

Full Length Research Paper

# Salt tolerance and mycorrhization of *Bacopa monneiri* grown under sodium chloride saline conditions

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Salinity of soil is a serious problem affecting plant growth and is increasing steadily in many parts of the world, particularly in arid and semi-arid areas. *Arbuscular mycorrhiza (A M)* is the most wide spread and significant mutualistic fungi having universal in their association including plants of agricultural and medicinal importance. *A. mycorrhiza* fungi have been shown to promote plant growth and salinity tolerance by various mechanisms. The effects of inoculation with two *A. mycorrhizal* fungi *Glomus mosseae* and *Glomus intraradices* have been investigated on *B. monneiri*, an important medicinal plant grown with five different levels of salinity (0.40, 80, 120 and 160 mM). Root colonization, leaf chlorophyll content and tolerance of the plants to salinity were determined. The results indicated that the *A. mycorrhizal* fungi could infect and colonize the roots effectively under high salinity levels and increased chlorophyll content. Dry mass production was significantly enhanced in the inoculated plants and the effect was more evident at the high salinity levels. More over, *A. mycorrhizal* colonization has increased  $\text{Na}^+$  and  $\text{Cl}^-$  uptake and reduced rhizosphere NaCl level significantly. *A. mycorrhizal* association significantly increased tolerance of plants to salinity and was found as an effective measure to enhance establishment of the plant and to decrease soil salinity.

**Key words:** *Arbuscular mycorrhiza*, *Glomus mosseae*, *Glomus intraradices*, *Bacopa monneiri*, salt tolerance, proline.

## INTRODUCTION

Arid and semi-arid lands constitute approximately one third of the world's land surface (Archibold, 1995). Environmental stresses, particularly salinity and drought are the two major abiotic factors restrict the distribution of plants in arid and semi-arid soils. It is estimated that nearly 19.5% of the irrigated agricultural lands are considered salt affected and in every year more than one million hectares of land are subjected to salinization (Munns et al., 1999). Accumulation of soluble salts in the rhizosphere will reduce the water potential and consequently affect the physiological processes of plants growing in these environments (Heyster and Nabors,

1982). Relatively high sodium chloride concentrations in soil can move into root cells by passive transport (Lazof and Bernstein, 1999) and therefore, the plant when exposed to high salinity, tends to change metabolic activity by the production of certain organic compounds to reduce the internal water potential and to maintain cell osmotic balance (Al-Khaliel, 2010).

Many technical and mechanical measures have been developed to alleviate the harmful impact of salt accumulation. However, these technologies became less attractive to the farmers due to economic viability and ecological concerns. Due to shortage of water in such land, it seems the more feasible strategy is to enhance the ability of the plant to tolerate the salinity or to produce salinity-resistant plant varieties.

Among the biological approaches to enhance plant growth in saline conditions, the role of *A. mycorrhizal* fungi has been well established. It has been suggested

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that *A. mycorrhizal* fungal colonization might enhance tolerance of plants (Tian et al., 2004) and their growth under saline condition (Al-Khaliel, 2010). Most native plants and crops of arid and semi-arid areas are *A. mycorrhizal* (Pond et al., 1984), and it has been reported that *A. mycorrhizal* fungal colonization might enhance salt tolerance of some plants (Tian et al., 2004).

*Bacopa monneiri* (L.) Pennell is a perennial creeping herb used in traditional treatment for epilepsy, asthma and has antioxidant properties and may improve intellectual activity (Stough et al., 2008). Since it is an important medicinal plant, application of *A. mycorrhizal* fungi may enhance its tolerance to salinity and early establishment in saline habitats. A study on the effect of soil salinity as well as the bio concentration of NaCl on these plants may be used to evaluate whether they can be grown in saline soil for phytoremediation purposes. Therefore, the present investigation was carried out to study the salt tolerance of *B. monneiri* and the accumulation of sodium chloride in the plant by the inoculation of two *A. mycorrhizal* fungi, *G. mosseae* and *G. intraradices* under induced saline conditions.

## MATERIALS AND METHODS

The experiment was conducted using autoclaved soil amended with original soil microbiota without *A. mycorrhizal* fungal spores and its electrical conductivity was (EC) 0.096 dSm<sup>-1</sup>. The substrate soil was apportioned into 2.0 kg plastic pots whose drainage holes were lined with perforated polyethylene bags. The experiment had a randomized complete factorial design with three mycorrhizal treatments (*G. mosseae*, *G. intraradices* and non- *A. mycorrhizal* inoculated) and five salt levels (0, 40, 80, 120 and 160 mM). Three replications per treatment were performed to give a total of 45 pots. The frequently observe *A. mycorrhizal* fungi, *G. mosseae* and *G. intraradices* were isolated from King Saud University, Riyadh were used as inoculants fungi and pot culture inoculum were propagated on sorghum plants (*Sorghum vulgare*). The mycorrhizal inoculum consisted of chopped roots and soil from a three months old pot culture of sorghum plants. The non-mycorrhizal inoculum consists of 10 ml of the inoculum filtrate which were sieved through a 30 µm

Tolerance index (Ti) of AM and non-AM plants to different NaCl sieve openings to remove mycorrhizal fungal spores and to assure similar microbial population. Five salinity levels of NaCl (0, 40, 80, 120 and 160 mM) were prepared on distilled water for the treatment pots.

