Influence of arbuscular mycorrhiza on osmotic adjustment compounds and antioxidant enzyme activity in nodules of salt-stressed soybean (*Glycine max*)

Omid YOUNESI[1*], Ali MORADI[2], Amin NAMDARI[1]

ABSTRACT

The influence of the colonization with arbuscular mycorrhizal (AM) fungus, *Glomus mosseae* (Nicolson and Gerdemann), on characteristics of growth, osmotic adjustment compounds and activity of antioxidant enzymes in nodules of salt-stressed soybean (*Glycine max* (L.) Merr.) was studied in this experiment. The pot experiment was arranged as a factorial in randomized complete block design with four replications at greenhouse of College of Agriculture, Tehran University, Iran. Results indicated that the contents of glycine betaine and proline in nodules were higher in inoculated than in non-inoculated plants. AM fungal colonization increased the activities of superoxide dismutase, catalase, and peroxidase in the nodules. The results indicate that the AM fungus is capable of alleviating the damage caused by salt stress on symbiotic nitrogen fixation of soybean plants by increasing the accumulation of compatible osmolytes and by increased antioxidant enzyme activity. Consequently, arbuscular mycorrhiza formation highly enhanced the salinity tolerance of soybean plant, which increased symbiotic nitrogen fixation and promoted plant growth.

Key words: antioxidants, nodules, osmolytes, salinity, soybean

IZVLEČEK

VPLIV ARBUSKULARNE MIKORIZE NA SPOJINE OSMOTSKE PRILAGODITVE IN ANTIOKSIDACIJSKO ENCIMSKO AKTIVNOST V NODULIH SOJE (*Glycine max* (L.) Merr.) V SLANOSTNEM STRESU

V poskusu je bil preučevan vpliv kolonizacije z arbuskularno mikorizno glivo (AM), *Glomus mosseae* (Nicolson and Gerdemann), na značilnosti rasti, snovi osmotskega prilagajanja in aktivnost antioksidacijskih encimov v nodulih soje (*Glycine max* (L.) Merr.) v slanostnem stresu. Lončni poskus je bil izveden kot naključni faktorski bločni poskus v štirih ponovitvah v rastlinjaku College of Agriculture, Tehran University, Iran. Izsledki so pokazali, da sta bili vsebnosti glicin betaina in prolina večji v inokoliranih kot v neinokuliranih rastlinah. Kolonizacija z AM glivo je povečala aktivnost superoksid dizmutaze, katalaze in peroksidaze v nodulih. Rezultati kažejo, da je AM gliva sposobna omiliti poškodbe, ki nastanejo ob slanostnem stresu v simbiontski vezavi dušika pri soji s povečano akumulacijo primernih osmotikov in povečano antioksidacijsko encimsko aktivnostjo. Posledično tvorba arbuskularne mikorize pri soji močno poveča toleranco na slanostni stres s povečano simbiontsko vezavo dušika, kar pospeši rast.

Ključne besede: antioksidanti, noduli, osmotiki, slanost, soja

1 INTRODUCTION

Symbiotic nitrogen fixation (SFN) in legumes such as soybean (*Glycine max*) is frequently limited, especially in semi-arid conditions by poor quality of soil and irrigation water. Soybean is classified as a salt-sensitive crop (Läuchli, 1984).The limitation in its productivity is associated with a decreased growth, poor symbiotic development of root-nodule bacteria (Georgiev and Atkins, 1993) and a consequent a reduction in the nitrogen-fixation capacity (Delgado et al., 1994).

[1] Ph. D., College of Agriculture & Natural Resources, Tehran University, Iran, e-mail: omidyounesi@gmail.com, *corresponding author

[2] Department of Agronomy and Plant Breeding, Faculty of Agriculture, Yasouj University, Yasouj, Iran

