

# Chloroplast pigments, water relationships, and growth of bell pepper under salt stress and nitrogen

Iara Almeida Roque<sup>1\*</sup>, Lauriane Almeida dos Anjos Soares<sup>2</sup>, Geovani Soares de Lima<sup>2</sup>,  
Iracly Amelia Pereira Lopes<sup>1</sup>, Luderlândio de Andrade Silva<sup>1</sup>, Maíla Vieira Dantas<sup>2</sup>,  
Rafaela Aparecida Frazão Torres<sup>1</sup>, Wesley Bruno Belo de Souza<sup>2</sup>

<sup>1</sup>Universidade Federal de Campina Grande, Pombal-PB, Brazil

<sup>2</sup>Universidade Federal de Campina Grande, Campina Grande-PB, Brazil

\*Corresponding author, e-mail: [yara.roque.sb@gmail.com](mailto:yara.roque.sb@gmail.com)

## Abstract

The use of saline water sources for irrigation in semi-arid regions is a challenge for horticultural production since plants are sensitive to salt stress conditions, thus requiring techniques that allow plant acclimation, including nitrogen fertilization. From this perspective, this study aimed to evaluate the chloroplast pigments, water relationships, and growth of bell pepper plants irrigated with saline water and subjected to nitrogen fertilization. The experiment was conducted under field conditions at the Federal University of Campina Grande, Pombal - PB, where a randomized block design was adopted with a 5 x 5 factorial arrangement corresponding to five electrical conductivity levels of irrigation water (0.3, 1, 1.7, 2.4, and 3.1 dS m<sup>-1</sup>) and five nitrogen levels (50, 75, 100, 125, and 150% of the dosage recommended for the crop), with three replications. The 125 and 150% N levels provided the highest relative water contents in bell pepper plants irrigated with electrical conductivity levels of up to 1.7 dS m<sup>-1</sup>. The contents of chlorophyll *a*, *b*, total chlorophyll, carotenoids, and stem diameter of bell pepper plants decreased when fertilized with 150% N and irrigated with the electrical conductivity of 3.1 dS m<sup>-1</sup>.

**Keywords:** *Capsicum annuum* L, mineral nutrition, saline water

## Introduction

Bell pepper (*Capsicum annuum* L.) is a vegetable of significant economic importance in Brazil, where it is used as a spice in several typical Brazilian dishes. The species belongs to the family Solanaceae and shows a short production cycle, thus bringing quick financial return (Casais et al., 2018; Silva et al., 2020). In Brazil, the states of Minas Gerais, São Paulo, Ceará, Rio de Janeiro, Espírito Santo, and Pernambuco stand out as the main bell pepper producers, with an estimated annual production of 290 thousand tons, the equivalent to 87% of the total production (Souza et al., 2019).

One of the main limitations in this crop's production in the semi-arid region of Brazil is water availability for irrigation, given the high evapotranspiration and low precipitation rates in the region, with rainfall events concentrated in some months, followed by long drought periods (Nobre et al., 2012; Silva et al., 2021). As a result,

this scenario requires the use of water sources with high salt contents for irrigation, which prevail in the Brazilian semi-arid region (Diniz et al., 2017).

In general, salt excess in the soil and/or water causes osmotic and ionic deleterious effects, especially in vegetables such as bell pepper (Lima et al., 2015; Soares et al., 2021). In irrigation-dependent crops, the use of high-salinity water can limit plant growth and production as it reduces the osmotic potential of the soil solution, causing ionic effects such as toxicity and nutritional imbalance (Lima et al., 2018; Barros et al., 2021).

However, plants developed a wide range of mechanisms to withstand stress conditions. In that regard, the evidence suggests that mineral nutrients, including nitrogen, play a key role in plant tolerance to salt stress due to its role as a constituent of amino acids, proteins, enzymes, and other molecules responsible for osmotic homeostasis in plants (Bezerra et al., 2018; Lacerda et

al., 2021), in addition to increasing the  $\text{NO}_3/\text{Cl}$  ratio, thus providing ionic homeostasis even under salt stress conditions (Silva et al., 2021; Roque et al., 2022).

However, there is little information available on the interactive effects of salinity and nitrogen fertilization on the morphophysiological responses of bell pepper, even though studies of this nature are important to improve production practices when only saline water sources are available (Lima et al., 2016). Therefore, the hypothesis of this study is that the increase in nitrogen levels can increase the synthesis of organic solutes and contribute to the osmotic adjustment of bell pepper plants under salt stress. From this perspective, this study aimed to evaluate the chloroplast pigments, water relationships, and the growth of bell pepper cultivated with saline water and nitrogen fertilization.

## Material And Methods

The experiment was developed from October to December 2020 in a field under 70% shading at the Center of Sciences and Agri-food Technology (CCTA) of the Federal University of Campina Grande (UFCG), located in the municipality of Pombal, Paraíba, at the following geographic coordinates:  $6^{\circ}47'20''$  S and  $37^{\circ}48'01''$  W, at an elevation of 194 m a.s.l. (Figure 1) the data referring to the maximum and minimum air temperatures, rainfall, and relative air humidity during the experimental period.

