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## Gas exchanges and photochemical efficiency of hydroponic bell pepper under salinity and plant density

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**ABSTRACT:** Gas exchanges and chlorophyll a fluorescence are ways to physiologically analyze the response of plants to salt stress. In this context, the present work was conducted between August and November 2016 in a greenhouse at the Federal University of Campina Grande (7°12'52" S, 35°54'24" W, mean altitude of 550 m), using bell pepper plants cv. All Big, exposed to levels of nutrient solution electrical conductivity (1.7, 3.7, 5.7, 7.7, 9.7 and 11.7 dS m<sup>-1</sup>) and cultivated in hydroponic system, spaced by 0.2 and 0.3 m, focusing on the analysis of gas exchanges and photochemical efficiency. The experimental design was completely randomized, in a 6 x 2 factorial scheme, with five replicates. Plant density influenced the sensitivity of the gas exchanges to salinity and, when reduced, mitigated its effects at higher salinity levels. Increasing plant density at high levels of electrical conductivity caused damage to the photosynthetic apparatus and even reduced the levels of efficiency of the photosystem II from 3.98 dS m<sup>-1</sup>.

**Key words:** *Capsicum annuum* L., low-cost hydroponics, photosynthesis

## Trocas gasosas e eficiência fotoquímica do pimentão hidropônico sob salinidade e densidades de plantio

**RESUMO:** As trocas gasosas e fluorescência da clorofila a são formas de analisar fisiologicamente a resposta das plantas ao estresse salino. Neste contexto, desenvolveu-se, entre agosto e novembro de 2016 em casa de vegetação da Universidade Federal de Campina Grande (7°12'52" Sul, 35°54'24" Oeste, altitude média de 550 m), o presente trabalho, utilizando-se plantas de pimentão cv. All Big, expostas a níveis de condutividade elétrica da solução nutritiva (1,7; 3,7; 5,7; 7,7; 9,7 e 11,7 dS m<sup>-1</sup>) e cultivadas em hidroponia com plantas espaçadas a cada 0,2 e 0,3 m, com foco na análise das trocas gasosas e eficiência fotoquímica. O delineamento experimental adotado foi inteiramente casualizado, em esquema fatorial 6 x 2, com cinco repetições. A densidade de plantas influenciou a sensibilidade das trocas gasosas à salinidade, inclusive, quando reduzida, mitigou seus efeitos nos níveis salinos mais elevados. O aumento da densidade de plantas em níveis de condutividade elétrica elevados provocou danos ao aparato fotossintético, reduzindo, inclusive, os níveis de eficiência do Fotossistema II a partir de 3,98 dS m<sup>-1</sup>.

**Palavras-chave:** *Capsicum annuum* L., hidroponia de baixo custo, fotossíntese



## INTRODUCTION

Bell pepper (*Capsicum annuum* L.) production in semi-arid regions is challenging, especially due to the limitation of water supply and salinity of the waters available for agriculture. Thus, the use of hydroponics has been mentioned (Soares et al., 2010; Santos Junior et al., 2013) as a form of cultivation which allows to equate various limits imposed by environmental and economic issues for bell pepper cultivation.

When associated with other forms of mitigation of salt stress, such as the increase in plant density (Santos Junior et al., 2015), low-cost hydroponics (Santos Junior et al., 2016) can even make viable the use of brackish waters, especially in communities of family farmers.

On the other hand, gas exchanges and photochemical efficiency have been used as parameters for analysis of plant growth and development by several authors (Silva et al., 2010; Suassuna et al., 2011), including in the bell pepper crop (Melo et al., 2017a). However, one of the relevant issues regarding the use of gas exchanges is that their measurements are significant to determine the photosynthetic rates (Santos et al., 2010).

In this context, about the ways to analyze physiologically bell pepper response to salt stress, Melo et al. (2017a) comment that research on gas exchanges provides important information, since the absorption of carbon dioxide ( $\text{CO}_2$ ) is fundamental for photosynthesis to occur. On the other hand, Yusuf et al. (2010) recommend the evaluation of fluorescence, because it represents an intrinsic sign emitted by the leaves about their physiological state, including changes in the photosynthetic apparatus and in the development process (Cha-Um & Kirmanee, 2011; Silva et al., 2011).

