

## EFFECT OF MYCORRHIZAL INOCULATION ON GROWTH, NITROGEN FIXATION AND NUTRIENT UPTAKE IN ALFALFA (*MEDICAGO SATIVA*) UNDER SALT STRESS

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**ABSTRACT.** Most legumes in natural conditions form a symbiosis with arbuscular mycorrhizal (AM) fungi. AM fungi in saline soils have been reported to improve salinity tolerance and growth in plants. In the present study, interaction between mycorrhizal fungus, *Glomus mosseae*, and salinity stress in relation to plant growth, nitrogen fixation, and nutrient accumulation was evaluated in alfalfa (*Medicago sativa*). Two alfalfa cultivars (Bami and Yazdi) were compared under different levels of salinity with and without mycorrhizal inoculations. Salt stress resulted in a noticeable decline in shoot and root dry matter accumulation, resulting in a decline in the shoot to root ratio (SRR) in all plants. However, Bami was found to be more tolerant to salinity than Yazdi. Inoculated plants exhibited better growth and biomass accumulation under stressed as well as unstressed conditions. Mycorrhizal colonization (MC) was reduced with increasing salinity levels, but the mycorrhizal dependency (MD) increased,

which was more evident in Yazdi. Nodulation was completely inhibited under salt stress conditions for both non - AM inoculated alfalfa varieties. Nodulation only occurred in inoculated plants. Nitrogenase activity was reduced with increasing salt concentrations. AM inoculated plants had considerably higher nodule numbers, dry weights, and nitrogenase activity under nonsaline environments. Bami had a comparatively lower Na<sup>+</sup> concentration and higher K<sup>+</sup> and Ca<sup>2+</sup> concentrations than Yazdi. Although nitrogen (N) and phosphorus (P) contents declined with increasing salinity, Bami had higher levels of N and P, as compared with Yazdi. Plants inoculated with *Glomus mosseae* had better plant growth and nitrogen fixation under salt stress.

**Key words:** Alfalfa; *Glomus mosseae*; Growth; Nitrogenase; Nodulation; Nutrients.

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## INTRODUCTION

One of the most severe and widespread problems as a result of the agricultural industry in some arid and semiarid regions is the degradation of soil quality due to desiccation and salinity. In fact, almost 40% of the world's land surface is affected by salinity-related problems (Zahran, 1999). The stresses imposed by salinity are mainly due to ion compositions and concentrations in the rhizosphere and in plant tissues (Volkmar *et al.*, 1998). In plants, salinity drastically affects photosynthesis (Soussi *et al.*, 1999), nitrogen metabolism (Santos *et al.*, 2002), and carbon metabolism (Balibrea *et al.*, 2003), and provokes disorders in plant nutrition that may lead to deficiencies of several nutrients and high levels of Na<sup>+</sup> (Mengel and Kirkby 2001). Such physiological changes result in decreased plant growth and consequently decreased crop yield (Singla and Garg 2005; Tejera *et al.*, 2006). Most legumes are known to be salt sensitive (Munns 2002), and the increasing worldwide use of irrigation has led to the prediction that, by 2050, 50% of all arable land will be salinized (Wang *et al.*, 2003). However, salt-affected soils can be utilized by growing salt-tolerant crops, because such crops would allow expansion of crop production to areas where conventional reclamation procedures are economically or technically limited (Ashraf and McNeily, 2004; Rejili *et al.*, 2007).

Vadez *et al.* (2006) and Mantri *et al.* (2007) reported that it is becoming increasingly important to produce genotypes/cultivars tolerant to high salinity for sustainable alfalfa production.

Plant roots are exposed to a range of soil microorganisms, with which they form a variety of interactions (Manchanda and Garg 2007). Associative and symbiotic nitrogen-fixing bacteria and arbuscular mycorrhizal (AM) fungi are common beneficial microorganisms of leguminous plants. It is frequently suggested that AM fungi may improve phosphorus nutrition and enhance nitrogen uptake, production of growth-promoting substances, or adaptation to various environmental stresses (Entry *et al.*, 2002; Saleh Al-Garni, 2006).

