Influence of Arbuscular Mycorrhiza on Membrane Lipid Peroxidation and Soluble Sugar Content of Soybean under Salt Stress

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Summary

The influence of the arbuscular mycorrhizal (AM) fungus, *Glomus mosseae*, on characteristics of growth, membrane lipid peroxidation and soluble sugar content in the shoots and roots of soybean (*Glycine max*) plants was studied in pot culture under salt stress. The experiment was arranged as a factorial in Randomized Complete Block Design (RCBD) with four replications in greenhouse of College of Agriculture, Tehran University, Iran. The plants inoculated with mycorrhiza had significantly greater shoot and root biomass than the nonmycorrhizal plants at all salinity levels. AM symbiosis decreased membrane relative permeability and malondialdehyde content in shoots and roots. The soluble sugar content in roots was higher in mycorrhizal than nonmycorrhizal plants, but there was no significant difference in soluble sugar content in shoots between mycorrhizal and nonmycorrhizal plants. The results indicate that the AM fungus is capable of alleviating the damage caused by salt stress on soybean plants by reducing membrane lipid peroxidation and increasing the accumulation of soluble sugar content. Consequently, arbuscular mycorrhiza formation highly enhanced the salinity tolerance of soybean plant, which increased host biomass and promoted plant growth.

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Introduction

Soil salinity limits particularly the production of both forages and grain legumes. Nitrogen (N) fixation has been found to be more sensitive than plant growth to salt (Delgado et al., 1994). Nevertheless, there is considerable genetic variability in salt tolerance among legume species and cultivars (Delgado et al., 1994).

Soybean are classified as a salt-sensitive crop (Läuchli, 1984) and the limitation in their productivity is associated with lower growth of soybean, 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).

The term "symbiosis" was first used by de Bary (1879) to refer to "the living together of differently named organisms". This definition encompasses both mutualistic and parasitic associations, but today, symbiosis is usually thought of in terms of a mutually beneficial association rather than the one in which one partner benefits more than the other (Lum and Hirsch, 2003). The arbuscular mycorrhizal (AM) symbiosis is one such highly evolved symbiotic association. In this association the fungal hyphae penetrate root cortical cells to form arbuscules to exchange nutrients and carbon.

When the plant is subjected to high or low salinity stress, the cell membrane is first affected with increased membrane permeability. At the same time, a variety of reactive oxygen species (ROS), such as superoxide anion radical (O_2^-), hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2), are induced, causing a loss in balance between production and scavenging in the cell or organism, which causes membrane lipid peroxidation (Apel and Hirt, 2004). As a consequence, plants protect themselves against oxidative injury by inducing osmotic adjustment and activity of antioxidant enzymes (Asada, 1999).

It is well-known that AM fungi not only stimulate the growth of plants but also contribute in enhancing plant tolerance to abiotic and biotic stresses such as salinity (Kaya et al., 2009) and drought (Augé, 2001). The AM symbiosis can alter plant physiology in a way to cope with stresses under stressful conditions (Miransari et al., 2008). However, the mechanisms by which the AM symbiosis influences the metabolism of host plants under salinity stress are not clear. Therefore, understanding the effect of AM fungi on lipid peroxidation and inducing osmotic adjustment of plants under salinity stress is of importance.

The purpose of this study is to evaluate the effect of *Glomus mosseae* on growth, membrane lipid peroxidation and osmotic adjustment in the shoots and roots of soybean plants under salt stress.

Materials and methods

The experiment was conducted in the 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). The experimental treatments consisted of three levels of salinity (0 (control), 6 and 12 dsm^{-1}) and two AMF inoculations (AMF and non-AMF) and were arranged as a factorial in completely randomized design. Each treatment was replicated four times.

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 pre-treated with a standard rhizobial inoculum of *Bradyrhizobium japonicum*. The AM spores were applied at 10 spores per seed (approximately 1500 spores/100 g of media). Seeds were inoculated by placing the AM inoculum 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 basic soil properties were as follows: organic matter content 1.08%, total N 0.062%, total K 740.8 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 and 12 dSm. The control plants 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 of EC meter, till the end of the experiments. Parameters such as mycorrhizal infection, nodule number and weight, membrane relative permeability, malondialdehyde (MDA), Soluble sugar content and chlorophyll content 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 and combined with the rest of the plant material. For dry weight measurements, the samples were dried in an oven at 70ºC for 72 h.