A randomized block design with a  $5 \times 5$  factorial arrangement was used in the experiment, referring to five electrical conductivity levels of irrigation water – ECw (0.3, 1.0, 1.7, 2.4, and  $3.1 \text{ dS m}^{-1}$ ) and five nitrogen doses – ND (50, 75, 100, 125, and 150% N), using as a reference the levels recommended by (Trani et al., 2014), resulting in 25 treatments, with three replications and one plant

per plot, totaling 75 experimental units. The different ECw levels were established based on the study developed by (Lima et al., 2016). The nitrogen doses were defined according to (Silva et al., 2019).

The bell pepper cultivar Yolo Wonder was used in the experiment. Two seeds were sown per cell in a polyethylene tray with a capacity of 50 mL and 162 cells. The cells were previously filled with a substrate composed of sand, soil, and manure at a ratio of 1:1:2, respectively. During this phase, the plants were daily irrigated with low-salinity water ( $0.3 \text{ dS m}^{-1}$ ), after which the seedlings were thinned to one plant per cell.

Transplanting was performed 15 days after sowing into 20-L pots adapted as drainage lysimeters, which received a 3-cm gravel layer under a geotextile fabric covering the bottom of the container to avoid obstruction by soil material. Next, each container received 22 kg of sandy-loam Fluvisol. The pots were then arranged in single rows spaced 0.60 m with a 0.40 m spacing between plants. The physical and chemical characteristics of the soil used in the experiment (Table 1) were determined according to Teixeira et al. (2017).

The different water electrical conductivity levels were prepared by dissolving sodium chloride (NaCl) in the local tap water from Pombal-PB ( $0.3 \text{ dS m}^{-1}$ ) by considering the relationship between the ECw and the concentration of salts (Richards, 1954):  $Q \text{ (mmol}_c \text{ L}^{-1}) = 10 \times \text{ECw}$ . In this study, soil chloride was used for this procedure as it is present at higher concentrations in the water sources of northeastern Brazil.

Irrigation was performed daily with low electrical conductivity water ( $0.3 \text{ dS m}^{-1}$ ) until 15 days after transplanting (DAT). After this period, irrigation with the different salinities began, and the water volume applied was determined based on the water requirements of the plants, according to Eq. 1:

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

Where:

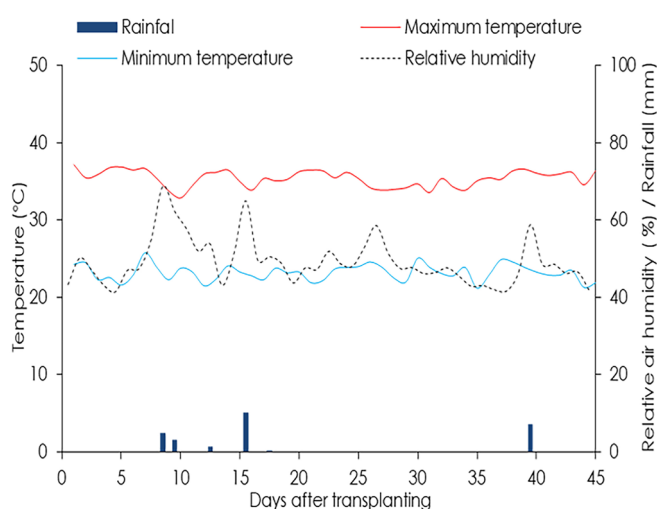
VC - water volume consumed (L);

VA - water volume applied on the previous day;

VD - drained water volume, quantified on the morning of the next day;

LF - 15% leaching fraction, applied every 15 days to minimize salt accumulation in the root zone. The leaching fraction and the frequency of application were established considering the crop's sensitivity level and the physical characteristics of the soil.

Fertilization was performed according to the crop requirements, as described by (Trani et al., 2014),



**Figure 1.** Meteorological data collected during the conduct of the experiment.

**Table 1.** Chemical and physical characteristics of the soil used in the experiment

		Chemical characteristics						
pH H <sub>2</sub> O)	OM	P	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H <sup>+</sup>
(1:2.5)	g kg <sup>-1</sup>	(mg kg <sup>-1</sup> )	.....cmol <sub>c</sub> kg <sup>-1</sup> .....					
5.58	2.93	39.2	0.23	1.64	9.07	2.78	0.0	8.61
.....Chemical characteristics.....			.....Physical characteristics.....					
EC <sub>se</sub>	CEC	SAR	ESP	Particle size (g kg <sup>-1</sup> )			Moisture (dag kg <sup>-1</sup> )	
(dS m <sup>-1</sup> )	cmol <sub>c</sub> kg <sup>-1</sup>	(mmolL <sup>-1</sup> ) <sup>0.5</sup>	%	Sand	Silt	Clay	33.42kPa <sup>1</sup>	1519.5kPa <sup>2</sup>
2.15	22.33	0.67	7.34	572.7	100.7	326.6	25.91	12.96

pH – Potential of hydrogen, MO. – Organic matter; Walkley-Black digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1 M KCl at pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted with 1 M NH<sub>4</sub>OAc at pH 7.0; Al<sup>3+</sup>+H<sup>+</sup> extracted with 0.5 M CaOAc at pH 7.0; CEes - electrical conductivity of the saturation extract; CEC - cation exchange capacity; SAR- sodium adsorption ratio of the saturation extract; ESP- exchangeable sodium percentage; <sup>1, 2</sup>referring to the field capacity and the permanent wilting point thresholds.

