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Response of cowpea bean (*Vigna unguiculata*) to the application of two levels of phosphorus and 7 levels of salinity in the soil

Respuesta de frijol caupí (*Vigna unguiculata*) a la aplicación de dos niveles de fósforo en el suelo y 7 niveles de salinidad

Enrique Combatt C.[1*], Jaime Hernández B.[1], Jaime Mercado L.[1]
María Quiroz B.[1], Eliana Burgo W.[1], Carlos Gómez G.[1], Jesús López P.[1]

1.Department of Agricultural Engineering and Rural Development, Universidad de Córdoba, avenue 6 No. 76-103, Montería, Colombia. Correspondence to author. emcombatt@correo.unicordoba.edu.co

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ABSTRACT

The cultivation of cowpea is important in the diet of the Caribbean region, where it is grown by small producers. The response of cowpea (*Vigna unguiculata*) to the application of 2 levels of phosphorus under 7 levels of soil salinity was evaluated. The experiment was carried out under greenhouse conditions at the Faculty of Agricultural Sciences of the University of Córdoba. It was carried out under a complete randomized design, with a factorial arrangement, 7 doses of salt in the soil (T1: 0, T2: 230, T3: 460, T4: 828, T5: 1500, T6: 1610 and T7: 2300 kg ha^{-1}) x 2 doses of phosphorus (30 and 60 kg ha^{-1}). The cowpea variety used was Caupicor 50. The variables evaluated were germination percentage, leaf area, number of nodes, plant height, number of leaves, leaf dry mass, stem dry mass, electrical conductivity and pH. Analysis of variance, Tukey's mean comparison test and interaction decomposition were performed. Phosphorus doses only showed significant differences for pH and plant height, while only Sal*P interaction was presented for the variables pH and stem dry mass. On the other hand, treatments equivalent to 1500, 1610 and 2300 kg ha^{-1} of Na were those that most affected the development of cowpea, and fertilization with high doses of phosphorus in the presence of NaCl contents in the soil did not influence the development of cowpea Caupicor 50. On the other hand, soil pH tends to decrease when phosphorus doses and NaCl contents in the soil increase.

Key words: Legume, soils, toxicity, nutritional absorption, nutritional elements.

RESUMEN

El cultivo de frijol caupí, es importante en la dieta alimentación en la región caribe, donde es sembradas por pequeños productores. Se evaluó la respuesta de frijol caupí (*Vigna unguiculata*) a la aplicación de 2 niveles de fósforo en el suelo bajo 7 niveles de salinidad. El experimento fue realizado bajo condiciones de invernadero en la Facultad de Ciencias Agrícolas de la Universidad de Córdoba. Se realizó bajo un diseño completo al azar, con arreglo factorial, 7 dosis de sal en el suelo (T1: 0, T2: 230, T3: 460, T4: 828, T5: 1500, T6: 1610 y T7: 2300 kg ha^{-1}) x 2 dosis de fósforo (30 y 60 kg ha^{-1}). La variedad de frijol caupí usada fue Caupicor 50. Las variables evaluadas fueron porcentaje de germinación, área foliar, número de nudos, altura de plantas, numero de hoja, masa seca de hojas, masa seca de tallo, conductividad eléctrica y pH. Se realizó el análisis de varianza, prueba de comparación de medias de Tukey y descomposición de las interacciones. Las dosis de fósforo solo presentaron diferencias significativas para pH y altura de planta, mientras que solo se presentó interacción Salinidad*P para las variables pH y masa seca de tallo. Por otra parte, los tratamientos que equivalen a 1500, 1610 y 2300 kg ha^{-1} de Na, afectaron el desarrollo del frijol caupí y la fertilización con dosis altas de fósforo en presencia de contenidos de NaCl en el suelo no influyo en el desarrollo de frijol Caupicor 50. Por otro lado, el pH del suelo tiende a disminuir cuando se incrementan las dosis de fósforo y los contenidos de NaCl en el suelo.

Palabras claves: Leguminosa, suelos, toxicidad, absorción de nutrición, elementos nutricionales.

INTRODUCTION

More than 800 million hectares around the world are affected by salts and more than 4% of cultivated areas have this problem (Munns et al., 2008). Likewise, many ions that make up the salts in the soil are toxic to plant cells when they are present in high concentrations externally or internally, and due to high concentrations of salt in the soil, a water deficit or osmotic stress can occur for plants due to the decrease in the osmotic potential in the soil. For this reason, salt stress is considered a growing threat to agriculture worldwide (Zhao, 2007).

