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Growth and physiological aspects of bell pepper (*Capsicum annuum*) under saline stress and exogenous application of proline

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This study aimed to evaluate growth and physiological aspects of ‘All Big’ bell pepper, under saline stress and exogenous application of proline on the leaves. The research was conducted in pots adapted as drainage lysimeters under greenhouse conditions, using sandy-loam eutrophic Regolithic Neosol, in the municipality of Campina Grande-PB, Brazil. The experiment was set in randomized blocks, in order to test two levels of irrigation water electrical conductivity - ECw (0.6 and 3.0 dS m^{-1}) associated with four proline concentrations (0, 10, 20 and 30 mmol L^{-1}). Plants were grown in 10-L pots for 50 days after transplantation. This period corresponds to the transition of vegetative and reproductive stages, in which plants were evaluated for growth, gas exchanges and chlorophyll *a* fluorescence. The increase in irrigation water salinity reduced growth, gas exchanges and the efficiency of photosystem II in bell pepper plants. Proline concentrations from 12.8 to 16.8 mmol L^{-1} incremented the activity of gas exchanges, reducing the effect of saline stress on bell pepper plants. Proline concentrations under study did not influence chlorophyll *a* fluorescence of ‘All Big’ bell pepper plants.

Key words: Saline water, gas exchanges, chlorophyll *a* fluorescence, proline.

INTRODUCTION

Bell pepper (*Capsicum annumm* L.) is one of the main Solanaceous crop cultivated in the world. In Brazil, the species is among the ten most important vegetables in the market, for being an attractive crop, with short period for the beginning of production, which promotes rapid return of investments, thus being widely explored by small- and medium-sized farmers, especially in the Northeast region of the country, where it is mainly

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consumed fresh (Leonardo et al., 2007; Campos and Cavalcante, 2009; Nascimento et al., 2011).

In Northeast Brazil, more precisely in the semiarid region, bell pepper is an important crop and its cultivation is located in irrigated areas, where family farming is practiced. However, due to the scarcity of water, caused by the low rainfalls and high atmospheric demand, which restrict the availability and use of good-quality water in agriculture, it is necessary to use waters of lower quality for irrigation, such as saline water (Cavalcante et al., 2011). Nevertheless, the use of saline water affects the development of sensitive crops, such as bell pepper, which tolerates, without significant yield losses, contents of salts in the soil between 1.3 and 3.0 dS m^{-1} in terms of electrical conductivity of the soil saturation extract or between 0.8 and 2.0 dS m^{-1} in terms of irrigation water salinity (Ayers and Westcot, 1999).

In general, the effects of salinity on plants are attributed to the osmotic effect and to the specific action of ion concentration. In bell pepper, countless disorders have been observed due to the excess of salts in the soil, among which: disorders in the permeability of cell membranes, alterations in stomatal conductance, photosynthesis and ionic balance, which lead to reduction in plant development, regardless of the cationic nature of the salts (Aktas et al., 2006; Leonardo et al., 2008). In sweet pepper, salinity causes deficiency in chlorophyll content, proline accumulation, increase antioxidant enzyme activity such as catalase both in roots and leaves (Chookhampaeng, 2011), in addition reduces shoot and root length, dry weight and leaf area (Ziaf et al., 2009). Al-Jasim et al. (2012) observed application of proline (0, 1, 5, 10 mM), sprayed exogenously, on seedlings of bell pepper seedlings caused decrease in almost all growth parameters of the non-stressed plant.

Thus, there appears the need to search for strategies regarding the management of saline waters in bell pepper cultivation, aiming to maintain the homeostasis when subjected to conditions of osmotic and ionic stress. Among the studies aiming at the establishment of plants under saline stress, the management of osmotic adjustment presents itself as the most promising strategy, since this physiological mechanism is the most effective for maintaining cell turgor, through the accumulation of compatible solutes (proline, glycine betaine, trehalose, sucrose, polyamines, mannitol, pinitol, etc.) in the vacuole or in the cytosol, promoting the maintenance of water balance inside the plant, under deficit conditions, caused by saline stress (Okuma et al., 2004; Ashraf et al., 2011; Lacerda et al., 2012; Marijuan and Bosch, 2013; Monteiro et al., 2014). The amino acid proline is the one of the most studied solute, due to its response, sensitivity and effectiveness under stress conditions (Trovato et al., 2008; Verbruggen and Hermans, 2008; Ashraf et al., 2011; Khan et al., 2015). Lacerda et al. (2012), evaluating exogenous application of proline in yellow melon plants under saline stress, reported that proline application at concentrations of up to 12 mmol L^{-1} was efficient at reducing the effects of stress caused by irrigation water salinity, promoting increase of 2.5 t ha^{-1} in the production of plants under stress. This study aimed to evaluate growth, gas exchanges, and chlorophyll *a* fluorescence and physiological aspects of 'All Big' bell pepper, as a function of saline stress and exogenous application of proline on the leaves.

