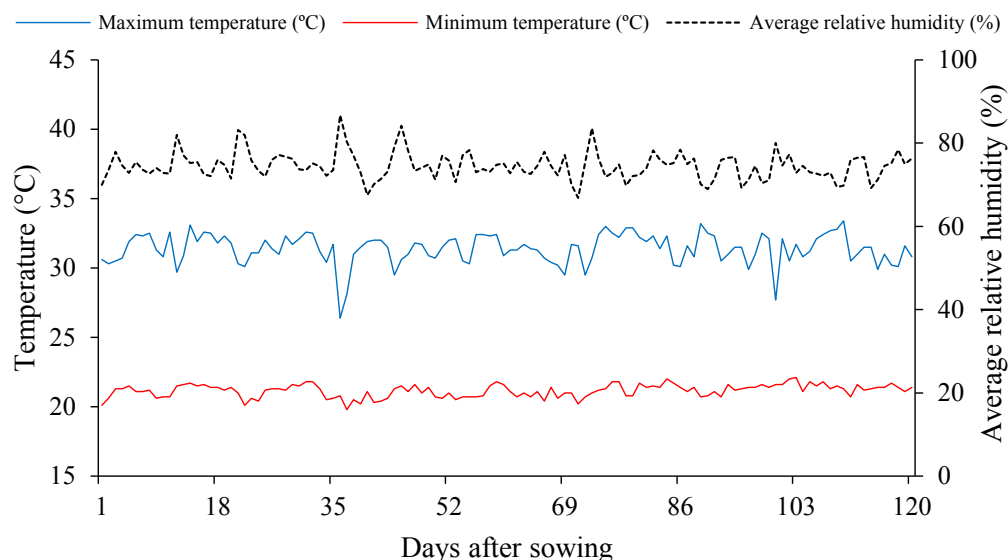
Additionally, SA acts as a signaling molecule that induces tolerance to biotic and abiotic stresses (Souri & Tohidloo, 2019; Horváth et al., 2015).

Salicylic acid can be applied through different methods (soaking, spraying, via the root, or associations between these), yet the approach that most reduces salt stress in plants is still not known. There are reports that foliar application of SA in Mulungu attenuated the initial fluorescence of the plants (Figueiredo et al., 2019). Soaking pumpkin seeds in SA improved germination kinetics and mitigated the effects of water salinity during the initial growth of pumpkin seedlings (Guirra et al., 2020). In tomato, foliar application of SA has been shown to generally induce tolerance to salt stress (Gharbi, Lutts, Dailly, & Quinet, 2018).

Given the above-described context, the present study was developed to evaluate the effects of different methods of application of SA on photosynthetic pigments, chlorophyll a fluorescence, gas exchange, and biomass accumulation of cherry tomato under salt stress.

## Material and Methods

The experiment was carried out from October 2020 to February 2021 in a protected environment (greenhouse) belonging to the Academic Unit of Agricultural Engineering (UAEA) at the Federal University of Campina Grande (UFCG), in Campina Grande - PB, Brazil (7°15'18" S, 35°52'28" W, 550 m asl). Figure 1 shows temperature (maximum and minimum) and average relative humidity data of the experimental site.



**Figure 1.** Air temperature (maximum and minimum) and mean relative humidity recorded inside the greenhouse during the experimental period.

Treatments resulted from the combination of two levels of electrical conductivity in the irrigation water ( $EC_w$ : 0.6 or 2.6  $dS\ m^{-1}$ ) and four SA application methods (M1 = no application of SA [control]; M2 = foliar spray; M3 = irrigation; or M4 = spray and irrigation), in a  $2 \times 4$  factorial arrangement distributed in a completely randomized design with five replicates. In both SA application methods, the concentration of 1 mM was used.

The SA concentrations adopted in both application methods were based on studies developed by Poursakhi, Razmjoo and Karimmojeni (2019) with the tomato crop. The water salinity levels, in turn, were defined according to Vieira, Nobre, Dias and Pinheiro (2016).

Cherry tomato cultivar Carolina was used. This material has indeterminate growth,

bears small, red fruits weighing between 10 and 12 g, and has a cycle of 110 to 120 days. The cultivar is resistant to verticillium wilt (*Verticillium albo-atrum* and *Verticillium dahliae*) and Fusarium (*Fusarium oxysporum*). In addition, the fruit has a longer shelf life, of around 18 days after harvest, and can be grown in small areas, reaching high yields with excellent financial returns (Matos et al., 2021).

For the experiment, polyethylene pots (Citropote<sup>®</sup>; 8  $dm^3$ ) were covered with a geotextile layer (Bidim OP 30) and filled with a layer of 0.3 kg of gravel (no. 0) followed by 8 kg of soil classified as Regosol (Psamments - United States, 2014). The soil was collected at a depth of 0-30 cm in the municipality of Riachão do Bacamarte - PB, Brazil. Physicochemical attributes of the soil (Table 1) were determined according to Teixeira, Donagemma, Fontana and Teixeira (2017).

**Table 1**  
**Chemical and physical attributes of the soil used in the experiment, before the application of treatments**

Chemical characteristics									
pH (H <sub>2</sub> O) (1:2.5)	OM dag kg <sup>-1</sup>	P (mg kg <sup>-1</sup> )	K <sup>+</sup> .....(cmol <sub>c</sub> kg <sup>-1</sup> )	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup> + H <sup>+</sup>	ESP (%)	ECse (dS m <sup>-1</sup> )
5.90	1.36	6.80	0.22	0.16	2.60	3.66	1.93	1.87	1.0
Physical-water characteristics									
Particle size fraction (g kg <sup>-1</sup> )			Texture class	Moisture (kPa)		AW .....	Total porosity %	AD	PD (kg dm <sup>-3</sup> )
Sand	Silt	Clay		33.42*	1519.5** dag kg <sup>-1</sup>				
732.9	142.1	125.0	SL	11.98	4.32	7.66	47.74	1.39	2.66

OM - organic matter: Walkley-Black Wet Digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1M KCl pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted using 1M NH<sub>4</sub>OAc pH 7.0; Al<sup>3+</sup> and H<sup>+</sup> extracted with 0.5M CaOAc pH 7.0; ESP - exchangeable sodium percentage; ECse - electrical conductivity of the saturation extract; SL - sandy loam; AW - available water; AD - apparent density; PD - particle density; \* - field capacity; \*\* - wilting point.