Mycorrhizal colonization

Mycorrhizal infection was estimated by the method of Phillips and Hayman (1970). 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 cotton blue dye was added. The samples were kept for 24 to 36 h. The roots were cut into small pieces of 2.5 cm approximately and observed under compound light microscope. Root pieces that contained even a single vesicle or arbuscules were considered as infected. The percentage of AM infection was calculated from the following equation: Percentage of AM colonization = (Root length infected/Root length observed) ×100.

Membrane relative permeability

Membrane relative permeability was measured according to Bai et al. (1996). Shoot and root samples were washed with deionized water, followed by the introduction of small excisions and then incubated in deionized water at room temperature. After 1 h, the electrical conductivity (L1) of the immersion solution was measured using a conductivity meter. The immersion solution was then placed in a boiling water bath for 10 min, and the electrical conductivity (L2) was measured after cooling. Membrane relative permeability was calculated by the formula L1/L2×100%.

Malondialdehyde (MDA)

Malondialdehyde (MDA) was measured according to the thiobarbituric acid (TBA) reaction as described by Zhang and Qu (2004). shoot and root samples were homogenized with 5% trichloroacetic acid and centrifuged at 4,000 g for 10 min. Two

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milliliters of extract was added to 2 ml 0.6% TBA placed in a boiling water bath for 10 min, and absorbance was read at 532, 600, and 452 nm. The MDA concentration was calculated according to the formula: $6.45 \times (A532 - A600) - 0.56 \times A450$.

Soluble sugar content

Soluble sugar content was determined by the anthrone method (Zhang and Qu, 2004) using sucrose as the standard. Leaf and root samples were homogenized with distilled water, placed in a volumetric flask for 1 h, and filtered with filter paper. The reaction mixture contained 1 ml extract and 5 ml anthrone (100 mg anthrone + 100 ml 72% H_2SO_4) and were placed in a boiling water bath for 10 min, and then absorbance was read at 620 nm.

Chlorophyll content

Two plants per replicate were used for chlorophyll determination at fruit set stage. Fresh tissue (1.0 g) was sampled from the youngest fully expanded leaf, extracted with 90% acetone and read using a UV/visible spectrophotometer (Shimadzu UV 1601) at 663, 645, and 750 nm. Absorbance at 750 nm was subtracted from the absorbance at the other two wavelengths to correct for any turbidity in the extract before chlorophyll concentrations were calculated using the following formulae (Strain and Svec, 1966).

Chl a (mgml^{-1}) = 11.64 × (A663) − 2.16 × (A645)

Chl b (mgml^{-1}) = 20.97× (A645) −3.94×(A663)

A663 and A645 represent absorbance values read at 663 and 645 nm wavelengths, respectively.

Results were analyzed statistically by analysis of variance using the Statistical Analysis System computer package (SAS Institute Inc., 1988). When analysis of variance showed significant treatment effects, the LSD test was applied to make comparisons among the means at the 0.05 level of significance (Steel and Torrie, 1980).

Results and discussion

Salinity stress significantly reduced the shoot and root 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). AM colonization significantly improved these parameters in the salt-stressed plants (Table 1). Enhancement of growth in mycorrhizal plants in saline conditions has been related partially to mycorrhizal-mediated enhancement of host plant nutrition (Aliasgharzadeh et al., 2001). It has been widely accepted that AM are able to adapt to edaphic conditions (Trimble and Knowles, 1995; Aliasgharzadeh et al., 2001; Giri et al., 2003). The effect of AM on dry matter was more pronounced in aerial biomass than root biomass which may be because of arbuscular mycorrhizal colonization caused a proportionally greater allocation of carbohydrates to the shoot than root tissues (Shokri and Maadi, 2009).

Nodule number and dry mass of the nodules decreased under all saline treatments (Figure 1). Similar decline in nodulation and nodule activity has also been reported earlier by Serraj et al., (2001); Tejera et al., (2005); Garg and Manchanda (2008). AM inoculation further boosted the nodulation under saline stress and the nodule number showed a significant increase in unstressed as well as stressed conditions. Evidences from the

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

Treatments	Shoot DM (g plant^{-1})	Root DM (g plant^{-1})	AMF colonization (%)
C - AMF	11.3b	4.6b	-
C + AMF	13.6a	5.8a	28.8a
S1 - AMF	7.9c	3.8c	-
S1 + AMF	10.6b	5.3a	26.3a
S2 - AMF	6.3c	2.1d	-
S2 + AMF	9.4bc	3.6c	18.8b