MATERIALS AND METHODS

The experiment was carried out during May and August 2015 in a greenhouse, at the Center of Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), in the municipality of Campina Grande-PB, Brazil, in the mesoregion of 'Agreste Paraibano', situated at the geographic coordinates of 7°15'18'' S, 35°52'28'' W and mean altitude of 550 m.

The treatments were distributed in a randomized block design, in 2 x 4 factorial scheme, with four replicates each consisting of one plant, corresponding to two levels of electrical conductivity of the irrigation water – ECw (0.6 and 3.0 dS m^{-1}) associated with three proline concentrations through foliar application (10, 20 and 30 mmol L^{-1}) and a control treatment without application of proline. Water with ECw of 3.0 dS m^{-1} was prepared using the salts of sodium chloride (NaCl), calcium chloride ($CaCl_2.2H_2O$) and magnesium chloride ($MgCl_2.6H_2O$) in order to have a equivalent proportion of 7:2:1 of Na:Ca:Mg, similar to observed in most water used in irrigation in the northeast region of Brazil (Medeiros et al., 2003). The amount of each salt was determined based on the relationship between ECw and the concentration of salts (10*$mmol_c$ L^{-1} = 1 dS m^{-1}).

The bell pepper hybrid used in the experiment was 'All Big', which belongs to the group known as 'cascadura'; this material has upright growth, small size, firm and thick pulp with sweet flavor, high yield and cycle of around 120 days (Araújo et al., 2009). In addition, the hybrid is tolerant to blight (*Phytophthora capsici*) and tomato mosaic virus (ToMV).

The seedlings of 'All Big' bell pepper were produced on expanded polystyrene trays with 128 cells, using the commercial substrate Plantmax®, and were transplanted to pots when they produced the second pair of definitive leaves.

Plants were grown in 10-L plastic pots filled with a layer of 0.3 kg of crushed stone (number zero), which covered the bottom of the pots, and 14 kg of a eutrophic Regolithic Neosol of sandy loam texture (layer of 0-20 cm), from the rural area of the municipality of Esperança-PB, properly pounded to break up clods, with the following physico-chemical characteristics: Sand = 656.6 g kg^{-1}; Silt = 175 g kg^{-1}; Clay = 168.4 g kg^{-1}; Total porosity = 53.64 m^3m^{-3}; Available water = 18.42 dag kg^{-1}; Apparent density = 1.27 kg dm^{-3}; $pH_{(1:2.5)}$ = 6.24;EC_{se} = 2.5 dS m^{-1};OM = 10.79 dag kg^{-1}; P = 48.0 mg kg^{-1}; K^+= 0.28 $cmol_c kg^{-1}$; Na^+= 1.82 $cmol_c kg^{-1}$; Ca^{+2} = 7.41 $cmol_c kg^{-1}$; Mg^{+2}= 5.23 $cmol_c kg^{-1}$; Al^{+3}= 0.0 $cmol_c kg^{-1}$and H^+= 3.07 $cmol_c kg^{-1}$. The analyses were performed at the Laboratory of irrigation and salinity of the UFCG, according to the methodologies proposed by Claessen (1997).

After filling the pots, soil water content was brought close to field capacity and, during the experiment, the moisture content in soil was maintained near to field capacity through daily irrigations, which consisted in the application of water corresponding to the treatment in each pot. The volume of water applied in each irrigation was estimated by the water balance in the previous irrigation, that is, water volume applied minus volume drained in the previous irrigation, plus a leaching fraction of 0.15, in order to avoid the excessive accumulation of salts in the soil, according to Ayers and Westcot (1999).

