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Effect of mycorrhizal inoculation on growth, nitrogen fixation, and nutrient uptake in *Cicer arietinum* (L.) 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 *Cicer arietinum* (L.) (chickpea). Two genotypes of chickpea (Pusa-329, salt tolerant, and Pusa-240, salt sensitive) 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, Pusa-329 was found to be more tolerant to salinity than Pusa-240. AM plants exhibited better growth and biomass accumulation under stressed as well as unstressed conditions. Mycorrhizal infection (MI) was reduced with increasing salinity levels, but the mycorrhizal dependency (MD) increased, which was more evident in Pusa-240. Salinity resulted in a marked decline in the nodule dry weights, whereas a surge in the nodule number was recorded. Nitrogenase activity was reduced with increasing salt concentrations. AM plants had considerably higher nodule numbers, dry weights, and nitrogenase activity under both saline and nonsaline environments. Pusa-329 had a comparatively lower Na^+ concentration and higher K^+ and Ca^{2+} concentrations than Pusa-240. Although nitrogen (N) and phosphorus (P) contents declined with increasing salinity, Pusa-329 had higher levels of N and P as compared with Pusa-240. Plants inoculated with *Glomus mosseae* had better plant growth and nitrogen fixation under salt stress.

Key words: *Cicer arietinum, Glomus mosseae,* growth, nitrogenase, nutrients

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 (Volkamar 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^+ (Mengel and Kirkby 2001). Such physiological changes result in decreased plant growth and

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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 chickpea 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).

Chickpea (*Cicer arietinum* L.) is an annual, self-pollinating, diploid (2n = 16) pulse crop and ranks third in world legume production (Ahmad et al. 2005). Chickpea is one of the most salt-sensitive legumes (Abdelly et al. 2005), and it has been stated that there is too little variability in chickpea 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 genotypes of *Cicer arietinum* (L.) on the basis of their relative plant growth, nitrogen fixation, and nutrient acquisition under salt stress and to investigate the role of AM fungi in conferring salinity tolerance.

Materials and methods

Plant growth conditions

The experiments were conducted from November 2005 to February 2006 in the greenhouse of the Department of Botany, Panjab University, Chandigarh, India (30.5°N, 76.5°E; elevation 305-366 m). The minimum temperature was 12.8 °C and the maximum temperature was 30.8 °C. The morning relative humidity was 28% and the afternoon relative humidity was 86%.

Treatments

The seeds were surface-sterilized with 0.2% mercuric chloride ($HgCl_2$) solution for 2 min and were pretreated with a standard rhizobial inoculum of *Mesorhizobium ciceri* strain F-75 and AM inoculum of *Glomus mosseae* obtained from the Division of Microbiology of the Indian Agricultural Research Institute (IARI), New Delhi, India. The AM spores were applied as 10 spores per seed (approximately 1500 spores per 100 grams of media). The AM inoculum was placed at a pot depth of 2-3 cm immediately prior to the sowing of the seeds to facilitate fungal colonization of plant roots.

The soil was obtained from nearby agricultural fields and was mixed with sand (v/v 1:1). It was fumigated with methyl bromide under airtight plastic sheets, and the fumigant was allowed to dissipate for 1 week (Al-Raddad 1991). In each pot, containing 7 kg of soil mixture with a pH of 7.2, electrical conductivity of 1.0 dS m^{-1}, and P content of 20 ppm, 8 seeds were sown. Seedlings were thinned to 4 after 6 days of emergence. Plants 15 days old were maintained under different saline concentrations, i.e. 4, 6, and 8 dS m^{-1} ECe (sodium chloride, calcium chloride, and sodium sulfate, 7:2:1 w/v as per Richards (1954)). Plants were harvested at 80 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 at S_x/TDW at S_i) × 100, where TDW = total dry weight, S_i = control treatment, and S_x = x treatment.

Mycorrhizal colonization

Mycorrhizal infection (MI) 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 cotton blue dye was added. The samples were kept for 24-36 h. 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 infected. The percent root infection was calculated as follows:

$$\text{Percent root infection} = \frac{\text{Total number of infected roots}}{\text{Total number of roots observed}} \times 100$$

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).

Rate of nitrogenase activity

Nitrogenase (EC 1.7.99.2) activity (ARA) was determined by acetylene reduction with nodulated root portions of plants, following the method of Herdina and Silsbury (1990). Gas samples were analyzed for ethylene produced in the reaction using a Shimadzu GC-14B gas chromatograph equipped with a Porapak R column (Ligero et al. 1986).