beginning at 10 DAT. The fertilization level corresponding to 100% of the N recommendation for bell pepper was 220 kg ha<sup>-1</sup>. The recommended phosphorus and potassium levels were 320 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 320 kg ha<sup>-1</sup> of K<sub>2</sub>O, supplied through 7.55 g of urea (45% N), 13.33 g of KCl (60% K<sub>2</sub>O), and 16 g monoammonium phosphate (60 g of P<sub>2</sub>O<sub>5</sub>) per plant, provided via irrigation water at 10, 20, and 30 DAT. The nitrogen applied as monoammonium phosphate was discounted from the urea previously provided. Micronutrients began to be provided at 10 DAT via foliar application on the adaxial and abaxial leaf surfaces through fortnightly applications using the commercial product Dripsol Micro Rexene®, which contained: Mg - 1.2%; B - 0.85%, Zn - 4.2%, Fe - 3.4%, Mn - 3.2%, Cu - 0.5%, and Mo - 0.06%. The crop management practices consisted of plant training, mulching (to provide milder thermal conditions for plant roots), and pest and disease control by chemical intervention.

At 45 DAT, leaf disks were taken from the middle third portion of the leaves of each experimental unit using a circular mold, after which the plant material was weighed, macerated, and placed in containers covered with aluminum foil, which received 25 mL of 80% acetone. Then, the containers were placed under refrigeration (8°C) for 24 hours, after which period the material was filtered through paper for five minutes. The quantification of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl<sub>T</sub>), and carotenoids (Carot) was performed by following the methodology of (Lichtenthaler, 1987), with spectrophotometry absorbance readings obtained at the wavelengths of 470 (A<sub>470</sub>), 647 (A<sub>647</sub>), and 663 nm (A<sub>663</sub>) using Eqs. 2, 3, and 4.

$$\text{Chlorophyll } a = (12.7A_{663} - 2.69 \times A_{645}) \quad (2)$$

$$\text{Chlorophyll } b = (22.9A_{645} - 4.68 \times A_{663}) \quad (3)$$

$$\text{Total chlorophyll} = 17.3A_{645} + 7.18A_{663} \quad (4)$$

$$\text{Carotenoids} = (1000 \times A_{470} - 1.82\text{Chl}a - 85.02\text{Chl}b) / 198 \dots \dots \dots (5)$$

At the same occasion (45 DAT), the relative water content (RWC) was determined by collecting two leaves

from each treatment and weighing them in a precision balance accurate to 0.001 g, after which the material was hydrated in plastic bags containing 200 mL of distilled water. Then, after 24 hours, the leaves were weighed again and oven-dried at 65 °C for 48 h to obtain their dry matter weight. The values obtained were employed in Eq. 6, according to the methodology of (Weatherley, 1950).

$$\text{RWC} = (P1 - P3) / (P2 - P3) \times 100 \quad (6)$$

Where:

RWC = relative water content (%);

P1 = fresh leaf mass (g);

P2 = turgid leaf mass (g) and

P3 = dry leaf mass (g).

The integrity of the cell membrane was evaluated by determining the electrolyte leakage in the leaf blade (EL). For this parameter, ten leaf disks (2.8 cm<sup>2</sup>) were taken from each experimental unit using an iron perforator, after which the samples were washed and placed in Erlenmeyer flasks containing 50 mL of distilled water. Then, the flasks were sealed with aluminum foil and kept at 25 °C for 90 minutes. Next, the electrical conductivity of the medium was determined, and the Erlenmeyer flasks were oven-dried at 90 °C for 90 minutes. Then, the electrical conductivity was measured again according to the methodology of (Scotti-Campos et al., 2013), using Eq. 7:

$$\text{EL} = (Xi / Xf) \times 100 \quad (7)$$

Where:

EL = electrolyte leakage in the leaf blade (%);

Xi = initial electrical conductivity (dS m<sup>-1</sup>);

Xf = final electrical conductivity (dS m<sup>-1</sup>).

After 35 DAT, the growth of bell pepper plants was evaluated through the number of leaves (NL), considering the leaves with lengths longer than 3 cm, stem diameter (SD), measured at 2 cm from the ground, and plant height (PH), measured from the base of the plant to the insertion of the apical bud in the main branch.

The data obtained were subjected to the normality test (Shapiro-Wilk) followed by analysis of

variance by the F-test at 0.05 of probability. When the isolated factors were significant, linear and quadratic polynomial regression were performed using the software SISVAR (Ferreira, 2019). For the significant effects of the interaction between factors (Saline levels and nitrogen doses), the data were analyzed following the multiple linear regression analysis procedures, and the respective response surfaces were plotted using the software Sigmaplot® v.12.5.

