Salt stress response of *Brachiaria* plants with and without inoculation of arbuscular mycorrhizal fungi

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INTRODUCTION. – Saline soils are considered a problem world-wide, especially in arid and semi-arid regions. These soils present high levels of salinity due to the soluble salts in irrigation water and fertilizers (COPEMAN *et al.*, 1996). One of the advantages that can be obtained with crops that are tolerant to salinity is a reduction in the costs of implantation when compared to the costs of physical-chemical recuperation of saline soils. Arbuscular mycorrhizal fungi (AMF) improve the osmotical adjustment of the leaf tissue in colonized plants through an increase in the concentration of soluble material (AUGÉ and STODOLA, 1990). Therefore, the tolerance of mycorrhizal inoculated plants to the salinity of the soil is probably related to their ability to osmotically adjust themselves to the solution potential of the soil. Several investigations have shown that AMF inoculation can increase the absorption and production of biomass of plants grown in saline soils (HIRREL and GERDEMANN, 1980; OJALA *et al.*, 1983). However, in some cases, mycorrhizal associations can have a negative impact on plants in conditions of high salinity (JOHNSON-GREEN *et al.*, 1995). The goal of this study was to evaluate the effect of different levels of NaCl on *Brachiaria humidicola* both with and without inoculation of the AMF *Glomus etunicatum*.

MATERIALS AND METHODS. – The experiment was carried out in a green house at a temperature 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 hour at a temperature of 121 °C under a pressure of 101 KPa in 24-hour intervals for 3 consecutive days. Fertilizers were applied 15 days prior to planting with: 275 mg of ammonia sulphate, 165 mg of simple super phosphate and 22 mg of potassium chloride (SANTOS and LIRA, 1998). Three kg of soil was used in plastic pots with a 4 L capacity. Chemical and physical analyses of the soil were performed at the Laboratory of soil of the Empresa Pernambucana de Pesquisa Agropecuária (Pernambuco Farming Research Company) (IPA) following the method of EMBRAPA (1979). The results 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 80 $g.kg^{-1}$; 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 particle density 2.7 $g.cm^{-3}$.

The plant used was the *Brachiaria* (*Brachiaria humidicola* Rendle cv. 409). The seeds were disinfected with hypochloride (20%) for two minutes, rinsed repeatedly in sterilized distilled water and placed in trays containing vermiculite substrate to germinate for a period of 7 days. Next, the transplant was effected using two $plants.pot^{-1}$. The AMF used was an isolate of *Glomus etunicatum* Becker and Gerdemann, supplied by the Centro Nacional de Pesquisa em Agrobiologia (National Agrobiology Research Center) EMBRAPA - RJ. The AMF was multiplied in pots with the host plant *Brachiaria humidicola*; 100 $spores.pot^{-1}$ were inoculated together with soil-colonized root pieces near the roots. The plants were irrigated with distilled water. The excess water was drained naturally to the collector vase and, whenever possible, replaced in the soil of the vase from which it had drained. The water used received a classification of low salinity (C_1S_1), with an electrical conductivity of 0.01 $dS.m^{-1}$ and, according to RICHARDS (1974), can be used for the irrigation of most plants, in any type of soil.

The levels of NaCl and electrical conductivity (EC) were determined (RICHARDS, 1974) using the soil saturation extract, with 0, 0.224, 1.09, 1.96 and 2.84 $g.kg^{-1}$ of soil. The electrical conductivity of the saturated extract was 2, 4, 8, 12 and 16 $dS.m^{-1}$ and the osmotic potential -0.08, -0.14, - 0.30, -0.46 and -0.60 MPa, respectively. Increased doses of NaCl were applied to the soil 15 days before planting. After the salinity treatment, the soil presented a pH of around 7.6. The relative values of temperature and humidity of the air were obtained using a thermohygrograph. The plants were harvested 60 days after planting (DAP), and the following parameters were evaluated: plant height (using a measuring tape); percentage of shoot and root dry matter (obtained from the relation between the weights of the dry matter and the green matter); percentage of root colonization was obtained after the staining of the roots (PHILLIPS and HAYMAN, 1970), using the grid-line intersect method described by GIOVANNETTI and MOSSE (1980); the number of spores was obtained after wet sieving and centrifugation (GERDEMANN and NICOLSON, 1963).

