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Effects of arbuscular mycorrhizal inoculation on biochemical parameters in *Capsicum annuum* grown under long term salt stress

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Abstract: Salt stress is an important environmental stress. Plants cope with salt stress with different strategies. In this study the effects of 2 different arbuscular mycorrhiza species (*Glomus mosseae* and *G. intraradices*) on some biochemical parameters in pepper plants (*Capsicum annuum* L. cv. Cumaovası) exposed to long term salt stress were studied. It was found that mycorrhizal inoculation increased RWC, P, total chlorophyll, and carotenoid content of pepper plants during salt application. The enzyme activities changed depending on the enzyme and salt stress application. The lowest MDA content was found in the plants inoculated with *G. intraradices*; however, there was no significant difference between the NaCl applications. It was found that plants inoculated with *G. intraradices* had less lipid peroxidation, and therefore it can be said that these plants have an advantage under salt stress.

Key words: *Capsicum annuum*, arbuscular mycorrhiza, salt, antioxidant enzymes, lipid peroxidation

Uzun dönem tuz stresinde yetiştirilen *Capsicum annuum* bitkisinde arbusküler mikorizanın biyokimyasal parametreler üzerine etkileri

Özet: Tuz stresi önemli bir çevresel strestir. Bitkiler tuz stresine karşı değişik stratejilere sahiptir. Bu çalışmada iki farklı mikoriza türünün (*Glomus mosseae* ve *G. intraradices*) uzun dönem tuz stresine maruz bırakılan biber bitkisinde (*Capsicum annuum* L. cv. Cumaovası) bazı biyokimyasal parametreler üzerine etkileri çalışıldı. Mikorizanın tuz uygulamalarında biber bitkisinin oransal su içeriği, fosfor, toplam klorofil ve karotenoid miktarlarını artırdığı belirlendi. Enzim aktivitelerinin tuz stresi uygulamalarına ve enzime göre değiştiği belirlendi. En düşük MDA içeriği *G. intraradices* ile enfekte olan bitkilerde bulundu, fakat tuz uygulamaları arasında önemli bir fark gözlenmedi. *G. intraradices* ile enfekte olan bitkilerde daha az lipid peroksidasyonu olduğu belirlendi, dolayısıyla *G. intraradices* ile enfekte edilmiş bitkilerin tuz stresinde daha avantajlı olabileceği söylenebilir.

Anahtar sözcükler: *Capsicum annuum*, arbusküler mikoriza, tuz, antioxidan enzimler, lipid peroksidasyonu

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Introduction

Salinity is currently one of the most severe abiotic factors limiting agricultural production. High rates of population growth and global warming are expected to further exacerbate the threat of salinity, especially in areas with a semi-arid climate in the Mediterranean region (Paranychianakis & Chartzoulakis, 2005).

The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis. Essential pathways include those that lead to synthesis of osmotically active metabolites, specific proteins, and certain free radical scavenging enzymes that control ion and water flux and support scavenging of oxygen radicals or chaperones (Parida & Das, 2005).

In recent years the use of biological methods as a practical way to alleviate soil stresses, including salinity, on plant growth has received increased attention (Daei et al., 2009; Miransari et al., 2009; Al-Khaliel, 2010). All plants studied in natural ecosystems are symbiotic with fungi that either reside entirely (endophytes) or partially (mycorrhizae) within plants. These symbioses appear to adapt to biotic and abiotic stresses and may be responsible for the survival of both plant hosts and fungal symbionts in high stress habitats (Rodriguez et al., 2004).

Arbuscular mycorrhizal fungi (AMF)—which are probably most abundant in agricultural soils and belong to the order *Glomales*—can form AMF association with the roots of 90% of the terrestrial plant species (Smith & Read, 2008) in which plant photosynthates are exchanged for water and mineral resources are acquired by the fungi from soils (Wu & Zou, 2010). Furthermore, it is extremely difficult to distinguish between direct and plant-mediated effects of salinity on AMF biology. Presumably, any environmental factor affecting the physiology of the host plant is likely to affect its fungal symbiont (Aliasgharzadeh et al., 2001). Some studies indicated that AMF can increase plant growth and the uptake of nutrients and decrease yield losses under saline conditions (Ruiz-Lozano et al., 1996; Al-Khaliel, 2010). In addition, degradation of reactive oxygen species in arbuscular mycorrhizas (AM) may be an efficient mechanism to attenuate the activation of plant defences (Lambais et al., 2003). However, the mechanism by which AMF improves salt resistance remains unclear (Kaya et al., 2009).

Once the basis of symbiotic communication is elucidated, it may be possible to develop predictive capabilities for establishing symbioses between specific fungi and plants in order to achieve desirable stress tolerance specific to geographic regions. Fungal symbiosis may ultimately provide an inexpensive and viable strategy for mitigating the impacts of global change on plants and plant communities (Rodriguez et al., 2004). Since global warming is expected to increase the amount of salt-affected land, the need for a thorough understanding of the mechanisms determining salt tolerance in plants is becoming a crucial component in maintaining agricultural production within economically viable levels (Paranychianakis & Chartzoulakis, 2005).

