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Growth and physical characterization of fruits of bell pepper (*Capsicum annuum* L.) cv. 'All Big' subjected to saline stress and exogenous application of proline

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Abstract

Salinity is considered as one of the main abiotic stresses affecting the growth and development of various solanaceous crops such as bell pepper. To reduce the adverse effects induced by saline stress, it is necessary to apply exogenous osmoprotectants such as proline. In this context, this study aimed to evaluate the growth and physical characteristics of fruits of 'All Big' bell pepper under saline stress and foliar application of proline, in an experiment conducted in pots using drainage lysimeters under greenhouse conditions. The study was carried out in randomized blocks, testing two levels of irrigation with low (control) and high water salinity (EC= 0.6 and 3.0 dS m^{-1}) associated with four doses of proline (0; 10; 20 and 30 mmol L^{-1}). Treatment effects on plant growth and phytomass accumulation were evaluated by measuring the following variables: plant height, stem diameter and number of leaves at 85 DAT, shoot and total dry phytomass at 95 DAT. At the end of the cycle, 100 DAT, bell pepper fruits were characterized based on transverse and longitudinal diameter and pulp thickness. Increasing irrigation water salinity reduced bell pepper growth and dry matter accumulation, and pulp thickness is more sensitive to irrigation water salinity. Proline doses did not inhibit the deleterious effect of irrigation water salinity on bell pepper development; increasing proline doses led to reduction in bell pepper growth and dry matter accumulation. The bell pepper cv. 'All Big' demonstrated sensitivity to irrigation water salinity with 3.8 dS m^{-1}.

Keywords: *Capsicum annuum* L., saline water, osmoprotectants.
Abbreviations: PH_plant height; SD_stem diameter; NL_number of leaves; SDP_shoot dry phytomass; TDP_total dry phytomass; FTD_transverse diameter, FLD_longitudinal diameter; PT_pulp thickness; ECw_water electrical conductivity.

Introduction

Bell pepper (*Capsicum annuum* L.), belonging to the Solanaceae family, is an agricultural crop that stands out due to not only its economic importance in the national horticultural market, but also the nutritional value of its fruits. Additionally, it is an excellent source of antioxidant compounds important for human health and one of the most consumed vegetables in Brazil. In the Northeast region, the states of Pernambuco, Paraíba, Ceará and Bahia stand out in descending order (Echer et al., 2002; IBGE, 2011).

Nevertheless, one of the main factors affecting bell pepper production in Northeast Brazil is the quality of the water used in irrigation, mostly with high concentrations of salts, because saline conditions usually lead to nutritional imbalance, reduction in germination, lower proportions in the rates of transpiration, photosynthesis and internal CO_2 concentration in the leaves, morphologically resulting in decrease of biomass accumulation, also compromising several characteristics of fruit quality and yield (Gammoudi et al., 2016; Lima et al., 2016).

Negative effects of salinity on plants are due to the reduction in the osmotic potential of the soil solution, and salinity may also lead to ionic toxicity, nutritional imbalance or both, due to the excessive accumulation of certain ions in plant tissues, especially chloride and sodium (Ben Taarit et al., 2010; Aydin et al., 2012). Nevertheless, plants may be able to acclimate to these stress conditions by accumulating compatible solutes, since they act as osmoprotectants, responsible for eliminating reactive oxygen species (ROS), minimizing dehydration in environments where water availability is limited, because cell turgor and physiological processes are maintained (Pang and Wang, 2008).

Among the organic solutes that accumulate in response to saline stress, proline stands out, and its exogenous application can lead to a preventive or recovery effect, but high doses may be harmful, causing metabolic imbalance (Hare et al., 2003). Various studies have demonstrated that exogenous proline application effectively regulates osmotic potential and plays a vital role in maintaining plant growth under osmotic stress (Ali et al., 2007; Ashraf and Foolad, 2007; Hoque et al., 2007). Lacerda et al. (2012), working with melon under saline water irrigation, employed proline concentrations of 0, 5, 10 and 20 mmol L^{-1}, and observed mitigation of the deleterious effects caused by irrigation water salinity, but the concentration of 20 mmol L^{-1} caused inhibitory effect on plants subjected to saline stress.

Several authors have studied the effect of irrigation with saline water on the cultivation of sweet pepper (Nascimento et al., 2011; Nascimento et al., 2015, Lima et al., 2018). However, in the literature there is no information regarding the use of exogenously applied proline, aiming at the attenuation or acclimatization of the pepper crop to the saline stress. Thus, it is imperative to conduct studies that explore the effects of the foliar application of this osmoprotectant, as a measure to reduce the impacts of saline stress on the growth and physical characteristics of the fruits of chili pepper cv. 'All Big', in Brazilian semiarid conditions.

