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ORIGINAL PAPER

Contribution of *Glomus intraradices* inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*

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Abstract The study aimed to investigate the effects of an AM fungus (*Glomus intraradices* Schenck and Smith) on mineral acquisition in fenugreek (*Trigonella foenum-graecum*) plants under different levels of salinity. Mycorrhizal (M) and non-mycorrhizal (NM) fenugreek plants were subjected to four levels of NaCl salinity (0, 50, 100, and 200 mM NaCl). Plant tissues were analyzed for different mineral nutrients. Leaf senescence (chlorophyll concentration and membrane permeability) and lipid peroxidation were also assessed. Under salt stress, M plants showed better growth, lower leaf senescence, and decreased lipid peroxidation as compared to NM plants. Salt stress adversely affected root nodulation and uptake of NPK. This effect was attenuated in mycorrhizal plants. Presence of the AM fungus prevented excess uptake of Na^+ with increase in NaCl in the soil. It also imparted a regulatory effect on the translocation of Na^+ ions to shoots thereby maintaining lower Na^+ shoot:root ratios as compared to NM plants. Mycorrhizal colonization helped the host plant to overcome Na^+-induced Ca^{2+} and K^+ deficiencies. M plants maintained favorable K^+:Na^+, Ca^{2+}:Na^+, and Ca^{2+}:Mg^{2+} ratios in their tissues. Concentrations of Cu, Fe, and Zn^{2+} decreased with increase in intensity of salinity stress. However, at each NaCl level, M plants had higher concentration of Cu, Fe, Mn^{2+}, and Zn^{2+} as compared to NM plants. M plants showed reduced electrolyte leakage in leaves as compared to NM plants. The study suggests that AM fungi contribute to alleviation of salt stress by mitigation of NaCl-induced ionic imbalance thus maintaining a favorable nutrient profile and integrity of the plasma membrane.

Keywords Arbuscular mycorrhiza · Lipid peroxidation · Micronutrient uptake · K^+:Na^+ · Ca^{2+}:Na^+ · Salinity

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Introduction

High concentrations of soluble salt in the soils of arid and semi-arid regions adversely affect plant growth and yield (Evelin et al. 2009; Hammer et al. 2011). Excessive salts in soil affect all major living processes such as growth, photosynthesis, protein, and lipid metabolism (Evelin et al. 2009). Salinization manifests largely as osmotic stress by disrupting the plant mineral relations, the outcome of which is the creation of ionic imbalance in the cell, that may result from the effect of salinity on nutrient availability, competitive uptake, transport, or partitioning within the plant or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for that essential element (Grattan and Grieve 1999). At the whole plant level, salinity frequently induces an increase in Na^+ and Cl^- ions as well as a decrease in K^+, Ca^{2+}, NO_3^-, and Pi concentrations (Shokri and Maadi 2009). Therefore, high concentrations of Na^+ and Cl^- ions in the soil solution may depress nutrient ion activities and produce extreme ratios of Na^+:K^+, Na^+:Ca^{2+}, Ca^{2+}:Mg^{2+}, and Cl^-:NO_3^-. As a result, the plant becomes susceptible to osmotic and specific ion injury as well as to nutritional

disorders (Grattan and Grieve 1999; Feng et al. 2002; Garg and Manchanda 2009).

Furthermore, salt stress-induced nutritional disorder may also challenge the structure and composition of plant cells. The cell membrane, being at the interface, is the first organelle to be affected by salt stress (Xu et al. 2010). The integrity of the membrane is disrupted due to peroxidation of lipids in the membrane system resulting in increased membrane permeability (Kaya et al. 2009). Polyunsaturated fatty acids are the main membrane lipid components susceptible to peroxidation and degradation by free radicals generated due to salt stress (Elkahoui et al. 2005). The salt-induced nutritional disorder, such as low N and Mg^{2+} concentration in the plant, may also damage macromolecules such as chlorophyll, resulting in loss in photosynthetic activity, thus subsequently leading to senescence of the leaves (De Michele et al. 2009). Moreover, the legume–*Rhizobium* symbioses and nodule formation on the legumes are affected by salinity (Aydi et al. 2008).

Arbuscular mycorrhizal (AM) fungi have often been employed to mitigate the effects of salt stress in various plants. A major objective of most of the studies examining salinity and mycorrhizal colonization is the overall performance of plants with agricultural and horticultural importance, such as pepper (Turkmen et al. 2008), wheat (Daei et al. 2009), tomato (Hajiboland et al. 2010), and citrus (Wu et al. 2010). Increased salt tolerance of mycorrhizal plants in these studies has been mainly attributed to AM fungal mediated enhanced uptake of nutrients, especially phosphorus. However, salinity disrupts the mineral nutrient acquisition of plants by reducing the activities as well as the availabilities of nutrient ions due to their competition with major ions (Na^+ and Cl^-) in the soil. Salinity effects are even more extreme in the case of micronutrients (Grattan and Grieve 1992). In this respect, AM fungal colonization aids the host plant in maintaining ionic balance by enhancing and/or selective uptake of nutrients (Evelin et al. 2009). Better understanding of the underlying mechanisms can be achieved if interactions between salinity levels and mycorrhization are studied taking other mineral nutrients affected by salinity such as Ca^{2+}, Mg^{2+}, Cu, Fe, Mn, and Zn^{2+} into consideration. Inoculation with AM fungi is also credited with minimizing the salt-targeted effects on macromolecules such as lipids, chlorophyll, and proteins and on the organelles of the cell. Mycorrhizal plants have been shown to possess higher activities of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase) and molecules (carotenoids, glutathione, and tocopherols; Porcel et al. 2003; Garg and Manchanda 2008; Latef and Chaoxing 2011) while maintaining a lower concentration of reactive oxygen species in the cell (Wu et al. 2010).

In the last decade, researchers have attempted to address the problem of salinity-induced nutrient disorder. However, the emphasis has been on the effect of mycorrhizal colonization on enhanced K^+ uptake and improved K^+:Na^+ ratio in plants (Giri et al. 2007; Colla et al. 2008; Daei et al. 2009), and few studies have addressed Ca^{2+} (Zuccarini 2007; Turkmen et al. 2008; Hajiboland et al. 2010) and Mg^{2+} uptake (Giri et al. 2003) under salinity stress. Although salinity studies have analyzed plant tissue for micronutrients (Cu, Fe, Mn^{2+}, and Zn^{2+}) in the plants grown in saline soils, they have directed little attention to micronutrients as affected by salinity and mycorrhization. Correspondingly, salt stress-induced lipid peroxidation and consequent increase in membrane permeability have not been discussed in relation to nutritional imbalance due to salt stress. We argue that much more can be learned if interactions between salinity levels and mycorrhizal colonization are studied taking more nutrients into consideration. Keeping this in view, the present study was undertaken to investigate the effects of different levels of salinity on nutrient acquisition, lipid peroxidation, and membrane permeability and to evaluate the role of an AM fungus (*Glomus intraradices* Schenck and Smith) in mitigation of NaCl-induced ionic imbalance in *Trigonella foenum-graecum* L. (fenugreek).

Materials and methods

Experimental design

The experiment was conducted in the Botanical Garden, Department of Botany, University of Delhi during growing season of *T. foenum-graecum* (November–February) under natural conditions of light, temperature, and humidity. The 2×4 factorial experiment was designed with two mycorrhizal conditions: inoculated or non-inoculated with the AM fungus (*G. intraradices* Schenck and Smith) combined with four concentrations of NaCl (0, 50, 100, and 200 mM NaCl). Thus, there were eight treatment combinations arranged in a randomized complete block design replicated three times.