Pepper is one of many horticultural crops grown primarily in mid-latitudes, and it is sensitive to high temperatures (Wheeler et al., 2000; Martin & Stutz, 2004); it is also an important vegetable in Turkey and the rest of the world (Sensoy et al., 2007). The mechanism of mycorrhiza with pepper plants during long term salt stress is not well understood.

We hypothesised that mycorrhizal species can increase pepper plant tolerance against long term salt stress. The effects of mycorrhiza on antioxidant enzyme activities and lipid peroxidation during long term salt stress are not well known. The objective of the present study was to compare the effects of 2 different AMF—*Glomus mosseae* and *G. intraradices*—in phosphorus content, relative water content, antioxidant defence systems, photosynthetic pigments, and lipid peroxidation in *Capsicum annuum* L. grown under long term salt stress conditions. This study may be helpful for understanding the mechanism of mycorrhiza during low but long term salt stress in pepper plants.

Materials and methods

Plant material and stress application

In this study, pepper plants (*Capsicum annuum* L.cv. Cumaovası) and 2 different arbuscular mycorrhizal fungi (AMF), *Glomus mosseae* (Nicolson and Gerdemann; Rothamsted Isolate, UK) and *G. intraradices* (Schenck and Smith; Nutri-

Link Isolate, USA), were used. A 100 g inoculation (approximately 1000 spores) of *G. mosseae* and *G. intraradices* inoculum was made both to the seeds and the seedlings as a 3 cm layer below. Control plants received mycorrhiza free inoculum. For each treatment 3 replicates were used. Plants were grown in pots. The soil medium was sterilised in an autoclave at 121 °C, 1 atm for 2 h. The mineral content of the soil was measured [P, 4.13 mg kg^{-1}; K, 2.44 mg kg^{-1}; Zn, 0.11 mg kg^{-1}; Fe, 2.33 mg kg^{-1}; Mn, 5.93 mg kg^{-1}; Cu, 0.92 mg kg^{-1}; pH, 7.96; salt, 0.24 mS; $CaCO_3$(%) = 16.6]. Plants were grown at 26/22 °C (day/night) on 65% RH in a growth chamber with 480 µmol m^{-2} s^{-1} of light. The salt treatments (0, 1, 2, 4, and 8 mM NaCl) were made 1 week after germination of the seedlings and applied for 2 months. Salt application was carried out on both mycorrhizal and nonmycorrhizal plants. The root samples were kept in alcohol for mycorrhizal inoculation analysis. Relative water content was analysed from the leaves immediately after harvest. The leaf samples were stored at −50 °C.

Relative water content (RWC): To determine relative water content (RWC) of plants, 4 leaf discs were weighed [fresh weight (FW)] immediately after harvesting. The same tissues were then placed in redistilled water for 2 h at 25 °C, and then their turgid weights (TW) were calculated (Sairam & Srivastava, 2002). The samples were dried in an oven at 110 °C for 24 h to obtain their dry weights (DW). RWC was calculated by the following equation:

$$RWC = (FW - DW)/(TW - DW) \times 100$$

Mycorrhizal colonisation: Root samples were washed and stained according to Koske and Gemma (1989). The samples of root material were taken and cleared in KOH solution and stained using trypan blue. Mycorrhizal colonisation was determined by the grid-line intersection method (Giovannetti & Mosse, 1980). The percentage of root infection was calculated as: Infection % = 100 × (total mycorrhizal root intercepts/total root intercepts).

Phosphorus analysis: The shoots and leaves of plants were dried at 65-75 °C for 48 h and then burned at 550 °C for 5 h in an ash oven. After the digestion of the plant material, the concentration of P was determined by spectrophotometer. The samples were measured at 882 nm (Murphy & Riley, 1962).

Extraction and analysis of pigments: The extraction of chlorophylls was carried out according to Porra et al. (1989). The leaves (0.5 g) were homogenised with 80% acetone. Chlorophyll *a* and chlorophyll *b* were measured at 647 and 664 nm by Perkin Elmer Lambda EZ 200 spectrophotometer, and then total chlorophyll content was calculated. For carotenoid analyses fresh leaf material (0.5 g) was ground in a pre-chilled mortar in 5 mL acetone containing 200 mg Na_2SO_4 and then filtered through glass fibre disks (Whatman GF/A). The samples were applied to silica gel (Sigma Type GF, 10-40 µm) TLC plates (20 × 20 cm, 0.5 mm thickness). The chromatograms were developed with hexane/diethyl ether/acetone (60:30:20, v/v/v) (Moore, 1974). Xanthophyll and β-carotene spots were scraped from the TLC plates and centrifuged in 5 mL acetone for 5 min at 5000 × *g*. The absorbance of supernatants was determined at a wavelength of 450 nm by Perkin Elmer Lambda EZ 200 spectrophotometer.