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 (leaves and roots) was taken in 50 mL volumetric flasks and 10 mL of vandomolybdate 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 one-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. Five diverse chickpea genotypes were evaluated in saline and nonsaline environments for their relative salt tolerance index (Table 1). On the basis of STI, it was concluded that genotypes Pusa-329, Pusa-72, and Pusa-212 had suitable salinity tolerance; on the other hand, genotypes Pusa-372 and Pusa-240 were comparatively salt-sensitive and had lower STIs. The results categorized genotype Pusa-329 as the most salt-tolerant genotype and Pusa-240 as the most salt-sensitive genotype. Salt stress decreased the root and shoot dry weights (Figures 1 and 2) in the nonmycorrhizal plants of both genotypes. 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) (Figure 3) was recorded in all plants with increasing saline dosages. Pusa-329 was able to maintain a higher SRR as compared to Pusa-240. Root colonization by AM fungi enabled the plants to grow better under similar stress conditions, and the shoot and root dry weights of stressed mycorrhizal plants were greater than those of their nonmycorrhizal counterparts. The results

Table 1. Effect of different levels of salinity on total dry weight (TDW) and salt tolerance index (STI) in the chickpea genotypes.

	TDW (g)					STI (%)				
	Pusa-329	Pusa-72	Pusa-212	Pusa-372	Pusa-240	Pusa-329	Pusa-72	Pusa-212	Pusa-372	Pusa-240
Control (C)	0.795	0.750	0.747	0.714	0.680	100	100	100	100	100
C + AM	0.990	0.952	0.911	0.816	0.764	124.528	126.933	121.95	114.285	112.352
4 dS m^{-1}	0.687	0.621	0.602	0.559	0.517	86.415	82.800	80.589	78.291	76.029
4 dS m^{-1} + AM	0.942	0.853	0.830	0.756	0.702	118.490	113.730	111.111	101.680	103.23
6 dS m^{-1}	0.616	0.568	0.550	0.463	0.465	77.484	75.733	73.627	64.845	68.382
6 dS m^{-1} + AM	0.806	0.752	0.703	0.600	0.587	101.383	100.266	94.109	84.033	86.323
8 dS m^{-1}	0.563	0.521	0.396	0.363	0.298	70.81	69.466	53.012	50.840	43.823

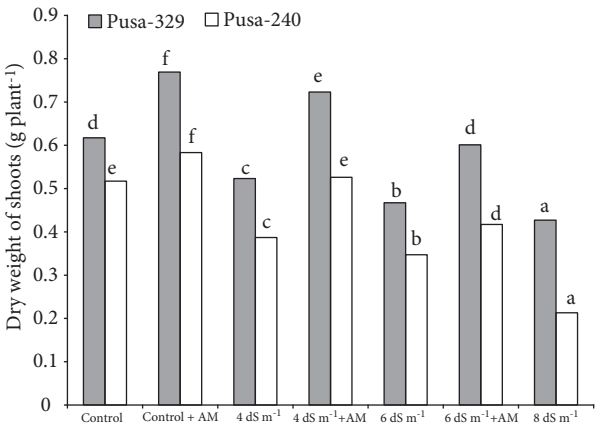

Figure 1. Effect of AM inoculation on shoot dry weight (grams per plant) in chickpea under salt stress. Treatments were designed as uninoculated controls, saline stress (4, 6, and 8 dS m^{-1}), and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly differently ($P < 0.05$) as determined by Duncan's multiple range test.

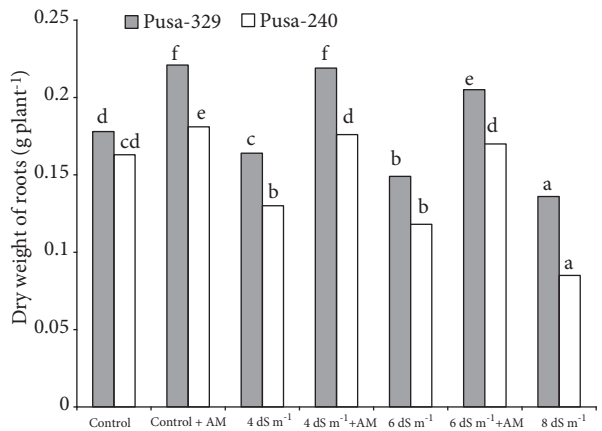

Figure 2. Effect of AM inoculation on root dry weight (grams per plant) in chickpea under salt stress. Treatments were designed as uninoculated controls, saline stress (4, 6, and 8 dS m^{-1}), and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly differently ($P < 0.05$) as determined by Duncan's multiple range test.

from the present study (Table 2) depict a decrease in the percentage of mycorrhizal infection (MI) with increasing salinity in both genotypes; the decrease in MI was more significant in the salt-sensitive genotype, Pusa-240. Mycorrhizal dependency (MD) increased in both genotypes with increasing salt concentrations. Pusa-240 showed greater dependence on its mycorrhizal partner and thus had higher MD than Pusa-329.

