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LEAF GAS EXCHANGE AND NUTRIENTS ACCUMULATION IN COWPEA PLANTS UNDER DIFFERENT MANAGEMENT STRATEGIES WITH BRACKISH WATER

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1 ABSTRACT

Many studies have shown that the use of strategies for the management of saline water in irrigation reduces impacts on the soil and crops. We aimed to evaluate the effect of management strategies of irrigation with brackish water on leaf gas exchange and mineral concentrations of cowpea *(Vigna unguiculata* L.Walp.). The experiment was conducted in randomized blocks with thirteen treatments and five replications. The treatments consisted of: T1 (control), T2, T3 and T4 using water of 0.5 (A1), 2.2 (A2), 3.6 (A3) and 5.0 (A4) dS m^{-1}, respectively, during the entire crop cycle; T5, T6 and T7, use of A2, A3 and A4 water, respectively, only in the flowering and fructification stage of the crop cycle; using different water in a cyclic way, six irrigations with A1 followed by six irrigations with A2 (T8), A3 (T9) and A4, (T10), respectively; T11, T12 and T13, using water A2, A3 and A4, respectively, starting at 11 days after planting (DAP) and continuing until the end of the crop cycle. Continuous application of the high salinity water (above 3.6 dS m^{-1}) over the whole cycle of cowpea inhibits leaf gas exchange, being stomatal conductance the most sensitive variable. The alternate use of brackish water or only in the salt tolerant growth stage reduces the accumulation of potentially toxic ions (especially chloride) and maintains the similar values of leaf gas exchange and total essential nutrients (K, Ca and Mg) extracted by plants, in relation to plants irrigated with canal water (low salinity).

Keywords: Salt stress, irrigation, stomatal conductance, photosynthesis, transpiration.

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2 RESUMO

Muitos estudos têm mostrado que a utilização de estratégias para o manejo de águas salobras na irrigação reduz os impactos sobre o solo e as culturas. Objetivou-se avaliar o efeito de estratégias de manejo de irrigação com água salobra sobre as trocas gasosas foliares e os teores de íons em feijão-caupi *(Vigna unguiculata* L.Walp.). O experimento foi conduzido em blocos ao acaso com treze tratamentos e cinco repetições. Os tratamentos consistiram de: T1 (controle), T2, T3 e T4, utilizando água de 0,5 (A1), 2,2 (A2), 3,6 (A3) e 5,0 (A4) dS m^{-1}, respectivamente, ao longo de todo o ciclo da cultura; T5, T6 e T7, utilizando águas salinas A2, A3 e A4, respectivamente, apenas na fase de floração e frutificação; uso de diferentes fontes de água de forma cíclica, com seis irrigações com A1 seguido por seis irrigações com A2 (T8), A3 (T9) e A4 (T10), respectivamente; T11, T12 e T13, usando água A2, A3 e A4, respectivamente, a partir de 11 dias após o plantio (DAP) e continuando até ao final do ciclo da cultura. A aplicação contínua de água de elevada salinidade (acima de 3,6 dS m^{-1}) ao longo do ciclo do feijão-caupi inibe as trocas gasosas foliares, sendo a condutância estomática a variável mais sensível. O uso alternado de água salobra ou apenas na fase de maior tolerância da cultura do feijão-caupi reduz o acúmulo de íons potencialmente tóxicos (especialmente o cloreto) e mantém os valores de trocas gasosas foliares e dos totais de nutrientes essenciais (K, Ca e Mg) extraídos pela cultura similares aos das plantas irrigadas apenas com água do canal (baixa salinidade).

Palavras-chave: Estresse salino, irrigação, condutância estomática, fotossíntese, transpiração.

3 INTRODUCTION

A high concentration of salts in the irrigation water can reduce crop productivity. The intensity of this effect depends on many factors, such as the species of plant or cultivar, phenological growth stage, composition of the saline water, intensity and duration of the stress, soil and climate conditions, and the type of irrigation management (MARSCHNER, 2012).

