



Initial development of cowpea plants under salt stress and phosphate fertilization

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ABSTRACT

The objective of this work was to study the effects of irrigation with saline water associated with phosphate fertilization on the emergence and early growth of cowpea plants. The assay was conducted in the greenhouse of the Department of Environmental Sciences and Technology of the Federal Rural University of the Semi-Arid (UFERSA) in Mossoró-RN, during October and November of 2015. The study adopted a randomized block with treatments arranged in a 5 x 3 grid, corresponding to five levels of water salinity (0.5, 1.5, 2.5, 3.5 and 4.5 dS m⁻¹) and three doses of superphosphate, based upon the soil analysis (60%, 100% and 140% of the recommended dose for the crop 60 kg P₂O₅ ha⁻¹), with five repetitions. The cowpea plants, cv. Paulistinha, were grown in lysimeters with capacity of 8 dm³. During the first 15 days of the initial stage of development the plants were evaluated for emergence, growth and biomass accumulation. The increase in water salinity above 1.5 dSm⁻¹ reduced the emergence, growth and dry matter accumulation of cowpea plants. The increase of 40% in the recommendation of phosphorus fertilization of cowpea increased the growth and biomass accumulation of shoot plants, regardless of salinity.

Keywords: saline water, soil and water management, *Vigna unguiculata*.

Desenvolvimento inicial de plantas de feijão-caupi sob estresse salino e adubação fosfatada

RESUMO

Objetivou-se estudar os efeitos da irrigação com água salina associada à adubação fosfatada com superfosfato simples na emergência e crescimento inicial de plantas de feijão-caupi. A pesquisa foi realizada em casa de vegetação do Departamento de Ciências Ambientais e Tecnológicas da Universidade Federal Rural do Semi-Árido (UFERSA), em

Mossoró-RN, no período de outubro a novembro de 2015, no delineamento experimental de blocos casualizados com tratamentos arranjos em esquema fatorial 5 x 3, relativos a cinco níveis de salinidade da água (0,5; 1,5; 2,5; 3,5 e 4,5 dS m⁻¹) e três doses de superfosfato simples, tendo como base a análise de solo (60%, 100% e 140% da dose recomendada para a cultura- 60 kg P₂O₅ ha⁻¹), com cinco repetições. As plantas de feijão-caupi cv. Paulistinha foram cultivadas em lisímetros com capacidade de 8 dm³. Durante os primeiros 15 dias da fase inicial de desenvolvimento, as plantas foram avaliadas quanto à emergência, crescimento e acúmulo de biomassa. O aumento da salinidade da água de irrigação acima de 1,5 dS m⁻¹ reduziu a emergência, o crescimento e o acúmulo de massa seca das plantas de feijão-caupi. O incremento de 40% na recomendação da adubação fosfatada do feijão-caupi promoveu incrementos no crescimento e no acúmulo de biomassa da parte aérea da planta, independente da salinidade.

Palavras-chave: água salina, manejo de solo e água, *Vigna unguiculata*.

1. INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp., is a socioeconomically important crop in Brazil, supplementing food supplies and employing farm workers, especially in the North and Northeast regions (Rocha et al., 2009). Although the Northeast is the main producing region, accounting for 90% of the Brazilian cowpea crop (Bezerra et al., 2010), the limited availability of water, particularly in the northeastern semi-arid portion, means the crops must be irrigated to achieve satisfactory yields. The high sensitivity of cowpea plants to water deficiencies in the soil, combined with differing amounts of variations in rainfall in different years and cultivation areas, causes great variations in the annual yields of this crop (Mousinho et al., 2008).

Irrigation is a necessary practice for the adequate development and production of the plants. However, besides quantitative aspects, the region also faces factors of qualitative nature, such as the excess of salts in the water (Medeiros et al., 2007). Thus, inadequate irrigation management increases the salt content of the soil, promoting its salinization, which in turn reduces the capacity of plants to absorb water, causing metabolic alterations identical to those caused by water stress (Munns and Tester, 2008). In addition, inadequate irrigation has indirect effects such as toxicity by specific ions, for example, salts of sodium and chlorine (Syvertsen and Garcia-Sanchez, 2014).