Considering these aspects, the present study aimed to evaluate the growth and physical characteristics of fruits of 'All Big' bell pepper under saline stress and foliar application of proline.

Results and Discussion

Effect of saline stress and exogenous application of proline on bell pepper growth

Based on the summary of the analysis of variance (Table 1), saline water irrigation had significant effect ($p<0.01$) on shoot and total dry phytomass and ($p<0.05$) on plant height, stem diameter, number of leaves, longitudinal diameter, transverse diameter and pulp thickness of bell pepper fruits. Regarding the proline doses, they had significant influence on plant height, stem diameter, shoot and total dry phytomass and longitudinal diameter of bell pepper fruits. The interaction between factors (SL x PD) did not affect significantly ($p>0.05$) any of the variables analysed. Sá et al. (2016), evaluating the growth of 'All Big' bell pepper cultivar as a function of saline stress and exogenous proline application, observed no interaction between factors.

In the comparison of means relative to the effect of salinity on the growth in height of 'All Big' bell pepper (Fig1A), PH decreased by 9.69 cm in plants irrigated with 3.0 dS m^{-1} water, a reduction of 13.93% compared with those subjected to the lowest water salinity level (0.6 dS m^{-1}). In salt-sensitive plants, such as bell pepper, growth is considerably reduced under such adverse condition, which has been widely reported in the literature (Nascimento et al., 2015; Özdemir et al., 2016). Such reduction in growth may result from the osmotic and ionic effects induced by the saline stress, which occur when toxic ions are absorbed in large amounts, along with the water absorbed by the roots, exceeding the plant's capacity to compartmentalize them in the vacuole. Consequently, the concentration of salts increases in the cytoplasm and inhibits the activity of enzymes of various metabolic pathways (Prisco and Gomes Filho, 2010).

Increased irrigation water salinity also significantly reduced bell pepper stem diameter, regardless of the proline doses, with greater reductions in plants irrigated with 3.0 dS m^{-1} water, equal to 8.54% (0.94 mm) compared with plants subjected to the low ECw (Fig 1B). Salinity inhibits photosynthesis by reducing the water potential (Toppa and Brambilla, 2011). Reduction of growth, as observed in the present study, was also found by Nascimento et al. (2011) in the bell pepper variety 'All Big' at 45 days after germination, subjected to different salt levels (0.3; 1.5; 2.5; 3.5 and 4.5 dS m^{-1}). In this study, stem diameter decreased by 0.51 mm per unit increase in ECw (dS m^{-1}).

In agreement with the results of plant height and stem diameter, the number of leaves in plants under salinity of 3.0 dS m^{-1} (55.62 leaves per plant) decreased by 17.97% compared with those irrigated with 0.8 dS m^{-1} water (67.81 leaves per plant). Such reduction in NL may be due to the accumulation of sodium chloride in cell walls and in the cytoplasm of leaves; in addition, it reduces the concentration of fibers within the cells, which eventually leads to death (Munns, 2002). These results agree with those found by Nascimento et al. (2015), who observed expressive reduction (26.2%) in the number of leaves of bell pepper irrigated with 5.0 dS m^{-1} water, compared with those irrigated with public-supply water (0.56 dS m^{-1}).

The same decrease occurred in shoot and total dry phytomass, and the highest accumulations of phytomass were found in plants under low salinity. As water salinity increased to 3.0 dS m^{-1}, the results were clearly reduced by 5.65 and 6.84 g per plant for shoot and total dry phytomass, respectively (Fig 2A and 2B). Saline stress affects phytomass accumulation, limiting the absorption of water and essential nutrients, with immediate effect on cell growth and expansion. In addition, high concentrations of salts can be extremely toxic to plants (Munns and Tester, 2008; Hussain et al., 2016). Sá et al. (2017), in a study with the same cultivar and ECw levels, observed at 20 DAS that irrigation water salinity increase from 0.6 to 3.0 dS m^{-1} led to reduction of 29.25% in total dry phytomass accumulation.