Healthy erect shoots with roots of *B. monneiri* were selected from the mother plants grown in hydroponic tubs. These were acclimatized in 1% Hoagland solution for 2 weeks under green house conditions. Cuttings of the plants consisting of 6 pairs of leaves were transplanted into pots containing autoclaved soil amended with the original soil microbiota and ten gram of inoculum of *A. mycorrhizal* fungus containing approximately 1500 infective propagules per gram substrate. Control plants received non-mycorrhizal inoculum of autoclaved soil amended with 10 ml of the inoculum filtrate of the original soil microbiota. All pots were fertilized with half strength Hoagland's solution on a biweekly basis.

After 20 days of *A. mycorrhizal* inoculation, the mycorrhizal and non-mycorrhizal pots received five levels of salinity treatment. To avoid salinity shock and to acclimatize the seedlings and *A. mycorrhizal* fungus to high NaCl concentrations, salinity stress was imposed on the seedlings by applying the saline irrigation water progressively. All the pots were leached with distilled water to prevent salt accumulation and to maintain optimum NaCl salinization level before applying subsequent saline irrigation.

After 20 days of salinity treatment, the whole plant harvested and then oven dried at 60°C for 48 h to determine the dry weight per plant (DW/P) for each NaCl salinization treatment. Concentrations of Na<sup>+</sup> and Cl<sup>-</sup> in root and leaves were extracted and measured separately. Na<sup>+</sup> was extracted using acid digestion with nitric acid and hydrogen peroxide (Plank, 1992) and Cl<sup>-</sup> was extracted with deionized water (Kalra, 1998). The extracts were analyzed by atomic absorption spectrophotometry (AAS). EC of soil was measured after the addition of NaCl and after 20 days of salinity treatment. Determination of *A. mycorrhizal* colonization on roots was carried out according to Phillips and Hayman (1970).

Leaf chlorophyll content was determined at harvest (n=3), by extraction of chlorophyll with acetone (Harborne, 1998). Physiologically mature leaves from randomly selected plants per treatment were collected. 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.

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

$$\text{Proline } (\mu\text{moles g}^{-1} \text{ fresh wt.}) = \frac{[(\mu\text{g proline/ml} \times \text{ml toluene})/115.5 \mu\text{g/} \mu\text{mole}]}{[(\text{g sample})/5]}$$

levels were determined according to Shetty et al. (1995).

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

The difference in DW/plant, proline content, Na<sup>+</sup> and Cl<sup>-</sup> contents between the salinity stressed mycorrhizal plants (SMP) and salinity stressed non-mycorrhizal plants (SNMP) within each treatment was expressed as relative response using the following equation:

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

Data were analyzed using simple regression analysis comparing

regression equations and elevations. Student t-test was used to compare mean pair wise of salinity stressed mycorrhizal plants and salinity stressed non-mycorrhizal plants of the same treatment (within each salinization level).

## RESULTS

Analysis indicated a significant negative relationship between amount of added NaCl and root colonization at higher level of treatments as indicated by decreased mycorrhizal colonization (Table 1). When NaCl concentration was at 40 and 80 mm, there was no

**Table 1.** Root colonization and dry weight of *Bacopa monneiri* inoculated with *G. mosseae* and *G. intraradices* at different levels.

NaCl (mM)	Control	<i>G. mosseae</i> inoculated plants		<i>G. intraradices</i> inoculated plants	
	Dry wt. (g)	Root colonization (%)	Dry wt. (g)	Root colonization (%)	Dry wt. (g)
0	0.92 <sup>a</sup>	86 <sup>a</sup>	1.39 <sup>b</sup>	70 <sup>a</sup>	1.24 <sup>a</sup>
40	0.9 <sup>a</sup>	84 <sup>ab</sup>	1.48 <sup>a</sup>	70 <sup>a</sup>	1.21 <sup>ab</sup>
80	0.79 <sup>c</sup>	80 <sup>b</sup>	1.32 <sup>cd</sup>	66 <sup>bc</sup>	1.05 <sup>cd</sup>
120	0.6 <sup>f</sup>	57 <sup>fg</sup>	1.07 <sup>fg</sup>	41 <sup>g</sup>	0.94 <sup>ef</sup>
160	0.42 <sup>i</sup>	42 <sup>i</sup>	0.79 <sup>i</sup>	32 <sup>i</sup>	0.68 <sup>i</sup>