Fertilizations with nitrogen (N), phosphorus (P) and potassium (K) were performed based on the recommendations of Novais et al. (1991), through top-dressing, along with the irrigation water, of 100, 150 and 300 mg kg^{-1} of soil of N, K_2O and P_2O_5, respectively, in three equal applications, at intervals of fifteen days, and the first application was performed 10 days after transplantation (DAT). Urea, monoammonium phosphate and potassium chloride were used as sources of N, P and K, respectively. Foliar application of proline was performed weekly from 15 DAT on, using a spray bottle in order to obtain the complete wetting of the plants, with a volume that ranged from 10 to 40 mL $plant^{-1}$, according to the development stage of the plant.

Gas exchanges were determined using the portable device "LCPro+" (ADC BioScientific Ltda.) for photosynthesis measurement, operating with control of temperature at 25ºC, irradiation of 1200 µmol photons m^{-2} s^{-1} and air flow of 200 mL min^{-1}, and CO_2 coming from the environment at a height of 3 m from the soil surface. The following variables were analyzed: CO_2 assimilation rate (*A*) (µmol m^{-2} s^{-1}), transpiration (*E*) (mol of H_2O m^{-2} s^{-1}), stomatal conductance (*gs*) (mol of H_2O m^{-2} s^{-1}) and internal CO_2 concentration (*Ci*) (µmol m^{-2} s^{-1}) on the third leaf from the apex. Based on these data, the intrinsic water use efficiency (*WUE*) (A/E) [(µmol m^{-2} s^{-1}) (mol H_2O m^{-2} $s^{-1})^{-1}$] and instantaneous carboxylation efficiency (A/Ci) (*EiCi*) (Silva et al., 2014) were quantified. These data were obtained in the transition of vegetative and reproductive stages, at 40 DAT.

In the same period, chlorophyll *a* fluorescence was determined using a pulse-modulated Fluorometer (Model OS5p - Opti Science). The Fv/Fm protocol was used in order to determine the variables of fluorescence induction: Initial fluorescence (Fo), Maximum fluorescence (Fm), Variable fluorescence (Fv = Fm-Fo) and Maximum quantum efficiency of photosystem II (Fv/Fm) (Sá et al., 2015). Such protocol was performed after leaf adaptation to the dark, and at sunrise, using a clip of the device, in order to guarantee that all primary acceptors are oxidized, that is, the reaction centers are open.

Also using the pulse-modulated fluorometer, two hours after fluorescence evaluations with adaptation to the dark, the evaluations under conditions of light were performed using the Yield protocol, by applying a source of actinic lighting with multi-flash saturation pulse, attached to a clip for the determination of photosynthetically active radiation (PAR-Clip) in order to determine the variables: Initial fluorescence before the saturation pulse (F'), maximum fluorescence after adaptation to saturating light (Fm'), electron transport rate (ETR) and quantum efficiency of photosystem II (Y_{II}).

The effects of the treatments on the growth of 'All Big' bell pepper were evaluated through the determination of plant height (PH), measured with a graduated ruler, stem diameter (SD), measured using a digital caliper and the number of leaves, through the count of mature leaves, at 50 DAT.

The collected data were subjected to analysis of variance by F test and, when significant, regression analysis was performed for the quantitative factor i.e. proline concentrations, while the Tukey's test at 0.05 probability level was applied for comparison of means of irrigation water salinity, using the statistical program SISVAR-ESAL (Ferreira, 2011).

RESULTS AND DISCUSSION

The plant height, stem diameter and number of leaves of bell pepper was reduced ($p<0.05$) by 16.4, 9.3 and 15.4%, respectively (Figure 1A, C and E) under irrigation water salinity of 3.0 dS m^{-1} in comparison to that of 0.6 dS m^{-1} at 50 DAT. Increment in irrigation water salinity increased soil salinity to levels above the threshold of the crop, causing physiological and nutritional alterations in the bell pepper plants, due to toxicity by specific ions; for example, the reduction in the permeability of cell membranes, photosynthesis and ionic balance, as observed by Leonardo et al. (2008) and Aktas et al. (2006), in bell pepper cultivation under saline water irrigation.

There was no effect of proline concentrations on the variable number of leaves ($p > 0.05$). The variables plant height and stem diameter tended to decrease as the proline concentrations increased from 0 to 30 mmol L^{-1}, regardless of the salinity condition (Figure 1B and D). The exogenous application of proline possibly induces the osmotic adjustment, even in the absence of water restrictions in the soil, thus causing an expenditure of energy, which compromises plant growth (Taiz and Zaiger, 2013).