Irrigation waters with different levels of electrical conductivity were prepared by dissolving the NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O, and MgCl<sub>2</sub>·6H<sub>2</sub>O salts at the equivalent proportion of 7:2:1, respectively, in water from local supply (EC<sub>w</sub> = 0.32 dS m<sup>-1</sup>). This proportion is commonly found in water sources used for irrigation on small farms in northeastern Brazil (J. F. D. Medeiros, Lisboa, Oliveira, Silva, & Alves, 2003). In the preparation of irrigation water, we considered the ratio between EC<sub>w</sub> and the salt concentration (Richards, 1954), according to Eq. 1:

$$C \text{ (mmol}_c\text{L}^{-1}) = 10 \times \text{EC}_w \text{ (dS m}^{-1}) \dots \dots (1),$$

where C - concentration of salts to be added (mmol<sub>c</sub> L<sup>-1</sup>); and EC<sub>w</sub> - electrical conductivity of water (dS m<sup>-1</sup>).

Thirty days after sowing (DAS), irrigation with saline water was started in a two-day irrigation shift, applying water so as to maintain soil moisture close to field capacity. The volume to be applied was determined

according to the water requirement of the plants, which was estimated by the water balance, according to Eq. 2:

$$V_i = \frac{(V_p - V_d)}{(1 - LF)} \dots \dots \dots (2),$$

where V<sub>i</sub> - volume of water to be applied in irrigation (mL); V<sub>p</sub> - volume applied in the previous irrigation event (mL); V<sub>d</sub> - volume drained after previous irrigation event (mL); and LF - leaching fraction of 0.10, applied every 30 days.

The SA concentration was obtained from a dilution in 30% of ethyl alcohol (95.5%), as it is a substance with low solubility in water at room temperature. To reduce the surface tension of the droplets on the leaf surface, the adjuvant Wil fix was used in the preparation of the solution at a concentration of 0.5 mL L<sup>-1</sup> of solution.

Applications were started five days before the application of saline water; i.e., at 25 DAS, sprays were performed on the abaxial

and adaxial surfaces. Subsequent applications were carried out at 15-day intervals, until 85 DAS (Table 2), using a sprayer, between 17h00 and 17h30. The average volume applied per

plant in each spray was 70 mL. The plants subjected to application via irrigation were irrigated with a SA volume of 50 mL on the same days of spraying.

**Table 2**  
**Dates of application of salicylic acid by different delivery methods during the experiment**

APPLICATION	Method 1 (Control)	Method 2 (Spray)	Method 3 (Irrigation)	Method 4 (Spray+Irrigation)
1st	-	25 DAS (10/25/2020)	25 DAS (10/25/2020)	25 DAS (10/25/2020)
2nd	-	40 DAS (11/09/2020)	40 DAS (11/09/2020)	40 DAS (11/09/2020)
3rd	-	55 DAS (11/24/2020)	55 DAS (11/24/2020)	55 DAS (11/24/2020)
4th	-	70 DAS (12/09/2020)	70 DAS (12/09/2020)	70 DAS (12/09/2020)
5th	-	85 DAS (12/24/2020)	85 DAS (12/24/2020)	85 DAS (12/24/2020)

The area was topdressed with nitrogen, potassium, and phosphorus, as recommended by Novais, Neves and Barros (1991). For this step, 0.76 g urea, 2.0 g potassium chloride, and 3.87 g monoammonium phosphate were applied, corresponding to 100, 150, and 300 mg kg<sup>-1</sup> of N, K<sub>2</sub>O, and P<sub>2</sub>O<sub>5</sub>, respectively. These rates were split into four application events via fertigation, at 15-day intervals, the first taking place at 40 DAS.

A micronutrient solution of the commercial product Dripsol<sup>®</sup> micro (concentration: 1.0 g L<sup>-1</sup>) was applied fortnightly. The product contained 1.1% Mg, 4.2% Zn, 0.85% B, 3.4% Fe, 3.2% Mn, 0.5% Cu, and 0.05% Mo and was applied foliarly, on the adaxial and abaxial surfaces, using a backpack sprayer.

During the experiment, all the cultivation and phytosanitary treatments recommended for the crop were adopted; the emergence of pests and diseases was monitored; and control measures were taken when necessary.

At 100 DAS, the levels of photosynthetic pigments (chlorophylls a and b, total chlorophyll, and carotenoids) were quantified by the method proposed by Arnon (1949), whereby plant extracts are made from samples of discs of the blade of the third mature leaf from the apex. With these extracts, the concentrations of chlorophyll and carotenoids in the solutions were determined using the spectrophotometer at the absorbance wavelengths (ABS) of 470, 647, and 663 nm, using the following equations:

$$\text{Chlorophyll } a \text{ (Cl } a) = (12.25 \times \text{ABS}_{663}) - (2.79 \times \text{ABS}_{647}) \dots\dots\dots(3)$$

$$\text{Chlorophyll } b \text{ (Cl } b) = (21.5 \times \text{ABS}_{647}) - (5.10 \times \text{ABS}_{663}) \dots\dots\dots(4)$$

$$\text{Total chlorophyll (Cl } T) = (7.15 \times \text{ABS}_{663}) + (18.71 \times \text{ABS}_{647}) \dots\dots\dots(5)$$

$$\text{Carotenoids (Car)} = ((1000 \times \text{ABS}_{470}) - (1.82 \times \text{Cl } a) - (85.02 \times \text{Cl } b)) / 198\dots(6)$$

The *Cl a*, *Cl b*, *Cl T*, and carotenoid values found in the leaves will be expressed in mg g<sup>-1</sup> of fresh matter.

Gas exchange was evaluated at 100 DAS, by determining stomatal conductance (gs), transpiration (E), CO<sub>2</sub> assimilation rate (A), and intercellular CO<sub>2</sub> concentration (Ci). From these data, instantaneous water use efficiency (*iWUE*) (*A/E*) and instantaneous carboxylation efficiency (*iCE*) (*A/Ci*) were quantified.