AM fungal inoculum

Inoculum of *G. intraradices* Schenck and Smith was obtained from The Energy and Resources Institute (TERI), New Delhi, India. The inoculum was bulked in an open-pot soil culture alternatingly using *Sorghum bicolor* L. and *T. foenum-graecum* L. as trap plants (Kapoor et al. 2002). Sorghum was cut at the ground level; the roots were chopped into small pieces and mixed with the soil mass of the culture pots. This soil-based inoculum was collected and used in the study. The AM fungal inoculum contained about 60–70 infectious propagules per 10 g soil, quantified according to the method of Sharma et al. (1996).

Soil and salt treatments

The soil used was sandy loam (sand 14.7%, silt 35.5%, and clay 22.8%) collected from the Botanical Garden of University of Delhi. The soil had the following physicochemical properties: loam texture, pH 7.5; electrical conductivity (EC), 0.19 dS m^{-1}; P, 3,100 mg kg^{-1}; K^+, 520 mg kg^{-1}; Ca^{2+}, 2,300 mg kg^{-1}; Mg^{2+}, 4,600 mg kg^{-1}; Na^+, 320 mg kg^{-1}; Cu, 4.43 mg kg^{-1}; Mn^{2+}, 200 mg/kg; and Zn^{2+}, 55.52 mg kg^{-1}. The soil was sieved and mixed with an equal volume of sand. The mixture was autoclaved for 15 min at 121°C and 15 psi to eliminate existing AM propagules. Two kilograms of the autoclaved soil was dispensed into each pot. An amount of 50 g of soil-based inoculum (containing 60–70 AM fungal propagules per 10 g soil) along with chopped AM sorghum roots was added to each pot, 3 cm deep, and mixed with soil. In the control plants, soil extract (prepared by suspending 50 g inoculum soil in 50 ml distilled water) filtered through Whatman No. 1 paper was added to reintroduce the native microbial population except for mycorrhizal propagules. Later, the same soil was autoclaved and added to the control.

Fenugreek (*T. foenum-graecum* L. var. Pusa Early Bunching)—a moderately salt-tolerant leguminous plant—was used as test plant. Seeds were procured from National Seeds Corporation, New Delhi. The seeds were surface sterilized in a 5% sodium hypochlorite solution for 15 min, and then washed several times with sterile water to remove any trace of chemical that could interfere with seed germination. Six sterilized seeds were sown in plastic pots, and later, seedlings were thinned to three per pot. The plants were allowed to grow in pots under natural conditions of temperature, light, and humidity.

The NaCl treatment started after 30 days of plant growth following the protocol of Sheng et al. (2008). In order to avoid salt effects on AM fungus establishment and osmotic shock to the fine fenugreek roots, NaCl was introduced gradually by successively adding 50 ml of prescribed NaCl solution in each pot for 7 days. A total volume of 350 ml of the corresponding saline solution was added per pot in this experiment. This brought the EC of saturated soil extracts to 0.19, 2.1, 5.2, and 9.1 dS m^{-1} in the 0-, 50-, 100-, and 200-mM NaCl treatments, respectively. Leaching was prevented by keeping the soil water below field capacity at all times. The plants were irrigated with autoclaved tap water twice in a week, and the EC of the soil extract was monitored using a conductivity meter (Decibel DB-1401) and adjusted once in 15 days with the respective NaCl solution.

Harvest

The plants were harvested 51 days after sowing when they were in the vegetative phase in all the treatments. At harvest, the plants were rinsed three times with deionized water, and then separated into root and shoot. Shoot and root length were measured. The number of nodules per plant was recorded. The dry weights of plant parts (root and shoot) were recorded after drying in an oven at 70°C for 72 h.

Mycorrhizal colonization

Root samples were cleared with 10% KOH, stained with 0.05% trypan blue in lactophenol as described by Philips and Hayman (1970), and microscopically examined for AM colonization by determining the percentage of root segments containing arbuscules and vesicles using gridline intersect method (Giovannetti and Mosse 1980).

Tissue elemental composition

Dried root and shoot tissues were analyzed for their nutrient composition. Oven-dried shoot and root samples were ground separately and sieved through a 0.5-mm sieve. Tissue sample of 0.2 g was digested in a tri-acid mixture (60% $HClO_4$:HNO_3:H_2SO_4 in the ratio 1:5:0.5). The acid digest were diluted with double-distilled water and made up to 50 ml and analyzed for the following macro- and micronutrients: P, K^+, Ca^{2+}, Mg^{2+}, Na^+, Cu, Fe, Mn^{2+}, and Zn^{2+}. Reagent blanks were prepared by carrying out the whole extraction procedure but in the absence of sample. Phosphorus concentration was determined following ammonium molybdate blue method (Allen 1989). The concentrations of K^+, Ca^{2+}, Mg^{2+}, Na^+, Cu, Fe, Mn^{2+}, and Zn^{2+} ions were determined in an atomic absorption spectrophotometer (AA-6300 Shimadzu) following Allen (1989). The concentration of nitrogen was determined in a CHNS analyser (Elementar Analysensysteme GmbH VarioEL III). Oven-dried root and shoot samples were oxidized in the combustion tube in the presence of oxygen at high temperature (1,150°C) using tungsten IV oxide as catalyst. Helium (He) gas was used to sweep out the combustion products out of the chamber and passed over high purity Cu to remove any oxygen not consumed in the initial combustion and to convert any oxides of nitrogen to nitrogen gas. Nitrogen gas was detected by a GC separation and quantified by thermal conductivity detection. Sulfanilic acid was used as a standard.

Lipid peroxidation

Lipid peroxidation in leaves was determined according to Heath and Packer (1968). Leaves (0.2 g, fifth and sixth leaves from the stem base) were homogenized in ice-cold extraction buffer (100 mM K-PO_4 buffer containing 0.1 mM EDTA).

The homogenate was centrifuged at 16,000×*g* for 15 min at 4°C. To 1 ml of the supernatant was added 0.5% thiobarbituric acid in 20% tri-chloro acetic acid. The mixture was heated at 95°C for 25 min and quickly cooled in an ice bath. The mixture was centrifuged at 3,000×*g* for 10 min. The absorbance of the supernatant was measured at 532 nm and corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. The amount of malondialdehyde (MDA) was calculated by using extinction coefficient of 155 mM^{-1} cm^{-1}.

Relative permeability

The plasma membrane permeability of leaf samples was calculated as described by Zwiazek and Blake (1991). Leaves (fifth and sixth leaves from the stem base) were washed with double-distilled water to remove surface adhered electrolytes. Twenty-five leaf discs of 5-mm diameter were cut out with the help of a cork borer and placed in closed vials containing 25 ml of double-distilled water. The leaf segments in the vials were incubated at room temperature for 1 h. The EC of the solution was measured using a conductivity meter (Decibel DB-1401). Thereafter, the leaf discs were heated to boiling in a water bath. The solution was cooled to room temperature, and the EC of the solutions were measured again:

$$\text{Relative permeability} = \text{EC of the solution before heating} / \text{EC of the solution after heating} \times 100$$

Chlorophyll concentration

The concentration of chlorophyll in leaves was determined according to Hiscox and Israeltam (1979). Fresh leaflets (0.1 g) from fifth and sixth mature leaves at the base of the plant were cut into small pieces and placed in a vial containing 7 ml dimethyl sulfoxide (DMSO). The vials were incubated at 65°C till the leaf tissue turn white in color. The extracts were transferred to a graduated cylinder and made up to a total volume of 10 ml with DMSO. The absorbance of the extract was taken at 645 and 663 nm in a UV-Vis spectrophotometer (Beckman Coulter DU730), and the concentrations of chlorophyll *a* and *b* were calculated according to Arnon (1949).

