Agrotrópica 28(2): 185 - 190. 2016.
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GAS EXCHANGE OF COWPEA VARIETIES SUBJECTED TO SALT STRESS

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Salt stress influences growth and development of the plants. In order to survive under these conditions, response mechanisms are developed and might be differently expressed by varieties of the same crop. This study aimed to evaluate physiological changes in the two cowpea varieties, subjected to salt stress conditions. The experiment was carried out in a greenhouse. A completely randomized design was adopted, with a 2 x 2 factorial considering 2 varieties (BRS Tapaihum and BRS Pujante) and two types of irrigation water (saline and non-saline). The water supply with saline solution of 10 dS m^{-1}, prepared with NaCl, started at 41 days after sowing and was applied daily for seven days. Chlorophyll and gas exchange indices were measured during the application of the treatments. At the end, shoot fresh and dry weight (leaves and stem), relative water content and leaf Na^+ and Cl^- concentrations were measured. The results showed that the varieties BRS Tapaihum and BRS Pujante resisted to salinity levels of 10 dS m^{-1} for seven days. BRS Pujante accumulated more Cl^- compared to BRS Tapaihum, which caused the reduction in chlorophyll contents and the greater drop in photosynthetic activity.

Key words: salinity, *Vigna unguiculata*, physiological exchanges, chlorophyll index

Trocas gasosas em variedades de Feijão submetidas a salinidade. O estresse salino interfere diretamente no crescimento e desenvolvimento das plantas. Para sobreviver a essas condições, são desenvolvidos mecanismos de resposta que podem se manifestar de forma diferenciada entre variedades de uma mesma cultura. O objetivo deste trabalho foi avaliar alterações fisiológicas em duas variedades de feijão caupi submetidas a condições de estresse salino. O trabalho foi conduzido em casa de vegetação, utilizando vasos de polietileno com capacidade de 3,0 L. O delineamento experimental utilizado foi o inteiramente casualizado organizado em esquema fatorial 2 x 2, considerando 2 variedades (BRS Tapaihum e BRS Pujante) e 2 tipos de água: não-salina e salina. As aplicações com solução salina de 10 dS m^{-1}, preparada com NaCl, foram iniciadas aos 41 dias após a semeadura, sendo realizada por sete dias. Durante a aplicação dos tratamentos foram realizadas as medidas dos índices de clorofila e das trocas gasosas. Ao final, foram avaliadas a massa fresca e seca da parte aérea (folha e caule), conteúdo relativo de água e as concentrações de Na^+ e Cl^- nas folhas. Os resultados mostraram que as variedades BRS Tapaihum e BRS Pujante resistiram a níveis de salinidade de 10 dS m^{-1} por sete dias. A variedade BRS Pujante acumulou maior Cl em relação à BRS Tapaihum, o que influenciou na redução dos teores de clorofila e em uma maior queda da atividade fotossintética.

Palavras-chave: salinidade, *Vigna unguiculata*, trocas fisiológicas, índice de clorofila.

Recebido para publicação em 24 de agosto de 2015. Aceito em 28 de julho de 2016.
DOI: 10.21757/0103-3816.2016v28n2p185-190

Introduction

Considering the fast population growth and the search for better life quality for people, the use of water with higher salt concentrations has become an alternative to irrigation of crops in areas with low availability of good quality water (Lima et al., 2007). However, its inadequate use may cause soil salinization, especially in the environmental conditions of Northeastern Brazil, where high evaporation rates and low rainfall occur, favoring salt accumulation through the process of capillary rise (Carneiro et al., 2004).

The excess of salts in soil may lead to negative effects on crops, changing metabolical, physiological and anatomical functions that contribute to the reduction of growth and interfere directly with productivity (Aragão et al., 2010). These changes in plant metabolism are usually caused by the osmotic effect and/or the excessive accumulation of ions in plant tissues, which may cause both ionic toxicity and nutritional imbalance (Soares et al., 2002).

Osmotic stress is a result of the reduction in water availability to plant, which favors stomatal closure and consequently leads to a series of problems, such as reduction in gas exchange by limiting CO_2 absorption (Bezerra et al., 2003). As for the accumulation of specific ions such as sodium and chloride, it can cause necrosis in plant tissues and accelerate senescence of older leaves, thus reducing the area used for photosynthesis (Munns, 2002). Neves et al. (2009) state that salt accumulation in chloroplasts can even cause damages in both the photosynthetic apparatus and the enzymatic system for CO_2 fixation.