In the evaluation of three cowpea cultivars, Patel et al. (2010) and Coelho et al. (2013) observed that there were drastic reductions in the biomass accumulation of the plants with the increase in irrigation water salinity. These authors claimed that the increase in water salinity led to increments of salts in the soil, making it unproductive for the crop. It becomes necessary to adopt strategies that go beyond irrigation management, such as the management of mineral and organic fertilizers along with irrigation management, to assist in the maintenance of soil salinity and sodicity.

Among the fertilization strategies, phosphate fertilization stands out, because it is crucial to obtain satisfactory crop yields and, additionally, phosphorus (P) is the most problematic nutrient for Brazilian soils, due to its low availability and mobility in the soil (Santos et al., 2008). Among the phosphate fertilizers, single superphosphate has the highest potential in soils with salinity and sodicity problems, because it is formulated based on P, calcium and sulfur. In addition to meeting the demand for P, the presence of calcium sulfate (gypsum) favors the supplementation of calcium and sulfur (Lopes et al., 2010), besides the competition between calcium and sodium ions in the exchange complex and in the soil solution, reducing the effects of sodicity (Sousa et al., 2012; Sá et al., 2013; 2015; Mesquita et al., 2015).

However, the interaction of salinity vs. phosphate fertilization is still little-studied for most crops, including cowpea. In studies conducted by Oliveira et al. (2010), the authors observed that the quality of irrigation water directly influences the determination of phosphate fertilization, which is more efficient for waters of low salinity. Thus, the objective of this work was to study the effects of irrigation with saline water associated with phosphate fertilization using single a superphosphate at the emergence and initial growth of cowpea plants.

2. MATERIAL AND METHODS

The study was carried out in a greenhouse of the Department of Environmental and Technological Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró-RN, from October to November 2015. The municipality of Mossoró is located in the semi-arid region of Northeast Brazil, at the local geographic coordinates of 5°11'31'' S and 37°20'40'' W, at an altitude of 18 m.

The experiment was conducted in a completely randomized design, in 5 x 3 grid scheme, formed by five levels of irrigation water salinity ($S_1=0.5$; $S_2=1.5$; $S_3=2.5$; $S_4=3.5$; $S_5=4.5$ dS m^{-1}) and three doses of phosphate fertilization with single superphosphate ($A_1 = 60\%$; $A_2 = 100\%$ and $A_3 = 140\%$ of the dose of 60 kg P_2O_5 ha^{-1} , recommended by Cavalcanti et al., 2008), with 5 replicates, totaling 75 experimental plots.

The P doses were calculated based on the soil analysis and the soil used in the experiment came from a virgin area of the experimental farm of the UFERSA, Campus of Mossoró, classified as latosolic Red-Yellow Argisol. The soil samples were collected in the layer of 0-30 cm and analyzed at the Laboratory of Analysis of Soils, Water and Plant - LASAP, of the UFERSA, following the methodology of EMBRAPA (2011) (Table 1).

Table 1. Physical and chemical characteristics of the soil collected in the layer of 0-30 cm and the bovine manure used in cowpea cultivation.

Soil														
Clay	Sand		Silt	DS	DP	Porosity			Textural Class					
-----g kg ⁻¹ -----			kg dm ⁻³	kg dm ⁻³	----%----									
100	890		10	1.57	2.51	37.45			Free Sand					
EC 1:2.5	pH	P	K ⁺	Ca ⁺²	Mg ⁺²	Na ⁺	Al ³⁺	H ⁺ +Al ³⁺	SB	T	OM	ESP		
dS m ⁻¹	H ₂ O	mg dm ⁻³	-----cmolc dm ⁻³ -----							g kg ⁻¹	%			
0.16	6.72	1.20	0.20	1.40	0.50	0.05	0.00	0.70	2.15	2.85	13.23	1.75		
Manure														
N	P	K	Ca	Mg	Na	Zn	Cu	Fe	Mn	pH	OC	T	C/N	CE
-----g kg ⁻¹ -----					-----mg kg ⁻¹ -----					H ₂ O	%	cmolc dm ⁻³	dSm ⁻¹	
14.85	3.25	1.16	16.11	3.07	0.66	65	15	3.77	121	6.53	10.70	34.24	7.21	2.56

Note: P, K⁺, Na⁺: extracted by Mehlich 1; Al³⁺, Ca²⁺, Mg²⁺: extracted by 1.0 mol L⁻¹ KCl; DS: Soil bulk density; DP: Soil particle density; EC: Electrical conductivity; SB: Sum of bases; T: Cation exchange capacity; OM: Walkley-Black wet digestion; ESP: Exchangeable sodium percentage; OC: Organic Carbon.