Statistical analysis

Experimental data were analyzed with SPSS 14.0 statistical program (SPSS Inc., Chicago, IL, USA). Two-way ANOVA was performed considering NaCl and AMF as independent factors. Differences between individual means were compared by Duncan's multiple comparison test.

Results

Mycorrhizal colonization and root nodulation

G. intraradices successfully colonized the roots of *T. foenum-graecum* at all levels of salinity. AM fungal colonization was not observed in plant roots that were not inoculated with *G. intraradices*. The percent root colonization was significantly ($P<0.001$) affected by NaCl level, *G. intraradices*, and the interaction between NaCl and AM fungus (Table 1). Mycorrhizal colonization declined gradually with increasing NaCl concentrations in the soil. At 200 mM NaCl, more than half of the colonization in plants was not subjected to salt stress.

Salinity had an adverse effect on nodulation of fenugreek plants. The number of nodules per plant decreased significantly with increasing NaCl concentration ($P<0.001$). The lowest number of nodules was observed in NM plants grown at 200 mM NaCl where there was a 93% decrease in the number of nodules as compared to non-stressed control. However, at all salinity levels, M fenugreek plants had a higher number of nodules as compared to NM plants (Table 1).

Plant growth and biomass

Growth parameters of *T. foenum-graecum* at four different NaCl levels with or without AM fungal inoculations are shown in Table 1. Fenugreek plants showed significant reduction in plant growth and biomass in response to NaCl stress. The reduction was dependent on the concentration of NaCl in the soil. M plants performed consistently better in all levels of salinity compared to NM plants. The shoots of M plants were significantly longer as compared to their corresponding NM plants. Mycorrhizal plants possessed longer roots at all NaCl levels; however, the differences between M and NM plants were not significant ($P<0.05$).

Shoot and root dry weights were significantly higher in M plants than in their NM counterparts at all salt levels. At the highest NaCl concentration, shoot dry weight in M plants was 32% higher than that of NM plants, and the value recorded was similar to that of NM shoot grown at 50 mM NaCl. Root dry weight decreased with increasing NaCl, and the lowest value was recorded for plants at 200 mM NaCl containing no AM fungal inoculum.

Concentrations of shoot and root mineral nutrients

The macro- and micronutrients concentrations of fenugreek shoot and root as a function of NaCl and *G. intraradices* are shown in Tables 2, 3, 4, and 5. The influence of AM colonization and salt stress on nutrient concentrations in shoot and root were variable. Two-way analysis of variance

Table 1 Influence of NaCl and *Glomus intraradices* inoculation on percent AM colonization, number of nodules, shoot and root lengths, and dry weights and in non-mycorrhizal (NM) and mycorrhizal (M) *Trigonella foenum-graecum* plants

Treatment NaCl (mM)	AM status	% AM colonization	No. of nodules per plant	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
0	NM		18.3±1.45 e	22.00±1.73 d	19.33±1.52 bc	0.36±0.02 c	0.08±0.01 c
	M	68±2 d	19.6±1.7 e	32.5±1.32 e	24.00±4.58 c	0.91±0.04 e	0.10±0.01 d
50	NM		8.6±0.6 c	16.66±0.57 b	18.83±0.763 ab	0.29±0.01 b	0.06±0.01 b
	M	52±2.6 c	12.6±1.20 d	22.5±1.32 d	23.00±5.00 bc	0.43±0.04 d	0.09±0.01 cd
100	NM		4.6±1.2 ab	15.50±0.50 ab	16.00±1.00 a	0.26±0.02 b	0.05±0.00 b
	M	44±1 b	8.0±1.1 bc	19.16±1.60 c	17.66±1.52 ab	0.32±0.01 bc	0.06±0.005 b
200	NM		1.3±0.8 a	12.16±1.75 a	14.83±1.25 a	0.19±0.01 a	0.03±0.01 a
	M	32±2 a	4.6±0.8 ab	16.83±0.76 b	16.66±1.15 a	0.28±0.03 b	0.05±0.005 b
Significance							
NaCl		–***	–***	–**	–**	–**	–**
AMF		–***	–***	–**	–*	–**	–**
NaCl×AMF		–***	NS	–*	NS	NS	NS

Values represent mean of replicates (±SD). Different letters indicate significant differences at $P<0.05$

NS not significant

*$P<0.05$; **$P<0.01$; ***$P<0.001$

showed that the NaCl levels and mycorrhizal treatments interacted with each other to bring significant changes in shoot Ca^{2+}, Na^{+}, and Zn^{2+} uptake (Tables 2 and 4) and root P, K^{+}, Na^{+}, Cu, Fe, Mn^{2+}, and Zn^{2+} uptake (Tables 3 and 5).

Sodium

Sodium concentration in root and shoot of both M and NM fenugreek plants showed a linear increase with increasing soil salinity (Tables 2 and 3). The accumulation of Na^{+} was more in root as compared to shoot, also evident in Na^{+} shoot:root ratio (Fig. 1). Mycorrhizal plants possessed significantly less Na^{+} as compared to NM plants in NaCl-supplemented soils. A close examination of the results (Fig. 1) showed that while there was a steady increase in Na^{+} shoot:root ratio with increasing salinity level in NM plants, in M plants, the increase was not significant, and at the highest concentration of NaCl (200 mM NaCl), a slight decrease was observed.

Table 2 Influence of NaCl and *Glomus intraradices* inoculation on the concentration of macronutrients in shoot of non-mycorrhizal (NM) and mycorrhizal (M) *Trigonella foenum-graecum* plants

NaCl (mM)	AM status	Macronutrients (mg kg^{-1})					
		Sodium	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
0	NM	1,200±50 a	66,800±780 cd	1,800±100 g	1,800±50 de	7,000±200 e	2,400±0.00 a
	M	1,100±100 a	70,900±100 d	2,200±100 f	2,000±100 e	7,400±200 f	2,350±100 b
50	NM	1,800±50 c	61,400±112 bc	1,400±100 cd	1,600±100 cd	6,300±200 d	2,300±200 b
	M	1,400±100 b	73,800±35,000 d	1,600±100 de	1,700±50 dc	7,100±200 ef	2,330±50 b
100	NM	2,000±100 c	33,100±14,900 a	1,200±100 bc	1,300±200 bc	5,300±200 c	2,350±100 b
	M	1,700±100 c	44,100±1320 ab	1,400±100 c	1,500±50 cd	6,100±100 d	2,330±100 b
200	NM	2,400±100 d	32,300±1,100 a	900±00 a	900±100 a	3,800±100 a	2,340±100 b
	M	1,700±100 cd	38,400±3,000 a	1,100±50 b	1,100±100 ab	4,600±100 b	2,320±60 b
Significance							
NaCl		–***	–***	–***	–***	–***	–*
AMF		–***	NS	–***	–**	–***	NS
NaCl×AMF		–**	NS	NS	NS	–***	NS

Values represent mean of replicates (±SD). Different letters indicate significant differences at $P<0.05$