Gas exchange measurements were performed on the third leaf, counted from the apex of the main branch of the plant. The irradiation of 1200 μmol photons m<sup>-2</sup> s<sup>-1</sup> and the air flow rate of 200 mL min<sup>-1</sup> were adopted, using the LCPro+ portable photosynthesis system (ADC BioScientific Ltd.).

Chlorophyll *a* fluorescence was also determined at 100 DAS on the same leaf used for gas exchange, using a OS5p pulse modulated fluorimeter (Opti Science) and applying the Fv/Fm protocol, to determine the following fluorescence induction variables: initial (Fo), maximum (Fm), and variable (Fv = Fm – Fo) fluorescence and quantum efficiency of photosystem II (Fv/Fm). This protocol was performed after adapting the leaves to the dark for a period of 30 min, using a clip of the equipment and ensuring that all acceptors were oxidized; i.e., with the reaction centers open.

At 120 DAS, the dry biomass of leaves (LDB), stems (SDB), and root (RDB) and the root/shoot ratio (R/S) were measured. To obtain the dry biomass, the stem of each plant was cut close to the ground and then the different parts (stem, leaf, and root) were separated and packed in a paper bag, which was then set to dry in a forced-air oven at a temperature of 65 °C until constant weight. Afterwards, the material was weighed, and the results represented LDB, SDB, and RDB.

Data were subjected to the distribution normality test (Shapiro-Wilk test) at a probability level of 0.05. Means referring to the levels of electrical conductivity in the water and SA application methods were compared by Tukey's test (p≤0.05), using SISVAR-ESAL statistical software (Ferreira, 2019).

## Results and Discussion

The interaction between salt levels (SL) and salicylic acid (SA) concentrations only significantly influenced chlorophyll *b* (Table 3). In contrast, the salt levels significantly affected all analyzed variables. Salicylic acid concentrations provided a significant effect on *Cl b*, *Cl T*, and Car.

**Table 3**

**Summary of analysis of variance for chlorophylls *a* (Cl *a*) and *b* (Cl *b*), total chlorophyll (Cl *T*), and carotenoids (Car) of cherry tomatoes irrigated with saline water and subjected to different methods of application of salicylic acid, at 100 days after sowing**

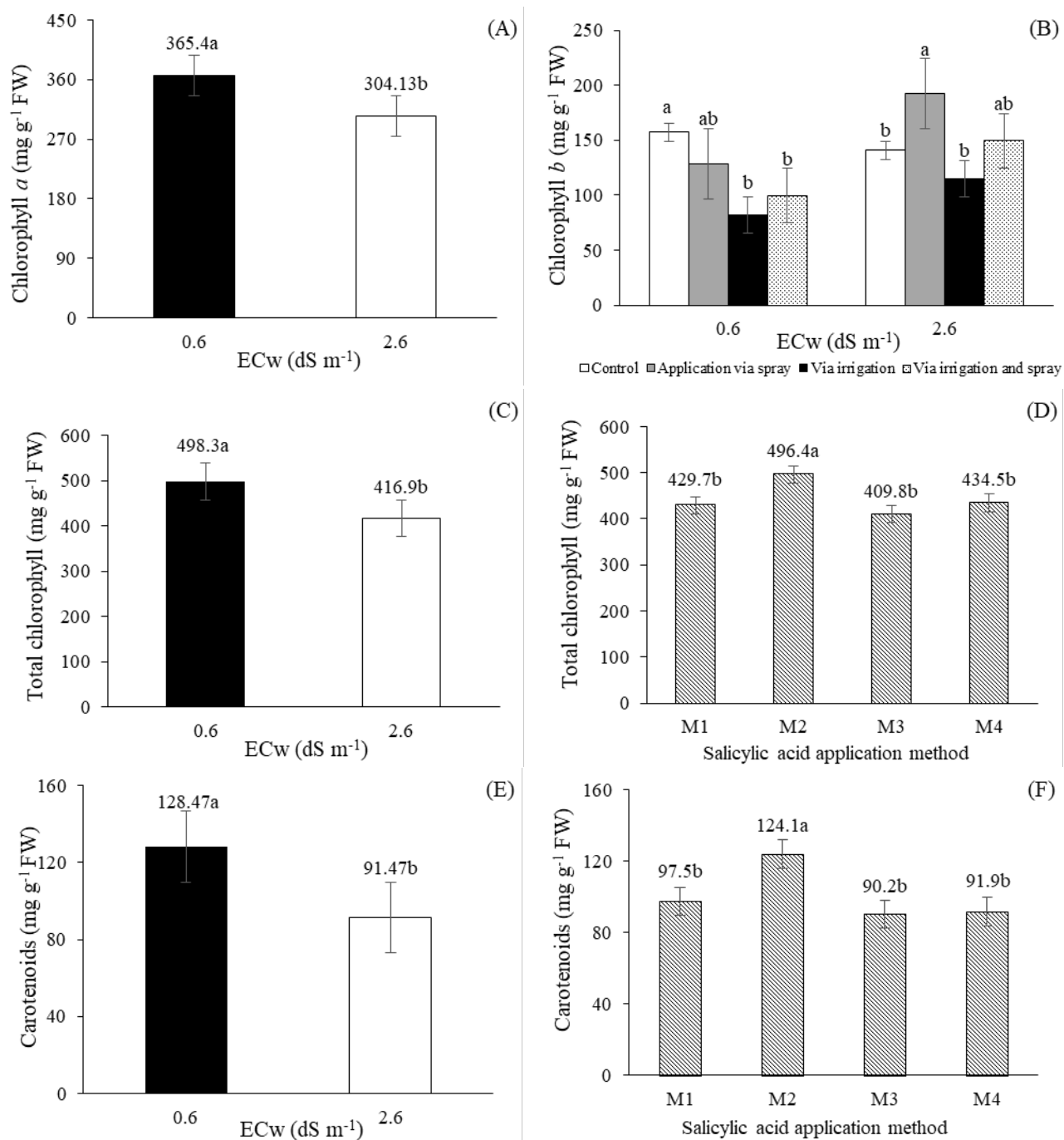
Source of variation	DF	Mean square			
		Cl <i>a</i>	Cl <i>b</i>	Cl <i>T</i>	Car
Salt level (SL)	1	37507.05**	10495.33**	66145.69**	3907.74*
Application method (AM)	3	10112.38 <sup>ns</sup>	3236.74**	17843.79*	2462.64*
Interaction (SL × AM)	3	6094.31 <sup>ns</sup>	7452.21**	1828.65 <sup>ns</sup>	1915.82 <sup>ns</sup>
Residual	32	3874.39	675.82	4333.53	651.41
CV (%)		18.59	19.51	14.39	24.78

ns, \*\*, \* not significant and significant at  $p \leq 0.01$  and at  $p \leq 0.05$ , respectively.