The establishment of the *Rhizobium*-legume symbiosis has been shown to be salt sensitive (Rao et al., 2002). Nodule initiation appears to be more sensitive to salt stress than nodule development (Zahran and Sprent, 1986). Cordovilla et al. (1994) and Soussi et al. (1999) found that the tolerance of the host plant to salt stress could be a determinant factor of symbiosis development. The effect of salt stress on symbiotic nitrogen fixation (SNF) and ion distribution in nodules has been studied in many crops such as soybean, common bean and alfalfa. It seems, the sensitivity of the symbiotic nitrogen fixation is not always associated with a high Na^+ accumulation in nodules. Salinity causes oxidative damage which affects nitrogen fixation and assimilation in nodules. Some studies have implicated reactive oxygen species (ROS) in nodule senescence (Becana et al., 2000; Garg and Manchanda, 2008). But plants are not defenseless; under salt stress some defense mechanisms are initiated which protect plants from harmful effects of oxidative stress. Reactive oxygen species (ROS) scavenging is one such common defense response against abiotic stress (Vranova et al., 2002). The major ROS scavenging system includes a complex enzymatic group such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and non-enzymatic molecules such as proline, glycine betain, sorbitol and manitol (Prochazkova et al., 2001).

Arbuscular mycorrhizal fungi (AMF) widely occur in saline soils (Aliasgharzadeh et al., 2001). These fungi exploit water and mineral salts from soils more effectively than plant roots (Kaya et al., 2003). Many studies have demonstrated that arbuscular mycorrhizal fungi (AMF) protected the host plants to improve the growth of plants under salt stress condition (Trimble and Knowles, 1995). Moreover, additive and sometimes synergistic effects on legume performance are frequently seen when both rhizobia and AMF are present (Goss and de Varennes, 2002; Sanginga et al., 1999; Fitter and Garbaye, 1995). Reports on the response of antioxidant defense system to stress factors in inoculated plants are contradictory; increase, no change, or even decrease in the activity of SOD, CAT, POD and APX were reported in mycorrhizal soybean (Porcel et al., 2003) subjected to drought and tomato subjected to salinity (He et al., 2007; Hajiboland et al., 2010).

The aim of this study was to evaluate the effect of root colonization with *Glomus mosseae* (Nicolson and Gerdemann) on growth parameters, nodulation, mineral uptake, osmotic adjustment compounds and antioxidant enzyme activity of soybean plants under salinity stress, in order to further understand salt tolerance mechanisms in inoculated plants.

2 MATERIALS AND METHODS

Experimental design
The experiment was conducted from 22th of April to 22th of November, 2011 in a greenhouse of the College of Agriculture, University of Tehran, Iran. Plants were grown in the greenhouse under natural sunlight with temperatures of 25 – 30°C (day) and 20 – 23°C (night). There were four replications for each treatment. The experiment was arranged as a factorial in completely randomized design.

Rhizobial and AM fungal inoculum
Mycorrhizal fungal and rhizobal inoculum were provided by the Institute of Soil and Water Research,Karaj, Iran. The AM fungal species used was *Glomus mosseae* (Nicolson and Gerdemann).The soybean 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 *Bradyrhizobium japonicum*. 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.

The soil used for pots was collected from the uncultivated site located in Qom province, Iran. The soil used in this experiment was not sterilized (autoclaved). The basic soil properties were as follows: organic matter content 1.08%, total N 0.062%, total K 740.80 $mg\,kg^{-1}$, total P 10.90 $mg\,kg^{-1}$, available P ($NaHCO_3$-extractable) 2.78 $mg\,kg^{-1}$, water-soluble K 13.43 $mg\,kg^{-1}$ and electrical conductivity 8.1 dSm^{-1}.

Five seeds were sown in each pot containing 2 kg of soil mixture. After 21 days, thinning was carried out to leave three uniform seedlings in each pot. When the seedlings were established (30 days after sowing), the plants were treated with saline solution with electrical conductivities 6 (S1 treatment) and 12 dSm (S2 treatment). The control plants (C) were treated with distilled water only. Pots were irrigated according to their weight at 80% field capacity moisture. 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. Parameters such as mycorrhizal colonization, nodule weight, leghemoglobin content, nitrogenase activity, osmolyte accumulation and antioxidant enzymes activities nodules were studied after 180 days of sowing. The plants and the adhering soil were transferred to the sieve and roots and nodules were collected from the sieve. For dry weight measurements, the samples were dried in an oven at 70°C for 72 h.