The irrigation management strategies, including use of brackish water during stages of increased tolerance, mixing water resources and cycling the use of different-quality waters, when associated with crop rotation, can help reducing the use of better-quality water and the accumulation of salts in the soil. This also reduces the impact of using such water resources, on both environment and plant development (MURTAZA; GHAFOOR & QADIR, 2006; BARBOSA, 2010; OSTER et al., 2012; YARAMI & SEPASKHAH, 2015; NEVES et al., 2015).

Many studies have shown that the use of strategies for the management of saline water in irrigation reduces impacts on the soil and crops (CALVET et al., 2013, SILVA et al., 2013; MESQUITA et al., 2015). For example, the alternate application of low and high salinity water may contribute to washing away excess of salts contained in the root zone, favoring crop development (BARBOSA et al., 2012). However, the effects of these strategies on physiological aspects of crops such as leaf gas exchange and the accumulation of ions have been little studied.

The osmotic stress associated with the total accumulation of salts in the soil reduces the plant-available water, thereby affecting leaf gas exchange and plant growth. In addition, in susceptible species, the accumulation of sodium and chloride may produce necrosis of the foliar tissue, possibly accelerating senescence of mature leaves and reducing the area available for photosynthesis (BEZERRA, LACERDA & GOMES FILHO, 2005; MUNNS & TESTER, 2008). If, therefore, the osmotic and ionic effects of salinity are reduced by the use of management strategies, the impact on physiological responses, which are important indicators of the effectiveness of such strategies, will be lessened.

Given the above, the aim was to evaluate leaf gas exchange and the concentration and total extracted K, Ca, Na, Cl and Mg in cowpea, under different strategies of irrigation management using water with different salt concentrations.

4 MATERIAL AND METHODS

The study was conducted during the dry season of 2011 (September-November 2011) in the municipality of Pentecoste, Ceará, Brazil (3º45'S, 39º15'W and altitude of 47 m). This region has semi-arid climate (BSw'h') according to Koeppen classification, and the soil of the area as classified as Fluvic Neosol (EMBRAPA, 1999), with loamy sand texture, EC1:1 (soil:distilled water) = 1.26 dS m^{-1}, and exchangeable sodium percentage 6.0%.

The experiment was conducted in randomized blocks with thirteen treatments and five replications, as previously described by Neves et al. (2015). In the composition of the treatments, four sources of water were used: A1 - Canal water with EC of 0.5 dS m^{-1}; A2 - Waste water from the desalination plant installed near to experimental area, with EC of 2.2 dS m^{-1}; A3 - Brackish water with EC of 3.6 dS m^{-1}, obtained by mixing the wastewater from a desalinization plant with NaCl and $CaCl_2.2H_2O$ salts in 7:3 equivalent ratio; and A4 - Brackish water with EC of 5.0 dS m^{-1}, also obtained by the addition of mentioned salts to wastewater from desalinization plant.

The treatments were as follows: T1 (control), T2, T3 and T4 using water of 0.5 (A1), 2.2 (A2), 3.6 (A3) and 5.0 (A4) dS m^{-1}, respectively, during the entire crop cycle; T5, T6 and T7, using A2, A3 and A4 water, respectively, only in the flowering and fructification stage of the crop cycle; using different types of water in a cyclic way, six irrigations with A1 followed by six irrigations with A2 (T8), A3 (T9) and A4, (T10), respectively**;** T11, T12 and T13, using A2, A3 and A4 water, respectively, starting at 11 days after planting (DAP) and continuing until the end of the crop cycle.

In all treatments, the water was applied using a drip irrigation system, and depth of irrigation water to be applied was based on evapotranspiration (ETo), estimated by Class A Pan method, and crop coefficients (Kc) recommended by Souza, Bezerra & Teófilo (2005). Seeds of cowpea cultivar EPACE 10, were sown in 0.8 x 0.3 m spacing, with two seeds per hole equivalent to plant density of 83,300 plants ha^{-1}. Each plot had dimensions of 6.6 x 4.0 m, with total of 65 plots (NEVES et al., 2015).