In agriculture, plants such as cowpea (*Vigna unguiculata*) present adaptive characteristics to salt stress, developing different mechanisms to reach ionic and osmotic homeostasis in cells (Praxedes et al., 2010, Sprent et al., 2010), and among them are, the increase in the synthesis of abscisic acid (ABA) (Raghavendra et al., 2010), the increase in the concentrations of K^+, Ca^{2+}, amino acids and other compatible ions and solutes (Maathuis et al., 2006), the intracellular compartmentalization of Cl (Turkan et al., 2009), the movement of these ions from the leaves to the roots (Munns et al., 2008, Manchanda et al., 2008) and recently some ultrastructural modifications in chloroplasts have been evidenced (Gómez et al., 2014). Likewise, Carmona et al. (2020) indicate that co-evolution between plants and symbiotic and asymbiotic environments has facilitated the development of defense mechanisms in plants that allow them to protect themselves against harmful effects by implementing strategies to overcome water deficit, regulations in stomatal conductance, and changes in photosynthesis.

On the other hand, among legumes, according to FAO (2012), 11.3 million hectares of cowpea are cultivated worldwide, with an approximate production of 5.7 million tons, and it is considered a very important legume. Mndzebele et al. (2020) states that, in addition to enriching the human diet, cowpea has the capacity to fix a considerable amount of atmospheric nitrogen, reaching up to 337 kg of nitrogen per hectare and can grow in low fertility soils. However, despite its adaptation to stressful conditions (Sprent et al., 2010) there are various factors that contribute to the low productivity of this species, among these factors the management of soil fertility stands out, especially if the supply of phosphorus is insufficient and the fixation capacity in the soil is high (De Olivera, 2012). Therefore, the use of genotypes that are efficient in phosphorus consumption and utilization can be a complementary solution to increasing production (Wang et al., 2010). According to Singh and Reddy (2011), knowledge of cowpea responses to salt stress conditions and the interaction between salinity and the application of available phosphorus in the soil is important because it can help improve the agronomic performance of new cultivars.

In Colombia, and specifically in the Caribbean region, most of the cowpea cultivation areas are located in the departments of Cesar (59.5%), Córdoba (19%) and La Guajira (16.6%), with average yields of 1700 kg ha^{-1}. However, in the Caribbean region, specifically in the department of Córdoba, estimates made by IDEAM (2002) indicate that there are approximately more than 7700 km^2 with salinization problems, and considering the great importance of cowpea in food security in tropical and subtropical developing countries (Nelson et al., 2009) and in the Caribbean region of Colombia, the present work aimed to evaluate the responses of cowpea to the application of two levels of phosphorus under different levels of soil salinity.

MATERIALS AND METHODS

The experiment was carried out under greenhouse conditions at the Faculty of Agricultural Sciences of the University of Córdoba located at 8° 45′ 0″ North, 75° 52′ 59″ West, with an altitude of 18 m.a.s.l., average annual temperature of 28 °C and average relative humidity of 78% (Palencia et al., 2006).

It was carried out under a complete randomized design (DCA) with a 7x2 factorial arrangement: 7 doses of salt in the soil (T1: 0, T2: 230, T3: 460, T4: 828, T5: 1500, T6: 1610 and T7: 2300 kg ha^{-1}) x 2 doses of phosphorus (30 and 60 kg ha^{-1}), the salt source was NaCl and the phosphorus source was P_2O_5. Each treatment was replicated 4 times (replications), and each experimental unit consisted of 5 kg of soil with a sandy loam texture deposited in a pot or plastic container. Seven days after applying the corresponding treatments (Salt and P), 5 cowpea bean seeds Caupicor 50 were planted in each pot. At 15 days after sowing (das), the less vigorous plants were cut, leaving 3 plants per pot. At 15 and 33 das, all treatments were fertilized with 20 ml of a KNO_3 solution, and irrigation was applied throughout the trial to maintain field capacity. Variables were measured at 40 das, except for germination percentage (PG: %) which was measured in the first 15 das. The variables measured in the plant were: leaf area (AF: cm^2), number of nodes (Nud), plant height (ALTP: cm), number of leaves (NH), leaf dry mass (MSH: g), stem dry mass (MST: g).

In addition, 1 kg soil samples were collected from each experimental unit and taken to the laboratory for electrical conductivity (EC: ds m^{-1}) and pH measurements, in order to determine how the applied treatments affected the soil conditions where the test was carried out. The collected data were analyzed using analysis of variance and Tukey's mean comparison test, using the SAS 9.1 statistical package. For the variables that presented Salinity*P interaction, the interaction decomposition was performed, and regressions were also performed for each of the variables with respect to the salt doses in each P treatment, to determine the trend and the adjusted model.

RESULTS AND DISCUSSION

Table 1 indicates that there was only a highly significant difference between the salt treatments for the variable EC. The mean comparison test indicates that the treatment with the highest EC was 2300 kg ha^{-1} of Na with a mean of 14.96 ds m^{-1}.