Given the above, this work was conducted to analyze the gas exchanges and photochemical efficiency of bell pepper plants grown in hydroponic system, at different population densities and exposed to brackish nutrient solutions.

## MATERIAL AND METHODS

The experiment was conducted in a greenhouse between the months of August and November 2016, at the Federal University of Campina Grande - UFCG, Campina Grande, PB, Brazil ( $7^\circ 12' 52''$  S,  $35^\circ 54' 24''$  W, at mean altitude of 550 m).

The hydroponic system adopted consisted of twelve 100-mm-diameter PVC pipes, leveled, with elbows at the ends, and one of them was connected to a faucet for water outlet, thus guaranteeing a 0.04-m-deep film of nutrient solution along the pipe. These pipes were fixed on a vertical wooden structure - 6 m long, 1.4 m wide and 1.8 tall (Santos Júnior et al., 2016).

Bell pepper was sown in 200-mL disposable plastic cups perforated on the sides and bottom, filled with coconut fiber. The seedlings were daily irrigated with rainwater, applying 20 mL in the morning and 20 mL in the afternoon, until 24 days after sowing (DAS). At 25 DAS, they were inserted into the pipes, in holes with 60 mm diameter, spaced according to previously established treatments.

The experimental design adopted was the completely randomized, with 12 treatments, in a 6 x 2 factorial scheme, with 5 replicates. Treatments were six levels of electrical conductivity in the nutritional solution (1.7, 3.7, 5.7, 7.7,

9.7 and 11.7  $\text{dS m}^{-1}$ ) and two densities of bell pepper plants (*Capsicum annuum* L.), cv. All Big, i.e., plants placed in cells spaced by 20 and 30 cm in the cultivation pipes.

The water used to prepare the nutrient solution came from the community dam of the Vitória Settlement ( $7^\circ 20' 47.49''$  S and  $36^\circ 2' 28.00''$  W), collected after six months without occurrence of precipitation, with the following physicochemical characteristics (EMBRAPA, 2011): pH = 8.24; EC = 29.15  $\text{dS m}^{-1}$ ; K = 0.012  $\text{g L}^{-1}$ ; Na = 5.50  $\text{g L}^{-1}$ ; Ca = 0.41  $\text{g L}^{-1}$ ; Mg = 1.2  $\text{g L}^{-1}$  and SAR = 30.74 ( $\text{mmol L}^{-1}$ )<sup>0.5</sup>. To allow this saline water to be used, it was diluted (Lacerda et al., 2010) in rainwater (EC  $\approx$  0), which resulted in the following salinity levels of the water mixture: 0.2; 2.2; 4.2; 6.2; 8.2 and 10.2  $\text{dS m}^{-1}$ , which reached the previously mentioned levels of electrical conductivity of the nutrient solution ( $\text{EC}_{\text{ns}}$ ) after the fertilizers (Furlani et al., 1999) were solubilized.

Nutrient solution management consisted of manual application of 40 L of nutrient solution twice a day (8 and 17 h) in each pipe, according to the treatments, aiming at the recirculation of nutrients, with daily monitoring of  $\text{EC}_{\text{ns}}$  and  $\text{pH}_{\text{ns}}$ . The evapotranspired volume in the respective container was replaced every week, with the respective mixture of water used to prepare the nutrient solution.

In relation to the gas exchanges, the internal carbon concentration ( $\text{Ci}$  -  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $\text{CO}_2$  assimilation rate ( $A$  -  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ), transpiration ( $E$  -  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and stomatal conductance ( $g_s$  -  $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) were evaluated. The instantaneous water use efficiency ( $\text{WUEi-A/E}$ ) was calculated by relating the  $\text{CO}_2$  assimilation rate with transpiration [ $(\mu\text{mol m}^{-2} \text{s}^{-1})/(\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1})$ ], and the instantaneous carboxylation efficiency ( $\text{CEi-A/Ci}$ ) [ $(\mu\text{mol m}^{-2} \text{s}^{-1})/(\mu\text{mol m}^{-2} \text{s}^{-1})$ ] was obtained from the relationship between the  $\text{CO}_2$  assimilation rate and internal carbon concentration. All these variables were measured at 49 and 60 DAS (fruiting stage), between 7 and 9 h, on the third leaf counted from the apex using an infrared gas analyzer - IRGA (ACD, model LCPro SD, Hoddesdon, UK).

