Morphophysiology and production of bell pepper grown under salt stress and salicylic acid foliar application

Morfofisiologia e produção de pimentão cultivado sob estresse salino e aplicação foliar de ácido salicílico

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Abstract

Considering the relevance of bell pepper and the limitations imposed by the deleterious effects of salt stress, especially in semi-arid regions, it is extremely important to establish strategies that can facilitate the use of saline water in vegetable production. In this scenario, this study aimed to evaluate the effect of the frequency of foliar application of salicylic acid on the morphophysiology and production of the bell pepper cv. "All Big" irrigated with saline water. The study was conducted at a greenhouse in Campina Grande - PB. The treatments were distributed in a completely randomized design and set up in a 4 × 4 factorial arrangement with three replications, corresponding to four application frequencies of salicylic acid (F1- No application of salicylic acid, F2 - Weekly application, F3- fortnightly application, and F4- monthly application) and four levels of electrical conductivity of irrigation water - ECw (0.8, 1.6, 2.4 and 3.2 dS m⁻¹). The fortnightly application of salicylic acid at a concentration of 1.0 mM mitigated the effects of salt stress on the morphophysiology and production components of bell pepper cv. All Big cultivated with ECw of up to 2.4 dS m⁻¹, which reinforces the hypothesis that salicylic acid can act as a signaling molecule and reduce the effects of saline stress in bell pepper, enabling the use of brackish water in agricultural activity, mainly in semi-arid regions of northeastern Brazil, which have a shortage of fresh water.

Keywords: Capsicum annuum, abiotic stress, brackish water, phytohormone.

Resumo

Considerando a relevância da cultura do pimentão e as limitações impostas pelos efeitos deletérios do estresse salino, principalmente em regiões semiáridas, é de suma importância o estabelecimento de estratégias que possam viabilizar o uso de águas salinas na produção de olerícolas. Neste contexto, objetivou-se com o presente estudo, avaliar o efeito da frequência de aplicação foliar de ácido salicílico sobre a morfofisiologia e a produção de pimentão cv. All Big irrigado com águas salinas. O estudo foi conduzido em casa de vegetação, em Campina Grande - PB. Os tratamentos foram distribuídos em delineamento inteiramente casualizados, em esquema fatorial de 4 × 4, com 3 repetições, sendo quatro frequências de aplicação de ácido salicílico (F1- Sem aplicação de ácido salicílico, F2 - Aplicação semanal, F3- quinzenal e F4- mensal) e quatro níveis de condutividade elétrica da água de irrigação - CEa (0,8, 1,6, 2,4 e 3,2 dS m⁻¹). A aplicação quinzenal de ácido salicílico na concentração de 1,0 mM amenizou os efeitos do estresse salino sobre a morfofisiologia e os componentes de produção do pimentão cv. All Big cultivado com CEa de até 2,4 dS m⁻¹, o que reforça a hipótese que o ácido salicílico pode atuar como uma molécula sinalizadora e reduzir os efeitos do estresse salino no pimentão, viabilizando o uso de água salobra na atividade agrícola, principalmente em regiões semiáridas do nordeste brasileiro, que possui escassez de água doce.

Palavras-chave: Capsicum annuum, estresse abiótico, águas salinas, fitormônio.

1. Introduction

Bell pepper (*Capsicum annuum* L.) is a species native to southern North America and northern South America, characterized as one of the most popular Solanaceae species worldwide and having significant economic importance due to its short cycle and easy cultivation, being easily produced by small, medium and large producers (Veloso et al., 2021; Rodríguez-Calzada et al., 2019). Its fruits contain several vitamins, such as vitamin C, provitamin A carotenoids, namely α - and β -carotene and β -cryptoxanthin, and oxygenated carotenoids that help

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protect the body against oxidative stress, thus preventing a series of chronic diseases- degenerative (Xavier and Pérez Gálvez, 2016; Li et al., 2017).

According to the Brazilian Horti & Fruit Yearbook, bell pepper production in Brazil totaled approximately 350 thousand tons in 2019 in a harvested area of 13 thousand hectares. However, irrigation water salinity is one of the most limiting factors for the production of several crops, especially in semi-arid regions (Silva et al., 2018), a situation commonly observed in northeastern Brazil.