Therefore, it is observed that the disturbances and injuries caused by the excess of salts affect plant metabolism and can cause death if the plant does not develop stress tolerance mechanisms.

In order to survive to these conditions, some species have developed mechanisms to minimize the negative effects of salt excess in the soil. In general, plants try to avoid excessive salt accumulation, especially in the photosynthetic tissue, or accumulate salt in a compartmentalized way, avoiding its interference with essential processes and functions (Oliveira, Gomes-Filho, Enéas-Filho, 2010).

Among the biochemical strategies used by plants, it can be listed selective ion accumulation or exclusion, control of ion uptake and transport to leaves, ion compartmentation in cellular (vacuole) and structural (leaves) levels, osmolyte synthesis, changes in photosynthetic pathways, changes in membrane structures, and induction of antioxidant enzymes and hormones (Esteves e Suzuki, 2008). Thus, the level of saline stress tolerance for plants will depend upon the adoption of one or more of these mechanisms and its/their efficiency in the reduction of damages caused by salts in excess, which may vary between different species, or even between varieties of the same species (Parida e Das, 2005).

Cowpea [*Vigna unguiculata* (L.) Walp] is moderately adapted to salinity, whose tolerance has been associated, at least in part, with the restriction of Na^+ accumulation in leaves, which is compartmentalized in the root system in the initial stages of salinity exposure (Cavalcanti et al., 2004). This crop plays an important role in the feeding of people who live in the Northeastern region of Brazil, for not only being part of the local agriculture, generating employment and income, but also for providing food with high nutritional value (Lima et al., 2007).

The objective of this study was to evaluate the cowpea varieties BRS Tapaihum and BRS Pujante, under saline stress conditions.

Materials and Methods

The experiment was carried out in a greenhouse of the Federal University of São Francisco Valley, Juazeiro-BA, Brazil (09°24’42’’S/40°30’59"W, 370 m).The region has semi-arid tropical climate, Koppen classification is BShw’, with average annual rainfall of 400 mm, average relative humidity of 67.8%, average temperature of 26.5 ºC and wind speed of 2.3 m s^{-1} (Reddy e Amorim Neto, 1983).

Two cowpea varieties, BRS Tapaihum and BRS Pujante, were cultivated in 3-L polyethylene pots, with three holes in the bottom to allow the drainage of excess irrigation water. The pots were placed on a 1-m high bench and filled with a substrate composed of clay soil and sand in 1:1 (v/v) ratio. Then, three seeds were placed at 2 cm depth in the substrate of each pot. The water supply was performed every day in the afternoon pouring 250 mL of water non-saline (EC_w = 0.05 dS m^{-1}) in each pot in order to increase soil moisture to its field capacity.

For maintaining plant growth, 200 mL of Hoagland e Arnon (1950) solution was applied from the 11th day after sowing on, twice a week. The thinning was performed at the 15th day after sowning, leaving only one plant per pot.

After 41 days, both varieties were subjected to two treatments for a period of seven days: water supply with and without the addition of saline solution, prepared with NaCl, presented 10 dS m^{-1} (250 mL per pot). The statistical design was completely randomized in a 2x2 factorial scheme, with six replicates.

Chlorophyll and gas exchange readings started 24h after treatments application, and were performed on a daily basis for seven uninterrupted days. The portable meter clorofiLOG, model CFL 1030 (Falker), was used for chlorophyll measurements, all performed in the afternoon 30 min before the first evaluation of gas exchange.

An infrared gas analyzer (IRGA – Model Li-6400) was used for gas exchange determinations. Measurements were performed in mature and healthy leaves close to the base of the plant, in the afternoon, from 14h:00min to 15h:00min. In the gas exchange measurements the following variables were analyzed: photosynthetically active radiation (PAR), CO_2 net assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s).

After chlorophyll and gas exchange measurements, the leaf relative water content (RWC) was determined. For this, discs of healthy leaves exposed to solar radiation were removed and immediately weighed in order to obtain fresh weight (FW). Then, the leaf discs were placed on Petri dishes and immersed in deionized water for 24 hours. After drying with paper towel, the discs were weighed for turgid weight (TW) and taken to an oven at 65 °C until constant mass for dry weight (DW) determination.