Pre-inoculated plants were grown in pots with different NaCl levels for 20 days. Data followed by same letter (s) in a column are not significant ( $P \leq 0.05$ ).

significant reduction in the root colonization in plants inoculated with both the *A. mycorrhizal* fungi. However, marked differences were observed in colonization between the two fungal symbionts. Percentage of root colonization was highest in *B. monneiri* inoculated with *G. mosseae* (86%) compared to the plants inoculated with *G. intraradices* (70%). In response to increased NaCl concentration from 120 to 160 mM, the root colonization has significantly reduced on an average by 34 and 51% in *G. mosseae* inoculated plants and 41 and 54% in plants inoculated with *G. intraradices*, respectively, compared to 0 mM treated *A. mycorrhizal* plants. All the growth parameters were adversely affected by the salinity treatments (Table 1).

However, there were differences, although not all significant, in plant biomass among NaCl treatments. There was an inverse relationship between relative shoot and root biomass and NaCl levels. The relative response in DW/plant between NaCl treated mycorrhizal and non-mycorrhizal plants tented to form a near-linear increase with increasing salinization levels. Salinity stressed non-*A. mycorrhizal* plants had significantly greater dry biomass compared with salinity stressed non-*mycorrhizal* plants grown at the same salinization levels. On average, plants inoculated with *A. mycorrhizal* exhibited a significantly ( $P \leq 0.05$ ) greater total plant dry weight compared to non-*A. mycorrhizal* plants. The mean dry weight of salinity stressed *G. mosseae* inoculated plants grown at the highest salinization levels (120 and 160 mM), was more than 80% that of salinity stressed non-*A. mycorrhizal* plants. Data showed that inoculation with *A. mycorrhizal* fungi significantly increases the chlorophyll content of plants as compared to non-mycorrhizal plants (Figure 1).

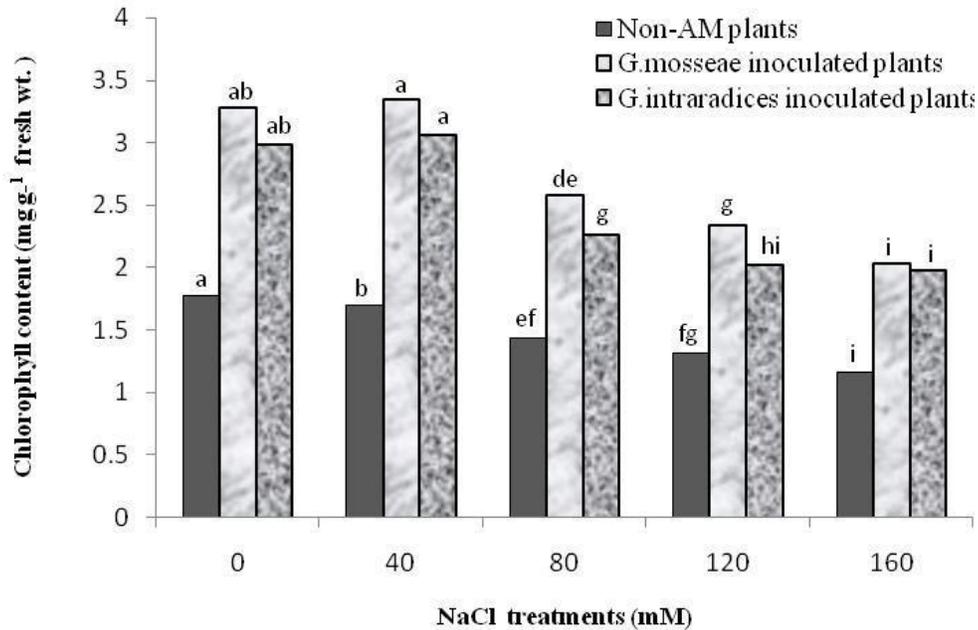
However, leaf chlorophyll content of *A. mycorrhizal* and non-*A. mycorrhizal* plants were significantly reduced at higher NaCl concentration. Among the mycorrhizal plants, *B. monneiri* inoculated with *G. mosseae* had greater leaf chlorophyll content than those inoculated with *G. intraradices* at all NaCl treatments.