Mycorrhizal colonization

Mycorrhizal colonization was estimated by the method of Phillips and Hayman (1970). For AMF colonization analysis, 2.5 -cm root segments from three plants per treatment were sampled at harvest and pooled to assess colonization percentage. The roots were cut and dipped in 8 % KOH solution for 24 h and then kept in 2% HCl solution for 15 to 30 min. Staining solution containing 0.05% (v/v) cotton blue dye was added. The samples were kept for 24 to 36 h at room temperature condition. Twenty 2.5 -cm stained root pieces were placed on each slide and three observations (the top, the middle, and the bottom) per 2.5-cm root piece were made with microscope. There were four slides per treatment. Root pieces that contained even a single vesicle or arbuscules were considered as colonized. The percentage of AM colonization was calculated from the following equation: Percentage of AM colonization = (Root length colonized/Root length observed) ×100.

Leghemoglobin

Leghemoglobin content was determined in fresh uniform sized root nodules measuring 0.5 cm or more diameter. Nodules were carefully removed from the roots with sharp edged blade. These were washed with prechilled double distilled water.

After washing, the nodules were blotted on filter paper, weighed and then finally crushed in prechilled sterilized pestle mortar containing 50.0 mM HCl, 5 mM MgCl2, 20 mM KCl and 5 mM mercapto ethanol. The slurry was centrifuged at 40°C at 8,000 rpm for 15 minutes. The pellets were discarded and supernatant (SN) was made to known volume i.e. 4 ml/ gm fresh weight of nodules. In this supernatant, lb content was estimated by using haemochromogen method (Hartree, 1955).

The 0.5 and 1 ml aliquot of clear extract was taken in the test tube. To each tube, 1.5 ml of 1 N NaOH was added and kept for half an hour at room temperature. After 30 minutes, 3 ml of pyridine solution and 1.5 ml of 10% (W/V) sodium bisulphide were added to each tube. Then distilled water was added to make the volume to 15 ml. The tubes were incubated for 30 minutes and the optical density was recorded at 535 and 556 nm. Calibration curve was prepared by using a standard solution of haemin 100 (μg/ml) by dissolving in 1N NaOH. Leghaemoglobin content is expressed as mg haemin / gm fresh weight of nodules. All observations were recorded in triplicates and data were subjected to statistical analysis of variance using three factorial randomized design methods (Bruning and Kintz, 1977).

Enzymeatic activity

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, n. 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 n. moles of ethylene produced per mg dry weight of nodules per hour.

Superoxide dismutase (SOD) activity

For Superoxide dismutase (SOD) activity analysis, the plant samples were brought to the laboratory and the roots were thoroughly washed under running tap water without damaging roots and nodules. The nodulated root sample from three plants per treatment were sampled at harvest and pooled to assess (SOD) activity. The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), according to Stewart and Bewley (1980). The reaction mixture (3 ml) contained 13 mM methionine, 75 mM NBT, 100 mM EDTA, 50 ml of enzyme extract within 50 mM phosphate buffer (pH 7.8). The reaction was started with 2 mM riboflavin by exposing the cuvette to a 15-W fluorescent tube for 10 min. The absorbance of each reaction mixture was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT.

Catalase activity

The activity of catalase (CAT) was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 (Chance and Meahly, 1955). The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2.

Peroxidase activity

Peroxidase (POD) activity was measured by following the change of absorption at 470 nm due to guaiacol oxidation. The activity was assayed for 1 min in a reaction solution (3 ml final volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 m MH_2O_2 and 0.15 ml enzyme extract (Polle et al., 1994).

Calcium, sodium and potassium content

Ground samples were ashed at 580°C for 6 h. The white ash was taken up in 2 M hot HCl, filtered into a 50 ml volumetric flask, and made up to 50 ml with distilled water. Na, K, and Ca were determined in these sample solutions. Na and K in the sample solution were analyzed using a flame photometer and Ca with an atomic absorption spectrophotometry (Chapman and Pratt, 1961).

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. Added 250 ml of concentrated HNO_3 and diluted to 1 L. A total of 0.5 g of material was taken in 50 ml volumetric flask and 10 ml of vandomolybdate reagent was added to each flask and made the volume by deionized water. The solution was kept for 30 min and took the absorbance at 420 nm with spectrophotometer. Appropriate standards were run simultaneously.

