Influence of NaCl on *Brachiaria humidicola* inoculated or not with *Glomus etunicatum*

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SUMMARY

An experiment was carried out to investigate the effects of different levels of NaCl on the growth of the grass *Brachiaria humidicola* inoculated or not with the arbuscular mycorrhizal fungus (AMF) *Glomus etunicatum.* The concentrations of NaCl utilized were 0, 0.22, 1.09, 1.96 and 2.84 g kg^{-1} of soil; corresponding to electrical conductivities of 2.22, 4.00, 8.13, 12.53 and 16.50 dS m$^{-1.}$ The salinity ratio of the soil reduced the dry matter in different parts of the plant when the electrical conductivity was above 8 dS m$^{-1.}$ Leaf area ratio and succulence increased at high salinity levels of the soil. The percentage of root colonization and the number of AMF spores in the rhizosphere were not affected by the increasing doses of NaCl added to the soil.

Key words: mycorrhiza, stress, inoculation, salinity.

INTRODUCTION

The ability of mycorrhizal plants to tolerate adverse environmental conditions is of great value in verifying the behaviour of these plants when subjected to salt stress. Several investigations have shown that some arbuscular mycorrhizal fungi (AMF) can increase the nutrient absorption and production of biomass in plants grown in saline soils (Hirrel and Gerdemann, 1980; Ojala *et al.*, 1983). AMF have improved the osmoregulation in colonized plants through the increase in the concentration of soluble substances in leaf tissue (Augé and Stodola, 1990). The introduction of AMF in saline soils could potentially

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improve the tolerance and development of the plants (Jain *et al.*, 1989). AMF inoculation has improved the growth of forage crops under saline conditions (Pfeiffer and Bloss, 1988). However, in some cases, the mycorrhizal fungi can have a negative impact on plants under high salinity conditions (Johnson-Green *et al.*, 1995).

According to Copeman *et al.* (1996), soils from arid and semi-arid regions have high salinity levels due to soluble salts in irrigation water and fertilizers. These areas show high levels of soil humidity, which can benefit forage crops, such as the grass *Brachiaria* spp. One of the advantages that can be obtained from the cultivation of crops tolerant to salinity is the reduction of costs when compared to those of the physical-chemical improvement of saline soils, when gypsum ($CaSO_4$) is used.

Osmotic potential variations are frequently found in both dry and wet soils, and they are often related to soil salinity, resulting in a low survival rate and a 50 % decline of the microbial community (Vlassak, 1996). The potential of AMF in easing saline stress has not been well studied, although there is evidence that mycorrhizal fungi increase the growth rate of blackgum cuttings *(Nyssa sylvatica* Marshall) (Keeley, 1980). Not all AMF isolates equally improve the growth of plants under saline stress (Hirrel and Gerdemann, 1980). The salinity of the soil also influences the symbiosis, as sodium and chlorine can reduce the germination of spores and the rate of root colonization (Hirrel, 1981).

The experiment presented was conducted to investigate the effect of different levels of NaCl on *Brachiaria humidicola* inoculated or not with *Glomus etunicatum* and also to evaluate plant growth.

MATERIAL AND METHODS

Preparation of the soil

The experiment was carried out in a greenhouse at temperatures of 29-33 °C (minimum-maximum) and a relative humidity of 81-89 % (minimum-maximum). The soil used, alluvial eutrophic (0-30 cm) (Barros Filho *et al.*, 1966), was collected from the municipality of Vitória de Santo Antão - Pernambuco, Brazil, air dried, sifted (5.00 mm) and autoclaved for 1 h at a temperature of 121 °C under a pressure of 101 KPa in a 24 h interval for 3 consecutive days. Soil fertilization 15 days prior to planting included 275 mg of ammonia sulfate, 165 mg of simple super phosphate and 22 mg potassium chloride. These fertilizer levels were based in the soil analysis recommendation (Santos and Lira, 1998). Three kg of soil were used in 4 L containers. The physical and chemical analyses of the soil were done at the Empresa Pernambucana de Pesquisa Agropecuária (IPA) following the method of Embrapa (1979). The results after soil analysis were: pH (water) 6.5; Ca^{2+} 13.5 $mmol_c\ kg^{-1}$; Mg^{+} 36.5 $mmol_c\ kg^{-1}$; K^{+} 2.6 $mmol_c\ kg^{-1}$; Na^{+} 6.0 $mmol_c\ kg^{-1}$; Al^{3+} 0.0 $mmol_c\ kg^{-1}$; P 64.8 mg kg^{-1}; N 0.9 g kg^{-1}; clay 80g kg^{-1}; silt 320 g kg^{-1}; fine sand 30 g kg^{-1}; coarse sand 570 g kg^{-1}; global density 1.4 g cm^{-3} and particular density 2.7 g $cm^{-3.}$