Table 1. Results of variance analysis for the application of salinity and phosphorus doses in bean cultivation.

FV	GL	CE	pH	PG	Alt P	Nud
Salinity	6	187.13**	0.14**	4380.95**	7632.42**	111.79**
P	1	6.04 ns	0.21**	178.57 ns	6668.62**	23.27 ns
Salinity*P	6	8.61 ns	0.04**	128.57 ns	254.94 ns	5.39 ns
Error	42	6.97	0.007	359.52	397.83	5.76
Total	55	26.78	0.03	769.74	1285.48	17.60
Mean		5.22	7.72	73.92	60.40	10.10
CV		50.50	1.10	25.64	33.01	23.75

FV: sources of variation, GL: degrees of freedom, CE: electrical conductivity, pH: soil reaction or pH, PG: grain weight, AltP: plant height, Nud: number of nodes. (*significant, ** highly significant, ns not significant at 5%).

The regression indicates a general tendency for EC to increase as more salt was applied to the soil, fitting a quadratic model. The equations that define the models can be seen in Figure 1A. As NaCl content in the soil increases, electrical conductivity increases because NaCl is a fairly soluble salt, while the application of fertilizers contributes little salt to the soil. However, other authors claim that the species is moderately sensitive to salt stress, since it supports a salinity of around 4.9 dS m^{-1} (49 mM NaCl) Fageria et al., 2011). In addition, the application of salinity at low doses presented electrical conductivities lower than those reported by Fageria in 2011, with EC of 0.73; 1.71; 2.85 and 3.42 ds m^{-1} in treatments T1, T2, T3 and T4, and the pH of all treatments was found to be below 8.5 (Figure 1B).

This shows that the treatments cannot be classified as highly saline soil. However, Pirasteh-Anosheh et al. (2014), consider that the effects that occur under salinity conditions on crop yield and its components are the result of a series of chemical, physiological and biochemical damages, and in turn a series of metabolic, enzymatic and hormonal responses that they experience from the moment the plants begin their germination process, until their biological cycle concludes. Leidi and Pardo (2002) explain that in highly saline solutions a change in electrical potentials is generated, which contributes to a maximum ionic conductivity, and where the electrochemical gradient of Na$^+$ is high.

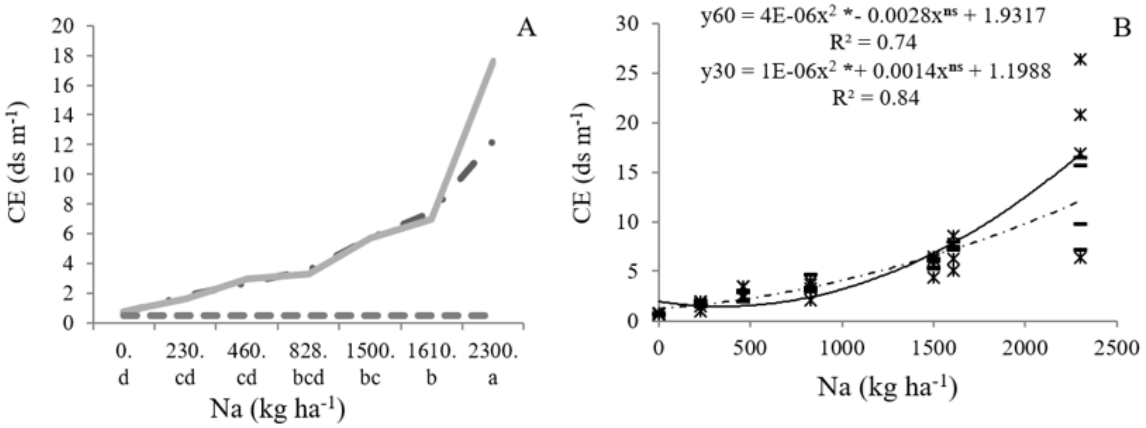

Figure 1. Electrical conductivity trend and regressions for different salinity levels, and phosphorus dose for salinity levels. P dose in kg/ha^{-1}: y – 30; y —— 60. Initial EC values ▬ ·

In this research, the pH variable presented a highly significant difference for Sal, P and Sal*P (Table 1), so the analysis of the decomposition of this interaction was carried out. In Table 2 it is observed that the interaction is given by highly significant differences in the phosphorus doses in the Na doses of 828, 1500, 1610 and 2300 kg ha^{-1}, where higher averages are recorded in the 30kg phosphorus treatment (pH= 7.86, 7.73, 7.72 and 7.72 respectively) compared to the 60kg phosphorus treatment (pH= 7.66, 7.51, 7.56 and 7.41).

