

Effect of salinity stress on mycorrhizal association and growth response of peanut infected by *Glomus mosseae*

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ABSTRACT

Arbuscular mycorrhiza is a mutualistic association between fungi and higher plants, and play a critical role in nutrient cycling and stress tolerance. However, much less is known about the mycorrhiza-mediated enhancement in growth and salinity tolerance of the peanuts (*Arachis hypogaea* L.) growing in the arid and semi-arid areas. Therefore, mycorrhizal status of *Glomus mosseae* in diverse salinity levels on original substrate soil conditions was investigated. Different growth parameters, accumulation of proline content and salt stress tolerance were studied. These investigations indicated that the arbuscular mycorrhizal fungi could improve growth of peanuts under salinity through enhanced nutrient absorption and photosynthesis. Chlorophyll content and leaf water content were increased significantly under salinity stress by the inoculation with mycorrhizal fungi. Tolerance of the plants to salinity was increased and the mycorrhizal association was found highly effective in enhancing peanut growth and establishment in soils under salinity and deficient in phosphorus.

Keywords: arbuscular mycorrhizae; *Glomus mosseae*; *Arachis hypogaea*; salt tolerance; proline

Arid and semi-arid soils make up 40% of the total global area (Fisher and Turner 1978) and the problem of high salinity that occurs in these soils may be a significant factor limiting agricultural productivity (Flowers et al. 1977). Accumulation of soluble salts in the rhizosphere will reduce the water potential and consequently reduce the availability of water to plants. In addition, the uptake of these salts will, doubtless, affect the physiological processes of plants growing in these environments (Heyster and Nabors 1982). Therefore, the plant when exposed to high salinity, tends to change metabolic activity to produce certain organic compounds such as sucrose, to synthesise amino acids especially proline and accumulate inside the cells to reduce the internal water potential to counterbalance the soil water potential to maintain cell osmotic balance.

Among the biological approaches to enhance plant growth in saline conditions, the role of arbuscular mycorrhizal (AM) fungi has been well established. Most native plants and crops of arid and semi-arid areas are mycorrhizal and it has been suggested that AM fungal colonization might enhance salt tolerance of some plants (Tain et al. 2004). Genetic diversity of isolates of *Glomus mosseae* from different geographical areas may have

different fertilization roles in their host plants (Giovannetti et al. 2003). Studies have indicated that some plants such as tomato (Al-Karaki 2006) and soybean (Sharifi et al. 2007) showed increased growth under saline conditions when their roots were colonized by AM fungi. Rosendahl and Rosendahl (1991) studied the role of AM fungi in protecting cucumber plants (*Cucumis sativus* L.) against the stress induced by sodium chloride.

Since peanut plant is known to benefit from mycorrhizal association with significant increase in dry matter yield, and increased uptake of P and other elements (Krishna and Bagyaraj 1982), it would be interesting to study the additional beneficial effects of AM fungi in reducing salinity stress imposed on them (*Arachis hypogaea* L. var. *hypogaea* cv. *Florunner*). Therefore, the objective of the present study was to understand the growth and physiological changes of peanut plants under induced saline conditions.

MATERIALS AND METHOD

Seedling preparation and AM inoculation. The experiment was conducted using the autoclaved

soil amended with the original soil microbiota formulated by Khaliel (2010). It was observed that the highest relative mycorrhizal dependency of peanut was at the original substrate soil with a P base line of 6 µg/g soil and therefore the substrate soil without further P fertilization was selected for this trial. The electrical conductivities of the salinized irrigation waters and the leachates were determined by a conductivity meter model LF 56 (Wis – Techn. Weksl Etten. D812 Weilheim). The concentration of the soluble salts of this soil was 280 µg/ml with electrical conductivity (EC_{soil}) of 0.094/dSm. The substrate soil was apportioned into 2.0 kg plastic pots whose drainage holes were lined with perforated polyethylene bags.