NS not significant

*$P<0.05$;**$P<0.01$; ***$P<0.001$

Table 3 Influence of NaCl and *Glomus intraradices* inoculation on the concentration of macronutrients in root of non-mycorrhizal (NM) and mycorrhizal (M) *Trigonella foenum-graecum* plants

NaCl (mM)	AM status	Macronutrients (mg kg^{-1})					
		Sodium	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
0	NM	3,400±100 a	17,600±1,400 cd	2,200±100 f	4,200±100 f	1,700±50 d	4,300±100 a
	M	3,200±1,000 a	26,100±1,400 e	2,300±50 g	4,500±100 g	1,800±100 d	4,600±100 bcd
50	NM	3,900±100 c	14,000±2,800 bc	1,800±50 d	3,100±100 d	1,100±200 c	4,400±100 ab
	M	3,700±0.00 b	20,000±3,900 d	2,000±50 e	3,800±100 e	1,100±300 c	4,700±50 cd
100	NM	4,100±100 d	6,000±1,800 a	1,400±1,100 b	2,700±100 b	800±50 bc	4,500±1,500 bc
	M	3,900±50 c	11,800±2,100 b	1,600±50 c	3,100±100 cd	1,000±100 c	4,800±1,000 d
200	NM	4,500±100 e	3,700±400 a	1,200±100 a	2,200±300 a	400±100 a	4,600±1,000 bcd
	M	4,200±50 d	7,600±1,200 a	1,300±50 b	2,800±100 bc	700±100 ab	4,730±1,500 cd
Significance							
NaCl		–***	–***	–***	–***	–***	–*
AMF		–***	–***	–***	–***	NS	–***
NaCl×AMF		–**	NS	–*	–*	NS	NS

Values represent mean of replicates (±SD). Different letters indicate significant differences at $P<0.05$

NS not significant

*$P<0.05$; **$P<0.01$; ***$P<0.001$

Nitrogen

Total N concentration in shoot and root of M and NM fenugreek plants was severely affected by NaCl-induced salinity in the soil. The N concentration was inversely proportional to the increase in NaCl level in the soil. At all salinity levels, M plants showed higher N concentration in shoot as compared to NM, though the difference was not significant at 100 and 200 mM NaCl. N concentration showed positive correlation with plant growth (shoot dry weight; $r^2=0.646$).

Phosphorus

The level of NaCl stress and AM fungal inoculation had significant effect ($P<0.001$) on P concentration in both shoot and root of fenugreek plants (Tables 2 and 3). Increasing NaCl concentration in the soil provoked a considerable decline in P concentration in both root and shoot. The shoot P concentration at 200 mM NaCl decreased to 50% of the P concentration at non-saline controls. However, as anticipated, M fenugreek plants possessed significantly higher P concentration than their controls at all levels of salinity. All the

Table 4 Influence of NaCl and *Glomus intraradices* inoculation on the concentration of micronutrients in shoots of non-mycorrhizal (NM) and mycorrhizal (M) *Trigonella foenum-graecum* plants

NaCl (mM)	AM status	Micronutrients (mg kg^{-1})			
		Copper	Iron	Manganese	Zinc
0	NM	19.78±0.65 e	900±50 c	2,300±200 a	75.17±0.37 f
	M	21.96±0.84 e	900±100 c	2,600±100 ab	94.60±0.86 g
50	NM	13.78±0.77 c	800±40 bc	3,100±700 bc	64.20±0.73 d
	M	17.89±0.87 de	980±180 c	3,900±100 d	72.81±0.68 e
100	NM	10.69±0.58 b	700±50 b	3,100±400 bc	48.33±0.58 b
	M	15.08±0.54 cd	800±100 bc	3,600±100 cd	61.25±0.03 c
200	NM	7.91±0.96 a	400±60 a	2,300±400 a	37.11±0.24 a
	M	10.85±0.56 b	400±100 a	2,600±100 ab	49.22±0.45 b
Significance					
NaCl		–***	–***	–***	–***
AMF		–***	–*	–**	–***
NaCl×AMF		NS	NS	NS	–***

Values represent mean of replicates (±SD). Different letters indicate significant differences at $P<0.05$

NS not significant

*$P<0.05$; **$P<0.01$; ***$P<0.001$

Table 5 Influence of NaCl and *Glomus intraradices* inoculation on the concentration of micronutrients in roots of non-mycorrhizal (NM) and mycorrhizal (M) *Trigonella foenum-graecum* plants

NaCl (mM)	AM status	Micronutrients (mg kg^{-1})			
		Copper	Iron	Manganese	Zinc
0	NM	46.37±0.89 e	6,600±300 e	4,300±300 bc	71.54±0.98 g
	M	51.96±0.38 g	8,200±200 f	5,100±100 d	78.63±0.72 h
50	NM	43.30±0.28 d	5,200±300 d	3,500±300 ab	65.82±1.18 e
	M	47.54±0.66 f	6,500±1,500 e	4,500±100 bc	69.93±0.77 f
100	NM	39.13±0.43 c	3,600±200 b	3,200±200 a	45.58±0.49 c
	M	42.61±0.54 d	4,400±0.10 c	4,000±100 bc	54.44±1.17 d
200	NM	32.99±0.86 a	3,100±200 a	3,200±700 a	38.05±0.29 a
	M	36.66±0.87 b	3,300±100 ab	3,300±200 a	41.49±0.62 b
Significance					
NaCl		–***	–***	–***	–***
AMF		–***	–***	–***	–***
NaCl×AMF		–*	–**	–*	–***

Values represent mean of replicates (±SD). Different letters indicate significant differences at $P<0.05$

NS not significant

*$P<0.05$; **$P<0.01$; ***$P<0.001$

readings on shoot P concentrations and shoot dry weight were pooled, and a positive linear correlation between the two parameters was found ($r^2=0.879$).

Potassium

Increased levels of NaCl in the soil induced a gradual decline in the concentration of K^+ in fenugreek plants. However, at the same NaCl level, plants inoculated with *G. intraradices* maintained higher shoot and root K^+ uptake than the NM plants (Tables 2 and 3). Potassium accumulation in root was more than two times in shoots, and these values were significantly higher in M roots as compared to NM roots. Consequently, salinity and root colonization by the AM fungus imparted a significant ($P<0.01$) effect on the K^+:Na^+ ratio (Fig. 2a, b). Salinization provoked a sharp decrease in the K^+:Na^+ ratio in both root and shoot of fenugreek plants. However, the concurrent presence of mycorrhiza resulted in a higher K^+:Na^+ ratio than in respective NM controls at all levels of salt stress.