Alfalfa (*Medicago sativa*) is a perennial flowering plant in the pea family fabaceae cultivated as an important forage crop in many countries around the world. The alfalfa is native to a warmer temperate climate such as that of Iran (where it is thought to have originated). Alfalfa is one of the most salt-sensitive legumes, and it has been stated that there is too little variability in alfalfa for salinity tolerance to allow successful breeding of salinity-tolerant varieties (Johansen *et al.*, 1990). The main aim of the present investigation was to study variability among different cultivars of alfalfa on the basis of their relative plant growth, nitrogen fixation and nutrient acquisition under salt stress and to

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investigate the role of AM fungi in conferring salinity tolerance.

### MATERIALS AND METHODS

#### Plant growth conditions

The experiment was conducted in soil microbiology laboratory and greenhouse of College of Agriculture, Tehran University, Iran, for eight months, from 10<sup>th</sup> of April to 10<sup>th</sup> of November, 2011.

#### Treatments

The alfalfa seeds were rinsed with water and surface sterilized by dipping in 0.1% sodium hypochlorite for 2 min and then washed three times with distilled water. Seeds were pretreated with a standard rhizobial inoculum of *Sinorhizobium meliloti*. The AM fungal spores were applied at 10 spores per seed (approximately 1500 spores/100 g of media). Seeds were inoculated by placing the fresh AM inoculum (30 g) in the hole under the seeds and covering with the soil.

Ten inoculated seeds along with control seeds (untreated) of two alfalfa cultivars were sown in 30 cm diameter pots. Each pot was filled with 2 kg non-sterilized soil. The soil used for pots was collected from the uncultivated site located in Qom province, Iran. The soil used in this experiment was sterilized (autoclaved). The basic soil properties were as follows: organic matter content 1.08%, total N 0.062%, total K 740.8 mg kg<sup>-1</sup>, total P 10.90 mg kg<sup>-1</sup>, available P (NaHCO<sub>3</sub>-extractable) 2.78 mg kg<sup>-1</sup>, water-soluble K 13.43 mg kg<sup>-1</sup> and electrical conductivity 8.1 dSm<sup>-1</sup>.

The plants were treated with saline solution with electrical conductivities 6 (S1 treatment) and 12 dSm<sup>-1</sup> (S2 treatment). The control plants (C) were treated with distilled water only. Pots

were irrigated according to their weight at 80% field capacity moisture. Plants were grown in the greenhouse under natural sunlight with temperatures of 25-30°C (day) and 20-23°C (night). After 15 days, thinning was carried out to leave five uniform seedlings in each pot. There were three replications for each treatment. Regular fortifications of saline solutions were made to maintain the desired soil salinity levels after monitoring the conductivity levels of the soils at weekly intervals, with the help of EC meter, till the end of the experiments. Plants were harvested at 180 days after sowing for detailed investigations.

#### Measurements

##### Salt tolerance index (STI)

The salt tolerance index was calculated as total plant (shoot + root) dry mass at different salt concentrations, compared to the total dry mass obtained for controls:  $STI = (TDW \text{ at } S_x / TDW \text{ at } S_i) \times 100$ , where TDW is the total dry weight;  $S_i$  is the control treatment and  $S_x$  is the x treatment.

##### Mycorrhizal colonization

Mycorrhizal colonization was estimated by the method of Phillips and Hayman (1970). The roots were cut and dipped in KOH solution for 24 h and then kept in HCl solution for 15-30 min. A staining solution containing 0.05% (v/v) cotton blue dye was added. The samples were kept for 24-36 h at room temperature condition. The roots were cut into small pieces of approximately 2.5 cm and observed under compound light microscope. Root pieces that contained even a single vesicle or arbuscule were considered colonized. The percentage of AM colonization was calculated from the following equation: Percentage of AM colonization = (Root length colonized / Root length observed) × 100.

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An index of mycorrhizal dependency (MD) was determined by expressing the dry weights of the plants concerned as a percentage of the dry weight of the control plants (Estaun *et al.*, 1987).