For Cl *a* (Figure 2A), Cl *T* (Figure 2C), and Car (Figure 2E), the cherry tomato plants irrigated with water with 2.6 dS m<sup>-1</sup> salinity showed reductions of 16.8% (61.3 mg g<sup>-1</sup> FW), 16.3% (81.4 mg g<sup>-1</sup> FW), and 28.8% (37 mg g<sup>-1</sup> FW), respectively, relative to the plants under irrigation with water with 0.6 dS m<sup>-1</sup> salinity. By analyzing chlorophyll *b* (Figure 2B), we observe that the plants of control treatment stood out in comparison with those that received the other SA application methods when cultivated

with low-salinity water (0.6 dS m<sup>-1</sup>). On the other hand, when exposed to salt stress, the plants of control treatment exhibited a 10.6% reduction (16.7 mg g<sup>-1</sup> FW) relative to those irrigated with water with 0.6 dS m<sup>-1</sup> salinity. However, SA application via foliar spray stood out when the cherry tomato plants were irrigated with higher-salinity water (2.6 dS m<sup>-1</sup>), significantly increasing Cl *b* by 36.8% (51.9 mg g<sup>-1</sup> FW) relative to the control plants irrigated with the same salt level.





**Figure 2.** Chlorophyll a (Cl a; A), total chlorophyll (Cl T; C), and carotenoids (Car; E) as a function of electrical conductivity in the irrigation water (ECw); chlorophyll b (Cl b; B) as a function of the interaction between salt levels and salicylic acid application methods; and total chlorophyll (Cl T; D) and carotenoids (Car; F) as a function of salicylic acid application methods, at 100 days after sowing.

Means followed by different letters differ significantly between treatments by Tukey's test ( $p \leq 5\%$ ). Vertical bars represent the standard error ( $n=5$ ). M1 - control (no application); M2 - application via spray; M3 - via irrigation; M4 - via irrigation and spray.

The reduction in chlorophyll levels in plants exposed to water salinity is probably due to the increase in the activity of the chlorophyllase enzyme, which degrades the molecules of this photosynthetic pigment, as observed by Dias, Lima, Pinheiro, Gheyi and Soares (2019) in the acerola tree. Salt stress reduces the production of photosynthetic pigments and induces the degradation of  $\beta$ -carotene, causing a decrease in the content of carotenoids, which are integrated components of thylakoids that act on the absorption and transfer of light to chlorophyll (A. A. R. Silva, Bezerra, Lacerda, Sousa, & Chagas, 2016).

The total chlorophyll (Figure 2D) and carotenoid (Figure 2F) levels of cherry tomatoes cultivated with foliar spray of SA (M2) were statistically superior to those that were subjected to the other SA application methods. Plants in the control treatment and those which received the application via spraying and irrigation simultaneously did not differ statistically from each other.

There was a significant SL  $\times$  SA interaction effect ( $p \leq 0.01$ ) on all gas exchange variables, except for the intercellular  $\text{CO}_2$  concentration (Table 4). Salt levels also significantly influenced gas exchange. The SA application methods significantly affected  $E$ ,  $A$ ,  $iCE$ , and  $iWUE$ .

**Table 4**

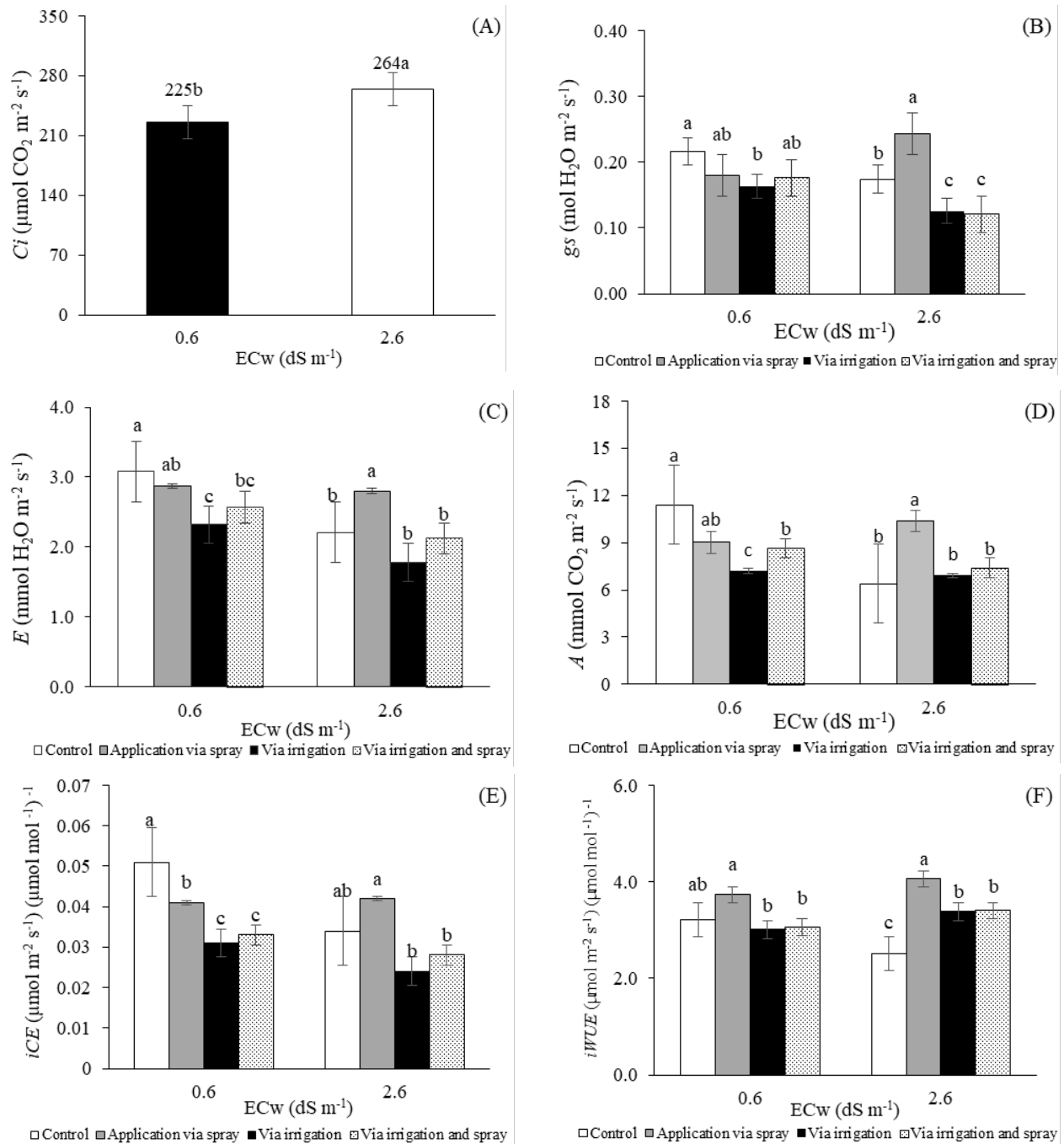
**Summary of analysis of variance for intercellular  $\text{CO}_2$  concentration ( $C_i$ ), stomatal conductance ( $g_s$ ), transpiration ( $E$ ),  $\text{CO}_2$  assimilation rate ( $A$ ), instantaneous carboxylation efficiency ( $iCE$ ), and instantaneous water use efficiency ( $iWUE$ ) of cherry tomato plants irrigated with saline water and subjected to different salicylic acid application methods, at 100 days after sowing**