There was no influence of application of proline in different concentrations on the net CO_2 assimilation rate (A) of bell pepper plants cultivated in the control treatment (ECw=0.6 dS m^{-1}), with a mean value of A of 21.98 µmol m^{-2} s^{-1}. However, plants subjected to saline stress responded quadratically to the increment in proline concentrations, with maximum A (22.75 µmol m^{-2} s^{-1}) at the concentration of 15.7 mmol L^{-1} (Figure 2A).

The results observed for CO_2 assimilation rate corroborate those of *Ci*, for which there was also no effect of proline concentrations in the control treatment. However, under high-salinity conditions, plants subjected to foliar applications from 0 to 30 mmol L^{-1}, where the lowest values of *A* were observed, showed the highest *Ci* values (Figure 2B). This indicates that the lower photosynthetic activity in this treatment is not related to limitations performed by the stomatal activity under the CO_2 inflow, but by the low activity of the ribulose 1,5-bisphosphate carboxylase (RuBisCo), inefficiently acting in the carboxylation of CO_2 (Machado et al., 2010; Lacerda et al., 2012).

The previously cited assumptions are confirmed by the results of instantaneous carboxylation efficiency (*EiCi*) (Figure 2C). There was low *EiCi* in plants at the highest and lowest studied concentrations of proline (0 and 30 mmol L^{-1}) under conditions of high salinity (Figure 2C). This denotes the low carboxylation efficiency of the RuBisCo enzyme, which may be related to the action of factors of non-stomatal nature, such as the low availability of ATP and NADPH from the electron transport chain of the photosystem II (PSII) (Silva et al., 2014; Sá et al., 2015).

The best photosynthetic activity observed at the intermediate proline concentrations may be related to the maintenance of cell turgor, through the accumulation of this solute (vacuole or cytosol), thus promoting the maintenance of water balance inside the plant, under deficit conditions, caused by saline stress (Okuma et al., 2004; Ashraf et al., 2011; Lacerda et al., 2012; Marijuan and Bosch, 2013; Monteiro et al., 2014; Khan et al.,

