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Mendes Mascena de Almeida, Aldênia; Freire Gomes, Vânia Felipe; Furtado Mendes
Filho, Paulo; Feitosa de Lacerda, Claudivan; Dias Freitas, Emanuel

Influence of salinity on the development of the banana colonised by arbuscular
mycorrhizal fungi

Influence of salinity on the development of the banana colonised by arbuscular mycorrhizal fungi[1]

Influência da salinidade no desenvolvimento de bananeiras colonizadas com fungos micorrízicos arbusculares

Aldênia Mendes Mascena de Almeida[2]*, Vânia Felipe Freire Gomes[2], Paulo Furtado Mendes Filho[2], Claudivan Feitosa de Lacerda[3] and Emanuel Dias Freitas[3]

ABSTRACT - This study evaluated the effect of salt stress on the growth of banana seedling colonized with mycorrhizal fungi (AMF) on a substrate from a Quartzipsamment. The experiment was conducted in a greenhouse, using a completely randomized design in split plots; the plots had 5 levels of salinity in irrigation water (0.5, 1.5, 2.5, 3.5 and 4 5 dS m^{-1}) and the subplots of four collection periods (40, 60, 80 and 100 days after transplanting), with 4 repetitions, totaling 80 experimental units. The seedlings of banana cv. "Prata" was produced by micropropagation and inoculated with arbuscular mycorrhizal and acclimatization for 40 days. Evaluations were made of leaf gas exchange, shoot dry mass, nutrient content, mycorrhizal root colonization and spore density. Increased levels of salinity caused reduction in dry matter production and photosynthetic rate, which may be associated with osmotic effects of salts in the soil, the increase in sodium and reduced the levels of N in leaves. Salinity reduced root mycorrhizal colonization, but did not influence the density of AMF spores under the conditions of this study.

Key words: *Musa* ssp.. Arbuscular Mycorrhizal Fungi. Salt stress.

RESUMO - Este trabalho teve o objetivo de avaliar o efeito do estresse salino no crescimento inicial de mudas de bananeiras colonizadas com fungos micorrízicos arbusculares (FMA) em um substrato proveniente de um Neossolo Quartzarênico. O experimento foi conduzido em casa de vegetação, utilizando-se delineamento inteiramente casualizado em parcelas subdivididas, sendo as parcelas representadas por cinco níveis de salinidade aplicados na água de irrigação (0,5; 1,5; 2,5; 3,5 e 4,5 dS m^{-1}) e as subparcelas por quatro períodos de coleta (40; 60; 80 e 100 dias após transplantio das mudas), com quatro repetições, totalizando 80 unidades experimentais. As mudas de bananeira cv. "Prata" foram produzidas por micropropagação e inoculadas com FMA e passaram por um período de micorrização e aclimatação de 40 dias. Foram realizadas avaliações das trocas gasosas foliares, massa seca da parte aérea, teores de nutrientes, colonização micorrízica arbuscular e densidade de esporos. O aumento nos níveis de salinidade provocou redução na produção de matéria seca e na taxa fotossintética, o que pode estar associado aos efeitos osmóticos dos sais no solo, ao aumento nos teores de Na e a redução dos teores de N nas folhas. A salinidade reduziu a colonização micorrízica, porém não influenciou na densidade de esporos no substrato.

Palavras-chave: *Musa* ssp.. Fungos micorrizicos arbusculares. Estresse salino.

INTRODUCTION

The banana is a fruit grown in all Brazilian states, from the coastal plain to the highlands of the interior, although there are restrictions on its cultivation due to such climatic factors as temperature and rainfall (GONDIM *et al.*, 2009). However, despite these limitations, the banana is one of the most exploited fruits in tropical regions, being among the most consumed and important in the diet of the global population (COSTA *et al.*, 2009), besides being a source of income for the population, and helping to maintain people in the countryside.

In 2012, the area planted with bananas in Brazil was approximately 480,800 ha, giving an estimated production of 7,134,609 t year^{-1}, with the northeast being the largest producer, especially the States of Rio Grande do Norte and Ceará (IBGE, 2014). The climatic conditions of the Brazilian Northeast are favourable to the development and production of the crop throughout almost the entire area (GONDIM *et al.*, 2009). However, productivity is still low in Brazil compared to other producing countries (SILVA *et al.*, 2005), as there are various problems presented by the Brazilian banana crop which result in lower productivity and in fruit of low quality (COSTA *et al.*, 2009); this is due to technologies which are available and suitable for exploitation of the crop not being used (MEDEIROS *et al.*, 2008).