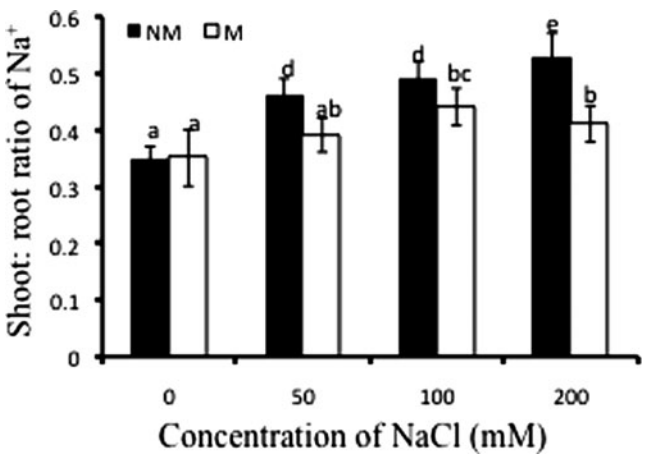

Fig. 1 Influence of NaCl and *Glomus intraradices* inoculation on shoot: root Na^+ ratio in non-mycorrhizal (*NM*) and mycorrhizal (*M*) *Trigonella foenum-graecum* plants. Values represent mean of replicates; ±SD. *Different letters* indicate significant differences at $P<0.05$

Calcium

There was a significant decrease in the concentration of Ca^{2+} with increasing NaCl levels in the soil (Tables 2 and 3). Calcium accumulation was higher in shoots than roots in M as well as NM plants. Shoot Ca^{2+} concentration was significantly higher in M plants compared to NM plants. Although the root Ca^{2+} concentration in M plants was higher than in NM plants, the increase was not significant at $P<0.05$. In shoots, although the Ca^{2+}:Na^+ ratio declined with increasing NaCl concentrations, M plants still displayed a significantly higher Ca^{2+}:Na^+ ratio than NM plants (Fig. 2c). In contrast to shoots, there was negligible change in the Ca^{2+}:Na^+ ratio in roots, and M roots maintained higher Ca^{2+}:Na^+ ratio than NM roots. However, the differences between M and NM plants were not significant (data not shown).

Magnesium

Uptake of Mg^{2+} in shoots was not influenced by NaCl or AM fungal inoculation (Tables 2 and 3). In shoots, except for the non-saline controls, the concentrations of Mg^{2+} were not significantly different between M and NM plants. The concentration of Mg^{2+} in roots of fenugreek plants was almost twofold higher than in shoots. Owing to insignificant changes in Mg^{2+} uptake and decreases in Ca^{2+} uptake, a decrease in the Ca^{2+}:Mg^{2+} ratio was observed with increasing levels of salt stress (Fig. 2d). Mycorrhizal plants showed a significantly higher Ca^{2+}:Mg^{2+} ratio compared to NM plants at all levels of salinity. However, in roots, despite M

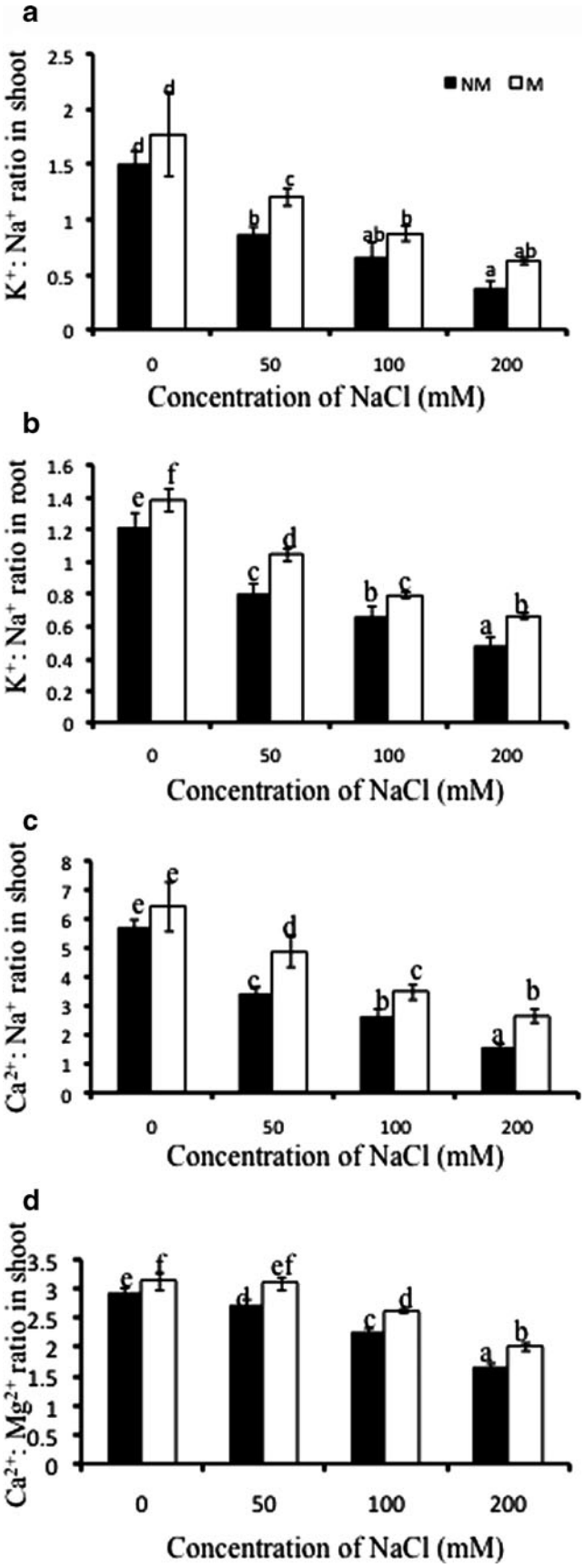

Fig. 2 Effects of NaCl and *Glomus intraradices* inoculation on **a** K^+:Na^+ ratio in shoot, **b** K^+:Na^+ ratio in root, **c** Ca^{2+}:Na^+ ratio in shoot, and **d** Ca^{2+}:Mg^{2+} ratio in shoot in non-mycorrhizal (*NM*) and mycorrhizal (*M*) *Trigonella foenum-graecum* plants. Values represent mean of dreplicates; ±SD. *Different letters* indicate significant differences at $P<0.05$

plants possessing a higher Ca^{2+}:Mg^{2+} ratio, the values were not statistically different to NM plants (data not shown).

Copper

Soil salinity had a negative effect on the accumulation of copper (Tables 4 and 5). The concentration of copper in shoots and roots of fenugreek plants decreased significantly with increasing NaCl levels in the soil. The root copper concentration, however, was less affected as compared to shoots. At 200 mM NaCl, the percent decrease in root Cu concentration was 28%, while in shoots there was almost a 60% decrease relative to the plants grown without any salt stress. Except for shoot Cu concentration at 0 mM NaCl, the M plants had significantly higher Cu concentrations than the NM plants in all saline treatments.

Iron

The shoot Fe concentration showed a slight decrease with increasing NaCl levels in the soil (Table 4). In contrast to this, there was a marked decline in root Fe concentrations with increasing salinity level (Table 5). At 200 mM NaCl, the concentration of Fe was almost half of the non-saline control. In all the treatments, Fe concentrations in roots and shoots continued to be greater in M plants as compared to respective NM plants.

Manganese

Manganese uptake showed inconsistent responses to salt stress both in the presence and the absence of *G. intraradices* colonization. While there was increase in uptake of Mn^{2+} into shoots up to 100 mM NaCl, it decreased thereafter at 200 mM NaCl (Table 4). In roots, a gradual decline in Mn^{2+} concentration was observed with increase in NaCl stress (Table 5). Uptake of Mn^{2+} in M roots was significantly higher at 0, 50, and 100 mM NaCl as compared to NM plants.

Zinc

NaCl and *G. intraradices* showed a significant interaction with respect to zinc uptake in both roots and shoots (Tables 4 and 5). The concentration of Zn^{2+} declined in both roots and shoots with increase in salinity, and these values were significantly higher in corresponding AM treatments.

Lipid peroxidation

Lipid peroxidation levels in leaves of fenugreek plants exposed to different NaCl levels, measured as the concentration of MDA, are given in Fig. 3a. Exposure to NaCl resulted in substantial increases in MDA concentration in fenugreek plants. Enhanced lipid peroxidation was observed in both M and NM leaves; however, the rate of MDA formation was higher in NM plants. Mycorrhizal plants maintained significantly lower MDA concentration at all saline treatments.

