International Journal of Plant Production 2 (3), July 2008
ISSN: 1735-6814 (Print), 1735-8043 (Online)
This is a refereed journal and all articles are professionally screened and reviewed.

GUASNR
www.ijpp.info

Vesicular-arbuscular (VA) mycorrhizae improve salinity tolerance in pre-inoculation subterranean clover (*Trifolium subterraneum*) seedlings

H.R. Asghari

Department of Agriculture, Shahrood University of Technology, Shahrood, Iran
E-mail: hamidasghari@gmail.com

Received 14 Jan. 2008; Accepted 15 March. 2008; Published online 01 June 2008

Abstract

Effects of the mycorrhizal fungus *Glomus intraradices* on establishment of subterranean clover (*Trifolium subterraneum L.*) seedlings in saline conditions were studied in a glasshouse experiment. Growth and nutrient uptake were determined 10, 20 and 30 days after transplanting of mycorrhizal and nonmycorrhizal matched seedlings into soils with five different levels of salinity. Mycorrhizal plants had greater shoot and root dry weight than nonmycorrhizal plants. The enhancement in seedling dry weight due to mycorrhizal fungi was greater under high salinity levels. The detrimental effects of salinity stress on plant growth were appeared immediately after application low salinity stress to nonmycorrhizal plants (3.5 dS/m), but it was only observed in mycorrhizal plants at 7.5 dS/m and above. Mycorrhizal fungi increased P concentrations in shoots and roots compared with nonmycorrhizal plants particularly at 12 dS/m. Root K/Na ratio was also increased in mycorrhizal plants, possibly contributing to salinity tolerance. Calculation of mycorrhizal responses in terms of plant dry weight, P and K contents showed that the beneficial effects of mycorrhizal fungi on seedling salinity tolerance are due to different mechanisms at different stage of growth: increased P uptake during early growth and increased K uptake at the later stages. Results are discussed in the context of application of mycorrhizal inoculation to revegetation of salt affected lands.

Keywords: *Trifolium subterraneum*; Mycorrhiza; Salinity; Nutrition; Growth

Introduction

Approximately 7% of the global land surface is covered with saline plant habitats (Ruiz-Lozano et al., 1996). Most importantly, about 20% of irrigated land has suffered from secondary salinization and 50% of irrigation schemes are affected by salt (Flowers et al., 1997). Many of the salt-affected areas remain unproductive for many years because of plant establishment problems. Despite using salt-tolerant plants and other techniques for plant establishment in these habitats, revegetation can be very difficult. Revegetation of

saline lands decreases soil erosion and produces forage for livestock and wildlife and can convert these degraded areas into productive lands.

Direct seeding and seedling transplantation are both possible methods to revegetate salt affected lands, but because of high levels of salinity, seed germination and plant establishment in saline environment need great care (Malcolm et al., 2003). Seedling establishment is a critical phase in revegetation of degraded lands, but it is particularly crucial in saline soils due to the effects of salinity on reduction of plant root growth and osmotic stress. Besides using soil amendments, use of salt-tolerant plants, conventional and modern plant breeding techniques are the prevalent methods to increase plant salinity tolerance in saline lands. In conjunction with breeding and using salt tolerant plants, it has been reported that mycorrhizal fungal colonization might further enhance tolerance (Asghari et al., 2005; Al-Karari et al., 2001; Cantrell and Linderman, 2001). Arbuscular mycorrhizas (AM) are the most common and widespread mycorrhizal associations and it is estimated that this symbiosis occurs in over two-thirds of terrestrial vascular plant species (Fitter and Merryweather, 1992). Their contributions to agriculture are well known, but their role in helping plants to establish in saline conditions has received less attention (Giri et al., 2002). Mycorrhizal seedlings of neem (*Azadirachta indica*) have been shown to have more dry matter accumulation as compared to non-mycorrhizal seedlings over a range of salinity levels (Pande and Tarafdar, 2002). Mycorrhizal wild bean plans (*Strophostyles helvola*) had greater vigour and enhanced growth because of increased chlorophyll contents, shoot dry weight, root available water and number of root nodules compared to nonmycorrhizal plants in saline conditions (Tsang and Maun, 1999). Increased plant growth and vigour via mycorrhizal symbiosis under saline conditions may be important in revegetation of salt affected lands.

Most previous research has used conditions in which plants were established into the soil and salt concentrations increased gradually or suddenly, followed by assessment of mycorrhizal development and function, but in this study pre-existing saline conditions were used, and preinoculated mycorrhizal transplants were used to overcome problem of initial AM colonization under saline conditions. This method can be of the practical importance in field conditions for saline land revegetation. Seedlings were grown in nursery conditions under non-saline conditions and sufficient time was allowed to elapse between inoculation and imposition of salinity stress to allow colonization to occur. The objective of this study was to investigate the effects of mycorrhizal fungi on plant dry weight, nutrient contents and seedling establishment at different harvests in saline conditions.