Source of variation	DF	Mean square					
		$C_i$	$g_s$	$E$	$A$	$iCE$	$iWUE$
Salt level (SL)	1	14630.63**	0.014*	2.32**	9.02*	$9.1 \times 10^{-4}$ **	2.58**
Application method (AM)	3	439.43 <sup>ns</sup>	0.007 <sup>ns</sup>	1.02**	11.29**	$2.4 \times 10^{-4}$ **	4.03**
Interaction (NS $\times$ AM)	3	77.16 <sup>ns</sup>	0.014**	0.41**	20.63**	$3.5 \times 10^{-4}$ **	7.16**
Residual	32	288.38	0.004	0.04	1.99	$3.4 \times 10^{-5}$	0.24
CV (%)		6.93	27.77	7.83	16.17	16.04	13.55

ns, \*\*, \* not significant and significant at  $p \leq 0.01$  and at  $p \leq 0.05$ , respectively.

The intercellular  $\text{CO}_2$  concentration (Figure 3A) of tomatoes grown under water with  $2.6 \text{ dS m}^{-1}$  salinity was statistically higher than those subjected to irrigation with  $0.6 \text{ dS m}^{-1}$ . When we compare the  $C_i$  of plants irrigated

with water with higher salinity ( $2.6 \text{ dS m}^{-1}$ ) and those cultivated with the lowest salinity level ( $0.6 \text{ dS m}^{-1}$ ) in relative terms, an increase of 17.3% ( $39 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) is observed.



**Figure 3.** Intercellular  $\text{CO}_2$  concentration ( $C_i$ ; A) as a function of the electrical conductivity of irrigation water (ECw); stomatal conductance ( $g_s$ ; B), transpiration ( $E$ ; C),  $\text{CO}_2$  assimilation rate ( $A$ ; D), instantaneous carboxylation efficiency ( $iCE$ ; E), and instantaneous water use efficiency ( $iWUE$ ; F) of tomato as a function of the interaction between salt levels and salicylic acid application methods, at 100 days after sowing.

Means followed by different letters differ significantly between treatments by Tukey's test ( $p \leq 5\%$ ). Vertical bars represent the standard error ( $n=5$ ).

Overall, by examining the variables of *gs*, *E*, *A*, *iCE*, and *iWUE* (Figure 3B to 2F), we note that the plants of control treatment stood out over those under the other SA application methods when grown under low-salinity water (0.6 dS m<sup>-1</sup>). When irrigated with water with electrical conductivity of 2.6 dS m<sup>-1</sup>, the plants of control treatment exhibited reductions of 19.4, 28.2, 43.9, 33.3, and 21.8% in *gs*, *E*, *A*, *iCE*, and *iWUE*, respectively.

Nonetheless, the foliar spray application method was highlighted when the tomato plants were irrigated with higher-salinity water (2.6 dS m<sup>-1</sup>), having their *gs*, *E*, *A*, *iCE*, and *iWUE* increased by 39, 6, 26.7, 62.0, 23.5, and 61.8%, respectively, in relation to the control plants irrigated with the same saline level. These results show that foliar application of SA can improve gas exchange in tomato plants under salt stress.

Stomatal closure is an early response of plants to salt stress, in an attempt to reduce water loss and, consequently, the absorption of salts from the soil solution without compromising photosynthetic activity (Dias et al., 2018; Bezerra et al., 2018a). The results of this study are in agreement with the reports by Martínez-Cuenca et al. (2020), who evaluated the effects of salt stress on cherry tomato plants and found reductions in gas exchange under salt stress conditions.