At 40, 80 and 120 mM NaCl concentrations, *A. mycorrhizal* plants inoculated with *G. mosseae* exhibited significantly increased (+96, +79 and +77%, respectively) chlorophyll content compared to non-*A. mycorrhizal*

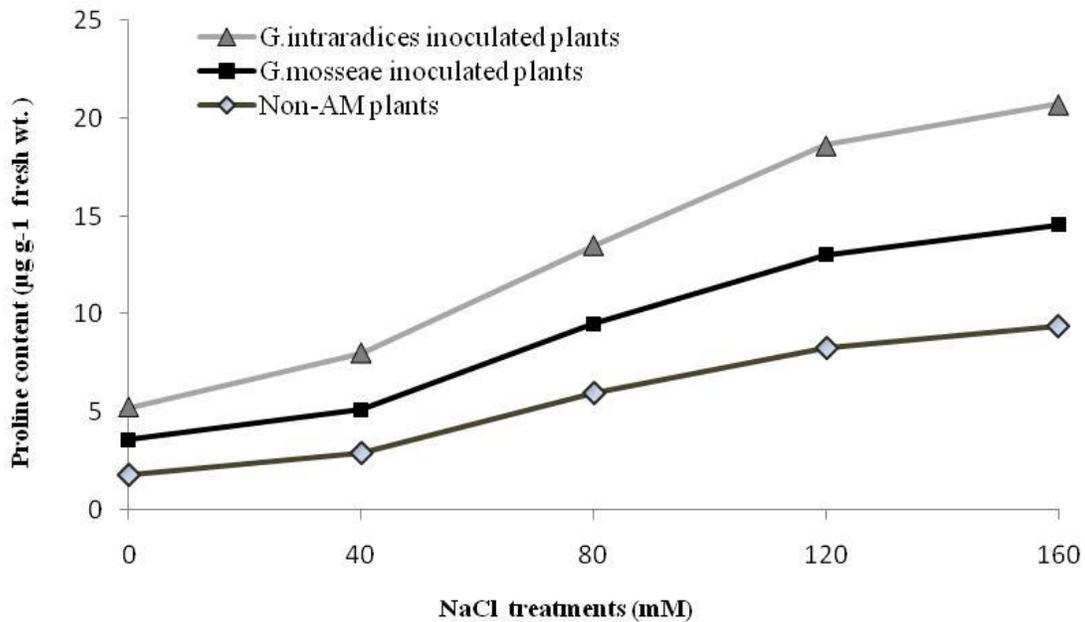
plants grown at respective NaCl levels. The NaCl and *A. mycorrhizal* interaction was significant at 40 mM NaCl treatment, where maximum chlorophyll content was observed compared to the other treatment levels (Figure 1). However, at 160 mM NaCl level there was a reduction of 38 and 34% in chlorophyll content in *G. mosseae* and *G. intraradices* inoculated plants respectively, compared to *A. mycorrhizal* plants treated with 0 mM NaCl level.

Proline accumulation of stressed non-mycorrhizal plants consistently increased with salinization treatments (Figure 2). Proline accumulation at 160 mM level was found very high compared to the plants treated with 0 mM NaCl. Among the plants inoculated with *A. mycorrhizal* fungi, the response to NaCl treatment showed variations along with the increase in salt concentration. On comparison, *G. mosseae* inoculated plants showed less accumulation of proline than plants infected by *G. intraradices*. Proline content of non-*A. mycorrhizal* plants showed 73 and 81% increase over the *G. mosseae* and 41 and 52% increase over *G. intraradices* inoculated plants at 120 and 160 mM NaCl levels, respectively (Figure 2). The tolerance index (Ti) to salinity in *A. mycorrhizal* plants was higher than that of the non-*A. mycorrhizal* plants. The tolerance index showed a gradual decrease as the salinity level increased from lower to higher concentration in all the treatments.

No visible symptoms of NaCl toxicity was observed in 40 and 80 mM treatments in *A. mycorrhizal* and non-*A. mycorrhizal* plants. After 20 days of NaCl treatment at 120 and 160 mM level, the non-*A. mycorrhizal* plants exhibited interveinal chlorosis. The toxicity symptoms were too severe in some plants at 160 mM level and caused plant injury or death after 20 days. Other symptoms of Cl stress observed in the study at 160 mM NaCl includes, premature yellowing of leaves, burning of leaf tips and margins, and bronzing followed by leaf abscission. The *A. mycorrhizal* plants appeared to be less affected by NaCl toxicity at 120 and 160 mM concentration compared to non-*A. mycorrhizal* plants. Analysis of the results showed that the  $\text{Na}^+$  and  $\text{Cl}^-$  contents were very high in the roots than in the leaves and increased proportionally to the increase in the NaCl levels.  $\text{Na}^+$  content in the roots of non-*A. mycorrhizal* plants increased by 8, 9, 11 and 13 folds over the 0 mM



**Figure 1.** Effect of NaCl on leaf chlorophyll content in *Bacopa monneiri* inoculated with *G. mosseae* and *G. intraradices*. Plants were grown in pots with different NaCl levels for 20 days. Chlorophyll content was determined by extraction with acetone. Data followed by same letter(s) are not significant ( $P \leq 0.01$ ).



