

Growth and gas exchange in cowpea plants under different managements and saline conditions¹

Crescimento e trocas gasosas em plantas de feijão-caupi sob diferentes manejos em condições salinas

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ABSTRACT - The cowpea is one of the main crops of economic and social importance in the northeast of Brazil. However, the growing need to increase crop yield has prompted the use of irrigation water with high levels of salts. The aim of this study therefore, was to evaluate the effects of different irrigation depths and rates of liquid biofertilizer on growth and gas exchange in cowpea plants under saline conditions. The experiment was conducted in a randomized block design, using a scheme of split lots consisting of four irrigation depths with saline water (75, 100, 125 and 150% of crop evapotranspiration - ETc) and sub-lots of four different doses of biofertilizer (0; 5,000; 10,000 and 15,000 L ha⁻¹). At 75 days after sowing (DAS), readings were taken of gas exchange in the plants (photosynthesis, stomatal conductance, internal CO₂ concentration and transpiration), and growth analyses were carried out (plant height, leaf area and total dry matter). An increase in irrigation depth gives an increase in total dry weight and leaf area. Increasing irrigation depth causes a significant increase in gas exchange, with quadratic behavior noted for photosynthesis and stomatal conductance, and linear behavior for internal CO₂ concentration and transpiration. The application of high doses of biofertilizer gives increased stomatal opening of the cowpea leaves.

Key words: *Vigna unguiculata* (L.) Walp. Water stress. Organic matter.

RESUMO - O feijão-caupi é uma das principais culturas de importância econômica e social para o Nordeste do Brasil. Entretanto, a crescente necessidade de aumentar a produção da cultura tem motivado a utilização de águas com elevados teores de sais na irrigação. Desta forma o objetivo deste trabalho foi avaliar os efeitos de diferentes lâminas de irrigação e doses de biofertilizante líquido sobre o crescimento e as trocas gasosas de plantas de feijão-caupi em condições salinas. O experimento foi conduzido no delineamento de blocos casualizados, utilizando o esquema de parcelas subdivididas, sendo as parcelas constituídas por quatro lâminas de irrigação com água salina (75; 100; 125 e 150% da evapotranspiração da cultura - ETc) e nas subparcelas quatro diferentes doses de biofertilizante (0; 5.000; 10.000 e 15.000 L ha⁻¹). Aos 75 dias após a semeadura (DAS) foram realizadas leituras das trocas gasosas das plantas (fotossíntese, condutância estomática, concentração interna de CO₂ e transpiração) e análises de crescimento (altura de planta, área foliar e matéria seca total). O aumento da lâmina de irrigação proporciona incremento na massa seca total e área foliar. Para as trocas gasosas, o aumento da lâmina de irrigação proporciona aumento significativo, sendo verificado comportamento quadrático para a fotossíntese e condutância estomática, e linear para concentração interna de CO₂ e transpiração. A aplicação de elevadas doses de biofertilizante proporciona aumento na abertura estomática das folhas de feijão-caupi.

Palavras-chave: *Vigna unguiculata* (L.) Walp. Estresse hídrico. Matéria orgânica.

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INTRODUCTION

The cowpea [*Vigna unguiculata* (L.) Walp] is one of the main subsistence crops in the north and northeast of Brazil, especially in the semi-arid region of the northeast (ANDRADE JUNIOR *et al.*, 2014). Considered as an excellent source of plant protein, the crop is marketed in the form of dry and mature grains, and therefore has great socioeconomic importance for the population of these regions, and enables the workforce to remain in the country (BASTOS *et al.*, 2012).

In the semi-arid region where long periods without rain are common during the rainy season, irrigation is seen as one of the technologies that contribute most to increases in food production, affording an adequate supply of water to the plants (SOUSA *et al.*, 2013). In some places, the use of water considered to be of low quality, especially having a high salt content, is emerging as a good alternative in irrigation. However, when used unwisely, especially under the conditions of a semi-arid climate (high temperatures and low rainfall), it can cause serious problems with salinization, reducing crop productivity and soil use capacity (RHOADES; KANDIAH; MASHALI, 2000).

The use of irrigation naturally entails incorporating salts into the soil profile, and from the continuous use of irrigation in the absence of leaching and water evaporation, the salts are deposited in the root zone and on the soil surface (CARVALHO *et al.*, 2012). However, soil salinization in agricultural areas around the world has generated concern and prejudiced the yields of various crops. Excess salts can compromise several physiological and biochemical functions in plants, mainly due to the water stress caused to the plants and by reducing nutrient absorption, thus lowering crop productivity (CALVET *et al.*, 2013). Sousa *et al.* (2013), evaluating the effect of saline water in the cowpea, saw a reduction in plant growth for an increase in the electrical conductivity of the irrigation water.