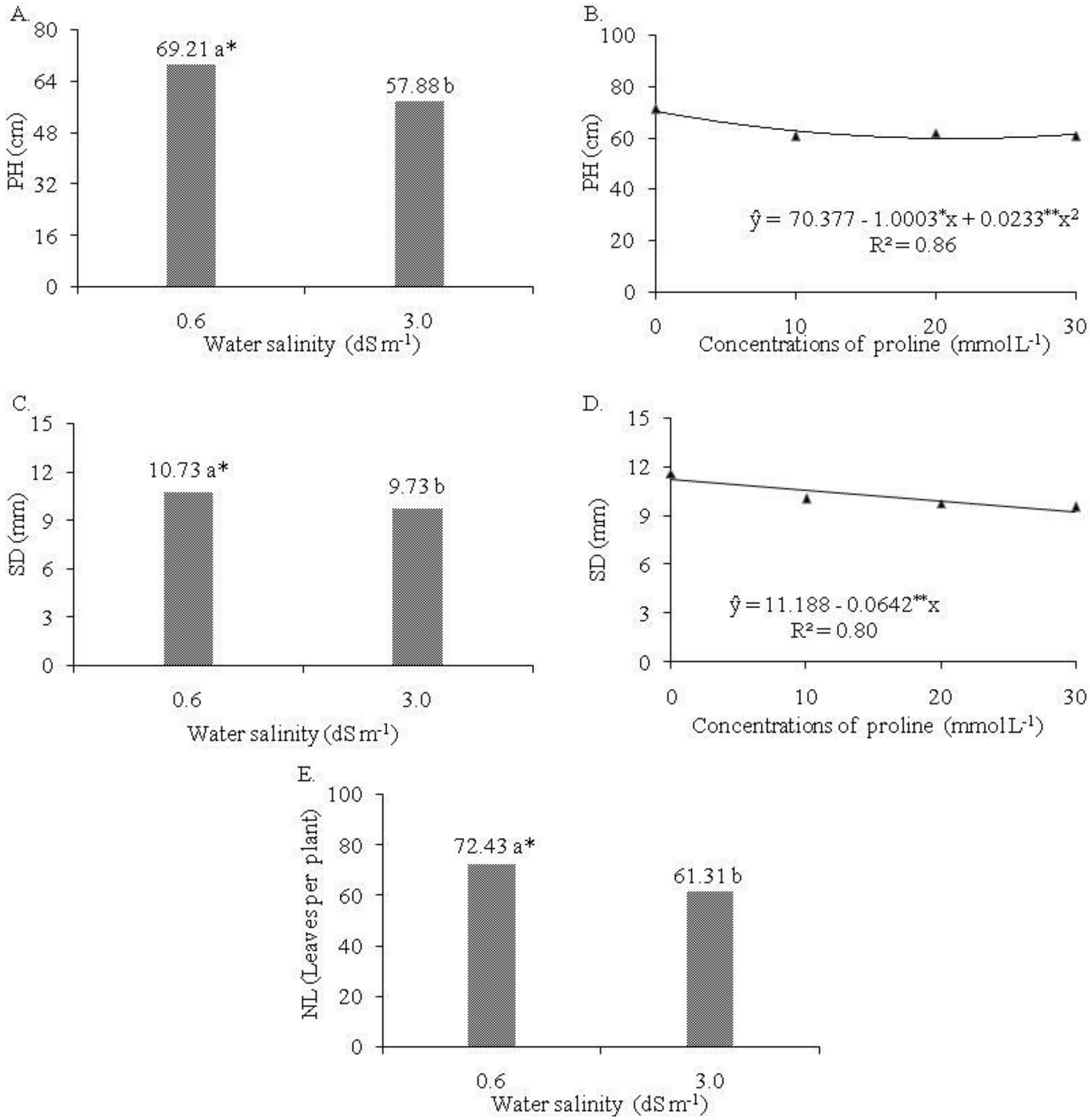

Figure 1. Plant height (PH) (A and B), stem diameter (SD) (C and D) and number of leaves (NL) (E) of 'All Big' bell pepper plants under different levels of water salinity and exogenous application of proline on the leaves.
(*) and (**) significant at 0.05 and 0.01 probability, respectively; (NS) not significant; Means followed by different letters indicate difference between treatments byTukey's test at $p < 0.05$.

2015). Believed to that the highest proline concentration (30 mmol L^{-1}) reduced the internal water potential of bell pepper plants to very low levels and, when associated with irrigation using water of higher ECw, stimulated greater absorption of water and consequently greater absorption of toxic ions by the plant (Aktas et al., 2006).

Exogenous application of proline on the leaves reduced stomatal conductance and, consequently, affected the transpiration of bell pepper plants irrigated with water of low salinity (Figure 1D and E). It should be pointed out that the reduction in stomatal conductance in plants cultivated under low salinity reached lower levels, compared with plants under high salinity. These results denote the efficiency of exogenous application of proline in the stomatal regulation of bell pepper plants, promoting higher transpiration without any damage to photosynthetic activity and with gains in water use efficiency.

Stomatal conductance and transpiration of bell pepper plants, when irrigated with water of high salinity, responded quadratically, with maximum values of