One of the main problems in cultivating the banana, especially in arid and semi-arid regions, is the salinity of the soil and water, since the crop shows significant limits on production when grown under salt stress (SILVA JUNIOR *et al.*, 2012; WILLADINO *et al.*, 2011). The species is considered to be sensitive to salinity (SILVA *et al.*, 2009) and in order for adequate vegetative growth requires values for the electrical conductivity (EC) of the irrigation water of up to 1.00 dS m^{-1}, and a sodium adsorption ratio (SAR) less than or equal to 10 (GONDIM *et al.*, 2009).

Salinity is a topic which is discussed all over the world, especially in arid and semi-arid regions, due to its negative effects on food production and on the physical, chemical and biological properties of the soil. According to Edelstein, Plaut and Ben-Hur (2011), in these regions, salinisation of the soil can occur due to i) a negative water balance (precipitation<evapotranspiration) in any one period of the year, ii) the source material, and iii) the use of inadequate systems of irrigation and drainage.

In the northeast of Brazil, problems of salinity have been noted in virtually all irrigated areas where the banana is one of the main crops (MEDEIROS *et al.*, 2008). Soil salinity therefore becomes a limiting factor on cultivation, being a serious problem that limits agricultural production and crop yield (LÚCIO *et al.*, 2013), due to the adverse effects on soil properties: physical (clay dispersion), chemical (soil pH and nutritional imbalance), and microbiological (arbuscular mycorrhizal colonisation, microbial biomass carbon and fungal spore density, among others). Despite these adverse effects on the microbiological properties of the soil, few reports are found of studies involving these variables and arbuscular mycorrhizal fungi (AMF) (LÚCIO *et al.*, 2013; YUAN *et al.*, 2007). The AMF provide the plants with many benefits, such as an increase in growth and nutrient acquisition (LÚCIO *et al.*, 2013), and promote an increased tolerance to adverse factors (temperature and salinity).

The aim of this study therefore, was to evaluate salt stress in the initial growth of seedlings of the banana, *Musa* ssp., colonised by arbuscular mycorrhizal fungi and grown in a sandy substrate under increasing conditions of salinity of the irrigation water.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Department for Soil Science of the Federal University of Ceará (UFC), in Fortaleza in the State of Ceará, Brazil (3°44' S, 38°33' W, at 20 m). The greenhouse has an area of 11.25 m^2 (4.5 m x 2.5 m), covered with 90% transparent PVC under 70% sombrite, closed at the sides with a screen to allow for natural ventilation.

The experimental design was completely randomised into split lots, the lots represented by five levels of salinity of the irrigation water (0.5, 1.5, 2.5, 3.5 and 4.5 dS m^{-1}), and the sub-lots by four collection periods for the banana seedlings (40, 60, 80 and 100 days after transplanting), with four replications, giving a total of 80 experimental units.

The solutions with different levels of salinity (0.5, 1.5, 2.5, 3.5 and 4.5 dS m^{-1}), which were applied to the pots (suspended 20 cm above the bench), were prepared by the addition of NaCl (PA) to water with the following characteristics: pH 6.5, EC = 0.3 dS m^{-1}, Ca^{2+} = 0.6 mmol$_c$ L^{-1}, Mg^{2+} = 0.8 mmol$_c$ L^{-1}, Na^+ = 1.5 mmol$_c$ L^{-1}, K^+ = 0.1 mmol$_c$ L^{-1}, Cl^- = 2.2 mmol$_c$ L^{-1} and HCO_3^{2-} mmol$_c$ L^{-1}. To calculate the amount of NaCl, the ratio between the electrical conductivity of the water (ECa) and the concentration of salts (mmol$_c$ NaCl L^{-1} = EC x 10) was used, taken from Rhoades and Loveday (1990).

The soil used in the experiment was collected at a depth of 0-20 cm in the irrigated area of the town of Tabuleiro de Russas, Ceará, and is classified as a

quartzarenic Neosol (EMBRAPA, 2006), with a pH of 5.0 and electrical conductivity of 0.27 dS m⁻¹.