Means followed by the same letter within a column are not significantly different at p<0.05, as determined by Duncan's Multiple Range test
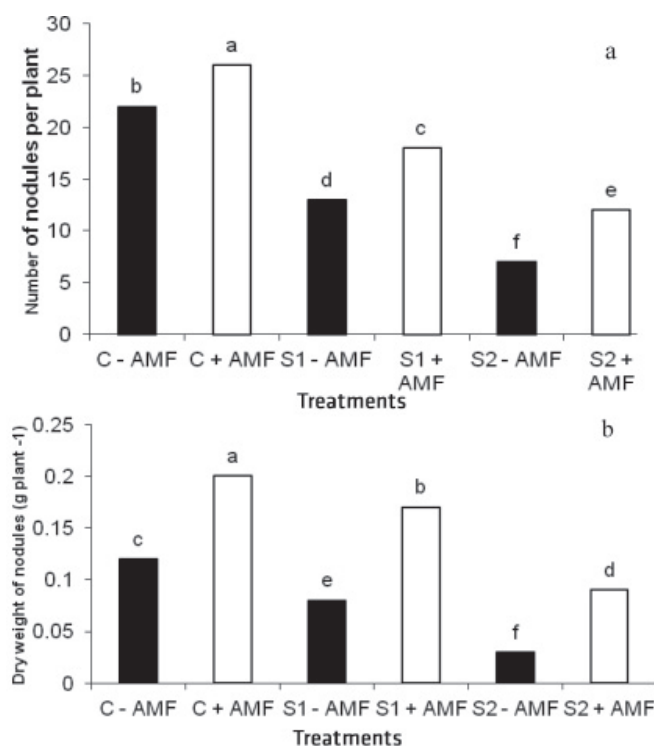
Figure 1. Effect of AM inoculation on number of nodules per plant (a) and dry weights of nodules per plant (b) of soybean under salt stress. Treatments are designed as uninoculated controls, saline stress (S1 = 6 and S2 = 12 dSm^{-1}) and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly different (p<0.05) as determined by Duncan's Multiple Range test

previous studies (Johansson et al., 2004; Rabie & Almadini, 2005; Garg & Manchanda, 2008) indicate that the presence of AM fungi enhances nodulation and nitrogen fixation by legumes.

AM colonization rates are shown in Table 1. Microscopie assessment confirmed that plants of the non-inoculation treatment were not colonized by AM. As it is evident from Table 1, the colonization rate declined with increasing NaCl level, indicating that salinity suppressed the growth of AM. Previous research has shown that salinity can reduce AM colonization by

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Table 2. Effect of salt stress on membrane relative permeability and MDA content in root and shoot of AM and non-AM soybean plants under salt stress

Treatments	Root membrane relative permeability (%)	Leaf membrane relative permeability (%)	Root MDA content (nmol. g^{-1}. FW)	Leaf MDA content (nmol. g^{-1}. FW)
C - AMF	12.5c	9.4bc	9.81a	7.45b
C + AMF	8.7a	5.1a	9.12a	5.23a
S1 - AMF	16.6d	11.8c	12.52b	8.33b
S1 + AMF	9.3b	5.8a	9.72a	6.24ab
S2 - AMF	23.6e	14.2d	14.66c	10.15c
S2 + AMF	11.4c	7.3b	11.23b	7.73b

Means followed by the same letter within a column are not significantly different at p<0.05, as determined by Duncan's Multiple Range test

inhibiting the germination of spores (Hajiboland et al., 2010), inhibiting growth of hyphae in soil and hyphal spreading after initial infection had occurred (McMillen et al., 1998), and reducing the number of arbuscules (Al-Karaki and Hammad, 2001).

Membrane permeability in the shoots and roots was lower in mycorrhizal than in nonmycorrhizal plants, which shows that the presence of the AM fungus could alleviate the peroxidation of membrane lipids (Table 2). Enhancement of membrane lipid peroxidation causes an increase in membrane permeability, exosmosis of electrolytes, and finally injures the cell membrane system. These results confirm the findings of a previous study in which it was shown that salt-stressed tomato plants inoculated with mycorrhizae had lower membrane permeability than noninoculated plants (Al-Karaki, 2000). Mycorrhizal soybean plants are supposed to have some special mechanisms to alleviate the cell membrane damage. One of the early responses of plants to pathogens, wounding, salinity and drought is the accumulation of reactive oxygen species (ROS) (Mittler, 2002). The ROS can in turn damage the cell membrane and hence promote electrolyte leakage. It is possible that mycorrhizal soybean plants may have accumulated some potential antioxidants to counteract ROS.