For photochemical efficiency, the initial fluorescence ( $F_0$ ) and maximum fluorescence ( $F_m$ ) of the dark-adapted leaf, maximum variable fluorescence ( $F_v$ ), maximum quantum efficiency of PSII ( $F_v/F_m$ ) from the relationship between maximum variable fluorescence and maximum fluorescence of the dark-adapted leaf were evaluated. The data were measured at 69 DAS at 8 h, using a portable fluorometer (LI-1600, USA), in intermediate leaves of the branches, pre-adapted to the dark using leaf clips after a 30 min period.

The data were subjected to analysis of variance by F test, at  $p \leq 0.05$  and  $p \leq 0.01$  probability levels. In the case of significance, the discussion of the interaction between treatments was prioritized and, in the other situations, polynomial regression analysis was performed for  $\text{EC}_{\text{ns}}$  and plant densities were compared by Tukey test. All analyses were carried out using a statistical program (Ferreira, 2011).

## RESULTS AND DISCUSSION

The gas exchanges of bell pepper plants were significantly influenced ( $p < 0.01$ ) by the interaction between salinity and

plant density at 49 and 60 DAS. However, at 60 DAS, stomatal conductance was only affected by the electric conductivity of nutritive solution separately.

For stomatal conductance (Figure 1A), at 49 DAS, there were reductions of 0.0674 and 0.0662 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> per unit increase in dS m<sup>-1</sup> in plants spaced by 20 and 30 cm, respectively. At 60 DAS, in relative terms, the reduction in gs per unit increase in salinity was 0.0834 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>. Regarding planting densities, the mean values observed were 0.0896 and 0.0793 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in plants spaced by 20 and 30 cm, respectively.