The current study (Table 3) revealed the accumulation of Na^+ ions with a decrease in the uptake of K^+ ions in both genotypes under salt stress. It was seen that most of the Na^+ was held up in the roots, and much less reached the leaves in both genotypes. A salinity-induced increase in the calcium content was observed in the leaves and roots of both genotypes, but this increase in calcium content was much less in Pusa-240. Pusa-329 had a comparatively lower Na^+ concentration and higher K^+ and Ca^{2+} concentrations than Pusa-240. The ion deficiency displayed by salinity stress, particularly by NaCl uptake, indicated an anionic imbalance, and as a result, the ratios of K^+ to Na^+ and Ca^{2+} to Na^+ declined with salinity. The leaves and roots of mycorrhizal plants accumulated less Na^+ and much more K^+ and Ca^{2+} than the corresponding

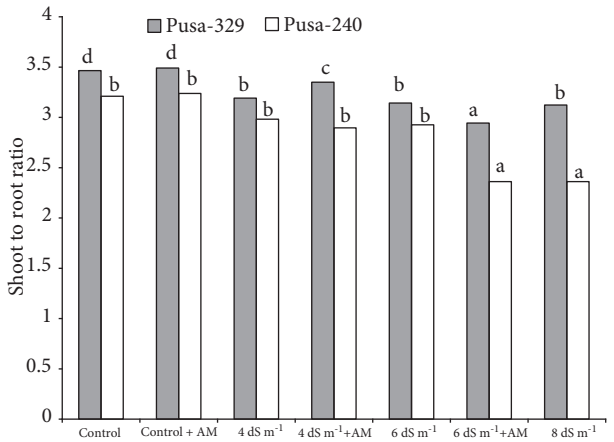

Figure 3. Effect of AM inoculation on shoot-to-root ratio (SRR) in chickpea under salt stress. Treatments were designed as uninoculated controls, saline stress (4, 6, and 8 dS m^{-1}), and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly differently ($P < 0.05$) as determined by Duncan's multiple range test.

nonmycorrhizal stressed plants. K^+ to Na^+ and Ca^{2+} to Na^+ ratios were significantly higher in mycorrhizal stressed plants than in stressed nonmycorrhizal Pusa-329 plants. A significant decline in the nitrogen (N) and phosphorus (P) contents was seen in the non-AM stressed plants of both genotypes. Mycorrhizal inoculations enhanced N and P acquisition by the roots of Pusa-329 plants under stressed as well as unstressed conditions in comparison to Pusa-240.

Nodule number (Figure 4) increased with increasing saline concentrations, and a decline in nodule dry weight and nitrogenase (ARA) activity (Figure 5) was observed in both genotypes. Pusa-329 had a higher nodule number, nodule dry weight, and nitrogenase activity than Pusa-240. AM inoculations boosted the nodulation and nitrogen fixation under saline and nonsaline conditions.

Discussion

Salinity-induced stress significantly reduced root and shoot dry weights and SRR in the non-AM plants of both genotypes, Pusa-329 and Pusa-240. 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 Sharifi 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 plants' 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 Pusa-329 through mycorrhizal inoculation at 4 dS m^{-1} 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 infection (MI) was reduced with increasing salinity levels, the mycorrhizal dependency (MD) of chickpea plants increased, and this increase was greater in Pusa-240 than in Pusa-329. It is indicated that symbiotic association between arbuscular mycorrhizal fungi and stress-tolerant chickpea 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 Na^+ was held up in the roots, and much less reached the shoots (leaves) in both genotypes. High concentrations of Na^+ inhibited K^+ and Ca^{2+} influx into the plants, resulting in a decline