## Results And Discussion

There was a significant effect of the interaction between irrigation water salinity (SL) and nitrogen doses (ND) on the contents of chlorophyll *a* (Chl *a*), *b* (Chl *b*), total chlorophyll (Chl  $\tau$ ), carotenoids (Carot), relative water content (RWC), and electrolyte leakage (EL) of the bell pepper plants (**Table 2**).

According to the regression equation (**Figure 2A**), the highest chlorophyll *a* content (Chl *a*) was observed at 45 DAT in bell pepper plants irrigated with low-salinity water (0.3 dS m<sup>-1</sup>) and fertilized with 100% of the N recommendation, corresponding to 20.30 mg g<sup>-1</sup> FM. In turn, fertilization with 50% N combined with irrigation with the ECw of 2.4 dS m<sup>-1</sup> provided the lowest Chl *a* value in bell pepper plants, with a mean of 12.09 mg g<sup>-1</sup> FM, representing a reduction of 8.20 mg g<sup>-1</sup> FM compared to the highest value. The reduction in the chlorophyll content due to irrigation with higher salinities is considered an indication of abiotic stress since, under these conditions, the plant can inhibit the synthesis of the chlorophyll-precursor molecule (5-aminolevulinic acid) or increase the enzyme responsible for chlorophyll degradation (chlorophyllase), thus reducing photosynthesis (Nóbrega et al., 2021).

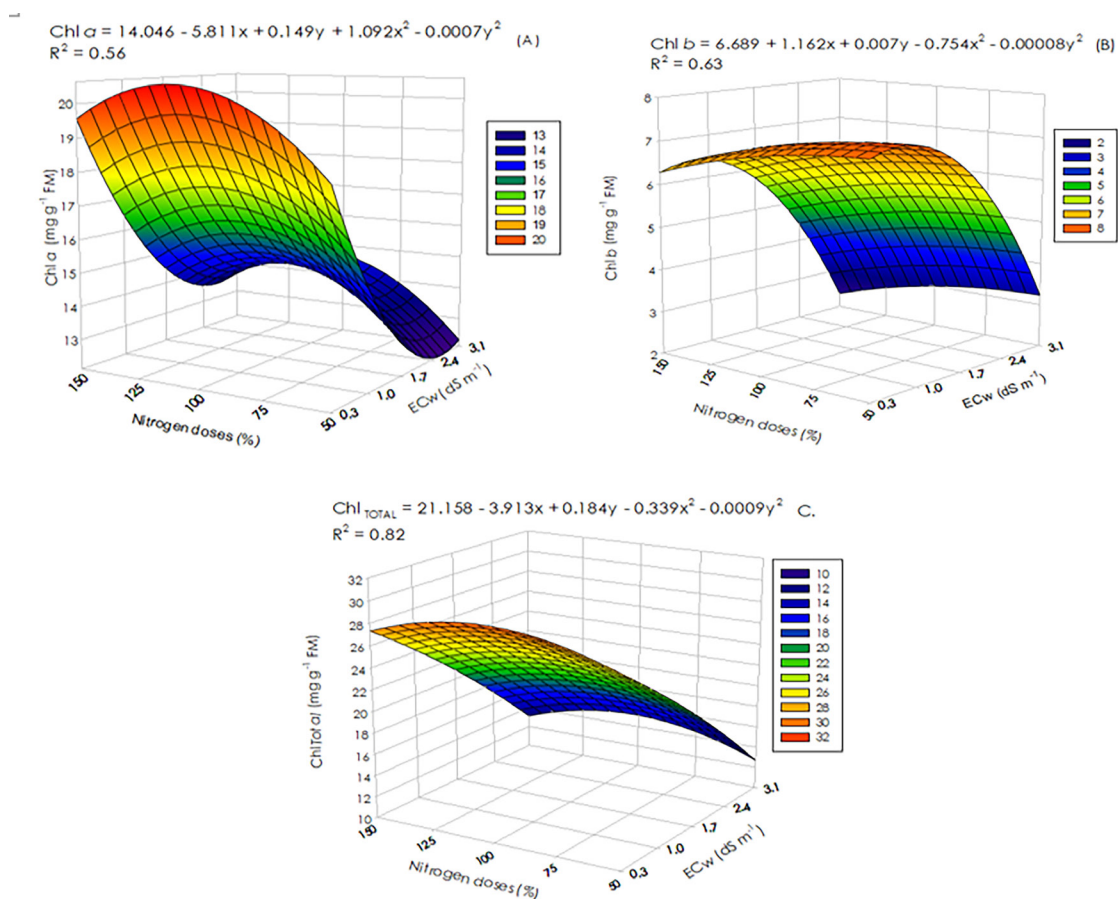
According to the regression equations for the contents of chlorophyll *b* (Chl *b*) as a function of water salinity and the nitrogen fertilization levels (**Figure 2B**), the highest CL *b* values were observed in plants irrigated with the ECw of 1 dS m<sup>-1</sup> and fertilized with 50% N (7.24 mg g<sup>-1</sup> FM). Fertilization with 150% combined with irrigation with the highest salinity (ECw of 3.1 dS m<sup>-1</sup>) provided the lowest Chl *b* in bell pepper plants. Therefore, there was a decrease of 31.27%, corresponding to an estimated loss of 4.95 mg g<sup>-1</sup> FM compared to the highest and lowest means estimated. These results indicate that higher N levels can intensify salt stress in plants since the supply of nitrogen fertilizers at inadequate amounts causes salt stress, toxicity, and increases the pH, thus harming the plant metabolism by reducing chlorophyll production (Santos et al., 2020), affecting photosynthesis and, consequently, plant growth. These effects occur because