### **Nitrogenase activity**

Nitrogenase was determined by the acetylene reduction activity test (ARA) on the nodulated root portion of three plants, following the method of Herdina and Silsbury (1990). Nitrogen fixing complex (nitrogenase) of legumes is able to reduce  $C_2H_2$  to  $C_2H_4$ . The nodulated root sample (1 g of root plus nodules) was immediately incubated at room temperature in vials containing acetylene ( $C_2H_2$ ) (10%, v/v) and sealed with serum caps. The sample of 1 ml of gas from the incubation mixture was analyzed for ethylene in a Perkin Elmer 8600 gas chromatograph equipped with a Porapak R column (Ligero *et al.*, 2007). From the standard values, the number of moles of ethylene produced in each case was calculated, the nodules were dried in an oven at 70°C for 24 h, and their dry weights were taken. The rate of enzyme activity was calculated as the number of moles of ethylene produced per mg dry weight of nodules per hour.

### **Calcium, sodium and potassium content**

Calcium, sodium and potassium contents were estimated using flame photometry and atomic absorption spectrophotometry by the method of Chapman and Pratt (1961). First, 10 mL of acid mixture consisting of nitric acid, sulfuric acid, and perchloric acid at a ratio of 9:4:1 was added to 2-5 g of ground samples and kept at 120°C overnight. The samples were then maintained at 70°C on a hot plate for 30 min; the temperature was increased to 120°C for 30 min and then to 250°C until only 3-4 mL of the sample was left. A final volume of 50 mL

was maintained with distilled water and left overnight.

The next day, it was filtered using Whatman No. 1 filter paper. Calcium, sodium and potassium contents were estimated on a flame photometer. A blank was run without plant samples.

### **Nitrogen content**

Nitrogen content was estimated by the colorimetric method of Lindner (1944) using Nessler's reagent following digestion in a mixture of concentrated sulfuric acid and perchloric acid.

### **Phosphorus content**

Phosphorus was estimated by the method given by Chapman and Pratt (1961). Vanadate solution was added to the molybdate solution and cooled to room temperature; 250 mL of concentrated  $HNO_3$  was then added and diluted to 1 L. Next, 0.5 g of plant material (shoots and roots) was taken in 50 mL volumetric flasks and 10 mL of vanadomolybdate reagent was added to each flask. The volume was achieved with deionized water. The solution sat for 30 min, and then the absorbance was taken at 420 nm with a spectrophotometer. Appropriate standards were run simultaneously.

All data were subjected to analysis of variance using two-way ANOVA, and means were compared with Duncan's multiple-range test (Duncan, 1955).

## **RESULTS**

The salt tolerance index (STI) is proposed as an indicator of the inherent salinity tolerance or resistance of agricultural crops to root-zone salinity. Two cultivars of alfalfa were evaluated in saline and non-saline environments for their relative salt tolerance index (*Table 1*). On the basis of STI, it was concluded

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that Bami cultivar had suitable salinity tolerance; on the other hand, Yazdi cultivar was comparatively salt-sensitive and had lower STI. The results categorized Bami cultivar as the most salt-tolerant cultivar and Yazdi as the most salt-sensitive cultivar. Salt stress decreased the root and shoot dry weights (*Table 1*) in the non - inoculated plants of both cultivars. In general, roots seemed to withstand salt stress better than the shoots, and their dry matter was significantly greater than that of the shoots. As a result, a decline in shoot to root ratio (SRR) (*Table 1*) was recorded in all plants with increasing saline dosages. Bami was able to maintain a higher SRR as compared to Yazdi. Root colonization by AM fungi enabled the plants to grow better under similar stress conditions, and the shoot and root dry weights of stressed inoculated plants were greater than those of their non-inoculated counterparts. The results from the present study (*Table 2*) depict a decrease in the percentage of mycorrhizal colonization with increasing salinity in both cultivars; the decrease in colonization was more significant in the salt-sensitive cultivar, Yazdi. Mycorrhizal dependency (MD) increased in both genotypes with increasing salt concentrations. Yazdi showed greater dependence on its mycorrhizal partner and thus had higher MD than Bami.