Relative permeability

NaCl and AM fungal inoculation showed significant effects on relative membrane permeability, measured in terms of electrolyte leakage from leaves of fenugreek plants grown under NaCl stress. There was a NaCl-dependent increase in electrolyte leakage in fenugreek plants. Although there were no significant differences in electrolyte leakage between M and NM leaves in non-saline controls and 200 mM NaCl, leaves of M plants maintained lower electrolyte leakage at all salinity levels (Fig. 3b).

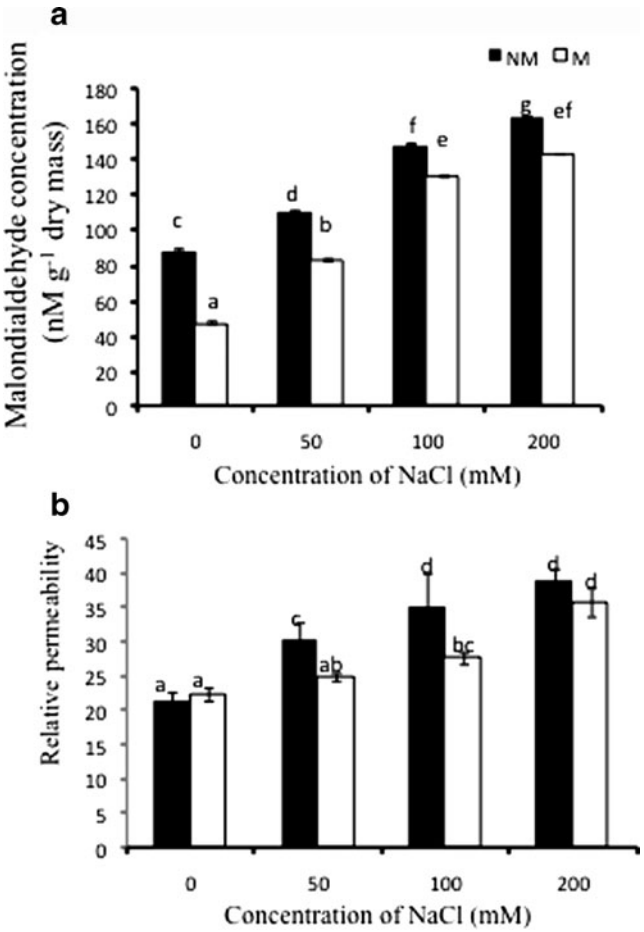

Fig. 3 Effects of NaCl and *Glomus intraradices* inoculation on **a** MDA concentration and **b** relative permeability of leaves of non-mycorrhizal (*NM*) and mycorrhizal (*M*) *Trigonella foenum-graecum* plants. Values represent mean of replicates; ±SD. *Different letters* indicate significant differences at $P<0.05$

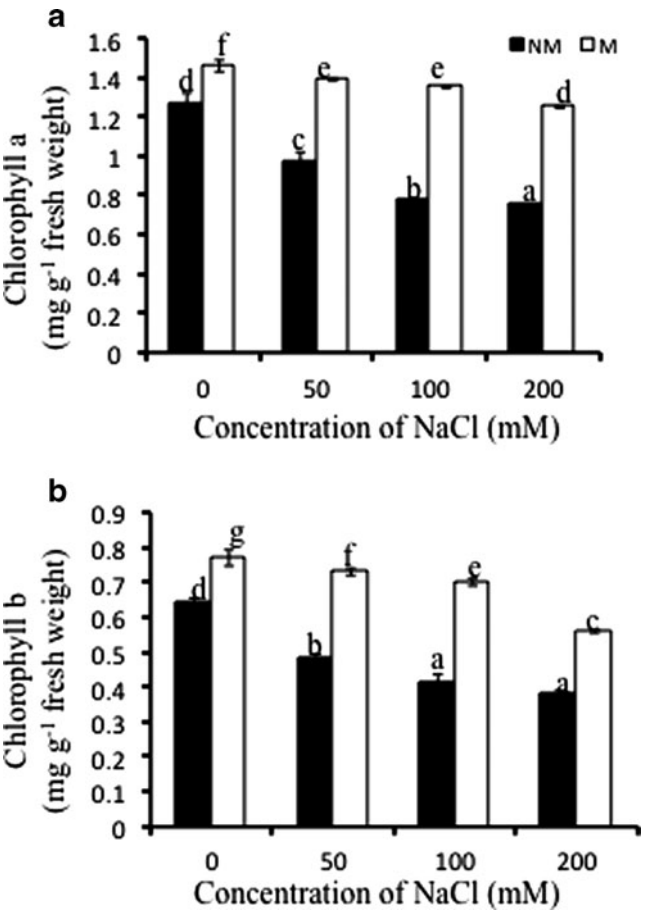

Fig. 4 Effects of NaCl and *Glomus intraradices* inoculation on **a** chlorophyll *a* and **b** chlorophyll *b* concentration in non-mycorrhizal (*NM*) and mycorrhizal (*M*) *Trigonella foenum-graecum* plants. Values represent mean of replicates; ±SD. *Different letters* indicate significant differences at $P<0.05$

Chlorophyll concentration

With increase in salinity levels, chlorophyll *a* and *b* concentrations decreased in plant leaves. However, at all levels of salinity, M plants possessed higher concentrations of both chlorophyll *a* and *b* as compared to NM plants. At the highest level of salinity (200 mM), chlorophyll concentration in M plants reached levels comparable to those of non-stressed NM plants (Fig. 4a, b).

Discussion

We observed that the ability of *G. intraradices* to colonize the roots of fenugreek plants declined with increasing NaCl levels. This is in agreement with earlier reports that addition

of salt to soil inhibits hyphal growth of *G. intraradices*, which subsequently decreases the spread of mycorrhizal colonization (McMillen et al. 1998; Jahromi et al. 2008). However, in spite of the decrease in AM colonization, the increase in shoot and root mass resulting from *G. intraradices* inoculation indicates that the beneficial effects of AM fungi on plant growth were, to some degree, independent of percent root colonization.

In the present study, we observed that in the presence of NaCl, the growth of fenugreek plants was reduced. This finding may be explained by nutritional imbalances in the plant due to the salt stress (Grattan and Grieve 1999). We observed that the M fenugreek plants had better growth and biomass as compared with NM plants under salinity stress. Our results are in accordance with earlier reports on other plant species (Giri et al. 2003, 2007; Giri and Mukerji 2004; Sannazzaro et al. 2007; Zuccarini and Okurowska 2008; Porras-Soriano et al. 2009; Shokri and Maadi 2009; Latef and Chaoxing 2011). We suggest that better growth and biomass in M plants is an indication of enhanced tolerance to salt stress and is a manifestation of improved uptake of nutrients and maintaining favorable ionic ratios than NM plants.

Root and shoot Na^+ concentrations in fenugreek plants increased proportionately to the level of NaCl added to the soil. However, the observation that Na^+ concentrations were higher in tissues of NM plants than M plants indicates that the symbiosis can control uptake of Na^+ when it becomes toxic to plants (Allen and Cunningham 1983). According to Hammer et al. (2011), AM fungi exclude Na^+ by discriminating its uptake from the soil or during its transfer to plants. The analysis of Na^+ shoot:root ratio also showed that while there was a continuous increase in the Na^+ shoot:root ratio in NM plants, in M plants, the increase in Na^+ shoot:root ratio was considerably lower, and it decreased at 200 mM NaCl. These findings substantiate that AM fungi induce a regulatory effect on the translocation of Na^+ to the aerial parts, thus maintaining favorable K^+:Na^+ and Ca^{2+}:Na^+ ratio in shoots over NM plants.