Due to the spatial and temporal variability in rainfall distribution, the semi-arid region of Northeastern Brazil favors water shortage and the increase in the concentration of dissolved salts in reservoirs, thus compromising the entire plant production cycle (Bezerra et al., 2018; Silva et al., 2019; Santana Júnior et al., 2020). High ion concentrations in the root region, especially Na⁺ and Cl⁻, cause a series of morphophysiological disorders due to the osmotic and ionic effects of salt stress, thus decreasing water uptake and inhibiting nutrient uptake (Oliveira et al., 2018; Sousa et al., 2023).

From this perspective, strategies aimed at allowing the use and/or mitigating the deleterious effects of irrigation water salinity on the cultivation of the bell pepper cv. All Big are of paramount importance. Such strategies include the use of eliciting substances (Veloso et al., 2021; Silva et al., 2020). In this scenario, salicylic acid (SA) is capable of inducing plant tolerance to both biotic and abiotic stresses (Silva et al., 2022a).

Salicylic acid is involved in plant growth and in physiological processes such as floral induction, stomatal regulation, ion absorption, photosynthesis, and respiration by intensifying the activity of antioxidant enzymes and providing protection against membrane damage (Silva et al., 2020; Esan et al., 2017). Studies have reported that the application of salicylic acid can mitigate the deleterious effects of salt stress in various vegetable crops, as observed in tomato (Silva et al., 2022b), basil (Silva et al., 2022c) and even bell pepper (Veloso et al., 2021). However, such studies have been limited to investigating concentrations and application methods, with no reports regarding the frequency of application of salicylic acid, especially in bell pepper.

From this perspective, this study aimed to evaluate the effect of the frequency of foliar salicylic acid application on the morphophysiology and production of the bell pepper cv. All Big irrigated with saline water.

2. Material and Methods

2.1. Location of the experiment

The experiment was conducted from May to October 2022 in a greenhouse at the Agricultural Engineering Academic Unit (UAEA) of the Federal University of Campina Grande (UFCG) in Campina Grande, Paraíba, Brazil (7° 13' 11" S, 35° 53' 31" W, and at an altitude of 550 m). The plant nursery was bow-shaped, with a length of 30 m, a width of 21 m, and a lateral height of 3.0 m, covered with low-intensity polyethylene (150 microns).

The data on air temperature (maximum and minimum) and relative air humidity during the experimental period are shown in Figure 1.

2.2. Plant material

The bell pepper cultivar All Big was used in the experiment. This cultivar is characterized as a high-yield hybrid with an erect growth habit, small size, a firm and thick pulp, sweet flavor, and a total cycle of 120 days, with high tolerance to *Pytophthora capsici* and the tomato mosaic virus (Araújo et al., 2009).

2.3. Treatments and experimental design

The treatments were composed of two factors, with four frequencies of salicylic acid application (F1- no salicylic acid application, F2- weekly application, F3-fortnightly application, and F4- monthly application) and four levels of electrical conductivity of irrigation water – ECw (0.8, 1.6, 2.4, and 3.2 dS m⁻¹) distributed in a completely randomized design set up in a 4 × 4 factorial arrangement with four replications and one plant per plot, totaling 48 experimental units. The electrical conductivity levels were based on a study conducted by Lima et al. (2017) and the salicylic acid concentration (1.0 mM) described by Veloso et al. (2021).

2.4. Setting up and conduction of the experiment

The experiment was conducted in 10-dm³ plastic pots adapted as drainage lysimeters filled with a 0.5 kg gravel layer, followed by 10 kg of soil classified as Entisol (USDA, 2014), collected at a depth of 0-30 cm in Lagoa Seca – PB, whose physicochemical characteristics (Table 1) were determined according to Teixeira et al. (2017).

The irrigation water with different electrical conductivity levels (ECw - 0.8, 1.6, 2.4, and 3.2 dS m⁻¹) was prepared by dissolving NaCl, $CaCl_{2^{2}}H_{2}O$, and $MgCl_{2^{*}6}H_{2}O$ at the equivalent proportion of 7:2:1 for Na: Ca: Mg, respectively, in local tap water (ECw: 0.4 dS m⁻¹). This salt proportion is commonly found in water sources used for irrigation in small properties in Northeastern Brazil (Medeiros et al., 2003). The preparation of the irrigation water concentrations considered the relationship between the ECw and the concentration of salts (Richards, 1954), according to Equation 1.

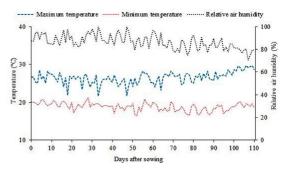


Figure 1. Maximum and minimum temperatures and air relative humidity inside the plant nursery.