Fertilization was based on the technical bulletin of recommendation of fertilization for the state of Pernambuco (Cavalcanti et al., 2008). The recommendation for the cowpea crop is 60 kg ha⁻¹ of P₂O₅, 20 kg ha⁻¹ of K₂O and 50 kg ha⁻¹ of N for one crop cycle, also adding 15 kg ha⁻¹ of Mg in the form of MgSO₄. The recommendation of fertilization was used to establish the doses of P₂O₅ (A₁= 36; A₂= 60 and A₃ = 84 kg ha⁻¹) applied as basal in the form of single superphosphate (A₁= 0.7; A₂ = 1.17 and A₃= 1.64 g pot⁻¹ of P₂O₅), while planting was performed after 20 days. During this period, the soil was maintained with moisture content close to its maximum water holding capacity.

After physical and chemical characterization of the soil and establishment of fertilizations, the soil was put in lysimeters with capacity for 8 dm³, of which 7 dm³ were filled by soil, 0.5 dm³ by bovine manure and 0.5 dm³ by crushed stone at the bottom, to facilitate drainage. The lysimeters were filled in the following sequence: screen, crushed stone; 2 dm³ of soil; and the mixture of soil (5 dm³), manure (0.5 dm³) and the P doses established for each treatment.

After preparation, the soil was irrigated to remain close to its maximum water holding capacity and subsequent irrigations were performed once a day to keep the soil close to its maximum retention capacity, based on the method of drainage lysimetry. The applied water depth was supplemented by a leaching fraction (LF) of 0.20 every seven days. The volume applied (V_a) per container was obtained by the difference between the previous water depth (L_{prev}) and the mean drainage (D), divided by the number of containers (n), as indicated in Equation 1.

$$V_a = \frac{V_{prev} - (D/n)}{1 - LF} \quad (1)$$

The waters with different saline levels were prepared by the addition of sodium chloride (NaCl), which accounts for 70% of the salt ions in water sources used for irrigation in small properties of Northeast Brazil (Medeiros et al., 2007). The solutions were prepared using water from the local supply system (EC_w = 0.53 dS m⁻¹), which was mixed with the salts as necessary. After preparation, the salinized waters were stored in 150-L plastic containers, one for each studied EC_w level, properly protected to avoid evaporation, entry of rain water and contamination with materials that could compromise their quality. To prepare the waters, with the specific values of electrical conductivity (EC), the salts were weighed according to the treatment and water was added until the desired EC level was achieved. The values were monitored with a portable conductivity meter, with conductivity adjusted to the temperature of 25°C.

After irrigation, the cowpea cv. 'Paulistinha' was sown on October 14, 2015, using 10 seeds per pot. Fifteen days after sowing, with total emergence of the plantlets, thinning was performed, leaving only three plants per pot. The number of emerged plantlets (cotyledons above the soil level) was counted, without discarding them, thus obtaining a cumulative value. Hence, the number of emerged plantlets referring to each count was obtained by subtracting the reading of the previous day from the value of the current day. The number of emerged plantlets referring to each reading was used to calculate the mean time of emergence (MTE), emergence speed index (ESI) and the emergence speed coefficient (ESC), according to the Equations 2, 3 and 4 described by Schuab et al. (2006).

$$MTE = \frac{(N_1 G_1) + (N_2 G_2) + \dots + (N_n G_n)}{G_1 + G_2 + \dots + G_n} \quad (2)$$

$$ESI = \frac{(N_1)+(N_2)+\dots+(N_n)}{G_1+G_2+\dots+G_n} \quad (3)$$

$$ESC = \frac{G_1+G_2+\dots+G_n}{(N_1G_1)+(N_2G_2)+\dots+(N_nG_n)} \times 100 \quad (4)$$

where:

MTE = Mean time of emergence (days);

G = number of emerged plantlets in each count;

N = number of days from sowing to each count;

ESI = emergence speed index; and

ESC = emergence speed coefficient.