Every six irrigations, corresponding to 15 days intervals, measurements of the rates of photosynthesis, transpiration and stomatal conductance were performed in fully mature leaves using a portable infrared gas analyzer (model LI6400XT, LICOR, USA). The measurements were taken between 09:00 and 12:00 h, using a coupled light source with an intensity of around 1,500 μmol m^{-2} s^{-1}, and under natural conditions of air temperature and CO_2 concentration.

The levels of Ca and Mg were determined by atomic absorption spectrophotometry, Na and K by flame photometry (MALAVOLTA; VITTI & OLIVEIRA, 1997), and Cl by colorimetry (GAINES; PARKER & GASCHO, 1984). From the data of dry matter production, planting density and mineral concentration, the total extracted for each mineral element was calculated in kg ha^{-1}.

The data were subjected to the analysis of variance (F test) and means were compared by Tukey's test at $p \leq 0.05$, using as a tool the program ASSISTAT 7.6 beta.

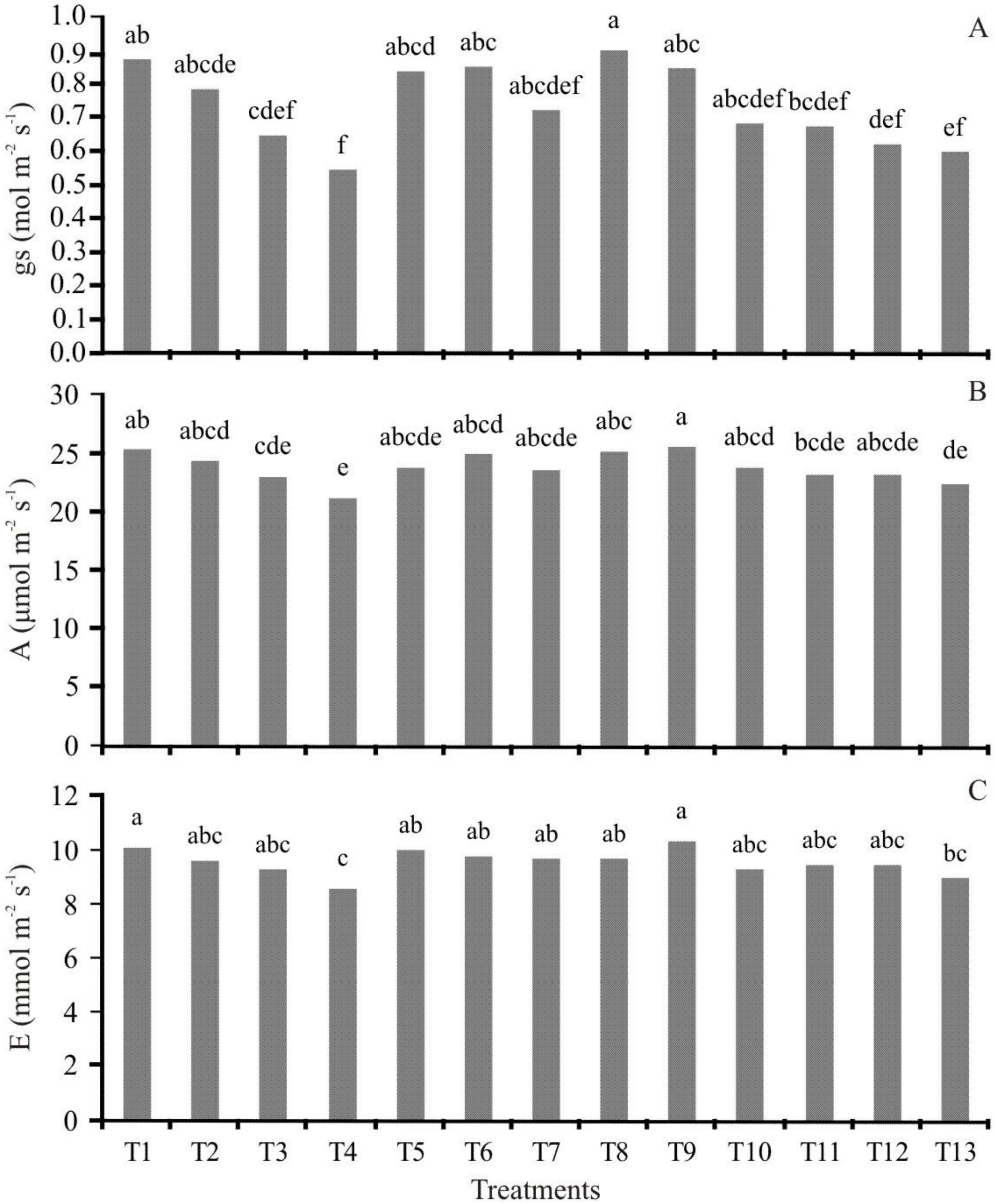
5 RESULTS AND DISCUSSION

The rates of stomatal conductance (g_s), net photosynthesis (A) and transpiration (E) were affected by the treatments and reading times throughout the crop cycle ($P<0.01$). No significant effects have been seen from the interaction treatment x time ($P>0.05$), indicating that the response to the treatments did not differ for measurements taken during the crop cycle.

Figure 1 shows the effects of the different treatments on leaf gas exchange: the values being the averages of the four readings taken over the cycle. It could be seen that stomatal conductance (Figure 1A) was the most sensitive variable to salt stress, with the lowest values observed for the treatments with a continuous application of brackish water, begun either before or after germination (T3, T4, T12 and T13). The treatments with brackish water applied alternately or only in the flowering and fructification stage of the crop cycle; (T5, T6, T7, T8, T9 and T10) presented similar results to the control (T1).

