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Attenuation of salt stress on the physiology and production of bell peppers by treatment with salicylic acid

Atenuação do estresse salino na fisiologia e produção de pimentões por tratamento com ácido salicílico

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Highlights

Salt stress reduces chloroplast pigments in plants and physical quality of fruits.
Gas exchanges are drastically affected by salinity in the water.
The 1.7 mM salicylic acid concentration minimizes deleterious effects of salinity.

Abstract

The use of saline water for irrigation in semi-arid regions has become a reality due to the water scarcity that occurs in most of the year. In this scenario, exogenous application of salicylic acid may be a strategy to mitigate the deleterious effects of salt stress on plants and ensure the production of socioeconomically important crops in the semiarid region of Northeast Brazil, such as bell pepper. Thus, this study examines the osmoprotective effect of salicylic acid on gas exchanges, chloroplast pigments and production components of 'All Big' bell pepper plants irrigated with water with different saline levels. The experiment was carried out in greenhouse conditions in Campina Grande - PB, Brazil. Treatments consisted of four levels of electrical conductivity on the irrigation water (0.8, 1.6, 2.4 and 3.2 dS m^{-1}) and four concentrations of salicylic acid (0, 1.2, 2.4 and 3.6 mM), which were distributed in a 4 × 4 factorial arrangement in a randomized block design with three replicates. Increases in irrigation water salinity from 0.8 dS m^{-1} resulted in changes in gas exchange and total chlorophyll levels of 'All Big' bell pepper plants. The estimated salicylic

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acid concentration of 1.7 mM reduced the effects of salinity on stomatal conductance, transpiration, CO_2 assimilation rate, instantaneous carboxylation efficiency, total chlorophyll and fruit diameters. Irrigation with water of 1.8, 0.8 and 1.6 dS m^{-1} salinity associated with the estimated salicylic acid concentration of 1.6 mM increased the biosynthesis of chlorophylls *a* and *b* and the number of fruits, respectively, in bell pepper plants.
Key words: *Capsicum annuum* L. Salt stress. Attenuation.

Resumo

A utilização de água salina para irrigação, em regiões semiáridas, tem se tornado uma realidade em função da escassez hídrica que ocorre na maior parte do ano. Assim, a aplicação exógena de ácido salicílico pode ser uma estratégia capaz de amenizar os efeitos deletérios do estresse salino sobre as plantas e garantir a produção agrícola de culturas socioeconomicamente importantes no semiárido do Nordeste brasileiro, como o pimentão. Deste modo, objetivou-se com esta pesquisa avaliar a capacidade osmoprotetora do ácido salicílico sobre as trocas gasosas, pigmentos cloroplastídicos e componentes de produção de plantas de pimentão 'All Big' irrigadas com águas de diferentes níveis salinos. O experimento foi conduzido em condições de casa de vegetação, em Campina Grande-PB. Os tratamentos foram distribuídos no delineamento de blocos casualizados, em esquema fatorial 4 x 4, correspondendo a quatro níveis de condutividade elétrica da água de irrigação - CEa (0,8, 1,6; 2,4 e 3,2 dS m^{-1}) e quatro concentrações de ácido salicílico - AS (0; 1,2; 2,4 e 3,6 mM), com três repetições. O aumento da salinidade da água de irrigação a partir de 0,8 dS m^{-1} resultou em alterações nas trocas gasosas e nos teores de clorofila total das plantas de pimentão 'All Big'. A concentração estimada em 1,7 mM de ácido salicílico amenizou os efeitos da salinidade sobre a condutância estomática, a transpiração, a taxa de assimilação de CO_2, a eficiência instantânea da carboxilação, clorofila total e os diâmetros dos frutos. A irrigação com água de 1,8, 0,8 e 1,6 dS m^{-1}, associada a concentração estimada de 1,6 mM de ácido salicílico incrementou respectivamente, a biossíntese de clorofila *a* e *b*, e o número de frutos em plantas de pimentão.
Palavras-chave: *Capsicum annuum* L. Estresse salino. Atenuação.

Introduction

Excess of salts in water and soil is one of the main abiotic restrictions in global agricultural production, especially in semiarid regions (Minhas, Ramos, Ben-Galc, & Pereira, 2020). Therefore, many of these regions use groundwater for irrigation, as is the case of the Brazilian Northeast. However, well waters with high salt concentrations are not unusually discovered, since 80% of the geological makeup of the northeast region consist of crystalline rocks (Cirilo, Montenegro, & Campos, 2010).

In general, plants irrigated with high-salinity water tend to exhibit morphological, physiological, biochemical and molecular alterations to survive in stressful environments, through mechanisms of accumulation of osmoprotectants, water use efficiency, enhanced photosynthesis, detoxification of reactive oxygen species (ROS) and phytohormone induction (Evelin, Devi, Gupta, & Kapoor, 2019; Dias et al., 2018a; Fricke, 2020). Nevertheless, these adaptive strategies are inefficient to cope with the osmotic and ionic effects, which induce a reduction in chloroplast pigments and in the photosynthesis, growth and production of crops (Yang et al., 2020).

Oliveira et al. (2018) found that irrigation with 4.5 dS m-1 saline water reduces growth and production in bell pepper. The authors stated that the highest fruit yields were achieved with the use of low-salinity water, a mixture of non-saline and saline waters and fortnightly alternation of high-salinity water.

Several strategies, including physico-chemical treatment methods, have been applied to increase the tolerance of plants to salinity, e.g., exogenous application of salicylic acid (SA) (Karlidag, Yildirim, & Turan, 2009; Nazar, Umar, Khan, & Sareer, 2015; Farhadi & Ghassemi-Golezani, 2020; Pizolato et al., 2020).

Salicylic acid (SA) is a water-soluble secondary metabolite and a phenolic compound produced by the plant organism (Souri & Tohidloo, 2019). It acts in the regulation of growth, development, ripening, and as a bio-messenger or signaling agent in plants that promotes tolerance to various biotic and abiotic stresses (Sheteiwy et al., 2019). According to Hara, Furukawa, Sato, Mizoguchi and Miura (2012), low SA concentrations can enhance antioxidant capacity in plants, whereas high concentrations can cause cell death or susceptibility to abiotic stresses such as salt stress.