After stabilization of emergence at 10 days after sowing, emergence percentage (EP) was determined using the relationship between the number of emerged plants and the number of planted seeds.

The initial growth of cowpea plantlets was monitored 15 days after sowing based on the evaluation of plant height (PH); stem diameter (SD) (mm), measured with a digital caliper at the base of the plants; and the number of leaves (NL), determined through the count of mature leaves. Three plants were collected, separated into shoots (leaves + stem) and roots, and dried in a forced-air oven at 65 °C until constant weight. Then, the material was weighed on an analytical scale with precision of 0.0001 g. These data were used to calculate leaf dry matter (LDM) (g), stem dry matter (StDM) (g) and total shoot dry matter (ShDM) (g).

The obtained data were subjected to analysis of variance by F test at 0.05 probability level. In case of significance, polynomial regression analysis was applied for the factor irrigation water salinity and test of means (Tukey) was applied for the factor phosphate fertilization, both at 0.05 probability level, using the statistical software SISVAR[®] (Ferreira, 2011).

3. RESULTS AND DISCUSSION

Irrigation water salinity affected the emergence of cowpea plants, with reductions of 21.1% in the emergence of plants cultivated at the lowest salinity level (0.5 dS m⁻¹) in comparison to those at the highest level (4.5 dS m⁻¹) (Figure 1A). For MTE, there was an increment in the time required for total emergence, so that plants irrigated with high saline level (4.5 dS m⁻¹) required 12.4% more time to emerge, in relation to the control (0.5 dS m⁻¹) (Figure 1B).

There was a decrease in the ESI of cowpea plants due to the increase in irrigation water salinity, with reductions of 7.2% per unit increase in water salinity (Figure 1C). Still regarding the emergence of cowpea plantlets, the decrease in the number of emerged seeds, as well as in the emergence speed, caused by the salt stress, linearly reduced the ESC (Figure 1D), which expresses the uniformity of emergence of the plants, indicating that the salt stress affected the physiological potential of the seeds and plantlets that emerged at a high salinity level, causing a reduction of vigor and possibly the death of the plants still in the germination stage. This probably occurred due to the reduction in the osmotic potential caused by the increase in the contents of NaCl in the soil, besides the toxicity caused by these ions, decreasing the viability of the seeds and the emergence of the plantlets (Munns and Tester, 2008; Voigt et al., 2009; Dantas et al., 2011; Taiz and Zeiger, 2013).

