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Irrigation strategies with saline water and phosphate fertilization in cowpea culture

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ABSTRACT: The use of saline water negatively affects gas exchange and plant growth. However, fertilization with a mineral source has been used to mitigate salt stress on agricultural crops. The objective of this study was to evaluate the gas exchange and the initial growth of cowpea beans submitted to different irrigation strategies with saline water and phosphate fertilization. The experiment was conducted from October to December 2019, in full sun in the experimental area belonging to the Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Redenção, CE, Brazil. The experimental design was entirely randomized in a 4 × 2 factorial scheme, referring to four irrigation strategies: E1 = irrigation with low-salinity water (0.3 dS m^{-1}) at germination, initial growth, and preflowering stages; E2 = saline water (3.2 dS m^{-1}) at preflowering only; E3 = saline water (3.2 dS m^{-1}) on germination and initial growth; E4 = saline water (3.2 dS m^{-1}) in the three phases, and two doses of phosphate fertilization (P1 = 50% of the recommended phosphorus dose and P2 = 100% of the recommended phosphorus dose), with four repetitions. Continuous salt stress in phenological phases negatively affects plant height, photosynthesis, transpiration, stomatal conductance, and leaf temperature of the cowpea crop. The dose of 100% phosphate fertilization mitigates the effects of continuous salt stress on chlorophyll pigment production.

Key words: fertilization management; salt stress; *Vigna unguiculata* L. Walp.

Estratégias de irrigação com água salina e adubação fosfatada na cultura do feijão caupi

RESUMO: O uso de água salina afeta negativamente as trocas gasosas e o crescimento das plantas. No entanto, a adubação com fonte mineral vem sendo utilizada para atenuar o estresse salino sobre as culturas agrícolas. O objetivo deste estudo foi avaliar as trocas gasosas e o crescimento inicial do feijão caupi submetido diferentes estratégias de irrigação com água salina e adubação fosfatada. O experimento foi conduzido de outubro a dezembro de 2019, a pleno sol na área experimental pertencente a Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Redenção, CE, Brasil. O delineamento experimental foi inteiramente casualizado em esquema fatorial 4 × 2, referentes a quatro estratégias de irrigação: E1 = irrigação com água de baixa salinidade (0,3 dS m^{-1}) nas fases de germinação, crescimento inicial e pré-floração; E2 = água salina (3,2 dS m^{-1}) apenas na pré-floração; E3 = água salina (3,2 dS m^{-1}) na germinação e crescimento inicial; E4 = água salina (3,2 dS m^{-1}) nas três fases, e duas doses de adubação fosfatada (P1 = 50% da dose recomendada de fósforo e P2 = 100% da dose recomendada de fósforo), com quatro repetições. O estresse salino contínuo nas fases fenológicas afeta negativamente a altura de planta, fotossíntese, transpiração, condutância estomática e a temperatura foliar da cultura do feijão caupi. A dose de 100% da adubação fosfatada mitiga os efeitos do estresse salino contínuo na produção do pigmento clorofila.

Palavras-chave: manejo da adubação; estresse salino; *Vigna unguiculata* L. Walp.

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Introduction

The cowpea bean (*Vigna unguiculata* L. Walp) is a major food and production source in the Northeast region of Brazil, as well as for other tropical and subtropical regions of the world, presenting itself as an important protein source of great social and economic importance, being adapted to the semi-arid regions of northeastern Brazil, with grain production in dry and irrigated crops (Prazeres et al., 2015; Oliveira et al., 2018).

The practice of irrigation allows agricultural production throughout the year, however, in many irrigated areas around the world as in northeastern Brazil, the supply of good quality water may not be sufficient to maintain irrigated agriculture, and lower quality waters are used in periods of scarcity, such as brackish waters (Carvalho et al., 2020; Silva et al., 2022).

Salt stress can restrict water and mineral nutrient uptake by plants, affecting metabolism, cell expansion, and photoassimilate production, which result in lower growth and decreased productivity of agricultural crops (Guilherme et al., 2021; Sousa et al., 2021). Living with this problem in semiarid regions presupposes the search for tolerant genotypes and the use of management strategies that reduce the impacts on plants and the environment (Goes et al., 2021).