In the scientific literature, studies focusing on the action of exogenous SA application on the physiology and production of bell pepper plants grown with saline water are incipient. The bell pepper (*Capsicum annuum* L.) is a vegetable of the nightshade family. This crop is considered to have great economic importance due to its wide consumption and production besides ease of cultivation in small areas and rapid return on investments due to its short cycle. According to Food and Agriculture Organization of the United Nations [FAOSTAT] (2020), Brazilian bell pepper production in 2017 was 79.37 t (Santos, Divincula, Santos, Vieira, & Carneiro, 2020).

Under the hypothesis that the exogenous foliar application of SA attenuates the deleterious effects of salinity in the cultivation of 'All Big' bell pepper, this study examines the effect of SA concentrations on the physiology and production of bell pepper irrigated with saline water.

Material and Methods

The experiment was conducted in greenhouse conditions, using 8-L plastic pots, at the Agricultural Engineering Academic Unit of the Federal University of Campina Grande (UAEA/UFCG), located in Campina Grande - PB, Brazil, (7°15'18" S and 35°52 '28" W, 550 m above sea level).

A randomized-block design was adopted with a 4 × 4 factorial arrangement corresponding to four levels of electrical conductivity of the irrigation water (ECw; 0.8, 1.6, 2.4 and 3.2 dS m^{-1}) and four concentrations of SA (0, 1.2, 2.4 and 3.6 mM), with three replicates, totaling 48 plants. The salinity levels were determined based on the study developed by Lima et al. (2016), whereas the SA concentrations were based on the study led by Abbaszadeh, Layeghhaghighi, Azimi and Hadi (2020).

The bell pepper used in the experiment was 'All Big', a high-yielding hybrid belonging to the *Casca dura* group with upright growth, small size, firm and thick pulp, a sweet flavor and a cycle of around 120 days. The hybrid is also tolerant to *Phytophthora capsici* rot and the tomato mosaic virus (Lima et al., 2016).

The pots used in the experiment were filled with 8 kg of Regosol of sandy-loam texture. In each pot, 300 g of gravel (no. 0), wrapped in non-woven geotextile (Bidim OP 30), were placed at the bottom to facilitate the passage of the drained water through the holes at the base of the pots. Beneath each pot was a stainless-steel gutter with a small slope that directed the drained water to a collector attached to the end of each gutter.

The soil used was collected at a depth of 0-30 cm, from the municipality of Lagoa Seca - PB. After grinding and sieving, the soil was characterized as to physico-chemical attributes (Table 1), following methodologies of Teixeira, Donagemma, Fontana and Teixeira (2017).

The irrigation waters were prepared by dissolving the NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ salts, at the equivalent ratio of 7:2:1, respectively, in local supply water (ECw = 0.38 dS m^{-1}). This ratio is commonly found in water sources used for irrigation on small farms in the northeast region of Brazil, based on the ratio between ECw and the concentration of salts (mmolc L^{-1} = 10*ECw dS m^{-1}) as established by Richards (1954).

Irrigation with the saline waters was carried out manually and daily, by applying the amount corresponding to that obtained by the water balance. The water volume to be applied on the plants was determined by Eq. 1:

$$VI = \frac{(Va - Vd)}{(1 - LF)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where VI = volume of water to be applied in the next irrigation event (mL); Va and Vd = volumes applied and drained, respectively, in the previous irrigation event (mL); and LF = leaching fraction of 0.15, applied every 15 days.

Table 1
Chemical and physical-water attributes of the soil used in the experiment before the treatments were applied

Chemical characteristics									
pH (H_2O) (1:2.5)	OM %	P (mg kg^{-1})	K+	Na+	Ca^{2+}	Mg^{2+}	Al^{3+} + H^+	ESP (%)	ECw (dS m^{-1})
			..............................(cmolc kg^{-1})						
5.90	1.36	6.80	0.22	0.16	2.60	3.66	1.93	1.87	1.0
Physical-water properties									
Physical-water properties			Texture class	Moisture (kPa)		AW	Total porosity %	AD	PD
Sand	Silt	Clay		33.42	1519.5 dag kg^{-1}	....		(g cm^{-3})	
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^{2+} and Mg^{2+} extracted with KCl 1 mol L^{-1} pH 7.0; Na^+ and K^+ extracted using NH_4OAc 1 mol L^{-1} pH 7.0; Al^{3+} and H^+ extracted with 1 mol L^{-1} calcium acetate, pH 7.0; ESP - exchangeable sodium percentage; ECe - electrical conductivity of the saturation extract; SL - sandy loam; AW - available water; AD - apparent density; PD - particle density.

The SA concentrations were obtained by diluting the SA in 30% alcohol and 70% distilled water. This alcohol percentage was insufficient to cause damage to plants, due to the high water volume used to dilute the solution. Only distilled water was used in the 0 mM concentration. Foliar applications were performed at 25, 40, 55 and 70 days after sowing (DAS), by spraying on the abaxial and adaxial faces of the leaves using a backpack sprayer, between 17h00 and 17h45. Once flower buds emerged, the applications of SA were interrupted.

The NPK fertilization recommendation of Novais, Neves and Barros (1991) for pot experiments was adopted, corresponding to 100 mg N, 300 mg P_2O_5 kg^{-1} and 150 mg K_2O kg^{-1} of soil. Urea was used as a source of N, monoammonium phosphate as phosphorus and potassium chloride as potassium. Fertilization with N and P_2O_5 was divided into five applications, which were performed at 15, 30, 45, 60 and 75 DAS; and K_2O in three applications, at 45, 60 and 75 DAS. To meet the micronutrient requirements, a nutrient solution containing 2.5 g L^{-1} of a commercial product with the following composition was applied to the adaxial and abaxial surfaces every two weeks: N (15%), P_2O_5 (15%), K_2O (15%), Ca (1%), Mg (1.4%), S (2.7%), Zn (0.5%), B (0.05%), Fe (0.5%), Mn (0.05%), Cu (0.5%) and Mo (0.02%).