In saline environments, the application of irrigation depths greater than the needs of the plant efficiently reduces the accumulation of salts in the soil, as the applied water fraction percolates below the root zone, leaching a part of the accumulated salts (ASSIS JÚNIOR *et al.*, 2007). Carvalho *et al.* (2012), growing maize irrigated with saline water under different leaching fractions, obtained high values for production components, applying a leaching fraction of 10% and using water of 3.3 dS m⁻¹.

Besides the application of irrigation depths greater than required by the plants, the use of biofertilizer is another management strategy that has recently been studied in plants grown in a saline environment. When applied via the soil and in liquid form, it releases humic substances into the soil, promoting the flocculation and formation of

aggregates, and improving the speed of water infiltration in the soil and thus the process of drainage and washing of the salts (SÁ *et al.*, 2015). Humic substances further induce an increase in osmotic adjustment in the plants by their accumulation, facilitating the absorption of water and nutrients in adverse saline environments (SOUTO *et al.*, 2013).

The aim of this work therefore, was to evaluate the effects of different irrigation depths and doses of liquid biofertilizer on growth and gas exchange in cowpea plants under saline conditions.

MATERIAL AND METHODS

The experiment was carried out from October to December 2015, in an experimental area of the Federal Institute for Education, Science and Technology of Ceará, in the town of Umirim, in the State of Ceará, Brazil, located between 3°41'09" S and 39°20'28" W, at an altitude of 61 m.

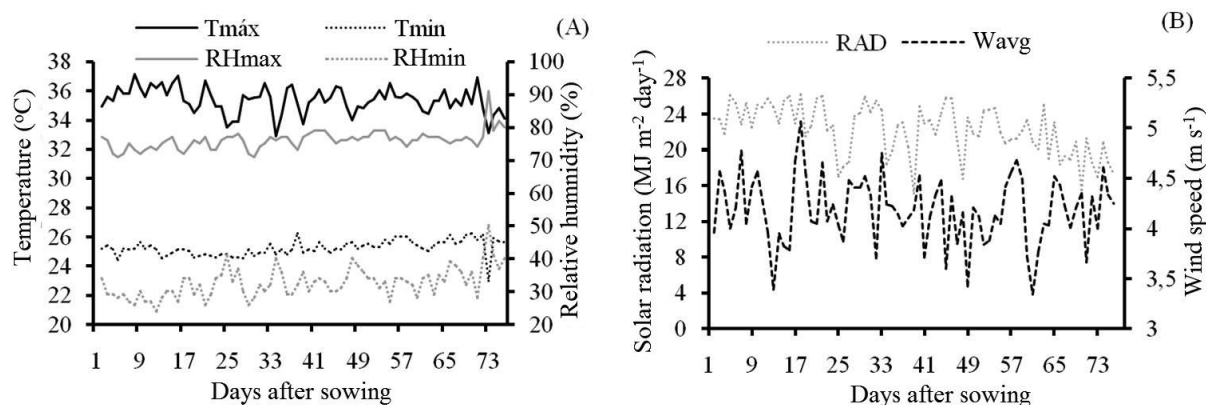
The climate in the region is classified as BSh, corresponding to a semi-arid climate with irregular rainfall, presenting two well-defined climatic seasons (ALVARES *et al.*, 2014). Average annual rainfall at the locality is 857 mm, and the average annual temperature is from 26 to 28°C (FUNCEME, 2015). Meteorological data relative to the period of the experiment were obtained from an automatic weather station of the National Institute of Meteorology, at Itapipoca, located near the locality. The data are shown in Figure 1.

The soil in the area is classified as a dystrophic Red-Yellow Argisol (EMBRAPA, 2013) of a clayey-sandy loam texture, presenting the following characteristics in the arable 0 to 20 cm layer: 238 g kg⁻¹ clay, 538 g kg⁻¹ sand and 224 g kg⁻¹ silt, pH (H₂O) 5.6, 1 mg dm⁻³ P (Mehlich1), 3.6 mmol_c dm⁻³ K, 18 mmol_c dm⁻³ Ca²⁺, 12 mmol_c dm⁻³ Mg²⁺, 39.6 mmol_c dm⁻³ H+Al and 16.96 g dm⁻³ organic matter. The soil was prepared by plowing followed by two cross harrowings.