In Figure 2A it can be observed that the pH increases in a similar way for the two doses of phosphorus in the first 3 salinity levels with respect to the initial pH, and then for the dose of 60 kg of phosphorus it begins to decrease as the salinity levels increase from treatment 4, but without reaching the initial pH value. This agrees with what is recorded in the regression (Figure 2B), where a greater tendency for the pH to decrease is observed in the 60kg phosphorus treatment, with respect to the trend of the 30kg treatment, adjusting to a linear model equation.

Table 2. Decomposition of the interactions for pH and stem dry mass in a soil with different doses of NaCl and phosphorus established with cowpea at greenhouse level.

Na (kg ha^{-1})	pH 30	pH60	CM-pH	*MST30	*MST60	CM-MST
0	7.92	7.98	0.0072 ^{ns}	2.0	2.1	0.0532 ^{ns}
230	7.76	7.81	0.0098 ^{ns}	2.0	2.8	1.4893**
460	7.80	7.72	0.0113 ^{ns}	1.8	2.3	0.5597*
828	7.86	7.66	0.0648**	1.4	1.7	0.2121 ^{ns}
1500	7.73	7.51	0.1013**	1.5	1.1	0.3787 ^{ns}
1610	7.72	7.56	0.0578**	1.2	0.5	0.7552*
2300	7.72	7.41	0.2048**	0.7	0.1	0.1138 ^{ns}

*pH 30 and 60 are phosphorus doses, CM: mean squares, MST 30 and 60: Dry stem mass at different phosphorus doses, *significant, **highly significant and ns not significant at 5%.

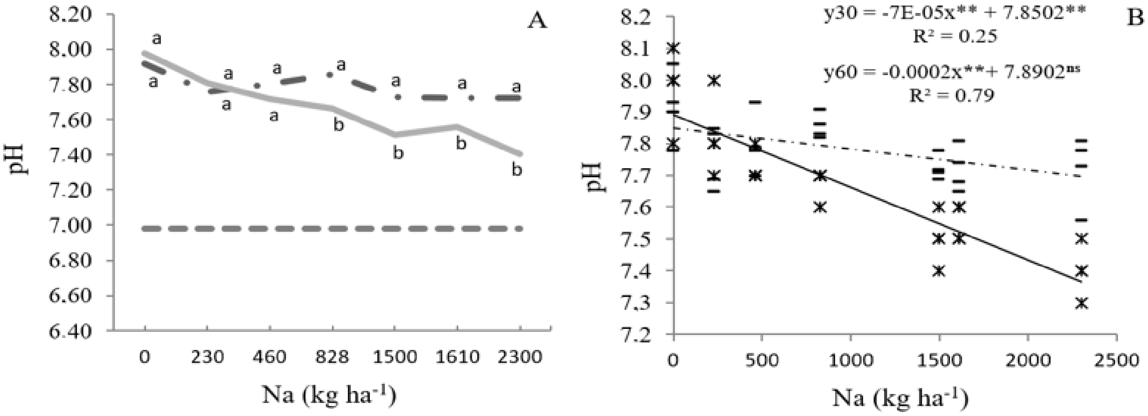

Figure 2. pH trend and regressions for different salinity levels and phosphorus doses for salinity levels. P doses in kg ha^{-1}: and 30; and 60. Initial pH values - - -

According to Zapata (2000), NaCl arrives dissociated to the soil, and Na^+ displaces H^+ from the exchange sites and these remain in solution, which decreases the pH. However, the decrease in pH in 60 kg of phosphorus can be explained by the reaction of P_2O_5 with the different doses of NaCl in the soil. When P_2O_5 reaches the soil, it interacts with H^+ and forms $H_2PO_4^-$ in a soluble diacid form and HPO_4^- in a soluble monoacid form, which should decrease the pH by increasing the free H^+ in solution. Likewise, the dissociated Na^+ reacts with either of the two soluble forms of phosphorus, forming $NaPO_4$, displacing the H^+ that will remain in the soil solution, decreasing the pH (Sanzano, 2000), and for this reason, when increasing the doses of P_2O_5 a lower pH was found.

Table 1 shows that the germination percentage (PG) showed a highly significant difference between the salt treatments. The mean comparison test shows that the highest germination percentages were between treatments 1 and 6 with a range between 72.5 and 90% (Figure 3A), and this trend can be better appreciated in figure 3B, where the regression shows a trend of lower PG as NaCl levels increase with a slight increase in T2 (230 kg ha^{-1}) and T3 (460 kg ha^{-1}), fitting a quadratic model.

These results agree with those recorded by Laynez-Garsaball et al. (2007) in a study evaluating the effect of salinity on corn germination, where the percentage and time of germination were affected in soils with higher salt concentrations. Other studies carried out by Madueño et al. (2006); Taffouo et al. (2009) and Kaymakanova (2008) show the same trends. In addition, Can et al. (2014) found that the decrease in germination under extreme salinity conditions is induced by a decrease in water and osmotic potentials, which are a quantitative expression of the free energy associated with water (Taiz and Zeiger, 2010) and also by the poor capacity of the seed to compartmentalize or exclude the toxic ions of Na+ and Cl- (Práxedes et al., 2011) which increases the concentration of these ions in the embryo, causing a toxic effect (Laynez-Garsaball et al., 2007).