Chl *b* is considered an accessory pigment that acts by widening and complementing the light range used in photosynthesis, capturing energy from other wavelengths and transferring it to Chl *a* (Silva et al., 2020). In a study that evaluated the Chl *b* content of bell pepper plants subjected to four levels of irrigation water salinity (0.8, 1.6, 2.4, and 3.2 dS m<sup>-1</sup>) and four concentrations of salicylic acid (SA) (0; 1.2, 2.4, and 3.6 mM), (Veloso et al., 2021) observed that the maximum estimated value of 144.85 mg g<sup>-1</sup> FM was obtained in plants subjected to 1.7 mM of SA and irrigated with the lowest water salinity (0.8 dS m<sup>-1</sup>). In contrast, the lowest value (60.46 mg g<sup>-1</sup> of FM) was observed in plants that received the SA concentration of 3.6 mM when irrigated with the ECw of 3.2 dS m<sup>-1</sup> (Veloso et al., 2021).

Following the trend of chlorophyll *a*, the total chlorophyll contents (Chl  $\tau_{Total}$ ) of the bell pepper plants were higher when the plants received 100% of the recommended N level and were irrigated with the lowest water salinity (ECw of 0.3 dS m<sup>-1</sup>), with an estimated mean value of 29.35 mg g<sup>-1</sup> FM. Irrigation with the highest salinity resulted in Chl  $\tau$  reductions at all fertilization levels studied, showing estimated mean values of 12.71, 14.50, 15.16, 14.70, and 13.11 mg g<sup>-1</sup> FM for the plants fertilized with 50, 75, 100, 125, and 150% of N, respectively (**Figure 2B**). Salt accumulation in the soil caused by irrigation salinity is responsible for the ionic effect in plants, characterized by the increase in the Na<sup>+</sup>: NH<sub>4</sub><sup>+</sup> ratio and generating a nutrient imbalance caused by the competition between sodium and nitrogen (Soares et al., 2018).

According to (**Figure 3**), the carotenoid content (Carot) decreased by 88.82% in bell pepper plants irrigated with the highest salinity (ECw of 3.1 dS m<sup>-1</sup>) and fertilized with 150% N, showing the lowest estimated mean, 0.43 mg g<sup>-1</sup> FM, compared to the bell pepper plants irrigated with low-salinity water and fertilized with 100% N, which showed a mean value of 3.90 mg g<sup>-1</sup> FM for this parameter (highest estimated value). Reductions in the carotenoid content caused by salt stress occur due to the degradation of  $\beta$ -carotene and the reduction in the formation of zeaxanthin due to the increase in reactive oxygen species (ROS), produced at higher amounts when plants are under stress and responsible for enzyme inactivation in plants (Silva et al., 2011; Capitulino et al., 2023). Carotenoids can act as antioxidant agents by protecting lipid membranes from the oxidative stress generated by ROS (Barbosa et al., 2014; Lima et al., 2017). In another study, (Tatagiba et al., 2014) observed reductions in the Chl *a* concentration in 34%, in the Chl *b* concentration in 56%, in the Chl  $\tau_{Total}$  concentration in





**Figure 2.** Contents of chlorophyll a – Chl a (A), chlorophyll b – Chl b (B), and total chlorophyll – Chl Total (C) as a function of the interaction between the electrical conductivity of irrigation water - ECw and nitrogen fertilization doses 45 days after transplanting.

**Table 2.** Summary of the analysis of variance for the contents of chlorophyll a (Chl a), b (Chl b), total chlorophyll (Chl<sub>T</sub>), carotenoids (Carot), relative water content (RWC), and electrolyte leakage (EL) of bell pepper plants 45 days after transplanting grown under different salinities and nitrogen doses

Sources of variation	DF	Mean Squares					
		Chl a	Chl b	Chl <sub>T</sub>	Carot	RWC	EL
Saline levels (SL)	4	109.25**	25.82**	238.18**	5.29**	568.33**	35.47 <sup>ns</sup>
Linear regression	1	323.41**	90.13**	856.24**	15.44**	769.67**	-
Quadratic regression	1	60.14*	10.11**	37.01 <sup>ns</sup>	0.00 <sup>ns</sup>	388.75**	-
Nitrogen doses (ND)	4	22.38 <sup>ns</sup>	9.73**	38.57*	3.02**	160.21**	46.43**
Linear regression	1	-	8.93**	856.24 <sup>ns</sup>	0.11 <sup>ns</sup>	16.33 <sup>ns</sup>	154.54**
Quadratic regression	1	-	0.25 <sup>ns</sup>	37.01 <sup>ns</sup>	10.21**	534.27**	2.27 <sup>ns</sup>
Interaction (SL × ND)	16	52.59**	20.02**	112.11**	1.56**	63.91**	15.35**
Blocks	2	25.03 <sup>ns</sup>	1.14 <sup>ns</sup>	28.16 <sup>ns</sup>	0.74 <sup>ns</sup>	28.43 <sup>ns</sup>	2.74 <sup>ns</sup>
CV (%)		21.81	15.17	15.33	19.12	4.68	9.49
Mean		15.70	5.62	21.47	2.98	79.56	20.14