**Figure 2.** Effect of NaCl on proline content in *Bacopa monneiri* inoculated with *G. mosseae* and *G. intraradices*. Plants were grown in pots with different NaCl levels for 20 days. Proline content was determined as per the method of Bates (1973).

NaCl level, while, 5, 6, 15 and 21 folds increase was observed over the 0 mM NaCl in the leaves at 40, 80, 120 and 160 mM NaCl levels, respectively (Table 2).

Among the mycorrhizal plants, *G. mosseae* inoculated plants showed higher  $\text{Na}^+$  and  $\text{Cl}^-$  contents compared to *G. intraradices* infected plants. Increase in  $\text{Na}^+$  content in

**Table 2.** Sodium content in root and leaf of *Bacopa monneiri* inoculated with *Glomus mosseae* and *G. intraradices* at different levels of NaCl treatments

NaCl (mM)	Na <sup>+</sup> content (mg/kg)					
	Root			Leaf		
	Control	<i>G. mosseae</i>	<i>G. intraradices</i>	Control	<i>G. mosseae</i>	<i>G. intraradices</i>
0	456 <sup>l</sup>	580 <sup>l</sup>	604 <sup>l</sup>	174 <sup>l</sup>	460 <sup>l</sup>	371 <sup>l</sup>
40	3648 <sup>de</sup>	4019 <sup>de</sup>	3840 <sup>de</sup>	886 <sup>gh</sup>	977 <sup>gh</sup>	860 <sup>gh</sup>
80	4122 <sup>c</sup>	4631 <sup>cd</sup>	4406 <sup>c</sup>	1074 <sup>g</sup>	1380 <sup>tg</sup>	1205 <sup>g</sup>
120	5058 <sup>b</sup>	5540 <sup>b</sup>	5281 <sup>bc</sup>	2590 <sup>cd</sup>	2445 <sup>d</sup>	2623 <sup>cd</sup>
160	5890 <sup>a</sup>	6406 <sup>a</sup>	6014 <sup>a</sup>	3578 <sup>a</sup>	3710 <sup>a</sup>	3694 <sup>a</sup>

Plants were grown in pots with different NaCl levels for 20 days. Na<sup>+</sup> contents were determined by tissue analysis. Data followed by same letter(s) in a column are not significant ( $P \leq 0.005$ ).

**Table 3.** Chlorine content in root and leaf of *B. monneiri* inoculated with *G. mosseae* and *G. intraradices* at different levels of NaCl treatments.

NaCl (mM)	Cl <sup>-</sup> content (mg/kg)					
	Root			Leaf		
	Control	<i>G. mosseae</i>	<i>G. intraradices</i>	Control	<i>G. mosseae</i>	<i>G. intraradices</i>
0	518 <sup>l</sup>	912 <sup>l</sup>	894 <sup>l</sup>	186 <sup>l</sup>	542 <sup>l</sup>	512 <sup>l</sup>
40	4994 <sup>tg</sup>	6684 <sup>tg</sup>	5960 <sup>tg</sup>	951 <sup>gh</sup>	1350 <sup>n</sup>	1134 <sup>n</sup>
80	8177 <sup>de</sup>	11320 <sup>de</sup>	10400 <sup>d</sup>	1926 <sup>f</sup>	2649 <sup>tg</sup>	2350 <sup>tg</sup>
120	11447 <sup>bc</sup>	15240 <sup>bc</sup>	14684 <sup>b</sup>	3774 <sup>c</sup>	5065 <sup>b</sup>	4769 <sup>bc</sup>
160	13982 <sup>a</sup>	18786 <sup>a</sup>	16972 <sup>a</sup>	4832 <sup>a</sup>	6430 <sup>a</sup>	5980 <sup>a</sup>

Plants were grown in pots with different NaCl levels for 20 days. Cl<sup>-</sup> contents were determined by tissue analysis. Data followed by same letter(s) in a column are not significant ( $P \leq 0.005$ ).