The current study (*Table 3*) revealed the accumulation of  $\text{Na}^+$  ions with a decrease in the uptake of  $\text{K}^+$  ions in both genotypes under salt stress. It was seen that most of the  $\text{Na}^+$

was held up in the roots, and much less reached the shoots in both cultivars. A salinity-induced decrease in the calcium content was observed in the shoots and roots of both cultivars, but this decrease in calcium content was much less in Bami. Bami had a comparatively lower  $\text{Na}^+$  concentration and higher  $\text{K}^+$  and  $\text{Ca}^{2+}$  concentrations than Yazdi. The ion deficiency displayed by salinity stress, particularly by NaCl uptake, indicated an anionic imbalance, and as a result, the ratios of  $\text{K}^+$  to  $\text{Na}^+$  and  $\text{Ca}^{2+}$  to  $\text{Na}^+$  declined with salinity. The shoots and roots of inoculated plants accumulated less  $\text{Na}^+$  and much more  $\text{K}^+$  and  $\text{Ca}^{2+}$  than the corresponding non-inoculated stressed plants.  $\text{K}^+$  to  $\text{Na}^+$  and  $\text{Ca}^{2+}$  to  $\text{Na}^+$  ratios were significantly higher in inoculated stressed plants than in stressed non- inoculated plants. A significant decline in the nitrogen (N) and phosphorus (P) contents was seen in the non-AM stressed plants of both cultivars (*Table 2*). Mycorrhizal inoculations enhanced N and P acquisition by the roots of Bami plants under stressed, as well as unstressed conditions, in comparison to Yazdi.

Salinity adversely affected nodule formation, so that nodulation was completely suppressed under salt stress conditions. However, AM inoculation significantly moderated the adverse effects of salinity on the evaluated treat (*Table 4*). In both varieties, the nodule formation only occurred in the AM inoculated plants. The nodule number and nodule dry weight was less in the Yazdi cultivar, compared to other cultivar.

Table 1 - Effect of different levels of salinity on total dry weight (TDW), salt tolerance index (STI), shoot dry weight, root dry weight and shoot to root ratio in the alfalfa cultivars

Treatments	TDW(G)		STI (%)		Shoot dry weight		Root dry weight		Shoot to root ratio	
	Bami	Yazdi	Bami	Yazdi	Bami	Yazdi	Bami	Yazdi	Bami	Yazdi
C - AMF	2.22	1.96	100	100	1.32	1.16	0.9	0.8	1.47	1.45
C + AMF	2.59	2.3	123	118	1.46	1.26	1.13	1.04	1.29	1.21
S1 - AMF	1.83	1.62	86.27	74.18	1.05	0.98	0.78	0.64	1.35	1.53
S1 + AMF	2.09	1.86	116	112	1.25	1.1	0.84	0.76	1.49	1.45
S2 - AMF	1.45	1.16	63.28	52.11	0.87	0.68	0.58	0.48	1.5	1.42
S2 + AMF	1.85	1.51	110	106	1.12	0.9	0.73	0.61	1.53	1.48
	LSD (0.05) 0.43		LSD (0.05) 12.6		LSD (0.05) 0.12		LSD (0.05) 0.15		LSD (0.05) 0.2	

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Table 2 - Effect of AM inoculation on mycorrhizal infection (MI, %), mycorrhizal dependency (MD, %), phosphorus content (P, mg g<sup>-1</sup> DW), and nitrogen content (N, mg g<sup>-1</sup> DW) in the shoots and roots of Bami and Yazdi under salt stress. Treatments were designed as uninoculated controls, saline stress (6 and 8 dSm<sup>-1</sup>), and arbuscular mycorrhizal (AM).