Studies on the interaction between salinity and phosphate fertilization have been carried out in some crops, on the assumption that increasing phosphate fertilization minimizes the effects of salinity. Phosphorus acts in the transfer of energy in the cell in the form of adenosine triphosphate (ATP) and participates in several processes, such as respiration and photosynthesis (Taiz et al., 2017). In cowpea crop, Sá et al. (2018) found higher net photosynthesis rates and Lima et. al. (2017) obtained higher initial growth under phosphate fertilization in saline environment. However, there are no studies relating phosphate fertilization and irrigation with saline water at different stages of bean crop development.

In this context, the present study aimed to evaluate the gas exchange and the initial growth of cowpea bean submitted to different irrigation strategies with saline water and phosphate fertilization.

Materials and Methods

The experiment was conducted from October to December 2019 in full sun in the experimental area belonging to the Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Redenção, CE, Brazil, with coordinates of latitude 4° 13' 33" and longitude 38° 43' 39", being evaluated only one cycle. The region climate is of type Aw', characterized as rainy tropical, very hot, with predominant rainfall in the summer and fall seasons.

The meteorological data obtained during the survey can be seen in Figure 1.

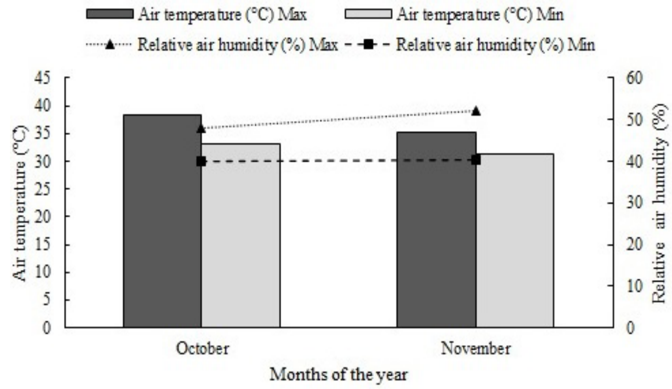


Figure 1. Meteorological data during the experimental conduction.

The experimental design was entirely randomized in a 4 × 2 factorial scheme, referring to four irrigation strategies with saline water until the pre-flowering stage and two doses of phosphorus (50% and 100% of the recommended dose for cowpea), with four repetitions.

The four strategies of were thus defined for the germination (0 to 10 days after sowing (DAS), initial growth (10 to 22 DAS), and pre-flowering (23 to 42 DAS) phases: E1 = irrigation with low salinity water (0.3 dS m^{-1}) in all three phases; E2 = irrigation with saline water (3.2 dS m^{-1}) only in preflowering; E3 = irrigation with saline water (3.2 dS m^{-1}) in the germination and initial growth phases; and, E4 = irrigation with high salinity water (3.2 dS m^{-1}) in all three phases.

The irrigation water was prepared by diluting the salts NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$, in the equivalent proportion of 7:2:1 between Na, Ca, and Mg, obeying the relation between ECw and its concentration ($mmol_c$ L^{-1} = EC × 10), according to the methodology contained in Rhoades et al. (2000). Irrigation with saline water was started according to the treatments, that is, based on the phenology of the crop, with a leaching blade of 15% according to Ayers & Westcot (1999), making use of a daily frequency, calculated according to the drainage lysimeter principle (Bernardo et al., 2019), in order to maintain the soil at field capacity.

The volume of water to be applied to the plants was determined according to Equation 1.

$$\mathrm{VI} = \frac{(\mathrm{Vp} - \mathrm{Vd})}{(1 - \mathrm{LF})} \tag{1}$$

where: VI - volume of water to be applied in irrigation (mL); Vp - volume of water applied in the previous irrigation (mL); Vd - volume of drained water (mL); and, LF - leaching fraction of 0.15.

We used cowpea seeds (*Vigna unguiculata* L. Walp) cv. Vita 7, which were grown in 8 L pots filled with Yellow Red Argissolo (Embrapa, 2018), which presented the following chemical attributes according to Table 1.

Sowing was done with five seeds per pot and after the establishment of the plants, seven days after sowing, manual thinning was done, leaving the two most vigorous plants in each pot. The use of irrigation strategies started on the day of sowing, alternating the irrigation waters according to the treatment used and the phenological phases of the cowpea bean.

Table 1. Chemical characteristics of the substrate used.