Proline content

Free proline content was determined following the method of Bates et al. (1973). Proline estimation is based on the formation of brick red colored proline- ninhydrin complex in acidic medium. Nodule sample (0.5 g) was homogenized in 5 ml of sulfosalicylic acid (3%) using mortar and pestle, the homogenate was filtered and filtrate was used for the estimation of proline content. Two milliliter of extract was taken in test tube and to it 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added and heated for 30 min. Six milliliter of toluene was added and then transferred to a separating funnel. The chromophore containing toluene was separated and its absorbance read was at 520 nm in spectrophotometer against toluene blank. Concentration of proline was estimated by referring to a standard curve made from known concentrations of proline.

Glycine betaine content

Glycine betaine estimation was done as per the method of Grieve and Grattan (1983). Betaine makes a betaine-periodite complex with iodide in acidic medium, which absorbs at 360 nm in UV range. Finely ground dry plant material (0.5 g) was mechanically shaken with 20 ml of deionized water for 48 h at 258°C. The samples were filtered. Thawed extracts were diluted 1:1 with 2 N sulphuric acid. Aliquot (0.5 ml) was cooled in ice water for 1 h and to it; cold potassium iodide-iodine reagent (0.2 ml) was added. The samples were stored at 0–48°C for 16 h and were centrifuged at 10,000 g for 15 min at 08°C. The supernatant was carefully aspirated. The periodite crystals were dissolved in 9 ml of 1, 2- dichloro ethane (reagent grade). After 2.0–2.5 h, the absorbance was measured at 365 nm with UV-visible spectrophotometer. Reference standards of

glycine-betaine (50–200 mg/ml) were prepared in 2 N sulphuric acid and the procedure for sample estimation was followed.

Statistical analysis

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

3 RESULTS

The results pointed out that different level of salt stress had inhibitory effects on mycorrhizal colonization, although high mycorrhizal colonization was observed at the moderate level of salinity stress.

Salinity stress significantly reduced the root and shoot dry matter compared with the control treatment (Table 1). However, AM fungal colonization mostly improved dry matter in the salt-stressed plants. This effect of AM on dry matter was more pronounced in shoot biomass than root biomass.

Table 1: Effect of salt stress on shoot length, root length, shoot DM, root DM and colonization in AM and non-AM soybean plants under salt stress.

Treatments	Shoot length (cm plant $^{-1}\pm$ SD)	Root length (cm plant $^{-1}\pm$ SD)	Shoot DM (g plant $^{-1}\pm$ SD)	Root DM (g plant $^{-1}\pm$ SD)	AMF colonization (%)
C - AMF	54.6±1.43[b]	34.8±1.64[a]	11.3±0.64[b]	4.6±0.7[b]	-
C + AMF	61.8±1.16[a]	36.6±1.65[a]	13.6±0.6[a]	5.8±0.51[a]	28.8± 1.05[a]
S1 - AMF	44.3±1.82[c]	31.2±0.48[b]	7.9±1.1[c]	3.8±0.67[c]	-
S1 + AMF	58.8±0.68[ab]	35.3±0.73[a]	10.6±0.75[b]	5.3±1.37[a]	26.3±1.63[a]
S2 - AMF	32.5±1.46[d]	30.7±1.33[b]	6.3±o.65[c]	2.1±0.28[d]	-
S2 + AMF	42.3±1.07[c]	34.5±1.02[a]	9.4±1.13[bc]	3.6±0.7[c]	18.8±0.23[b]

Results represent the average of three experiments ± SD. Different letters represent significant differences ($p < 0.05$) between treatments at each column.

nodulation of vicia faba L. plants by Rhizobium leguminosarum. Planta, 167: 303-309.