Parameter	Treatments					
	C - AMF	C + AMF	S1 - AMF	S1 + AMF	S2 - AMF	S2 + AMF
	Bami					
MC	-	91.2	-	86.23	-	78.45
MD	-	25.7	-	30.25	-	33.8
Shoot	2.2	2.68	1.87	2.27	1.25	1.96
Root	1.78	2.12	1.33	1.5	0.98	1.23
Shoot	13.17	14.5	11.53	12.83	9.63	10.74
Root	11.87	12.62	8.27	10.26	6.53	8.93
	Yazdi					
MC	-	89.76	-	80.71	-	68.4
LSD (0.05)	6.27					
MD	-	24.65	-	34.54	-	37.2
LSD (0.05)	7.13					
Shoot	2.12	2.53	1.6	2.1	1.1	1.6
LSD (0.05)	0.33					
Root	1.35	2.13	1.15	1.37	0.86	1.2
LSD (0.05)	0.31					
Shoot	12.65	13.55	10.88	11.74	8.3	10.17
LSD (0.05)	1.58					
Root	11.37	12.1	7.9	10.17	6.5	8.24
LSD (0.05)	1.47					

**Table 3 - Effect of AM inoculation on sodium content ( $\text{Na}^+$ ,  $\text{mg g}^{-1}$  DW), potassium content ( $\text{K}^+$ ,  $\text{mg g}^{-1}$  DW), calcium content ( $\text{Ca}^{2+}$ ,  $\text{mg g}^{-1}$  DW),  $\text{K}^+$  to  $\text{Na}^+$  ratio, and  $\text{Ca}^{2+}$  to  $\text{Na}^+$  ratio in the shoots and roots of Bami and Yazdi under salt stress. Treatments were designed as uninoculated controls, saline stress (6 and 8  $\text{dSm}^{-1}$ ) and arbuscular mycorrhizal (AM).**

Parameter	Treatments						
	C - AMF	C + AMF	S1 - AMF	S1 + AMF	S2 - AMF	S2 + AMF	
<b>Bami</b>							
$\text{Na}^+$	Shoot	1.48	1.4	2.45	1.86	3.76	2.55
	Root	2.63	2.47	3.87	3.25	5.7	4.28
$\text{K}^+$	Shoot	32.5	37.45	15.75	24.12	9.15	22.1
	Root	30.16	34.8	12.06	18.6	6.4	11.8
$\text{Ca}^{2+}$	Shoot	3.55	3.87	3.18	3.6	2.57	3.16
	Root	4.11	4.28	3.74	4	3.2	3.67
$\text{K}^+$ to $\text{Na}^+$ ratio	Shoot	21.96	26.75	6.43	12.97	2.43	8.67
	Root	11.47	14.09	3.12	5.72	1.12	2.76
$\text{Ca}^{2+}$ to $\text{Na}^+$ ratio	Shoot	2.40	2.76	1.30	1.94	0.68	1.24
	Root	1.56	1.73	0.97	1.23	0.56	0.86
<b>Yazdi</b>							
$\text{Na}^+$	Shoot	2.11	1.66	3.2	2.28	4.44	3.7
	LSD (0.05) 0.27						
$\text{K}^+$	Root	2.6	2.33	4.16	3.42	6.3	5.38
	LSD (0.05) 0.72						
$\text{Ca}^{2+}$	Shoot	28.8	32.11	11.27	17.14	7.5	16.55
	LSD (0.05) 2.11						
$\text{K}^+$ to $\text{Na}^+$ ratio	Root	26.73	29.5	10	15.48	4.28	8.83
	LSD (0.05) 2.45						
$\text{Ca}^{2+}$ to $\text{Na}^+$ ratio	Shoot	3.48	3.37	2.66	2.87	1.84	2.47
	LSD (0.05) 0.16						
$\text{K}^+$ to $\text{Na}^+$ ratio	Root	4.16	4.2	3.5	3.74	2.64	3.21
	LSD (0.05) 0.13						
$\text{Ca}^{2+}$ to $\text{Na}^+$ ratio	Shoot	13.65	19.34	3.52	7.52	1.69	4.47
	LSD (0.05) 3.1						
$\text{Ca}^{2+}$ to $\text{Na}^+$ ratio	Root	10.28	12.66	2.40	4.53	0.68	1.64
	LSD (0.05) 1.2						
$\text{Ca}^{2+}$ to $\text{Na}^+$ ratio	Shoot	1.65	2.03	0.83	1.26	0.41	0.67
	LSD (0.05) 0.35						
$\text{Ca}^{2+}$ to $\text{Na}^+$ ratio	Root	1.60	1.80	0.84	1.09	0.42	0.60
	LSD (0.05) 0.19						