Table 2. Effect of AM inoculation on mycorrhizal infection (MI, %), mycorrhizal dependency (MD, %), phosphorus content (P, mg g^{-1} DW), and nitrogen content (N, mg g^{-1} DW) in the leaves (L) and roots (R) of Pusa-329 and Pusa-240 under salt stress. Treatments were designed as uninoculated controls, saline stress (4, 6, and 8 dS m^{-1}), and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Parameter		Treatments						
		Control (C)	C + AM	4 dS m^{-1}	4 dS m^{-1} + AM	6 dS m^{-1}	6 dS m^{-1} + AM	8 dS m^{-1}
		Pusa-329						
MI		-	93.57 b	-	87.29 ab	-	85.30 a	-
MD		-	23.46 a	-	33.53 b	-	37.58 b	-
P	L	4.272 c	7.124 e	3.565 b	5.482 d	3.275 b	5.122 d	2.327 a
	R	6.485 cd	12.240 f	5.325 bc	8.215 e	4.505 ab	7.615 de	3.902 a
N	L	133.25 d	175.75 g	121.00 c	150.00 f	110.25 b	140.75 e	86.25 a
	R	79.25 d	105.20 f	69.75 bc	95.52 e	62.35 b	89.20 e	58.32 a
		Pusa-240						
MI		-	79.97 b	-	68.20 a	-	66.13 a	-
MD		-	11.04 b	-	35.38 b	-	44.06 b	-
P	L	4.082 e	6.072 f	2.154 c	2.800 d	1.824 b	2.210 c	1.132 a
	R	5.975 e	10.025 f	2.905 c	4.000 d	2.320 b	3.070 c	1.570 a
N	L	121.45 f	149.75 g	82.25 c	102.05 e	60.50 b	86.25 d	31.50 a
	R	70.05 f	83.05 g	48.85 c	58.25 e	38.55 b	51.25 d	20.00 a

in K^+ to Na^+ and Ca^{2+} to Na^+ ratios in the non-AM stressed plants. The shoots and roots of AM plants accumulated less Na^+ and much more K^+ and Ca^{2+} than the corresponding non-AM plants, resulting in significantly higher K^+ to Na^+ and Ca^{2+} to Na^+ ratios. Therefore, prevention of Na^+ accumulation in the plant and enhancement of K^+ concentrations in roots could be part of the general mechanism of the salt stress alleviation of chickpea by *Glomus mosseae*. It has been generally accepted that AM fungi would enhance nutrient uptake by infected 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 leaves, from Na^+ toxicity either by regulating Na^+ uptake from the soil or by accumulating it in roots, thereby delaying its translocation onto the shoot system of infected plants (Rabie and Almadini 2005).

Nitrogen (N) and phosphorus (P) levels in the leaves and roots were reduced with increasing salinity in both the chickpea genotypes; the decline was more pronounced in Pusa-240 than Pusa-329.

Table 3. Effect of AM inoculation on sodium content (Na^+, mg g^{-1} DW), potassium content (K^+, mg g^{-1} DW), calcium content (Ca^{2+}, mg g^{-1} DW), K^+ to Na^+ ratio, and Ca^{2+} to Na^+ ratio in the leaves (L) and roots (R) of Pusa-329 and Pusa-240 under salt stress. Treatments were designed as uninoculated controls, saline stress (4, 6, and 8 dS m^{-1}), and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Parameter		Treatments						
		Control (C)	C + AM	4 dS m^{-1}	4 dS m^{-1} + AM	6 dS m^{-1}	6 dS m^{-1} + AM	8 dS m^{-1}
		Pusa-329						
Na^+	L	0.583 a	0.605 b	0.665 c	0.650 c	0.760 e	0.720 d	0.889 f
	R	1.947 a	2.138 ab	2.275 b	2.099 ab	2.935 cd	2.845 c	3.090 e
K^+	L	20.095 e	21.775 f	19.235 bc	19.475 d	18.154 a	18.735 b	17.237 a
	R	13.095 e	14.995 f	12.170 d	12.450 d	9.395 b	11.075 c	8.890 a
Ca^{2+}	L	3.540 a	4.258 cd	3.698 a	3.998 b	4.205 c	4.255 d	4.494 e
	R	4.025 a	5.020 e	4.295 b	4.697 c	4.990 d	5.492 g	5.145 f
K^+ to Na^+ ratio	L	34.468 f	35.991 g	28.924 d	29.961 e	23.886 b	27.551 c	19.490 a
	R	6.725 f	7.013 f	5.349 d	5.931 e	3.201 b	3.892 c	2.877 a
Ca^{2+} to Na^+ ratio	L	6.072 e	7.180 g	5.560 c	6.150 f	5.532 b	5.909 d	5.032 a
	R	2.067 c	2.390 d	1.887 b	2.366 d	1.700 a	1.930 b	1.665 a
		Pusa-240						
Na^+	L	0.675 a	0.725 b	0.892 d	0.792 c	0.942 f	0.912 e	1.250 g
	R	2.400 a	2.681 b	4.955 d	3.905 c	5.875 f	5.150 e	6.110 g
K^+	L	18.037 d	19.448 e	8.800 c	8.893 c	6.855 b	8.725 c	4.910 a
	R	9.692 f	10.995 g	5.000 d	6.350 e	3.934 b	4.462 c	1.875 a
Ca^{2+}	L	1.978 a	2.295 b	1.990 a	2.000 a	2.016 a	2.022 a	2.046 a
	R	2.292 a	2.752 b	2.315 a	2.340 a	2.374 a	2.388 a	2.420 a
K^+ to Na^+ ratio	L	26.721 e	26.824 e	9.865 c	11.228 d	7.277 b	9.566 c	3.928 a
	R	4.038 f	4.101 g	1.009 d	1.475 e	0.669 b	0.789 c	0.316 a
Ca^{2+} to Na^+ ratio	L	2.930 e	3.165 f	2.230 c	2.525 d	2.140 b	2.214 c	1.638 a
	R	0.955 d	1.026 e	0.467 b	0.599 c	0.404 a	0.463 b	0.396 a

