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Full Length Research Paper

Effects of two different AMF species on growth and nutrient content of pepper seedlings grown under moderate salt stress

Turkmen O.[1]*, Sensoy S.[2], Demir S.[3] and Erdinc C.[2]

[1]Selcuk University, Agriculture Faculty, Department of Horticulture, 42090 Konya, Turkey.
[2]Yuzuncu Yil University, Agriculture Faculty, Department of Horticulture, 65080 Van, Turkey.
[3]Yüzüncü Yıl University, Agriculture Faculty, Department of Plant Protection, 65080 Van, Turkey.

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This study was carried out to determine the effects of different Arbuscular Mycorrhizal Fungi (AMF) species on the growth and nutrient contents of pepper seedlings (cv. Demre) grown under moderate salt stress. Two different mychorrhizas (*Glomus intraradices* and *Gigaspora margarita*) were tested on a growing media containing moderate salt stress (75 ppm NaCl). The study was replicated four times with 8 plants in each replicate. At the end of the study, some nutrients such as P, K, Ca, and Na and plant growth parameters such as shoot height, stem diameter, root length, and dry and fresh weights of shoots and roots were investigated. Saline condition had negative effects on the seedlings. Both AMF species had positive effects on salt tolerance based on the plant growth parameters and nutrient contents. *G. intraradices* caused better response in seedling development compared to *G. margarita*, though insignificantly.

Key words: Arbuscular mychorrhizal fungi, NaCl nutrient, pepper seedling growth.

INTRODUCTION

Soil salinity is one of the limiting environmental factors for agricultural productivity and more than one third of the world's agricultural land faces with this problem (Tekinel and Çelik, 1983; George et al., 1997; Wang et al., 2003). Salinity may occur when there is irregular irrigation, inadequate drainage, wrong fertilizer application, and it extremely increases especially in protected cultivation.

Arbuscular Mycorrhizal Fungi (AMF) are the most widespread root fungal symbiont and are associated with the vast majority of higher plants. AMF have been shown to improve soil structure (Miller and Jastrow 2000) and have great importance due to their great capability to increase the plant growth and yield through efficient nutrient uptake (Smith and Read, 1997). AMF also enable plants to cope with biotic and abiotic stresses; AMF might help to fight against verticillium wilt (Garmendia, et al., 2004). AMF might alleviate some nutrient deficiencies, improve drought tolerance, overcome the detrimental effects of salinity, and enhance tolerance to pollution (Brundrett, 1991; Declerck et al., 1995; Turkmen et al., 2005).

Pepper (*Capsicum annuum* L.) is an important vegetable both in the world and in Turkey (Anonymous, 2005). This vegetable species is quite susceptible to salinity stress. Recent studies suggested that AMF inoculated pepper could benefit from its association with AMF (Davies et al., 2002; Salami, 2002; Demir, 2004; Garmendia et al., 2004; Turkmen et al., 2005). On the other hand, it was shown that the effectiveness of different AMF species might have varied based on the various environmental conditions as well as plant and mychorrhiza genotypes (Parke and Kaeppler, 2000; Linderman and Davis, 2004; Sensoy et al., 2007). Therefore, the present study aimed to investigate the effects of two different AMF on the growth and nutrient contents of pepper seedlings (cv. Demre) grown under moderate salt stress which is an important environmental

*Corresponding author. E-mail: turkmenonder@hotmail.com. Tel.: +90-546-875 06 42.

Table 1. Probabilities of significance for analyses of variance of seedling growth, root colonization and some shoot and root mineral contents in pepper grown under different salinity levels and inoculated with two different AMF species.