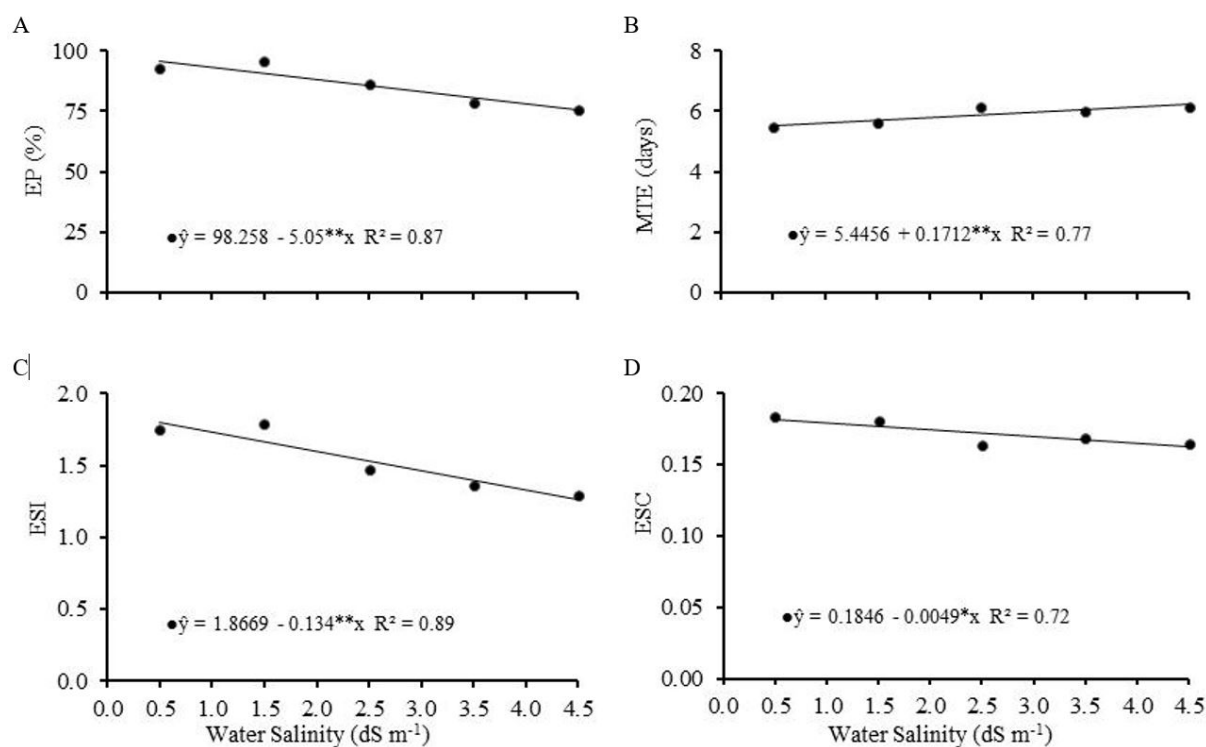


Figure 1. Emergence percentage (EP) (A), mean time of emergence (MTE) (B), emergence speed index (ESI) (C) and emergence speed coefficient (ESC) (D) of cowpea plantlets, cv. Paulistinha, under different levels of water salinity, at 15 days after sowing.

Note: * and ** = significant at 5 and 1% probability ($p < 0.05$ and $p < 0.01$).

The increase in salinity linearly reduced the height and stem diameter of cowpea plants at rates of 0.76 cm and 0.17 mm per dS m^{-1} , respectively (Figure 2A and C). For plant height, the management of phosphate fertilization also had a significant effect, so that plants showed the highest growth when fertilized with 40% more of P_2O_5 (Figure 2B). These results express the positive effect of phosphate fertilization on the nutritional aspects of cowpea plants, promoting increments in the growth in height, regardless of the salinity condition. Similar results were reported by Oliveira et al. (2010), who observed that the higher P availability also promoted the growth of radish plants, regardless of the studied level of salinity. In addition, the levels of 60 and 100% of the P recommendation did not differ, possibly because of the low P concentrations existing in the soil of these treatments, which were not sufficient to promote satisfactory plant growth (Figure 2B).

For the number of leaves, there was significant interaction between the levels of irrigation water salinity and phosphate fertilization, so that plants cultivated with 60 and 100% of the P recommendation linearly reduced the number of leaves by 28.1 and 30.7% at the high salinity level (4.5 dS m^{-1}) in relation to the control (0.5 dS m^{-1}). Plants cultivated with 140% of the P recommendation showed mean production of 4.06 leaves per plant, at all salinity levels studied, which indicates the previous lack of salt stress on the leaf production of these plants (Figure 2D).

The reduction of growth in cowpea plants when subjected to salt stress can be related to the decrease in the water potential of the tissues caused by the excess of salts in the soil solution, leading to restrictions in the rates of cell elongation and division, which directly depend on the extensibility of the cell wall. Consequently, the failures occurring in the adjustment resulted in injuries similar to those caused by drought, such as loss of turgor and growth reduction (Silva

et al., 2009; Sousa et al., 2011; Taiz and Zeiger, 2013). In addition, the low water content in the cells, caused by the restriction imposed by salts due to the soil salinity, promotes metabolic alterations and indirect effects, such as toxicity by specific ions, for example salts of sodium and chlorine (Munns and Tester, 2008; Syvertsen and Garcia-Sanchez, 2014). However, plants fertilized with higher P doses obtained greater growth in height and their number of leaves was not affected by salinity during the first 15 days of cultivation, indicating that the plants responded to phosphate fertilization even under saline conditions (Figure 2B and D).