The experimental design was in randomized blocks with a factorial arrangement of 5 x 2. The levels of NaCl were: 0, 0.22, 1.09, 1.96 and 2.84 $g.kg^{-1}$, corresponding to electrical conductivity of 2, 4, 8, 12 and 16 of the saturated extract of the soil in both the presence and absence of *Glomus etunicatum* AMF with 4 replicates. The STEEL and TORRIE (1960) mathematical model was applied for statistical analysis. The variance and regression analyses were determined using the F and Tukey tests ($p < 0.05$), through the SANEST program (ZONTA *et al.*, 1984).

RESULTS AND DISCUSSION. – The variance analysis showed that the salinity had a significant effect on the percentage of shoot dry matter (%SDM) in *Brachiaria* plants (Table 1). Regardless of the presence or absence of AMF, a reduction from 18.02% (control) to 13.25% was detected in the %SDM with an increasing in the level of EC. The absence of any alteration in the %SDM up to the level of 8 $dS.m^{-1}$ of EC in the soil indicated that the NaCl did not interfere with the growth and

TABLE 1. – *Percentage of shoot (SDM) and root (RDM) dry matter at 60 DAP, plant height (PH) 30 and 60 DAP of* Brachiaria *with and without mycorrhizal inoculation in soil with increasing levels of electrical conductivity.*

EC ($dS.m^{-1}$)	With AMF				Without AMF			
	SDM (%)	RDM (%)	PH (cm)[1]	PH (cm)[2]	SDM (%)	RDM (%)	PH (cm)[1]	PH (cm)[2]
2	18.57	11.40	70.37	115.50	17.47	10.33	67.30	107.37
4	18.23	11.48	65.72	101.25	17.58	10.11	60.17	97.00
8	16.04	11.40	39.92	80.00	16.23	9.56	36.10	71.37
12	13.62	10.21	19.67	71.00	12.88	7.20	25.75	71.75
16	13.79	5.62	17.87	65.83	12.90	6.55	15.67	46.00
Average	16.05	10.0a	42.71	86.72	15.41	8.75b	41.00	78.70
F (Fungi)	0.29 n.s.	0.04*	0.58 n.s.	0.06 n.s.				
F (Salinity)	0.00*	0.00*	0.00*	0.00*				
F (Fungi x Salinity)	0.96 n.s.	0.37 n.s.	0.54 n.s.	0.63 n.s.				
SMD (0.05)	2.73	1.25	9.64	19.47				

* Significant at the 0.05 probability level. n.s. not significant. Averages followed by the same letter do not differ statistically ($p < 0.05$) according to F and Tukey's test. Average from four replicates.

[1] Plant height at 30 DAP.

[2] Plant height at 60 DAP

that the *Brachiaria* is therefore tolerant to salinity. However, there are no references to the behaviour of *Brachiaria* in salinity environments among the research material on AMF. This grass, according to TABOSA (1982) demonstrated itself to be superior, in terms of tolerance, to *Cynodon dactylon* («Grama de Burro»), which, according to CARTER (1975) and BOGDAN (1977), is a species with recognizable tolerance to salinity.

The %SDM was reduced with the increase in soil salinity (Fig. 1A). Plants subjected to 16 $dS.m^{-1}$ showed a decrease of 27% in the relative production, in comparison to control plants (Fig. 1B).

The percentage of root dry matter (%RDM) could be described by a quadratic equation (Fig. 1C). Significant differences were found, resulting not only from the levels of salinity, but also from the AMF inoculation (Table 1). In comparison with the control, there was a decrease of