OM (g kg^{-1})	P (mg kg^{-1})	Mg	K	Ca	Na	H^++Al^{3+}	pH	ECse
		(cmol$_c$ d^{-3})					H_2O	(dS m^{-1})
4	2	0.3	0.06	2.5	0.57	0.33	7.6	0.37

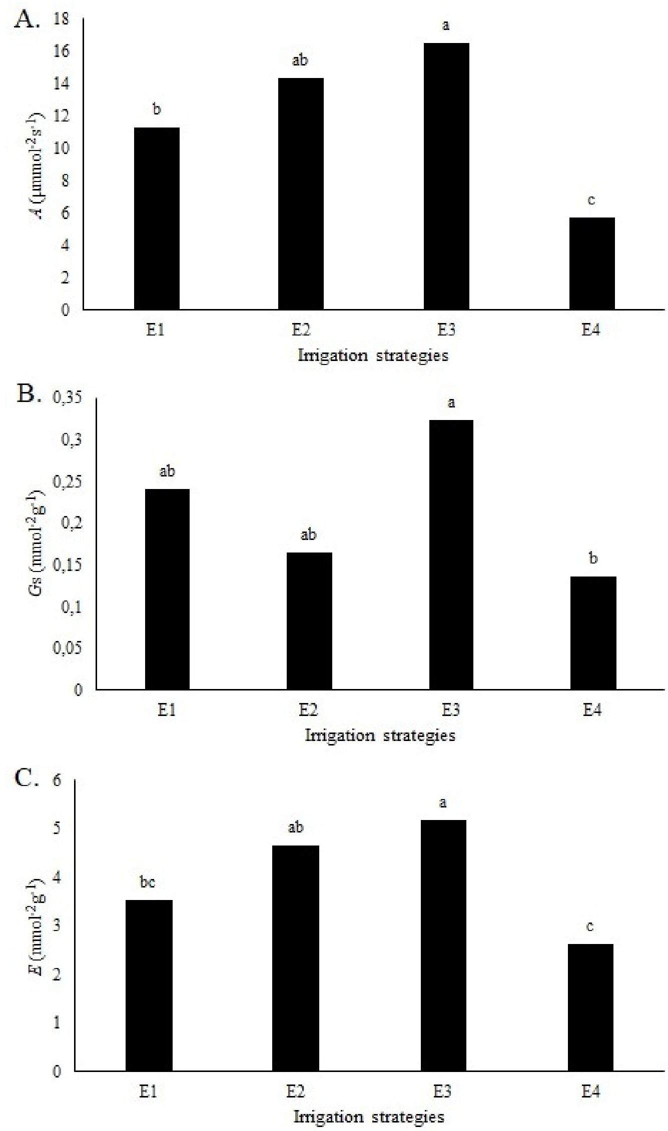
OM = organic matter; ECse = electrical conductivity of the saturation extract.

Mineral fertilization followed the fertilization proposal for the cowpea crop (60 kg ha^{-1} of P_2O_5, 30 kg ha^{-1} of K_2O, and 30 kg ha^{-1} of N for one cycle of the crop) following the recommendation of Melo et al. (2017). Phosphorus fertilization was done with simple superphosphate fertilizer, using 6 g for the treatment with 100% and 3 g for 50% of the recommended dose, respectively, of P in each pot.

At 45 days after sowing (DAS), net CO_2 assimilation rate (A - µmol CO_2 m^{-2} s^{-1}), transpiration (E - mol H_2O m^2 s^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}) internal CO_2 concentration (C_i - µmol CO_2 m^{-2} s^{-1}), and leaf temperature (LT) were evaluated in fully expanded leaves, using an infrared gas analyzer (IRGA, LI-6400XT, LI-COR, Inc, Lincoln, Nebraska, USA) equipped with an artificial radiation source with intensity set to 2,000 µmol m^{-1} s^{-1}. The measurements were taken between 9:00 and 11:00 am. Then, relative chlorophyll index (RCI) measurements were performed on the same leaves, using a non-destructive method with a portable meter (SPAD - 502 Plus, Minolta, Japan).

Then, the following growth assessments were performed: plant height (PH) measured with a ruler graduated in centimeters; stem diameter (SD) measured with a digital caliper (WORKER, model 940534) at the base of the stem; leaf number (LN) by direct counting of whole leaves; and, leaf area (LA) was estimated using an image digitizer (scanner).

The variables studied and measured during the study were analyzed by the Kolmogorov-Smirnov test ($p \leq 0.05$) to assess normality. The data were submitted to analysis of variance (ANOVA) by the F test. When significant the data regarding saline water use strategies and phosphate fertilization were subjected to Tukey test at 0.05 significance using the statistical program ASSISTAT 7.7 (Silva & Azevedo, 2016).