The experimental design was of randomized blocks in a scheme of split lots, with 16 treatments and four replications, giving a total of 64 experimental plots. The lots consisted of four different irrigation depths (75, 100, 125 and 150% of crop evapotranspiration - ET_c). The sub-lots were represented by different doses of liquid cattle biofertilizer applied to the soil (0, 60, 120 and 180 mL plant⁻¹), at values equivalent to 0; 5,000; 10,000 and 15,000 L ha⁻¹.

The plots were of 40 m² (2.0 x 20.0 m); for application of the different doses of biofertilizer, they were divided into 10 m² sub-plots (2.0 x 5.0 m)

Figure 1 - (A) - Maximum temperature (Tmax); Minimum temperature (Tmin); Maximum relative humidity (RHmax) and minimum relative humidity (RHmin); (B) - Solar radiation (RAD) and average wind speed (Wavg), measured during the experiment (10/07/2015 to 12/21/2015)



containing 5 rows spaced 0.4 m apart. The three central rows were considered as the working area of each plot, with one meter from each end being discarded.

Irrigation of the crop was localized using a drip system. The drippers were self-compensating, with a flow of 1.8 L h⁻¹ at a pressure of 98 kPa, and emitters spaced 0.3 m apart. The water used for irrigation came from a deep well; its physicochemical characteristics can be found in Table 1.

After sowing, the experimental area was irrigated with a depth equivalent to 100% of crop evapotranspiration up to 8 DAS. After this period, the irrigation depths were applied with reference to each treatment. In order to control the volume of water applied in each treatment, a meter was installed at the beginning of each lateral irrigation line.

The meteorological data were used to calculate the daily reference evapotranspiration (ET_o), according to the Penman Monteith methodology, parameterized in FAO Bulletin 56 (ALLEN *et al.*, 1998), Equation 1:

$$ET_o = \frac{0,408(R_n - G) + \gamma \frac{900}{t_m + 273} u_2 (e_s - e_a)}{\Delta + \gamma (1 + 0,34u_2)} \quad (1)$$

where: *R_n* - balance of radiation on the surface of the reference crop (MJ m⁻² d⁻¹); *G* - soil heat flow (MJ m⁻² d⁻¹);

γ - psychrometric constant (kPa °C⁻¹); *t_m* - air temperature (°C); *u₂* - wind speed (m s⁻¹); (*e_s* - *e_a*) - water vapor pressure deficit (kPa); *Δ* - slope of the water vapor saturation pressure curve (kPa °C⁻¹).

Crop evapotranspiration (ET_c) was obtained with formula (2), by Allen *et al.* (1998):

$$ET_c = K_c \times ET_o \quad (2)$$

where: *ET_c* - crop evapotranspiration (mm day⁻¹); *ET_o* - reference evapotranspiration (mm day⁻¹); *K_c* - crop coefficient (dimensionless).

The biofertilizer was obtained by aerobic fermentation, using a container with a capacity for 1,000 liters and adding fresh cattle manure and non-saline water at a ratio of 1:1 (PENTEADO, 2007). During the preparation period, more specifically at 30 days, homogenization was carried out by agitation to improve fermentation efficiency.

After the anaerobic fermentation process and stabilization of the product, a sample of the biofertilizer was collected, diluted in water at a ratio of 1:1 and analyzed for its physicochemical properties (Table 2).

The biofertilizer was diluted in water to a concentration of 50% for application. The application

Table 1 - Physicochemical analysis of the irrigation water

pH	EC	Cations (mmol L ⁻¹)				Anions (mmol L ⁻¹)				SAR	Classification
	dSm ⁻¹	Ca	Mg	Na	K	Cl ⁻	SO ₄ ²⁻	HCO ₃ ⁻	CO ₃ ²⁻		
6.4	3.81	12.6	17.2	6.4	2.1	35.0	-	3.6	-	1.65	C4S1

C₄ - Water salinity very high. Unsuitable for ordinary irrigation. If however, crops with a very high salt tolerance are grown on highly permeable and well-drained soils, when an excess of irrigation water is used in order to provide copious leaching, its use should be taken into account; S₁ - Water with a low sodium content. Can be used for irrigation in almost all types of soil. However, certain crops that are highly sensitive to sodium may be affected