Seedlings of the banana cv. Prata were produced by micropropagation. After being selected and standardised, the plants were transplanted from the in-vitro culture medium to the pots containing the substrate, where an established population of mycorrhizal fungi already existed, properly identified by microscopic analysis which gave a mean value of 525 spores per 100 g of soil, predominantly of *Glomus sp*. and *Gigaspora sp*.

The seedlings spent a period of 40 days in the greenhouse with a view to acclimatising the plant as well as establishing the AMF. After this period of 40 days, irrigation of the pots with applications of saline water (750 mL) was begun, following an irrigation schedule of 24 h intervals, keeping the soil at field capacity, and applying a leaching fraction of 0.15 of the percolated water to the pots (AYERS; WESTCOT, 1985). During the experiment, the plants received 50 ml of Hewitt's nutrient solution (Hewitt, 1952) weekly, excluding P, so that the element did not interfere with fungal colonisation.

The plants were first collected 40 days after transplanting (DAT), before application of the salinity levels; the remaining samples were taken after application of the treatments at 60, 80 and 100 DAT. For each collection, leaf gas exchange, shoot dry mass, arbuscular mycorrhizal colonisation and the spore density of the arbuscular mycorrhizal fungi (AMF) were determined.

To characterise the gas exchange, net photosynthetic rate (A), stomatal conductance (gs) and transpiration (E) were evaluated. These measurements were taken from the first fully expanded leaf from the apex of the banana plant, using a plant gas exchange analyser (IRGA, ADC System). The photosynthetically active radiation (PAR) measured inside the greenhouse showed average values of 246, 240, 239 and 240 µmol m⁻² s⁻¹ for the measurements taken at 40, 60, 80 and 100 DAT respectively.

After the measurements of gas exchange and plant height, the shoots and root system of the banana plants were collected. The shoots were then placed to dry in a forced air circulation oven at a temperature of around 65 °C to constant weight. This material was later ground in a Wiley mill and used in the preparation of nitro-perchloric digestion extracts to determine the levels of P, K and Na. The levels of Na and K were analysed by flame photometry, and of P by colorimetry using the ascorbic acid reduction method. Total nitrogen (TN) was measured in an extract from sulphuric acid digestion by the semimicro Kjeldahl method, as described by Sarruge and Haag (1974).

For analysis of the arbuscular mycorrhizal colonisation, the roots were bleached by heating in a 10% solution of KOH. They were then stained, following a method adapted by Vierheilig *et al*. (1998). Quantification of root colonisation was by observation, on slides with a light-field optical microscope, of the presence of typical AMF fungal structures in the region of the root cortex of the stained fragments. Different classes of colonisation were later assigned by grouping samples according to estimates of the percentage of colonised root: class 1: 0-5%, 2: 6-25%, 3: 26-50%, 4: 51-75%, and 5: 76-100% (KORMANICK; MCGRAW, 1982).

The density of AMF spores in the substrate was evaluated by extraction of the spores using the technique of wet sieving, as per Gerdemann and Nicholson (1963). Samples of the sieved material were examined with the aid of a stereoscopic microscope to verify the presence of AMF.

The data were submitted to analysis of variance (F-test) and regression. Correlation analysis was also carried out for photosynthesis and stomatal conductance. Choice of regression models was based on the significance of the coefficients of the regression equations at a level of 5% probability. The SAEG/UFV (2007) statistical software was used as a tool to aid in the statistical analysis.

RESULTS AND DISCUSSION

Table 1 shows the results of the analysis of variance for SDM, stomatal conductance (*gs*), rate of transpiration (*E*), net photosynthetic rate (*A*), levels of N, P, K, Na, mycorrhizal colonisation and AMF spore density in banana plants under different conditions of salinity. A significant effect ($p > 0.01$) was seen on SDM for salinity level, period and interaction. There was no significant effect for salinity level on stomatal conductance (*gs*) or rate of transpiration (E*),* however a significant effect ($p > 0.05$) was found on the net photosynthetic rate (*A*). For period of evaluation, a significant effect ($p > 0.01$) was found on the variables under analysis, with a decrease in all three variables (*gs*, *E* and *A*). There was no significant effect for the interaction.