Table 1. Chemical and physical soil attributes, at the 0-0.30 m layer, of the soil used in the experiment before the application of treatments.

Chemical characteristics										
pH (H ₂ O)	O M	Р	K*	ESP	ECse					
(1:2.5)	dag kg-1	(mg kg-1)	(cmol _c kg ⁻¹)						(dS m ⁻¹)	
6.7	12.75	5.96	0.89	0.09	3.72	0.95	0.91	1.87	1.0	
Water-physical characteristics										
Particle size (g kg-1)			Textural	Moisture (kPa) Total porosity		AD	PD			
Sand	Silt	Clay	class	33.42* 1519.5** dag kg-1		AW	%	(kg	dm-3)	
727	211	62	SL	13.07	5.26	7.66	41.9	2.68	1.56	

O M - Organic matter: Walkley-Black wet digestion; Ca²⁺ and Mg²⁺ extracted with KCl 1 M pH 7.0; Na⁺ and K⁺ extracted with NH₄OAc 1 M pH 7.0; Al³⁺ and H⁺ extracted with CaOAc 0.5 M 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.

$$Q \cong 10 \times ECw \tag{1}$$

Where:

Q – Salt content to be added (mmol_c L^{-1});

ECw –Electrical conductivity of irrigation water (dS m⁻¹). Sowing was performed by placing three bell pepper seeds at a depth of 2 cm. Later, the seedlings were thinned to one plant per pot 15 days after sowing (DAS).

Irrigation was performed always at 7:00 a.m. to maintain the soil moisture content close to the maximum water retention capacity. Irrigation with saline water began at 35 DAS by applying, in each container, the water corresponding to the plant water requirements, estimated by the water balance volume determined using Equation 2.

$$VI = \frac{(Va - Vd)}{(1 - LF)}$$
(2)

Where:

VI - water volume to be applied in irrigation;

Va – water volume applied in the previous irrigation event (mL);

Vd - drained volume (mL);

LF – 0.15 leaching fraction applied every 30 days.

The concentration of salicylic acid was obtained by dilution in ethyl alcohol (30%) since this is a substance with low solubility in water at ambient temperature, with the preparation occurring always on the same day of application. The foliar applications began at 30 DAS and continued at their respective application frequencies (Table 2) in order to achieve the full wetting of the leaf area using a backpack sprayer. Daily spraying began at 5:00 p.m. The Wil fix adjuvant was used to reduce the surface tension of droplets on the leaf surfaces (0.5 mLL⁻¹) using a manual sprayer.

Initial fertilization with NPK was performed according to the recommendations of Novais et al. (1991) by applying 100 mg of N, 300 mg of P_2O_{5} , and 150 mg of K_2O per kg of soil using calcium nitrate (15.5% N), monoammonium

phosphate (60% P_2O_5 , 12% N), and potassium chloride as sources (60% K_2O), respectively, split into 15 applications. Fortnightly, the Dripsol micro® solution at the concentration of 1.0 g L⁻¹ was applied on the leaves using a backpack sprayer. This solution contained Mg (1.1%). Zn (4.2%), B (0.85%), Fe (3.4%), Mn (3.2%), Cu (0.5%), and Mo (0.05%).

During the experimental period, all crop management and phytosanitary practices recommended for the crop were performed to prevent problems with pests and diseases, adopting preventive measures whenever necessary using chemical insecticides of the pyranose and imidacloprid groups at the concentrations of 0.4 mL L⁻¹ and 0.1 mL l⁻¹, respectively.

2.5. Variables analyzed

The physiological variables were analyzed at 90 DAS based on the relative water content (RWC), electrolyte leakage (% EL) stomatal conductance (gs), transpiration (E), CO₂ assimilation rate (A), and internal carbon concentration (Ci). From 90 to 110 DAS, the following production variables were evaluated: total number of fruits (TNF), equatorial fruit diameter (EFD), polar fruit diameter (PFD), total production (TP), and mean fruit weight (MFW). At 110 DAS, the variables shoot dry phytomass (SDP), root dry phytomass (RDP), and the ratio of root to shoot dry phytomass (R/SH) were evaluated. Finally, the water consumption (WC) was measured at 110 DAS.