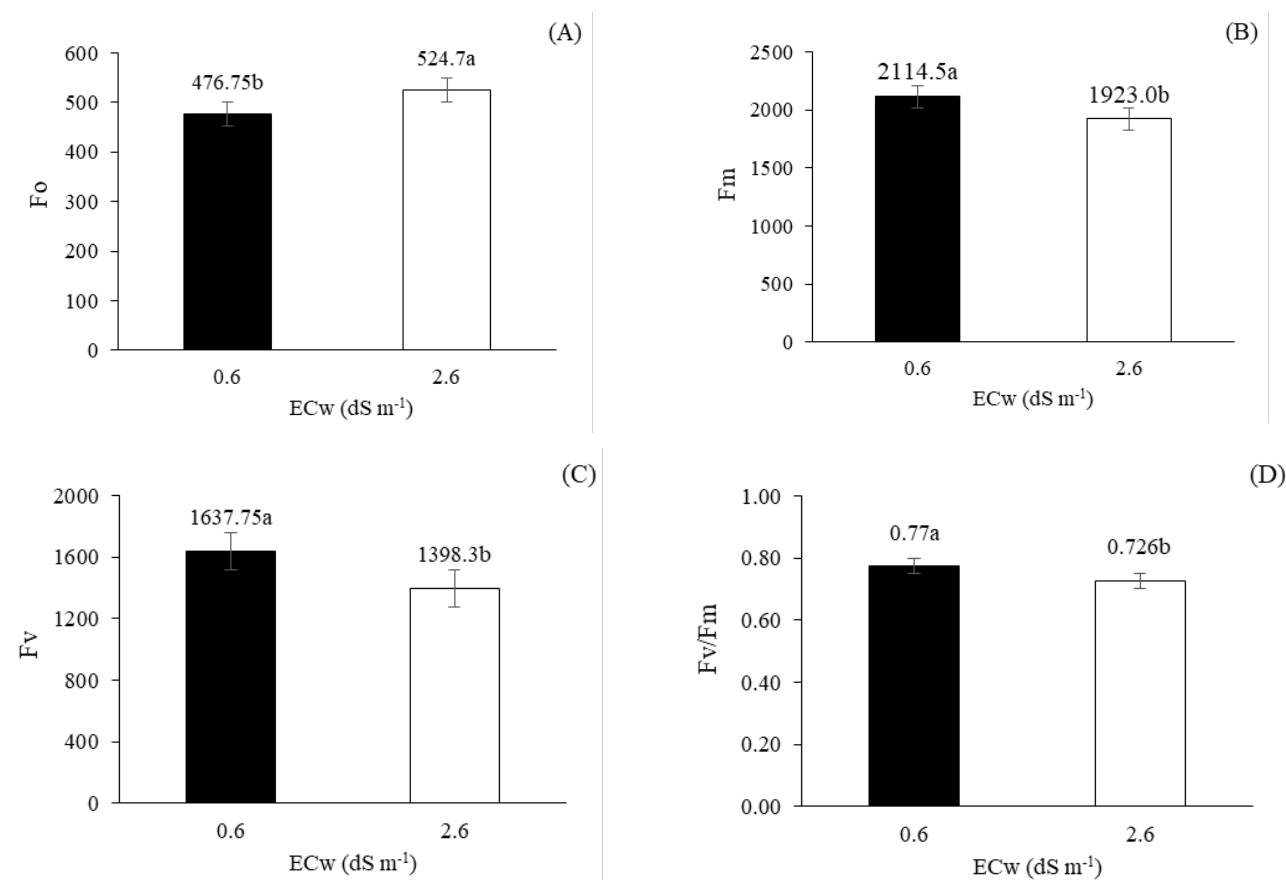
The reductions in gas exchange under salt stress conditions can be attributed to the degradation of photosynthetic pigments (Figure 2), as they are an integral part of the light capture complex for the photosynthesis

process, reductions in chlorophyll fluorescence (Figure 4), PSII photoinhibition, changes in the membrane-bound ATPase enzyme complex, and a decrease in Rubisco activity (Lawlor & Cornic, 2002).

However, the application of SA via foliar spray was able to attenuate the deleterious effects of salinity on chlorophyll *b* (Figure 2B) and on the gas exchange variables (Figure 3) of the cherry tomatoes. Khan, Asgher and Khan (2014) and Ahmad et al. (2018) described that SA increases pigment content, improves transpiration rates and photosynthetic electron transport, maintains antioxidant enzyme activities, and improves photosynthetic activity under salt stress.

The application of SA via foliar spray increased the *iWUE* of the cherry tomato plants regardless of the irrigation water salinity level, although the effect of SA on the increase in *iWUE* under higher salinity (2.6 dS m<sup>-1</sup>) was greater than under low salinity (0.6 dS m<sup>-1</sup>). Salicylic acid is an endogenous phenolic-type regulator that regulates the physiological and biochemical processes of plants to alleviate the deleterious effects of various stresses, such as salinity (Ghassemi-Golezani, Farhangi-Abriz, & Bandehagh, 2018).

There was a significant difference in the irrigation water salinity levels for the chlorophyll *a* fluorescence variables (Table 5). On the other hand, SA application methods and the interaction between application methods and SL did not significantly influence the studied variables, at 100 days after sowing.



**Figure 4.** Initial fluorescence (Fo; A), maximum fluorescence (Fm; B), variable fluorescence (Fv; C), and quantum efficiency of photosystem II (Fv/Fm; D) of tomato as a function of the electrical conductivity in the irrigation water (ECw), at 100 days after sowing. Means followed by different letters differ significantly between treatments by Tukey's test ( $p \leq 5\%$ ). Vertical bars represent the standard error ( $n=5$ ).

**Table 5**

**Summary of analysis of variance for initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum efficiency of photosystem II (Fv/Fm) in cherry tomato plants irrigated with saline water and subjected to different salicylic acid application methods, at 100 days after sowing**

Source of variation	DF	Mean square			
		Fo	Fm	Fv	Fv/Fm
Salt level (SL)	1	22992.03**	366722.50**	573363.02**	$2.29 \times 10^{-2**}$
Application method (AM)	3	3720.89 <sup>ns</sup>	14685.9 <sup>ns</sup>	10182.29 <sup>ns</sup>	$6.9 \times 10^{-4ns}$
Interaction (NS × AM)	3	365.09 <sup>ns</sup>	19389.37 <sup>ns</sup>	17032.63 <sup>ns</sup>	$2.6 \times 10^{-4ns}$
Residual	32	838.81	6449.85	7909.72	$3.5 \times 10^{-4}$
CV (%)		5.78	3.98	5.86	2.48

ns, \*\*, \* not significant and significant at  $p \leq 0.01$  and at  $p \leq 0.05$ , respectively.