Averages followed by the same letter do not differ by Tukey test at 5% significance level.

Figure 2. Photosynthesis (A), stomatal conductance (B), and transpiration (C) of cowpea plants under different irrigation strategies with high and low salinity water.

Results and Discussion

The highest average values for net CO_2 assimilation rate (A) were observed in E2 and E3 (Figure 2A). The best result was obtained by treatment E3 (good quality water only in pre-flowering), which differed statistically from treatments E1 and E4, with lower photosynthetic rates. This result shows that the application of low salinity water in the pre-flowering stage results in the dilution of salts in the soil and better physiological performance of the plants, as well as that the presence of salts in the irrigation water in the germination and initial growth stages contributed to a higher photosynthetic activity in bean plants, considering that treatment E1, which does not use lower salinity water, had a lower photosynthetic rate compared to E3.

Concordant results were found by Oliveira et al. (2017) when studying gas exchange in cowpea plants irrigated with saline water continuously, where they detected a reduction in photosynthesis rates with increasing soil saturation extract electrical conductivity. Prazeres et al. (2015) when evaluating the physiological responses of two cowpea cultivars irrigated with saline water, observed the reduction of photosynthesis rate with the use of high salinity water until 55 DAS.

On the other hand, continuous salt stress (E4) provided lower stomatal conductance (Figure 2B), indicating the occurrence of higher accumulation of soil salts and greater stomatal limitation to the carbon assimilation process (Taiz et al., 2017). Salt stress contributes directly to the reduction of leaf area, by its osmotic, nutritional and toxic effects. Once the leaf area is reduced, there is a reduction in the photosynthesizing area and consequently in stomatal conductance (Rodrigues et al., 2021).

Salt stress provides stomata closure and consequently reduced transpiration to prevent water loss to the medium (Pereira Filho et al., 2019). Negative effects of salinity were observed by Oliveira et al. (2017), where they found that increasing irrigation water salinity reduced stomatal conductance values in the cowpea crop.

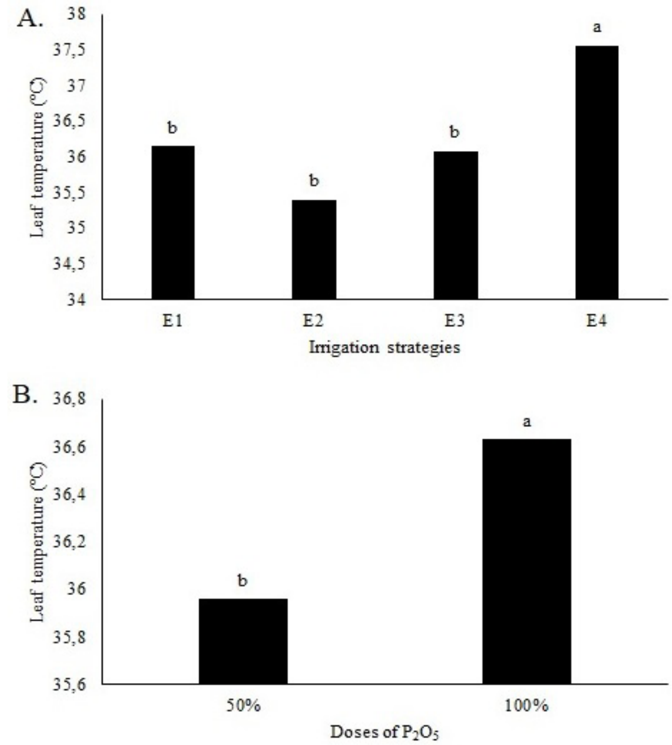
Similarly, treatment E4 (use of saline water in all three phases) reduced transpiration of cowpea plants (Figure 2C). Reduced transpiration is a strategy in which the plant performs in response to water loss, as uptake is impaired by the accumulation of salts in the soil (Andrade et al., 2018). Stress caused by excess ions generally decreases CO_2 assimilation, stomatal conductance, and plant transpiration (Taiz et al., 2017).