Effect of saline stress and exogenous application of proline on the physical characterization of bell pepper fruits

According to the results of the means comparison test, referring to the transverse (FTD) and longitudinal diameter (FLD) of bell pepper fruits as a function of the salt levels (Fig 3A and 3B), these variables were negatively affected by the increase in water salinity. Fruit transverse diameter decreased by 5.56 mm when plants were irrigated with 3.0 dS m^{-1} water, and the lowest FTD (55.10 mm) occurred at this level, corresponding to a total reduction of 9.16% compared with the value obtained at 0.6 dS m^{-1} salinity (15.5 mm) (Fig 3A). Likewise, fruit longitudinal diameter had total reduction of 10.81% in plants subjected to the highest salinity level (3.0 dS m^{-1}). At this salinity level, mean FLD was equal to 54.60 mm, while the lowest salinity level (0.6 dS m^{-1}) led to FLD of 61.22 mm (Fig 3B). This may have occurred because high irrigation water salinity results in decrease in the osmotic potential of the soil solution, leading to restrictions in the sap flow inside the xylem and in the water accumulation rate during cell expansion, consequently reducing fruit size (Navarro et al., 2002; Yamaguchi and Blumwald, 2005).

Table 1. Summary of analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), shoot dry phytomass (SDP), total dry phytomass (TDP), fruit longitudinal diameter (FLD), fruit transverse diameter (FTD) and pulp thickness (PT) of 'All Big' bell pepper irrigated with saline water under different doses of proline.

Source of variation	DF	Mean squares							
		PH	SD	NL	SDP	TDP	FLD	FTD	PT
Saline levels (SL)	1	750.78*	7.03*	1188.28*	255.54**	373.60**	350.26*	246.53*	5.77*
Doses of proline (DP)	3	217.11*	7.86*	28.36^{ns}	118.72**	182.50**	161.40*	5.97^{ns}	1.19^{ns}
Linear regression	1	357.00*	17.55*	2.75^{ns}	25.05**	436.98**	36.33^{ns}	10.55^{ns}	2.80^{ns}
Quadratic regression	1	166.53^{ns}	5.28^{ns}	81.28^{ns}	79.60*	88.51*	421.00**	6.71^{ns}	0.22^{ns}
Interaction (SL*DP)	3	14.53^{ns}	1.69^{ns}	446.61^{ns}	2.52^{ns}	2.17^{ns}	138.81^{ns}	35.07^{ns}	0.35^{ns}
Blocks	2	398.03*	0.03^{ns}	170.61^{ns}	18.72^{ns}	22.91^{ns}	41.56^{ns}	70.07^{ns}	0.79^{ns}
CV (%)		11.43	11.00	16.69	16.98	15.11	10.45	9.19	14.60

ns, **, * respectively, not significant, significant at $p < 0.01$ and $p < 0.05$.