The relative water content (RWC) was determined according to the methodology of Weatherley (1950) by obtaining the fresh mass – FM (g) of five 12-mm wide leaf disks removed from the middle-third portion of the main branch and putting this material into beakers containing 50 mL of distilled water for 24 hours. After this period, the excess water was removed from the disks with a paper towel, thus obtaining the turgid mass – TM (g) of the samples. Next, the disks were dried at \approx 65 ± 3 °C to obtain their dry mass – DM (g) (90 DAS), according to Equation 3.

$$RWC = \left(\frac{FM - DM}{TM - DM}\right) \times 100$$
(3)

Total salicylic acid application per treatment – (mL per plant)								
Dates of application	F1 – Control	F2 – Weekly	F3 – Forthnightly	F4 – Monthly				
20/06/2022 (33 DAS)	0	14	14	14				
27/06/2022 (40 DAS)	0	20	0	0				
04/07/2022 (47 DAS)	0	18.75	18.75	0				
11/07/2022 (54 DAS)	0	18.75	0	0				
18/07/2022 (61 DAS)	0	44.4	44.4	44.4				
25/07/2022 (68 DAS)	0	44.4	0	0				
01/08/2022 (75 DAS)	0	44.4	44.4	0				
Total applied	0	204.7	121.55	58.4				

Table 2. Volume of salicylic acid applied per treatment throughout the experiment.

Where:

RWC - relative water content (%);

FM – fresh leaf mass (g);

TM- turgid mass (g);

DM – dry mass (g).

Electrolyte leakage (EL) was determined using a copper perforator to obtain five leaf disks with an area of 1.54 cm² per experimental unit. The disks were put into an Erlenmeyer containing 50 mL of distilled water. Soon after, the containers were closed with aluminum foil and subjected to ambient temperature ($25 \,^{\circ}$ C) for 24 hours. Next, the electrical conductivity (90 DAS) initial – Xi (dS m⁻¹) was measured using a benchtop conductivity meter (MB11, MS Techonopon[®]. After this procedure, the containers were subjected to the temperature of 85 °C in a drying oven for 120 minutes (SL100/336, SOLAB[®]). After the material cooled, the final electrical conductivity – Xf (dS m⁻¹) was measured. Electrolyte leakage in the leaf blade was determined according to Scotti-Campos et al. (2013), based on Equation 4.

$$EL = \frac{Xi}{Xf} \times 100$$
 (4)

Where:

EL – electrolyte leakage (%);

Xi – initial electrical conductivity (dS m⁻¹);

Xf – final electrical conductivity (dS m⁻¹).

Stomatal conductance – $gs (mol H_2O m^2 s^{-1})$, transpiration – $E (mmol H_2O m^{-2} s^{-1})$, CO_2 assimilation rate – $A (\mu mol CO_2 m^2 s^{-1})$, and the internal carbon concentration– Ci ($\mu mol CO_2 mol air^{-1}$) were measured using an irradiation of 1200 μmol photons $m^{-2} s^{-1}$ and an airflow of 200 mL min⁻¹, determined through the photosynthetic light saturation curve (Fernandes et al., 2021) using a portable photosynthesis meter (LCPro+ from ADC BioScientific Ltd.).

The ripe fruits were harvested weekly from 90 DAS to 110 DAS by counting the number of fruits – NF, the equatorial fruit diameter – EFD (mm), the polar fruit diameter – PFD (mm), the total production – TP (g per plant), and the mean fruit weight– MFW (g per fruit). The NF was measured by counting all fruits produced by the plants. The equatorial and polar diameters were measured using a digital caliper. The total production was measured by summing the weights of harvested

fruits and the mean weight per fruit, obtained through the relationship between the total production and the total number of fruits.

The phytomass variables were obtained through by cross-sectioning the stem of each plant, which was cut close to the soil at 110 DAS, and separating the stem, leaves, and roots, which were stored in paper bags and dried to constant weight at 65°C in a forced-air oven (SL100/336, SOLAB®). Soon after, the material was weighed, thus obtaining the dry shoot phytomass - DSP (g per plant), dry root phytomass - DRP (g per plant), and the ratio of root to shoot dry phytomass - R/SH (g g⁻¹).

Water consumption – WC (mm) was determined by summing the total water volume applied and subtracting the water volume drained during the crop cycle (110 days).

2.6. Statistical analysis

The data collected were subjected to multivariate analysis by principal component analysis (PCA) by synthesizing the amount of relevant information contained in the original data sets into a lower number of dimensions, resulting from linear combinations of the original variables generated based on the eigenvalues ($\lambda \ge 1.0$) in the correlation matrix, explaining percentages higher than 10% in the total variance (Govaerts et al., 2007). After reducing the dimensions, the original data on the variables of each component were subjected to analysis of variance by the 'Hotelling' test t p ≤ 0.05 .