Cultivation practices consisted of manual weeding and, whenever necessary, application of insecticides of the Neonicotinoid chemical group, fungicide of the Triazole chemical group and acaricide of the Abamectin chemical group.

At 80 days after sowing (DAS), stomatal conductance (gs; mol H_2O m^{-2} s^{-1}), transpiration (E; mmol H_2O m^{-2} s^{-1}), CO_2 assimilation rate (A; μmol CO_2 m^{-2} s^{-1}) and internal CO_2 concentration (Ci; μmol CO_2 mol^{-1}) were determined using a portable infrared gas analyzer (IRGA; LCPro + Portable Photosynthesis System®, ADC BioScientific Ltd., London, United Kingdom), between 07h00 and 08h00. These data were used to quantify the instantaneous water use efficiency (IWUE = *A/E*; μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) and instantaneous carboxylation efficiency (ICE = *A/Ci*) (μmol CO_2 m^{-2} s^{-1}/μmol CO_2 mol^{-1}).

Chlorophyll *a* and *b*, total chlorophyll and carotenoid levels were also determined at 80 DAS, according to the methodology of Arnon (1949), using five leaf discs collected from the third mature leaf of the apex, which were immersed in 80% acetone and stored in the dark for 48 h. The obtained extracts were read on a spectrophotometer at the wavelengths of 470, 646 and 663 nm. The readings were entered in the following equations: Chlorophyll a (Chl *a*) = (12.21 × ABS663) - (2.81 × ABS646); Chlorophyll *b* (Chl *b*) = (20.13 × ABS64) - (5.03 × ABS663); and Carotenoids (Car) = [(1000 × ABS470) - (1.82 × Chl *a*) - (85.02 × Chl *b*)]/198. The sum of the Chl *a* and Chl *b* data was used to quantify the total chlorophyll. The Chl *a*, Chl *b,* total chlorophyll and Car contents measured in the leaves were expressed in mg g^{-1} FW (fresh weight).

Production components were determined at 80 DAS, by evaluating the number of fruits (NF), equatorial diameter of the fruit (EDF) and polar diameter of the fruit (PDF). The number of fruits was determined by counting the fruits produced per plant. The equatorial and polar diameters were measured with a digital caliper.

Data were subjected to analysis of variance by the F test and, when significant, linear or quadratic regression analysis was performed for the water salinity levels and SA concentrations. When there was a significant interaction effect between the factors, the salinity factor was subsequently analyzed considering each SA concentration, using SISVAR-ESAL statistical software (Ferreira, 2019).

Results and Discussion

As shown in Table 2, there was a significant effect of irrigation water salinity levels on *gs, E, A, Ci,* ICE and IWUE. The SA concentrations significantly influenced *gs, E, A* and ICE. However, there was no significant interaction effect between saline levels and SA concentrations (SL × SA) for any of the variables analyzed in the 'All Big' bell pepper at 80 DAS.

Table 2

Summary of the F test for stomatal conductance (*gs*), transpiration (*E*), CO_2 assimilation rate (*A*), internal CO_2 concentration (*Ci*), instantaneous carboxylation efficiency (ICE) and instantaneous water use efficiency (IWUE) of 'All Big' bell pepper plants irrigated with saline water and salicylic acid concentrations, 80 days after sowing

Source of variation	F test					
	gs	E	A	Ci	ICE	IWUE
Salinity levels (SL)	**	**	**	**	**	*
Linear equation	**	**	**	**	**	**
Quadratic equation	ns	ns	ns	ns	ns	ns
Salicylic acid (SA)	**	**	**	ns	**	ns
Linear equation	*	ns	ns	ns	ns	ns
Quadratic equation	**	**	**	ns	**	ns
SL × SA interaction	ns	ns	ns	ns	ns	ns
Block	ns	ns	ns	ns	ns	ns
CV (%)	7.87	10.88	5.10	5.75	7.28	12.63

ns, *, ** not significant and significant at $p<0.05$ and $p<0.01$, respectively. CV: coefficient of variation.

The stomatal conductance of 'All Big' bell pepper decreased linearly, by 11.65% per unit increase in irrigation water salinity (Figure 1A). Plants grown under irrigation with 0.8 dS m^{-1} water had higher *gs* (0.298 mol H_2O m^{-2} s^{-1}) than those irrigated with 3.2 dS m^{-1} water (0.206 mol H_2O m^{-2} s^{-1}), that is, there was a 27.96% reduction in the *gs* of the plants irrigated with the highest salt level relative to those under the lowest ECw level. The same trend also occurred for leaf transpiration (Figure 1B). With the increase in water salinity, E declined by 7.92% per unit increase in ECw. In other words, plants irrigated with 3.2 dS m^{-1} water exhibited a 19.02% lower E than those irrigated with 0.8 dS m^{-1} water. The first phenomenon that occurs in plants under salt stress is stomatal closure, caused by the decrease in osmotic potential of water in the soil, which hinders water absorption by the roots. Thus, stomatal closure ensures less water loss to the atmosphere, but also becomes a physical barrier to the entry of CO_2 into the mesophilic cells, reducing carboxylation rates to the lowest level (Branco et al., 2020). The findings described here corroborate Melo, Souza, Duarte, Cunha and Santos (2017), who reported a reduction in *gs* of bell pepper in response to the increase in ECw from 0 to 9 dS m^{-1} and highlighted that the gas exchange parameters were efficient to identify the deleterious effects of salinity on the crop.