Enzyme extraction and assays: Fresh leaves (1 g) were homogenised in 5 mL of 0.1 mol/L potassium phosphate buffer (pH 6.8) containing 0.1 mmol/L EDTA. The homogenate was centrifuged at 16,000 × *g* for 5 min at 4 °C, and the supernatant was immediately used for the following enzyme assays. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reaction of nitro blue tetrazolium (NBT), as described by Beyer and Fridowich (1987). The amount of enzyme required to cause 50% inhibition of the reduction of NBT at 560 nm was defined as 1 unit of SOD activity. Catalase (CAT, EC 1.11.1.6) activity was assayed by measuring the rate of decomposition of H_2O_2 at 240 nm, as described by Aebi et al. (1983). Glutathione reductase (GR, EC 1.8.1.7) activity was measured by following the change in 340 nm as oxidised glutathione (GSSG)-dependent oxidation of NADPH, according to the method of Carlberg and Mannervik (1985). To determine ascorbate peroxidase (APX, 1.11.1.11) activity, a fall in absorbance at 290 nm was measured as ascorbate was oxidised. APX activity (unit/g FW) was calculated using an extinction coefficient of 2.8 mmol L^{-1} cm^{-1} for ascorbate at 290 nm (Bonnet et al., 2000). Protein concentrations were determined by a modified Lowry method (Hartree, 1972) with bovine serum albumin as a standard protein.

Lipid peroxidation: Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation using the thiobarbituric acid method described by Ohkawa et al. (1979). For MDA extraction, 0.2 g of leaf samples was homogenised with 1 mL of 5% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 12,000 rpm for 15 min at room temperature. Equal volumes of supernatant and 0.5% thiobarbituric acid (TBA) in 20% TCA solution (freshly prepared) were placed into a new tube and incubated at 96 °C for 25 min. The tubes were transferred to an ice bath and then centrifuged at 10,000 rpm for 5 min. The absorbance of the supernatant was recorded at 532 nm and corrected for nonspecific turbidity by subtracting the absorbance at 600 nm, and 0.5% TBA in 20% TCA solution was used as the blank. MDA content was determined using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Statistical analysis

Experimental data were statistically analysed by Statistica 6.0. Tukey's Honestly Significant Difference (HSD) was calculated in the determination of different groups ($P < 0.050$).

Results

It was found that mycorrhizal inoculation increased the RWC ratio of the leaves ($P < 0.001$). Mycorrhizal plants inoculated with *G. intraradices* had higher RWC than plants inoculated with *G. mosseae* and nonmycorrhizal plants. However, salt application decreased the RWC ratio in mycorrhizal and nonmycorrhizal plants (Figure 1). Mycorrhizal plants had a higher P concentration than nonmycorrhizal plants ($P < 0.001$). The highest P content was found in the plants inoculated with *G. intraradices*, however salt application decreased P content significantly ($P < 0.001$) (Figure 2).

There was no infection in the nonmycorrhizal plants because the pots were used separately. The highest root infection was determined in the plants inoculated with *G. intraradices* ($P < 0.001$). Salt application decreased the infection ratio. However, the infection ratio in plants inoculated with *G. intraradices* was higher than in plants inoculated with *G. mosseae* (Figure 3).

According to our results mycorrhiza—especially *G. intraradices*—increased total chlorophyll. However, salt application generally decreased total chlorophyll content ($P < 0.001$), especially in nonmycorrhizal plants and plants inoculated with *G. intraradices* (Table). Similar to chlorophyll content, mycorrhiza also increased total carotenoid content ($P < 0.001$). Salt application produced a different effect on the mycorrhizal and nonmycorrhizal plants ($P < 0.01$). An application of 8 mM NaCl increased the carotenoid content in plants inoculated with *G. mosseae* and nonmycorrhizal plants; this content decreased in plants inoculated with *G. intraradices* (Table).

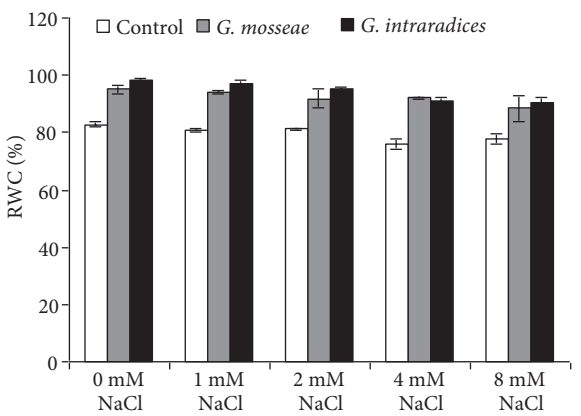

Figure 1. RWC (%) content of leaves of pepper plants grown under salt stress; bars represent standard errors.