Material and methods

Soil

A soil with a loamy sandy surface texture was collected from Ferries McDonald Conservation Park, Monarto area of South Australia. This area is located 60 km south- east of Adelaide on the eastern flank of the Mount Lofty Ranges. Soil was passed through a 2 mm mesh sieve, mixed thoroughly and autoclaved (110 °C, 1 h, twice at 48 h intervals) to remove indigenous AM propagules. Soil properties were 82.5% sand, 7.5% silt, 10% clay,

1.8% organic matter, pH 8.2, electrical conductivity (EC_e) 2.2 dS/m; 13.5 mg/kg P (Colwell, 1963). The soil total cation exchange capacity (CEC) was 9.7 meq/100g (Mg, Ca, Na and K were 1.5, 6.2, 0.6 and 1.2 meq/100g respectively). Seedlings for transplanting into test soils were raised in autoclaved Ferries McDonald soil (see below). Five levels of salinity (2.2, 3.5, 5, 7.5 and 12 dS/m) were produced by adding 0, 0.25, 0.5, 1 and 2g NaCl per kg of the soil. Soils were incubated for one week, and then pots were filled with 1.4 kg of soil.

Seedling production and transplanting

The subterranean clover (*Trifolium subterraneum*) seeds were soaked for 10 minutes in a mixture of 1 part sodium hypochlorite (NaOCl 12.5% w/v), 2 parts of reverse-osmosis water (R.O), rinsed with R.O water three times and germinated on moist filter paper at 23°C in a germinator. Seeds were coated with a suspension of *Rhizobium leguminosarum* biovar *trifolii* to promote nodule development, prior to planting. Pre-germinated seeds were sown in autoclaved Ferries McDonald soil (one per 60 cm^3 pot) inoculated or not with *Glomus intraradices* Schenk and Smith (DAOM 181602). The inoculum was dried pot culture material, consisting of soil/sand mix plus colonized root fragments, spores and external hyphae. This was mixed with Ferries McDonald soil in the ratio 10% inoculum to 90% soil. Non-inoculated pots received an additional 10% Ferries McDonald soil. R.O water was added to maintain soil moisture at 80% field capacity. Long Ashton nutrient solution (Hewitt, 1966) without P was added (10 ml per pot) once per week for 2 weeks during which time mycorrhizal colonization became established (approx 20% root length colonized). At this stage no major differences in growth or P nutrition had occurred. One mycorrhizal or nonmycorrhizal seedling was then transplanted into the salinity treatments there were three replicates. The plants were grown in a glasshouse with natural light and mean diurnal temperatures of 22°C day and 14°C nights and watered with R.O. water thrice weekly to maintain soil moisture at 80% field capacity.

Measurements

Plants were harvested 10, 20 and 30 days after transplanting. They were washed thoroughly and leaf number, shoot and root fresh and dry weights, shoot and root P, Na and K concentrations and root mycorrhizal colonization were measured at each harvest. Plant P concentration was determined colorimetrically after nitric-perchloric acid digestion (Hanson, 1950) and K and Na were determined using flame photometry (Ryan et al., 1996). Visual observation of mycorrhizal colonization was made by clearing washed roots with 10% KOH for 5 days at room temperature and staining with trypan blue using a modification of the method of (Phillips and Hayman, 1970) omitting phenol from the reagents. After root staining mycorrhizal colonization was determined by a gridline intersect method (Giovannetti and Mosse, 1980).

Calculations and data analysis

This experiment had a randomized complete block design with three replications per treatment and harvest. The treatments in this experiment were 2 levels of AM inoculation (inoculated and non-inoculated), 5 levels of salinity (2.2, 3.5, 5, 7.5 and 12 dS/m) and 3 harvest times (10, 20 and 30 days after transplanting). Data were analysed statistically using analyses of variance with GenStat 6 Release 6.1 (2002) software. Probabilities of significance among treatments and interactions and LSDs ($P \leq 0.05$) were used to compare means within and among treatments.

Salinity response of shoot dry weight was calculated in mycorrhizal (M) or nonmycorrhizal (NM) plants as follows:

$$\%\,\text{Salinity response} = \frac{\text{DW (+salt) - DW (-salt)}}{\text{DW (-salt)}} \times 100 \quad (1)$$

Mycorrhizal growth response (MGR) was calculated using the individual total plant dry weight (DW) of M and the mean dry weight of NM plants at each harvest as follows:

$$\%\text{MGR} = \frac{\text{DW(M) - mean DW (NM)}}{\text{mean DW (NM)}} \times 100 \quad (2)$$

Mycorrhizal P response (MPR) was calculated as follows:

$$\%\text{MPR} = \frac{\text{P content (M) - mean P content (NM)}}{\text{mean P content (NM)}} \times 100 \quad (3)$$

Mycorrhizal potassium (K) response (MKR) was calculated similarly.

Results

Plant growth

A trail experiment showed that 15 day after planting M plants had 20% colonization and there were no significant differences between M and NM seedlings on SDW and shoot P concentration (data not shown). The average of SDW at transplanting time was 25 mg and shoot P concentration was 0.1% in both M and NM seedlings. At 10 days after transplanting colonization had increased from 20% to between 50 and 65%, with no significant differences between salinity treatments (Figure 1). Differences in colonization within salinity treatments between the first and the second harvest were not significant, but colonization significantly decreased at the third harvest particularly at 7.5 dS/m and above.