Five salinity levels for the irrigation of pots were prepared by adding sodium chloride (NaCl) to distilled water. The NaCl concentrations were 0, 0.1, 0.2, 0.3 and 0.5M. Seeds of *A. hypogaea* were surface-sterilized with 0.01% mercuric chloride. Pre-germinated seeds were transplanted into each of 50 pots (one seed/pot) that constituted the five levels of salinity-stressed mycorrhizal treatments on top of a bed of 5 g of inoculum. The crude inoculum consisted of chopped roots and soil from a four-month old pot culture of *Glomus mosseae* propagated on Sudan grass. Pre-germinated seeds were also transplanted (one seed/pot) to make 50 salinity-stressed non-mycorrhizal peanuts pots. Each of the salinity-stressed non-mycorrhizal peanuts experimental pots received 5 ml of the crude inoculum filtrate which were sieved through a 37 µm sieve openings to remove mycorrhizal fungal spores to assure similar microbial population in all pots. The plants were established for 2 weeks prior to irrigation with the salt solutions in the greenhouse. The experiment was conducted in a completely randomized design with 10 salinity-stressed mycorrhizal and 10 salinity-stressed non-mycorrhizal peanuts at each of the five salinity treatments. Twenty pots with peanuts watered with distilled water were used as control. All pots were fertilized with half strength Hoagland's solution on a biweekly basis.

Acclimatization and salinization procedures. To avoid salinity shock and to acclimatize peanut seedlings and the candidate AM fungus to high NaCl concentrations, salinity stress was imposed on the seedlings by applying the saline irrigation water progressively. Each treatment was watered with the lowest NaCl concentration then with the next higher concentration until each treatment reached its designed irrigation concentration. The acclimatization took 2.5 weeks to get to 0.5M

treatment. The treatments were irrigated twice a week with appropriate saline concentration at the level avoiding the leaching. To maintain NaCl salinization at the correct level, before applying each subsequent saline irrigation, all the pots were leached with distilled water (approx. 700 ml/pot) to prevent salt accumulation beyond the experimental concentration. The experiment was terminated after 9 weeks of salt treatment.

Determination of growth and physiological parameters. The whole plant including shoot and root system was weighed to determine the fresh weight per plant (FW/plant), and then oven dried at 60°C for 48 h to determine the dry weight per plant (DW/plant) for each NaCl salinization treatment.

Tolerance index (T_i) of AM and non-AM peanut plants to different NaCl levels were determined according to Shetty et al. (1995):

$$T_i = \frac{\text{DW plant at salinity level} \times 100}{\text{DW plant at 0.0 level of salinity}}$$

Leaf nutrient analysis. Physiologically mature leaves from randomly selected plants per treatment were collected at harvest; leaves were pooled and ground to pass through a 40 µm mesh screen. Nitrogen is estimated by calorimetric method and the other nutrients (K, P and Na) were determined by atomic absorption spectrophotometry (AAS).

Leaf chlorophyll content. Leaf chlorophyll content was determined at harvest ($n = 3$), by extraction of chlorophyll with acetone (Harborne 1998). Semi-mature leaflets were collected and surface area was determined. Chlorophyll was extracted in 80% acetone and the supernatant was quantified with a spectrophotometer at 645 and 663 nm, and compared to an 80% acetone blank standard. Total chlorophyll content was expressed as mg/g fresh weight.