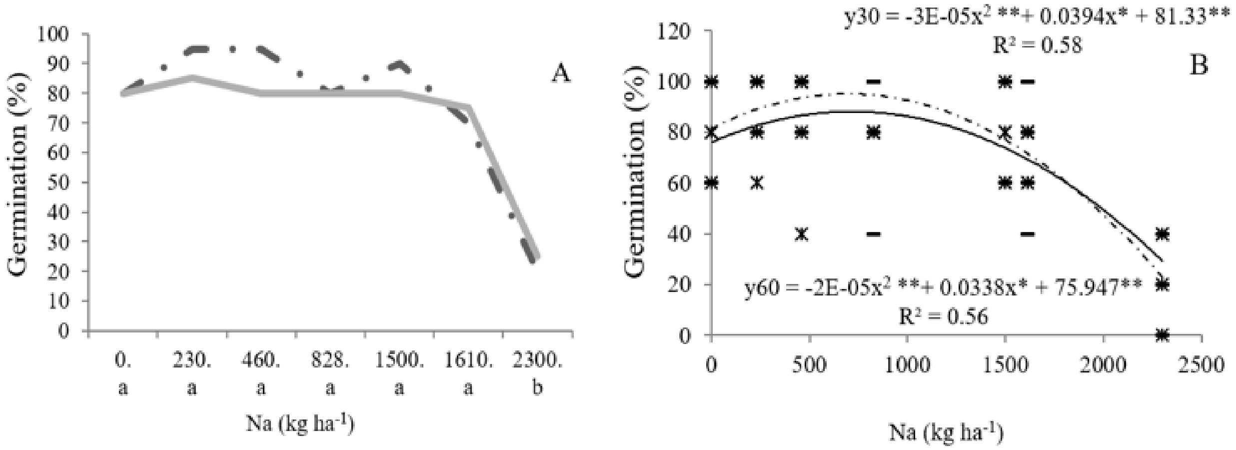

Figure 3. Behavior of the germination percentage variable under different doses of sodium in kg ha^{-1}: T1: 0, T2: 230 T3: 460 T4: 828, T5: 1500, T6: 1610, T7: 2300 and doses of P in kg ha^{-1}: ▬ ▪ and ▬ 30; ▬▬ and 60.

The variable plant height presented statistically significant differences between salinity treatments and also for the phosphorus doses (Table 1). According to the mean comparison test, the salinity treatment where the highest plant height was found was T2 (230 kg ha^{-1}) with an average of 96 cm and the phosphorus dose where there was the highest height was 30 kg ha^{-1}, with an average of 71.3 cm compared to 60 kg ha^{-1}, which was 49 cm (Figure 4A). In addition, in relation to the phosphorus doses and independent of the salinity treatments, the phosphorus dose where the highest height was found was 30 kg (Figure 4B). This response to 30 kg of phosphorus on height gain contrasts with the response to phosphorus fertilization in a study conducted by Serna et al. (2011) in corn crops, where the variable plant height was higher with higher doses of P. Furthermore, according to Perez et al. (2024), under these salinity conditions, the use of foliar application of amino acids is a viable strategy to increase crop productivity.

On the other hand, when analyzing the trend in height gain at each dose of phosphorus, within the salinity levels, an inverse relationship between plant height and salinity is observed in graph 4C, finding greater plant height at the dose of 30 kg of phosphorus, which fits a quadratic model, while with 60 kg of phosphorus, the trend fits better to a linear model. According to Aiazzi (2005), the application of salinity in Atriplex cordobensis plants produced a significant decrease in the length of the aerial part in the plants.

According to Martínez (2011), the obvious effect of saline stress can be manifested in the loss of turgor, that is, a plant cell exposed to a saline medium balances its water potential by losing water, which produces a decrease in the osmotic potential and turgor, which are responsible for the mechanical force for cell elongation (Taiz and Zeiger, 2010).