Only the variables with a correlation coefficient equal to or higher than 0.6 remained in the PCA. The variables that showed a correlation coefficient lower than 0.6 were subjected to analysis of variance by the F-test at 0.05 of probability. In cases of significance, the Tukey test of means ($p \le 0.05$) was performed for the application frequencies of salicylic acid, whereas the quantitative data were subjected to regression. All analyses were performed using the statistical software R-Studio (V.4.1.0).

3. Results and Discussion

Following the recommendations of Hair et al. (2009), the internal CO_2 concentration (*Ci*), number of fruits (NF),

Courses of an electric e	DE	Mean square							
Sources of variation	DF	Ci	NF	PFD	DSP				
Salinity levels (SL)	3	483.17**	4.08**	285.86*	14.44**				
Linear regression	1	2768.01**	1.67**	254.05**	8.70**				
Quadratic regression	1	131.12**	0.33 ^{ns}	31.54**	1.11 ^{ns}				
Application frequencies (FA)	3	511.98**	1.75 ^{ns}	104.54 ^{ns}	18.23**				
Interaction (SL × FA)	9	409.08**	22.75**	449.61 ^{ns}	9.14**				
Residual	32	371.0	10.67	840.59	27.45				
CV (%)		2.44	17.11	13.03	6.71				

Table 3. Summary of the analysis of variance for the internal carbon concentration (*Ci*), number of fruits (NF), polar fruit diameter (PFD), and dry shoot phytomass (DSP) of bell pepper plants irrigated with saline water and subjected to application frequencies of salicylic acid.

CV – Coefficient of variation; DF – Degree of freedom. ^{ns}Non-significant. *Significant at $p \le 0.05$. **Significant at $p \le 0.01$.

polar fruit diameter (PFD), and the dry shoot phytomass (DSP) were removed from the dataset of the multivariate analysis and analyzed by univariate analysis for showing correlation coefficients lower than 0.6. There was a significant effect of the interaction between the electrical conductivity levels of irrigation water and the application frequencies of salicylic acid (Table 3) on the *Ci*, NF, and DSP. The electrical conductivity levels of irrigation water significantly affected all variables. On the other hand, the application frequencies of salicylic acid caused significant effects on *Ci* and DSP.

The increment in the electrical conductivity of irrigation water increased the internal CO₂ concentration regardless of the application frequency of salicylic acid (Figure 2). There was no significant difference between the control plants and those that received the weekly and monthly application of SA, and the highest Ci values were obtained in the control treatment (182.16 µmol CO₂ m⁻² s⁻¹), weekly application (174.40 μ mol CO₂ m⁻² s⁻¹), and monthly application (170.14 µmol CO, m⁻² s⁻¹) in plants irrigated with the ECw of 3.2 dS m⁻¹. On the other hand, the plants that received the fortnightly foliar application of salicylic acid showed Ci reductions in relation to the other application frequencies. When comparing the plants that received the fortnightly SA application and irrigated with the ECw of 3.2 dS m⁻¹ with the controls irrigated with the same ECw level, there was a reduction of 35.78% in this variable $(64.90 \,\mu mol \, CO_2 \, m^{-2} \, s^{-1}).$

The results obtained in this study revealed that salt stress caused by the increase in the electrical conductivity of irrigation water reduced the relative water content in the leaves, influenced stomatal closure, and reduced transpiration (Table 4). The increase in the internal CO₂ concentration could be related to the reduction in RuBisCO in the Calvin cycle but also to the increased production of reactive oxygen species due to the incomplete oxygen recovery, which reduces the carboxylation efficiency (Roumani et al., 2022).

On the other hand, the beneficial effect of the fortnightly application of salicylic acid could be related to its role in increasing the activity of RuBisCO, the uptake of potassium, and the ATP content, thus maintaining an adequate

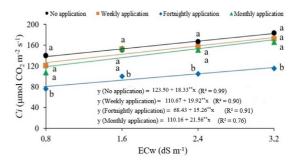


Figure 2. Internal carbon concentration (*Ci*) of bell pepper plants as a function of the interaction between the electrical conductivity of irrigation water (ECw) and the application frequencies of salicylic acid at 90 DAS. Means with the same letters indicate no significant difference between the application frequencies of salicylic acid (Tukey test, $p \le 0.05$).