Free proline content. The determination was conducted as per the method of Bates (1973). The proline concentration (µmol/g FW) was determined from a standard curve and calculated as follows:

$$\text{Proline} = \frac{[(\mu\text{g proline/ml} \times \text{ml toluene})/115.5 \mu\text{g}/\mu\text{mol}]}{[(\text{g sample})/5]}$$

Leaf relative water content. A leaf from a third branch of the main stem from 3 salt-stressed mycorrhizal and 3 salt-stressed non-mycorrhizal plants at each salinity level was selected and removed at 10:00 h in the morning. The fresh weight was recorded. The leaves were submerged in distilled water in Petri dishes and kept for 5 h in the dark. The saturated weight (SW) was determined. The leaves were oven dried at 60°C for 48 h and then dry weight was determined. The leaf relative water content (LRWC)

was calculated according to the formula devised by Turner and Kramer (1980):

$$\text{LRWC} = \frac{(\text{FW} - \text{DW}) \times 100}{\text{SW} - \text{DW}}$$

Mycorrhizal assessment. Determination of colonization percentage of roots and determination of number of spores per gram was carried out according to Phillips and Hayman (1970).

Relative response. The difference in FW/plant, DW/plant, proline content and leaf relative water content between the salinity-stressed mycorrhizal peanuts (SMP) and salinity-stressed non-mycorrhizal peanuts (SNMP) within each treatment was exposed as relative response (= % increase) using the following equation:

$$\text{Relative response (\%)} = (\text{SMP} - \text{SNMP}) \times 100/\text{SNMP}$$

Data analysis. Data were analysed using a simple regression analysis comparing regression equations and elevations. Student *t*-test was used to compare mean pair wise of salinity-stressed mycorrhizal peanuts and salinity-stressed non-mycorrhizal peanuts of the same treatment (within each salinization level).

RESULTS

Plant growth characteristics. All the physical parameters were highly adversely affected by the salinity treatments. A growth analysis indicated a significant ($P \geq 0.05$) negative relationship that accounted for 93.5% of the variation in FW/plant for salinity-stressed and 86.1% of variation in FW/plant for salinity-stressed non-mycorrhizal peanuts in response to added NaCl (Figure 1).

Similarly a negative linear relationship ($P \geq 0.05$) in DW/plant was observed for salinity-stressed mycorrhizal plants (86.6%) and salinity-stressed non-mycorrhizal peanuts (63.8%) in response to NaCl treatments. Comparison of regression of slopes of FW/plant and DW/plant of salinity-stressed mycorrhizal peanuts and salinity stressed non-mycorrhizal peanuts indicated no significant difference ($P \geq 0.05$). However, comparison of elevation indicated that salinity-stressed mycorrhizal peanuts had significantly greater ($P \geq 0.05$) FW/plant and DW/plant than salinity-stressed non-mycorrhizal peanuts. The relative response in FW/plant and DW/plant between salinity-stressed mycorrhizal peanuts and non-mycorrhizal peanuts tented to form a near-linear increase with increasing salinization levels.

Salinity-stressed mycorrhizal peanuts had significantly greater fresh and dry biomass compared with salinity-stressed non-mycorrhizal peanuts grown at the same salinization levels at all NaCl treatments. Increasing concentrations of NaCl significantly ($P \leq 0.05$) reduced total plant DM (Figure 1). On average, plants inoculated with AM exhibited a significantly ($P \leq 0.05$) greater total plant DM (+53%) compared to non-AM plants. The mean dry weight per plant (DW/plant) of salinity-stressed mycorrhizal peanuts grown at the highest salinization level (0.5M) was more than 87% that of salinity-stressed non-mycorrhizal peanuts grown in the same salinization level (Figure 1). In non-AM plants treated with 0.2 and 0.3M NaCl, there was a reduction (–20% and –47%) in total plant DM compared to control (0M NaCl) plants. Non-AM plants treated with 0.5M NaCl exhibited a maximum decrease in total plant DM (–66%).