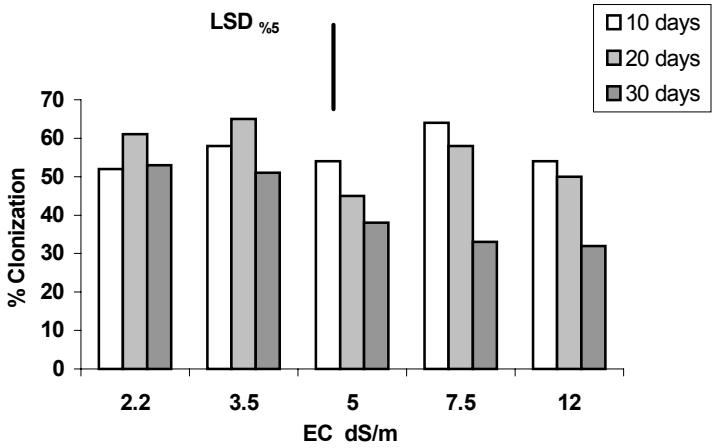

Figure 1. Colonization of roots of *Trifolium subterraneum* grown in different levels of salinity at different harvests.

Analysis of variance on total shoot and root dry weight indicated highly significant effects due to mycorrhizal fungi and, salinity and harvest (Figure 2). M plants were larger than NM plants in all treatments. There were no significant differences in shoot and root dry weight between M and NM plants at 10 days after transplanting, but significant differences were apparent at 20 and 30 days at all salinity levels.

The effects of different levels of salinity on plant shoot dry weight are shown in terms of salinity responses (Eqn 1) at the first and third harvests (Figure 3). With increasing salinity SDW decreased in M and NM plants at both harvests. Reduction of shoot dry weight was apparent at all salinity levels in NM plants, but only at 7.5 dS/m and above in M plants at 30 days after transplanting. Salinity responses in M plants were higher at high levels of salinity at 10 days after transplanting compared to NM plants, but lower at 30 days at the same salinity level. The same trend was apparent for root dry weight (results not shown).

Mycorrhizal growth responses (Eqn 2) in terms of total dry weight at different salinity levels and different harvests are shown in Fig 4. The effect of salinity and plant age on MGR was significant. As salinity increased MGR decreased. However, MGR increased with time, so that the greatest effect of mycorrhizal fungi in increasing plant dry weight occurred at 3.5 dS/m at the third harvest.

P uptake

Salinity and mycorrhizal inoculation had some effects on plant nutrient concentration (Tables 1 and 2). P concentrations were significantly higher in M plants than NM plants at all salinities in shoots and roots at the first harvest. There was a trend towards decreased P concentrations in shoots and roots of M and NM plants with increasing salinity, but it was non significant. M plants had significantly higher shoot P concentration at first harvest, but

not at the third harvest. Root P concentration was significantly higher in M plants than NM at all harvests.

P contents of the shoots were used to calculate MPR (Figure 5), using Eqn 3. MPR increased significantly with increasing salinity up to 5 dS/m, but subsequently decreased to a minimum at 12 dS/m. MPRs were significantly higher at first harvest than second and third harvests. The same trend was found for MPR in roots (results not shown).