Inoculation and planting

The plant used was *B. humidicola* Rendle cv. 409. The seeds were sterilized with sodium hypochlorite (20 %) for two minutes, rinsed repeatedly in sterilized distilled water and placed in trays containing vermiculite as substrate (mineral formed by aluminum and magnesium hydrated silicates) for seven days. Two seedlings per container were transplanted. The AMF used was an isolate of *Glomus etunicatum* Becker, Gerdemann, supplied by the Centro Nacional de Pesquisa em Agrobiologia EMBRAPA - RJ. The spores were extracted using Gerdemann and Nicolson's (1963) technique. After extraction, the spores were selected, separated, and individually counted, and 100 spores per container were used as inoculum. The plants were irrigated with distilled water and the excess water was drained naturally to a collector, and whenever possible, replaced into the soil from which it had drained. The water utilized had low salinity (C_1S_1), with an electrical conductivity of 0.01 dS m^{-1} and, according to Richards (1974), it could be used for irrigation of most plant species in any type of soil.

Application of saline stress and analysis

The levels of NaCl and electrical conductivity were determined using the soil saturation water extract with 0, 0.22, 1.09, 1.96 and 2.84 g kg^{-1} of soil. The electrical conductivities of the saturated extract were 2, 4, 8, 12 and 16 dS m^{-1} and the osmotic potentials –0.08, –0.14, –0.30, –0.46 and –0.60 MPa, respectively (Richards, 1974). Increased doses of NaCl were applied to the soil 15 days before planting. After the salinization process, all treatments had a soil pH of 7.6. Temperature and relative humidity of the air were recorded using a thermohygrograph. The plants were harvested 60 days after planting, and the following parameters were evaluated: leaf area ratio [LAR=Leaf Area (dm^{2})/Total Plant Dry Matter (g)] and succulence (g H_2O/g dry matter) calculated in accordance with Benincasa (1988); shoot + root dry weight of the seedlings was recorded after maintaining them in a heated greenhouse at 65 °C for 72 h; the percentage of mycorrhizal root colonization after staining the roots (Phillips and Hayman, 1970) using the method described by Giovannetti and Mosse (1980), and number of spores in the soil was quantified using the method by Gerdemann and Nicolson (1963).

Statistical Analysis

The experimental design was a randomized block factorial arrangement of 5 salt levels and 2 mycorrhizal treatments with 4 blocks (using 20 plants in each block). The Steel and Torrie (1960) mathematical model was applied for the statistical analysis. The significance of the variance and regression analyses were determined with the F and Tukey tests ($p \leq 0.05$), using the SANEST program (Zonta *et al.*, 1984).

RESULTS

Influence of salinity on plant growth

With the increase of salinity, stem + sheath dry matter of *B. humidicola* plants was lower. Significant differences were already detected at a dose of 1.09 g NaCl kg^{-1} of soil, corresponding to an electrical conductivity of 8 dS m^{-1} (Table 1).

Table 1

Mean leaf (LDM), stem + sheath (SSDM), root (RDM) dry matter, and leaf area ratio (LAR) in *Brachiaria humidicola* plants inoculated or not with *Glomus etunicatum* on soil with increasing levels of electrical conductivity (EC)

EC (dS m^{-1})	*Glomus etunicatum*				Not-inoculated			
	LDM(g)	SSDM(g)	RDM(g)	LAR (dm^2 g^{-1})	LDM(g)	SSDM(g)	RDM(g)	LAR (dm^2 g^{-1})
2	12.23	3.83	2.24	0.69	10.24	3.79	2.07	0.81
4	10.41	3.16	2.23	0.71	8.56	3.45	1.63	0.88
8	4.03	1.60	0.77	0.90	3.37	1.42	0.83	0.96
12	1.96	0.72	0.23	1.01	1.68	0.76	0.25	1.30
16	1.00	0.42	0.15	1.07	0.46	0.24	0.10	1.44
Average	5.91	1.95	1.12	0.88 b	4.86	1.93	0.98	1.08 a
(Salinity)	*	*	*	*				

* Significant at $p \leq 0.05$. Means, in the row, followed by same letter, do not differ ($p \leq 0.05$) after Tukey's test. (4 blocks, 20 plants/block).

There were significant differences in leaf dry matter in accordance to the salinity levels of the soil (Table 1). The greatest leaf weigths were obtained at 0 and 0.22 g NaCl kg^{-1} soil, corresponding to electrical conductivities of 2 and 4 dS m^{-1}, respectively. Similar results were found for shoot and root dry matter data. The control (2 dS m^{-1}) and the 4 dS m^{-1} treatments produced more biomass than plants grown at 16 dS m^{-1}. The leaf dry matter was only significantly reduced when the electrical conductivity of the soil reached 8 dS m^{-1}. Plants grown at 16 dS m^{-1} were only 8 % of the mass of the control.