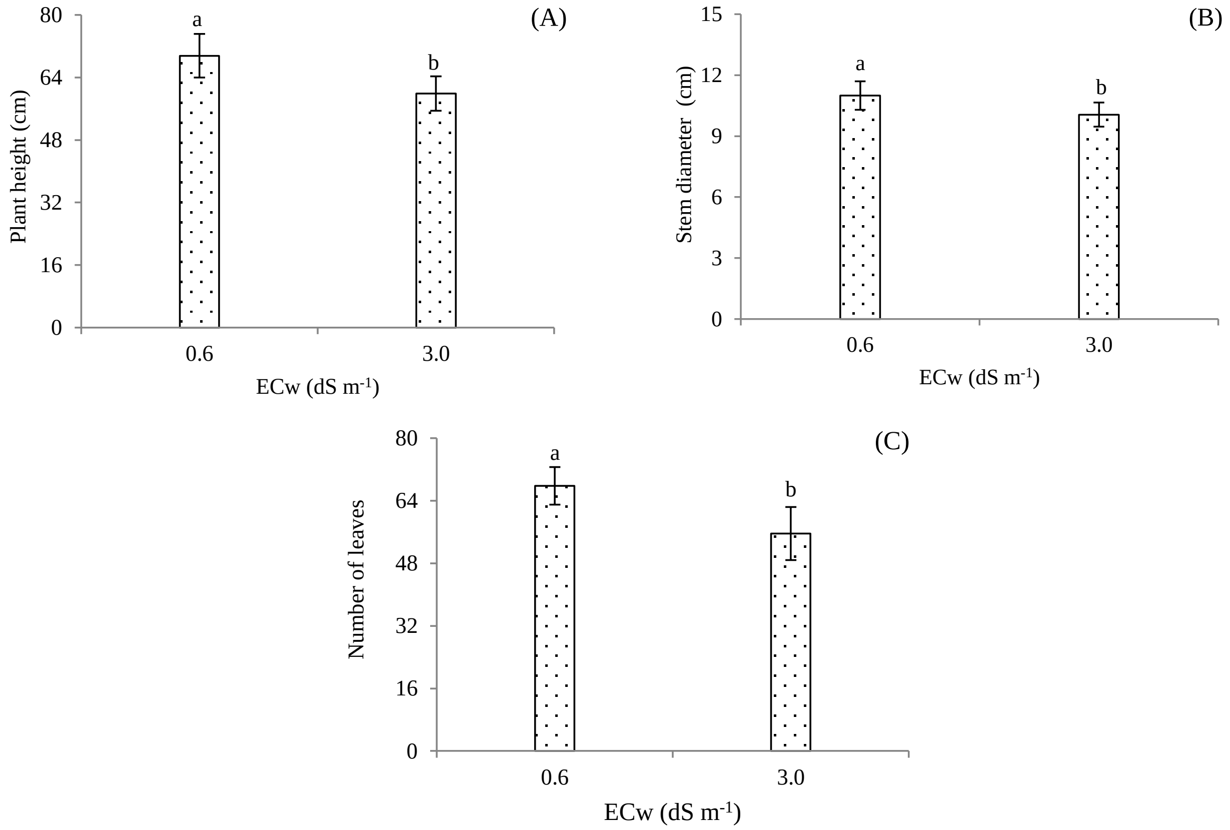

Fig 1. Plant height (A), stem diameter (B) and number of leaves (C) of 'All Big' bell pepper as a function of water electrical conductivity - ECw.

Table 2. Chemical and physical characteristics of the eutrophic Regolithic Neosol used in the experiment.

Chemical characteristics									
pH (H_2O) (1:2.5)	O.M. dag kg^{-1}	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+	ECse (dS m^{-1})
			..............................(cmol$_c$ kg^{-1})...................................						
6.24	10.79	48.00	0.28	1.82	7.41	5.23	0.00	3.07	2.50

Physical characteristics									
Size fraction (g kg^{-1})			Textural class	Water content - (kPal)		AW	Total porosity	AD	DP
				33.42	1519.5				
Sand	Silt	Clay		..........dag kg^{-1}...		....	m^3 m^{-3}		(kg dm^{-3})
656.6	175.0	168.4	SL	28.84	10.42	18.42	53.64	1.27	2.74

O.M. – Organic matter: Walkley-Black wet digestion; Ca^{2+} and Mg^{2+} extracted with 1 mol L^{-1} KCl at pH 7.0; Na^+ and K^+ extracted with 1 mol L^{-1} NH4 OAc at pH 7.0; H^+ and Al^{3+} extracted with 1 mol L^{-1} CaOAc at pH 7.0; ECse – Electrical conductivity of the saturation extract; SL – Sandy loam; AW – Available water; AD - Apparent density; DP –particle density.

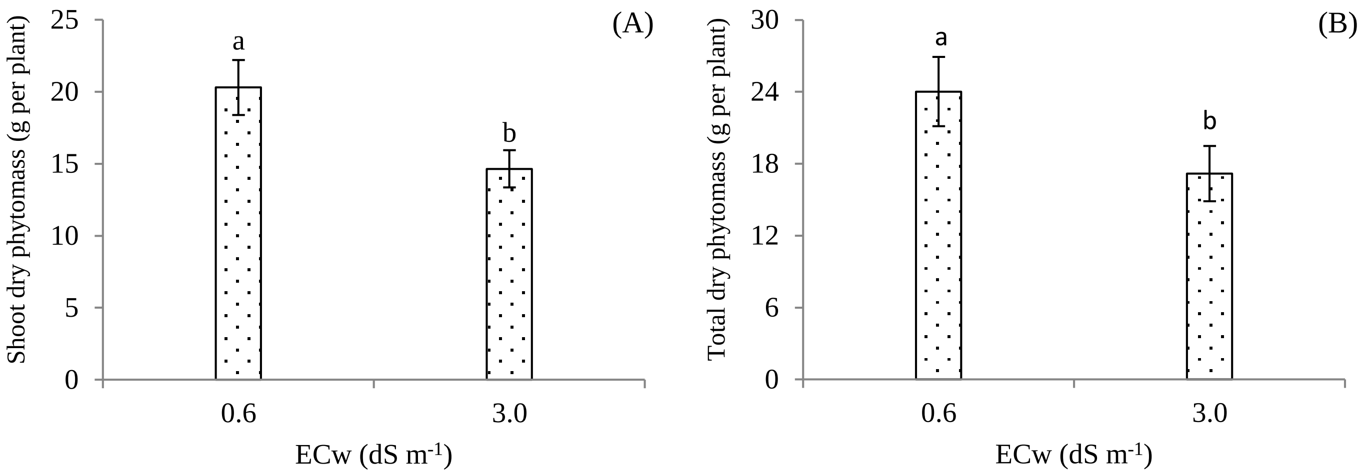

Fig 2. Shoot dry phytomass (A) and total dry phytomass (B) of 'All Big' bell pepper as a function of water electrical conductivity - ECw.