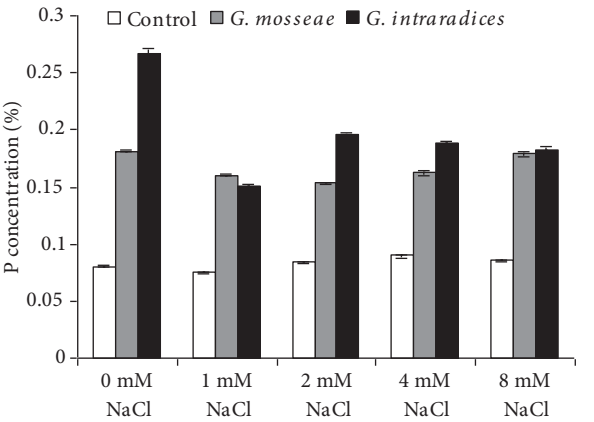

Figure 2. Phosphorus content of leaves and shoots of pepper plants grown under salt stress; bars represent standard errors.

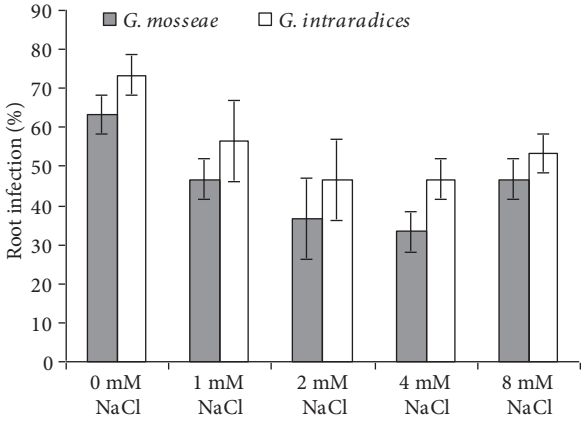

Figure 3. The root colonisation mycorrhizal plants with *G. mosseae* and *G. intraradices* grown under salt stress; bars represent standard errors.

In our study mycorrhizal inoculation increased the antioxidant system in some of the salt applications. Applications of NaCl at 2 and 4 mM increased the SOD activity of nonmycorrhizal plants; however, mycorrhizal plants were not affected by low salt concentrations. An 8 mM NaCl application increased SOD activity of plants inoculated with *G. intraradices* ($P < 0.05$) (Figure 4).

According to our results, mycorrhizal application did not increase GR activity. The highest GR activity was found in nonmycorrhizal plants. In addition, long term salt application did not affect GR activity significantly in the plants inoculated with *G. mosseae* and *G. intraradices* (Figure 5). In contrast to GR activity, the highest APX activity was found in the plants inoculated with *G. intraradices* under salt application (Figure 6); it was also found that mycorrhiza significantly affected APX activity ($P < 0.01$). The highest CAT activity was found in plants inoculated with *G. intraradices* without salt treatment. However, an 8 mM NaCl application decreased the CAT activities of both mycorrhizal and nonmycorrhizal plants ($P < 0.001$) (Figure 7).

In our study it was found that mycorrhizal inoculation decreased the MDA content of pepper plants. As a result of these findings, it can be said that mycorrhizal plants had less lipid peroxidation than nonmycorrhizal plants. The lowest MDA content was found in the plants inoculated with *G. intraradices*, but there was no significant difference between the NaCl applications (Figure 8).

Table. Total chlorophyll and carotenoid content in the leaves of pepper plants.

Treatments	Salt (mM NaCl)	Total chlorophyll (mg g^{-1})	Total carotenoid (μg g^{-1})
Non-mycorrhiza	0	3.936 ± 0.035	7.847 ± 0.051
	1	3.888 ± 0.029	7.953 ± 0.240
	2	3.930 ± 0.006	7.841 ± 0.042
	4	3.873 ± 0.010	7.736 ± 0.010
	8	3.900 ± 0.057	7.897 ± 0.010
G. mosseae	0	3.948 ± 0.025	7.922 ± 0.063
	1	3.955 ± 0.012	7.953 ± 0.042
	2	3.935 ± 0.024	7.810 ± 0.035
	4	3.908 ± 0.007	7.847 ± 0.135
	8	3.965 ± 0.020	8.060 ± 0.063
G. intraradices	0	4.006 ± 0.065	8.151 ± 0.017
	1	3.968 ± 0.064	8.039 ± 0.058
	2	3.950 ± 0.065	7.897 ± 0.042
	4	3.983 ± 0.049	7.953 ± 0.019
	8	3.712 ± 0.077	7.940 ± 0.010

Mean of 3 replicates and ± is standard error.