Salinity reduced the net photosynthetic rate (Figure 1A) and shoot dry matter production (SDM) in the banana plants (Figure 1B). The net photosynthetic rate displayed linear behaviour over time, noting that differences between treatments were seen from 60 days after transplanting (DAT) and intensified with time (Figure 1A). According to Taiz and Zeiger (2009), among the physiological processes affected by salinity, mainly photosynthesis stands out, as this can be inhibited by the accumulation of ions (Na⁺ and/or Cl⁻) in the chloroplasts or by stomatal effects. It is important to point out that although there was

Table 1 - Summary of analysis of variance for the effect of salinity of the irrigation water on SDM, stomatal conductance (*gs*), rate of transpiration (*E*), net photosynthesis (*A*), nutrient content (N, P, K and Na), mycorrhizal colonisation and AMF spore density in banana seedlings at 40, 60, 80 and 100 days after transplanting

SV	Mean square					
	Level of Salinity (S)	Residual (A)	Period of evaluation (T)	Interaction S x T	Residual (B)	Cv (%)
DF	5	15	3	15	45	-
SDM	1.99**	0.86	130.91**	0.81**	0.14	10.40
Gs	0.169[ns]	0.143	105.15**	0.151[ns]	0.933	11.02
E	3.732[ns]	0.547	338.47**	1.237[ns]	0.323	15.38
A	33.29*	1.950	130.92**	6.588[ns]	1.598	12.20
N	50.44**	1.1981	16.27*	15.98**	4.159	5.44
P	0.025[ns]	0.027[ns]	0.054[ns]	0.049[ns]	0.036	18.77
K	420.31	7.80	20.28*	33.19	15.84	17.77
Na	10.48**	0.083	1.86**	0.940**	0.064	20.64
Mycorrhizal colonisation	1810.18**	109.26	195.77*	293.27**	87.83	20.20
AMF spore density	105.21[ns]	39.78	102.95[ns]	93.49[ns]	40.25	16.50

SV= source of variation; SDA= Shoot dry matter (g); gs=stomatal conductance; E=transpiration; A= photosynthetic rate (mmol $m^{-2}s^{-1}$); N= nitrogen (g kg^{-1}); P = phosphorous (g kg^{-1}); K = potassium (g kg^{-1}); Na = sodim (g kg^{-1}); mycorrhizal colonisation (%) and AMF spore density (100 g soil). ** and * = Significant at 1% probability (p>0.01) and significant at 5% probability (p>0.05) respectively by F-test; ns = not significant

Figure 1 - Net photosynthetic rate (A) and shoot dry weight (B) in the banana submitted to five levels of salinity (■0.5, ▲1.5, ♦2.5, □3.5 and ○4.5 m^{-1}) for four periods of evaluation (40, 60, 80 and 100 DAT): (1A) (1B). * significant at 5% by F-test
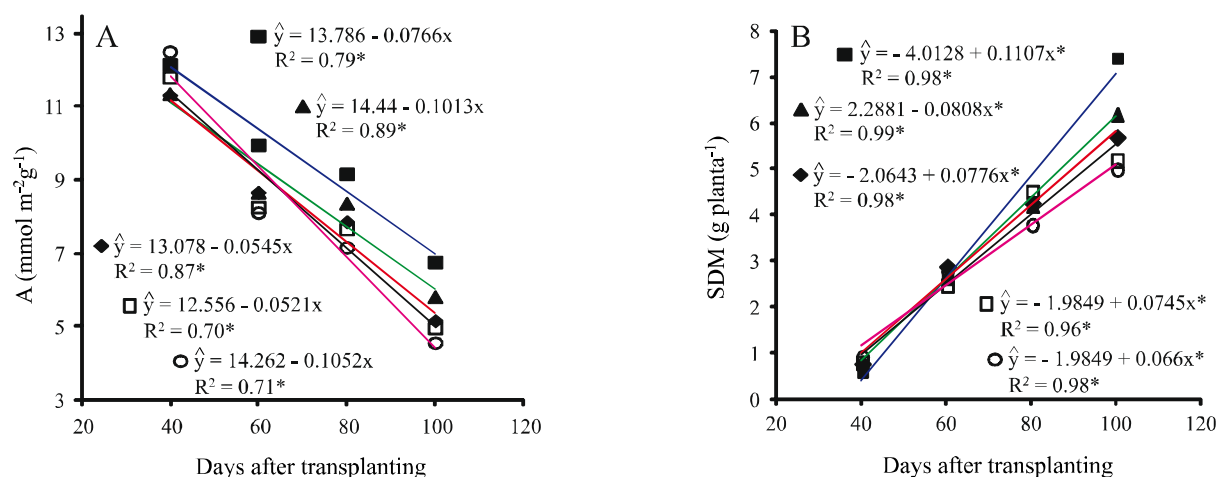
no significant effect for salinity on stomatal conductance (*gs*), the correlation coefficient between photosynthesis and stomatal conductance was high (gs = 0.0195A + 0.1084, r = 0.8).