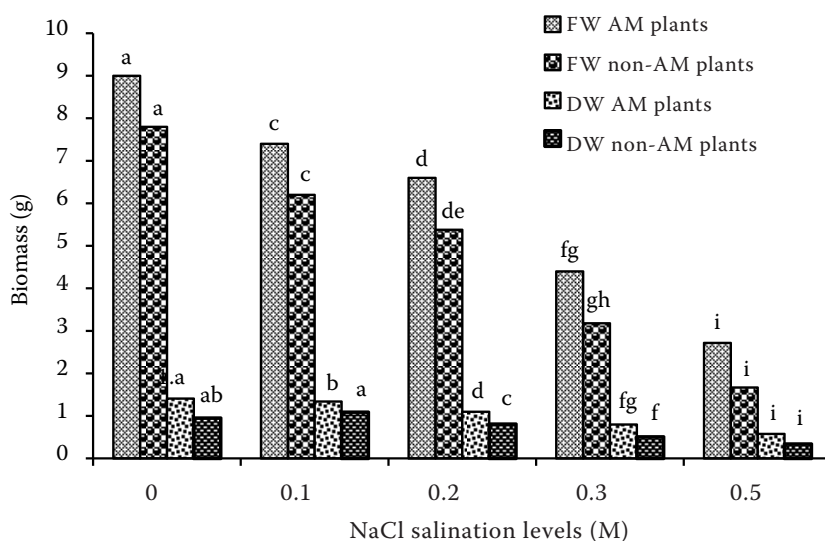


Figure 1. Effect of salinity stress on fresh and dry weights of mycorrhizal and non-mycorrhizal peanuts

Table 1. Effect of salinity stress on leaf macronutrients, Na and chlorophyll content in mycorrhizal and non-mycorrhizal peanuts

NaCl (M)	AM inoculation	N	P	K	Na	Chlorophyll (mg/g FW)
(%)						
0	–AMF	2.54	0.12	1.25	0.02	1.98
	+AMF	2.98*	0.22*	1.75*	0.03*	3.88*
0.1	–AMF	2.46	0.10	1.18	0.02	1.90
	+AMF	3.26**	0.26**	1.90**	0.03*	4.02**
0.2	–AMF	2.28	0.10	1.02	0.02	1.81
	+AMF	3.02*	0.20	1.40	0.03	3.70*
0.3	–AMF	2.12	0.09	1.00	0.02	1.52
	+AMF	2.51	0.20	1.26	0.04**	3.16
0.5	–AMF	2.02	0.09	0.98	0.02	1.30
	+AMF	2.38	0.18	1.20	0.04**	2.84

* $P \leq 0.05$ or ** $P \leq 0.01$; data not followed by asterick (s) are not significant

The tolerance index of salinity in AM plants was higher than that of the non-AM plants. The T_i showed a gradual decrease as the salinity level increased from lower to higher concentration in all the treatments. However, non-AM plants at 3 and 5M NaCl showed decreased T_i compared to other salinity levels.

Leaf nutrients. Leaf nutrient content of N, P, and K was significantly ($P \leq 0.05$) reduced by increasing NaCl concentration, whereas the level of Na increased with higher NaCl concentrations (Table 1). At selected concentrations of NaCl, plants inoculated with AM fungi has a significantly ($P \leq 0.01$) increased leaf nutrient content of N, P and K compared to non-AM plants. In non-AM plants at 0.5M of NaCl applications, leaf N, P and K content was reduced to 25%, 33% and 27%, respectively. In AM plants, even though there was a decrease in the leaf nutrient contents at higher NaCl concentrations, the maximum N, P and K content was observed at 0.1M NaCl application. The AM-inoculated plants at the above concentration showed 9%, 18% and 9% of increase in N, P and K contents, respectively. Concentration of Na in the leaves was found increasing along with the increase in the NaCl treatment in both AM and non-AM plants. However, higher Na level was found in the leaves of AM peanuts compared to the leaves of non-AM peanuts at all salination levels.