Table 2 - Physicochemical attributes of the liquid cattle biofertilizer

Variable	Biofertilizer	Unit
pH	7.1	-
Electrical conductivity	8.41	dS m ⁻¹
Organic matter	207.2	g L ⁻¹
N	1.5	g L ⁻¹
P	0.3	g L ⁻¹
P ₂ O ₅	0.7	g L ⁻¹
K	2.6	g L ⁻¹
K ₂ O	3.2	g L ⁻¹
Ca	1.0	g L ⁻¹
Mg	0.9	g L ⁻¹
S	Absent	g L ⁻¹
C/N	80.1	-
Fe	238.8	mg L ⁻¹
Cu	35.6	mg L ⁻¹
Zn	45.5	mg L ⁻¹
Mn	23.7	mg L ⁻¹

of each dose of biofertilizer was divided into three, the same volume being applied for each. The first application of biofertilizer was made at 10 DAS, the second at 40 DAS and the third at 70 DAS, distributed over the soil in the projected area of the plant canopy.

In order to evaluate growth, plants within the working area of each experimental plot were collected at 75 DAS and taken to the Chemistry Laboratory on the Umirim Campus of IFCE, where the following were analyzed: main stem length (MSL), determined by measuring the stem with the aid of a graduated tape; total dry matter (TDM), by drying the shoots of the plant in a closed air circulation oven at a temperature of 65°C to constant weight, the weight later being determined with the aid of a semi-analytic balance; and leaf area (LA), each foliole of the plant was separated and the length and width measured. After this procedure, the values obtained were applied to Equation 3, obtained by Lima *et al.* (2008):

$$AF = \sum [0,9915 \times (C \times L)^{0,9134}] \quad (3)$$

where: LA = leaf area (cm²); \sum = summation; L = length (cm) e W = width (cm).

To determine net CO₂ assimilation (A), stomatal conductance (g_s), transpiration (E) and internal CO₂ concentration (C_i), a model LI-6400XT IRGA infrared gas analyzer from LI-COR, Inc (USA) was used. Data were collected from the central foliole of the third leaf counting

from the apex of the plant, between 8 am and 11 am, with an artificial light source, under saturating radiation and ambient conditions of temperature and CO₂ concentration.

The results were submitted to analysis of variance, applying the F-test at 5% significance. Regression analysis of the irrigation depths and doses of biofertilizer was performed. The SISVAR 5.6 software was used for all the analyses.

RESULTS AND DISCUSSION

The interaction between the factors, irrigation depth and dose of biofertilizer, had a statistically significant effect (p > 0.05) for the variable, leaf-area. Main stem length and total dry weight individually responded significantly to irrigation depth. The doses of biofertilizer did not show a significant effect for any of the variables under analysis (Table 3).

For main stem length (Figure 2), it can be seen that the regression equation significantly fit (p > 0.05) the quadratic model under the effect of the different irrigation depths, showing the estimated maximum point for a depth of 112% of ETc (corresponding to 42.5 cm), 7.2% higher than the lowest estimated value.

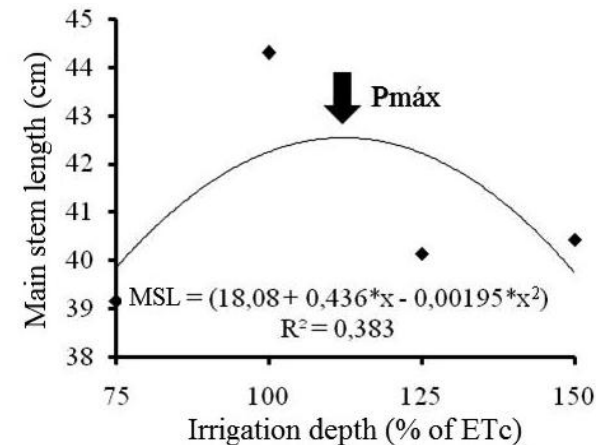
According to Mesquita *et al.* (2013), the excessive application of water can promote greater leaching of nutrients essential to growth, causing physiological disturbances and a consequent reduction in plant growth, in addition to saturating the soil and causing less oxygenation of the root system.

Table 3 - Summary of analysis of variance for main stem length, leaf area and total dry matter obtained from cowpea plants irrigated with different depths of saline water and doses of biofertilizer

S.V.	D.F.	Mean square		
		MSL	LA	TDM
Block	3	972.2**	263473 ^{ns}	103.36*
Depth (L)	3	82.17*	1270931*	141.99*
Residual (L)	9	17.72	293164	25.69
Biofertilizer (B)	3	122.3 ^{ns}	449896 ^{ns}	10.5 ^{ns}
L x B	9	71.21 ^{ns}	491175*	8.23 ^{ns}
Residual (B)	36	48.74	174446	29.65
CV% (L)	-	10.26	18.53	19.74
CV% (B)	-	17.02	17.17	21.21