roots of *G. mosseae* plants was 7, 8, 9 and 10 folds over 0 mM and 2, 3, 5 and 8 folds over 0 mM NaCl in leaf at 40, 80, 120 and 160 mM NaCl levels, respectively. Cl<sup>-</sup> content was higher than Na content in both roots and leaves at all levels of NaCl treatments. Cl<sup>-</sup> content increases in non- *A. mycorrhizal* plants were 5, 10, 20 and 26 folds over the 0 mM in leaf and 10, 17, 22 and 27 folds increase over the 0 mM NaCl in roots at 40, 80, 120 and 160 mM NaCl levels, respectively (Table 3). Significantly, higher Cl<sup>-</sup> content ( $p < 0.005$ ) was noticed in the roots and leaves of plants treated with *A. mycorrhizal* fungi and the Cl<sup>-</sup> concentration was comparatively higher in *G. mosseae* inoculated plants. The increase was over 34, 38, 33 and 34% in the root Cl<sup>-</sup> concentration in *G. mosseae* inoculated plants compared to non- *A. mycorrhizal* plants at 40, 80, 120 and 160 mM NaCl levels, respectively. Similar results were also observed in leaf Cl<sup>-</sup> content in *G. mosseae* inoculated plants compared to non- *A. mycorrhizal* plants. However, a significantly higher increase in Na<sup>+</sup> content was not recorded in the *A. mycorrhizal* treatments compared to the control plants supplemented with NaCl.

NaCl concentration in untreated soil was relatively low and electrical conductivity was 0.9 to 1 dS/m. EC

measured before the experiments in NaCl treated soil was between 3.2 to 6.2 dS/m. After the experiments, the EC in the rhizosphere soils was less than 1.7 dS/m. Comparison of the EC in the rhizosphere soil indicated a significant decrease of over 73% in the soil after 20 days duration of experiment.

## DISCUSSION

The decline in root colonization in the experiment may probably due to the direct effect of NaCl on fungal infection by inhibiting the hyphal spreading (McMillen et al., 1998) and suppressing the formation of *A. mycorrhiza* (Tian et al., 2004). The high infection percentage and growth enhancement of *A. mycorrhizal* inoculated plants at 40 to 80 mM NaCl indicated that the *A. mycorrhizal* fungi are more active at low or moderate level of salinity. The trends in colonization recorded in this study agree with the reports of Zuccarini and Okurowska (2008) and Al-Khaliel (2010). Differential response of *A. mycorrhizal* fungi in colonizing same host in identical conditions observed in this study corroborates with the report of Wu and Zou (2009) and this was attributed to the ability of the

fungi to colonize the host root quickly and extensively. High level of colonization may be the prime determinant of the efficacy of symbiosis and *G. mosseae* was observed as the potential inoculant fungus for *B. monneiri*.

*A. mycorrhizal* efficiency can be measured in terms of the host growth enhancement under different salinity conditions. *A. mycorrhizal* mediated improvement in growth under salt stress in this study may be due to increased mineral absorption, photosynthetic efficiency and better adaptation of the plants to tolerate salinity levels. Beneficial effects of *A. mycorrhizal* inoculation on plant growth under salinity were reported earlier by Al-Khalil (2010). The reduced plants growth of non-*A. mycorrhizal* and *A. mycorrhizal* under high salinity is mainly attributed to the negative effect of the high osmotic potential of the saline soil solution which tend to reduce nutrient and water uptake as well as reduce the plant root growth. *A. mycorrhizal* fungi are found to exhibit differences in increasing the fitness of the host plants when supplied with saline solutions. Root colonization of *G. mosseae* was significantly higher than *G. intraradices* and caused better response on *B. monneiri* in plant biomass and salt tolerance. Similar responses with *A. mycorrhizal* fungi were reported earlier by Wu and Zou (2009). It is noteworthy that the inhibitory effect of salinity on plant growth was significantly reduced by pre-inoculation of these plants with *A. mycorrhizal* fungi.

The most remarkable effect of mycorrhization in relation to salinity stress was observed in terms of the chlorophyll content. Inoculation of *A. mycorrhizal* fungi could enhance chlorophyll production significantly in non-saline conditions compared to non-*A. mycorrhizal* plants and found minimizing the deleterious effects of salinity through higher chlorophyll production. Similar result was observed in a previous study by Murkute et al. (2006) on citrus rootstock. In our study it was observed that the chlorophyll content decreased significantly by the salination at higher levels but under moderate level of salt stress chlorophyll content was found superior to those of non-stressed plants, showing that in this respect mycorrhization is capable to fully counter balance salt stress (Zuccarini, 2007). Chlorophyll content decreased under stress may be due to the suppression of specific enzymes responsible for the synthesis of photosynthetic pigments (Murkute et al., 2006) or by a decrease in the uptake of minerals needed for chlorophyll biosynthesis (El-Desouky and Atawia, 1998). Higher chlorophyll content may reflect the higher photosynthetic rate necessary to support the *A. mycorrhizal* associations (Wright et al., 1998) and the increased photosynthesis may be mediated by increased P nutrition, which obviously could have given rise to an increase in plant growth. However, at high NaCl concentration chlorophyll formation was retarded and this result corroborates with the study of Singh et al. (2000), who reported degradation of