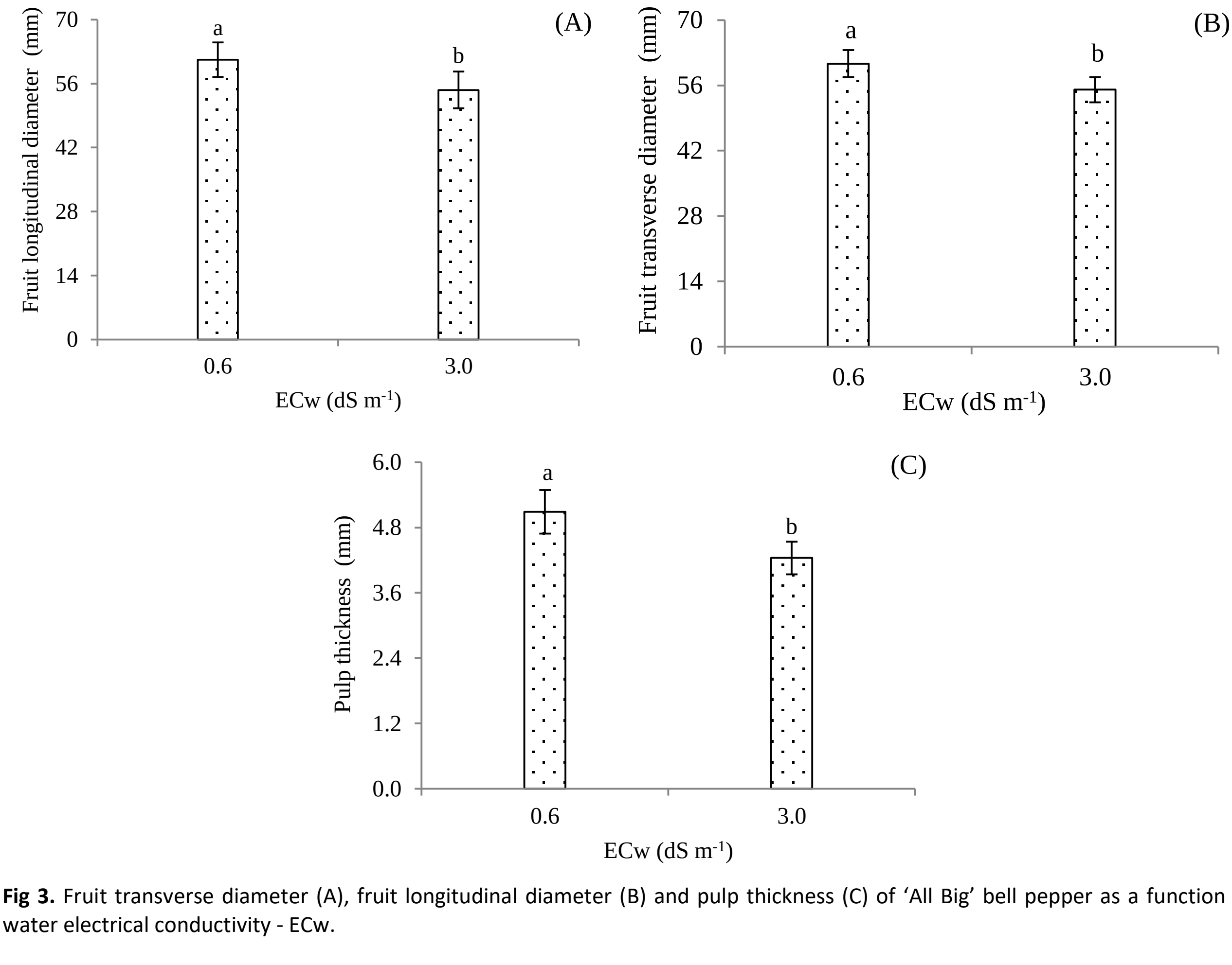

Fig 3. Fruit transverse diameter (A), fruit longitudinal diameter (B) and pulp thickness (C) of 'All Big' bell pepper as a function of water electrical conductivity - ECw.