The *Ci* of the bell pepper plants increased with salt stress, by 6.50% per unit increase in the irrigation ECw (Figure 1C). Comparatively, the plants irrigated with 3.2 dS m^{-1} water had a 37.168 μmol m^{-2} s^{-1} increase in *Ci* relative to those subjected to water with the salinity level of 0.8 dS m^{-1}. Despite the reduction in *gs* with increased salinity (Figure 1A), the influx of CO_2 into the substomatal chamber was not impaired. However, A (Figure 1D) decreased by 6.0% per unit increase in water salinity, meaning plants irrigated with 0.8 dS m^{-1} water showed a higher A (13.93 mmol H_2O m^{-2} s^{-1}) than those grown with 3.2 dS m^{-1} water (11.82 mmol H_2O m^{-2} s^{-1}). Considering that there was no restriction in *A* in the substomatal chamber (Figure 1C), this reduction in A may be related to non-stomatal factors, such as accumulation of Na^+ ions and/or Cl^- in the chloroplasts, which causes alterations in the state of the thylakoid membranes of chloroplasts that. These alterations, in turn, induce changes in the biochemical and photochemical processes involved in photosynthesis (Braz et al., 2019), causing the CO_2 available for photosynthesis not to be synthesized during the carboxylation phase (Morais et al., 2018). The results found in this study corroborate Lima, Fernandes, Soares, Gheiy and Fernandes (2020), who observed an increase in *Ci* and a reduction in *A* with the increase in irrigation water salinity from 0.3 to 3.5 dS m^{-1}, in passion fruit plants. Cavalcante, Santos, Furtado and Chaves (2019) also found that the *A* of 'All Big' bell pepper decreased with the increase in water salinity from 1.7 to 11.7 dS m^{-1}, in a hydroponic system.

The instantaneous carboxylation efficiency of 'All Big' bell pepper plants was also negatively affected by the increase in water salinity. According to the regression equation (Figure 1E), plants irrigated with 3.2 dS m^{-1} water had a 22.12% reduction in ICE relative to those cultivated under of 0.8 dS m^{-1} salinity. This reduction is influenced by the internal CO_2 concentration, which tends to increase in salinity conditions, as illustrated in Figure 1C, to the detriment of *A* (Figure 1D). Thus, the reduction in *A* with a consequent increase in Ci induces a reduction in ICE (Dias et al., 2018b).

A similar effect occurred for IWUE (Figure 1F), which decreased linearly, by 5.71% per unit increase in irrigation-water salinity. This decrease in IWUE may be related to the reduction in *gs,* E and A of the plants (Figure 1A, 1B and 1D). In addition, it can be stated that 'All Big' bell pepper is considered moderately sensitive to salinity, since plants that are not able to increase their water use efficiency under salinity conditions possibly have greater sensitivity to salt stress, as reductions in water intake result in decreased absorption of specific ions, preventing toxic effects (especially Na^+ and Cl^-) on the plant (Soares et al., 2018).

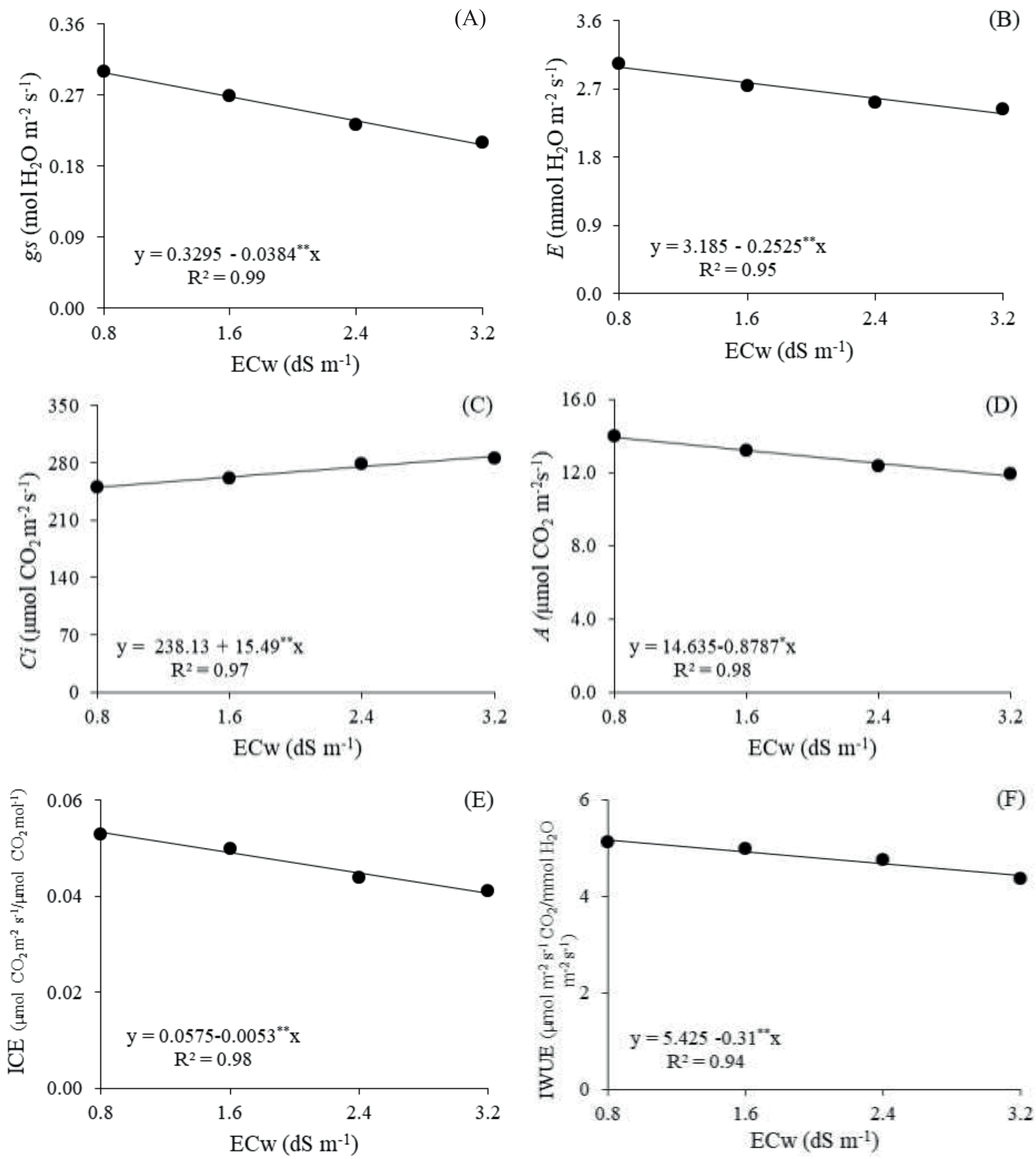

Figure 1. Stomatal conductance (*gs*; A), transpiration (*E*; B), internal CO_2 concentration (*Ci*; C), CO_2 assimilation rate (*A*; D), instantaneous carboxylation efficiency (ICE; E) and instantaneous water use efficiency (IWUE; F) of 'All Big' bell pepper plants as a function of irrigation water salinity, at 80 days after sowing.