chlorophyll at high salinity. Although root colonization was inhibited at high salinity, *A. mycorrhizal* could promote plant growth through improvement of plant nutrition and production of osmoregulators (Vijayan, 2009). Proline is one of the most prominent osmolyte in plants that accumulates for osmotic adjustment under salt stress therefore, proline accumulation may be a symptom of stress in plant species (Yoshida et al., 1997). *A. mycorrhizal* inoculated plants showed less accumulation of proline compared to the non-*A. mycorrhizal* plants and are higher salt tolerant than non-*A. mycorrhizal* plants. Similar results were also reported in faba plants by Rabie and Almadini (2005). Improved tolerance of *A. mycorrhizal* plants may be related to enhanced mineral nutrition and by the improved physiological processes (Ruiz-Lozano and Azcón, 2000). Proline is also known to induce expression of salt stress responsive genes (Chinnusamy et al., 2005) that may promote the plants to achieve tolerance under salinity. Stressed *A. mycorrhizal* plants osmotically adjust better than non-*A. mycorrhizal* plants and the reduced foliar proline in *A. mycorrhizal* plants suggests that the fungus was able to alleviate the damage due to salt stress.

Our results indicated that  $\text{Na}^+$  and  $\text{Cl}^-$  contents in the leaves and roots increased linearly as the NaCl level increased. Although, root  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations increased proportionally to salt treatments, they appeared to reach a plateau at the 120 mM level and no further differences were found in case of  $\text{Na}^+$  and  $\text{Cl}^-$  contents at 120 and 160 mM NaCl levels. While mycorrhizal plants accumulated more  $\text{Na}^+$  in their roots with increasing salinity, leaves of these plants had lower  $\text{Na}^+$  content and showed limited accumulation with increasing salinity. These results are in consistent with the work of Zandavalli et al. (2004) and suggest that *A. mycorrhizal* fungi may protect shoot system, mainly leaves, from  $\text{Na}^+$  toxicity either by regulating  $\text{Na}^+$  uptake or by accumulating it in root thereby delaying its translocation into shoot system (Rabie and Almadini, 2005). The increase in  $\text{Cl}^-$  concentrations evidently had some detrimental impact on non-*A. mycorrhizal* plants since it was noted that the leaves turned yellow, brown followed by leaf abscission by the end of the experiment. However, Valencia et al. (2008) found no visible toxicity symptoms on plants grown at high concentrations of NaCl. Chloride accumulation was more in *G. mosseae* inoculated plants and that  $\text{Cl}^-$  content was higher than  $\text{Na}^+$  content in both roots and leaves at all salt levels. Valencia et al. (2008) showed similar results while screening the salt tolerance in soybean. In spite of high saline environments, *A. mycorrhizal* plants grew without much toxic symptoms and could survive even after 20 days of NaCl treatments reflecting their adaptation to high salinity. These results suggest that control of ion uptake and compartmentalization of ions are the most effective strategies of *A. mycorrhizal* fungi for adaptation of plants to salinity stress (Rabie and Almadini, 2005).

Sodium chloride concentration in untreated soil in this study was relatively low and after NaCl treatment the salinity level increased and measurement of EC of the soil became 3.8 to 6.2 dS/m. Soil conductivity is related to the sodium chloride content (Wentz, 2001) and the high EC recorded was due to the addition of NaCl, which leads to the increased absorption of Na<sup>+</sup> and Cl<sup>-</sup> from the soil. It is also noteworthy that a decline in the NaCl content was observed after 20 days of treatment and the measurement of EC in the rhizosphere of NaCl treated plants were found decreased to 1.7 dS m<sup>-1</sup>. Mycorrhizal colonization has enhanced the absorption of NaCl as evidenced by the increase in Na<sup>+</sup> and Cl<sup>-</sup> content in salinity stressed plants and the decrease in the NaCl content in the rhizosphere. For areas contaminated with sodium chloride in many parts of the world such as arid and semi arid areas, *B. monneiri* may also be used to reduce salinity to a certain extent. Analysis on the concentration of NaCl in the rhizosphere revealed that *A. mycorrhizal* inoculated *B. monneiri* could absorb more than 73% of NaCl from the soil. This suggests that the *A. mycorrhizal* colonized *B. monneiri* could reduce sodium chloride levels and toxicity and can be used to improve soil conditions in saline areas