MDA is often regarded as the product and a reflection of the degree of membrane lipid peroxidation (Ali et al., 2005). Therefore, MDA content in the shoots and roots of soybean plants was measured under saline stress. MDA content in the shoots and roots was lower in mycorrhizal than in nonmycorrhizal plants at all salinity treatments, although the difference between mycorrhizal and nonmycorrhizal plants was not significant for the root MDA content in control plants (Table 2). As the salinity stress was increased, the membrane relative permeability and MDA content in the shoots and roots of both AM inoculated and non-inoculated plants increased, and at similar conditions, the root membrane relative permeability was higher than that in shoots.

Mycorrhizal plants had higher soluble sugar content in the roots at all saline stress treatments, compared with nonmycorrhizal plants (Figure 2). There was also no significant difference in soluble sugar content in shoots between mycorrhizal and nonmycorrhizal plants regardless of salt stress treatments. Soybean plants had similar shoot soluble sugar content at 6 and 12 dSm, but the root soluble sugar content was higher at 12 dSm than that at 6 dSm.

Chlorophyll concentrations were significantly reduced by salinity treatments (Figure 3). The adverse effects of high NaCl
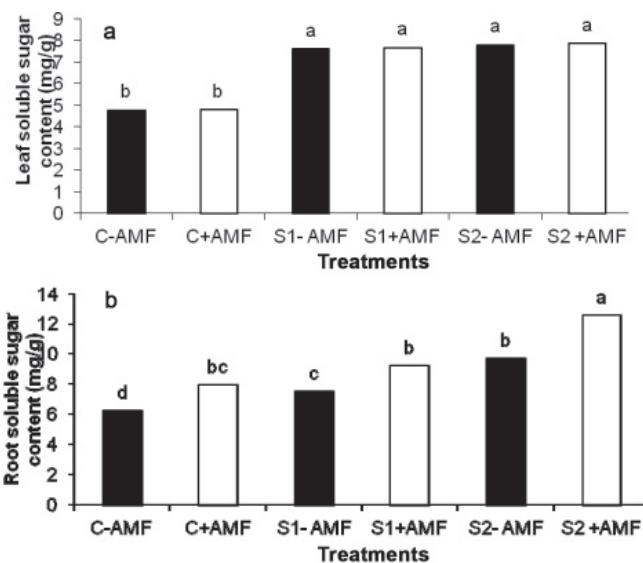
Figure 2. Effect of AM inoculation leaf soluble sugar content (a) root soluble sugar content (b) of soybean under salt stress. Treatments are designed as uninoculated controls, saline stress (S1 = 6 and S2 = 12 dSm^{-1}) and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly different (p<0.05) as determined by Duncan's Multiple Range test
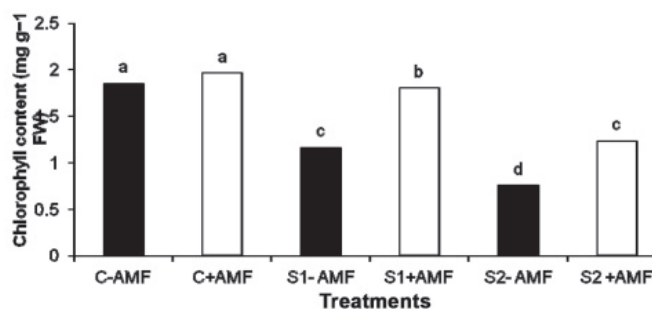
Figure 3. Effect of AM inoculation on chlorophyll content in the shoots of soybean under salt stress. Treatments are designed as uninoculated controls, saline stress (S1 = 6 and S2 = 12 dSm^{-1}) and arbuscular mycorrhiza (AM). Means followed by the same letter are not significantly different (p<0.05) as determined by Duncan's Multiple Range test

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on chlorophyll concentration have previously been shown in tomato (Kaya et al., 2001) so the present data are in agreement with these findings. Mycorrhizal colonization significantly improved chlorophyll concentration, but it did not significantly change chlorophyll concentration in non-stressed plants. Similar results have also been reported that mycorrhizal colonization increased chlorophyll content in mungbean (Rabie, 2005) and in *Sesbania aegyptiaca* and *Sesbania grandiflora* (Giri and Mukerji, 2004) plants grown at high salinity. This suggests that salt interferes with chlorophyll synthesis more in non-mycorrhizal than in mycorrhizal plants. There may be several reasons for low chlorophyll content in plant tissues under salinity stress. One explanation might be that NaCl has an antagonistic effect on N absorption (Pandey and Saxena, 1987; Paul et al., 2000), a nutrient which is an essential component of the structure of chlorophyll molecule.

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. Enhanced osmotic adjustment of compounds in mycorrhizal plants may contribute to better maintenance of the ion balance the photochemical reactions in plants under salinity.

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