The production of root dry matter was reduced as well with the increase in salinity levels. A greater yield of root dry matter was observed when the plants were subjected to electrical conductivities of 2 and 4 dS m^{-1} (Table 1).

There were significant responses in the leaf area ratio at all levels of NaCl added to the soil (Table 1). There was a significant increase in the leaf area ratio as the level of salinity in the soil increased. When the plants were subjected to an electrical conductivity of 16 dS m^{-1}, their leaf area ratio was 68 % greater than that of the control. The highest leaf area ratio was found in plants grown at electrical conductivities of 2, 4 and 8 dS m^{-1}.

Concerning the leaf area ratio there were significant differences between mycorrhizal and non–mycorrhizal plants. Mycorrhizal *B. humidicola* plants had a higher leaf area ratio (Table 1).

Succulence in leaves and root

The NaCl concentration in the soil solution had an influence on the succulence of the different parts of the plants. At the highest level of salinity applied to the soil, significant differences were verified in leaves and roots (Table 2). There was an increase in the succulence of the leaf and the root when soil salinity increased with a greater significant increase in the roots. Succulence in the roots was 4 to 10 times greater than that in the leaves.

Table 2

Succulence in leaf (SL) and succulence in root (SR) in *Brachiaria humidicola* plants inoculated or not with *Glomus etunicatum* on soil with increasing levels of soil electrical conductivity (EC)

EC (dS m^{-1})	Succulence (g H_2O/g dry matter)			
	Glomus etunicatum		Non-inoculated	
	SL	SR	SL	SR
2	4.97	8.11	4.81	9.03
4	5.01	7.87	4.84	9.70
8	5.23	7.92	4.64	10.03
12	5.72	9.71	5.95	13.60
16	6.69	16.96	5.95	15.53
Average	5.52	10.11	5.34	11.58
(Salinity)	*	*		

* Significant at $p \leq 0.05$ according Tukey's test (4 blocks, 20 plants/block).

Number of spores and percentage of mycorrhizal root colonization

Regarding the number of spores recovered from the soil, there was no significant difference among NaCl treatments. However, the percentage of root colonization did not show either any significant difference in relation to the levels of NaCl applied to the soil (Table 3).

Table 3

Spore density and percentage of root colonization in *Brachiaria humidicola* plants inoculated or not with *Glomus etunicatum* on soil with increasing levels of soil electrical conductivity (EC)

EC (dS m^{-1})	*Glomus etunicatum*	
	N 0 spore/150g soil [1]	Root Col. (%) [2]
2	196.50	5.50
4	201.75	3.00
8	246.00	4.00
12	240.25	10.00
16	239.75	5.00
Average	224.85 a	5.50 a
(Salinity)	n.s.	n.s.

n.s. not significant according Tukey's test. (4 blocks, 20 plants/block).
[1] Original data transformed to Log (X) to normalize variance for Anova.
[2] Original data transformed to $\sqrt{(X + 1)}$ to normalize variance for Anova.

DISCUSSION

The results obtained for the production of dry matter of the plants were similar to those found by Hassan *et al.* (1970). Ruiz-Lozano *et al.* (1996) verified that both the dry matter of shoots and roots of lettuce plants were reduced with the increase of salinity and this reduction was greater in non-mycorrhizal plants.

In the evaluation of dry matter production of the different parts of the *B. humidicola* plants, it was observed that the root proved to be most sensitive to saline stress, followed by leaves and shoot. Hassan *et al.* (1970) stated that the dry matter production of the leaves, stem and ears of maize decreased with the increase in the levels of salinity in the soil. This reduction was particularly evident starting at 8 dS m^{-1}. Boursier and Lauchli (1990) verified that the reduction of leaf dry matter of sorghum with salinity was approximately 6 % per dS m^{-1} of electrical conductivity in the extract.

Concerning the leaf area ratio, *B. humidicola* plants showed a high photosynthetic efficiency per leaf area unit, even in soil with no added sodium chloride (2 dS m^{-1}). The rates of cell and tissue expansion are regulated by the extension of the cell wall, which is driven by the turgor pressure. Therefore, the osmotic effects of salinity are associated with a loss in cell wall extension and cell expansion, leading to growth arrest (Lewis *et al.*, 1989). Hardie and Leyton (1981) verified, in red clover, that mycorrhizal inoculated plants recuperated the turgescence more quickly than non-inoculated plants.