S.V. - Source of Variation; C.V. - Coefficient of Variation; D.F. - Degrees of Freedom; MSL - Main stem length (cm); LA - Leaf area (cm²); TDM - Total dry matter (g); *Significant by F-test at 5% probability; **Significant by F-test at 1% probability; ns = not significant

Figure 2 - Main stem length (MSL) in cowpea plants for the application of different irrigation depths



* significant at 5% probability by F-test. Pmax = maximum point

For the leaf area, it was seen that without the use of biofertilizer (0 mL plant⁻¹) and at the dose of 60 mL plant⁻¹ there was a significant mathematical fit ($p > 0.01$) to the linear model, showing an increase in leaf area of the order of 59.7 and 45.7% respectively. These values were obtained between the minimum (75% of ETc) and maximum (150% of ETc) depths applied (Figure 3A).

According to Taiz and Zeiger (2013), when plants are exposed to water stress, there is a decrease in photosynthetic rate, mainly due to a reduction in stomatal opening and consequently the entry of CO₂, thereby reducing the production of photoassimilates and the leaf area of the plants. It should also be noted that the use of saline water for irrigation, promotes the accumulation of salts in the soil profile; however, when a volume of

water greater than that recommended is applied, there is a reduction in salt accumulation in the surface layer, resulting in a better distribution of salts in the profile (ASSIS JÚNIOR *et al.*, 2007).

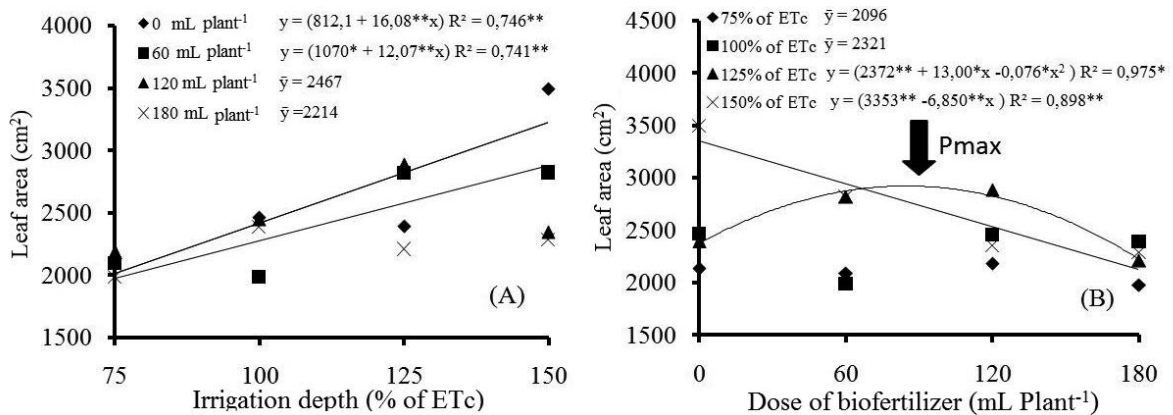
For the different irrigation depths within the doses of biofertilizer (Figure 3B), a significant effect can be seen in the plants for depths of 125% and 150% of ETc. For the irrigation depth of 125% of ETc, there is a significant effect ($p > 0.05$), fitting the regression equation to the quadratic model and showing maximum point at an estimated dose of 85.5 mL plant⁻¹, equivalent to a leaf area of 2,927.9 cm², followed by a decrease of 23.2% up to the maximum dose applied. For the depth of 150% of ETc, it can be seen that the equation was significant ($p > 0.01$) and fit the linear model, presenting a leaf area of 3,353 cm² without the use of biofertilizer, and of 2,120 cm² at the dose of 180 ML plant⁻¹, a reduction of 36.77%.

The reduction in leaf area with the increase in doses of biofertilizer may have resulted from the phytotoxic effect of the high EC of the biofertilizer (8.41 dS m⁻¹); when the plant is exposed to an excess of salts, low osmotic adjustment capacity occurs in the plant, which leads to low water absorption and a consequent reduction in leaf growth.

Andrade Júnior *et al.* (2014), evaluating different irrigation depths in cowpea cultivars, found a drop in the leaf area of plants exposed to excess water. Under conditions of excess water, the carbohydrate assimilation efficiency is lower, as under such conditions, plants usually display a smaller number of leaves due to the process of foliar senescence starting early.