Nodule number and dry mass of the nodules decreased under all saline treatments (Figure 1). AM fungal inoculation further boosted the nodulation under saline stress and the nodule number showed a significant increase in unstressed as well as stressed conditions.
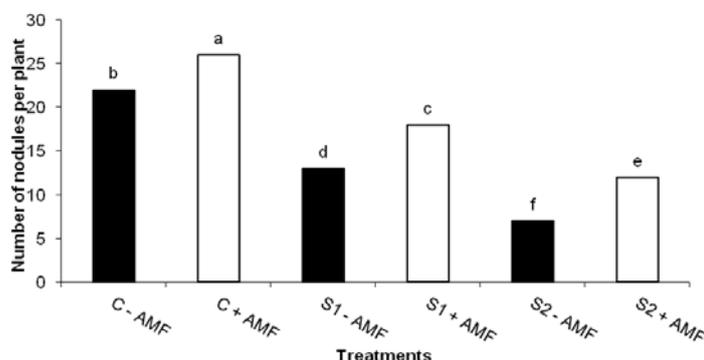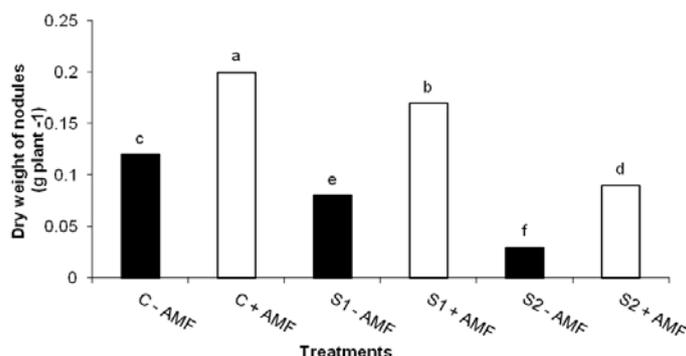
Figure 1: Effect of AM inoculation on number of nodules per plant (a) and dry weights of nodules per plant (b) of soybean under salt stress. Treatments are designed as uninoculated controls, saline stress (S1 = 6 and S2 = 12 dSm^{-1}) and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly different ($p<0.05$) as determined by Duncan's Multiple Range test.

Marked decline in the leghemoglobin content was observed in plants exposed to salt stress (Figure 2). The decrease in leghemoglobin content was smaller in inoculated plants, when compared to corresponding uninoculated-stressed plants. AM fungi conferred an advantage on the plants under saline stress and at 12 dSm salinity, inoculated plants had higher leghemoglobin content than the corresponding non-inoculated plants.
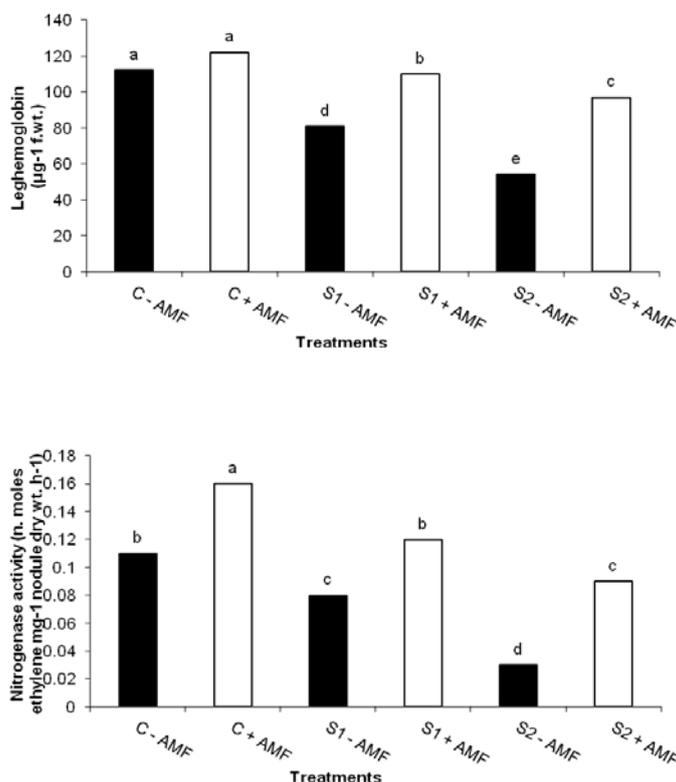

Figure 2: Effect of AM inoculation on leghemoglobin content (a) and nitrogenase activity (b) in the nodules of soybean under salt stress. Treatments are designed as uninoculated controls, saline stress (6 and 12 dSm^{-1}) and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly different ($p<0.05$) as determined by Duncan's Multiple Range test.

Nodule activity in terms of acetylene-dependent ethylene production was severely damaged by the presence of salt stress. The presence of fungi proved to be favorable and nitrogenase activity was significantly higher in AM inoculated plants, than in non-inoculated plants, regardless of the saline treatments.