Ruiz-Lozano *et al.* (1996) obtained a greater yield of root dry matter in lettuce plants inoculated with AMF in comparison to the non-inoculated treatments. In our experiment the greater values of leaf area ratio were recorded in mycorrhizal plants, indicating that mycorrhizal inoculated plants were more efficient and, therefore, had a lower leaf area ratio (Table 1) but the other growth parameters were not affected by mycorrhizal inoculation. Totawat and Mehta (1985) noted that in genotypes of maize and sorghum, regardless of the species, the leaf area, height and dry matter production were greater in plants that exhibited less leaf area ratio. The same result was observed in this experiment for mycorrhizal inoculated plants. Augé *et al.* (1986) also showed that the leaf area ratio was greater in *Rosa hybrida* L. plants not colonized by AMF.

Brachiaria humidicola plants presented a greater leaf area ratio when the electrical conductivity of the soil was 12 and 16 dS m^{-1}, indicating that transpiration was probably greater in these treatments, simultaneously increasing both the demand for water and the concentration of Na^+ and/or Cl^- ions in the shoots dry matter. According to Akita and Cabuslay (1990) the leaf area ratio and the selectivity of the root against the Na^+ ion are the main characteristics when selecting for plant tolerance in high salinity conditions.

The greater succulence in the leaves when plants were subjected to the highest level of NaCl, may have led to the dilution of ions (Na^+ and Cl^-) that were found in potentially toxic concentrations (Table 2). According to Azevedo Neto *et al.* (1996), less succulence in the roots of inoculated maize plants may have been favorable to the concentration of organic and inorganic soluble substances, contributing to its osmotic adjustment in relation to the external environmental conditions. Marcum and Murdoch (1994) working with grass forage crops both tolerant and sensitive to salinity, observed greater succulence in the shoot tissues of the tolerant plants. The authors discuss that the greater growth of the tolerant plants may be the result of ion dilution in the dry matter shoots of the plants.

When analyzing the succulence in leaves and roots, it was observed that roots showed much higher values than leaves. This fact explains the utilization of the root as a storage place for Na^+ and/or Cl^- ions, excluding them from metabolically active spots situated in the leaf.

The lower mean root succulence observed in the treatments with mycorrhizal plants, in comparison to the non-mycorrhizal treatments, was probably due to the high water content in the root tissues. This may have inhibited the action of the fungi, since AMF does not develop well under conditions of high soil humidity (Sieverding, 1979). Lewis *et al.* (1989) verified that root water content of maize was superior to that of the shoots dry matter. Greater succulence observed in leaves and root when the *B. humidicola* plants were subjected to an electrical conductivity of 16 dS m^{-1}, indicated that the plant performed an osmotic adjustment to the increased salinity of the soil. Therefore, succulence represented a fundamental mechanism utilized by *B. humidicola* for tolerance to salinity. The higher succulence detected in the root, could be expected in accordance to the statement by Bernstein (1961), showing that the roots make an osmotic adjustment more rapidly and lose turgescence more slowly than the seedlings dry matter.

The high level of available phosphorus in the soil (64.83 mg Kg^{-1}) most probably influenced both the number of *Glomus etunicatum* spores produced and the low percentage of mycorrhizal root colonization, despite the salinity level (Table 3). Copeman *et al.* (1996) confirmed the adaptation capacity of this AMF species to saline soils.

Authors such as Hirrel and Gerdemann (1980), Duke *et al.* (1986), Ojala *et al.* (1983) and Pfeiffer and Bloss (1988) noted that the percentage of root colonization by AMF decreased with an increase in the salinity of the soil as a result of the toxic effect of the ions. According to Ruiz-Lozano *et al.* (1996), the root colonization in lettuce plants was not affected when subjected to the median level of salinity (3g NaCl kg^{-1}). Furthermore, Chambers *et al.* (1980) verified that the addition of various levels of salinity in the soil had no negative influence on mycorrhizal colonization.

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RESUMEN

Efecto del cloruro sódico en *Brachiaria humidicola* inoculada con el hongo formador de micorrizas arbusculares *Glomus etunicatum*

El experimento fue realizado con el objetivo de investigar los efectos de niveles crecientes de NaCl en la gramínea *Brachiaria humidicola* inoculada con la micorriza *Glomus etunicatum.* Las concentraciones de NaCl utilizadas fueron 0, 0,22, 1,09, 1,96 y 2,84 $g.kg^{-1}$ de suelo; que corresponden a conductividades eléctricas de 2,22, 4,00, 8,13, 12,53 y 16,50, respectivamente. La salinidad del sustrato redujo la materia seca en distintas partes de la planta cuando la conductividad eléctrica del suelo alcanzó 8 $dS.m^{-1}$. El área foliar y la suculencia aumentaron proporcionalmente a la salinidad en el sustrato. El porcentaje de colonización micorrícica en las raíces y el número de esporas del hongo micorrícico arbuscular (AMF) en la rizosfera no fueron afectados por la concentración de NaCl en el suelo.

Palabras clave: micorrizas, estrés, inoculación, salinidad

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