In relation to SDM in the banana (Figure 1B), it can be seen that there was a linear increase with time, and that the differences between treatments became evident after 80 and 100 DAT, showing a reduction at the higher levels of salinity at a significance level of 5% (p>0.05). Similar results were found by Willadino *et al.* (2011) in tetraploid genotypes of the banana, where the same authors report that this reduction is directly associated with the osmotic and ionic component of the substrate solution, which are both inseparable from salt stress. Correia *et al.* (2009) also saw a reduction in shoot dry mass in peanut plants with increases in the salinity of the irrigation water of 0.4, 1.5, 3.0, 4.5 and 6 dS m^{-1}.

The effects of salt stress on the growth and productivity of crops are generally related mainly to nutritional imbalance, ion toxicity (Na$^+$ and Cl$^-$) and

low osmotic potential (EPSTEIN, BLOOM, 2006). One explanation for the reduction in SDM is attributed to the reduction in photosynthetic rate, and the diversion of energy destined for growth to activation and maintenance of the metabolic activity associated with adaptation to salinity (FLOWERS, 2004; MUNNS *et al.*, 2002).

Total nitrogen levels in the shoots of the banana plants were significantly influenced by the increasing levels of salinity ($p<0.01$), the period ($p<0.05$) and the interaction of salinity and period ($p<0.01$) (Table 1). However, according to regression analysis, it was not possible to fit an equation model. At 40 DAT, total nitrogen levels in shoots of the banana seedlings were similar for all treatments, with a mean value of around 30 g kg^{-1}, since for this period, the banana seedlings had not yet received any levels of salinity (period for acclimatisation and mycorrhizal colonisation), as shown in Figure 2A. For collections made at 60, 80 and 100 DAT, the levels of total nitrogen in the shoots at the lowest level of salinity remained unchanged and higher than in the remaining treatments. The reduction was greater for the treatment with the greatest salt concentration of the irrigation water, reaching values close to 20 g N kg^{-1} at 100 DAT. Some authors have attributed this decrease in nitrogen content to antagonism of the Cl- ion towards the absorption of NO_3^-, as the Cl^- ions are in direct competition with the NO_3^- for the same transporter in the plasma membrane (ARAGÃO *et al.*, 2010; BARHOUMI *et al.*, 2010; FEIJÃO *et al.*, 2011).

P levels in the shoots of the banana plants were not affected by the increasing levels of salinity, period of sampling or the interaction between salinity and period of sampling (Table 1 and Figure 2B). Different responses were obtained by Lacerda *et al.* (2006) in sorghum plants,