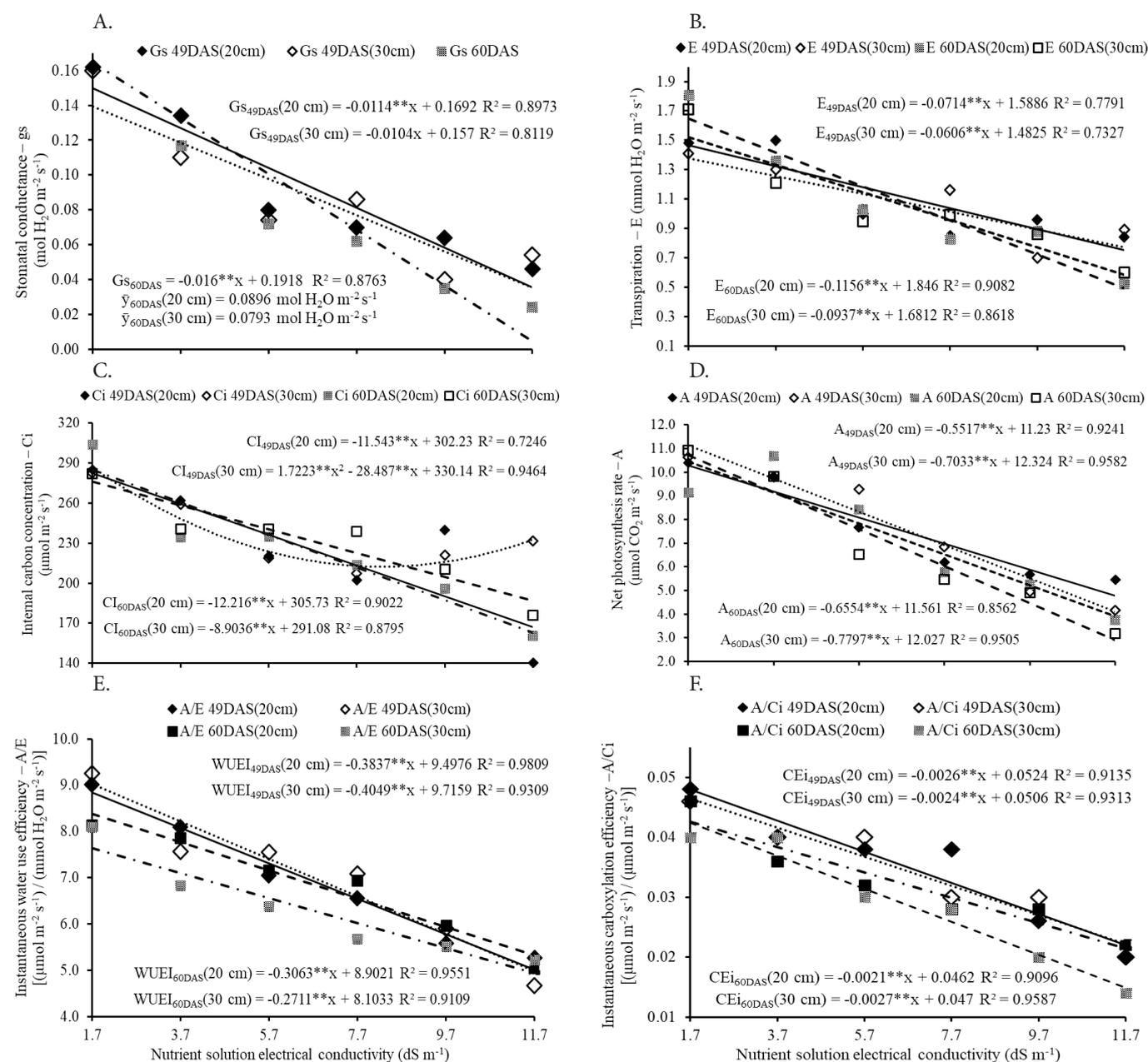
Thus, it was possible to observe that stomatal closure was one of the first defense mechanisms of plants exposed to salt stress and, specifically in the present study, at EC<sub>ns</sub> of 11.5 dS m<sup>-1</sup>, for example, there were very low levels of gs (0.035 and 0.046 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> at 49 for both spacings and 60 DAS, respectively), whose impact triggered limitations in

transpiration and CO<sub>2</sub> assimilation and, consequently, in the photosynthetic process (Campos et al., 2014; Freire et al., 2014).

For transpiration (Figure 1B), it was observed that at 49 DAS, plants exposed to solutions with EC<sub>ns</sub> estimated at 11.7 dS m<sup>-1</sup> transpired more (0.7532 and 0.7735 in plants spaced by 20 and 30 cm, respectively) than those at 60 DAS (0.4935 and 0.5849 in plants spaced by 20 and 30 cm, respectively).

It should be highlighted that there was greater transpiration rate in plants at higher density. Probably, the influence of the higher number of plants on variables such as temperature, radiation, among others, allowed a better adjustment to salt stress and allowed higher levels of transpiration (Lima et al., 2010).

At 49 DAS, in plants at spacing of 20 cm, the Ci (Figure 1C) decreased by 0.0382 μmol m<sup>-2</sup> s<sup>-1</sup> per unit increase in the EC<sub>ns</sub> used in the present study. As the distance between plants increased to 30 cm, a minimum Ci of 212.346 μmol m<sup>-2</sup> s<sup>-1</sup> was found



**Figure 1.** Analysis of the interaction between treatments for stomatal conductance (A), transpiration (B), internal carbon concentration (C), net photosynthesis rate (D), instantaneous water use efficiency (E) and instantaneous carboxylation efficiency (F) in bell pepper plants (cv. All Big) under salt stress and different densities at 49 and 60 days after sowing (DAS)

at estimated  $EC_{ns}$  of approximately  $8 \text{ dS m}^{-1}$ . It was observed that in the higher values of  $EC_{ns}$  the internal concentration of  $CO_2$  in the plants was higher when 30 cm of spacing was adopted, that is, under higher  $EC_{ns}$  the use of lower plant densities showed a higher internal  $CO_2$  concentration.