Nitrogen is the mineral element that plants require in greatest amounts. It serves as a constituent of many plant cell components, including amino acids and nucleic acids. Deficiency of N in the plant tissue rapidly inhibits plant growth, inducing chlorosis in leaves. Soil salinity affects total nitrogen uptake and soil nitrogen contribution leading to reduced plant growth (van Hoorn et al. 2001). This negative effect of salt on N uptake is clearly seen in our study. The total N concentration in both shoots and roots showed a significant decline with increasing NaCl levels in the soil. Our findings are concurrent with previous reports (Cantrell and Linderman 2001; Silveira et al. 2001; Frechilla et al. 2001; Colla et al. 2008). However, plant growth was not strongly correlated to N concentration in *T. foenum-graecum*.

The decrease in tissue N concentrations in a legume due to salt stress results from a decrease in root nodulation of the plants, as well as from interference by salinity in N acquisition and utilization. In the present study, we observed that the number of nodules in fenugreek plants declined with increasing NaCl concentrations in the soil. Inhibition of nodule formation in legumes under saline stress has been reported by various authors (Rao et al. 2002; Garg and Manchanda 2008). However, M plants of *T. foenum-graecum* had a consistently higher number of nodules in roots as compared to NM plants. Similar reports in previous studies have attributed the mycorrhizal moderation of nodulation to improved water uptake (Ruiz-Lozano et al. 2001) and higher antioxidative capacity in M plants (Garg and Manchanda 2008). However, better nutrient uptake and maintenance of ionic balance in M plants may also play a significant role in nodulation under salt stress conditions. For example, Ca^{2+}, which facilitates infection of root nodules by *Rhizobium*, is required for the initiation of symbiosis and activates downstream events such as Cl^- efflux and membrane depolarization (Felle et al. 1999). A higher Ca^{2+} concentration in M plants may partly account for the higher number of nodules on the *G. intraradices*-colonized roots. Also, the fact that both mycorrhizal and *Rhizobium* symbiosis follow common mechanisms supports our finding (Parniske 2008). Manganese is also known to affect nodulation, symbiotic efficiency, and N metabolism (Izaguirre-Mayoral and Sinclair 2009; Pelaez et al. 2010). Mn^{2+} ions act as a cofactor for NAD-malic enzymes, involved in nitrogen metabolism in legumes (Chen et al. 1998). However, the role of *G. intraradices* in improving the uptake of Mn^{2+} ions is not evident in our study, although ion levels remained higher in M plants up to 100 mM NaCl. Addition of salt (NaCl) increases the plant available Mn^{2+} in soil (Khattak and Jarrell 1989) so that an adverse effect on N_2 fixation due to decreased Mn^{2+} uptake can be ruled out in this case.

Under stress conditions, the otherwise mobile forms of N are rendered immobile (Hodge and Fitter 2010; Miransari 2010). Salinity interferes with the uptake of both forms of available N, nitrate, and ammonium ions (Frechilla et al. 2001). Uptake of nitrates by plants is affected by salinity at two levels: (1) by direct competition of chloride with nitrate and (2) at the level of the membrane and/or the membrane proteins by changing plasmalemma integrity (Cramer et al. 1985; Frechilla et al. 2001). Inhibition of ammonium uptake by salinity could be due to direct competition with sodium and to the depolarizing effect of NaCl on the plasmalemma (Hawkins and Lewis 1993). These changes in N uptake influence the different steps of N metabolism, such as uptake, reduction, and protein synthesis (Frechilla et al. 2001). Although the role of AM fungi in absorbing inorganic N is considered minor in most situations, as N is

mobile and readily absorbed by plants by diffusion and mass flow, AM fungi can function as a facilitator for N uptake through activation of a plant ammonium transporter in the presence of AM fungi (Guether et al. 2009). Thus, the improved uptake of N in M plants under salt stress may be due to better nutrient uptake and maintenance of ionic balance observed in this study that translates into increased nodulation and N_2 fixation, and better acquisition of N (both nitrate and ammonium ions) from the soil.

Phosphorus is an essential macronutrient and forms an integral component of several key plant structure compounds of plant cells, including the sugar–phosphate intermediates of respiration and photosynthesis, and the phospholipids that make up the plant membranes (Taiz and Zeiger 2006). Under saline stress, there is reduction in the uptake and concentration of P in plant tissues resulting in reduced and stunted growth, dark green coloration of the leaves, production of slender stems, and death of older leaves (Giri et al. 2003, 2007; Taiz and Zeiger 2006; Giri and Mukerji 2004; Al-Karaki 2006; Hajiboland et al. 2010; Latef and Chaoxing 2011). This is further corroborated in our study where plant growth is directly correlated to shoot P concentration. The NaCl-induced high ionic strength in the soil reduces the activity of P and decreases availability of Ca phosphates in soil (Grattan and Grieve 1999). In the present study, root colonization by *G. intraradices* was found to enhance P uptake and increase its concentration in *T. foenum-graecum* plants under salt stress. The extensive hyphal network of the AM fungus explores more soil volume and increase the absorption surface of roots, thus contributing to the enhanced P concentration in M plants (Ruiz-Lozano and Azcon 2000) while NM plants lack this benefit. Mycorrhizal hyphae also have a higher affinity for phosphate ions and a lower threshold concentration for absorption than do plant roots. The ability of the mycorrhizal hyphae to store larger amounts of absorbed P than the plant roots also facilitates the continued movement of P into the hyphae (Bolan 1991). Improved P uptake by AM fungus in plants grown under saline conditions may contribute to the maintenance of vacuolar membrane integrity and facilitate the compartmentalization of Na^+ ions within vacuoles. This prevents Na^+ ions from interfering in metabolic pathways of growth, thereby reducing the negative impacts of salinity (Cantrell and Linderman 2001).

Our study revealed that NaCl treatment reduced the level of K^+, an antagonist of Na^+. Since Na^+ and K^+ have similar physiological properties, it is inevitable that there exists a competition between Na^+ and K^+ in all processes. Foremost, Na^+ competes with K^+ for entry sites at the root plasma membrane for ingression into the symplast (Grattan and Grieve 1999). Thereafter, cytoplasmic Na^+ competes for K^+ binding sites and hence inhibits metabolic processes that crucially depend on K^+. Potassium is essential for (1) charge balancing in the cytoplasm—a counter ion for the large excessive negative charge on proteins and nucleic acids, (2) activation of crucial enzymatic reactions such as occurring in formation of pyruvate, and (3) maintaining osmotic pressure of the vacuole and cell turgor (Maathuis 2009). Besides, interfering with K^+ acquisition by the roots, Na^+ ions may also disrupt the integrity of root membranes and alter the selectivity (Grattan and Grieve 1999). Therefore, maintenance of a high cytosolic K^+:Na^+ ratio is indispensable in order to enhance plant salt tolerance. In this study, K^+ concentration in plant tissues decreased as the NaCl concentration in the soil solution increased bringing about a NaCl-induced K^+ deficiency. This may be one of the primary reasons accounting for the reduction in growth of *T. foenum-graecum* plants under salt stress (Grattan and Grieve 1999). However, our observation that *G. intraradices*-colonized plants resulted in higher foliar and root K^+ concentrations compared to NM plants under salinity stress shows the potential of mycorrhiza in preventing the disruption of K^+ homeostasis. The capacity of plants to maintain a high cytosolic K^+:Na^+ ratio is one of the key determinants of plant salt tolerance (Maathuis and Amtmann 1999). The efficacy of *G. intraradices* in maintaining favorable K^+:Na^+ ratio was also reported by other authors (Shokri and Maadi 2009; Porras-Soriano et al. 2009).