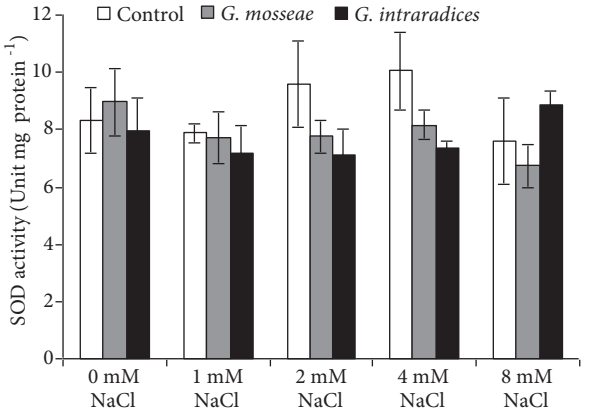

Figure 4. SOD activity in leaves of pepper plants grown under salt stress; bars represent standard errors.

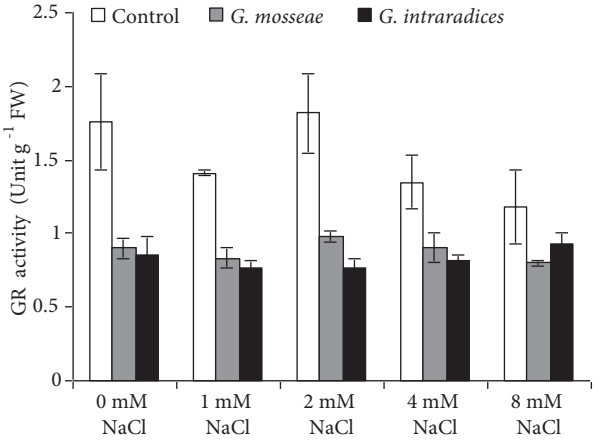

Figure 5. GR activity in leaves of pepper plants grown under salt stress; bars represent standard errors.

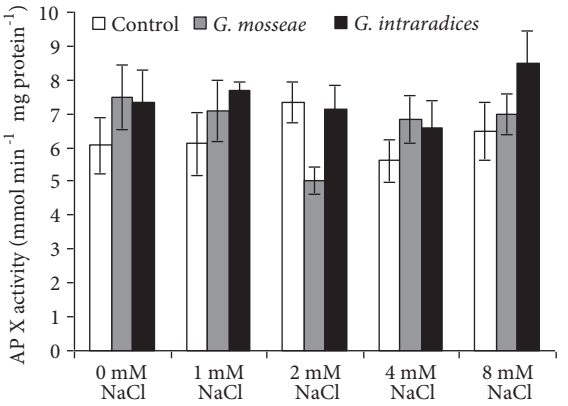

Figure 6. APX activity in leaves of pepper plants grown under salt stress; bars represent standard errors.

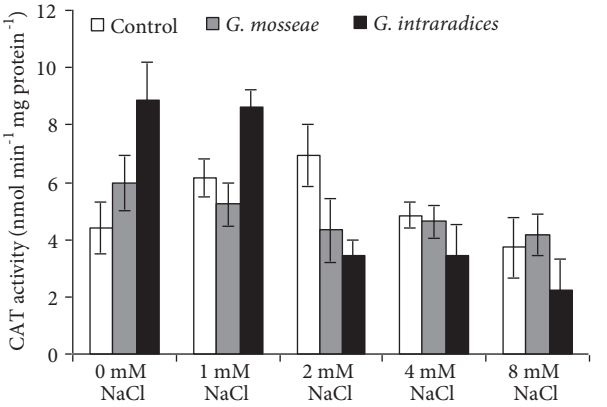

Figure 7. CAT activity in leaves of pepper plants grown under salt stress; bars represent standard errors.

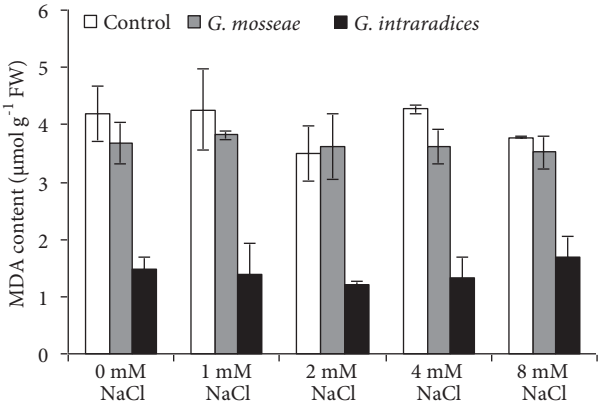

Figure 8. MDA content in leaves of pepper plants grown under salt stress; bars represent standard errors.

Discussion

Salinity is the most serious threat to agriculture and the environment in many parts of the world (Parida & Das, 2005). Previously it was reported that some chemicals can improve salt resistance in some plants (Munir & Aftab, 2009). AM is a biological strategy. Cho et al. (2006) indicated that AM-induced salinity resistance might help explain the observation that AM plants are often more resilient to drought stress than their nonAM counterparts. Due to the different AM symbionts and different ways of salinising soils, the presence of excess salt in soils occasionally widens the difference in drought responses between AM and nonAM plants.