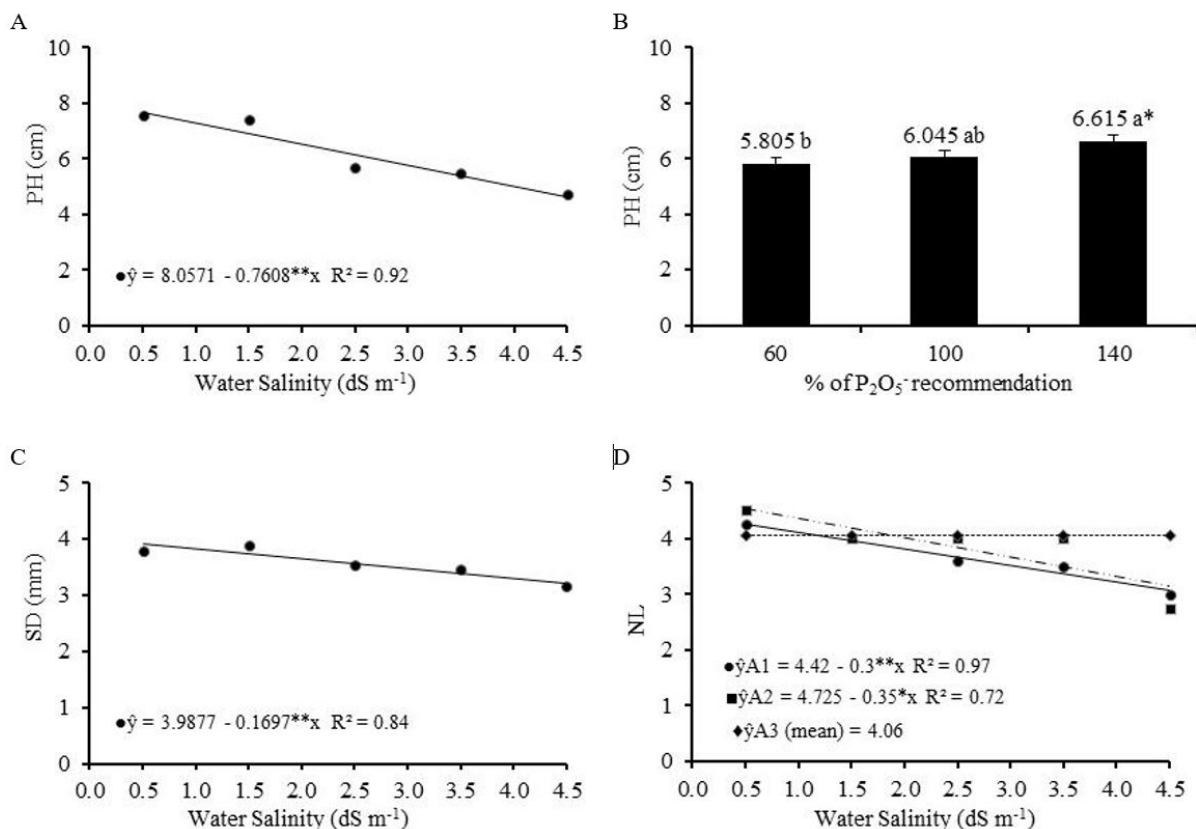


Figure 2. Plant height (PH) (A and B), stem diameter (SD) (C) and number of leaves (NL) (D) of cowpea, cv. Paulistinha, under different levels of water salinity and phosphate fertilization (A1= 60, A2=100% and A3 140% of the recommendation of P₂O₅⁻ of 60 kg ha⁻¹), at 15 days after sowing.

Note: * and ** = significant at 5 and 1% probability ($p < 0.05$ and $p < 0.01$). Equal letters do not differ from the Tukey test at the 5% probability level.

Ferreira et al. (2007) observed that high soil salinity decreases the P contents in the tissue of maize plants, because the ionic force reduces the phosphate activity in the soil. Possibly, the addition of P beyond the recommendation promoted the higher availability of the nutrient, thus leading to better growth in relation to plants cultivated under the lowest doses.

StDM was influenced by water salinity, with linear reductions in the stem phytomass accumulation of 14.2% per dS m⁻¹ (Figure 3). According to Munns and Tester (2008), plant growth is restricted by both the water deficit induced by the high osmolarity of the solution and the ionic toxicity, involving metabolic and physiological damages, thus affecting the phytomass accumulation of the plant.