Unlike stomatal conductance, the rates of photosynthesis (Figure 1B) and transpiration (Figure 1C) were affected only when continuous application of water with an EC of 5.0 dS m^{-1} during the entire crop cycle (T4) or after sowing until the end of the cycle (T13). On the other hand, the application of brackish water both cyclically and at the end of the crop cycle (T5, T6, T7, T8, T9 and T10) did not affect either photosynthesis or transpiration, when compared to control plants. Maintaining these important physiological responses indicates the possibility of partially substituting the fresh water for brackish irrigation water without compromising plant development.

Figure 1. Stomatal conductance (gs), net photosynthesis (A) and rate of transpiration (E), in mature leaves of cowpea plants as a result of the treatments (T1 to T13)



* Bars with the same letter do not differ statistically by Tukey's test at 5%

T1-low-salinity water (A1) throughout the cycle (Control); T2-Water with an EC of 2.2 dS m^{-1} (A2) throughout the cycle; T3-Water with an of EC 3.6 dS m^{-1} (A3) throughout the cycle; T4-Water with an EC of 5.0 dS m^{-1} (A4) throughout the cycle; T5-Water with an EC of 2.2 dS m^{-1} in the final phase of the cycle (flowering and fruiting); T6-Water with an EC of 3.6 dS m^{-1} in the final phase of the cycle (flowering and fruiting); T7-Water with an EC of 5.0 dS m^{-1} in the final phase of the cycle (flowering and fruiting); T8-Cyclic use of A1 and A2 (6 irrigations with A1 followed by 6 irrigations with A2), beginning with A1 at planting; T9- Cyclic use of A1 and A3 (6 irrigations with A1 followed by 6 irrigations with A3), beginning with A1 at planting; T10- Cyclic use of A1 and A4 (6 irrigations with A1 followed by 6 irrigations with A4), beginning with A1 at planting; T11-The use of water with an EC of 2.2 dS m^{-1}, applied 11 days after planting (DAP) until the end of the cycle; T12-The use of water with an EC of 3.6 dS m^{-1}, applied 11 days after planting (DAP) until the end of the cycle; T13-The use of water with an EC of 5.0 dS m^{-1}, applied 11 days after planting (DAP) until the end of the cycle.