The increasing SA concentrations elicited a quadratic response from the gs of the 'All Big' bell pepper plants. According to the regression equation (Figure 2A), the highest estimated gs value (0.2702 mol H_2O m^{-2} s^{-1}) was observed in the plants subjected to the SA concentration of 1.5 mM, which declined thereafter and reached a lower value in those that received the concentration of 3.6 mM (0.2285 mol H_2O m^{-2} s^{-1}). Leaf transpiration (Figure 2B) was higher (2.839 mol H_2O m^{-2} s^{-1}) in plants under application of 1.6 mM SA. After this concentration, E decreased, reaching its minimum value in the plants under the SA concentration of 3.6 mM (2.447 mol H_2O m^{-2} s^{-1}). Adequate concentrations of SA improve the physiological performance of 'All Big' bell pepper, as this compound acts by activating the antioxidant system, reducing oxidative stress and causing the leaching of ions from membranes (Pizolato et al., 2020). In a study investigating the effect of SA application on pepper plants under salinity, Kaya, Ashraf, Alyemeni and Ahmad (2020) concluded that SA is capable of reversing the negative effects of salinity on the plants, because the disturbances caused to the physiological activities in plants under salt stress can be reversed by the external supply of adequate concentrations of SA, which is able to prevent the destructive effect of ROS.

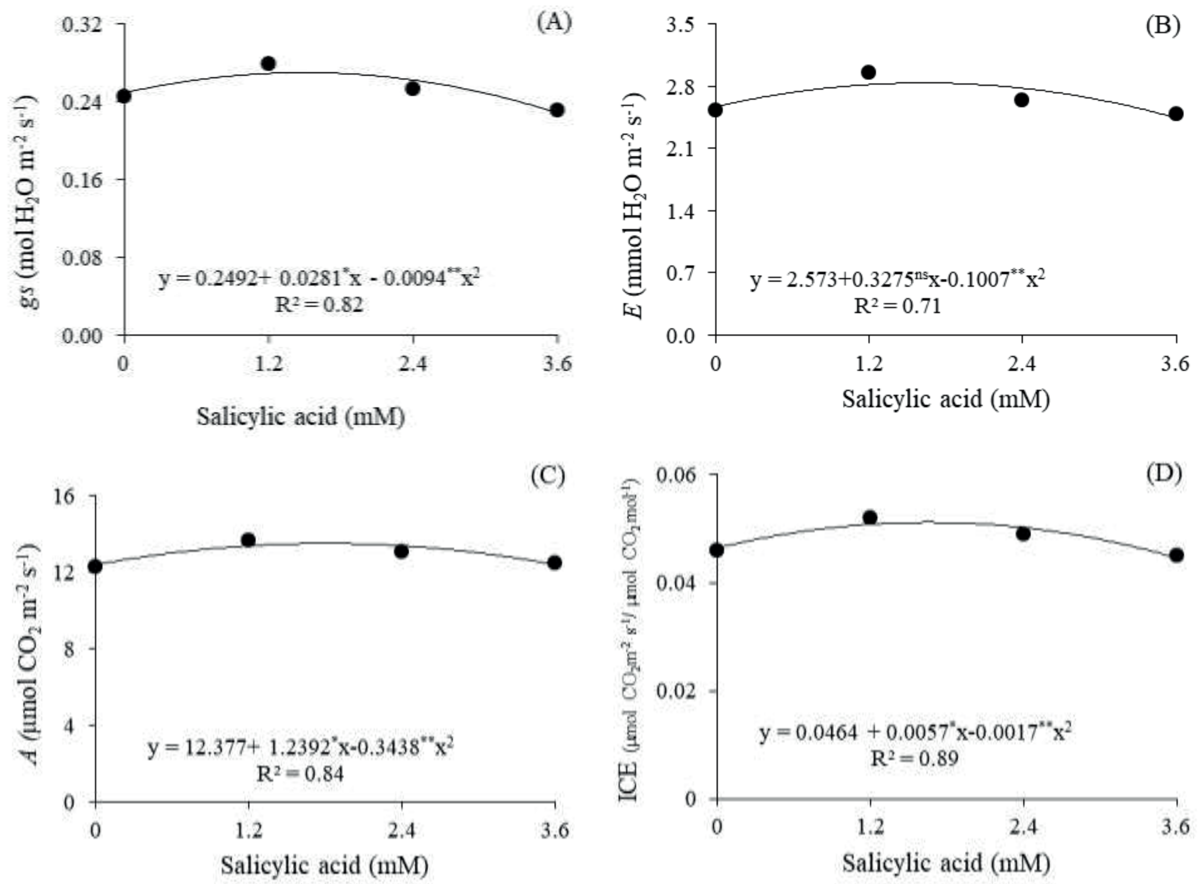

Figure 2. Stomatal conductance (*gs*), transpiration (*E*), CO_2 assimilation rate (*A*) and instantaneous carboxylation efficiency (ICE) of 'All Big' bell pepper plants as a function of salicylic acid concentrations (A, B, C and D), at 80 days after sowing.