The initial fluorescence of cherry tomatoes increased as a function of irrigation, with a 10% increase (48) achieved at  $2.6 \text{ dS m}^{-1}$  relative to the plants cultivated with  $\text{EC}_w$  of  $0.6 \text{ dS m}^{-1}$  (Figure 4A). In contrast to  $F_o$ , the maximum fluorescence of cherry tomatoes decreased significantly with increasing salinity of irrigation water (Figure 4B). Plants irrigated with water with  $2.6 \text{ dS m}^{-1}$  salinity showed a reduction of 9.06% compared with those irrigated with low-salinity water ( $0.6 \text{ dS m}^{-1}$ ); i.e., a decrease of 191.5. Fluorescence (Figure 4C) and the quantum efficiency of photosystem II (Figure 4d) followed the same trend observed for maximum fluorescence. Plants irrigated with water with  $0.6 \text{ dS m}^{-1}$  salinity were superior by 17.12% (239.5) in Fv and by 6.61% (0.048) in Fv/Fm (Figure 4C and 4D) compared with those irrigated with high-salinity water ( $2.6 \text{ dS m}^{-1}$ ).

Initial fluorescence is associated with the oxidation capacity of quinone; therefore, the greater the increase in  $F_o$ , the lower the ability of quinone to receive electrons and consequently transfer energy to photosystem II (PSII) (Sá et al., 2018). Thus, the increase in  $F_o$  values under salt stress conditions may be a consequence of damage to the PSII reaction center or a reduction in the excitation capacity to transfer energy to the reaction center (Baker, 2008).

The decrease in maximum fluorescence with increasing water salinity may be an indication of little efficiency in quinone photoreduction and in the flow of electrons between the photosystems. This results in

low activity of PSII in the thylakoid membrane, which directly influences the electron flow between photosystems (M. M. P. Silva et al., 2006; Tatagiba, Moraes, Nascimento, & Peloso, 2014). According to Zanandrea et al. (2006), the fluorescence variable represents the flow of electrons from the reaction center of photosystem II to plastoquinone. Any change in  $F_o$  or  $F_m$  causes a decrease in the primary electron acceptor quinone of photosystem II.

Irrigation water salinity also negatively affected the quantum efficiency of photosystem II of the cherry tomato plants, as measured by the Fv/Fm ratio. As stated by Melo et al. (2010), Fv/Fm refers to the excitation energy transfer capacity and expresses the efficiency of capturing this energy by the open reaction centers of PSII. It is thus clear that there was damage to PSII, since the Fv/Fm ratio found in plants irrigated with  $2.6 \text{ dS m}^{-1}$  salinity was below the index ( $0.75 \text{ electrons quantum}^{-1}$ ) considered as the threshold to damage the photosynthetic apparatus of plants (Santos et al., 2010). When the photosynthetic apparatus is intact, Fv/Fm values vary between 0.75 and  $0.85 \text{ electrons quantum}^{-1}$  (Reis & Campostrini, 2011).

There was a significant effect of the interaction between salt levels and SA application methods only on the dry biomass of leaves and roots (Table 6). Salinity levels significantly affected all analyzed variables. On the other hand, the SA application methods significantly affected all variables, except stem dry biomass.

**Table 6**

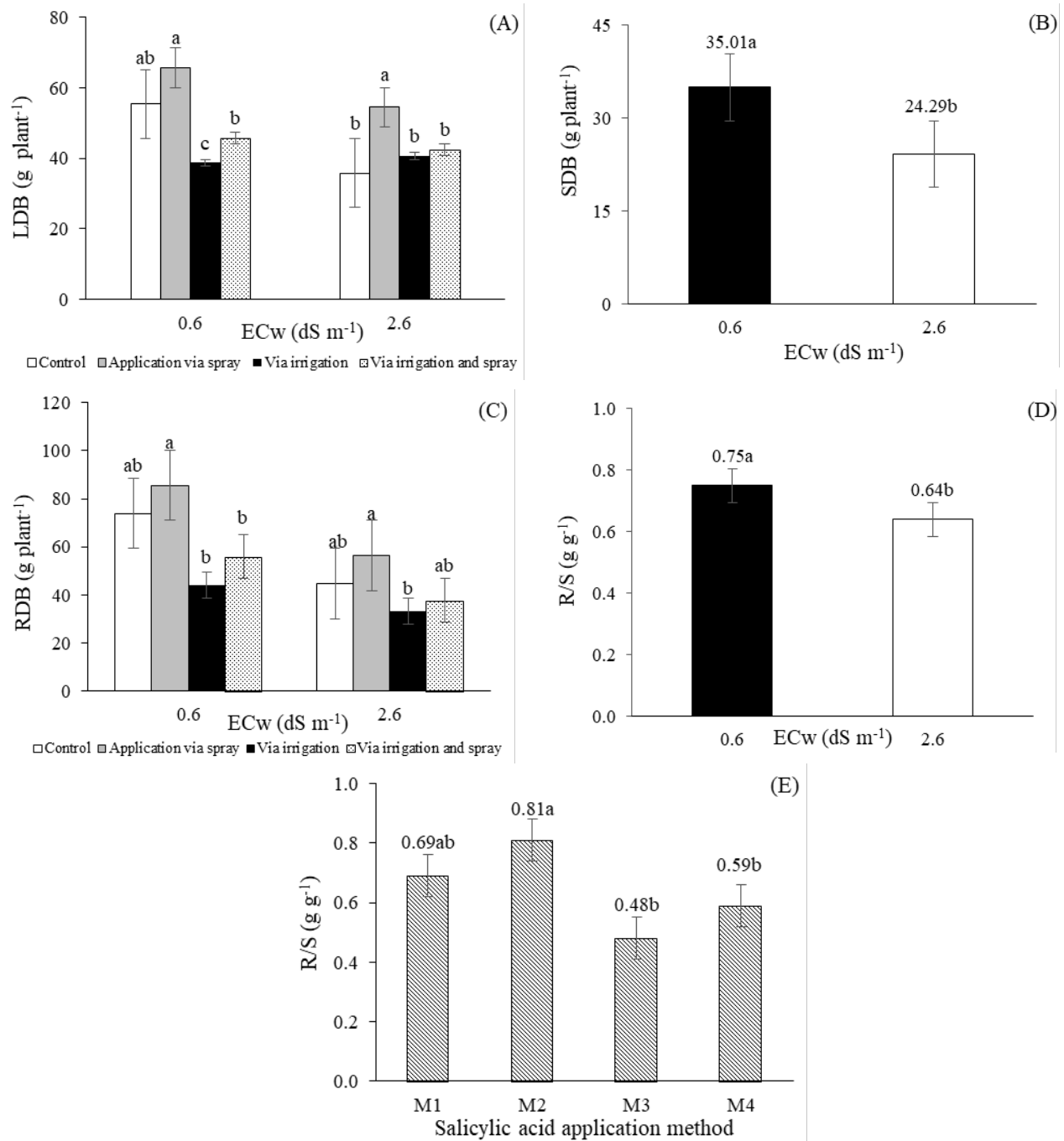
**Summary of analysis of variance for dry biomass of leaves (LDB), stems (SDB), and roots (RDB) and root/shoot ratio (R/S) of cherry tomato plants irrigated with saline water and subjected to different salicylic acid application methods, at 120 days after sowing**