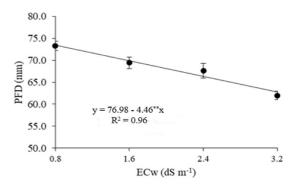


Figure 3. Polar fruit diameter (PFD) of bell pepper as a function of the electrical conductivity of irrigation water (ECw).

Na⁺/K⁺ relationship in plants and favoring a higher tolerance to salt stress (Lee et al., 2014).

The polar fruit diameter was negatively affected by the increase in the electrical conductivity of irrigation water (Figure 3). Plants irrigated with the ECw of 3.2 dS

										Principal components		
										PC1	PC2	
Eigenvalues (2	λ)									8.59	1.18	
Percentage of total variance (S ² %)										78.13	10.76	
Hotelling test (T ²) for the application frequencies of salicylic acid (F)									0.01	0.01		
Hotelling test (T ²) for the electrical conductivity of irrigation water (ECw)									0.01	0.01		
Hotelling test (T ²) for the interaction (F× ECw)										0.01	0.01	
Correlation coefficients (r)												
PCs	RWC	EL	gs	Е	Α	DRP	R/SH	DEF	PT	MFW	WC	
PC1	-0.92	0.91	-0.97	-0.81	-0.98	-0.78	-0.46	-0.98	-0.95	-0.97	-0.85	
PC2	-0.03	0.27	-0.07	0.17	-0.01	0.53	0.83	-0.13	-0.14	-0.11	-0.24	
Tractication	Mean values											
Treatments	RWC	EL	gs	Е	Α	DRP	R/SH	EFD	ТР	MFW	WC	
S1F1	77.27	23.69	0.14	2.04	22.68	2.84	0.20	69.83	237.93	32.45	278.77	
S1F2	78.97	22.06	0.17	2.73	26.71	3.62	0.21	72.05	236.39	33.42	278.77	
S1F3	81.44	20.94	0.19	3.09	30.68	4.18	0.22	74.51	276.17	39.29	278.77	
S1F4	75.17	22.78	0.16	2.21	24.58	3.31	0.19	71.11	223.28	34.13	278.77	
S2F1	76.02	25.08	0.12	1.90	20.41	2.49	0.20	66.33	207.31	29.21	254.86	
S2F2	77.03	24.85	0.15	2.54	24.04	2.44	0.20	68.45	224.20	30.08	254.86	
S2F3	77.95	23.12	0.16	2.85	27.61	3.94	0.24	70.78	233.38	35.36	254.86	
S2F4	77.35	24.61	0.14	2.06	22.12	2.94	0.22	67.55	212.19	30.72	254.86	
S3F1	74.45	26.44	0.11	1.96	18.37	2.34	0.19	63.02	186.27	26.28	220.76	
S3F2	75.75	25.28	0.13	2.36	21.64	2.95	0.20	65.03	195.49	27.07	220.76	
S3F3	74.92	23.84	0.15	2.51	24.85	3.43	0.21	67.25	203.54	31.82	220.76	
S3F4	74.07	24.09	0.12	1.91	19.91	2.80	0.17	64.18	184.95	27.65	220.76	
S4F1	68.87	28.69	0.10	1.64	14.70	2.84	0.22	59.87	171.42	23.66	198.02	
S4F2	72.68	26.06	0.12	2.20	17.31	2.90	0.24	61.77	177.12	24.36	198.02	
S4F3	71.19	24.88	0.13	2.61	19.88	2.45	0.17	63.88	182.92	28.64	198.02	
S4F4	70.13	25.78	0.11	1.78	15.93	1.33	0.10	60.97	177.56	24.88	198.02	

Table 4. Eigenvalues, percentage of total variance explained in the multivariate analysis of variance (MANOVA), and correlations (r) between original variables and principal components.

S1 (0.8 dS m⁻¹); S2 (1.6 dS m⁻¹); S3 (2.4 dS m⁻¹); S4 (3.2 dS m⁻¹); F1 – Without salicylic acid); F2 – Weekly application; F3 – Fortnightly application; F4 – Monthly application; RWC – Relative water content - %; EL – Electrolyte leakage - %; gs – Stomatal conductance - mmol H₂O m⁻² s⁻¹; *E* – Transpiration - mmol H₂O m⁻² s⁻¹; A – CO₂ assimilation rate - μ mol CO₂ m⁻² s⁻¹; DRP – Dry root phytomass - g per plant; R/SH – Root to shoot ratio; EFD – Equatorial fruit diameter - mm; TP – Total production – g per plant; MFW – Mean fruit weight – g per fruit); WC – Water consumption – L per plant.

m⁻¹ showed a minimum estimated value of 62.73 mm for PFD, corresponding to a 14.53% reduction (10.66 mm) compared to plants irrigated with the ECw of 0.8 dS m⁻¹.