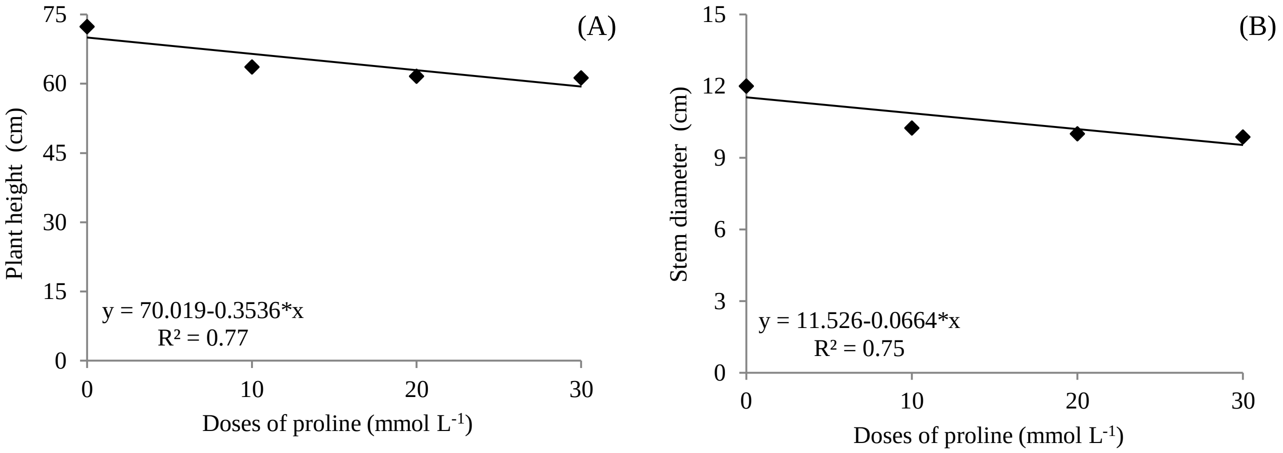

Fig 4. Plant height (A) and stem diameter (B) of 'All Big' bell pepper as a function of exogenous proline application.

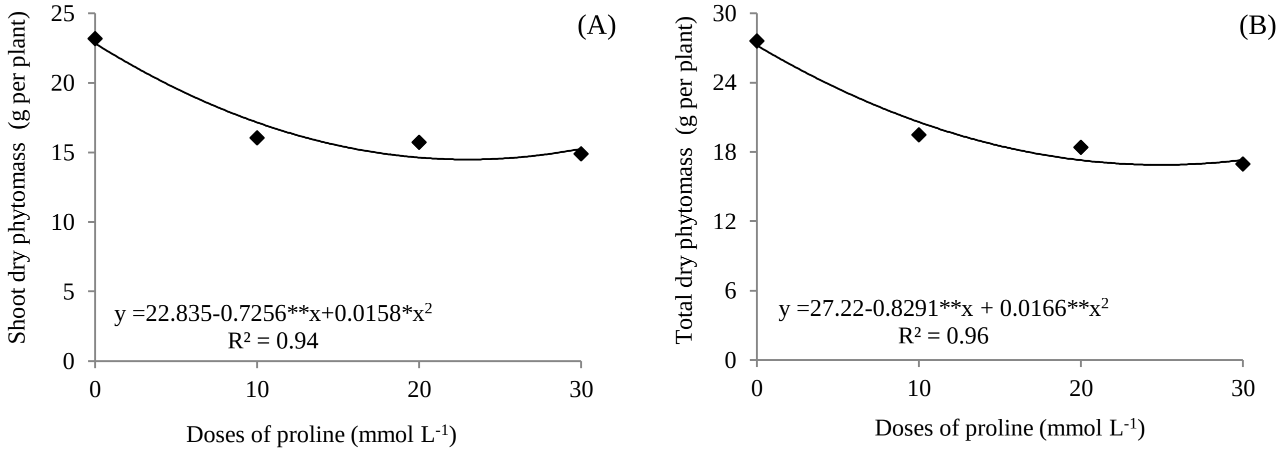

Fig 5. Shoot dry phytomass (A) and total dry phytomass (B) of 'All Big' bell pepper as a function of exogenous proline application.