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## REFERENCES

- Al-Khaliel AS (2010). Effect of salinity stress on mycorrhizal association and growth response of peanut infected by *Glomus mosseae*. *Plant Soil Environ.*, 56: 318-324.
- Archibald OW (1995). *Ecology of world vegetation*, Chapman and Hall, London.
- Bates LS (1973). Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207.
- Chinnusamy V, Jagendorf A, Zhu JK (2005). Understanding and improving salt tolerance in plants. *Crop Sci.*, 45: 437-448.
- El-Desouky SA, Atawia AAR (1998). Growth performance of some citrus rootstocks under saline conditions. *Alexandria J. Agric. Res.*, 43: 231-254.
- Harborne JB (1998). Nitrogen compounds. In Harborne JB (eds) *Phytochemical methods: A guide to modern techniques of plant analysis*, Chapman & Hall, London, pp. 187-234.
- Kalra YP (1998). *Handbook of reference methods for plant analysis*. CRC Press, Boca Raton, FL.
- Lazof DB, Bernstein N (1999). The NaCl induced inhibition of shoot growth: the case for distributed nutrition with special consideration of calcium. *Adv. Bot. Res.*, 29: 113-189.
- McMillen BG, Juniper S, Abbott LK (1998). Inhibition of hyphal growth of a vesicular-arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. *Soil Biol. Biochem.*, 30: 1639-1646.
- Munns R (2003). Comparative physiology of salt and water stress. *Plant Cell Environ.*, 25: 239-250.
- Murkute AA, Sharma S, Singh SK (2006). Studies on salt stress tolerance of citrus rootstock genotypes with arbuscular mycorrhizal fungi. *Hort. Sci.*, (Prague) 33: 70-76.
- Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, 55: 158-161.
- Plank CO (1992). *Plant analysis reference procedures for the southern region of the United States*. Southern Cooperative Series Bull. Univ. of Georgia, Athens., p. 368.
- Pond EC, Menge JA, Jarrell WM (1984). Improved growth of tomato in salinized soil by vesicular arbuscular mycorrhizal fungi collected from saline soils. *Mycologia*, 76: 74-84.
- Rabie GH, Almadini AM (2005). Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. *Afr. J. Biotechnol.*, 4: 210-222.
- Ruiz-Lozano JM, Azcón R (2000). Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity. *Mycorrhiza*, 10: 137-143.
- Shetty G, Hetrick D, Schwat P (1995). Effects of mycorrhizal fertilizers amendments on zinc tolerance of plants. *Environ. Pollut.*, 88: 308-314.
- Singh SK, Sharma HC, Goswami AM, Datta SP, Singh SP (2000). In vitro growth and leaf composition of grapevine cultivars as affected by sodium chloride. *Biol. Plant.*, 43: 283-286.
- Stough C, Downey LA, Lloyd J, Silber B, Redman S, Hutchison C, Wesnes K, Nathan PJ (2008). Examining the nootropic effects of a special extract of *Bacopa monniera* on human cognitive functioning: 90 day double-blind placebo-controlled randomized trial. *Phytother. Res.*, 22: 1629-1634.
- Tian CY, Feng G, Li XL, Zhang FS (2004). Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline on salinity tolerance of plants. *Appl. Soil. Ecol.*, 26: 143-148.
- Valencia R, Chen P, Ishibashi T, Conatser M (2008). A rapid and effective method for screening salt tolerance in soybean. *Crop Sci.*, 48: 1773-1779.
- Vijayan K (2009). Approaches for enhancing salt tolerance in mulberry (*Morus L*) -A review. *Plant Omics J.*, 2: 41-59.
- Wentz D (2001). *Salt tolerance of plants*. Agriculture and Food, Alberta, California, U.S.A.  
[http://www1.agric.gov.ab.ca/\\$department/deptdoc.nst/all/agdex3303](http://www1.agric.gov.ab.ca/$department/deptdoc.nst/all/agdex3303)
- Wright DP, Read DJ, Scholes JD (1998). Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. *Plant Cell Environ.*, 21: 881-891.
- Wu QS, Zou YN (2009). Arbuscular mycorrhizal symbiosis improves growth and root nutrient status of citrus subjected to salt stress. *Sci. Asia*, 35: 388-391.
- Yoshida Y, Kiyosue T, Nakashima K, Yamaguchi-shinozaki K (1997). Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiol.*, 38: 1095-1102.
- Zandavalli RB, Dillenburg LR, Paulo VD (2004). Growth responses of *Araucaria angustifolia* (Araucariaceae) to inoculation with the mycorrhizal fungus *Glomus clarum*. *Appl. Soil Ecol.*, 25: 245-255.
- Zuccarini P (2007). Mycorrhizal infection ameliorates chlorophyll content and nutrient uptake of lettuce exposed to saline irrigation. *Plant Soil Environ.*, 53: 283-289.
- Zuccarini P, Okurowska P (2008). Effects of mycorrhizal colonization and fertilization on growth and photosynthesis of sweet basil under salt stress. *J. Plant Nutr.*, 31: 497-513.