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**Table 4 - Effect of AM inoculation on number of nodules per plant and nitrogenase activity (n. moles ethylene mg<sup>-1</sup> nodule dry wt. h<sup>-1</sup>) in the shoots and roots of Bami and Yazdi under salt stress. Treatments were designed as uninoculated controls, saline stress (6 and 8 dSm<sup>-1</sup>) and arbuscular mycorrhizal (AM).**

Treatments	Parameter			
	Number of nodules per plant		Nitrogenase activity	
	Bami	Yazdi	Bami	Yazdi
C - AMF	0	0	0.12	0.093
C + AMF	21	16	0.18	0.13
S1 - AMF	0	0	0.084	0.065
S1 + AMF	14	7	0.14	0.09
S2 - AMF	0	0	0.05	0.033
S2 + AMF	8	5	0.11	0.06
	LSD (0.05) 4		LSD (0.05) 0.014	

AM inoculations boosted the nodulation and nitrogen fixation under saline and non-saline conditions. With increasing saline concentrations, decline in nodule dry weight and nitrogenase (ARA) activity (Table 4) was observed in both cultivars. Bami had a nodule dry weight and nitrogenase activity than Yazdi.

### DISCUSSION

Salinity-induced stress significantly reduced root and shoot dry weights and SRR in the non-AM plants of both cultivars, Bami and Yazdi. The results are in good agreement with those reported by Al-Karaki and Hammad (2001), Tain *et al.* (2004), Singla and Garg (2005), Juniper and Abbott (2006), Ghazi and Al-Karaki (2006), Tufenkci *et al.* (2006), Sannazzaro *et al.* (2006), and Sharii *et al.* (2007). The root dry matter was not affected as severely as the aerial organs (shoots). The same results were shown by Rejili *et al.* (2007) and Khadri *et al.* (2007), who

considered this behavior profitable, since it could improve plant water status. Both shoot as well as root dry weights were significantly greater in AM than in non-AM plants, both under stressed and unstressed conditions. Maximum salinity tolerance was achieved in Bami through mycorrhizal inoculation at 6 dSm<sup>-1</sup> in the rooting medium, where complete amelioration of negative effects of salinity was observed and the shoot and root biomass were even greater than in the untreated controls. The results from this study agree with previous data (Rabie and Almadini 2005; Sannazzaro *et al.*, 2006; Tufenkci *et al.*, 2006; Sharifi *et al.*, 2007).

Salinity may reduce mycorrhizal colonization in the roots by inhibiting the germination of spores (Hirrel, 1981), inhibiting the growth of hyphae in soil and hyphal spreading after initial infection has occurred (McMillen *et al.*, 1998), and reducing the number of arbuscules (Tain *et al.*, 2004; Rabie and Almadini, 2005;

Juniper and Abbott, 2006; Sannazzaro *et al.*, 2006; Sharifi *et al.*, 2007). In this study, although mycorrhizal colonization was reduced with increasing salinity levels, the mycorrhizal dependency (MD) of alfalfa plants increased, and this increase was greater in Yazdi than in Bami. It is indicated that symbiotic association between arbuscular mycorrhizal fungi and stress-tolerant alfalfa plants was strengthened in the saline environment once the association was established. This may be a sign of the ecological importance of AM association for plant growth and survival under salinity stress (Rabie and Almadini, 2005).