The polar and equatorial fruit diameters are variables of great interest to consumers as they define the size of the edible part and fruit classification (Oliveira et al., 2023). The decrease in these parameters occurred due to the restrictions occurred in the gas exchange and the low translocation of photoassimilates, possibly because of the limitations in water and nutrient uptake, also including the competition between Na⁺ and K⁺, thus limiting fruit growth (Ferreira et al., 2020). With regard to the number of fruits (Figure 4), there were reductions as a function of the increase in the electrical conductivity of irrigation water regardless of the application frequencies of salicylic acid. Also, the plants subjected to fortnightly applications of SA stood out from the other frequencies by showing the highest number of fruits (7.83 fruits) when irrigated with the ECw of 0.8 dS m⁻¹ compared to the control irrigated with the same salinity, with an increase of 1.32 fruits (20.3%).

The increase in the number of fruits observed in the present study could be related to the eliciting role of salicylic acid, inducing an increase in photosynthetic activity and

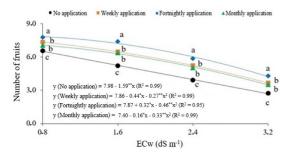


Figure 4. Number of fruits – NF(B) as a function of the interaction between the electrical conductivity of irrigation water– ECw and application frequencies of salicylic acid – SA. Means with the same letters indicate no significant difference between the application frequencies of salicylic acid (Tukey test, $p \le 0.05$).

improving the translocation of photoassimilates for fruit production (Diaz et al., 2016). Similar results were obtained by Silva et al. (2022b) with cherry tomato under different application methods of salicylic acid irrigated with saline waters (ECw ranging from 0.6 to 2.6 dS m⁻¹), reaching the highest value for the number of fruits (37 fruits per plant) under foliar application of salicylic acid (1 mM) and the ECw of 0.6 dS m⁻¹, corresponding to an increase of 42.31% (11 fruits per plant) compared to the control irrigated with the same salinity.

When analyzing the dry shoot phytomass accumulation (Figure 5), there was a similar effect to NF, i.e., reductions as a function of the increase in electrical conductivity regardless of the application frequency of salicylic acid. Moreover, the plants sprayed fortnightly stood out from the other frequencies, showing the highest DSP values. The highest DSP value (17.56 g per plant) was obtained in the plants irrigated with the ECw of 0.8 dS m⁻¹. On the other hand, the plants of the control treatment exposed to salt stress showed the lowest DSP values (9.85 g per plant) under irrigation with the ECw of 3.2 dS m⁻¹.

At adequate concentrations and depending on the manner and time of application, salicylic acid can regulate physiological and biochemical plant processes, minimizing the oxidative damage caused by abiotic stresses and favoring plant growth (Farhadi and Ghassemi-Golezani, 2020).

The multidimensional space of the original variables was represented for the two principal components (PC1 and PC2) with eigenvalues higher than $\lambda \ge 1.0$, following Kaiser (1960). The eigenvalues and the percentage of explained variance for each component (Table 4) represented, together, 88.89% of the total variation. PC1 explained 78.13% of the total variance, formed by most variables analyzed, except the root/shoot ratio (R/SH). PC2 represented 10.76% of the remaining variance, formed by the R/SH variable. According to the multivariate analysis of variance (Table 4), there was a significant effect ($p \le 0.01$) of the interaction between the application frequencies of salicylic acid (F) and the electrical conductivity levels of irrigation water (ECw) for the two principal components (PC1 and PC2). There was also a significant effect ($p \le 0.01$) of the factors when analyzed individually.

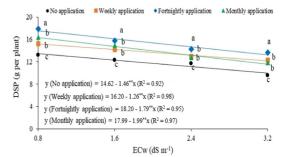


Figure 5. Dry shoot phytomass – DSP of bell pepper plants as a function of the interaction between the electrical conductivity levels of irrigation water– ECw and application frequencies of salicylic acid– SA at 90 DAS. Means with the same letters indicate no significant difference between the application frequencies of salicylic acid (Tukey test, $p \le 0.05$).

