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Gas exchange and photosynthetic pigments in bell pepper irrigated with saline water

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Key words:
salt stress
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ABSTRACT
The tools that evaluate the salinity effects on plants have great relevance as they contribute to understanding of the mechanisms of tolerance. This study aimed to evaluate gas exchanges and the contents of photosynthetic pigments in bell peppers cultivated with saline solutions (0, 1, 3, 5, 7 and 9 dS m^{-1}) prepared using two sources: NaCl and a mixture of Ca, Mg, K, Na and Cl salts, in randomized blocks with a 6 x 2 factorial scheme and 4 replicates, totaling 48 experimental plots. The net photosynthesis (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci), instantaneous carboxylation efficiency (A/Ci) and water use efficiency (WUE), besides chlorophyll a, b and carotenoids were evaluated. The gas exchange parameters were efficient to indicate the effects of salinity. All photosynthetic pigments decreased with increased electrical conductivity, and the chlorophyll a is the most sensitive to salinity, while the water use efficiency increased with the increment of electrical conductivity.

Palavras-chave:
estresse salino
fotossíntese
clorofila
semiárido brasileiro

Trocas gasosas e pigmentos fotossintéticos em pimentão irrigado com água salina

RESUMO
As ferramentas que avaliam os efeitos da salinidade nos vegetais apresentam grande relevância uma vez que contribuem para compreender os mecanismos de tolerância. Objetivou-se avaliar as trocas gasosas e os teores dos pigmentos fotossintéticos em pimentão cultivado com água salina (0, 1, 3, 5, 7 e 9 dS m^{-1}) elaboradas de duas formas: NaCl e uma mistura de sais de Ca, Mg, K, Na e Cl. O delineamento experimental foi em blocos ao acaso com arranjo fatorial 6 x 2, com 4 repetições totalizando 48 parcelas experimentais. Foram avaliadas a fotossíntese líquida (A), a condutância estomática (gs), a transpiração (E), a concentração interna de CO_2 (Ci), a eficiência instantânea de carboxilação (A/Ci) e a eficiência do uso da água (EUA) além da clorofila a, clorofila b e carotenoides. Os parâmetros de trocas gasosas foram eficientes para indicar os efeitos da salinidade. Todos os pigmentos fotossintéticos reduziram com o aumento da condutividade elétrica sendo a clorofila a de maior sensibilidade à salinidade enquanto a eficiência do uso da água aumentou com a elevação da condutividade elétrica.

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Introduction

Bell pepper (*Capsicum annumm* L.) is one of the ten economically most important vegetables, with national production of 249,000 t year^{-1} (IBGE, 2006). It is cultivated mainly by small and medium farmers in the semi-arid region, making the Northeast region the second largest producer, responsible for 31% of the national production (Nascimento et al., 2015).

Soil salinity affects the development of the vegetables through the inhibition of water absorption, ionic imbalance and toxicity, causing metabolic alterations and inhibitions (Ahmed et al., 2012; Souza et al., 2012), constituting one of the main abiotic stress factors that limit the development of crops in the semi-arid region. Thus, studies involving bell pepper development under these environmental conditions become necessary (Glenn et al., 2012; Souza et al., 2014; Nascimento et al., 2015).

Plants under salt stress initially show reductions in stomatal conductance, photosynthetic rate and in the biosynthesis of chlorophyll (Rhein et al., 2015), besides changes in water use efficiency and water status of the plant, leading to the inhibition of growth (Flowers & Colmer, 2008; Santos et al., 2013).

In this context, it is essential to evaluate the effects of solutions with different ionic compositions on the metabolism of plants under salt stress (Duarte & Souza, 2016; Melo et al., 2016), since most studies with this focus are based on solutions using only NaCl (Belkheiri & Mulas, 2013; Nedjimi, 2014).

Therefore, the present study aimed to evaluate the gas exchanges and contents of photosynthetic pigments in bell pepper irrigated with water of six levels of electrical conductivity, using two sources: sodium chloride and a mixture of calcium, magnesium, sodium, potassium and chloride, similar to the composition observed at field.

Material and Methods

The soil used in the experiment was collected in the municipality of Pesqueira, PE (8° 34' 11" S; 37° 48' 54" W; 630 m), semi-arid region of Northeast Brazil, in the layer of 0-30 cm and classified as Fluvic Neosol (EMBRAPA, 2013). Then, the soil was air-dried, pounded to break up clods, homogenized and sieved through a 4-mm mesh, thus preserving its microaggregates.