The net A and the ICE of bell pepper were significantly affected by the SA concentrations. According to the regression equations (Figure 2C and 2D), the estimated maximum values of A (13.493 mmol H_2O m^{-2} s^{-1}) and ICE (0.511 [(μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$]) were obtained by plants grown under the concentration of 1.8 mM, which declined thereafter, with the lowest values of 12.382 mmol H_2O m^{-2} s^{-1} for A and 0.0449 [(μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] for ICE seen in the plants that received the SA concentration of 3.6 mM. The increase in *A* and ICE in the plants that received the SA concentration of 1.8 mM may be related to the ability of SA to reduce the production of H_2O_2, preventing oxidative stress, as well as its role as a defense activator, inducing the plant's tolerance to stress (Jayakannan, Bose, Babourina, Rengel, & Shabala, 2015). Miao et al. (2020) added that exogenous application of SA on cucumber seedlings under salt stress considerably improved plant growth and the photosynthetic rate of leaves under stress conditions.

There was a significant SL × SA interaction effect for the Chl *a*, Chl *b* and number of fruits per plant (NF) (Table 3). The saline levels significantly affected the total chlorophyll content (Chl_{total}). The SA concentrations, in turn, significantly influenced Chl_{total}, the equatorial diameter of the fruit (EDF) and the polar diameter of the fruit (PDF) of the 'All Big' bell pepper plants, at 80 DAS. The carotenoid content (Car) was not significantly influenced by any of the studied sources of variation.

Table 3

Summary of F test for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl_{total}), carotenoids (Car), number of fruits (NF), equatorial diameter of fruit (EDF) and polar diameter of fruit (PDF) of 'All Big' bell pepper plants irrigated with saline water and acid applications, 80 days after sowing

Source of variation	Teste F						
	Chl *a*	Chl *b*	Chl_{total}	Car	NF	EDF	PDF
Salinity levels (SL)	*	**	**	ns	**	ns	ns
Linear equation	*	**	**	ns	**	ns	ns
Quadratic equation	ns	ns	ns	ns	ns	ns	ns
Salicylic acid (SA)	ns	**	*	ns	**	**	**
Linear equation	ns	**	**	ns	ns	ns	ns
Quadratic equation	ns	ns	ns	ns	**	**	**
SL × SA interaction	**	*	ns	ns	**	ns	ns
Block	ns	ns	ns	ns	ns	ns	ns
CV (%)	17.89	29.19	16.670	40.94	7.77	6.42	7.38

^{ns}, *, ** not significant and significant at p<0.05 and p<0.01, respectively. CV: coefficient of variation.

According to the regression equation (Figure 3A), it appears that the levels of chlorophyll *a* (Chl *a*) in the 'All Big' bell pepper plants that were not subjected to the application of SA decreased when they were irrigated with water with ECw greater than 1.7 dS m^{-1}, whereas the lowest Chl a value (203.3704 mg g^{-1} FW) was obtained at the highest water salinity level (3.2 dS m^{-1}). On the other hand, SA application up to the concentration of 1.7 mM provided an increase in the levels of Chl *a* in plants subjected to salinity, the highest value of which (286.112 mg g^{-1} FW) was shown by the plants grown under the SA concentration of 1.7 mM and irrigated with 1.8 dS m^{-1} water, corresponding to a 10.35% increase (29.640 mg g^{-1} FW) as compared to plants grown under 1.8 dS ECw m^{-1} and 0 mM SA.

The chlorophyll b (Chl *b*) levels (Figure 3B) of the leaves of the plants subjected to SA concentrations of up to 1.7 mM increased when compared with those that did not receive SA application, regardless of the salinity of the irrigation water. The maximum estimated Chl b content was 144.859 mg g^{-1} FW, in the plants subjected to 1.7 mM SA and irrigated with 0.8 dS m^{-1} water. However, the increase in SA concentrations above 1.7 mM caused reductions in the Chl *b* levels in the plants, resulting in the lowest value (60.465 mg g^{-1} FW) in those that received the concentration of 3.6 mM and irrigated with 3.2 dS m^{-1} water, corresponding to a 30.71% reduction (26.13 mg g^{-1} FW) when compared with the plants under 1.7 mM SA and irrigated with water of 3.2 dS m^{-1}.