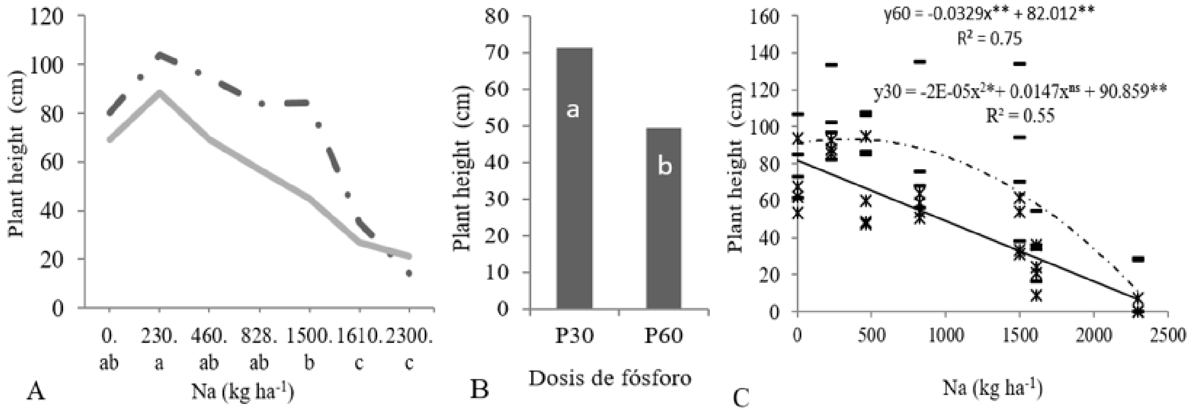

Figure 4. Behavior of the plant height variable under different doses of sodium in kg ha^{-1}: T1: 0, T2: 230 T3: 460 T4: 828, T5: 1500, T6: 1610, T7: 2300 and doses of P in kg ha^{-1}: ▬ · and ▬ 30; ━━ and 60

The variables leaf number, leaf area, stem dry mass and leaf dry mass (Table 3) showed significant differences for the salinity treatments, and the stem dry mass showed a significant interaction (Salinity *P). When analyzing the results of the mean comparison test, it is observed that the highest number of nodes was present in T2 (13.3), T3 (12.7), and T4 (12.4), the highest number of leaves was present in treatments T1 (36.3), T2 (33.3), and T3 (28.7), while the largest leaf area (cm^2) was found in treatments T2 (661.3), T1 (597.5), and T3 (550.2) (Figure 5 A, C, E).

Table 3. Results of variance analysis for the variables number of leaves (NH), leaf area (AF), stem dry mass (SDM), and leaf dry mass (LDM) in a soil with different doses of NaCl and phosphorus established with cowpea at greenhouse level.

FV	GL	NH	AF	MST	MSH
Salinity	6	900.07**	345961.16 **	4.62 **	3.86 **
P	1	156.44 ^{ns}	9959.11 ^{ns}	0.10 ^{ns}	3.49 ^{ns}
Salinity*P	6	94.19 ^{ns}	17724.94 ^{ns}	0.58 **	1.15 ^{ns}
Error	42	89.10	15794.79	0.11	1.09
Total	55	179.35	519171.95	0.65	1.44
Mean		23.00	414.56	1.47	1.54
CV		41.02	30.31	22.84	67.79

The general trend in the regression is inverse (Figure 5 A, C, D, E, F) with the soil salinity contents of these three variables, but the nodes evaluated fit a quadratic model (Figure 5 B). In general, it can be said that there was greater development of these variables in the first three treatments, and this is corroborated by the decrease in the number of leaves and nodes, caused by the fact that the soil water is not usable in the treatments with a higher amount of salinity, due to the decrease in water potential. In addition, with high levels of water stress, senescence and leaf abscission increase (Ichi et al., 2013), and there is a reduction in the size of the leaves and the leaf area (Okon, 2013). In addition, according to Mudgal et al. (2010), in some plants there is activation of some mechanisms, such as the accumulation of compatible solutes (proline and sucrose), and also some structural adaptations such as the swelling of the cell cortex, which are used as a barrier to prevent the entry of Na+ ions (Hussain et al., 2010).