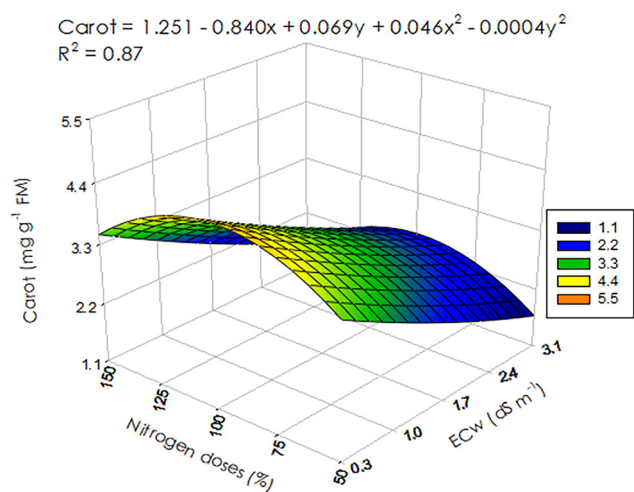
<sup>ns</sup>, \*; \*\* respectively non-significant and significant at  $p \leq 0.05$  and  $\leq 0.01$ ; DF - Degree of freedom; CV= coefficient of variation;

40%, and in the carotenoid content in 41% of tomato leaves subjected to the NaCl concentration of 150 mmol L<sup>-1</sup> compared to plants not subjected to salt stress (0 mmol L<sup>-1</sup> of NaCl).

(Silva et al., 2020) evaluated four biochar levels (0, 7, 14, and 21 m<sup>3</sup> ha<sup>-1</sup>) and four nitrogen doses (0, 40, 80, and 120 kg ha<sup>-1</sup>) and observed 2188.18 for Chl<sub>T</sub> and 423.13 mg g<sup>-1</sup> FM for Carot in bell pepper plants that received 70 kg ha<sup>-1</sup> of N and 10 m<sup>3</sup> ha<sup>-1</sup> of biochar. In contrast, the

plants non-fertilized with N (0 kg ha<sup>-1</sup>) and biochar (0 m<sup>3</sup> ha<sup>-1</sup>) reached 1408.85 for Chl<sub>T</sub> and 273.42 mg g<sup>-1</sup> FM for Carot, corresponding to 35.62 and 34.67% reductions in the contents of Chl<sub>T</sub> and Carot, respectively, in relation to the highest values.

With regard to the relative water content (RWC), the 125% level of nitrogen associated with irrigation with the ECw of 1 dS m<sup>-1</sup> stood out for providing the highest value for this variable, with an estimated mean of 93.63%.



**Figure 3.** Carotenoid content (Carot) of bell pepper leaves as a function of the interaction between the electrical conductivity of irrigation water - ECw and nitrogen fertilization doses 45 days after transplanting.

This value corresponds to a 23.84% increase in relation to the lowest RWC value (69.81%) in plants that received a treatment composed of 50% N and water with the highest salinity (ECw of 3.1 dS m<sup>-1</sup>). However, it should be noted that, when the plants received water with the electrical conductivity of 1.7 dS m<sup>-1</sup> and were fertilized with 125% N, the mean value of this parameter was 92.75%, i.e., a reduction of only 0.90%, similar to fertilization with 150%, which provided the plants irrigated with 1 and 1.7 dS m<sup>-1</sup> with the means of 92.98 and 92.08%, with reductions of 0.67 and 1.57% compared to the highest values (**Figure 4A**). This behavior could be due to the acclimation of bell pepper plants irrigated with high-salinity water caused by higher nitrogen doses as this nutrient is part of several organic compounds such as enzymes, proteins, amino acids, and nucleic acids that act in the osmotic adjustment of plants under salinity conditions, favoring the water uptake by plants (Dias et al. 2020; Roque et al., 2022b).

According to (Figure 4B), the highest N level studied (150%) provided the highest mean value of electrolyte leakage (EL) in bell pepper plants irrigated with the electrical conductivity of 3.1 dS m<sup>-1</sup>, corresponding to 24.01%. This value represents an increase of 6.37% compared to the lowest value, observed in plants irrigated with 0.3 dS m<sup>-1</sup> and fertilized with 50% N (17.64%). The accumulation of ions such as Cl<sup>-</sup>, Na<sup>+</sup>, and B, supplied in the saline irrigation water, destabilizes the cell membrane, which can be intensified by the increase in the nitrogen supply, with higher N doses causing competition with K and Ca, nutrients that provide resistance to the cell membrane (Sousa et al., 2017).