The experiment was carried out in a greenhouse at the Federal Rural University of Pernambuco (UFRPE) during the period of 90 days. Seedlings of bell pepper (*Capsicum annuum* L.), cv. 'Itamara', were cultivated in pots with capacity for 8 L, filled with sieved soil, with one plant each.

Soil chemical characterization (Table 1) was performed using air-dried fine earth (ADFE), which was evaluated for pH_{H2O} using the soil:water ratio of 1:2.5 and the exchangeable cations Ca^{2+}, Mg^{2+}, Na^+ and K^+, extracted with 1 mol L^{-1} ammonium acetate (Thomas, 1982). The saturation paste was prepared (Richards, 1954) to obtain the saturation extract, analyzed for electrical conductivity and pH, with the soluble bases and the chloride ion determined through the method of titration with $AgNO_3$ (EMBRAPA, 1997).

The cation exchange capacity (T) was determined through the index cation method (Richards, 1954). The results of the exchange complex were used to calculate the values of sum of bases (SB) and exchangeable sodium percentage (ESP). Based on the chemical characterization, fertilization was performed according to the manual of recommendation of fertilization for the state of Pernambuco (IPA, 2008).

The physical characterization (Table 2) consisted of analyses of granulometry and clay dispersed in water in the ADFE through the densimeter method, estimating the indices of dispersion and flocculation. Soil bulk density was determined through the graduated cylinder method and soil particle though the volumetric flask method (EMBRAPA, 1997). Field capacity and permanent wilting point were determined based on the characteristic soil-water retention curve (CSWRC). The total porosity was estimated using the values of soil bulk and particle density.

The seeds were placed to germinate on polystyrene trays and, at 40 days after sowing, the seedlings were transplanted to the pots based on their health, height and vigor, and trained using single trellis.

After transplanting, the plants were subjected to a moisture content equivalent to 80% of field capacity. The moisture

Table 1. Initial chemical characterization of the Fluvic Neosol used to fill the pots in the greenhouse experiment

Saturation extract		Exchange complex		Ratio (soluble bases)	
Variables	Values	Variables	Values	Variables	Values
pH_{se}	8.17	$pH_{(1:2.5)}$	7.70	Na/Ca	5.34
EC (dS m^{-1})	1.26	Ca^{2+} (cmol$_c$ kg^{-1})	5.53	Na/Mg	5.13
Ca^{2+} (mmol L^{-1})	1.00	Mg^{2+} (cmol$_c$ kg^{-1})	2.22	Na/K	4.76
Mg^{2+} (mmol L^{-1})	1.04	Na^+ (cmol$_c$ kg^{-1})	0.26	Na/Cl	0.78
Na^+ (mmol L^{-1})	5.34	K^+ (cmol$_c$ kg^{-1})	0.50	Cl/Ca	6.80
K^+ (mmol L^{-1})	1.12	SB (cmol$_c$ kg^{-1})	8.51	Cl/Mg	6.53
Cl^{-1} (mmol L^{-1})	6.80	ESP (%)	3.00	Cl/Na	1.27
				Cl/K	6.07

pH_{se} - pH determined in the saturation extract; ESP – Exchangeable sodium percentage; SB – Sum of bases

Table 2. Initial physical characterization of the Fluvic Neosol used to fill the pots in the greenhouse experiment

Sand			Silt	Clay	CDW	Ds	Dp	IF	ID	P_T	FC	PWP
Fine	Coarse	Total								%		
g kg^{-1}						kg dm^{-3}					g g^{-1}	
312	117	429	422	149	102	1.24	2.52	0.31	0.69	50.79	0.23	0.05

CDW – Clay dispersed in water; Dp – Soil particle density; Ds – Soil bulk density; ID – CDW/Clay; IF: (1 – ID); ID – Index of dispersion; IF – Index of flocculation; P_T – Total porosity; FC – Field capacity; PWP – Permanent wilting point content was selected based on the characteristic soil-water retention curve (CSWRC). The saline solutions used for irrigation were prepared in the laboratory using two sources of salts: NaCl and a mixture of NaCl, KCl, $CaCl_2$ and $MgCl_2$ (Table 3), with the following levels of electrical conductivity: 0, 1, 3, 5, 7 and 9 dS m^{-1}. In order to justify the composition and the proportion of salts used to prepare the mixture, a water sample was collected for characterization from an artesian well located inside the area where the soil was collected.