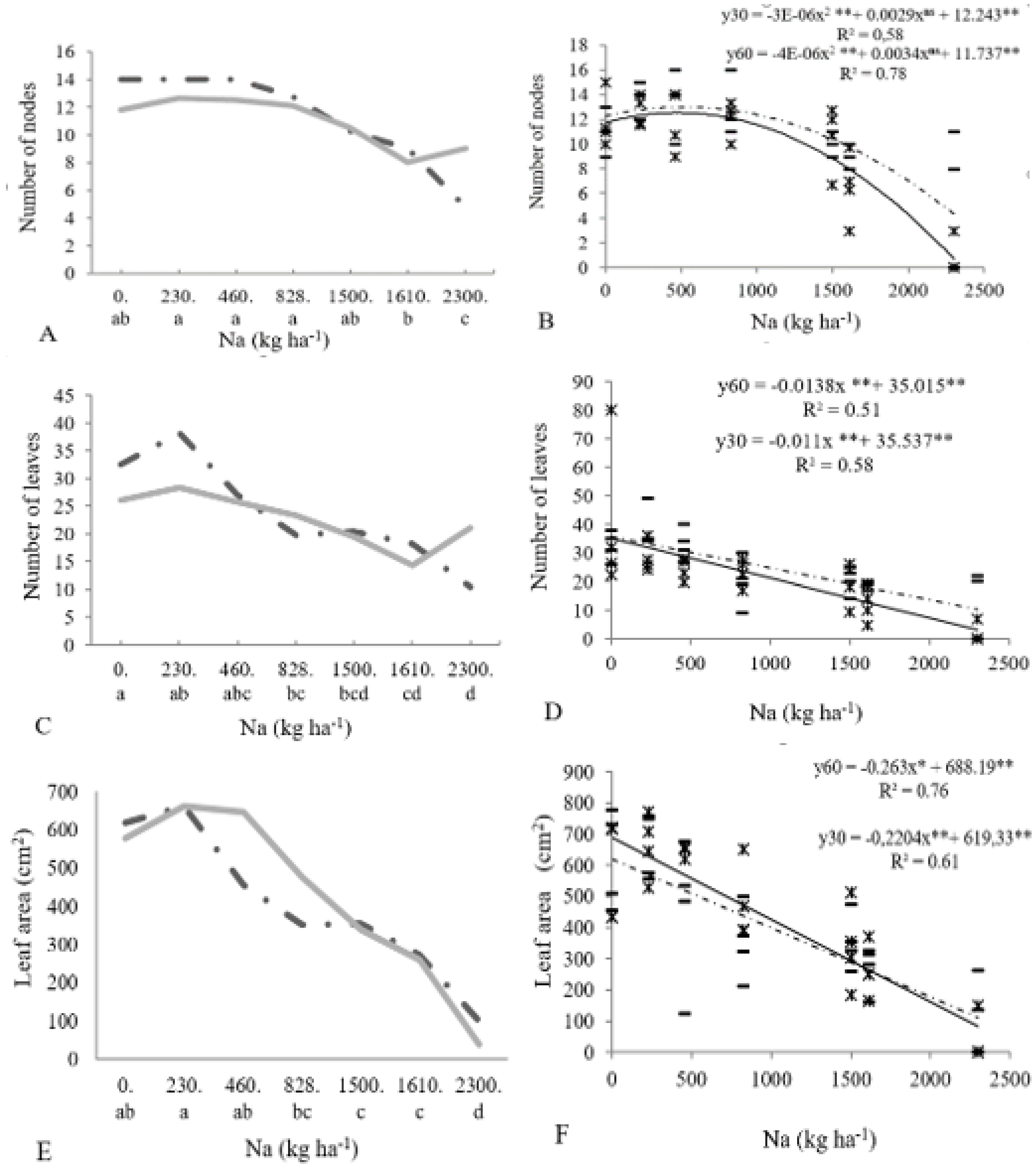

Figure 5. Behavior of the variables, number of nodes, number of leaves and leaf area at different doses of phosphorus and salinity. Na dose in kg ha^{-1}: T1: 0, T2: 230 T3: 460 T4: 828, T5: 1500, T6: 1610, T7: 2300. P dose in kg ha^{-1}: ▬ · and ▬ 30; ━━ and 60.

The variable dry stem mass presented highly significant differences for Salinity and Salt*P, so the interaction was broken down. Table 2 shows that the interaction is given by the significant difference between the phosphorus doses in the treatments of 230, 460 and 1610 kg ha^{-1} of Na, presenting a highly significant response only in 230 kg ha^{-1}, where the highest average with 2.8 g was found in the dose of 60 kg phosphorus, while for the dose of 30 kg it was 2.0 g. Similar results were found with 460 kg ha^{-1}, although the difference between the averages was smaller with 2.3 g in the dose of 60 kg phosphorus and 1.8 g for the dose of 30 kg. The opposite case occurs in the treatment of 1610 kg ha^{-1} of Na, where a significant difference was found in favor of the dose of 30 kg of phosphorus with 1.1 g. This response was higher than 0.5 g found at the dose of 60 kg (Figure 6 A). These results can also be seen in Figure 6 B, where the regression shows the trends of the two doses of phosphorus, fitting a cubic model for the 60 kg dose and a linear model for the 30 kg dose. These results agree with those found by Can et al. (2014) who report a gradual decrease in stem dry weight as the NaCl dose increased.