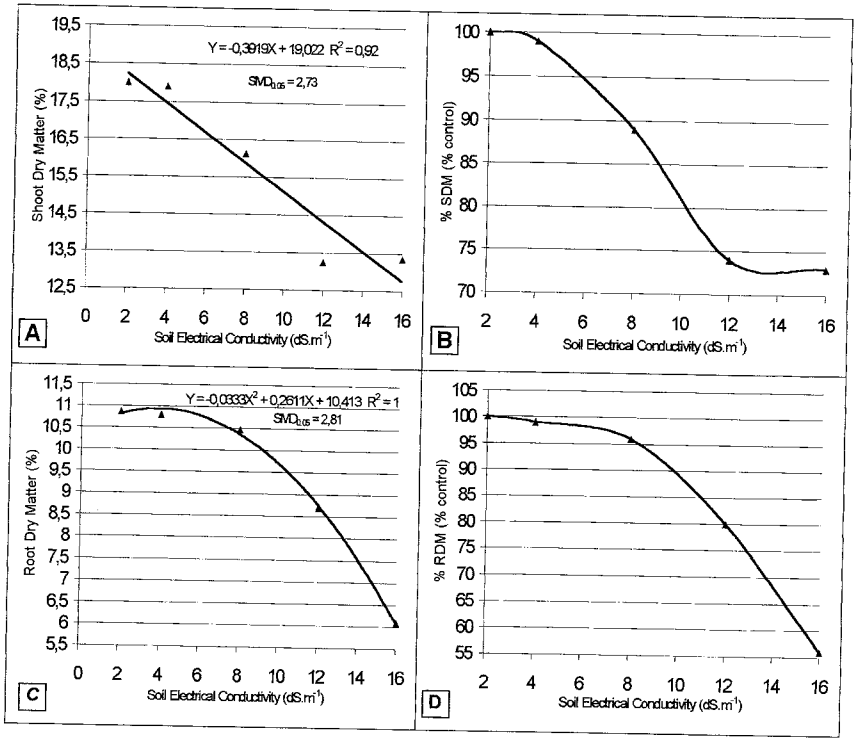

FIG. 1. – Percentage of shoot (A, B) and root (C, D) dry matter in *Brachiaria* plants in soil with increasing levels of electrical conductivity. Absolute (A, C) and relative (B, D) values.

up to 44% in the relative production when the plants were subjected to the EC at 16 $dS.m^{-1}$ (Fig. 1D). The AMF had a significant influence on the %RDM in the *Brachiaria* plants, with a gain of 14% in the production, when compared to the non-inoculated plants.

Plant height, at 30 and 60 DAP, diminished with the increase of electrical conductivity in the soil solution (Figs. 2A e 2C). At 60 DAP, *Brachiaria* plants showed a better physical aspect at all levels of EC of the soil, when compared with those at 30 DAP (Table 1). In comparison to the control samples, the reductions in the height of the *Brachiaria* plants at the EC level of 16 $dS.m^{-1}$ at 30 and 60 DAP were 76 and 50%, respectively (Figs. 2B e 2D). These results are in partial accordance with those observed by MAAS *et al.* (1986), where decreases in the height of *Sorghum* during the vegetative stage were 76 and 82%. Reductions in the height of maize plants produced by elevated levels of salinity have been reported by several researchers.

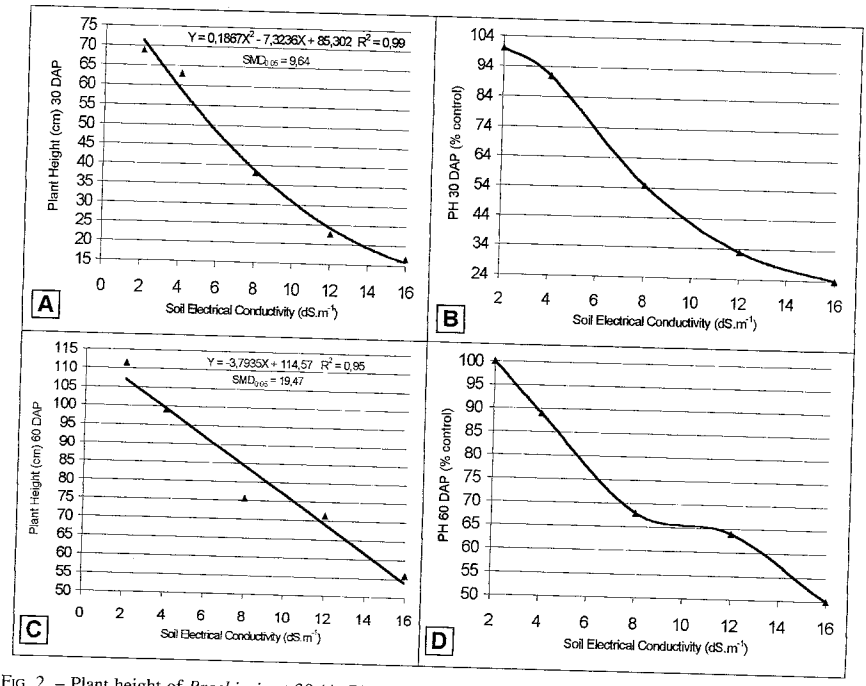

FIG. 2. – Plant height of *Brachiaria* at 30 (A, B) and 60 DAP (C, D), in soil with increasing levels of electrical conductivity. Absolute (A, C) and relative (B, D) values.