Total chlorophyll content. Data showed that inoculation with AM fungi significantly increases the total leaf chlorophyll content of peanut plants as compared to non-mycorrhizal plants (Table 1). However, leaf chlorophyll contents of

AM and non-AM plants were significantly ($P \leq 0.01$) reduced by increasing NaCl concentration. Arbuscular mycorrhizal plants had greater leaf chlorophyll content than non-AM plants at all NaCl treatments. At 0M, 0.1M, 0.2M, 0.3M, and 0.5M NaCl concentrations, AM plants exhibited significantly higher (+95%, +103%, +86%, +59% and +43%, respectively) chlorophyll content compared to non-AM plants. The NaCl \times AM interaction was significant at 0.1M NaCl treatment, where the maximum chlorophyll content was observed compared to the other treatment levels. In non-AM plants treated with 0.3 and 0.5M NaCl, there was a reduction (–23% and –34%) in chlorophyll content compared to control (0M NaCl) plants. In AM plants treated with 0.5M NaCl there was a

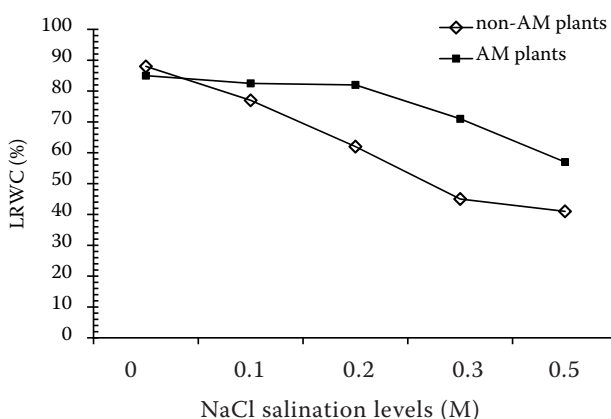


Figure 2. Effect of salinity stress on leaf relative water content of mycorrhizal and non-mycorrhizal peanuts

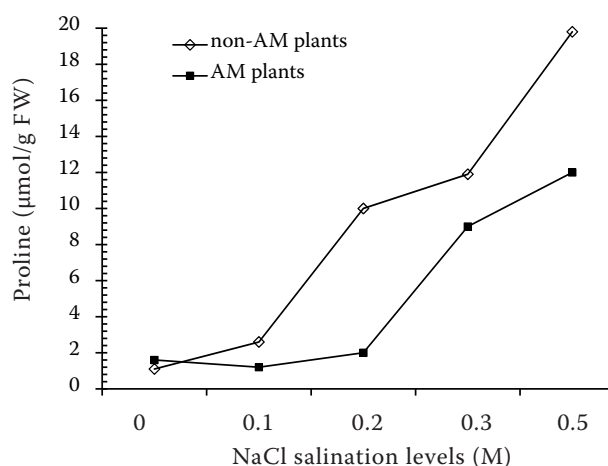


Figure 3. Effect of salinity stress on proline content of mycorrhizal and non-mycorrhizal peanuts

reduction (–27%) in chlorophyll content compared to AM control (0M NaCl) plants.

Leaf relative water content. The leaf relative water content (LRWC) of salinity-stressed mycorrhizal peanuts showed a gradual decrease with respect to the increasing salinization (Figure 2). Analysis indicated a significant ($P \geq 0.05$) negative relationship that accounted for 85.7% of the variation in LRWC in response to added NaCl. The LRWC of salinity stressed non-mycorrhizal plants also decreased with the salinity treatment (Figure 2), which showed 96.3% of variation in response to added NaCl.

The LRWC of salinity-stressed mycorrhizal plants was significantly greater than salinity stressed non-mycorrhizal peanuts at all salinization levels. The relative responses in LRWC between salinity-stressed mycorrhizal and salinity-stressed non-mycorrhizal peanuts tend to increase with increased concentration of NaCl.

Free proline content. Proline content of salinity-stressed mycorrhizal peanuts increased with respect to the increase in salinity levels (Figure 3). Linear regression analysis indicated a significant ($P \geq 0.05$) positive relationship that accounted for 86.4% of the variation in response to added NaCl.

Mean proline accumulation of stressed non-mycorrhizal peanuts consistently increased with gradual salinization (Figure 3). Linear regression analysis indicated a significant ($P \geq 0.05$) positive relationship in proline content (95.6%) in response to added NaCl.