The plants were initially irrigated with distilled water, for 10 days, and the electrical conductivity was gradually increased, in order to avoid osmotic shock on the recently transplanted plants. Then, daily irrigations were performed using the saline solutions of different levels of electrical conductivity (Table 3).

Along the entire experiment, the moisture content in the pots was maintained gravimetrically. The pots were daily weighed and irrigated with a volume corresponding to the daily evapotranspiration. Weighing and irrigation were performed always at the end of the day to allow an equilibrium of the soil moisture during the night, without losses through evaporation.

The net photosynthesis (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci), instantaneous carboxylation efficiency (A/Ci) and water use efficiency (A/E) were determined 15 days after induction of salinity from 8 and 11 a.m., using the Infra-Red Gas Analyzer (IRGA - Model LICOR XT 6400).

The photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted according to the procedures described in Arnon (1949) and quantified according to Lichtenthaler & Buschmann (2001), also at 44 days after induction of salinity.

The treatments were arranged in randomized blocks, formed by four blocks in a 2 x 6 factorial scheme, which corresponded to two sources of salts (NaCl and mixture) and six levels of electrical conductivity, totaling 48 plots. The data obtained for the gas exchanges were subjected to descriptive statistical analyses through the mean and standard deviation and the data obtained for the photosynthetic pigments were analyzed through analysis of variance and fit of regression equations using the software SAEG.

Table 3. Salt concentrations (g L^{-1}) required to obtain the values of electrical conductivity (dS m^{-1}) used in the irrigation water of both salt sources

EC	Sources of salt				
	Sodium chloride	Mixture of salts			
	NaCl	NaCl	KCl	$CaCl_2$	$MgCl_2$
0	-	-	-	-	-
1	0.5000	0.3770	0.0020	0.0860	0.1090
3	1.6110	1.1364	0.0071	0.2519	0.3200
5	2.8417	1.8950	0.0134	0.4260	0.5337
7	3.3422	2.9414	0.0170	0.6553	0.8267
9	6.0049	4.1826	0.0211	0.9312	1.1830

Results and Discussion

There were no differences between the solutions of NaCl and mixture of salts for the gas exchanges in bell pepper plants subjected to salinity. However, plants irrigated using water with EC of 1 dS m^{-1} showed increase in net photosynthesis (48.86%), transpiration (38.10%), stomatal conductance (105.55%), internal CO_2 concentration (6.59%) and instantaneous carboxylation efficiency (39.45%), in comparison to the control (Figures 1A, B, C, D, E).

As the irrigation water EC increased, from 3 dS m^{-1} on, the gas exchange variables decreased and reached values of 6.75 μmol CO_2 m^{-2} s^{-1} for A, 3.58 mmol H_2O m^{-2} s^{-1} for E, 0.09 mol H_2O m^{-2} s^{-1} for gs, 229.80 μmol CO_2 mol^{-1} for Ci and 0.03 for instantaneous carboxylation efficiency (A/Ci) in the treatment with 9 dS m^{-1} (Figures 1A, B, C, D, E).

WUE showed the opposite response, increasing for all saline treatments, being 37.36% higher in comparison to the control at the EC of 9 dS m^{-1} (Figure 1F).

The observed reductions in net photosynthesis due to the increase in EC levels, above 3 dS m^{-1}, are probably correlated with the increase in toxicity caused by the salts and dehydration of the cell membranes, which reduces the permeability for the inflow of CO_2. In addition, the decrease in CO_2 assimilation rate along with the decrease in gs and Ci suggests that such reduction of CO_2 in the leaves is due to the stomatal closure (Silva et al., 2010).

Stomatal closure is one of the first defense mechanism of the plants under stress, reducing leaf transpiration, besides causing reduction in the normal CO_2 flow towards the carboxylation site, and gs is one of the main responsible for the reduction of photosynthesis in plants cultivated under saline conditions (Cruz et al., 2003).