TOTAWAT and MEHTA (1985) cited that at 12 $dS.m^{-1}$ of EC, the height of maize plants was reduced to about 43%. In the present work, at this same level, *Brachiaria* plants at 30 DAP presented reductions in height of up to 67%. However, at 60 DAP, the reduction was only 36%, less than that found by TOTAWAT and MEHTA (1985). Significant differences in plant height, both at 30 as well as 60 DAP, were only detected when the EC of the soil reached 8 $dS.m^{-1}$. The reductions were of 45 and 32%, respectively, when compared to the control plants (Figs. 2B e 2D).

On the other hand, the multiplication of spores was not affected by the salt in the soil (Table 2). COPEMAN *et al.* (1996) confirmed the adaptation capacity of this species of AMF to salt soils. Furthermore, the high level of phosphorus in the soil (64.83 $mg.kg^{-1}$) may have contributed to the low production of *Glomus etunicatum* spores.

It was also verified that the percentage of root colonization was not affected by the increased doses of NaCl in the soil (Table 2). The *Brachiaria* plants did present low rates of root colonization, but this fact may also be attributed to the high level of phosphorus in the soil which

TABLE 2. - Spore density of Glomus etunicatum and percentage of root colonization (Col. Root) in Brachiaria plants with and without mycorrhizal inoculation in soil with increasing levels of electrical conductivity, 60 DAP.

EC (dS.m^{-1})	With AMF		Without AMF	
	Spore (150 g soil)[1]	Col. Root (%)[2]	Spore (150 g soil)[1]	Col. Root (%)[2]
2	196.50	5.50	0.00	0.00
4	201.75	3.00	0.00	0 00
8	246.00	4.00	0.00	0.00
12	240.25	10.00	0.00	0.00
16	239.75	5.00	0.00	0.00
Average	181.70a	4.99a	0.00b	0.00b
F (Fungi)	0.00*	0.00*		
F (Salinity)	0.06 n.s.	0.10 n.s.		
F (Fungi x Salinity)	0.40 n.s.	0.10 n.s.		
SMD (0.05)	0.38	0.28		

* Significant at the 0.05 probability level. n.s. not significant. Averages followed by the same letter do not differ statistically ($p < 0.05$) according to F and Tukey's test. Average from four replicates.

[1] Original data, in variance analysis were transformed in Log (X).

[2] Original data, in variance analysis were transformed in $\sqrt{(X+1)}$.

had a negative influence on the rate of root colonization of the *Brachiaria* plants. Researchers such as HIRREL and GERDEMANN (1980), DUKE *et al.* (1986), OJALA *et al.* (1983), and PFEIFFER and BLOSS (1988) noted that the percentage of colonization by AMF diminishes 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 radicular colonization in lettuce plants was not affected when subjected to a medium level of salinity. However, CHAMBERS *et al.* (1980) noted that the addition of elevated levels of salinity in the soil had a negative influence on mycorrhizal colonization.

CONCLUSIONS. – *Brachiaria humidicola* showed a tolerance to salinity when subjected to an EC of the soil of 8 dS.m^{-1}. The salinity of the soil reduced both plant height and percentage of shoot and root dry mat-

ter. The arbuscular mycorrhizal fungi had a significant influence on the percentage of root dry matter in the *Brachiaria* plants at different levels of NaCl in the soil. Root colonization and the number of *Glomus etunicatum* spores were not affected by the increased doses of NaCl in the soil.

REFERENCES

Augé R.M. and Stodola A.J.W.: Apparent increase in symplastic water contributes to greater turgor in mycorrhizal roots of droughted *Rosa* plants. *New Phytologist. 115*, 285 (1990).

Barros Filho A., Albuquerque I. de A., Torres C. de A. and Oliveira L.B. de: Levantamento pedológico e conservacionista da Estagio Experimental do Cedro-Vitória de Santo Antão-Pernambuco, p. 43. Instituto de Pesquisas Agronômicas de Pernambuco. *Boletim Técnico 15*, Recife (1966).

Bogdan A.V.: Tropical pastures and fodder plants, Londres, Longman Group Ltda, 475 p. (1977).

Carter D.L.: Problems of salinity in agriculture. p.25-35. In: Poljakoff-Mayber A., Gale J. (Ed.) Plants in saline environments. Berlin Springer-Verlag (1975).

Chambers C.A. Smith S.E. and Smith F.A.: Effects of ammonium and nitrate ions on mycorrhizal infection, nodulation and growth of Trifollum subterraneum. *New Phytologist. 85*, 47 (1980).