The reduction in the rate of photosynthesis in treatments with both a continuous application of water at an EC of 5.0 dS m^{-1} (T4) and applied after planting until the end of the cycle (T13), may be due to the partial closing of the stomata resulting from the osmotic and ionic effects of the salinity, and to the effects of ion toxicity on the metabolism (BEZERRA, LACERDA & GOMES FILHO, 2005; WILSON et al., 2006; GUIMARÃES et al., 2012; TORRES et al., 2012; TAIZ & ZEIGER, 2013). However, reductions in the rates of photosynthesis were less significant than the reductions observed for vegetative biomass production (results previously published by NEVES et al., 2015), confirming that instantaneous rates of leaf gas exchange are less sensitive to salt stress than plant growth (LACERDA et al., 2006; ASSIS JÚNIOR et al., 2007; SILVA, 2015).

The irrigation management treatments using waters at different salt concentrations affected the levels of Ca, Na and Cl in the vegetative parts of the cowpea plants ($p<0.01$ or $p<0.05$); no effect has been seen on the K concentration ($p>0.05$).

The levels of potassium in the vegetative part (leaves and stems) did not differ significantly between treatments with the use of brackish water, even when applied continuously throughout the cycle (Table 1). These results are different to those obtained by other authors, which showed a reduction in K concentration with increasing salinity (LACERDA et al., 2003; AQUINO et al., 2007). According to these authors, this reduction in K concentration is due to the competition of K and Na ions for the absorption sites on the cell membrane of the root system. However, this response varies between species and is more common in plants stressed only with NaCl, a different condition from that used in this study.

Table 1. Levels of mineral elements in the vegetative part (leaves and stems) of cowpea plants irrigated with brackish water

Treatments*	K	Ca	Na	Cl	Mg
	g kg^{-1}				
T1	28.89a**	14.87bcde	4.11ab	15.01f	9.08ab
T2	28.45a	14.45cde	3.13b	20.13e	9.65ab
T3	29.44a	22.15ª	3.79ab	20.41e	10.64a
T4	26.73a	16.88bc	4.64a	32.03b	10.37a
T5	28.45a	14.65bcde	4.04ab	28.01bc	9.56ab
T6	26.98a	12.94e	3.84ab	26.66cd	9.87ab
T7	28.95a	15.42bcde	3.40b	27.28c	8.94ab
T8	27.39a	13.35de	3.25b	22.80e	10.02ab
T9	27.34a	16.35bcd	3.22b	20.80e	9.83ab
T10	25.42a	13.95cde	3.33b	27.55c	8.10ab
T11	27.14a	14.49cde	3.15b	30.48bc	7.05b
T12	26.81a	14.60bcde	3.18b	28.99bc	8.31ab
T13	25.65a	17.93b	3.18b	37.51a	8.05ab

*For a description of the treatments, see the legend in Figure 1. ** Mean values with the same letters in the columns do not differ statistically by Tukey's test at 5%. n = 5

The Ca concentration of the shoot (leaves and stems) of the cowpea varied for all treatments, being greater for treatments where water at higher salinity levels was applied (T3, T4 and T13). These higher values can be explained by the calcium, which was added when preparing water with an electrical conductivity of 3.6 and 5.0 dS m^{-1}, and also by the continuos using of water throughout almost the entire plant cycle. Although Ca levels were lower for treatments when brackish water was applied, only at the stage of greater crop tolerance (T5, T6 and T7), or applied cyclically (T8, T9 and T10), it could be seen that they did not differ from the control (T1), being limiting neither to plant development (NEVES et al., 2015) nor to leaf gas exchange (Figure 1).

Sodium levels in the vegetative parts showed small variations between treatments (Table 1), with a variation of 4.64 g kg^{-1} DM for the highest value, and 3.13 g kg^{-1} DM for the smallest value; the highest value being found in plants continuously irrigated with water at a higher salinity (T4). Moreover, the Cl concentration varied from 37.51 to 15.01 g kg^{-1}, the highest values being seen for those treatments where water at higher salinity levels was applied in almost in the entire crop cycle (T4 and T13). The accumulation of Na and Cl in the leaf tissue of plant species under salt stress is one of the main inherent effects of this agent on plant metabolism, given that the high concentrations of these ions can cause permanent damage to the cell structures, which can also compromise metabolic efficiency and may lead to cell death (MUNNS & TESTER, 2008).