Comparison of the slopes of the salinity-stressed non-mycorrhizal peanuts and mycorrhizal peanuts indicated a highly significant ($P \geq 0.05$) proline content in salinity stressed non-mycorrhizal pe-

nuts at all salinization levels with the exception at the control (0.0M).

Mycorrhizal assessment. Analysis showed a significant ($P \geq 0.05$) negative relationship between the amount of added NaCl and root colonization at higher level of NaCl treatments as indicated by decreased spore production and percentage of colonization (PC). Colonization and spore density [number of spores per gram soil (NOS/g S)] decreased from an average of 93% and 12 spores/g, respectively, at highest salinization level (Figure 4). At the highest salinization level spore formation was very much decreased but AM colonization was only reduced to half compared to 0M NaCl application.

DISCUSSION

Salt stress adversely affected the growth parameters of both mycorrhizal and non-mycorrhizal plants, however a more pronounced reduction in biomass was observed in non-mycorrhizal peanuts. The indices of fresh and dry biomass production indicate that *Glomus mosseae* markedly improved the growth characteristics of the plants under the saline conditions. Tain et al. (2004) have demonstrated that inoculation with AM fungi could improve growth of plants under a variety of salinity stress conditions. Enhancement in growth and salt tolerance observed in mycorrhizal peanuts may be due to the better nutritional status of the plants (Zandavalli et al. 2004). To some extent, these AM fungi have been considered as bio-ameliorators of saline soils (Tain et al. 2004).

In the present investigation, the tolerance capacity of AM peanuts was higher than that of non-AM plants at all levels of NaCl application. The data recorded in Figure 1 revealed the tolerance of mycorrhizal plants to salt stress as indicated by the increase in the fresh and dry weight. The tolerance index of AM plants at 0.3 and 0.5M NaCl were 55% and 42.3% compared to the T_i of non-AM plants (51% and 32%) at the same level of salinity. The beneficial effects of mycorrhiza on the growth under saline conditions have been studied in various plant species and families (Sánchez-Blanco 2004). But when the plants were exposed to the higher concentrations of sodium chloride, the biomass of the plants was substantially reduced irrespective of the presence or absence of the mycorrhizal fungi. The major reason for the detrimental effects may be the negative osmotic pressure caused by the salt in the root zone (Jacoby 1994) or the growth inhibition due to injury of cells in transpiring