A large number of studies have demonstrated that salinity reduces nutrient uptake and accumulation, or affects nutrient partitioning within the plant (Essa 2002; Smykalova and Zamecnikova, 2003; Fernandez-Garcia *et al.*, 2004). The ionic composition seems to provide a useful diagnostic indication of the reduction in growth associated with salinity. In the current study, most of the  $\text{Na}^+$  was held up in the roots, and much less reached the shoots in both cultivars. High concentrations of  $\text{Na}^+$  inhibited  $\text{K}^+$  and  $\text{Ca}^{2+}$  influx into the plants, resulting in a decline in  $\text{K}^+$  to  $\text{Na}^+$  and  $\text{Ca}^{2+}$  to  $\text{Na}^+$  ratios in the non-AM stressed plants. The shoots and roots of AM plants accumulated less  $\text{Na}^+$  and much more  $\text{K}^+$  and  $\text{Ca}^{2+}$  than the corresponding non-AM plants, resulting in significantly higher  $\text{K}^+$  to  $\text{Na}^+$  and  $\text{Ca}^{2+}$  to  $\text{Na}^+$  ratios. Therefore, prevention of  $\text{Na}^+$  accumulation in the plant and enhancement of  $\text{K}^+$

concentrations in roots could be part of the general mechanism of the salt stress alleviation of alfalfa by *Glomus mosseae*. It has been generally accepted that AM fungi would enhance nutrient uptake by colonized plants under salinity conditions (Rao and Tak 2002; Yano-Melo *et al.*, 2003; Zandavalli *et al.*, 2004; Rabie and Almadini 2005; Sannazzaro *et al.*, 2006; Sharifi *et al.*, 2007). It is suggested that AM fungi protect the shoot system, mainly shoots, from  $\text{Na}^+$  toxicity either by regulating  $\text{Na}^+$  uptake from the soil or by accumulating it in roots, thereby delaying its translocation onto the shoot system of colonized plants (Rabie and Almadini, 2005).

Nitrogen (N) and phosphorus (P) levels in the shoots and roots were reduced with increasing salinity in both the alfalfa cultivars; the decline was more pronounced in Yazdi than Bami. AM inoculated-stressed plants showed more increment in their N and P content than the corresponding non-AM inoculated plants. The main mechanism for enhanced salinity tolerance in inoculated plants seems to be due to an improvement in nutrient uptake and translocation under both stressed and unstressed environments (Yano-Melo *et al.*, 2003; Tain *et al.*, 2004; Rabie and Almadini 2005).

Reduction of nodulation and inhibition of nitrogen fixing activity in legumes are typical effects of salinity. The nodule number decreased with the increase in salt levels in all plants, with Yazdi showing greater decrease than Bami. Also, nodule dry

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mass accumulation and nitrogenase activity declined at all stages with increasing salt dosages, and the negative effects were more severe in Yazdi. Although reductions in these parameters were reported by L'taief *et al.* (2007) and Khadri *et al.* (2007), an decrease in the average nodule number and dry mass with increasing salinity levels have also been observed for chickpea (Soussi *et al.*, 1999; Garg and Singla 2004) and for faba bean (Yousef and Sprent 1983; Cordovilla *et al.*, 1999). The detrimental effect on nitrogen fixation was less severe in mycorrhizal Bami plants, as compared to Yazdi. Evidence from previous studies (Amora-Lazcano *et al.*, 1998; Johansson *et al.*, 2004; Rabie and Almadini 2005) indicates that the presence of AM fungi is known to enhance nodulation and nitrogen fixation by legumes. The increased phosphorus uptake conferred by the AM symbiosis is beneficial for the functioning of the nitrogenase enzyme of the bacterial symbionts, leading to increased nitrogen fixation and consequently promotion of root and mycorrhizal development.

### CONCLUSION

On the basis of the results presented here, our results support the view that AMF can contribute to protect plants against salinity. Mycorrhizal inoculations enhanced N and P acquisition by the roots of plants under stressed as well as unstressed conditions. Also, AM

inoculations boosted the nodulation and nitrogen fixation under saline and non-saline conditions. Thus, the present study indicates a possible correlation between increased salt tolerance of alfalfa cultivars and the presence of a fungal endophyte in the rooting medium.

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