Trait	AMF	Salinity level	AMF x Salinity
Shoot heigth	**	**	ns
Root length	**	ns	ns
Stem-neck-diameter	*	ns	ns
Shoot fresh matter	**	*	ns
Root fresh matter	*	*	ns
Shoot dry matter	**	**	ns
Root dry matter	*	*	ns
AMF colonization	ns	ns	*
Shoot P concentration	ns	*	ns
Root P concentration	**	ns	ns
Shoot K concentration	ns	ns	*
Root K concentration	ns	ns	ns
Shoot Na concentration	**	**	ns
Root Na concentration	*	ns	ns
Shoot Ca concentration	ns	*	*
Root Ca concentration	*	**	ns
Shoot K:Na rate	**	**	ns
Root K:Na rate	*	ns	ns
Shoot Na:Ca rate	**	**	ns
Root Na:Ca rate	ns	**	ns

Ns, *, and **: non-significant, significant at P <0.05 and P <0.01, respectively.

problem.

MATERIALS AND METHODS

An autoclaved mixture of sand, farmyard manure and soil was used as a growth medium. The composition of growth medium was as follows: 0.03% total salt, 2.65% total nitrogen, 17.5 ppm available phosphorous, and pH 7.65. Associations of Official Analytical Chemists' methods (AOAC 1990) were used to analyze certain soil characteristics. The growth medium was placed into the plastic pots having 300 cc of volume.

The experiment was arranged as a 3 x 2 randomly factorial design. (AMF applications were (*Glomus intraradices* (*Gi*), *Gigaspora margarita* (*Gm*), and a control; salinity treatments were: (control and 75 ppm NaCl) with four replications each having eight pots without any drainage (total 192 pots). Inoculums consisted of spores, extraradical mycelium and mycorrhizal roots of *Gi* and *Gm*. The five g (25 spores g^{-1}) of inoculum was placed in the growth medium before the seeds were sown (Demir and Onoğur 1999). The same amount of sterilized growth medium was laid into the control pots. Three pepper seeds were sown in each pot having 250 cc volumes of growth medium and seedlings were thinned to one per pot shortly after seed emergence. The pots were placed in a growth chamber at a temperature of 22 ± 1°C, with 12 h fluorescent illumination with 8000 lx light intensity, and the seedlings were irrigated with the distilled water. Plants were harvested 10 weeks after the seed sowing and inoculation.

Pepper roots were dyed to detect AMF presence, which was determined using a modification of Phillips and Hayman's (1970) method, and the percentage and intensity of mycorrhizal colonization was estimated by Grid Line Intersect Method (Giovanetti and Mosse, 1980).

At the end of the study, some plant growth parameters such as shoot height stem diameter, root length, and fresh weights of shoots and roots were determined. Dry weights of shoots and roots and some nutrients such as P, K, Ca, and Na were also determined after these samples were oven-dried at 68ºC for 48 h and ground. The vanadate-molybdate-yellow procedure with spectrophotometer was used for P analysis (Kacar, 1984). K, Ca, and Na were measured by atomic absorption spectrometry using the AOAC method (AOAC, 1990).

The data were analyzed by using SAS statistical program, all data were submitted to variance analysis, and LSD values were given to differentiate the treatments (SAS Software 1982).

RESULTS

AMF and salinity treatments had significant effects on most of the seedling growth and nutrient acquisition traits (Table 1), but the only significant AMF x salinity interactions were for AMF colonization, shoot K concentration, and shoot Ca concentration.

Seedling growth traits

Almost all of the seedling growth parameters were higher for mycorrhizal than nonmycorrhizal plants regardless of salinity levels (Table 2). Compared to the control treatments, both AMF significantly (P <0.01) increased the

Table 2. Seedling growth parameters and root colonization in pepper inoculated with two different AMF species and grown under moderate salinity stress.