Copeman R.H., Martin C.A. and Stutz J.C.: Tomato growth in response to salinity and mycorrhizal fungi from saline or nonsaline soils. *Hortscience. 31*, 341 (1996).

Duke E.R., Jhoson C.R. and Koch K.E.: Accumulation of phospharus, dry matter and betaine during NaCl stress of split-root citrus seedlings colonized with vesicular-arbuscular mycorrhizal fungi on zero, one or two halves. *New Phytologist. 104*, 583 (1986).

EMBRAPA Serviço Nacional de Levantamento e Conservação de Solos, Manual de Análises do Solo, Rio de Janeiro v. 1 (1979).

Gerdemann J.W. and Nicolson T.H.: Spores of mycorrhizal Endogone species extracted for soil by wet sieving and decanting. *Transactions of the British Mycological Society. 46*, 235 (1963).

Giovannetti M. and Mosse B.: An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist. 84*, 489 (1980).

Hirrel M.C. and Gerdemann J.W.: Improved growth of onion and bell pepper in saline soils by two vesicular-arbuscular mycorrhizal fungi. *Soil Science Society American Journal 44*, 654 (1980).

Johnson-Green P.C., Kenkel N.C. and Booth T.: The distribution and phenology of arbuscular mycorrhizae along an inland salinity gradient. *Canadian Journal of Botany. 73*, 1318 (1995).

Maas E.V., Poss J.A. and Hoffman G.J.: Salinity sensitivity of sorghum at three growth stages. Irrigation Science, New York, V. 7 n. 1: 1 (1986).

Ojala J.C., Jarrel W.M., Menge J.A. and Johnson E.L.: V. Influence of mycorrhizal fungi on the mineral nutrition and yield of onion in saline soil. *Agronomy. 75*, 225 (1983).

Pfeiffer C.M. and Bloss H.E.: Growth and nutrition of guayule (Parthenium argentatum) in saline soil as influenced by vesicular-arbuscular mycorrhiza and phosphorus fertilization. *New Phytologist. 108*, 315 (1988).

Phillips J.M. and Hayman D.S.: Improved produces for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society. 55*, 158 (1970).

Richards L.A.: Suelos salinos y sodicos: diagnostico y rehabilitacion, Ed. 6 Mexico: Editorial Limusa, 172 p. (1974).

Ruiz-Lozano J.M. Azcón R. and Gómez M.: Alleviation of salt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. *Physiologia Plantarum. 98*, 767 (1996).

Santos D.C. dos and Lira M. de A.: Pastagens: *Brachiaria* spp.; *Digitaria decumbens*; *Panicum maximum*; *Cenchrus ciliares*; *Urochica moçambicencis*. p. 168. In: Recomendações de adubações para o Estado de Pernambuco. Ed. A. de F.J. Cavalcanti *et al.*, 2a aproximação. Recife-IPA (1998).

Steel R.G.O. and Torrie J.H.: Principles and procedures of statistics. McGraw, Hill. New York, 481 p. (1960).

Tabosa J.N.: Teste de tolerância à salinidade em gramíneas forrageiras tropicais. p. 135 UFRPE, Recife, Dissertação de Mestrado (1982).

Totawat K.L. and Mehta A.K.: Salt tolerance of maize and sorghum genotypes. *Annals of Arid Zone. 24*, 229 (1985).

Zonta E.P., Machado A.A. and Silveira Junior P.: Sistemas de análise estatística para microcomputadores (SANEST). Pelotas: Departamento de Matemática e Estatística, 151 p. (1984).

ABSTRACT. – The experiment was carried out to investigate the effects of different levels of NaCl in *Brachiaria humidicola* with and without inoculation of the arbuscular mycorrhizal fungi (AMF) *Glomus etunicatum*. The soil used was alluvial eutrophic with a pH of 7.6 and the plant was the *Brachiaria humidicola* cv 409. The levels of NaCl and electrical conductivity of the saturated extract were 0, 0.22, 1.09, 1.96 and 2.84 $g.kg^{-1}$ of soil with 2, 4, 8, 12 and 16 $dS.m^{-1}$, respectively. *Brachiaria humidicola* showed tolerance to salinity when subjected to an EC of the soil of 8 $dS.m^{-1}$. The soil salinity reduced plant height, as well as the percentage of root and shoot dry matter. The AMF had a significant influence on the percentage of root dry matter in the *Brachiaria* plants under different levels of NaCl in the soil. The percentage of root colonization and the number of AMF spores were not affected by the increased doses of NaCl in the soil.