The results obtained in this study corroborate the information from Rodrigues et al. (2021) that salt stress reduces the amount of transpired water, and may contribute to a reduction in the absorption and loading of toxic Na^+ and Cl^- ions into the plant. Sá et al. (2018) observed a linear reduction in transpiration in bean plants as a function of increasing irrigation water salinity. Working with cowpea crop under salt stress in protected environment conditions, Prazeres et al. (2015) found reductions in transpiration.

The highest leaf temperature values were obtained using high salinity water until pre-flowering (E4), as shown in Figure 3A. Salt stress leads to lower water uptake by the plant, which tends to close its stomata, preventing water loss to the environment and reducing the latent heat dissipation associated with water vaporization in the leaf tissues.

Thus, the boundary layer around the leaf from transpiration is affected, leading to increased leaf temperature (Taiz et al., 2017). Similar results to this study were described by Sousa et al. (2021), when they found that salt stress elevated the leaf temperature of peanut plants.

The 100% dose of P recommendation was statistically superior to the 50% dose for leaf temperature in the bean crop (Figure 3B). This result may be associated with the adsorption of the applied P by the soil colloids, which prevented the total absorption of the doses. P is an element that is poorly available to plants in the soil and this fact, associated with the



Averages followed by the same letter do not differ by Tukey test at 5% significance level.

Figure 3. Leaf temperature in cowpea plants at 45 DAS under different irrigation strategies (A) and phosphate doses (B), under different irrigation strategies with high and low salinity water.

high adsorption characteristics of this element by soils, means that the efficiency of phosphate fertilizers is low, with 10 to 20% of the nutrient being made available to crops in the year of application (Vieira et al., 2021).

Diverging from this study, Sousa et al. (2012) when studying the physiological responses in jatropha submitted to phosphate fertilization, observed no significant effect of phosphate fertilization on leaf temperature.

According to Table 2, only in E2 (saline water only at pre-flowering) the phosphate fertilization with 50% of the recommended dose was higher than the 100% dose significantly for internal CO_2 concentration. There was no statistical difference between the irrigation strategies. The bean plants expressed higher C_i with the presence of phosphate fertilization even under salt stress, that is, in this

Table 2. Internal CO_2 concentration, chlorophyll of cowpea bean at 45 DAS under different irrigation strategies with high and low salinity water and phosphate doses.

Treatments	C_i (µmol (CO_2) m^{-2} s^{-1})		Chlorophyll (µg cm^{-2})	
	Phosphate fertilization (% of recommended dose)			
	50%	100%	50%	100%
E1	216.33 aA	246.66 aA	73.95 aA	65.30 abB
E2	259.33 aA	225.33 aB	63.85 bA	62.30 bA
E3	260 aA	246 aA	71.45 aA	65.95 abB
E4	249.33 aA	228.66 aA	61.35 bB	72.10 aA
	MG = 241	CV (%) = 7.78	MG = 67.03	CV (%) = 4.68

Averages followed by the same lower case letter in the column and capital in the row do not differ by Tukey test at 5% significance. (Internal CO_2 concentration - Dms row = 32.52 and Dms column = 43.90; Chlorophyll - Dms row = 5.43 and Dms column = 7.33).

phenological phase the phosphorus uptake was not impaired by salt stress.

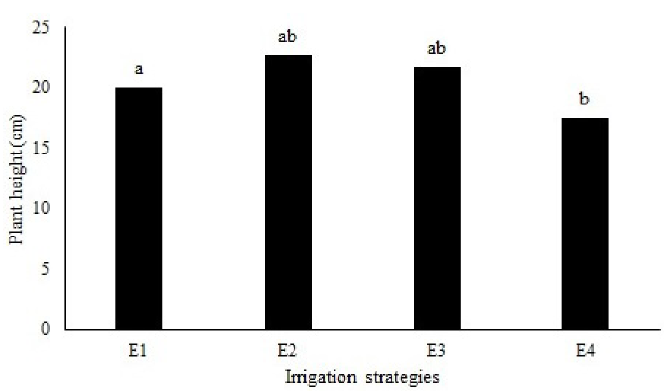
Values considered high in C_i inside the leaves indicate that CO_2 is not being used for the synthesis of sugars by the photosynthetic process, with accumulation of this gas, indicating that some non-stomatic factor is interfering in this process (Sousa et al., 2021). The data found in this study are opposite to that of Sá et al. (2018) that evaluating the interaction between salinity of irrigation water and phosphate fertilization in the culture of cowpea, did not observe significant responses of the interaction of the aforementioned factors on the internal concentration of CO_2.