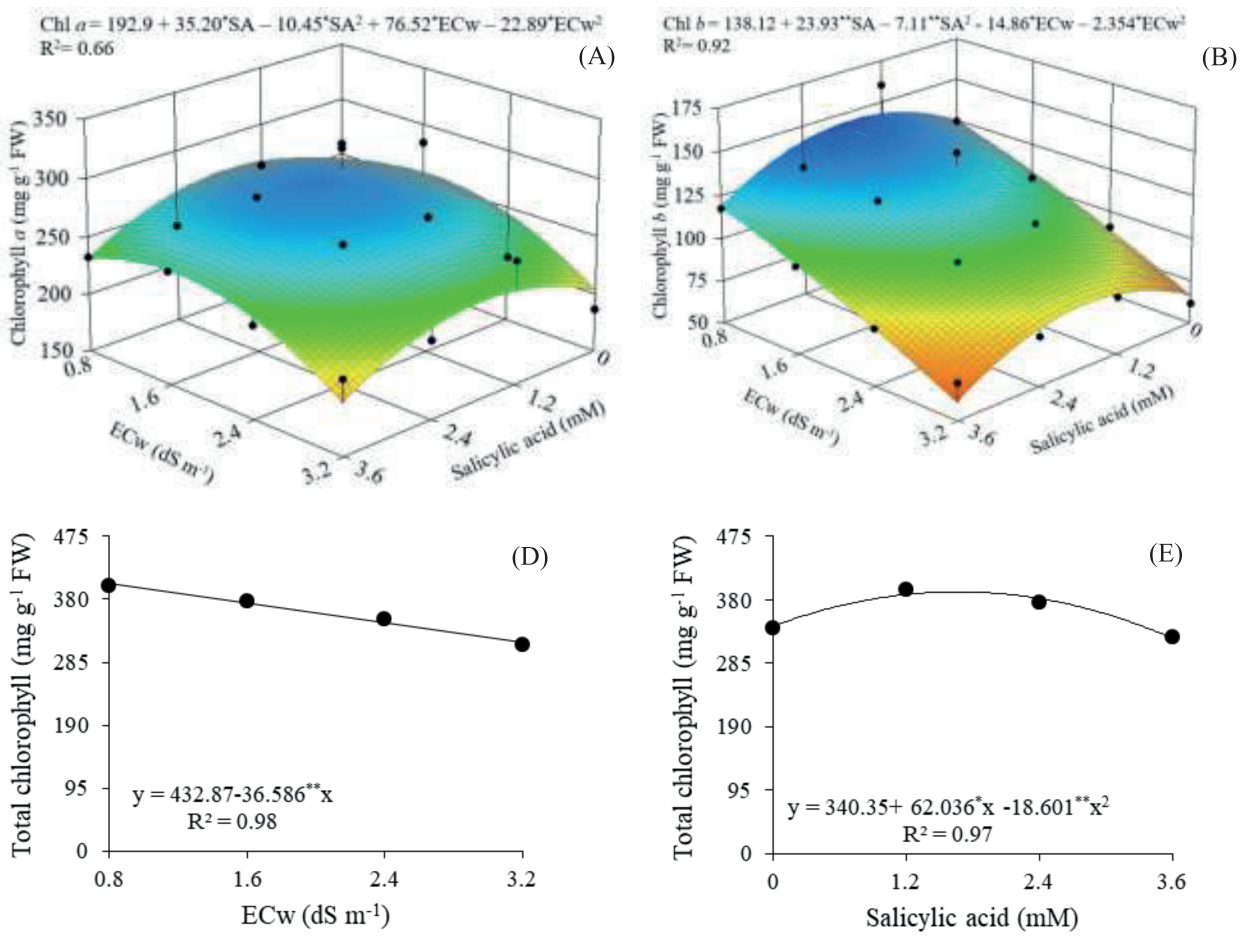

Figure 3. Chlorophyll *a* (A) and *b* (B) contents of bell pepper plants as a function of the interaction between water salinity levels (ECw) and concentrations of salicylic acid; and total chlorophyll content of 'All Big' bell pepper plants as a function of ECw levels (C) and salicylic acid concentrations (D), at 80 days after sowing.

Under stress conditions, such as high salinity, plants produce ROS that, in high amounts in the cell, can cause peroxidation and consequently destroy photosynthetic pigments (Perkins-Veazie, Collins, & Howard, 2008). However, oxidative damage can be mitigated by the exogenous application of adequate concentrations of SA, through its antioxidant action and its protective role of membranes that increase the plant's tolerance to damage (Gunes et al., 2007). The results of the present study corroborate Amirinejad, Sayyari, Ghanbari and Kordi (2017), who observed that exogenous application of 0.75 mM SA contributed to the maintenance of the chlorophyll content of pepper plants under salt stress and stated that antioxidant action of salicylic acid benefits the chlorophyll content of bell pepper plants under stress conditions. Rashed, Mahmoud, El-Tantawy, Fouad and El-Kassas (2017) found that the exogenous application of 400 ppm SA provided an increase in the chlorophyll contents of 'All Big' bell pepper subjected to salt stress and pointed out that SA acts as one of the antioxidant substances concentrated in the chloroplast that protects the photosynthetic apparatus when a plant is subjected to stress, eliminating excess free radicals.

The total chlorophyll levels in the bell pepper plants decreased linearly with the salinity levels of the irrigation water, at 80 DAS. According to the regression equation (Figure 3C), Chl_{total} decreased by 8.45% with each unit increase in ECw, that is, chlorophyll synthesis in the plants irrigated with 3.2 dS m^{-1} reduced by 87.80 mg g^{-1} FW (20.28%) relative to those under water with 0.8 dS m^{-1} salinity. This reduction in chlorophyll contents may be related to the accumulation of salts in the leaf tissue, where concentrations above that tolerated by plants stimulate the activity of the enzyme chlorophyllase, promoting the degradation of chlorophylls and chloroplasts. As a result, the plant has losses in photosynthetic activity, as observed in Figure 1D, and in pigmentation proteins (Munns & Tester, 2008). The obtained results agree with the findings of Melo et al. (2017), who observed a linear decrease in the levels of photosynthetic pigments in bell pepper in response to the increase in irrigation ECw up to 9 dS m^{-1}. Those authors highlighted that the reduction in photosynthetic pigment levels may be associated with changes in metabolic activities and damage caused to the membrane structure.

Total chlorophyll levels in the 'All Big' pepper plants were also influenced by the concentrations of SA (Figure 3D). Based on the regression equation, the maximum estimated chlorophyll content occurred in the plants subjected to the SA concentration of 1.7 mM (392.054 mg g^{-1} FW), and the lowest in those that received the SA concentration of 3.6 mM (322.611 mg g^{-1} FW). It is noteworthy that SA may favor the maintenance of chlorophyll levels in the pepper plant due to its effects on biological processes of the plant, e.g., stimulation of the photosynthetic apparatus and increased chlorophyll content (Khodary, 2004). Abdelaal et al. (2020) observed that exogenous application of 1 mM SA on pepper plants significantly increased the chlorophyll levels, and stressed that this action could be related to the antioxidant action of SA on the ROS that degrade chlorophyll in saline conditions, making exogenous SA application an effective method to overcome the harmful effects of salt stress on pepper plants.

As illustrated in Figure 4A, the highest number of fruits on the bell pepper plants

(8.23 fruits per plant) that were not subjected to SA application was found in those irrigated with 1.6 dS m^{-1} water. However, as ECw was increased, this number was reduced to a minimum of 4.85 fruits on plants irrigated with 3.2 dS m^{-1} water. On the other hand, SA application at a concentration of 1.5 mM on plants irrigated with 1.6 dS m^{-1} water provided the highest number of fruits per plant, that is, a 11.24% increase in those irrigated with 1.6 dS m^{-1} water and 0 mM SA. In general, high levels of salinity can cause fruit abortion due to physiological and/or biochemical changes induced by the high concentration of salts, directly affecting the number of fruits (Giuffrida et al., 2014). In addition, salt stress acts on microsporogenesis and elongation of the filaments of the stamen, increasing tissue cell death and causing ovule abortion and senescence of fertilized embryos (Oliveira et al., 2018). Nonetheless, although exogenous application of SA can lead to an increase in photosynthetic activity and, consequently, in the translocation of photoassimilates for fruit production, high SA concentrations result in oxidative stress in plants, which culminates in a marked decrease in the number and size of fruits (Diaz et al., 2016).