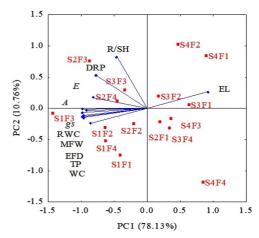


Figure 6. Two-dimensional projections of the principal component scores for the electrical conductivity of irrigation water (ECw) and application frequencies of salicylic acid (F) and the variables analyzed in the two principal components (PC1and PC2).

The two-dimensional projections of the effects of the treatments and the variables in the first and second principal components (PC1 and PC2) are shown in Figure 6. In the first principal component (PC1), a process was identified, possibly characterized by the effect of the interaction between the application frequencies of salicylic acid and the electrical conductivity of irrigation water. Moreover, the correlation coefficients for the RWC, EL, *gs*, *E*, *A*, DRP, R/SH, EFD, TP, MFW, and WC parameters were higher than 0.75 (Table 4).

When analyzing PC1, the bell pepper plants cultivated under the ECw of 0.8 dS m⁻¹ and sprayed fortnightly with salicylic acid (S1F3) stood out with the highest values (Table 4) of RWC (81.44), gs (0.19 mmol H₂O m⁻² s⁻¹), *E* (3.09 mmol H₂O m⁻² s⁻¹), *A* (30.68 µmol CO₂ m⁻² s⁻¹), DRP (4.18 g per plant), EFD (74.51 mm), TP (276.17 g per plant), MFW (39.29 g per fruit), and WC (278.77 mm). Furthermore, the plants subjected to the S1F3 treatment recorded the lowest electrolyte leakage (20.94%) in the leaf blade, highlighting the beneficial effect of salicylic acid on these variables when applied fortnightly.

When comparing the results obtained in the plants of treatment S1F3 with those grown under S1F1, increases of 5.40 (4.17%), 35.71 (0.05 mmol $H_20 \text{ m}^2 \text{ s}^{-1}$), 51.47 (1,05 mmol $H_20 \text{ m}^{-2} \text{ s}^{-1}$), 35.27 (8.0 µmol CO₂ m⁻² s⁻¹), 47.18 (1.34 g per plant), 6.70 (4,68 mm), 16.07 (38.24 g per plant), and 21.08% (6.84 g per fruit) were observed in the RWC, *gs*, *E*, *A*, DRP, EFD, TP, and MFW, respectively. Furthermore, electrolyte leakage was reduced by 2.75%.

The results obtained in this study also indicated that the fortnightly foliar application of salicylic acid had a beneficial effect on the variables analyzed regardless of the electrical conductivity of irrigation water, i.e., even the plants irrigated with the ECw of 3.2 dS m⁻¹ (S4F3) showed increases in the variables analyzed compared to the absence of salicylic acid (S4F1).

The beneficial effect of salicylic acid is possibly associated with its role in enzymatic and photosynthetic activities, thus maintaining the balance between the production and elimination of reactive oxygen species (Batista et al., 2019). Furthermore, SA acts as a regulator in physiological and biochemical plant processes, preventing reductions in the levels of auxins and cytokinins and favoring higher cell division in the root apical meristem (Osama et al., 2019). When evaluating application methods of salicylic acid, Silva et al. (2022b) observed that the fortnightly foliar application of SA (1 mM) resulted in the highest values for MFW (3.3 g per fruit), TP (114.5 g per plant), and EFD (16.6 mm) in cherry tomato plants (*Solanum lycopersicum* L.) irrigated with saline water.

When analyzing principal component 2 (PC2), the bell pepper plants cultivated under the ECw of 3.2 dS m⁻¹ associated with a weekly application frequency (S4F2) obtained the highest value for the R/SH (0.24 g g⁻¹). The increase in the R/SH ratio could be a plant tolerance mechanism to salt stress since the growth of the root system could be associated with a response to salt stress due to the increase in the water uptake area and the decrease in the area responsible for evapotranspiration (Santos et al., 2020). Salt excess in the water negatively interferes with the water flow from roots to leaves due to osmotic and toxic effects (Hafez et al., 2020).

4. Conclusions

The fortnightly application of salicylic acid at a concentration of 1.0 mM mitigates the effects of salt stress on the morphophysiology and production components of bell pepper cv. All Big cultivated with ECw of up to 2.4 dS m⁻¹, which reinforces the hypothesis that salicylic acid can act as a signaling molecule and reduce the effects of saline stress in bell pepper, enabling the use of brackish water in agricultural activity, mainly in semi-arid regions of northeastern Brazil, which have a shortage of fresh water.

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