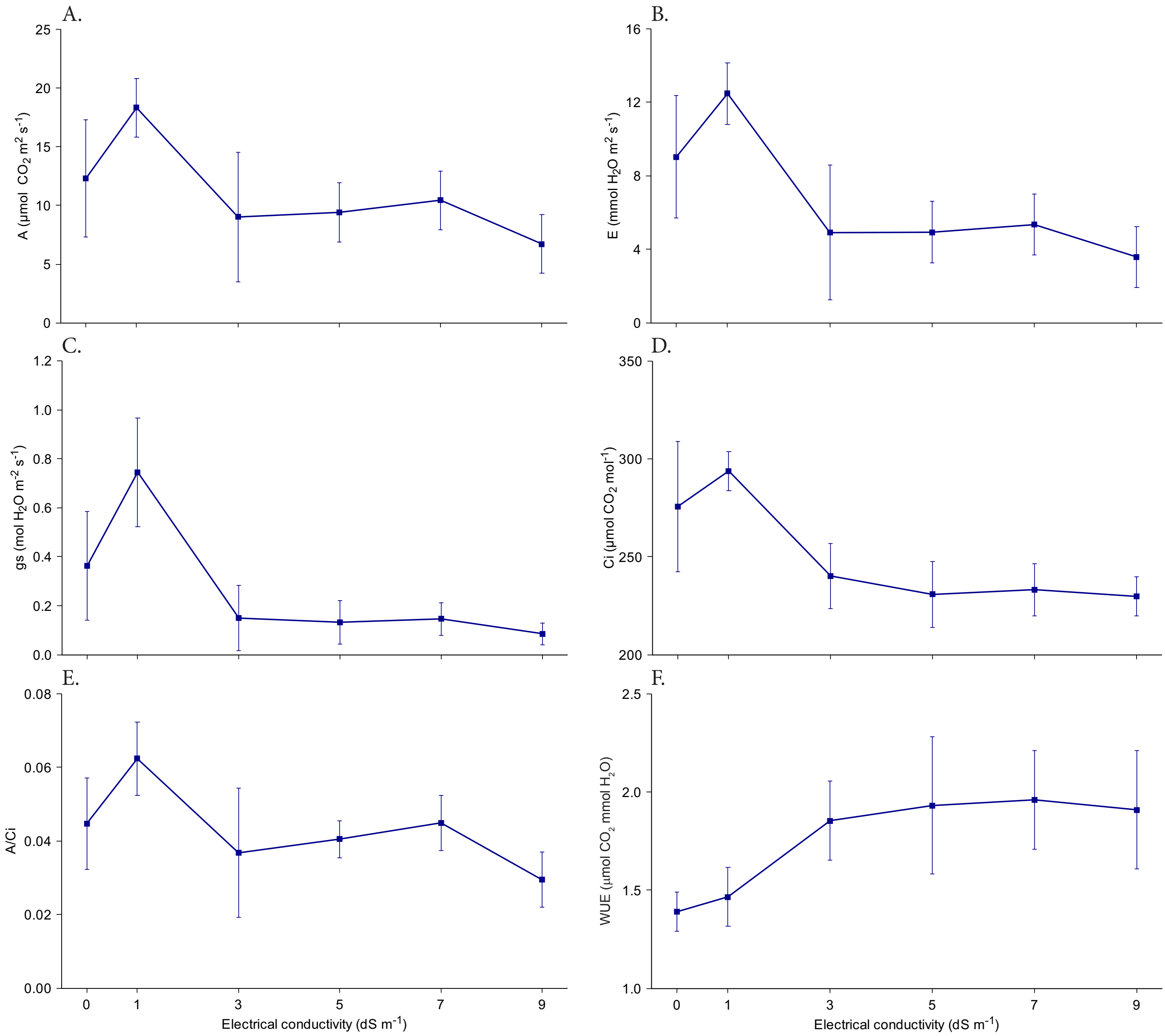
The internal CO_2 concentration decreased in plants subjected to EC above 3 dS m^{-1} (Figure 1D). This behavior, however, does not reflect reductions in the metabolization of the carbon, but instead it reflects stomatal restrictions, since the gs values remained low for the other EC levels (Campos et al., 2014). If the Ci values are very low, the entry of CO_2 in the mesophyll cells is limited. Thus, the plant uses CO_2 from the respiration to maintain a minimum level of photosynthetic rate, making it limited.