NaCl salinity significantly reduced the calcium uptake into roots as well as shoots of *T. foenum-graecum.* NaCl-induced Ca^{2+} deficiency has been reported in different plants, such as strawberry (Kaya et al. 2002), tomato (Novarro et al. 2000; Tuna et al. 2007), and beans (Cabot et al. 2009). Calcium, owing to its ability to form intermolecular linkages, plays an essential role in processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, and regulate ion transport and selectivity (Munns 2002; Maathuis 2009). High Na^+ concentrations in the root zone inhibit uptake and transport of Ca^{2+}, and thus subsequently, salt-stressed plants have a lower Ca^{2+}:Na^+ ratio (Grattan and Grieve 1999). Also, the Ca^{2+}:Na^+ ratio is an important measure of salinity stress since Na^+ replaces Ca^{2+} in the plasma membrane and cell wall when Na^+ levels are high, reducing cell turgor and hydraulic conductivity, and disturbing Ca^{2+} signaling (Laüchli and Lüttge 2002). In this study, M plants showed higher uptake of Ca^{2+} ions and therefore maintained a higher Ca^{2+}:Na^+ ratio than NM plants. Our results showing higher Ca^{2+}:Na^+ ratios in M plants are in line with those of Garg and Manchanda (2009). However, in our earlier study on *Acacia aurculiformis*, we observed no visible change in Ca^{2+} uptake under salinity stress (Giri et al. 2004). It has been suggested that mycorrhiza may not be important to nutrients, such as Ca^{2+}, moving to plant roots by mass flow as compared with nutrients moving by diffusion. It is

currently not clear how AM fungi affect plant transport and uptake systems for Ca^{2+} ions.

Magnesium is a macronutrient and forms an integral part of the chlorophyll molecule. Although reports of decreased chlorophyll concentration and photosynthesis in response to salinity and their increase in AM plants are widely reported (Sheng et al. 2008; Latef and Chaoxing 2011), the question of Mg^{2+} nutrition as affected by salinity and mycorrhization has drawn little attention (Cantrell and Linderman 2001; Colla et al. 2008; Shokri and Maadi 2009). In our earlier investigation on *Acacia nilotica* (Giri et al. 2003), we reported that NaCl imparted negative effects on Mg^{2+} uptake, while inoculation with AM fungi alleviated this effect. However, in the present investigation, we found that NaCl and mycorrhizal inoculation had little or no effect on Mg^{2+} concentration in plant tissues. This may be attributed to the low availability of Ca^{2+} under NaCl stress (as discussed earlier). Ca^{2+} is strongly competitive with Mg^{2+}, and the binding sites on the root plasma membrane appear to have less affinity for highly hydrated Mg^{2+} than for Ca^{2+} (Marschner 1995). Therefore, in this experiment with NaCl salinity where Na^+:Ca^{2+} ratio is high in soil, the availability of Ca^{2+} to plants is less. The elimination of the competition between Mg^{2+} and Ca^{2+} may account for the little or no effect of salinity on Mg^{2+} concentration.

A crucial question addressed in the present study was the effect of NaCl stress and mycorrhization on the uptake of micronutrients. Unfortunately, in most of the salinity–plant mineral nutrition studies, the micronutrients have drawn the least attention. In our study, we observed that the concentration of Cu, Fe, Mn^{2+}, and Zn^{2+} in roots as well as shoots reduced with increases in salinity. However, the extent of the decline varied with the plant tissue, levels of salinity, and the type of micronutrient. The availability of most micronutrients depends on pH and pE of the soil solution, as well as the nature of binding sites on organic and inorganic particle surfaces. The mobility of Cu, Fe, and Zn^{2+} in the soil solution is low. In saline soils, the solubility of these micronutrients also decreases (Grattan and Grieve 1999). As available Cu, Zn^{2+}, and Fe levels decrease with increase in salinity, a depletion zone of these metal nutrients is formed around the roots. As a result, the uptake of these nutrients is limited in NM plants. As discussed above for poorly mobile macronutrients, the effectivity of mycorrhizal plants depends upon the spread of extraradical hyphae in soil (Burkert and Robson 1994), and decreased Cu, Zn^{2+}, and Fe in mycorrhizal plants with rises in soil salinity levels may be due to direct effects of salinity on hyphal growth and spread.

Prevention of lipid peroxidation and maintenance of membrane integrity has been considered as one of the key processes in salinity tolerance (Juan et al. 2005; Feng et al. 2002; Garg and Manchanda 2009). In our study, lipid peroxidation in leaves increased under salt stress in both M and NM plants. This, together with the corresponding increase in electrolyte leakage from leaves, suggests injury to the leaf plasma membrane due to peroxidation of membranes under salt stress. However, the amplitude of the increase in both MDA concentration and electrolyte leakage in M plants was lower than that of NM plants, which is in accordance with earlier reports (Feng et al. 2002; Garg and Manchanda 2009). Our findings provide evidence that the presence of AM fungi in roots can prevent cell membrane injury in shoots caused by salt stress, and maintain integrity and stability of the plasma membrane by increasing P levels in tissues and maintaining higher Ca^{2+}:Na^+ ratios under salt stress. Decreased electrolyte leakage in leaves of M plants may be related to P-induced changes in membrane phospholipid levels and associated changes in permeability properties (Graham et al. 1981).

Leaf senescence is characterized by a progressive yellowing due to chlorophyll degradation and loss of photosynthetic activity (De Michele et al. 2009). It is most often quantified by decreases in chlorophyll concentration and by increases in membrane permeability (Lutts et al. 1996). Chlorophyll concentration severely declined in NM plants compared to M plants, indicating more leaf senescence in salt-stressed NM plants. Specific effects of salt stress on leaf senescence have been related to accumulation of toxic ions (Na^+ and Cl^-) or to K^+ and Ca^{2+} depletion (Yeo et al. 1991). This is in accordance with our observation that M plants accumulated less Na^+ ions. The deleterious effect of NaCl on chlorophyll concentration is also reported to be attenuated by potassium fertilization (Bohra et al. 1995) so that higher concentrations of chlorophyll in M plants of *T. foenum-graecum* may be due to improved K^+ and Ca^{2+} uptake. Previous studies have also attributed salt stress effects to decreases in Mg^{2+} absorption (Giri and Mukerji 2004); however, this is not in concordance with our observations on Mg^{2+}.

In conclusion, the results of this study confirm that NaCl stress disrupts uptake of nutrients resulting in reduced plant growth and biomass. However, plant tolerance to salt stress is improved by AM fungal colonization. Greater nutrient acquisition resulted in better growth and biomass in mycorrhizal plants as compared to their non-mycorrhizal counterparts. Mycorrhizal colonization also helps in mitigating NaCl-induced ionic imbalance by maintaining favorable K^+:Na^+, Ca^{2+}:Na^+, and Ca^{2+}:Mg^{2+} ratios than the non-mycorrhizal plants. Lower leaf senescence, lipid peroxidation, membrane permeability, and higher nodulation in mycorrhizal plants also demonstrate the potential of AM fungi in alleviating the effects of salt stress.

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