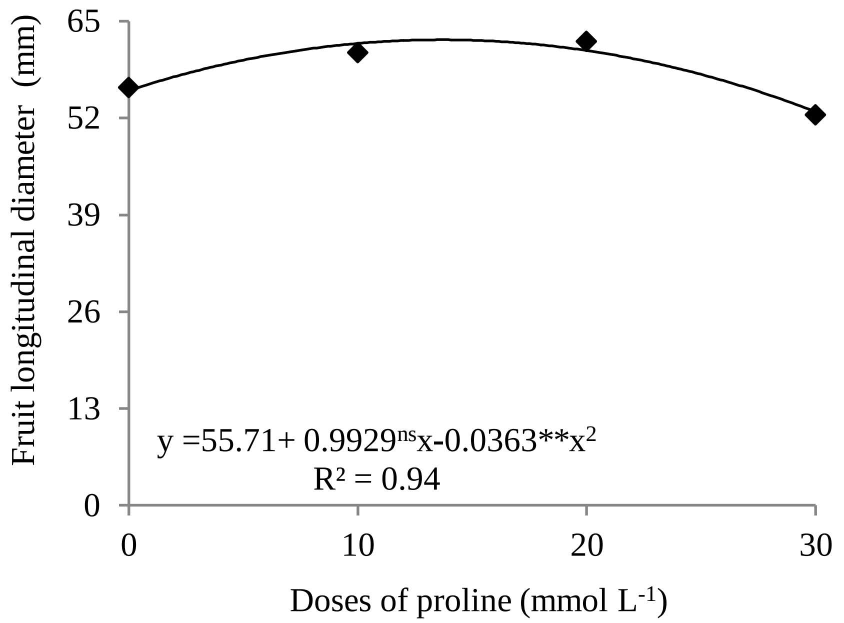

Fig 6. Fruit longitudinal diameter of 'All Big' bell pepper as a function of exogenous proline application.

For bell pepper pulp thickness, the exposure to salinity led to a 16.69% reduction at the highest salinity level, i.e., plants irrigated with 3.0 dS m^{-1} water showed reduction of 0.85 mm in pulp thickness, compared with those subjected to the lowest salinity level, 0.6 dS m^{-1} (Fig 3C). The alterations caused by saline stress on plant physiology and mainly expressed in the vegetative growth can also affect fruit quality (Purqueiro and Cecilio Filho, 2005). Modifications in the pulp thickness of bell pepper fruits have also been reported by Navarro et al. (2002), Rubio et al. (2009), and Navarro et al. (2010), caused by the saline irrigation water applied to the plants.

For the effect of proline doses on bell pepper growth and stem diameter (Fig 4A and 4B), according to the regression equation, there were linear reductions of 5.03 and 5.72% for every 10 mM increment in the proline concentration, respectively. In other words, although exogenous proline application in plants exposed to abiotic stress usually leads to prevention or recovery from saline stress, when the highest proline dose was applied (30 mM), plant height and stem diameter decreased by 16.92 and 22.15%, i.e., reductions of 10.59 cm and 1.98 mm, respectively, in comparison to plants under the lowest level (0 mM), without proline application. Likewise, Dawood et al. (2014) reported that exogenous proline application at 25 mM concentration mitigated the toxicity of diluted seawater with electrical conductivity of 3.13 and 6.25 dS m^{-1} on *Vicia faba* plants, whereas the 50 mM dose resulted in increased toxicity to the plants.

For shoot (SDP) and total dry phytomass (TDP) of 'All Big' bell pepper, the effect of exogenous proline application was quadratic, and highest SDP and TDP (22.83 and 27.22 g, respectively) were found at the lowest proline dose (0 mM), (Fig 5A and 5B). At this level, SDP and TDP decreased by approximately 36.13 and 38.46%, respectively, compared with the highest level of proline (30 mM). As observed in the present study, Yamada et al. (2005) reported inhibition in the growth of Petunia plants, due to early leaf senescence, when they were subjected to different doses of proline (5, 10 and 50 mM). Such toxicity of proline may be caused by the reduction in the activity of the pyrroline-5-carboxylate synthase (P5CS) in the proline degradation pathway, which suggests inhibition of its synthesis due to excess proline (Hellmann et al., 2000).

According to the regression equation for fruit longitudinal diameter (FLD), the data fitted best to a quadratic model, and maximum diameter (62.53 mm) was obtained at proline dose of 14 mM (Fig 6). Based on the results of 'All Big' bell pepper FLD, these reductions are possibly related to the disruptive effects of high proline concentrations on chloroplast and mitochondria membranes, inducing the increase in the levels of reactive oxygen species (Hare et al., 2002).

Materials and Methods

Localization, experimental procedure, treatments and plant material

The experiment was carried out in protected environment at the Center of Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), in the municipality of Campina Grande, Paraíba, in the '*Agreste Paraibano'* Mesoregion, situated at the geographic coordinates 7°15'18'' S, 35°52'28'' W and mean altitude of 550 m. A randomized complete block design was used, in 2 x 4 factorial arrangement, testing two levels of irrigation with low (control) and high salinity water (EC= 0.6 and 3.0 dS m^{-1}) associated with four levels of weekly foliar applications of proline (0; 10; 20 and 30 mmol L^{-1}), with four replicates.