The instantaneous carboxylation efficiency, for having a close relationship with the CO_2 assimilation rate and with the intracellular CO_2 concentration, showed results similar to those of these two variables and was higher in plants under EC of 1 dS m^{-1} and lower in plants under EC of 9 dS m^{-1}.

The reduction in stomatal conductance is part of the response of the plants subjected to stresses to reduce the loss of available water of the environment (Chaves et al., 2009). As a consequence, the water use efficiency increases with the reduction of gs, since the use of water molecules becomes more efficient (Campos et al., 2014).

Similar to results obtained in the present study, Bosco et al. (2009) found reductions of A, gs, E and Ci due to the increasing level of electrical conductivity caused by NaCl in eggplant. Among the ten salinity levels applied, the treatment with EC of 14 dS m^{-1} led to the highest damages to the eggplant plants, while the variable stomatal conductance was the most sensitive to this salinity level, with reduction of 57.5%. This result is similar to that found in the present study, in which gs was also the variable most susceptible to the increase in salinity.

Tatagiba et al. (2014), studying the photosynthetic behavior of tomato plants subjected to four doses of salinity in the



Standard deviation of the means represented by the bars

Figure 1. Net photosynthesis (A) [A], transpiration (E) [B], stomatal conductance (gs) [C], internal CO_2 concentration (Ci) [D], instantaneous carboxylation efficiency(A/Ci) [E] and water use efficiency (WUE) [F] of leaves of bell pepper (*Capsicum annuum* L.) at 15 days after induction of salinity

cultivation water, observed that, from 50 mmol L^{-1} of NaCl on, the gas exchanges showed significant reductions, decreasing in the most saline treatment (150 mmol L^{-1}) by 59, 67 and 60% for net photosynthesis, stomatal conductance and transpiration, respectively, compared with the control, without the addition of NaCl. These reductions are similar to those found in the most saline treatment (9 dS m^{-1}) of the present study: 45.41, 76.32 and 60.29%, respectively.

Although they did not differ between the solutions of NaCl and the mixture of salts, the photosynthetic pigments exhibited linear reduction with the increase in irrigation water EC (Figure 2), especially for chlorophyll a, decreasing its content by 50% at the EC of 9 dS m^{-1} in relation to the control.

The reduction in the contents of photosynthetic pigments in response to the salinity levels may be associated with the modifications of the metabolic activities, damages on the membrane and reduction in the development and

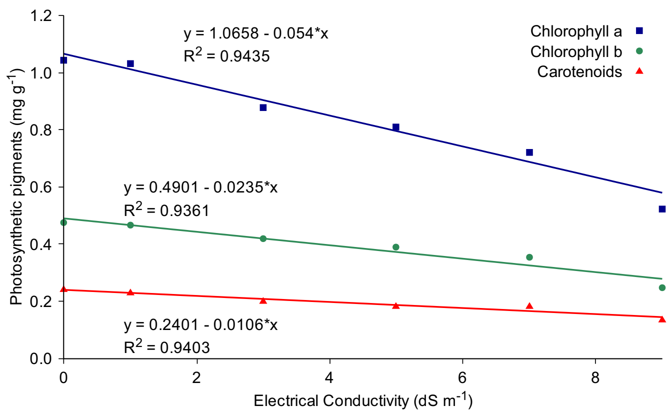


Figure 2. Mean contents of chlorophyll a, b and carotenoids at different levels of electrical conductivity (n = 4) evaluated 44 days after the stress

differentiation of tissues in the chloroplasts (Ali et al., 2004). This reduction may also be associated with the decrease in the biosynthesis of chlorophyll a and with the increment in the activity of the chlorophyllase enzyme, and with the instability of the protein complexes caused by the effects of the salt stress (Houimili et al., 2010).

The lower reduction in the content of carotenoids may be related to its activity as antioxidant, mitigating the oxidative effects and resulting in more constant contents, even with the increase in salinity (Ziaf et al., 2009; Ashraf & Harris, 2013).

Conclusions

1. The gas exchange parameters were efficient to indicate the deleterious effects of salinity on bell pepper plants.

2. The sources of salt did not influence the reductions of gas exchange parameters and the responses were due to the increase in the electrical conductivity.

3. All photosynthetic pigments decreased with the increase in electrical conductivity, and chlorophyll a was the variable most sensitive to salinity.

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