Source of variation	DF	Mean square			
		LDB	SDB	RDB	R/S
Salt level (SL)	1	728.80**	1145.97**	5447.79**	0.03*
Application method (AM)	3	712.71**	26.79 <sup>ns</sup>	1687.53**	0.26**
Interaction (NS × AM)	3	309.65*	21.14 <sup>ns</sup>	757.17**	0.01 <sup>ns</sup>
Residual	32	109.54	24.51	111.37	0.005
CV (%)		21.86	16.70	19.32	9.98

ns, \*\*, \* not significant and significant at  $p \leq 0.01$  and at  $p \leq 0.05$ , respectively.

Irrigation with water with  $2.6 \text{ dS m}^{-1}$  salinity negatively affected the leaf (Figure 5A) and root (Figure 5B) dry biomass accumulation of the cherry tomatoes, regardless of SA application method. However, SA application via foliar spray stood out relative to the other methods, especially when the plants were irrigated with water with  $2.6 \text{ dS m}^{-1}$  salinity, as they showed increases of 52.4% ( $18.8 \text{ g plant}^{-1}$ ) in LDB and 26.1% ( $11.7 \text{ g plant}^{-1}$ ) in SDB compared with the control plants grown at the same salt level.

Fariduddin, Khan, Yusuf, Aafaqee and Khalil (2018) obtained similar results in an evaluation of the effect of foliar spray with SA ( $10^{-5} \text{ M}$ ) on tomato plants under salt stress ( $200 \text{ mM NaCl}$ ). The authors found that the application of SA provided an increase in the dry and fresh matter of both roots and shoots when compared with the control treatment. Salicylic acid is a natural phenolic compound that is involved in plant growth and in physiological processes that contribute to acclimatization to salt stress (A. A. R. Silva et al., 2021).



**Figure 5.** Dry biomass of leaves (LDB; A) and root (RDB; C) of tomato as a function of the interaction between the levels of electrical conductivity in irrigation water (ECw) and salicylic acid application methods, stem dry biomass (SDB; B) and root/shoot ratio (R/S; D) as a function of ECw, and R/S (E) as a function of salicylic acid application methods, at 100 days after sowing.

Means followed by different letters differ significantly between treatments by Tukey's test ( $p \leq 5\%$ ). Vertical bars represent the standard error ( $n=5$ ). M1 - control (no application); M2 - application via spray; M3 - via irrigation; M4 - via irrigation and spray.



For stem dry biomass (Figure 5B), the cherry tomato plants irrigated with water with  $0.6 \text{ dS m}^{-1}$  salinity stood out with the largest accumulation relative to those subjected to the EC<sub>w</sub> of  $2.6 \text{ dS m}^{-1}$ . Between the plants irrigated with low-salinity water ( $0.6 \text{ dS m}^{-1}$ ) and those irrigated with higher salinity ( $2.6 \text{ dS m}^{-1}$ ), a superiority of  $10.72 \text{ g plant}^{-1}$  is observed in SDB for the former.

The decrease in the biomass accumulation in the plants irrigated with water with  $2.6 \text{ dS m}^{-1}$  salinity may be related to the high concentrations of salts present in the irrigation water, which caused ionic, osmotic, hormonal, and nutritional changes, thereby restricting water absorption and negatively affecting the growth and development of crops (Lima et al., 2016; Bezerra et al., 2018b).

In this study, spray application of SA acid mitigated the deleterious effects of salinity on *gs*, *E*, *A*, *iCE*, and *iWUE*. This response resulted in the accumulation of LDB and RDB. Increases in salinity hinder the absorption of water by the roots and, consequently, the entry of diluted SA in solution. However, the plants probably had a higher uptake of SA via foliar application. According to Farhadi and Ghassemi-Golezani (2020), foliar spray with SA is an easy and important way to improve the growth and yield of plants under salt stress. The enhancing effects of SA in different plant species under biotic and abiotic stresses may be due to its role in improving the plant's antioxidant capacity and protecting membranes against deterioration (Esan, Masisi, Dada, & Olaiya, 2017).

The root/shoot ratio of the cherry tomatoes (Figure 5D) differed statistically when the plant was irrigated with different salinity

levels. Irrigation with water with  $2.6 \text{ dS m}^{-1}$  salinity caused a 14.7% decrease ( $0.11 \text{ g g}^{-1}$ ) in R/S compared with plants under irrigation with EC<sub>w</sub>  $0.6 \text{ dS m}^{-1}$ . The R/S of the cherry tomato plants (Figure 5E) cultivated without SA application (M1) and with foliarly sprayed with SA (M2) differed significantly relative to the other SA application methods. Application via irrigation (M3) and simultaneous use of irrigation and spraying (M4) did not differ statistically from each other.

## Conclusions

Irrigation with water with  $2.6 \text{ dS m}^{-1}$  salinity negatively affects chlorophyll a fluorescence and the chlorophyll *a*, total chlorophyll, and carotenoid contents, besides inhibiting dry stem biomass production and the root/shoot ratio. The method of application of salicylic acid via foliar spray minimizes the deleterious effects of salt stress on gas exchange and chlorophyll *b* and induces greater accumulation of leaf and root dry biomass as well as increases the root/shoot ratio of cherry tomatoes, at 120 days after sowing.

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