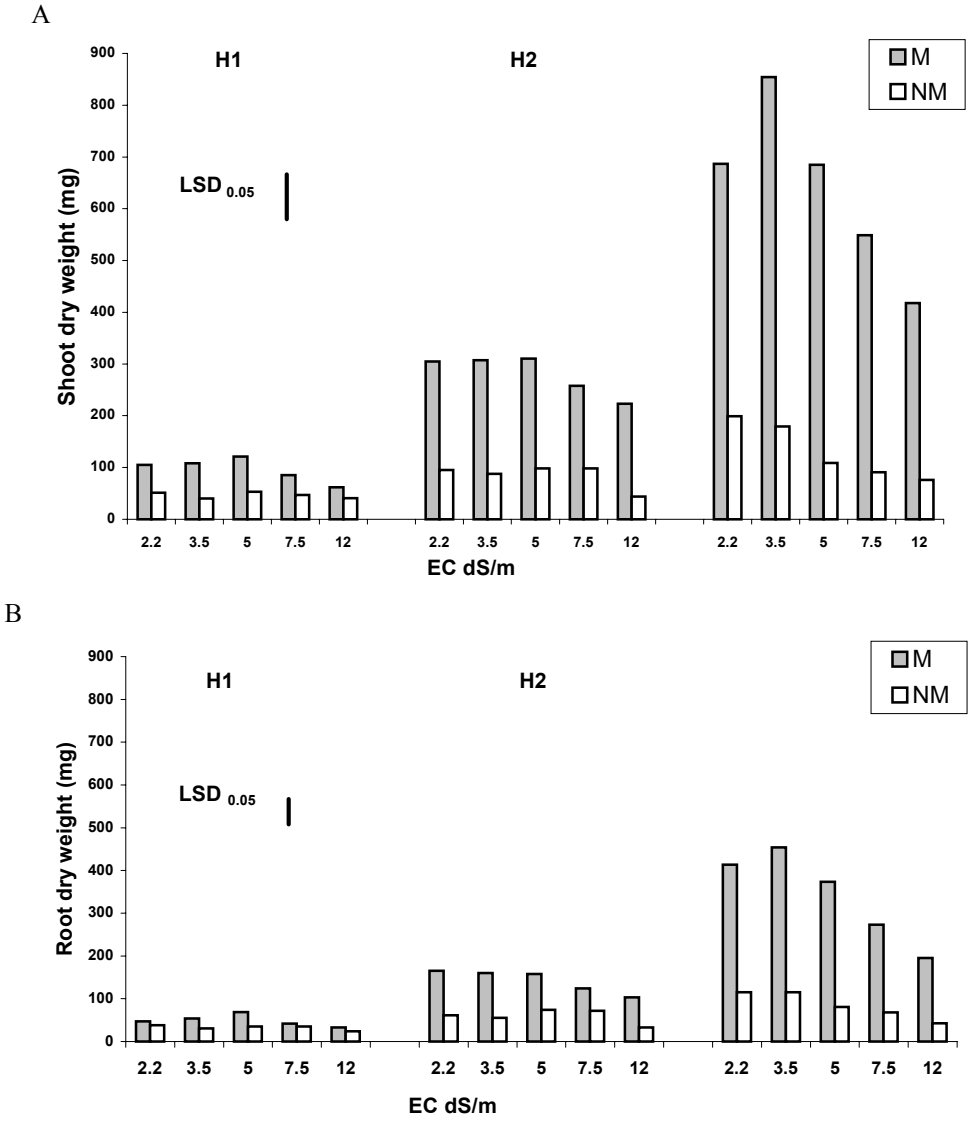

Figure 2. Shoot (A) and root (B) dry weights growth at different salinity levels in mycorrhizal and nonmycorrhizal *Trifolium subterraneum.*

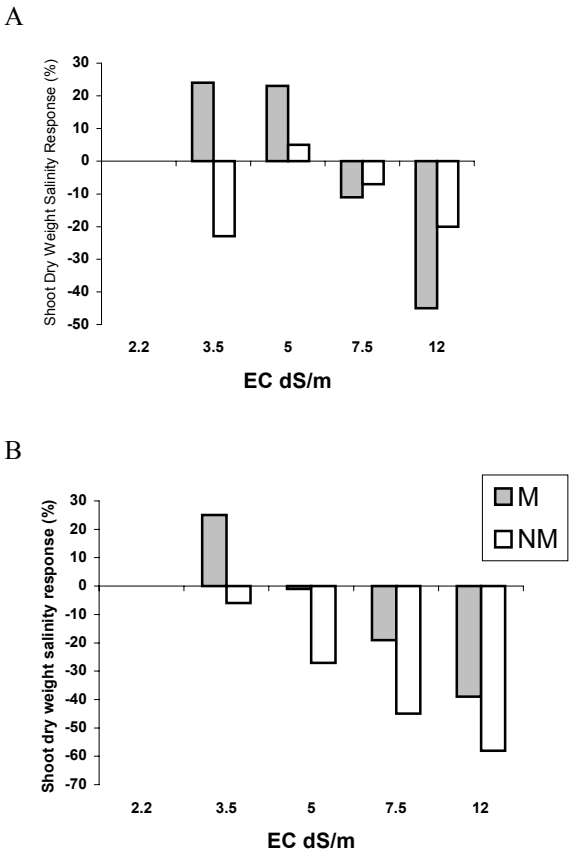

Figure 3. Salinity responses of shoot dry weight in mycorrhizal and nonmycorrhizal *Trifolium subterraneum* at first (A) and third (B) harvests.

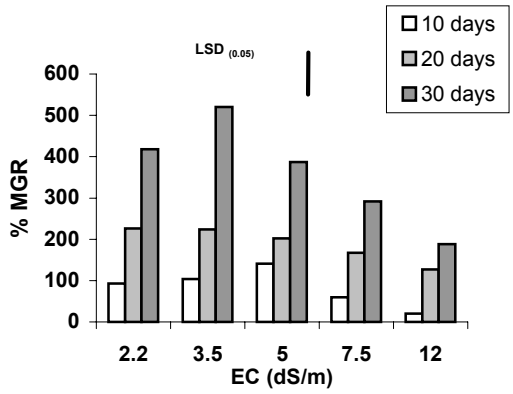

Figure 4. Mycorrhizal growth response (MGR) on *Trifolium subterraneum* total dry weight at different salinity levels and different harvests.

Table 1. Shoot and root nutrient concentrations of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at 10 days after transplanting to different salinity levels. Means of 3 replicates ± standard error.