Previous studies show the positive effects of mycorrhiza on plant growth (Ortas & Akpınar, 2006; Miransari et al., 2009; Wu & Zou, 2010). In our study AMF positively affected the growth of pepper plants; mycorrhizal plants had higher P content than nonmycorrhizal plants. Similarly, Roldán et al. (2008) reported that mycorrhizal inoculation significantly increased growth, foliar nutrients, and shoot water content of the plants, independent of the water regime.

However, highly available soil P often limits AM colonisation and causes the C-costs to the host to outweigh the benefits of colonisation (Ryan & Graham, 2002). In our study, soil with a low P level was used. Even when P availability is low and AM colonisation levels are high, AMF may not always contribute to plant growth for reasons not yet understood (Ryan & Graham, 2002). Therefore, soil P availability is an important factor for determining the effects of mycorrhiza.

Wu and Zou (2010) indicated that the beneficial effects of mycorrhiza could contribute to high chlorophyll and therefore high photosynthetic activity. Demir (2004) reported that *G. intraradices* increased the chlorophyll concentrations of *Capsicum annuum*. Similarly, in our study mycorrhizal plants had higher chlorophyll content than nonmycorrhizal plants.

The inhibition of photosynthesis under low to moderate salinity stress appears to be mainly attributed to diffusional limitations (stomatal and mesophyll conductance), and biochemical limitations to photosynthesis appear to occur only when stress becomes heavy (Paranychianakis & Chartzoulakis, 2005). In addition, the amounts of some minerals accumulated in the soil result in low water potential. This, in turn, blocks the water absorbance of roots and results in a lack of water supply to the mesophyll, thus influencing stomatal opening and the photosynthetic biochemical reactions (Yang et al., 2010).

In our study long term salt stress generally decreased chlorophyll and total carotenoid contents. Al-Khaliel (2010) reported that chlorophyll content and leaf water content of peanut increased significantly under salinity stress after inoculation with *G. mosseae*. Previously it was reported that mycorrhizal colonisation of wheat under water stress conditions had beneficial effects on water status (Beltrano & Ronco, 2008). In our study it was found that mycorrhizal plants, especially plants with *G. intraradices*, had higher RWC than other plants. However, salt application decreased the RWC ratio in mycorrhizal and nonmycorrhizal plants.

Roots of the pepper normally form symbiotic associations with AMF (Davies et al., 1992; Martin & Stutz, 2004). The infection ratio of *G. intraradices* was found to be higher than that of *G. mosseae*. Some researchers mentioned that infection could be altered by environmental stresses, for example temperature (Martin & Stutz, 2004). Similarly, in our study it was found that salt application decreased mycorrhizal inoculation. Previously it was reported that germination of spores and the subsequent hyphal growth of some AMF are reduced by increasing the concentration of salt (Juniper & Abbott, 1993).

In most of the previous studies mycorrhizal application enhanced the antioxidant system (Ruiz-Lozano et al., 1996; Lambais et al., 2003). Malan et al. (1990) found a correlation between antioxidant activities, SOD, GR, and environmental stress tolerance in inbred maize. In our study there was a correlation between mycorrhiza and antioxidant systems and salt stress. However, the activities of the enzymes changed depending on the enzyme and the salt stress application.

Previously it was reported that SOD activity was enhanced by drought in nonAM plants (Caravaca et al., 2005). Ruiz-Lozano et al. (1996) reported that increased SOD activity in mycorrhizal plants is not related to their nutritional status, but is the direct effect of AM association in response to drought treatment of the host plant. Similar to drought stress, the effect of salt stress on SOD activity was significantly higher in nonmycorrhizal plants exposed to 2 mM and 4 mM NaCl stress. Increasing the antioxidant activity plays an important role in scavenging oxidants. With these strategies plants can find protection from the deleterious effects of oxidants and cope with salt stress.

Koca et al. (2006) reported that the salinity tolerance of *L. pennellii* is associated with higher SOD and POX activities and a lower level of lipid peroxidation than in *L. esculentum*. It was also reported that CAT activity in nodulated soybean

roots inoculated with *G. mosseae* was induced when the plants were well watered but not under drought stress (Porcel et al., 2003). Cekic and Unyayar (2006) mentioned that higher SOD, APX, and CAT activities in tomato plants may be associated with the enhanced growth and salt tolerance during salt exposure. In our study the highest CAT activity was detected in the plants inoculated with *G. intraradices* without salt application; however, during salt application—especially 8 mM NaCl application—CAT activity decreased significantly in mycorrhizal plants.

Blilou et al. (2000) stated that APX and CAT activities were induced in tobacco plants inoculated with *G. mosseae* in the early stages of symbiosis development. Porcel and Ruiz-Lozano (2004) reported that under well watered or drought stress conditions soybean plants inoculated with *G. intraradices* had lower APX activity. ZhongQun et al. (2007) indicated that APX activity was induced gradually by AMF. In our study the highest APX activity was found in the plants inoculated with *G. intraradices* under an 8 mM NaCl application.