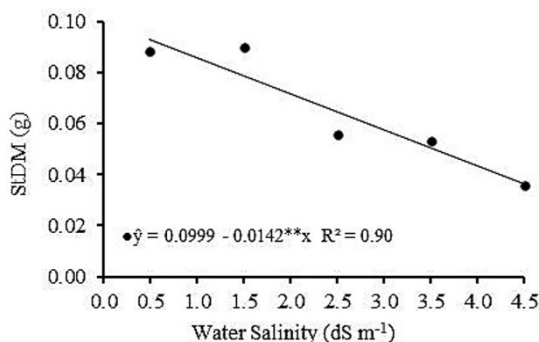


Figure 3. Stem dry matter (StDM) of cowpea plants, cv. Paulistinha, under different levels of irrigation water salinity and phosphate fertilization (A1= 60, A2=100% and A3 140% of the recommendation of P_2O_5 of 60 $kg\ ha^{-1}$), at 15 days after sowing.

Note: ** = significant at 0.01 probability level ($p < 0.01$).

For LDM and ShDM, as observed for StDM, there were linear reductions in the phytomass accumulation, as the irrigation water salinity increased, respectively to 0.062 and 0.76 g per $dS\ m^{-1}$ (Figure 4A and C). The observed behavior denotes the severity of the salt stress on cowpea plants, which affected the synthesis of carbohydrates and, consequently, biomass accumulation. However, plants cultivated in soil containing 140% of the recommended dose of P showed higher accumulations of LDM (on average, 25.5%) and ShDM (on average, 19.2%) in relation to the other treatments, indicating that under this condition the reduction in phytomass accumulation by the salt stress was attenuated (Figure 4B and D). Possibly, with the application of P in the form of single superphosphate, besides meeting the requirement of P, the release of calcium sulfate favored the supply of calcium and sulfur.

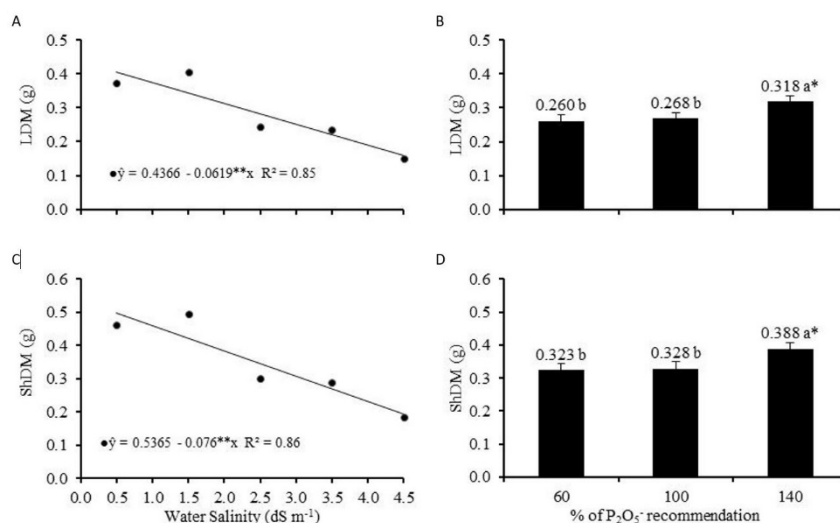


Figure 4. Leaf dry matter (LDM) (A and B) and shoot dry matter (ShDM) (C and D) of cowpea plants, cv. Paulistinha, under different levels of irrigation water salinity and phosphate fertilization (A1= 60, A2=100% and A3 140% of the recommendation of P_2O_5 of 60 $kg\ ha^{-1}$), at 15 days after sowing.

Note: * and ** = significant at 5 and 1% probability ($p < 0.05$ and $p < 0.01$). Equal letters do not differ from the Tukey test at the 5% probability level.

4. CONCLUSIONS

The increase in irrigation water salinity above 1.5 dSm⁻¹ reduced emergence, growth and dry matter accumulation of cowpea plants.

The increment of 40% in the recommendation of phosphate fertilization for the cowpea crop promoted increments in the growth and biomass accumulation of the shoots, regardless of the salinity level.

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