When comparing findings for sodium and chloride in the shoot, it was observed that values for the Cl^- were much higher than Na^+ concentration. A similar tendency was obtained by Guimarães et al. (2012) and Sousa et al. (2007), especially for leaf tissues, who found Cl concentrations up to five times higher than Na concentration. These results can be explained by the greater ability of this species to limit the absorption and transport of Na^+ from the root zone to the shoots, compared to the chloride (TRINDADE et al., 2006). It is worth noting that both the accumulation of Na^+ and Cl^- may have contributed to inhibit growth and yield in the crop, since cultivated plants generally have a low ability to compartmentalize these ions in the vacuoles, with accumulation occurring in the cytosol and other organelles, and then causing toxicity to cellular structures and cellular processes such as photosynthesis (ASSIS JÚNIOR et al., 2007).

Chloride levels were generally lower in those treatments where brackish water was used only at the stage of greater crop tolerance (T5, T6 and T7), or used cyclically (T8, T9 and T10), a fact that may have contributed to the lesser effect on leaf gas exchange (Figure 1) and plant development. Similar results have been seen by Barbosa et al. (2012). According to these authors, the cyclic use makes possible the replacement of about 50% of the irrigation depth with water of high salinity, without negative impacts on productivity of maize and presenting a decrease in the accumulation of potentially toxic ions (Na and Cl).

Totals of K, Ca, Na and Cl extracted by the cowpea plants were influenced by the irrigation management treatments ($p<0.01$), a consequence of variations in concentration and/or in the production of total dry biomass (vegetative and reproductive parts).

Continuous application of brackish water (T4) and application after planting until the end of the cycle (T13), at a level of 5.0 dS m^{-1}, reduced the extracted totals of K, Ca and Na. This reduced extraction of nutrients by the plants under these treatments is mainly due to a reduction in plant growth, and shows that the amount of fertilizer applied to crops irrigated with saline water should be less than that applied to plants irrigated with non-saline water (GRATTAN & GRIEVE, 1999; NEVES et al., 2009; LACERDA et al., 2016). For the essential elements K and Ca, it could be seen that the management strategies under test (alternating or only at the stage of greater crop tolerance) resulted in similar values to those for the control plants, being a result of maintaining the levels of these elements (Table 1) as well as of the growth and productivity of the crop, as observed on previously published results (NEVES et al., 2015).

Table 2. **Extracted quantities of mineral elements in cowpea plants irrigated with brackish water**

Treatments*	K	Ca	Na	Cl	Mg
	kg ha^{-1}				
T1	114.46a**	45.31ab	13.38a	59.82de	35.22a
T2	93.23abc	34.28bcde	8.31bcd	63.81cde	29.41abc
T3	91.49abc	53.77[a]	10.16abcd	65.21cde	30.35abc
T4	52.22d	21.66e	6.64cd	56.87e	17.87de
T5	116.35a	43.08abc	12.97ab	88.94abc	34.54ab
T6	107.46ab	39.03bcd	12.50ab	91.59ab	36.51a
T7	114.46a	45.50ab	11.17abc	98.40a	33.23ab
T8	104.53ab	39.74abcd	10.46abc	83.40abcd	36.02a
T9	92.31abc	40.67abc	18.96abcd	69.88bcde	31.02abc
T10	89.11abc	34.66bcde	9.30abcd	79.35abcde	25.96bcd
T11	103.82ab	37.73bcd	9.43abcd	95.42a	25.88bcd
T12	78.74bcd	30.48cde	7.41cd	75.08abcde	23.08cde
T13	58.46cd	26.03de	5.45d	69.94bcde	16.29e

* For a description of the treatments, see the legend in Figure 1. ** Mean values with the same letters in the columns do not differ statistically by Tukey's test at 5%. n = 5

6 CONCLUSIONS

Continuous application of high-salinity water throughout the cowpea cycle inhibits leaf gas exchange, with stomatal conductance being the most sensitive variable.

The alternating use of brackish water, or only at the stage of greatest tolerance of the cowpea crop, reduces the accumulation of potentially toxic ions (especially chloride) and holds the rates of leaf gas exchange and total essential nutrients (K, Ca and Mg) extracted by the crop at similar values to those observed of plants irrigated with canal water.

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