According to our results mycorrhizal application did not increase GR activity. In nonmycorrhizal plants GR activity was higher than in mycorrhizal plants. GR activity was found to be different than other antioxidant enzymes. Marulanda et al. (2007) indicated that *G. intraradices* increased GR activity under drought conditions. The response of antioxidant enzymes can be changed according to mycorrhiza, plant, and stress conditions. There are several different reports about AMF and antioxidant systems. According to our data plant reaction can be changed in response to AMF.

In previous studies it was reported that AM plants had lower MDA content than nonAM plants; AMF decreased oxidative damage to lipids during stress conditions (Porcel & Ruiz-Lozano, 2004; Wu et al., 2006; Rahmaty & Khara, 2011). These findings are similar to our results. Low MDA content is an advantage in alleviating the deleterious effects of lipid peroxidation for the pepper plant.

Kaya et al. (2009) suggested that under saline conditions pepper plants need mycorrhiza, not only for acclimatisation but also for continued nutrient uptake during the progressive growth stages. Similarly, according to our results mycorrhiza can be a strategy for alleviating the deleterious effects of salt stress in pepper plants.

The right combination of AM species and host plant can partially or completely alleviate the stress of salinity and make the use of saline soil and water for the cultivation of crop plants even more effective than before (Daei et al., 2009). According to our results we can conclude that *G. intraradices* would be more effective as a defence strategy during long term salt stress conditions in the pepper plant. Further studies should be done to determine the mechanisms for effects of AMF, at high doses, on the growth of pepper plants under long term salt application.

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References

Aebi HE, Bergmayer J & Grabl M (1983). Catalase In: *Methods of Enzymatic Analysis*, pp. 273-286. Weinheim: Verlag Chemie.

Al-Khaliel AS (2010). Effect of salinity stress on mycorrhizal association and growth response of peanut infected by *Glomus mosseae*. *Plant Soil Environ* 56: 318-324.

Aliasgharzadeh N, Rastin SN, Towfighi H & Alizadeh A (2001). Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza* 11: 119-122.

Beltrano J & Ronco MG (2008). Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and rewatering by the arbuscular mycorrhizal fungus *Glomus claroideum*: Effect on growth and cell membrane stability. *Brazilian Journal of Plant Physiology* 20: 29-37.

Beyer WF & Fridowich I (1987). Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Anal Biochem* 161: 559-566.

Blilou P, Bueno JA & Ocampo Garcia-Garrido J (2000). Induction of catalase and ascorbate peroxidase activities in tobacco roots inoculated with the arbuscular mycorrhizal *Glomus mosseae*. *Mycol Res* 104: 722-725.

Bonnet M, Camares O & Veisserie P (2000). Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll a flurescence and antioxidant enzyme activities of ryegrass. *J Exp Bot* 51: 945-953.

Caravaca F, Alguacil MM, Hernández JA & Roldán A (2005). Involvement of antioxidant enzyme and nitrate reductase activities during water stress and recovery of mycorrhizal *Myrtus communis* and *Phillyrea angustifolia* plants. *Plant Sci* 169: 191-197.

Carlberg I & Mannervik B (1985). Glutation Reductase. *Method Enzymol* 113: 484-490.

Cekic FO & Unyayar S (2006). Interactive effects of NaCl and $CdCl_2$ on the antioxidant enzyme activities and some biochemical compounds in two tomato genotypes. *Fresen Environ Bull* 15: 633-639.

Cho K, Toler H, Lee J, Ownley B, Stutz JC, Moore JL & Auge RM (2006). Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. *J Plant Physiol* 163: 517-528.

Daei G, Ardekani MR, Rejali F, Teimuri S & Miransari M (2009). Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. *J Plant Physiol* 166: 617-625.

Davies Jr FT, Potter JR & Linderman RG (1992). Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independent of plant size and nutrient content. *J Plant Physiol* 139: 289-294.

Demir S (2004). Influence of arbuscular mycorrhiza on some physiological growth parameters of pepper. *Turk J Biol* 28: 85-90.

Giovannetti M & Mosse B (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhiza in roots. *New Phytol* 84: 489-500.

Hartree EF (1972). Determination of protein: A Modification of Lowry Method that Gives a Linear Photometric Response. *Anal Biochem* 48: 422-427.

Juniper S & Abbott L (1993). Vesicular arbuscular mycorrhizas and soil salinity. *Mycorrhiza* 4: 45-57.

Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna AL & Cullu MA (2009). The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. *Scientia Horticulturae* 121: 1-6.

Koca H, Ozdemir F & Turkan I (2006). Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii*. *Biol Plantarum* 50: 745-748.

Koske RE & Gemma JN (1989). A modified procedure for staining roots to detect vam. *MycolRes* 92: 486-505.

Lambais MR, Rios-Ruiz WF & Andrade RM (2003). Antioxidant responses in bean (*Phaseolus vulgaris*) roots colonized by arbuscular mycorrhizal fungi. *New Phytol* 160: 421-428.