AMF status	Salinity level (NaCl)	Shoot height (cm)	Root length (cm)	Stem-neck-diameter (mm)	Shoot fresh matter (g plant^{1})	Root fresh matter (g plant^{-1})	Shoot dry matter (g plant^{1})	Root dry matter (g plant^{1})	AMF colonization
Control	Control	9.41	11.47	2.37	2.025	0.875	0.163	0.053	-
	75 ppm	7.04	9.94	2.27	1.570	0.735	0.135	0.040	-
G. intraradices	Control	12.34	14.19	2.49	2.910	1.315	0.245	0.073	0.288
	75 ppm	10.79	13.51	2.25	2.573	0.893	0.183	0.053	0.278
G. margarita	Control	11.44	13.38	2.72	2.590	1.095	0.223	0.068	0.243
	75 ppm	10.30	13.35	2.78	2.473	1.008	0.193	0.060	0.390
LSD (0.05)		1.23	1.29	0.30	0.213	0.285	0.012	0.026	0.071

Table 3. Nutrient acquisition parameters in pepper inoculated with two different AMF species and grown under moderate salinity stress.

AMF status	Salinity level (NaCl)	Shoot						Root					
		P	K	Na	Ca	K:Na	Na:Ca	P	K	Na	Ca	K:Na	Na:Ca
Control	Control	0.605	5.11	0.893	1.710	5.788	0.520	0.610	2.43	1.435	2.198	1.698	0.670
	75 ppm	0.643	4.06	1.105	1.373	3.485	0.910	0.625	2.23	1.255	1.500	1.795	0.923
G. intraradices	Control	0.603	4.16	0.580	1.490	7.173	0.388	0.518	2.33	1.085	1.530	2.178	0.720
	75 ppm	0.710	4.73	0.960	1.593	5.033	0.607	0.620	2.43	1.203	1.293	2.063	0.927
G. margarita	Control	0.658	4.33	0.608	1.513	7.185	0.400	0.423	2.24	1.103	1.748	2.038	0.648
	75 ppm	0.747	3.85	0.677	1.297	5.717	0.517	0.397	2.20	1.100	1.397	2.003	0.790
LSD (0.05)		0.059	0.40	0.112	0.139	0.649	0.066	0.066	0.17	0.152	0.252	0.205	0.141

the shoot heights of pepper seedlings whereas salt application significantly (P <0.01) decreased this trait. Both AMF also significantly (P <0.01 and P <0.05, respectively) increased the root lengths and as well as stem-neck diameters of pepper seedlings.

Fresh and dry weights of shoots and roots were significantly affected by either AMF or salinity applications. Salinity application significantly decreased the mentioned traits whereas AMF species significantly increased these traits.

AMF colonization

Control plants showed no AMF colonization; therefore, statistical differences were dwelled on the inoculated ones. Either AMF or salinity had no significant effects on AMF colonization, but there was a significant (P <0.05) effect of the AMF x Salinity interaction on this trait. While the *Gm* inoculation in control soil had the lowest AMF colonization, the *Gm* inoculation in saline treatment had the highest AMF colonization (Table 2).

Nutrient acquisition traits

Most of the nutrient acquisition traits were positively affected by AMF treatments (Table 3). The salinity application significantly (P <0.05) increased the shoot P concentration. The root P contents of *Gm* inoculated pepper seedlings were significantly (P<0.05) lower than either those of the control or *Gi* treatments. Either AMF or salinity treatments had no effects on the K contents of shoots and roots, but there was significant effect of the AMF x Salinity interaction on the shoot K concentration.

The salinity application significantly (P <0.01) increased the shoot Na contents of pepper seedlings whereas both AMF species significantly decreased the Na contents of both shoots and roots. The salinity application significantly (P <0.05) decreased the shoot Ca contents of pepper seedlings, and there was also significant (P <0.05) effect of the AMF x Salinity interaction on this trait. Compared to *Gi* treatments, the Ca contents of the control and *Gm* treatments decreased in the salinity condition.

The salinity significantly (P <0.01) decreased especially the shoot K:Na ratio in pepper seedlings. On the other hand, both AMF species significantly (P <0.01) increased K:Na ratios in both shoots and roots of pepper seedlings. The salinity significantly (P <0.01) increased the Na:Ca ratios in both shoots and roots of pepper seedlings. On the other hand, both AMF species significantly (P<0.01) decreased especially the Na:Ca ratio in shoots of pepper seedlings.