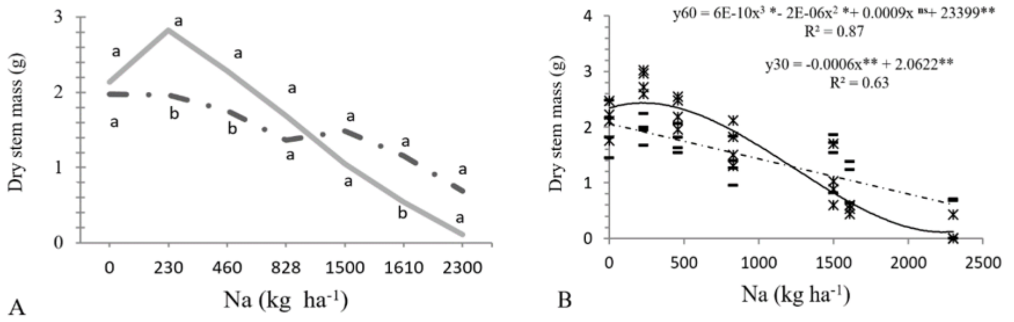

Figure 6. Behavior of the dry stem mass variables at different doses of phosphorus and salinity. Na dose in kg ha^{-1}: T1: 0, T2: 230, T3: 460, T4: 828, T5: 1500, T6: 1610, T7: 2300. P dose in kg ha^{-1}: ▬ · and ▬ 30; ▬▬ and 60.t

The variable stem dry mass showed highly significant differences between salinity treatments (Table 2.). According to the mean comparison test, the treatments with the highest leaf dry mass were 460 and 230 kg ha^{-1} with an average of 2.29 g and 2.13 g respectively. The behavior of this variable generally showed a tendency to decrease as the soil salinity content increased (Figure 7 A). In addition, at both phosphorus doses the response was explained by a linear model, similar to the results found in leaf area. Taffouo et al. (2009) found that dry weight is significantly reduced due to osmotic stress and the effect of the specific ions Cl$^-$ and Na$^+$. The decrease in dry mass would be linked to the decrease in net photosynthesis as soil salinity levels increase (Wilson et al., 2006). This can be explained by the accumulation of starches in chloroplasts due to the inactivation of alpha amylase under saline conditions, which produces a change in the structure of the grains, thus reducing their light-capturing capacity (Humme et al., 2010). This is compounded by the difficult extraction of water by the roots, which donates the electrons necessary for the Hill reaction to occur (Taiz and Zeiger, 2010) and the accumulation of toxic ions whose action influences the production of reactive oxygen species, which affect various organic substances such as lipids, proteins and nucleic acids, culminating in membrane depolarization (Maia et al., 2010).

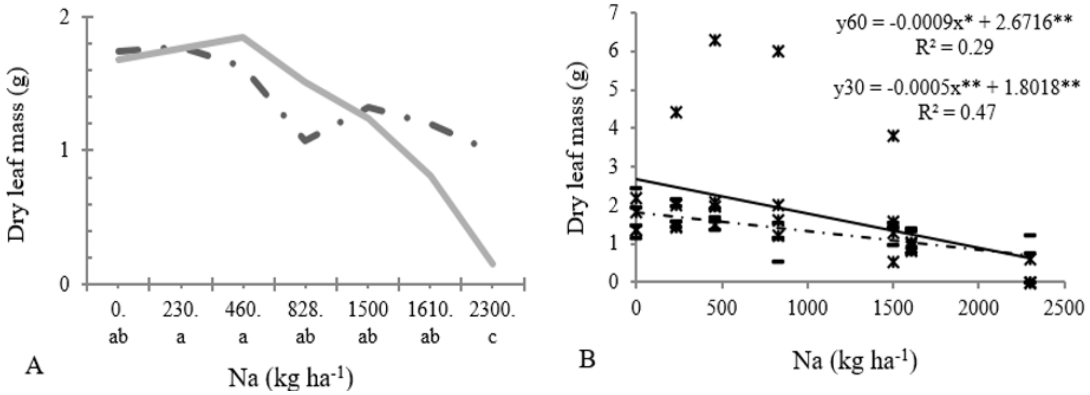

Figure 7. Behavior of the variables dry leaf mass at different doses of phosphorus and salinity. Na dose in kg ha^{-1}: T1: 0, T2: 230, T3: 460, T4: 828, T5: 1500, T6: 1610, T7: 2300. P dose in kg ha^{-1}: ▬ · and ▬ 30; ▬▬ and 60.

CONCLUSIONS

- The increase in soil salt content affected all growth variables in the cowpea crop.
- In the soil, pH tends to decrease when phosphorus doses and NaCl contents in the soil increase.
- The bean variety used (Caupicor 50) showed tolerance to salinity conditions, being affected only by high concentrations of salt in the soil (1610 and 2300 kg ha^{-1} of Na) where electrical conductivity values of 7.25 and 15 ds m^{-1} were presented respectively.
- The germination of the Caupicor 50 bean variety was affected by excessive NaCl contents in the soil (2300 kg ha^{-1} of Na), showing good germination capacity below these salt levels.
- Fertilization with high doses of phosphorus in the presence of NaCl contents in the soil does not influence the development of Caupicor 50 beans.

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