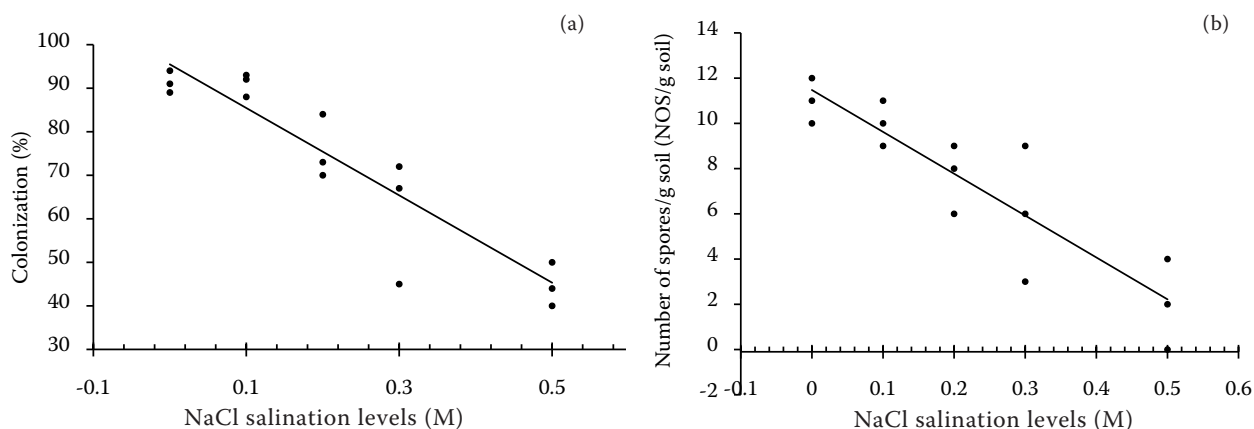


Figure 4. Effect of salinity stress on colonization and sporulation in peanuts

leaves (Tuteja 2007). In general, AM fungi helped to partially alleviate NaCl stress, as indicated by greater plant growth (total plant DM and leaf nutrients) compared to non-AM plants.

The beneficial effect of AM symbiosis on plant growth has been largely attributed to higher uptake of phosphorus (Moyersoen et al. 1998). In this study AM plants showed higher values of phosphorus at all salinity levels (Table 1). This result indicates that the effect of AM fungi on phosphorus uptake constitutes one of the main mechanisms for increasing plant tolerance of salinity. This type of relationship between host and endophyte was earlier reported by Tain et al. (2004) and Rabie and Almadini (2005). Although P is the most commonly reported nutrient enhanced, N and K contents were also found increased by AM association (Rabie and Almadini 2005).

Results in Table 1 show increased chlorophyll content in the leaves of AM plants compared to non-AM plants. Higher chlorophyll content may reflect the higher photosynthetic rate necessary to support the carbon cost of AM associations (Wright et al. 1998). Increased photosynthesis of AM plants may be mediated by increased P nutrition, which obviously could have given rise to an increase in plant growth. But at higher NaCl concentration, total chlorophyll content was reduced. Singh et al. (2000) reported degradation of chlorophyll at salinity.

Sodium chloride stress reduced the leaf relative water content (LRWC) in both salt-stressed mycorrhizal and non-mycorrhizal peanuts, but the LRWC was significantly higher in salt-stressed AM peanuts than in salt-stressed non-AM plants at salinization level ≥ 0.2 M. The index of LRWC indicates that *G. mosseae* has improved water content of stressed peanuts. This supports the view that symbiotic relationship between AM fungi

and host plants improve water uptake (Abdella and Abdel-Fattah 2000). Host mycorrhization can influence plant-water relations by altering the osmotic balance of the cells (Rosendahl and Rosendahl 1991).

Proline accumulation is reported as a symptom of stress in less salinity-tolerant plants and it plays multiple roles in stress tolerance as a mediator of osmotic adjustment (Yoshiba et al. 1997) and to protect macromolecules during dehydration (Sanchez et al. 1998). In this study, both salt-stressed mycorrhizal and non mycorrhizal peanuts accumulated free proline but foliar increases of free proline in salt stressed-mycorrhizal plants was significantly higher at 0.2M salination level compared to the other cases. Stressed mycorrhizal plants osmotically adjust better than non-AM plants with a greater concentration of solutes of Na or synthesise more solutes such as proline. The higher accumulation of Na in AM peanuts in this study was for the osmoregulation purpose, while the reduced foliar proline in AM peanuts suggests that the fungus was able to alleviate the damage due to salt stress.

Sporulation and colonization levels were inversely correlated with NaCl concentration (Figure 4). The decreased colonization by the AM fungi under salt stress may be because of the reduced germination of fungal spores or due to the high pH associated with increasing NaCl concentrations (van Aarle et al. 2002). The reduction in biomass production with NaCl stress is likely to correlate with the decrease in AM colonization. This is consistent with previous finding that the peanut is dependent upon mycorrhizal association for nutrient uptake and growth (Krishna and Bagyaraj 1982).

Results of the measured indices in this experiment indicated that inoculation of salt-stressed peanuts with the AM fungus *Glomus mosseae* is having a role in relieving stress induced by NaCl. This microbial

inoculant could enhance peanut growth and tolerance to salt stress through improved mineral nutrition in phosphorous deficient calcareous soils.

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