Treatments	Salinity (dS/m)	Root (mg/g)			Shoot (mg/g)		
		P	K	Na	P	K	Na
M	2.2	3.3±0.2	2.1±0.1	8.9±1.2	2.7±0.0	14.1±3.5	6.3±1.3
	3.5	4.1±0.1	35.2±0.9	12.8±1.1	2.4±0.2	16.5±2.3	6.9±0.7
	5	3.9±0.3	37.8±2.3	17.2±0.4	3.1±0.4	12.6±1.8	8.2±1.2
	7.5	4.5±0.3	37.4±3.5	14.9±0.8	2.9±0.2	10.6±1.7	11.0±1.1
	12	3.7±0.4	46.4±0.3	13.0±1.6	2.5±0.3	15.0±2.3	16.5±2.2
NM	2.2	0.9±0.1	34.6±1.2	10.7±0.8	0.5±0.1	16.4±2.9	5.8±0.3
	3.5	0.5±0.1	37.6±1.8	13.4±0.6	0.7±0.1	17.3±0.6	4.6±0.2
	5	0.6±0.2	32.0±1.8	12.0±0.1	0.6±0.1	17.3±1.4	6.1±0.4
	7.5	0.4±0.1	33.4±1.1	17.2±1.4	0.5±0.1	12.0±1.3	6.3±1.1
	12	0.2±0.0	28.2±2.0	19.3±2.4	0.4±0.1	14.9±2.2	1.7±1.2
Inoculation		***	**	ns	***	ns	***
Salinity		ns	ns	ns	ns	ns	***

, * significant at the 0.01, 0.001 probability levels, respectively
ns not significant at P≤0.05

Table 2. Shoot and root nutrient concentrations of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at 30 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity (dS/m)	Root (mg/g)			Shoot (mg/g)		
		P	K	Na	P	K	Na
M	2.2	2.1±0.7	7.2±2.4	8.4±.0.9	2.2±0.3	16.1±5.9	4.7±1.3
	3.5	2.1±0.7	23.7±3.4	10.5±1.1	1.9±0.4	18.8±1.8	8.1±0.7
	5	2.7±0.2	21.1±1.9	9.5±0.8	1.8±0.4	21.2±1.4	8.5±2.4
	7.5	3.3±0.0	28.8±1.7	10.3±0.7	1.4±0.4	10.7±0.9	10.9±1.7
	12	3.4±0.2	26.4±1.1	12.1±0.6	1.8±0.1	8.8±1.4	17.6±2.3
NM	2.2	1.8±0.3	27.4±1.3	8.1±0.4	1.7±0.4	32.4±12.1	4.1±0.6
	3.5	1.6±0.1	28.4±2.2	10.0±0.4	1.8±0.4	29.2±10.9	5.3±1.0
	5	1.7±0.2	29.8±1.3	10.5±0.1	1.2±0.1	16.2±6.0	10.3±2.2
	7.5	1.2±0.0	22.6±1.4	9.8±0.1	1.6±0.0	14.8±3.7	9.9±1.5
	12	1.6±0.1	26.4±5.1	16.5±1.5	1.8±0.1	14.0±3.7	23.1±2.2
Inoculation		***	*	ns	ns	ns	ns
Salinity		ns	ns	***	ns	ns	***

*, *** significant at the 0.05, 0.001 probability levels, respectively
ns not significant at P≤0.05

K and Na uptake

Root K concentration was significantly reduced with increasing salinity in NM plants, but there was an increase in M plants (Tables 1 and 2). Increased salinity also decreased K concentration in roots of NM plants, but there was a significant increase in roots of M plants. There were no significant differences in shoot K concentrations by increasing salinity. The MKR in shoots shows that mycorrhizal effects on K uptake were generally higher at 30 days than at 10 days, but decreased with increasing soil salinity at all harvests, although the extent of this difference decreased with increasing salinity between 3.5 and 12 dS/m (results not shown). Values at 20 days varied rather less between treatments. MKR in roots showed the same trend as shoots (results not shown). With increasing salinity shoot Na concentration was significantly increased in both M and NM plants (Tables 1 and 2). Na concentration significantly increased in roots of NM plants against a reduction in M plants (particularly at 10 days). However at 12 dS/m root Na concentration in M plants was significantly lower than NM plants.

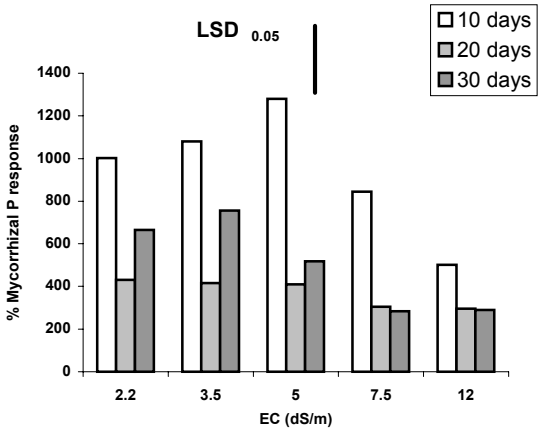

Figure 5. Mycorrhizal P response (MPR) of Trifolium subterraneum shoots at different salinity in three harvests.

The K/Na ratio in terms of concentration in shoots of both M and NM plants decreased greatly as salinity increased with values for NM plants generally higher than for M plants. Differences decreased as salinity increased. In roots salinity had little effects on K/Na ratio in M plants, but in NM plants values declined significantly as salinity increased. Major differences between M and NM plants were observed at 2.2 (NM>M) and 12 dS/m (M>NM) (results not shown).