Evaluating different doses of aerobic biofertilizer on the leaf area of cowpea plants, Silva *et al.* (2013) found lower values when the plants were submitted to high doses

Figure 3 - Breakdown of the interaction of irrigation depth and saline water vs doses of biofertilizer on the leaf area of cowpea plants in Umirim



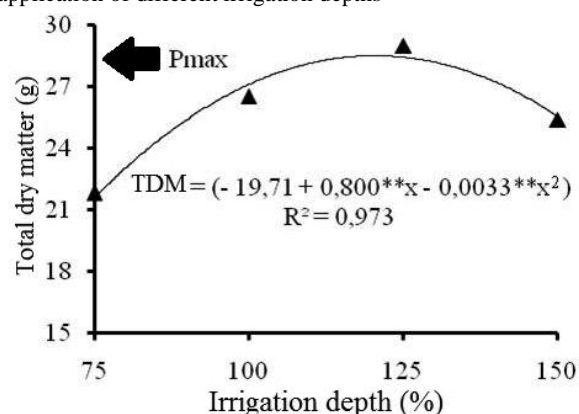
**, * significant by F-test respectively at 1% and 5% probability. Pmax = maximum point

of the organic product. Biofertilizer is an organic fertilizer rich in macro- and micronutrients, originating from the fermentation of organic matter, and acting nutritionally on the plant metabolism. However, although biofertilizer is usually used as a source of nutrients, some crops may display phytotoxicity to the product due to nutritional imbalance, which depends on its chemical composition, rate of mineralization and nitrogen content, or especially on the high salt concentration of some formulations (GONÇALVES; SCHLEDECK; SCHWENGBER, 2009).

In relation to total dry matter (Figure 4), according to the regression analysis the data fitted the quadratic model, showing a high coefficient of determination ($R^2 = 97\%$), where an increase in irrigation depth resulted in increases in the value for TDM, with 21.72 g for the smallest depth applied (75% of ETc), and reaching the estimated maximum value of 28.77 g per plant when submitted to an irrigation depth of 121.2% of ETc, a difference of 32.4%.

Similar results were obtained by Silva (2014), who found that an increase in applied irrigation depth gave higher values for shoot dry matter in cowpea plants, fitting the quadratic model. The reduction in total dry matter under conditions of water deficit and water surplus may be associated with two factors: first, a reduction in transpiration rate, due to a decrease in stomatal opening, or even the paralysis of photosynthesis, and a reduction in biomass production (CHAVARRIA; SANTOS, 2012); and second, the application of greater volumes of saline water, which may have led to salt accumulation in the soil, reducing its osmotic potential and inhibiting the absorption of water and nutrients by the plant,

Figure 4 - Total dry matter (TDM) in cowpea plants for the application of different irrigation depths



** significant by F-test at 1% and 5% probability; Pmax = maximum point

and consequently the production of photoassimilates (CAVALCANTE *et al.*, 2010).

According to the analysis of variance shown in Table 4, there was no significant interaction between factors for any of the analyzed gas exchange variables. For net photosynthesis (A), internal CO_2 concentration (C_i) and transpiration (E), there was a significant effect only from irrigation depth. This was different from stomatal conductance (g_s), that also showed a significant effect for the doses of biofertilizer.

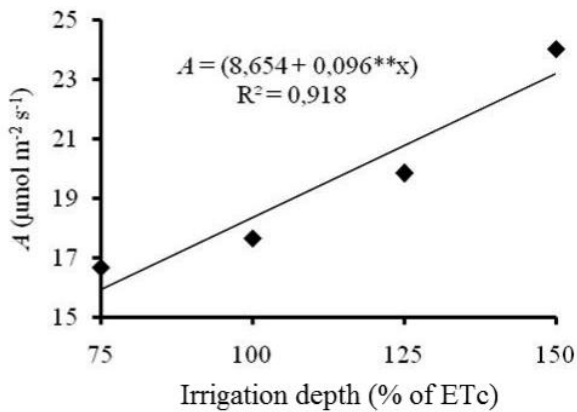
It can be seen that for A (Figure 5) there was a significant effect ($p > 0.05$) for the linear model, with a minimum value of $15.85 \mu\text{mol m}^{-2} \text{s}^{-1}$ seen for the irrigation depth of 75% of ETc, and a maximum value of $23.04 \mu\text{mol m}^{-2} \text{s}^{-1}$, an increase of 45.35%.