AM-stressed plants showed more increment in their N and P content than the corresponding non-AM plants. The main mechanism for enhanced salinity tolerance in AM 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 increased with the increase in salt levels in all plants, with Pusa-329 showing greater increase than Pusa-240. However, nodule dry mass accumulation and nitrogenase activity declined at all stages with increasing salt dosages, and the negative effects were more severe in Pusa-240. Although reductions in these parameters were reported by L'taief et al. (2007) and Khadri et al. (2007), an increase 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 Pusa-329 plants as compared to Pusa-240. 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

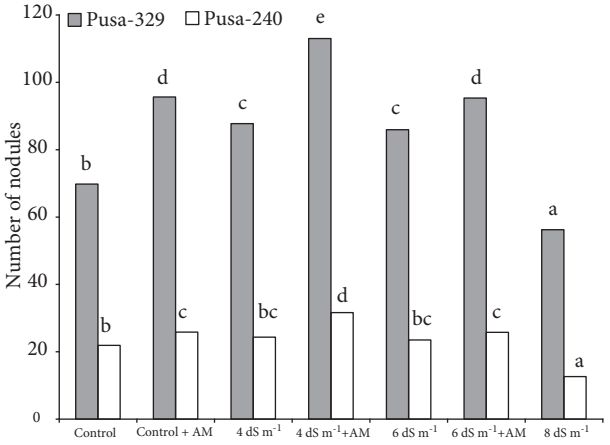

Figure 4. Effect of AM inoculation on number of nodules in chickpea under salt stress. Treatments were designed as uninoculated controls, saline stress (4, 6, and 8 dS m^{-1}), and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly differently ($P < 0.05$) as determined by Duncan's multiple range test.

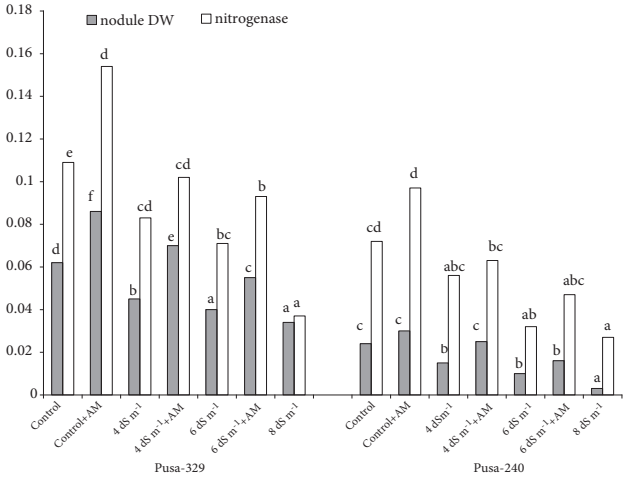

Figure 5. Effect of AM inoculation on dry weight of nodules and nitrogenase activity (nmole ethylene mg^{-1} nodule DW h^{-1}) in chickpea under salt stress. Treatments were designed as uninoculated controls, saline stress (4, 6, and 8 dS m^{-1}), and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly differently ($P < 0.05$) as determined by Duncan's multiple range test.

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.

Thus, the present study indicates a possible correlation between increased salt tolerance of chickpea genotypes and the presence of a fungal endophyte in the rooting medium.

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