Discussion

Overall the results show that seedling growth and hence establishment was increased by preinoculation of subterraneum clover with *Glomus intraradices* (Schenk and Smith) in saline conditions compared with non-inoculated conditions. Preinoculation increased mycorrhizal effectiveness and ensured root colonization under salt stress. There was no

significant effect of salt on root colonization at 10 days after transplanting (Figure 1). Initial mycorrhizal colonization depends on germination of spores or other fungal propagules in the soil, growth of hyphae through the soil and finally hyphal entry to the roots (Bowen, 1987). Previous studies in which seeds or seedlings were planted into the soil and the salt concentration increased gradually or suddenly (Azcón and Elatrash, 1997; Al-Karaki, 2000; Al-Karari et al., 2001), showed that mycorrhizal colonization was negatively affected by increased soil salinity, possibly via the effects of salinity on initial colonization. In this investigation seedling preinoculation allowed the fungi to become established before salinity stress was imposed, and the percentage of mycorrhizal colonization was not significantly affected by salt level. It is concluded that preinoculation in the nursery would increase mycorrhizal colonization and consequently improved ability of seedlings to benefit from any mycorrhizal effects (tolerate salinity stress after transplanting to the field).

The results present are in agreement with previous work showing that plant growth in saline soils is increased by AM inoculation (Al-Karaki, 2006). Nonmycorrhizal plants showed an immediate dry weight reduction after salinity stress, but there were deferred up to 7.5 dS/m in M plants. The reason for lack of significant differences in shoot and root dry weight between M and NM plants at 10 days after transplanting could be related to the low level of carbon resources available in M plants at this early stage of growth. Carbon allocated to the developing fungi may have decreased allocation of necessary resources for salinity tolerance and hence decreased survival in salt stressed conditions (Johnson et al., 1997).

Increased growth of M plants in saline conditions was at lease partly related to enhance P concentration in shoots and roots, as suggested by other researchers (Asghari et al., 2005; Al-Karaki, 2006). A positive mycorrhizal P response was observed at all three harvests and all salinity levels, although the magnitude of the response decreased at 7.5 and 12 dS/m.

Shoots and roots P concentration significantly increased in M than NM plants grown at 12 dS/m salinity (Tables 1 and 2), this results is in contrast to a study where the effects of mycorrhizal fungi on tomato shoot P concentration were not significant at the highest salinity level (7.4 dS/m) (Al-Karaki, 2000). Despite of a few results indicating an increase in P content with increasing soil salinity (Villora et al., 2000), there are many reports that demonstrate reduction in plant P uptake in saline conditions in different plants (Pond et al., 1984; Martinez et al., 1996; Navarro et al., 2001).

Less attention has been paid to the effects of mycorrhizal fungi on plant nutrient uptake under salt stress during plant growth. Calculation of MPR shows that the efficiency of mycorrhizal fungi to increase P uptake is highest at 10 days after transplanting and decreased as plants aged (Figure 5). This is consistent with previous observations that mycorrhizal colonization increased P inflow at an early stage of plant growth, compared to nonmycorrhizal plants, and declined with increasing plant age (Smith et al., 1986; Jakobsen et al., 1992). Furthermore the critical role of P in energy reactions in the plant and high importance of phosphorus nutrition early during crop plant growth has been demonstrated (Grant et al., 2001).

In this study increased salinity generally decreased shoot K concentrations in both M and NM plants. Lower shoot K concentration in M plants could be related to increased

plant biomass via increased P uptake and consequent dilution of K particularly at third harvest. Increased K concentration in roots of M plants with increasing salinity was another important effect of AM fungi, which may be related to salinity tolerance. Potassium is an essential element for turgor-pressure-driven solute transport in the xylem and water balance in plants (Marschner, 1995) and its acquisition is a cellular process and critical for increasing salt tolerance in glycophytic plants (Wu et al., 1996; Zhu et al., 1998). Potassium deficiency induced by Na is a major problem for plants under salt stress (Grattan & Grieve, 1999). Some studies have reported that AM fungal colonization had no significant effects on plant K contents and concentration (Poss et al., 1985; Rozema et al., 1986; Al-Karaki, 2000; Mohammad et al., 2003), but results of this study indicate root K concentration increase at high salinity level. Calculation of MKR indicates that M plants take up more K than NM plants and that response increased with increasing plant age. Maximum MKRs were observed at 30 days at moderate salinity level. It is concluded that increased K uptake at a later stage of plant establishment could be another mechanisms to combat salinity stress in M plants.

The results of this study show that shoot Na concentrations in M plants were greater than NM plants (Tables 1 and 2). A significant increase in Na concentration was found in roots of NM plants, but there was a reduction in M plants. Previous reports have been inconsistent. Some researchers found that concentration of Na in shoot tissue of non halophytic plants following addition of NaCl to the soil was higher in M plants compared to NM plants (Allen and Cunningham, 1983; Pfeiffer and Bloss, 1988; Cantrell and Linderman, 2001). In contrast, M plants of halophytic *Aster tripolium* had less Na in shoots than NM plants when grown in saline conditions (Rozema et al., 1986). Mycorrhizal inoculation decreased shoot Na concentration in barley plants grown in a soil with a high level of salinity (16.6 dS/m), but had no effect when plants were grown in low salinity (Mohammad et al., 2003).