Table 4 - Summary of the analysis of variance for gas exchange in cowpea plants irrigated with different depths of saline water and doses of biofertilizer, at 75 days after sowing

S.V.	D.F.	Mean square			
		A	g_s	C_i	E
Block	3	2.18 ^{ns}	0.0004 ^{ns}	143.83 ^{ns}	4.15 ^{ns}
Depth (L)	3	169.94*	0.058*	3340.29**	22.01**
Residual (L)	9	37.01	0.008	434.88	2.21
Biofertilizer (B)	3	30.72 ^{ns}	0.014*	785.24 ^{ns}	4.67 ^{ns}
L x B	9	35.97 ^{ns}	0.009 ^{ns}	814.24 ^{ns}	3.12 ^{ns}
Residual (B)	36	25.78	0.004	482.6	1.68
CV% (L)	-	31.14	42.28	9.68	26.78
CV% (B)	-	25.99	32.53	10.2	23.38

S.V. - Source of Variation; C.V. - Coefficient of Variation; A - Net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$); g_s - Stomatal conductance ($\text{mol de H}_2\text{O m}^{-2} \text{s}^{-1}$); C_i - Internal CO_2 concentration ($\mu\text{mol m}^{-2} \text{s}^{-1}$); E - Transpiration ($\text{mol de H}_2\text{O m}^{-2} \text{s}^{-1}$); D.F. - Degrees of Freedom; *Significant by F-test at 5% probability; **Significant by F-test at 1% probability; ns = not significant

Figure 5 - Photosynthesis (*A*) in cowpea plants for different irrigation depths



** significant by F-test at 1% and 5% probability

Under limited conditions of soil water supply, inhibition of photosynthesis occurs due to a reduction in stomatal opening, which is the first protection mechanism in avoiding excessive water loss and maintaining plant turgescence (HU; WANG; HUANG, 2010). In the photosynthetic process, water is required for the release of protons and electrons at the photochemical stage, as well as for regulation of the stomatal opening, allowing the absorption of carbon dioxide and the mobilization of photoassimilates (CHAVARRIA; SANTOS, 2012).

Fitting the regression equation to the linear model (Figure 6A), a significant effect was seen ($p > 0.05$) for stomatal conductance, with a minimum value of $0.152 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for the irrigation depth of 75% of ETc, this value being 80.9% lower than the maximum obtained ($0.276 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) with the depth of 150% of ETc.

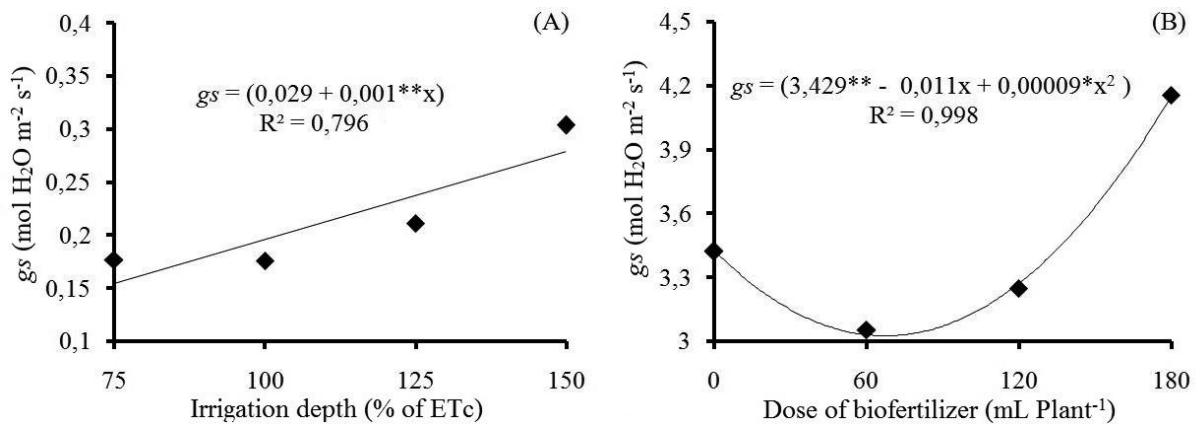
Moreira *et al.* (2015) obtained similar results in the cowpea under different irrigation depths, finding values between $0.27 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for an irrigation depth of 40% of ETc, and $0.49 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for a depth corresponding to 120% of ETc. When under water stress, the plant undergoes several physiological and morphological changes; a reduction in stomatal opening is one of these changes, which occurs due to the increase in the concentration of abscisic acid (ABA), a hormone produced in the roots that has as one of its functions to control the opening and closing of the stomata, reducing or increasing transpiration and as a consequence water loss (TAIZ; ZEIGER, 2013).