At the end, plant shoot was removed and stem and leaves were separated. Then, the material was placed in paper bags and immediately weighed in order to determine fresh weight. The bags were later taken to an oven at 65 °C, left there for one week and then the dry weight determined.

Soil samples were also collected from each pot, and a compound sample of each treatment was used for electrical conductivity determination through soil saturation extract in order to verify the soil salinity level at the end of the experiment.

Later, Na^+ and Cl^- concentrations were determined in dry leaf samples, which were separately ground and accommodated in properly identified flasks. Sodium was extracted from the leaves through the digestion of 0.5 g of the material in 6 mL of nitric-perchloric solution (5:1), for later analysis on a flame photometer (EMBRAPA, 2011).

The concentration of Cl^- was determined through the Mohr's method; placing 1 g of leaf sample in 100 mL of a $Ca(NO_3)_2.4H_2O$ solution at 0.085 mol.L^{-1} under agitation for 15 minutes. Then, samples were filtered, 10 mL of the extract were removed and it was added 1 mL of the indicator potassium chromate (K_2CrO_4) at 5% (w/v) for later titration with silver nitrate ($AgNO_3$).

The results were subjected to analysis of variance and treatment means were compared through Tukey test at 0.05 of probability.

Results and Discussion

In general, regardless of the evaluated parameters and the variety, cowpea plants showed resistance to salinity, since they could resist to levels of 10 dS m^{-1} for a period of seven days without significant leaf losses or death.

As for gas exchange evaluations, it was observed a steep decrease in photosynthetic rates in plants under saline conditions right in the first two days of the stress imposition (Figura 1). Plants of BRS Tapaihum, under saline conditions, showed the highest photosynthetic rates; however, it was less resistant to saline stress showing a reduction of 76% in photosynthetic activity under such conditions.

It was also observed a significant decrease in photosynthetic rates of both varieties during the fifth day of measurements. This reduction might be related to the drop of the photosynthetically active radiation (PAR) that occurred in the same period (Figure 1). The reduction of light intensity may often be below the light saturation point, thus reducing the photosynthetic process, as stated by Lopes et al. (1986).

The decrease in the photosynthetic rate for both varieties under saline stress was correlated with the reduction of stomatal conductance and consequently transpiration, which was caused by stomatal closure in response to the limitation of water uptake caused by the decrease of the soil osmotic potential (Bezerra et