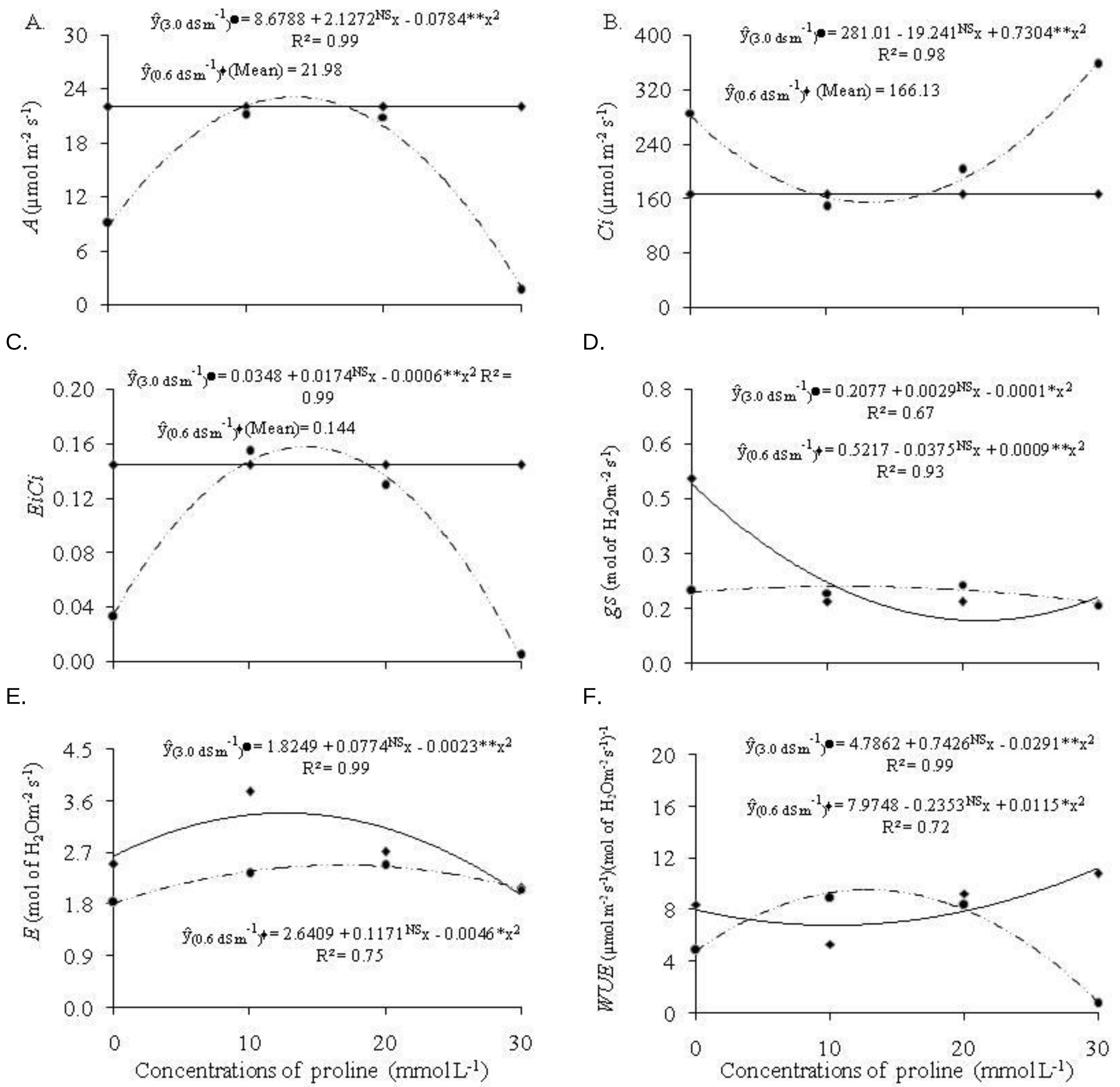

Figure 2. CO_2 assimilation rate (*A*, μmol m^{-2} s^{-1}) (A), internal CO_2 concentration (*Ci*, μmol m^{-2} s^{-1}) (B), instantaneous carboxylation efficiency (*EiCi*) (C), stomatal conductance (*gs*) (D), transpiration (*E*) (E), water use efficiency (*WUE*) (F) of 'All Big' bell pepper plants under levels of water salinity and concentrations of proline applied on the leaves.
(*) and (**) significant at 0.05 and 0.01 probability, respectively; (NS) not significant.

approximately 0.23 and 2.48 mol of H_2O m^{-2} s^{-1}, at the estimated proline concentrations of 14.5 and 16.8 mmol L^{-1} (0.21 and 1.82 mol of H_2O m^{-2} s^{-1}), respectively (Figure 2D and E). It is observed that, despite the small variation in stomatal conductance, there was a significant increase in the transpiration rate of the plants, when subjected to the concentrations of proline. These responses may be related to the osmotic adjustment promoted by the exogenous application of proline on the leaves, so that transpiration guaranteed the occurrence