Melo et al. (2017a), studying bell pepper plants (cv. Itamara) exposed to up to  $9.0 \text{ dS m}^{-1}$ , commented on the efficiency of these parameters as indicators to evaluate salt stress, which ratifies the feasibility of adopting dense configurations to mitigate the osmotic effect imposed by the use of brackish waters.

At 49 DAS, the  $CO_2$  assimilation rate - A (Figure 1D) was higher for both plant densities ( $4.7751$  and  $4.0954 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$  for 20 and 30 cm, respectively) in relation to the plants at 60 DAS ( $3.8928$  and  $2.9045 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$  for 20 and 30 cm, respectively) in the  $EC_{ns}$  of  $11.7 \text{ dS m}^{-1}$ .

Thus, it was verified that with the increase of the  $EC_{ns}$  there was a decrease of the net photosynthesis, in which the values of "A" of the plants with a spacing of 20 cm stood out those of 30 cm.

The increase in plant density burdened the photosynthetic process, probably in response to the reduction in leaf stomatal conductance, transpiration and  $CO_2$  assimilation, stimulated by the increased salinity (Silva et al., 2011). In another analysis, the increased toxicity caused by the salts and the dehydration of cell membranes also reduce the permeability to  $CO_2$  influx, compromising its assimilation (Melo et al., 2017a).

In relation to the instantaneous water use efficiency (Figure 1E) at 49 DAS, it was possible to estimate reductions of 0.0404 and 0.0417 [ $(\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1})/(\text{mmol } H_2O \text{ m}^{-2} \text{ s}^{-1})$ ] per unit increase in nutrient solution salinity for plants spaced by 20 and 30 cm, respectively.

At 60 DAS, the spacing of 20 cm led to higher instantaneous water use efficiency at all salinity levels in comparison to the treatments with spacing of 30 cm, but the estimated reduction per unit increase was 0.0344 [ $(\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1})/(\text{mmol } H_2O \text{ m}^{-2} \text{ s}^{-1})$ ]. In most situations, the increase in plant density caused higher intrinsic water use efficiency. This result is probably associated with the fact that the reduction in stomatal conductance induces a more efficient use of water molecules (Chaves, et al., 2009; Campos, et al., 2014).

The instantaneous carboxylation efficiency (Figure 1F) was negatively influenced at 49 DAS, with decreases of 0.0496 and 0.0474 [ $(\mu\text{mol m}^{-2} \text{ s}^{-1})/(\mu\text{mol m}^{-2} \text{ s}^{-1})$ ] per unit increase in  $EC_{ns}$ . At 60 DAS, the highest instantaneous carboxylation efficiency was observed in plants cultivated at spacing of 20 cm, and the difference tended to increase in relation to plants spaced by 30 cm, as a function of the increase in  $EC_{ns}$ .

The reductions in  $CO_2$  concentrations imposed by the increase of  $EC_{ns}$  lead to restriction on the influx of this component into the mesophyll cells. Thus, the plant uses  $CO_2$  from respiration to maintain a minimum level of photosynthetic rate, making it limited (Taiz & Zeiger, 2013).

In studies with bell pepper plants (cv. Itamara) exposed to water salinity of up to  $9.0 \text{ dS m}^{-1}$ , Melo et al. (2017a) found results similar to those of the present study, in which the

highest values were obtained in plants under water  $EC$  of  $1.0 \text{ dS m}^{-1}$  and the lowest values in plants under water  $EC$  of  $9 \text{ dS m}^{-1}$ . According to the authors, the instantaneous carboxylation efficiency is closely related to  $CO_2$  assimilation rate and its intracellular concentration.

There was significant interaction ( $p \leq 0.01$ ) between the factors  $EC_{ns}$  and plant densities for the variables: initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum efficiency of the PSII, by the Fv/Fm ratio.