The experiment used the bell pepper cultivar 'All Big', which belongs to the group known as 'Casca Dura'. This material has upright growth habit, short size, firm and thick pulp with sweet flavor, high yield and cycle of approximately 120 days (Araújo et al., 2009). Bell pepper seedlings were produced on expanded polystyrene trays with 128 cells, using the commercial substrate Plantmax®, and transplanted when they produced the second pair of true leaves.

Establishment and management of the experiment

Plants were transplanted to 10-L plastic pots, which were perforated at the bottom and connected to drains and a container to collect the drained water, to monitor the drained volume and crop water consumption. The pots were filled with a 0.3-kg layer of crushed stone, which covered the bottom, and 14 kg of material of a eutrophic Regolithic Neosol with sandy loam texture (0-20 cm depth), properly pounded to break up clods, from the rural area of the municipality of Esperança, PB. Its physical and chemical characteristics (Table 2) were determined at the UFCG's Laboratory of Irrigation and Salinity, according to methodologies proposed by Claessen (1997).

Fertilization with nitrogen (N), phosphorus (P) and potassium (K) was carried out according to recommendation for pot experiments found in Novais et al. (1991). Along with the irrigation water, 100, 150 and 300 mg kg^{-1} of soil of N, K_2O and P_2O_5, respectively, were split into three applications at 15-day intervals, with the first application at 10 days after transplanting (DAT). Urea, monoammonium phosphate and potassium chloride were used as sources of N, P and K, respectively. Foliar application of proline was weekly performed according to the treatments, from 15 DAT on, using a sprayer, in such a way to thoroughly wet the plants, and a volume of 10 to 40 mL per plant, according to the development stage.

The low-salinity water (0.6 dS m^{-1}) used in irrigation was obtained by diluting water from the public supply system of Campina Grande-PB in rainwater. The level corresponding to the high ECw (3.0 dS m^{-1}) was prepared in such a way to obtain an equivalent proportion of 7:2:1 between Na:Ca:Mg, respectively. The quantities of sodium, calcium and magnesium chlorides were determined using the equation of Richards (1954), considering the relationship between ECw and the concentration of salts ($10*mmol_c\ L^{-1} = 1\ dS\ m^{-1}$). Soil moisture was increased to a level corresponding to field capacity in all experimental units along the experiment, with daily irrigations, by applying solutions corresponding to the treatments in each pot. The applied volume was estimated by water balance: applied water volume minus water volume drained in the previous irrigation, plus a leaching fraction of 0.15, to avoid excessive accumulation of salts in the soil (Ayers and Westcot, 1999).

Traits measured

Treatment effects on plant growth and phytomass accumulation were evaluated by measuring the following variables: plant height (PH), stem diameter (SD) and number of leaves (NL) at 85 DAT, and shoot (SDP) and total dry phytomass (TDP) at 95 DAT. Plant height was measured from the base to the apex of the plants. Stem diameter was measured at 5 cm above the base, using a digital caliper, whereas the number of leaves was determined by direct count, considering leaves larger than 3 cm.

To obtain the dry phytomass, the stem of each plant was cut close to the soil and the shoots were separated into different parts (stem and leaves), which were dried in an oven at 65 ºC. The material was then weighed to obtain leaf and stem phytomass, which were summed to obtain shoot phytomass and total phytomass. At the end of the cycle, 100 DAT, bell pepper fruits were harvested and characterized based on transverse diameter (FTD), longitudinal diameter (FLD) and pulp thickness (PT), measured using a digital caliper, with values expressed in 'mm'.

Statistical analysis

The obtained data were subjected to analysis of variance by F test at 0.05 and 0.01 probability levels. In cases of significance, regression analysis was carried out for the proline levels and comparison of means (Tukey test at 0.05 probability level) was performed for the irrigation water salinity levels, using the statistical program SISVAR-ESAL (Ferreira et al., 2011).

Conclusion

Irrigation water salinity reduces the growth and phytomass accumulation of bell pepper, cultivar 'All Big', and pulp thickness is more sensitive to irrigation water salinity. Proline doses do not inhibit the deleterious effect of irrigation water salinity on bell pepper development. Increasing proline doses lead to reduction in bell pepper growth and dry matter accumulation. The bell pepper cv. 'All Big' demonstrated sensitivity to irrigation water salinity with 3.8 dS m^{-1}.

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