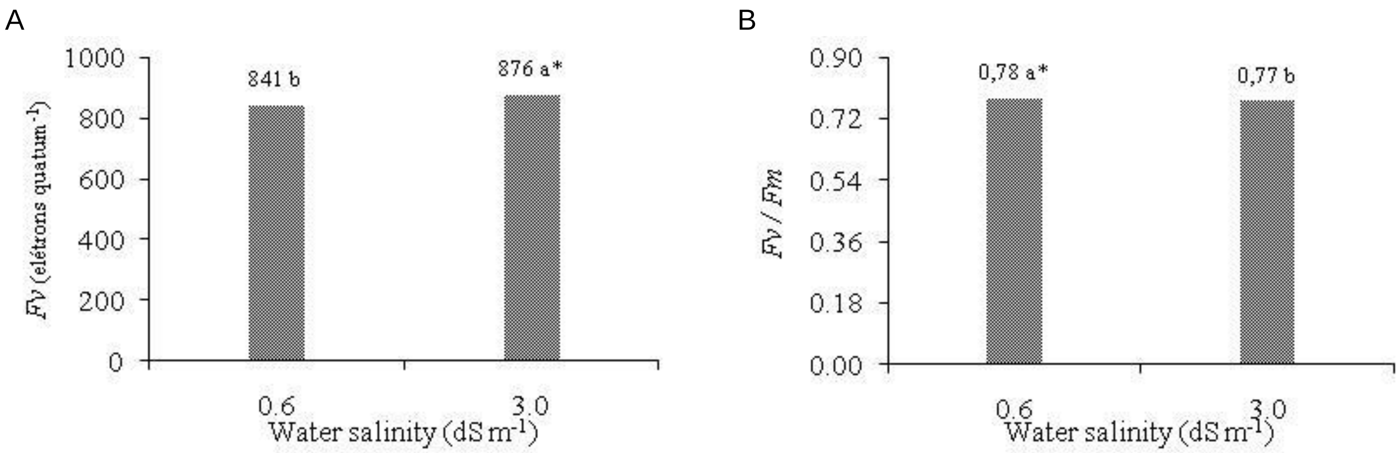

Figure 3. Variable fluorescence (*Fv*) (A) and quantum efficiency of photosystem II (*Fv/Fm*) (B) of 'All Big' bell pepper plants under levels of water salinity and concentrations of proline applied on the leaves.
Means followed by different letters indicate significant difference between treatments byTukey's test, $p < 0.05$.

of absorption and transport of water inside the plant, maintaining its water relationships active, through stomatal regulation (Shimazaki et al., 2007; Taiz and Zaiger, 2013). Thus, the increase in transpiration is an indication of the efficiency of exogenous application of proline in the osmotic regulation of bell pepper plants, especially because there was an increase in the water use efficiency of these plants, with increment in proline concentration until the estimated value of 12.8 mmol L^{-1} (Figure 2F).

As to chlorophyll fluorescence, there was significant influence ($p<0.05$) of the levels of irrigation water salinity on variable fluorescence and quantum efficiency of PSII; in comparison to low salinity water, there was an increase of 4.0% in *Fv* and reduction of 1.3% in *Fv/Fm*, when subjected to irrigation with water of high salinity (3.0 dS m^{-1}) (Figures 3A and B). The increase in variable fluorescence is possibly a strategy of the species to mitigate the effects of saline stress on the photosynthetic activity, increasing photochemical activity in order to meet the necessity of ATP and NADPH in the biochemical stage of photosynthesis and, consequently, maintain the activity of gas exchanges at satisfactory levels, considering the reductions observed in these variables with the increase in irrigation water salinity. This result indicates that saline stress is acting on the photochemical activity of bell pepper plants in the beginning of the reproductive stage at 40 DAT, because the reduction in the quantum efficiency of PSII is an indication of photo inhibitory damages, which result in the loss of efficiency in the transfer of energy from the photosystem II (P_{680}) to the photosystem (P_{700}) and, consequently, reduction in the synthesis of ATP and NADPH (Baker and Rosenqvst, 2004; Silva et al., 2014; Sá et al., 2015). However, the observed damages are still incipient, considering that there was no influence of the treatments on the electron transport rate (ETR).

Conclusions

Irrigation with high salinity water (EC=3.0 dS m^{-1}) reduce growth, gas exchanges and efficiency of the photosystem II in'All Big' bell pepper plants.

Exogenous applications of proline in concentrations from 12.8 to 16.8 mmol L^{-1} increase the activity of gas exchanges, reducing the effect of saline stress on bell pepper plants.

Proline concentrations influence positively the variable fluorescence and reduce quantum efficiency of photosystem II of chlorophyll *a* of 'All Big' bell pepper plants.

Conflict of interest

The authors have not declared any conflict of interest.

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