Malan C, Greyling MM & Gressel J (1990). Correlation between CuZn superoxide dismutase and glutathione reductase and environmental and xenobiotic stress tolerance in maize inbreds. *Plant Sci* 69: 157-166.

Martin CA & Stutz JC (2004). Interactive effects of temperature and arbuscular mycorrhizal fungi on growth, P uptake and root respiration of *Capsicum annuum* L. *Mycorrhiza* 14: 241-244.

Marulanda A, Porcel R, Barea JM & Azcón R (2007). Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive *Glomus* species. *Microb Ecol* 54: 543-552.

Miransari M, Bahrami HA, Rejali F & Malakouti MJ (2009). Effects of soil compaction and arbuscular mycorrhiza on corn (*Zea mays* L.) nutrient uptake. *Soil Till Res* 103: 282-290.

Moore TC (1974). *Research Experiences in Plant Physiology*. New York: Springer Verlag.

Munir N & Aftab F (2009). The role of polyethylene glycol (PEG) pretreatment in improving sugarcane's salt (NaCl) tolerance. *Turk J Bot* 33: 407-415.

Murphy Y & Riley JP (1962). A modified single solution method for determination of phosphate in natural waters. *Anal Chim Acta* 27: 31-36.

Ohkawa H, Ohishi N & Yagi Y (1979). Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem* 95: 351-358.

Ortas I & Akpınar C (2006). Response of kidney bean to arbuscular mycorrhizal inoculation and mycorrhizal dependency in P and Zn deficient soils. *Acta Agr Scand B-S P* 56: 101-109.

Paranychianakis NV & Chartzoulakis KS (2005). Irrigation of Mediterranean crops with saline water: from physiology to management practices. *Agriculture, Ecosystems and Environment* 106: 171-187.

Parida AK & Das AB (2005). Salt tolerance and salinity effects on plants: a review. *Ecotox Environ Safe* 60: 324-349.

Porcel R, Barea JM & Ruiz JM (2003). Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. *New Phytol* 157: 135-143.

Porcel R & Ruiz-Lozano M (2004). Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *J Exp Bot* 55: 1743-1750.

Porra RJ, Thompson RA & Kriedemann PE (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvent verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta* 975: 384-394.

Rahmaty R & Khara J (2011). Effects of vesicular arbuscular mycorrhiza *Glomus intraradices* on photosynthetic pigments, antioxidant enzymes, lipid peroxidation, and chromium accumulation in maize plants treated with chromium. *Turk J Biol* 35: 51-58.

Rodriguez RJ, Redman RS & Henson JM (2004). The role of fungal symbioses in the adaptation of plants to high stress environments. *Mitigation and Adaptation Strategies for Global Change* 9: 261-272.

Roldán A, Díaz-Vivancos P, Hernández JA, Carrasco L & Caravaca F (2008). Superoxide dismutase and total peroxidase activities in relation to drought recovery performance of mycorrhizal shrub seedlings grown in an amended semiarid soil. *J Plant Physiol* 165: 715-722.

Ruiz-Lozano JM, Azcón R & Gómez M (1996). Alleviation of salt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. *Physiol Plantarum* 98: 767-772.

Ryan MH & Graham JH (2002). Is there a role for arbuscular mycorrhizal fungi in production agriculture? *Plant Soil* 244: 263-271.

Sairam RK & Srivastava GC (2002). Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Sci* 162: 897-904.

Sensoy S, Demir S, Turkmen O, Erdinc C & Savur OB (2007). Responses of some different pepper (*Capsicum annuum* L.) genotypes to inoculation with two different arbuscular mycorrhizal fungi. *Scientia Horticulturae* 113: 92-95.

Smith SE & Read DJ (2008). *Mycorrhizal Symbiosis*. San Diego, CA: Academic Press.

Wheeler TR, Crawford PQ, Ellis RH, Porter JR & Vara Prasad PV (2000). Temperature variability and the yield of annual crops. *Agr Ecosyst Environ* 82: 159-167.

Wu QS, Zou YN & Xue Xia R (2006). Effects of water stress and arbuscular mycorrhizal fungi on reactive oxygen metabolism and antioxidant production by citrus (*Citrus tangerine*) roots. *Eur J Soil Biol* 42: 166-172.

Wu QS & Zou YN (2010). Beneficial roles of arbuscular mycorrhizas in citrus seedlings at temperature stress. *Scientia Horticulturae* 125: 289-293.

Yang X, Wang X & Wei M (2010). Response of photosynthesis in the leaves of cucumber seedlings to light intensity and CO_2 concentration under nitrate stress. *Turk J Bot* 34: 303-310.

ZhongQun H, Chao Xing H, Zhi Bin Z, Zhi Rong Z & Huai Song W (2007). Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhizae under NaCl stress. *Colloids and Surfaces B: Biointerfaces* 59: 128-133.