Potassium and phosphorus contents in the nodules declined with increase in the salt concentrations in the soil in all the stressed plants, whereas an increase in the sodium and calcium contents was observed in all the stressed plants (Table 2). Nodules of AM inoculated plants maintained significantly higher ion (potassium, phosphorus, and calcium) contents than the corresponding non-inoculated plants under all saline treatments.

Table 2: Effect of salt stress on potassium, sodium, calcium, and phosphorus content in nodules of AM and non-AM soybean plants under salt stress.

Treatments	Potassium (mg g^{-1} d.wt.± SD)	Sodium (mg g^{-1} d.wt.± SD)	Calcium (mg g^{-1} d.wt.± SD)	Phosphorus (mg g^{-1} d.wt.± SD)
C - AMF	22.22±1.08[b]	1.52±0.08[a]	2.61±0.65[a]	8.32±1.27[b]
C + AMF	27.11±1.64[a]	1.34±0.09[a]	3.41±0.76[bc]	12.6±1.2[a]
S1 - AMF	8.11±0.48[d]	5.77±0.0.39[c]	2.56±0.21[a]	5.1±1.04[d]
S1 + AMF	12.18±0.9[c]	4.05±0.93[b]	3.78±0.92[c]	8.05±0.88[b]
S2 - AMF	5.26±1.02[e]	10.3±1.16[e]	3.18±1.12[bc]	4.21±0.64[e]
S2 + AMF	8.92±0.47[d]	9.1±0.77[d]	4. 2±0.83[d]	6.73±0.78[c]

Results represent the average of three experiments ± SD. Different letters represent significant differences ($p < 0.05$) between treatments at each column.

Proline concentration increased in the nodules with salinity, however, in non-inoculated stressed plants, the increase was not significant at 6 dSm

(Figure 3). Nodular proline levels in inoculated salt stressed plants were higher than in non-inoculated salt-stressed plants.
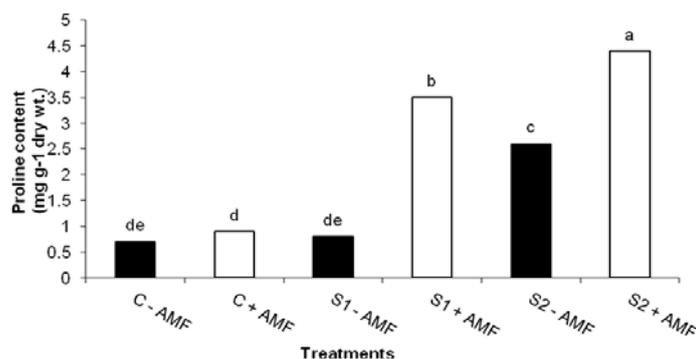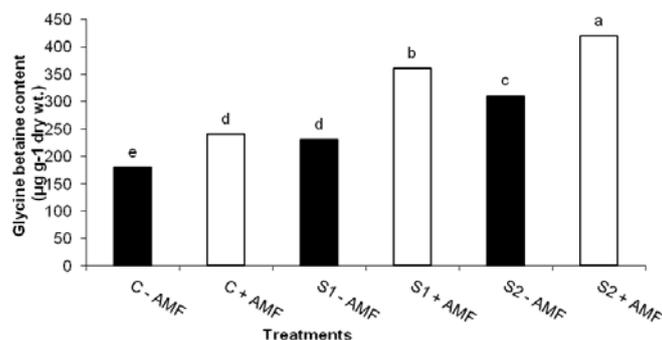
Figure 3: Effect of AM inoculation on the proline (a) and glycine betaine (b) content in the nodules of soybean under salt stress. reatments are designed as uninoculated controls, saline stress (6 and 12 dSm^{-1}) and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly different ($p<0.05$) as determined by Duncan's Multiple Range test.

Glycine betaine content increased significantly in nodules of all the stressed plants, however, stress induced increase in glycine betaine content was higher in nodules of AM inoculated plants than those of non- AM inoculated plants.