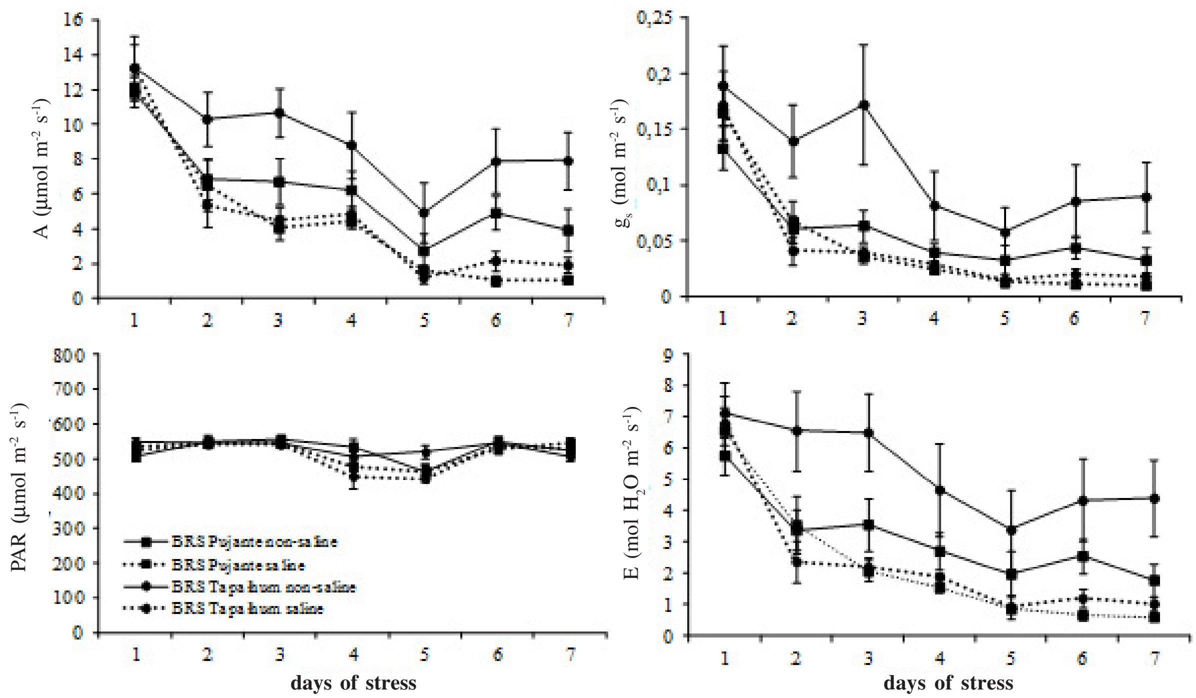

Figure 1. Net assimilation rate (A), stomatal conductance (g_s), photosynthetically active radiation (PAR) and transpiration rate (E) in varieties of cowpea BRS Pujante and BRS Tapaihum subjected to saline and non-saline water supply. The vertical bars represent the standard error of the mean.

al., 2005).This can be confirmed by Munns (2002), who states that during short-term saline stress the osmotic effects of salts prevails, causing the water potential in the root zone to decrease and restrict water uptake.

Regarding the evaluations of chlorophyll indices, it was possible to observe a reduction of chlorophylls only in the variety BRS Tapaihum when subjected to saline stress (Figura 2). This may have been another factor that allowed a greater drop in the photosynthetic activity of this variety. Chlorophylls are pigments that activate the process of electron transfer through the photosystems, which are important protein complexes for the performance of the photochemical and biochemical phases of the photosynthetic activity.

Reductions of chlorophylls a and b were also observed in rice (Lima et al., 2004), and castor bean (Silva et al., 2001) under saline stress. This reduction of chlorophyll content is probably associated with the possible inhibition of the synthesis of 5-aminolevulinic acid, a chlorophyll precursor (Santos, 2004), or the increase of the chlorophyllase activity which degrades chlorophyll (Lima et al., 2004).

According to the variance analysis, significant interaction between varieties and salinity was observed for leaf Na^+ and Cl^- content. In this case, saline solution application enhanced Na^+ content only in BRS Tapaihum, with a percentage increase of 47% compared to non-stressed plants (Table 1).

As for Cl^- content (Table 1), it was observed a significant increase in the leaves of both varieties subjected to saline conditions. However, Cl^- content was higher in the leaves of BRS Pujante than for BRS Tapaihum. This increase in Cl^- content may be the main

Table 1. Concentration of Na^+ and Cl^- in leaves of the cowpea varieties BRS Pujante and BRS Tapaihum subjected to saline and non-saline water supply

Varieties	Treatments	
	Non-Saline	Saline
	Na^+ (g kg^{-1} of DW)	
BRS Pujante	0,48 aA*	0,53 aB
BRS Tapaihum	0,57 bA	0,84 aA
	Cl^- (g kg^{-1} of DW)	
BRS Pujante	0,75 bA	14,37 aA
BRS Tapaihum	0,93 bA	7,47 aB

* Same lowercase letter between treatments and same uppercase letter between varieties do not differ by Tukey test at .05 probability.