The initial fluorescence (Fo) (Figure 2A) with the increase  $EC_{ns}$  was minimal in plants spaced by 20 and 30 cm, respectively, at the estimated  $EC_{ns}$  levels of  $8.62 \text{ dS m}^{-1}$  ( $191.53$  electrons quantum $^{-1}$ ) and  $10.05 \text{ dS m}^{-1}$  ( $185.95$  electrons quantum $^{-1}$ ).

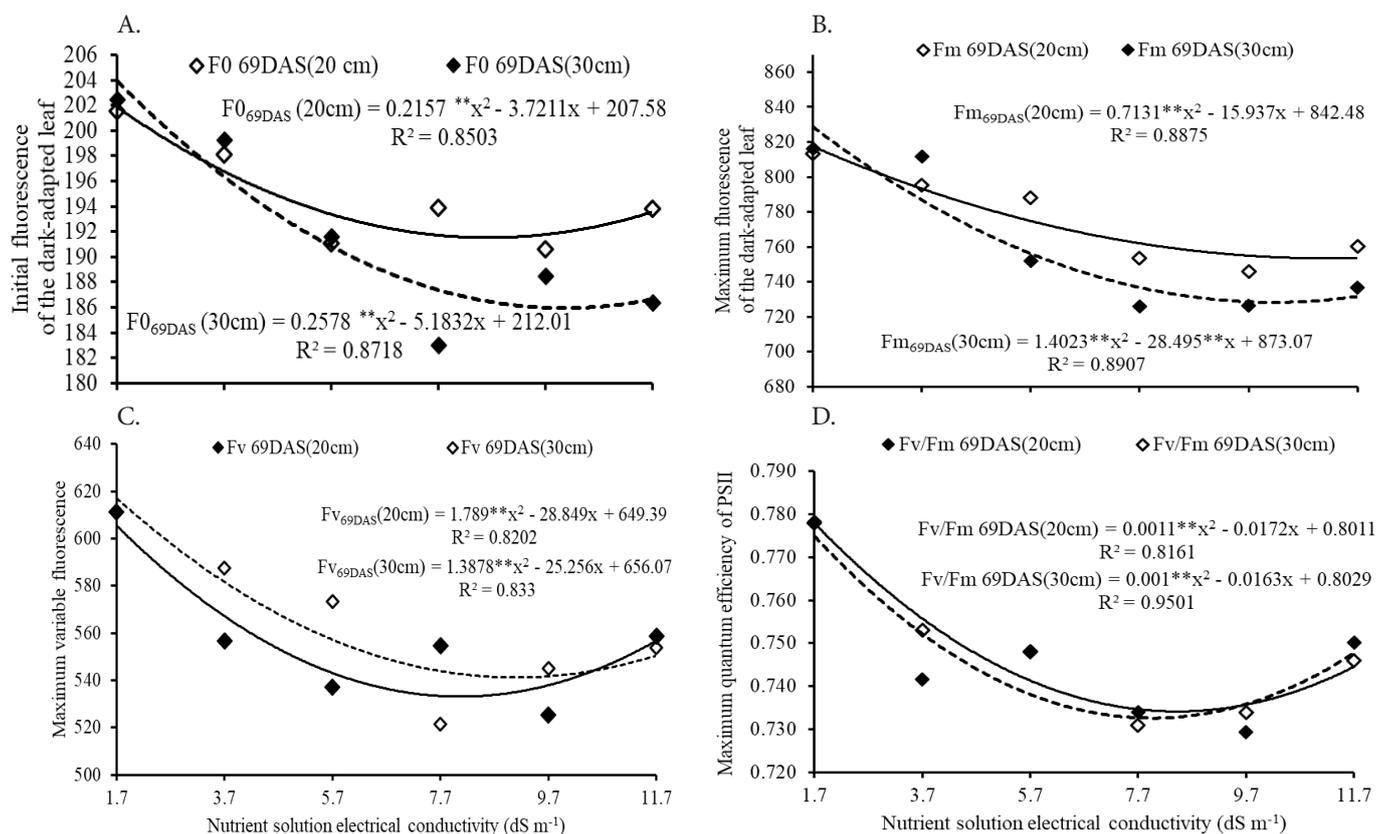
In studies with bell pepper plants (cv. Itamara) exposed to salt stress, Duarte (2015) also observed, at 54 DAT, reduction in Fo between plants under 0 and  $9.0 \text{ dS m}^{-1}$ , corroborating that, within this range of salinity, the maximum efficiency of light energy utilization has been compromised.