Figure 2 - Levels of nitrogen (A), phosphorus (B), potassium (C) and Na (D) in shoots of banana plants subjected to five levels of salinity (■0.5, ▲1.5, ♦2.5, □3.5 and ○4.5 dS m^{-1}) for four periods of evaluation (40, 60, 80 and 100 DAT). * Significant at 5% by F-test
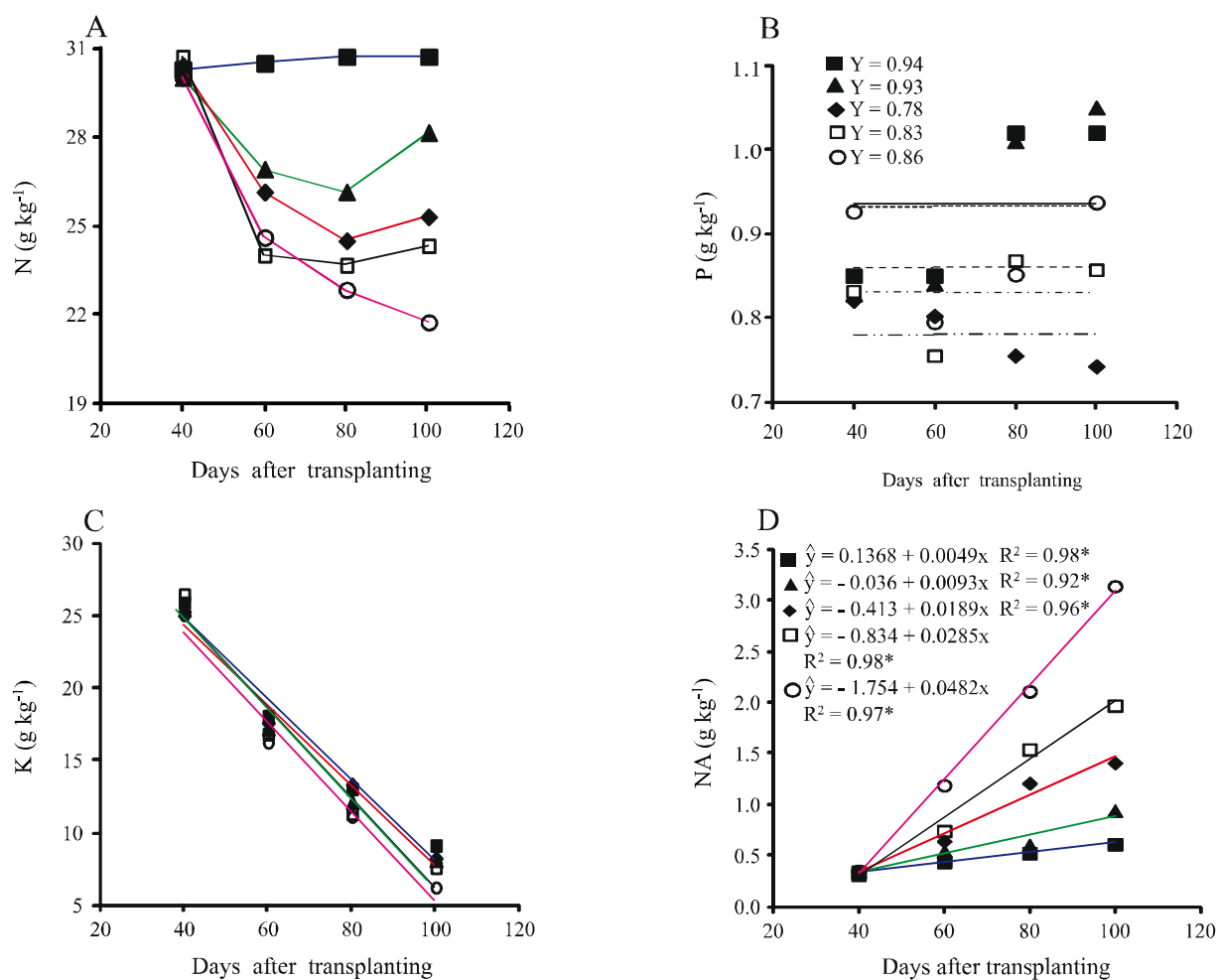
with the plants presenting greater values when subjected to salt stress. Levels of K in shoots of the banana plants were not affected by the levels of salinity, nor by the interaction between factors, but decreased linearly over time (Table 1 and Figure 2C).

Sodium levels in shoots of the banana plants were significantly influenced by the application of increasing levels of salinity ($p<0.01$), by the period of sampling ($p<0.01$) and the interaction between salinity and period of sampling ($p<0.01$) (Table 1), results which were expected, due to addition of the treatments. The N content of the shoots of the banana seedlings increased linearly with time after application of the salinity levels, noting that differences between treatments were first seen at 60 DAT and intensified over time, especially for those treatments with higher levels of salinity (Figure 2D).

The greater accumulation of Na may have contributed to the decrease in photosynthetic rate and dry matter production that was also found in samples taken after 60 DAT, since the element is considered to be a potentially toxic ion, particularly in the case of the banana (SANTOS; GHEYI, 1994; SILVA *et al.*, 2005).

The high levels of Na may explain the visual symptoms of toxicity (necrosis on the edges of the older leaves) observed in the banana seedlings during the experiment, especially at the salinity level of 4.5 dS m^{-1}. Rodrigues *et al.* (2012) mention that the damage caused by high salinity in soils is mainly attributed to the toxic effects of sodium on the plant metabolism.

For the variables associated with the arbuscular mycorrhizal fungi, a significant effect was found for level of salinity ($p<0.01$), period of evaluation ($p<0.05$) and the interaction salinity x time on arbuscular mycorrhizal colonisation (AMC) ($p <0.01$) (Table 1). However, no significant effects where seen for the treatments on the spore density of the arbuscular mycorrhizal fungi (AMF).