DISCUSSION

It was clearly seen from the seedlings parameters of pepper in the present study that salinity had negative effects on pepper growth. It is in line with the other studies showing the adverse effects of salt on vegetable crops including pepper (Martinez and Lauchli, 1991; Al-Karaki, 2000; Turkmen et al., 2005). Salinity affects soil fertility by increasing soil osmotic pressure and harmful ions such as Na and Cl, decreasing water potential, and disturbing ion balance concentration; therefore, the uptake, transportation and usage of plant nutrients are negatively affected by salinity (Roberts et al., 1984). For example, an increase in soil Na concentration reduces the K uptake of plants (Siegel et al., 1980).

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In the salt stress studies, the ameliorative effects of K and Ca have been stated in various vegetable species (Turkmen et al., 2000, 2002). K ameliorates the transport of nutrients in plants and Ca reduces the toxic effects of salt, presumably by facilitating higher K:Na selectivity (Rabie and Almadini, 2006). Moreover, salinity tolerance studies could utilize the K:Na and Na:Ca ratios as an important selection criteria (Dasgan et al., 2002; Sensoy et al., 2005). Mycorrhizal symbiosis is an important factor in helping plants to cope with adverse environmental conditions. In the present study, pepper seedlings inoculated with AMF had apparently better seedling growth results. Major reason for the increase of the growth can be attributed to the ability of plants in associations with AMF to uptake some nutrients efficiently (Smith et al., 1992). In the present study, the enhanced growth of mycorrhizal seedlings in saline condition could have been related partly to uptake of Na, K, Ca, and their ratios. The effect of AMF on the plant Na content was clearly observed that AMF decreased the Na uptake of plants. AMF could protect plants from Na toxicity either by regulating Na uptake from soil or by accumulating it in root (Rabie and Almadini, 2006). This is important because the lower the Na uptake is, the higher the salinity tolerance is in pepper. Aktas et al. (2006) had some supportive results in their study showing that the severity of salinity symptoms was much correlated with the Na concentration in various pepper genotypes. In the present study, moreover, AMF decreased the Na:Ca ratio and increased the K:Na ratio in pepper seedlings; therefore, AMF protected the pepper seedlings form the adverse effects of salinity, which was clearly seen from the seedling growth parameters. Similar results found in a study that AMF decreased the Na:Ca ratio and increased the K:Na ratio in *Vicia faba* (Rabie and Almadini, 2006). The overall results obtained from the present study are in line with the other studies showing the positive effects of AMF on pepper (Davies et al., 2002; Salami, 2002; Demir, 2004; Garmendia et al., 2004; Turkmen et al., 2005).

In plants grown in P deficit growth medium, mycorrhizal-mediated enhancement of host plant P nutrition could be clearly seen (Al-Karaki, 2000; Demir, 2004). In the present study, however, AMF did not cause significant increases in P content compared to control because there was adequate P in the growing medium. Sari et al. (2002) observed that under no sterile condition and high P levels, the desired effects of AMF could not be obtained in crops. Moreover, P uptake by mycorrhizal plants might be comparatively reduced under high salinity levels (Al-Karaki, 2000).

There could be other mechanisms explaining the role of AMF in salt tolerance. Some researchers revealed that AMF increased the proline (a nontoxic and protective osmolyte), acid phosphatase, alakaline phosphatase activities in salt stressed *V. faba* plants as well as Mg and N levels (Rabie and Almadini, 2006). In P deficit soils, AMF on P uptake might represent one of the main mechanisms for enhanced salinity tolerance (Rabie and Almadini, 2006).

As a conclusion, AMF have importance due to their capabilities on the increase in plant growth and yield under certain conditions. From the results obtained from the present study, it could be concluded both AMF have an ameliorative effects on pepper seedlings grown under moderate salinity and they clearly lessen the negative aspects of salinity stress conditions. *G. intraradices* caused even better response in seedling development compared to *G. margarita*, though insignificantly.

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