For the relative chlorophyll content, the 50% P dose was statistically superior in treatments E1 (irrigation with low salinity water in the three phases) and E3 (irrigation with high salinity water in the germination and initial growth phases), while in E4 (irrigation with high salinity water in the three phases) it was the 100% P dose that resulted in the highest value (Table 2).

This result shows that the 100% recommended dose of P performed best and mitigated the salt stress when associated with continuous irrigation with saline water (E4). It is pointed out that higher P availability in the early stages provides better potential for growth, development, ATP production and photosynthetic pigments for later stages such as flowering and fruiting (Taiz et al., 2017).

The irrigation strategy that used saline water only at flowering (E2) showed the lowest chlorophyll contents at both 100% and 50% P. The reduction in chlorophyll pigment production was impaired by salt stress in the most sensitive phase of the crop. Similar results to this study, were recorded by Pereira Filho et al. (2019) when they concluded that the stimulation of chlorophyllase, an enzyme that degrades chlorophyll, with the accumulation of salts from irrigation water, reduced this pigment in the bean crop subjected to salt stress.

The irrigation strategies presented in Figure 4 reveal that treatment E1 differed statistically from E4 in the plant height variable, obtaining the highest values. This result reflects the continuous use of saline water applied until the pre-flowering stage in the culture of cowpea cv. Vita 07.



Averages followed by the same letter do not differ by Tukey test at 5% significance level.

Figure 4. Height of cowpea plants under different irrigation strategies with high and low salinity water.

The accumulation of salts in the root zone, generated by the use of saline water reduced water uptake by the osmotic effect, impairing mitotic processes of multiplication and cell expansion, reducing plant height (Sá et al., 2018). Studies describing a similar trend of reduction in plant height of cowpea irrigated with high salinity water were also reported by Lima et al. (2017).

According to the stem diameter data presented in Table 3, only in S2 the phosphate fertilization with 100% of the recommended dose in association with the use of saline water in preflowering was higher than the 50% dose in the same treatment. This result may be related to the increased uptake of this nutrient by the roots due to the use of low salinity irrigation water during the phenological phases of germination and initial growth of the bean plant. With the use of low-salinity water in these phases, the bean plants effectively absorbed the 100% dose of the recommendation, which, being higher than the 50% dose, contributed to a greater stem expansion. It is also pointed out that phosphorus deficiency in plants leads to reduced growth in young plants and the production of slender stems (Taiz et al., 2017).

Similar results were observed by Sá et al. (2018) when they recorded that the use of saline water until 30 DAS, negatively reduced the stem diameter of the cowpea crop. Silva et al. (2022) when analyzing the stem diameter of peanut under salt stress and mineral fertilization with a phosphate source, also recorded a reduction similar to that of this study.

Table 3. Stem diameter of bean cv. Vita 7 as a function of irrigation strategies and doses of phosphorus at 45 DAS.

Treatments	Stem diameter (mm)	
	Phosphate fertilization (% of recommended dose)	
	50%	100%
E1	6.08 aA	5.38 bA
E2	5.30 bB	6.58 aA
E3	5.60 aA	6.32 abA
E4	5.48aA	5.04 bA
	MG = 5.72	CV (%) = 12.04

Averages followed by the same lower case letter in the column and capital in the row do not differ by Tukey test at 5% significance (Dms row = 0.88 and Dms column = 1.17).

Conclusions

Continuous salt stress in phenological phases negatively affects plant height, photosynthesis, transpiration, stomatal conductance, and leaf temperature of the cowpea crop.

The dose of 100% phosphate fertilization mitigates the effects of continuous salt stress on chlorophyll pigment production.

The irrigation strategy with the use of saline water only in the pre-flowering period associated with a dose of 100% phosphate fertilization was more efficient in increasing stem diameter, while for a higher internal CO_2 concentration it was the phosphate dose of 50% that stood out the most.

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Compliance with Ethical Standards

Author contributions: Conceptualization: RMRR, GGS; Data curation: RMRR, ASB, MHCF; Formal analysis: RMRR, GGS, ASB, MHCF; Funding acquisition: GGS; Investigation: RMRR; Methodology: RMRR, GGS; Project administration: RMRR; Resources: CFL, JGLM; Supervision: GGS; Validation: GGS, CFL, JGLM; Writing – original draft: RMRR, ASB, MHCF; Writing – review & editing: CFL, JGLM.

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