Exposure of the plants to salt stress resulted in general increment in the antioxidant enzyme activities of the nodules. Mycorrhizal inoculation further increased the antioxidant enzyme activities. Saline stress led to enhanced SOD activity in nodules of all the plants. SOD activity was higher in inoculated plants than in non- inoculated-stressed plants at 6 and 12 dSm, respectively. CAT activity increased in the nodules at 12 dSm, while the salt levels of 6 dSm did not bring a significant increase in CAT activity in comparison with the control. Symbiosis with the mycorrhizal fungi significantly increased the CAT activity at 6 and 12 dSm and it was higher than corresponding nodules of stressed non-AM inoculated plants. POX activity increased with application of saline doses of 6 and 12 dSm. Salinity induced increase in POX activity of non- inoculated nodules. A higher increase was observed in nodules of inoculated plants at 6 and 12 dSm, respectively.

Table 3: Effect of salt stress on SOD, CAT, POX activities in nodules of AM and non-AM soybean plants under salt stress.

Treatments	SOD (units mg^{-1}prot. min^{-1}± SD)	CAT (s mol H$_2$O$_2$ red. mg^{-1}prot. min^{-1}± SD)	POX (n mol tetra- guaiacol formed min^{-1} g^{-1} f.wt.± SD)
C - AMF	16.05± 0.48[e]	7.86±0.23[e]	204.23±3.22[f]
C + AMF	20.4± 1.11[d]	9.84±0.85[d]	217.54±1.78[e]
S1 - AMF	27.87±2.3[c]	8.03±0.45[e]	257.44±2.1[d]
S1 + AMF	47.04±o.78[b]	15.11±1.33[c]	321.02±0.76[b]
S2 - AMF	29.85±1.3[c]	22.12±0.65[b]	279.6±1.28[c]
S2 + AMF	53.87±1.42[a]	36.7±1.37[a]	349.77±1.48[a]

Results represent the average of three experiments ± SD. Different letters represent significant differences ($p < 0.05$) between treatments at each column

4 DISCUSSION

The colonization rate declined with increasing NaCl level, indicating that salinity suppressed the growth of AM fungi. It has been reported that addition of various salts to soil inhibits hyphal growth with a subsequent decrease in the spread of mycorrhizal colonization (Ruiz-Lozano and Azcon, 1996; Hajiboland et al., 2010).

Salinity stress significantly reduced the root and stem dry matter compared with the control treatment due to direct effects of ion toxicity or indirect effects of saline ions that cause soil /plant osmotic imbalance (Abdel Latef, 2010). Colonization with AMF significantly improved dry matter in the salt-stressed plants. This effect of AM fungi on dry matter was more pronounced in aerial biomass than root biomass which may be because of arbuscular mycorrhizal colonization can cause a proportionally greater allocation of carbohydrates to the shoot than root tissues (Shokri and Maadi, 2009). Enhanced growth of mycorrhizal tomato plants grown in saline environments has been related partly to mycorrhiza-mediated enhancement of host plant nutrition (Kaya et al., 2009). Cantrell and Linderman (2001) reported that AM fungi improved growth under salt stress condition. These findings indicated the benefits of AM fungi and the important role they play in increasing salinity tolerance.

Size and dry weights of root nodules decreased in soybean plants grown in saline environment. Our results indicated that the reductions in dry weights under salt-stressed conditions were more closely linked to the reductions in the size of nodules, rather than to the initiation of the nodules.

The process of nitrogen fixation was affected negatively by salt stress, as revealed by declined leghemoglobin content and reduced nitrogenase activity. Similar decline in nodulation and nodule activity has also been reported earlier by Serraj et al. (2001); Tejera et al. (2005); Bolanos et al. (2006); Garg and Manchanda (2008). Despite a decline in the functional efficiency of nodules, AM inoculated plants had considerably higher leghemoglobin content and nitrogenase activity than corresponding non-AM inoculated plants under salt stress. AM inoculation markedly increased nodulation at low saline concentration. Evidences from the previous studies (Johansson et al. 2004; Rabie and Almadini, 2005; Garg and Manchanda, 2008) indicate that the presence of AM fungi enhances nodulation and nitrogen fixation by legumes.