Decreased Na concentration in roots of M plants accompanied by increased root K concentration could be important factors to increase the ratio of K to Na and hence plant salinity tolerance due to AM fungi at high salinity. The ratio of K to Na in saline soils is often extremely low, so that Na^+ ions can inhibit uptake of K^+ ions, which inhibit enzyme functions due to ion imbalance (Brain et al., 1999).

In summary, the results indicate that preinoculation with mycorrhizal fungi had a significant role in promoting seedling growth and establishment of *Trifolium subterraneum* in saline conditions, with best results obtained at moderate levels of salinity (3.5-5 dS/m) at 30 days after transplanting. Improved mineral nutrition (Marschner and Dell, 1994; Al-Karaki and Al-Raddad, 1997), improved water potential (Hildebrandt et al., 2001; Marulanda et al., 2003), improved physiological processes (increased carbon dioxide exchange rate, transpiration, stomatal conductance and water use efficiency) (Ruiz-Lozano et al., 1996) are the most important salinity tolerance mechanisms in mycorrhizal plants that have been reported. The results of this study show that improved nutrient uptake is probably the major mechanism involved in increasing seedling salinity tolerance. Increased P uptake plays the main role for salt stress tolerance at early stages and increased K uptake at later stages after transplanting. These results may be of practical importance as they highlight the potential of using preinoculated mycorrhizal seedlings to revegetate saline

lands. Future research is needed to evaluate the role of mycorrhizal fungi on uptake of other elements in saline conditions in other plant species.

Acknowledgements

The author thanks professors S.E. Smith and F.A. Smith for their supervisions in the project. The author gratefully acknowledges Iranian Ministry of Science, Research and Technology for scholarship grants. Mr. Colin Rivers and Ms. Debbie Miller for their excellent technical assistance, and thanks other members of soil biology group of Soil and Land Systems of School of Earth and Environmental Sciences at Adelaide University.

References

Al-Karaki, G.N., 2006. Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Scientia Horticulturae 109:1-7.

Al-Karaki, G.N., 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza* 10: 51-54.

Al-Karaki, G.N, Al-Raddad A., 1997. Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of wheat genotypes differing in drought resistance. Mycorrhiza 7, 83-88.

Al-Karari, G.N, Hammad, R., Rusan, M., 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. Mycorrhiza 11, 43-47.

Asghari, H.R., Marschner, P., Smith, S.E., and Smith, F.A., 2005. Growth response of *Atriplex nummularia* to inoculation with arbuscular mycorrhizal fungi at different salinity levels. Plant and Soil 373, 245-256

Allen E.B., Cunningham, G.L., 1983. Effects of vesicular-arbuscular mycorrhizae on Distichlis spicata under three salinity levels. New Phytologist 93, 227-236.

Azcón, R,, Elatrash, F., 1997. Influence of arbuscular mycorrhizae and phosphorus fertilization on growth, nodulation and N_2 fixation (N^{15}) in *Medicago sativa* at four salinity levels. Biology and Fertility of Soils 24, 81-86.

Bowen, G.D., 1987. The biology and physiology of infection and its development. In:eds. Ecophysiology of VA Mycorrhizal Plants. Boca Raton Florida., CRC Press., 27-70.

Brain, J., Atwell, B., Kriedemann, P., Paul, E., Collin, G.N., 1999. Plants in action, edn.Macmillan Education Australia.

Cantrell, I.C., Linderman, R.G., 2001. Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant and Soil* 233: 269-281.

Colwell, J.D., 1963. The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales. Australian Journal of Experimental Agricultural and Animal Husbandry 3, 190-197.

Fitter, A.H., Merryweather, J.W., 1992. Why are some plants more mycorrhizal than others? an ecological enquiry. In: eds. Mycorrhizas in Ecosystems. Wallingford, UK., CAB International., 26-36.

Flowers, T.J., Garcia, A., Koyama, M., Yeo, A.R., 1997. Breeding for salt tolerance in crop plants - the role of molecular biology. Acta Physiologiae Plantarum 19, 427-433.

Giovannetti. M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologistl, 84: 489-500.

Giri, B., Kapoor, R., Mukerji, K.G., 2002. VA Mycorrhizal techniques/VAM technology in establishment of plants under salinity stress conditions. In: Mukerji KG, Manoharachary C, Chamola BP, eds. Techniques in mycorrhizal studies. Netherlands: Kulwer, 313-327.

Grant, C.A., Flaten, D.N., Tomasiewicz, D.J., Sheppard, S.C., 2001. The importance of early season phosphorus nutrition. Canadian Journal of Plant Science 81, 211-224.

Grattan, S.R., Grieve, C.M., 1999. Salinity mineral nutrient relations in horticultural crops. *Scientia Horticulturae* 78: 127-157.

Hanson, W.C., 1950. The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. Journal of the Science of Food and Agriculture 1: 172-173.