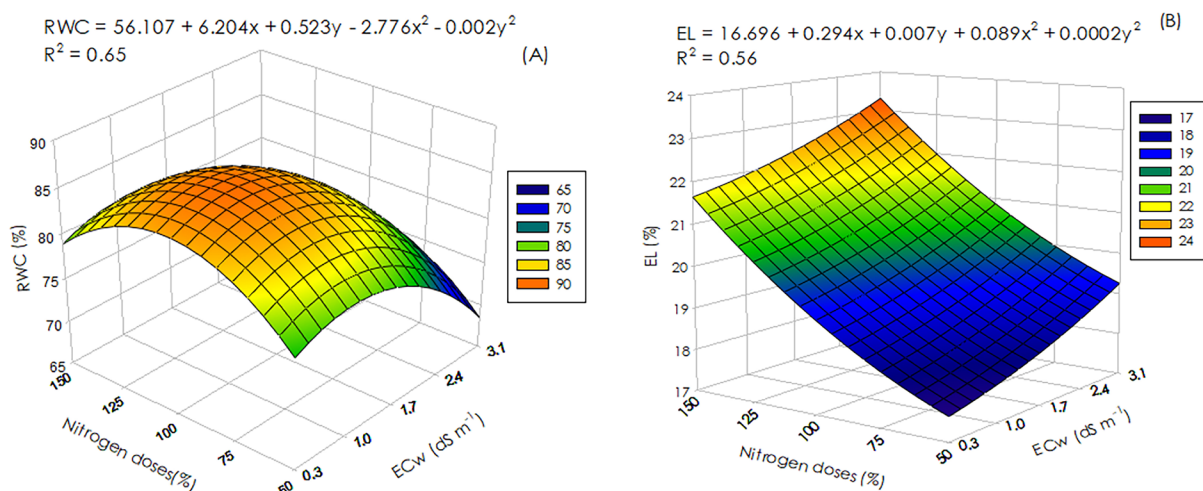
There was a significant effect of the interaction

between salinities and nitrogen doses (SL × ND) on plant height (PH), stem diameter (SD), and number of leaves (NL) (**Table 3**).

(**Figure 5A**) shows that plant height (PH) was higher when irrigation was performed with the highest salinity (3.1 dS m<sup>-1</sup>) and fertilization occurred with 100% N, achieving a mean value of 25.60 cm. Fertilization with 75% N and irrigation with the ECw of 3.1 dS m<sup>-1</sup> resulted in plants with 25.12 cm, i.e., representing a reduction of only 0.48 cm compared to the highest value. In contrast, the lowest AP value was estimated in plants irrigated with the ECw of 1.7 dS m<sup>-1</sup> and fertilized with 150% N. Adequate nitrogen doses can provide ion homeostasis in plants, favoring the uptake of this nutrient even under salinity conditions. In contrast, high N levels increase the soil EC and pH, possibly intensifying the deleterious effects of salinity (Alvarenga et al., 2019; Sá et al., 2021), which is confirmed by the Chl b (Figure 2B) and electrolyte leakage (Figure 4B).

For stem diameter (SD), the highest value was observed in plants irrigated with low-salinity water (ECw of 0.3 dS m<sup>-1</sup>) and fertilized with 75% N, with a mean of 7.17 mm. This value was higher by 1.85 mm compared to the lowest value observed (5.32 mm) in plants irrigated with the highest salinity (ECw of 3.1 dS m<sup>-1</sup>) and fertilized with 150% of the N recommendation (Figure 5B). The SD reduction occurred as a function of irrigation with high-salinity water, which negatively affected the photosynthetic pigments (Figure 1 and 2) and thus decreased photosynthesis, producing smaller plants (Lima et al., 2020). (Roque et al., 2022) studied the effects of different salinities (0.3, 1.3, 2.3, 3.3, and 4.3 dS m<sup>-1</sup>) and nitrogen doses (50, 75, 100, 125, and 150% of the N recommendation) in tomato plants and observed maximum values of 11.98 and 11.62 mm in the SD of plants irrigated with the ECw levels of 1.3 and 2.3 dS m<sup>-1</sup>, respectively.

The number of leaves increased by 29.27% (7.94 leaves) in plants that received 150% N and irrigated with the ECw of 3.1 dS m<sup>-1</sup>, with a mean of 27.12 leaves compared to the lowest value (19.18 leaves) observed in plants irrigated with 1 dS m<sup>-1</sup> and fertilized with 75% N. The 125% N level also stood out when the plants received the ECw of 3.1 dS m<sup>-1</sup>, with a mean value of 24.98 leaves, corresponding to a reduction of only 2.14% compared to the highest value. This behavior is similar to the RWC (Figure 4C), for which the 125 and 150% levels promoted the acclimation of bell pepper plants and a higher water uptake occurred even under high salinity conditions, which may have favored leaf formation. The management of nitrogen fertilization favors ion