In this study, the contents of potassium and phosphorus declined under saline conditions. Phosphorus concentration of nodules was significantly lowered in salt-affected compared with control plants. Reduction of P uptake in saline soils was attributed to precipitation of $H_2PO_4 -$ with Ca^{2+} ions in soil and of K^+ and Ca^{2+} to a competition with Na^+ (Marschner, 1994). A marked effect of AMF on the uptake of P was observed even in the control plants. The enhancement of plant P (Giri et al., 2007) uptake by AMF has been reported and was considered one of the main reasons for amelioration of growth in salt-affected plants colonized by AMF (Ruiz-Lozano and Azcon, 1996).

The nodules of AM inoculated plants accumulated lesser Na^+ than the corresponding non-inoculated-stressed plants. Nodular potassium and calcium contents were higher in inoculated-stressed plants than in stressed non-inoculated plants, which could have been an important factor in maintaining higher nodulation and nitrogen fixation in these plants. Higher K^+ accumulation by inoculated plants in saline soil could be beneficial by maintaining a high K^+/Na^+ ratio and by influencing the ionic balance of the cytoplasm or Na^+ efflux from plants (Giri et al., 2007; Aleman et al., 2009). Improved ionic ratios in the aerial parts of inoculated-stressed plants have been reported earlier by Giri et al. (2003); Rabie (2005); Rabie and Almadini (2005).

Many plants accumulate proline as a nontoxic and protective osmolyte under saline conditions Parida et al., 2003). Marked increase in free proline occurs in many plants during moderate or severe water or salt stress; this accumulation, mainly as a result of increased proline biosynthesis, is usually the most outstanding change among the free amino acids (Hurkman et al., 1989). Data reported here revealed that proline and glycine betaine contents increased under salt stress. Synthesis and accumulation of both the osmolytes were significantly higher in the nodules of AM inoculated- stressed plants than the corresponding non-inoculated ones. The results suggested that higher accumulation of proline and glycine betaine contents in the nodules of inoculated-stressed plants was correlated with enhanced nitrogen fixing ability of these plants. High proline concentration was suggested to protect nodule metabolism by avoiding protein denaturalization and maintaining cell pH levels (Irigoyen et al., 1992).

A constitutively high antioxidant capacity under stress conditions can prevent damages due to ROS formation (Harinasut et al., 2003). There are reports showed that a greater SOD activity in salt tolerant plants (Benavides et al., 2000). Our results showed that moderate and high salinity caused a significant increase in SOD activity in nodules of both inoculated and non-inoculated soybean plants. These results are similar in part to results obtained by Garratt et al. (2002) who found enhanced SOD activity under salinity condition in cotton. Based on the induced SOD activity in the nodules of soybean plants grown under salinity, it could be concluded that SOD is important for soybean to tolerate salinity. Furthermore, enhanced SOD activity in inoculated plants as compared to non-inoculated plants supports the view that increased antioxidative enzyme activities could be involved in the beneficial effects of mycorrhizal colonization on the performance of plants grown under semi-arid conditions (Alguacil et al., 2003). Gradual exposure of the AM fungus to salinity enhanced its ability to increase SOD activity in the host plants. The great SOD activity in inoculated plants could increase the capacity of nodules to scavenge superoxide radicals.

Plant possesses hydrogen peroxide scavenging enzymes POD and CAT. Detoxifications of the reactive oxygen protect cells against harmful concentration of hydroperoxides (Castillo, 1992). The increased POD in response to salinity has been reported (Harinasut et al., 2003). In tolerant plants, POD activity was found to be higher to protect plants against the oxidative stresses (Sreenivasulu et al., 1999). Pacovsky et al. (1991) studied POX activity in *Phaseolus vulgaris* colonized by *Glomus etunicatum* and found that peroxidase activity increased in the mycorrhizal plants. Alguacil et al. (2003) reported that mycorrhizal inoculation increased CAT activity in *Olea europaea* grown under semi-arid conditions. On the other hand, since CAT is involved in decomposition of H_2O_2 in peroxisomes, similar increases in CAT activity of non-inoculated and inoculated plant at moderate and high NaCl indicate that under these conditions H_2O_2 is probably produced in higher concentrations in the peroxisome.

On the basis of the results presented here, our results support the view that AMF can contribute to protect plants against salinity by alleviating the salt induced oxidative stress. This ameliorative effect of mycorrhizal colonization shows significant interactions with salt exposure. Enhanced antioxidant enzymes activity in AM inoculated plants may contribute to better maintenance of the ion balance the reactions in nodules under salinity.

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