AMC varied from 30% to 80% according to regression analysis, values which were classified as low and high respectively (Figure 3A). These values are included in classes 3 (26-50%) and 5 (76-100%) according to Kormanick and Mcgraw (1982). At 40 DAT, the values for AMC were similar, since for this period the pots did not receive any level of salinity. However, for the application time of the treatments, there were significant differences between the lowest (0.5 and 1.5 dS m^{-1}) and the highest levels of salinity (2.5, 3.5 and 4.5 dS m^{-1}), with significant reductions in AMC over time in those plants under moderate and high salinity.

This result confirms those obtained by Tavares *et al.* (2012) and Li Fan *et al.* (2011) when studying mycorrhizal colonisation under different levels of salinity in plants of the sabiá and strawberry respectively. Lúcio *et al.* (2013) also saw a reduction in AMC in melon plants at levels of salinity higher than 1.63 dS m^{-1}. Evelin, Kapoor and Giri (2009) explain that the reduction in AMC under saline conditions may be associated with the direct action of the salts on the AMF having a negative effect on the formation and performance of symbiosis, with a decrease in spore germination and mycelial growth.

Figure 3 - Arbuscular mycorrhizal colonisation (A) and spore density of arbuscular mycorrhizal fungi (B) in a substrate of a quartzarenic Neosol cultivated with banana subjected to five levels of salinity (■0.5, ▲1.5, ♦2.5, □3.5 and ○4.5 dS m^{-1}) for four periods of evaluation (40, 60, 80 and 100 DAT). * Significant at 5% by F-test
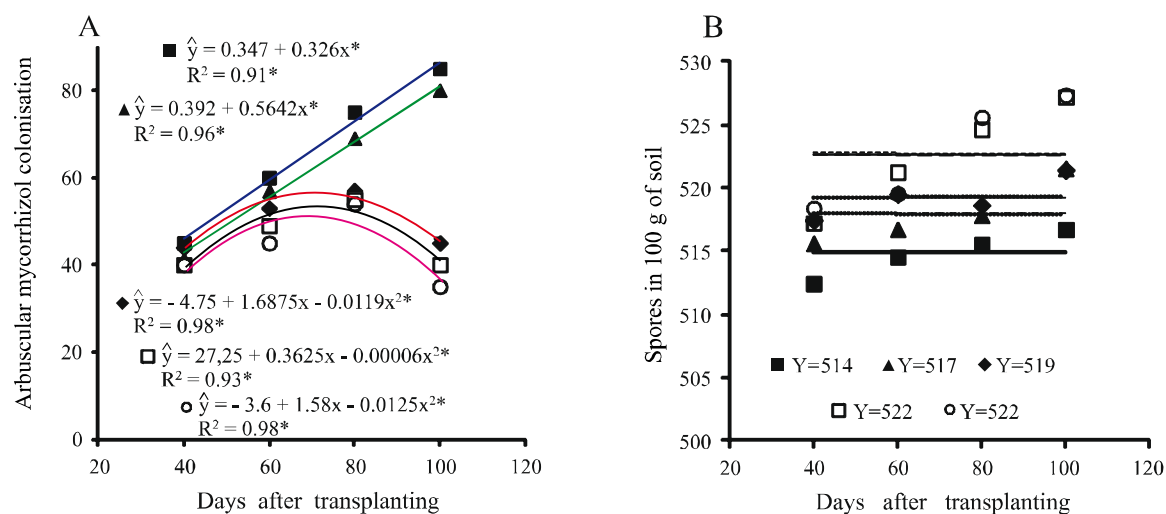
Figure 3B shows the values for AMF spore density in the soil. After application of the treatments with increasing levels of salinity, a predominance was seen of fungal spores from the genera *Glomus* and *Scutellospora* (data not shown). The occurrence of these genera confirms the distribution pattern of these fungi in tropical ecosystems, because they display a high adaptive capacity to wide ranges of arid and semi-arid conditions. Unlike the results found in the present work, Bezerra *et al.* (2010) found that the density of AMF spores in the soil increased with increases in the salinity of the irrigation water.

CONCLUSION

The increase in levels of salinity caused a reduction in dry matter production and net photosynthetic rate. Salinity reduced mycorrhizal root colonisation, but did not influence the density of the AMF spores.

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