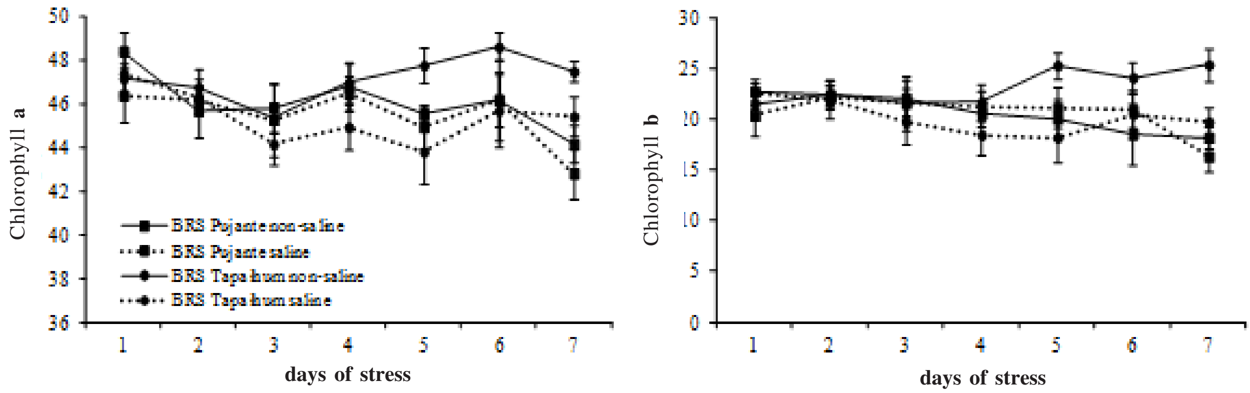

Figure 2. Chlorophyll a and b in cowpea varieties BRS Pujante and BRS Tapaihum subjected to saline and non-saline water supply. The vertical bars represent the standard error of the mean.

factor that has influenced photosynthetic activity, since the variety BRS Pujante was the one with the lowest photosynthetic rates in the last days of measurement.

When compared to the Cl^- content, appears that there was a smaller increase in Na^+ content in cowpea leaves. Assis Junior et al. (2007) and Souza et al. (2011) observed that in bean leaves Cl^- content was much higher than Na^+ content, which disagrees with the results in this study. Dutra et al. (2011) showed that Cl^- content in cowpea leaves is higher than Na^+ content, and that the opposite happens in the roots.

This result indicates that the varieties probably used some sort of active mechanism that restricted the significant accumulation of this element in the leaves, and it may be an exclusion mechanism or a mechanism for retaining Na^+ in other plant organs, such as the roots (Cavalcanti et al., 2004; Trindade et al., 2006). Praxedes et al. (2010) reported that the mechanism of restriction of Na^+ transport to the shoot is a characteristic associated to salinity tolerance.

As shown in Table 2, it was possible to observe significant differences between plants subjected to saline and non-saline conditions, regardless of the evaluated variety, for leaf relative water content, and fresh and dry weights of leaves and stem.

The leaf relative water content (RWC) was reduced for both varieties, showing that the salt stress influence the absorption and water acumulation in plant tissues. The mechanisms of osmotic adjustment possibly developed by the varieties were not efficient to reestablish the water status of the plants (Martínez et al., 2004).

Water status in plants may vary from species to species when subjected to saline stress. Silva et al. (2009) verified that leaves of Physic nuts (*Jatropha curcas*) showed increases in relative water content when plants were subjected to saline solutions of 75 and 100 mmol L^{-1} of NaCl, revealing an inverse effect, in which the excess of accumulated ions has contributed to water retention in leaf tissues.

Shoot fresh and dry weight of both cowpea varieties was significantly reduced by salinity. Similar results were found by Costa et al. (2003) for varieties of *Vigna unguiculata*. Lima et al. (2007) verified expressive reduction in shoot dry weight from the saline level correspondent to 2.13 dS m^{-1} on, decreasing in 22.6% compared to control plants; however, the greatest reduction was found for the highest saline level (5.0 dS m^{-1}), with a decrease of 67%.

These results are associated with the osmotic, toxic and nutritional effects resulting from the availability and accumulation of salts in the plant root zone, which affect CO_2 net assimilation, inhibit leaf expansion and accelerate senescence of mature leaves, consequently reducing the area destined for the photosynthetic process and the total production of photoassimilates (Munns, 2002; Lacerda et al., 2006).

Table 2. Relative water content (RWC), dry and fresh weight of the leaves and ste

Treatments	RWC (%)	Dry Weight (g)		Fresh Weight (g)	
		Leaves	Stem	Leaves	Stem
Non-Saline	80,43 a	2,46 a*	1,35 a	16,21 a	8,67 a
Saline	72,84 b	1,68 b	1,08 b	11,47 b	6,57 b

* Same letter between treatments do not differ by Tukey test at .05 probability.

Conclusions

Both varieties BRS Tapaihum and BRS Pujante were severely affected by salinity level of 10 dS m^{-1} of the water applied for seven days, with significant reduction of shoots fresh and dry mass.

Photosynthetic activity in BRS Tapaihum was more affected by salinity that BRS Pujante and may be associated with toxic effects of sodium accumulated in greater quantity in this variety.

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