Hewitt, E.J., 1966. Sand and water culture methods used in the study of plant nutrition, edn. Farnham Royal, United Kingdom,: Commonwealth Agric. Bureaux.

Hildebrandt, U., Janetta, K., Ouziad, F., Renne, B., Nawrath, K., Bothe, H., 2001. Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes. Mycorrhiza 10, 175-183.

Hirrel, M.C., Gerdemann, J.W., 1980. Improved growth of onion and bell pepper in saline soils by two vesicular-arbuscular mycorrhizal fungi. Soil. Science. Society. American. Journal 44, 654-658.

Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytologist 120. 371-380.

Johnson, N.C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist 135, 575-586.

Malcolm, C.V., Lindley, V.A., O'Leary, J.W., Runciman, H.V., Barrett-Lennard, E.G., 2003. Halophyte and glycophyte salt tolerance at germination and the establishment of halophyte shrubs in saline environments. Plant and Soil 253, 171-185.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd edn. London San Diego: Academic Press.

Marschner, H., Dell, B., 1994. Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159, 89-102.

Martinez, V., Bernstein, N., Lauchli, A., 1996. Salt-induced inhibition of phosphorus transport in lettuce plants. Physiologia Plantarum 97, 118-122.

Marulanda, A., Azcon, R., Ruiz-Lozano, J.M., 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by Lactuca sativa plants under drought stress. Physiologia Plantarum 119, 1-8.

Mohammad, M.J., Malkawi, H.I., Shibli, R., 2003. Effects of arbuscular mycorrhizal fungi and phosphorus fertilization on growth and nutrient uptake of barley grown on soils with different levels of salts. Journal of Plant Nutrition 26: 125-137.

Navarro, J.M., Botella, M.A., Cerda, A., Martinez, V., 2001. Phosphorus uptake and translocation in salt-stressed melon plants. Journal of Plant Physiology 158, 375-381.

Niu, X.M., Bressan, R.A., Hasegawa, P.M., Pardo, J.M., 1995. Ion Homeostasis in Nacl Stress Environments. *Plant Physiology* 109: 735-742.

Ojala, J.C., Jarrell, W.M., Menge, J.A., Johnson, ELV., 1983. Influence of mycorrhizal fungi on the mineral nutrition and yield of onion in saline soil. Agronomy Journal 75: 255-259.

Pande, M., Tarafdar, J.C., 2002. Effect of phosphorus, salinity and moisture on VAM fungal association in neem (Azadirachta indica Linn.). Symbiosis 32, 195-209.

Pfeiffer, C.M., Bloss, H.E., 1988. Growth and nutrition of guayule (Parthenium argentatum) in a saline soil as influenced by vesicular-arbuscular mycorrhiza and phosphorus fertilization. New Phytologist 108, 315-321.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55, 158-161.

Pond, E.C., Menge, J.A., Jarrell, W.M., 1984. Improved growth of tomato in salinized soil by vesicular-arbuscular mycorrhizal fungi collected from saline soils. Mycologia 76, 74-84.

Poss J.A., Pond, E., Menge, J.A., Jarrell, W.M., 1985. Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. Plant and Soil 88, 307-319.

Powell C.L., 1975. Potassium uptake by endotrophic mycorrhizas. In: Sanders F.E., Mosse B., Tinker, P.B., eds. Endomycorrhizas. London: Academic Press, 461-468.

Rozema, J., Arp, W., Vandiggelen, J., Vanesbroek, M., Broekman, R., Punte, H., 1986. Occurrence and Ecological Significance of Vesicular Arbuscular Mycorrhiza in the Salt-Marsh Environment. Acta Botanica Neerlandica 35, 457-467.

Ruiz-Lozano, J.M., Azcón, R., Gomez, M., 1996. Alleviation of salt stress by arbuscularmycorrhizal Glomus species in Lactuca sativa plants. Physiologia Plantarum 98, 767-772.

Ryan, J., Garabet, S., Harmsen, K., Rashid, A., 1996. A soil and plant analysis manual adapted for the West Asia and North Africa region, edn. ICARDA, Aleppo, Syria:

Smith, S.E., St John, B.J., Smith, F.A., Bromley, J.L., 1986. Effects of mycorrhizal infection on plant growth, nitrogen and phosphorus nutrition of glasshouse-grown *Allium cepa* L. New Phytologist 103, 359-373.

Tsang, A., Maun, M.A., 1999. Mycorrhizal fungi increase salt tolerance of Strophostyles helvola in coastal foredunes. Plant Ecology 144, 159-166.

Villora, G., Moreno, D.A., Pulgar, G., Romero, L., 2000. Salinity affects phosphorus uptake and partitioning in zucchini. Communications in Soil Science and Plant Analysis 31, 501-507.

Wu. S.J., Ding, L., Zhu, J.K., 1996. SOS1, a genetic locus essential for salt tolerance and potassium acquisition. Plant Cell 8, 617-627.

Zhu, J.K., Liu, J.P., Xiong, L.M., 1998. Genetic analysis of salt tolerance in Arabidopsis: Evidence for a critical role of potassium nutrition. Plant Cell 10, 1181-1191.