The equatorial diameter of the bell pepper fruits (Figure 4B) was also significantly influenced by the concentrations of SA, with the estimated maximum value (50.98 mm) observed in the plants subjected to the application of 1.8 mM SA, whereas those under 3.6 mM SA had the lowest EDF value (47.22 mm). This represents a 7.38% decrease in comparison to plants that received the application of 1.8 mM SA. The polar diameter of the bell pepper fruits (Figure 4C) fitted the quadratic model as a function of the increase in SA concentrations, with the highest value (47.46 mm) occurring in plants that received the SA concentration of 1.8 mM and the lowest (43.67 mm) in those under 3.6 mM SA. In a study with Jalapenõ pepper, Díaz et al. (2020) found that the SA concentration of 0.2 mM provided the largest fruit diameters. The increase in fruit diameters is related to the functions SA plays in plant growth, as it acts in cell division and stimulates the accumulation of abscisic and indole-acetic acids in the plant, and these, in turn, increase the size of the fruit, fostering cell division (Javid, Sorooshzadeh, Moradi, Sanavy, Allahdadi, 2011).

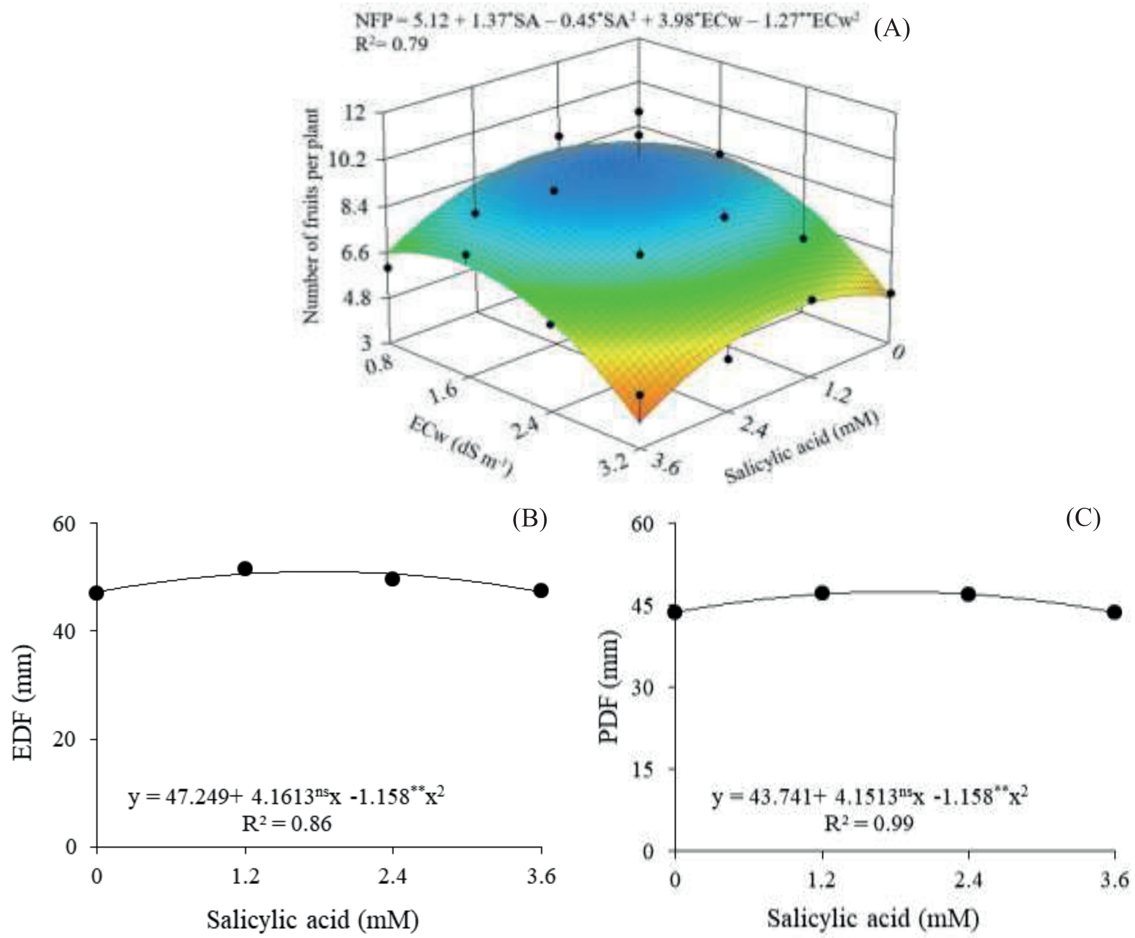

Figure 4. Number of fruits per plant (NFP; A) according to the interaction between water salinity levels (ECw) and salicylic acid concentrations; equatorial diameter of the fruit (EDF; B); and polar diameter of the fruit (PDF; C) of 'All Big' bell pepper plants as a function of salicylic acid concentrations, at 80 days after sowing.

Conclusion

Increases in irrigation water salinity from 0.8 dS m^{-1} lead to changes in gas exchange and total chlorophyll levels in 'All Big' bell pepper plants at 80 days after sowing.

The average salicylic acid concentration estimated at 1.7 mM attenuates the deleterious effects of water salinity on stomatal conductance, transpiration, CO_2 assimilation rate and instantaneous carboxylation efficiency, in addition to increasing total chlorophyll biosynthesis and the polar and equatorial diameters of 'All Big' bell pepper fruits.

Irrigation with water of 1.8, 0.8 and 1.6 dS m^{-1} electric conductivity, associated with salicylic acid at the concentration of 1.6 mM, stimulates the biosynthesis of chlorophylls *a* and *b* and number of fruits in bell pepper plants, respectively.

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