However, the increase in plant density (20 cm) from  $8.62 \text{ dS m}^{-1}$  possibly caused a reduction in the maximum efficiency of light energy utilization, although the values of Fo have increased. Melo et al. (2017b) exposed *Atriplex nummularia* plants to irrigation water from 0 to  $40 \text{ dS m}^{-1}$  under soil conditions and stated that under high salinity levels, the increase of Fo could mean the possible loss of the reaction centers of photosystem II as well as in oxidative damage (Baker, 2008).

Plant density also influenced the effect of salinity on maximum fluorescence in the dark (Figure 2B) where the highest values with increasing  $EC_{ns}$  were obtained for the spacing 20 cm. The minimum values of the maximum fluorescence at the dark were obtained in the  $EC_{ns}$  of  $11.17 \text{ dS m}^{-1}$  ( $753.43$ ) for the spacing 20 cm and  $10.16 \text{ dS m}^{-1}$  ( $728.31$ ) for the spacing 30 cm.

Since this trend was also observed for Fo, the increase in salinity has possibly caused a lower flux of electrons between the photosystems, resulting in less activity or inactivity of the photosystem II reaction centers (Melo et al., 2017b) in plants spaced by 20 cm, i.e., the increase in plant density associated with increased salinity probably compromised the photoreduction of quinone A (QA) (Tatagiba et al., 2014).

The variable fluorescence, which represents the plant's ability to transfer the energy of the ejected electrons from the pigment molecules to the formation of the reducing agent NADPH, ATP and Fdr was affected by the increase in  $EC_{ns}$  and plant density (Figure 2C). According to this figure, the minimum values 533.09 and 541.16 electrons quantum $^{-1}$  in relation to the spacings 20 and 30 cm corresponded to the estimated  $EC_{ns}$  of 8.06 and  $9.10 \text{ dS m}^{-1}$ , respectively. It is evident that the increase in plant density can limit the interception and use efficiency of solar radiation, leading to several physiological and morphological changes (Brachtvogel et al., 2012). However, when associated with salt stress, as is the case in this study, in which there was increased reduction in the variable fluorescence of chlorophyll a in plants at lower density of cultivation, it becomes evident that there were damages in



**Figure 2.** Analysis of the interaction between nutrient solution electrical conductivity and plant density for initial fluorescence of the dark-adapted leaf (A), maximum fluorescence of the dark-adapted leaf (B), maximum variable fluorescence (C) and maximum quantum efficiency of PSII (D) in bell pepper plants (cv. All Big) under salt stress and different densities at 49 and 60 days after sowing (DAS)

the PSII and, consequently, reduction in CO<sub>2</sub> assimilation for the different densities evaluated (Baker, 2008).

The quantum efficiency of PSII (Figure 2D) was minimal at EC<sub>ns</sub> levels of 7.82 and 8.15 dS m<sup>-1</sup>, with values estimated at 0.734 and 0.736 at the plant spacings of 20 and 30 cm, respectively.

According to Bolhar-Nordenkampf et al. (1989), the ideal F<sub>v</sub>/F<sub>m</sub> ratio should be between 0.75 and 0.85. Specifically in the case of the present study, at EC<sub>ns</sub> levels above 3.98 and 4.47 dS m<sup>-1</sup>, in plants spaced by 20 and 30 cm, respectively, the estimated values were lower than 0.75, i.e., they revealed photoinhibition damage in the PSII reaction centers, probably due to the decline in net photosynthesis (Silveira et al., 2010).

## CONCLUSIONS

1. Plant density influenced the sensitivity of gas exchanges to salinity and, when reduced, mitigated its effects at higher salinity levels.

2. The increase in plant density at high levels of electrical conductivity caused damages to the photosynthetic apparatus and even reduced the levels of efficiency of the photosystem II from 3.98 dS m<sup>-1</sup>.

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