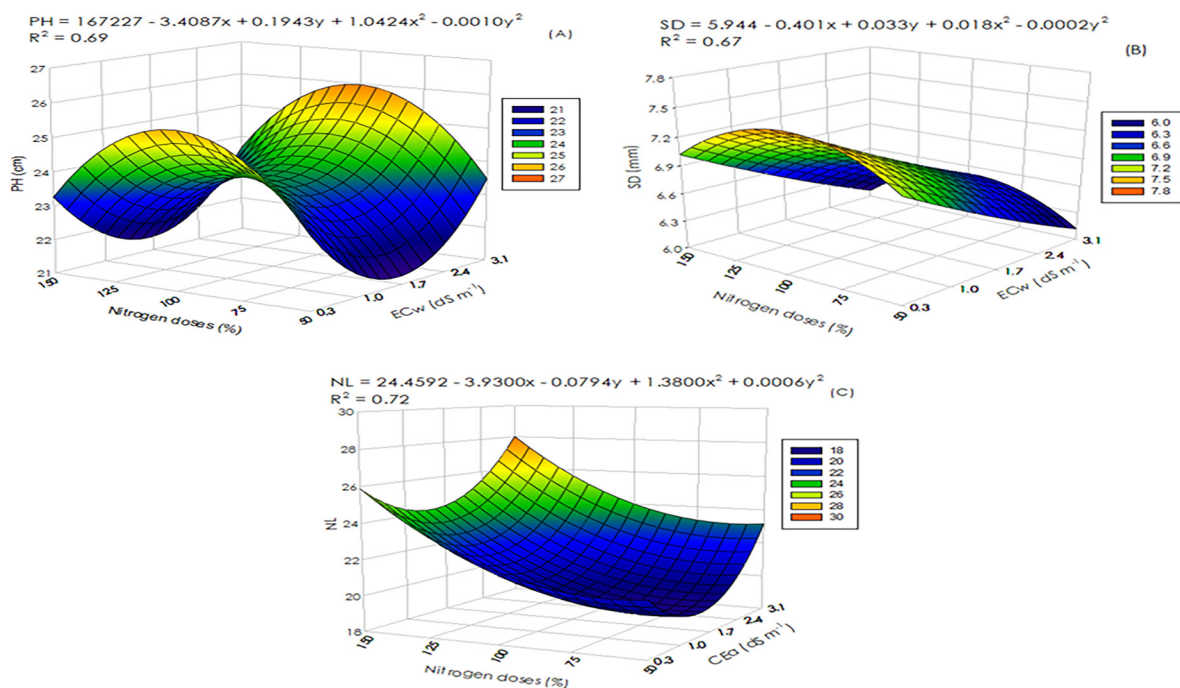


**Figure 4.** Relative water content (RWC) and electrolyte leakage (EL) of bell pepper leaves as a function of the interaction between the electrical conductivity of irrigation water - ECw and nitrogen fertilization doses 45 days after transplanting.

**Table 3.** Summary of the analysis of variance for plant height (PH), stem diameter (SD) and number of leaves (NL) of bell pepper plants 35 days after transplanting under different salinities and nitrogen doses

Sources of variation	DF	Mean squares		
		PH	SD	NL
Saline levels (SL)	4	17.14**	0.69*	39.75**
Linear regression	1	1.35 <sup>ns</sup>	0.36 <sup>ns</sup>	42.66**
Quadratic regression	1	54.77**	1.09*	96.01**
Nitrogen doses (ND)	4	21.57**	2.01**	71.92**
Linear regression	1	0.24 <sup>ns</sup>	0.01 <sup>ns</sup>	220.82**
Quadratic regression	1	76.20**	5.54**	33.60**
Interaction (SL × ND)	16	7.37**	1.35**	19.26**
Blocks	2	0.10 <sup>ns</sup>	0.06 <sup>ns</sup>	6.41 <sup>ns</sup>
CV (%)		4.14	6.50	7.63
Mean		23.55	6.83	22.37

<sup>ns</sup>, <sup>\*</sup>, <sup>\*\*</sup> respectively non-significant and significant at  $p \leq 0.05$  and  $\leq 0.01$ ; DF = Degree of freedom; CV = coefficient of variation.



**Figure 5.** Plant height (PH) – A, stem diameter (SD) – B, and number of leaves (NL) – C of bell pepper as a function of the interaction between irrigation water salinity - ECw and nitrogen fertilization doses 45 days after transplanting.



homeostasis in plants under salt stress by increasing the  $\text{NO}_3/\text{Cl}$  ratio and consequently increasing cell division since N is part of the protoplast structure, where cell division occurs, thus increasing leaf formation under these circumstances (Bittar et al., 2021).

### Conclusions

The N doses of 125 and 150% favored a higher relative water content in bell pepper plants irrigated with electrical conductivity levels of up to  $1.7 \text{ dS m}^{-1}$ .

Irrigation with the electrical conductivity of  $3.1 \text{ dS m}^{-1}$  combined with the N fertilization levels of 100 and 125% increased the number of leaves in bell pepper plants.

The contents of chlorophyll *a*, *b*, total chlorophyll, carotenoids, and stem diameter of bell pepper plants decreased when the plants were fertilized with 150% N and irrigated with the electrical conductivity of  $3.1 \text{ dS m}^{-1}$ .

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