Figure 6B shows the effect of different doses of biofertilizer on stomatal conductance in cowpea plants at 75 DAS. It can be seen that there was a significant effect ($p > 0.05$) when fit to the quadratic model. Increases in the dose of biofertilizer increased the stomatal opening from the minimum point at a dose of $62 \text{ mL biofertilizer plant}^{-1}$, equivalent to $3.07 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$.

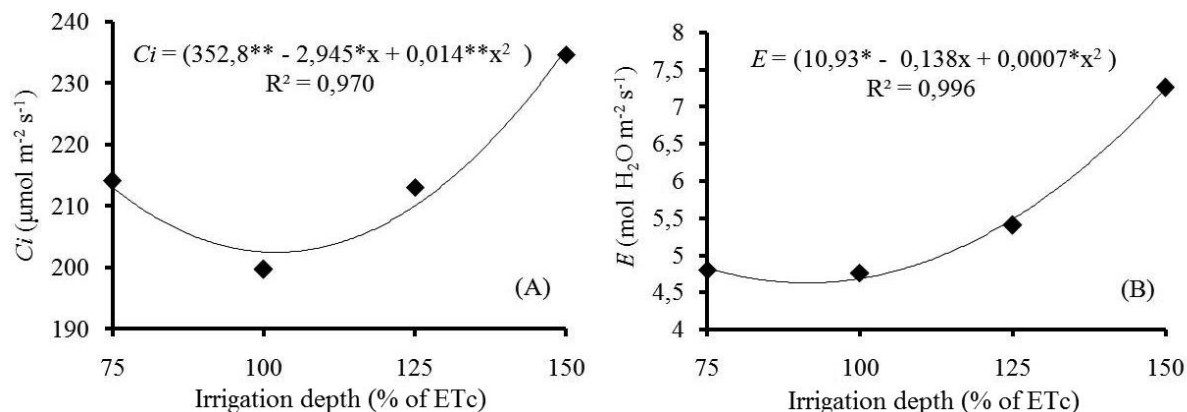
Biofertilizer is a product obtained from the aerobic and anaerobic fermentation of organic materials; as it is rich in macro- and micronutrients, it is considered a good alternative for plant nutrition (ALVES *et al.*, 2009). The inadequate supply of essential elements causes disturbances in the metabolic and physiological processes of plants (TAIZ; ZEIGER, 2013). Evaluating physicochemical characteristics is therefore recommended to avoid possible problems with toxicity in the plants.

For internal CO_2 concentration (Figure 7A), the regression equation fitted the quadratic model. A minimum point was observed for the irrigation depth of 105.2% of ETc, equivalent to $197.9 \text{ μmol m}^{-2} \text{ s}^{-1}$, followed by a 14.1% increase up to the maximum irrigation depth applied. For transpiration (Figure 7B), the different

Figure 6 - Stomatal conductance (*gs*) in cowpea plants for different irrigation depths (A) and doses of biofertilizer (B)



**, * significant by F-test respectively at 1% and 5% probability

Figure 7 - Internal CO₂ concentration (*C_i*) (A) and transpiration (*E*) (B) in cowpea plants for different irrigation depths

**, * Significant by F-test respectively at 1% and 5% probability

irrigation depths showed a significant effect ($p > 0.05$) where the regression equation fitted the quadratic model, with a minimum of $58 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for an irrigation depth of 92% of ETc. This value is 54.9% smaller than the maximum value ($7.09 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), which was for an irrigation depth of 150% of ETc.

Such behavior is expected due to the availability of water in the soil resulting in a less-negative water potential, ensuring that the plant does not need to spend much energy in absorbing water, and maintains a greater flow by transpiration to guarantee the influx of CO₂ (MOREIRA *et al.*, 2015). In general, an increase in the value of *C_i* is accompanied by increases in the value of *g_s*; stomatal limitation would be the main factor limiting photosynthetic performance, as the greater the stomatal opening, the greater the CO₂ diffusion to the substomatal chamber (NASCIMENTO, 2009).

Stomatal closure and the consequent reduction in the normal flow of CO₂ towards the carboxylation site, make up one of the main factors responsible for the reduction in photosynthesis, where water is one of the main components responsible for the process that regulates the opening or closing of the stomata (BOSCO *et al.*, 2009).

CONCLUSIONS

1. The application of large irrigation depths interferes with growth in cowpea plants, resulting in an increase in leaf area and total dry matter;
2. Leaf area in the cowpea is reduced with increases in the dose of biofertilizer applied;
3. An increase in the volume of water applied increases gas exchange in cowpea plants;

4. The application of biofertilizer interacts negatively with increases in irrigation depth for stomatal conductance.

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