Scientia Horticulturae 121 (2009) 1–6

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity

Cengiz Kaya [a,*], Muhammed Ashraf [b], Osman Sonmez [a], Salih Aydemir [a], Atilla Levent Tuna [c], Mehmet Ali Cullu [a]

[a] Harran University. Agriculture Faculty. Soil Science and Plant Nutrition Department, Sanliurfa, Turkey
[b] Department of Botany, University of Agriculture, Faisalabad, Pakistan
[c] Mugla University, Biology Department, Mugla, Turkey

ARTICLE INFO

Article history:
Received 8 August 2008
Received in revised form 18 November 2008
Accepted 1 January 2009

Keywords:
Salinity
Mycorrhizae
Inorganic nutrients
Membrane permeability

ABSTRACT

This study investigated the effects of arbuscular mycorrhizal (AM) colonisation by *Glomus clarum* on growth and fruit yield of pepper (*Capsicum annum* cv. 11B 14) grown at high salinity. The experiment was conducted in pots containing a mixture of perlite and sand (1:1, v/v) under glasshouse conditions. Treatments were: (1) no added NaCl without arbuscular mycorrhizae (NS-AM), (2) no added NaCl with arbuscular mycorrhizae (NS + AM), (3) added 50 mM NaCl without arbuscular mycorrhizae (S1-AM) and (4) added 100 mM NaCl without arbuscular mycorrhizae (S2-AM), (5) added 50 mM NaCl with arbuscular mycorrhizae (S1 + AM) and (4) added 100 mM NaCl with arbuscular mycorrhizae (S2 + AM). The NaCl treatments reduced pepper shoot and root dry matter, and fruit yield compared with the non-saline treatments. The concentrations of N, P and K, in the leaves were significantly reduced by salinity stress, however, mycorrhizal colonisation of the salt-stressed plants restored leaf nutrient concentrations to the levels in non-stressed plants in most cases. AM inoculation improved pepper growth under salt or saltless conditions and reduced cell membrane leakage.

1. Introduction

Pepper (*Capsicum annuum* L.) is one of the main crops for greenhouse cultivation, and high-quality yield is an essential prerequisite for its economical success in the Mediterranean region. Recent studies have reported varied responses of pepper to salinity. For greenhouse peppers thresholds ranging from 0 to 2 dS/m and slopes defining linear decrease in yield due to subsequent increase in salinity ranging from 8 to 15% have been reported (Sonneveld, 1988; Chartzoulakis and Klapaki, 2000; Navarro et al., 2002).

Soils in the arid and semiarid regions have excessive concentrations of soluble salts, which adversely affect plant growth (Cerda and Martinez, 1988). However, one of the cost-effective strategies for counteracting salinity stress involves growing crops possessing inherent ability to tolerate saline conditions (Steppuhn and Curtin, 1992; Ashraf and Harris, 2004; Sabir and Ashraf, 2008). Furthermore incorporating factors that enable plants to tolerate salt stress could improve growth and production under saline conditions (Ashraf and Foolad, 2007; Sharifi et al., 2007; Ashraf et al., 2008). However, alleviation of salinity problem is expensive and often represents only a temporary solution.

Arbuscular mycorrhizal fungi (AMF) widely exist in salt-affected soils (Juniper and Abbott, 1993). In recent years, some studies indicated that AMF can increase plant growth, uptake of nutrients, and decrease yield losses in tomato under saline conditions (Ruiz-Lozano et al., 1996; Al-Karaki, 2000). Root colonization by AMF involves a series of morpho-physiological and biochemical events that are regulated by the interaction of plant and fungus, as well as by environmental factors. To some extent, these fungi have been considered as bio-ameliorators of saline soils (Azcon-Aguilar and Barea, 1997; Singh et al., 1997; Rao, 1998). Therefore, knowledge of the relationship between plants and the fungi is of considerable importance for the successful utilization of AMF under particular conditions (Tian et al., 2004). The mechanism by which AMF improve salt resistance remains unclear. However, a number of studies have revealed that mycorrhizal-mediated enhancement of host mineral nutrient uptake, especially of immobile soil nutrients such as P, Cu and Zn is one of the major factors responsible for improving plant growth under saline conditions (Al-Karaki, 2000; Al-Karaki et al., 2001).

Many of the studies on mycorrhizal infection have been conducted under heavy-metal contaminated and drought hit soils (Weissenhorn et al., 1993; del Val et al., 1999; Azcon et al., 1996;

* Corresponding author. Tel.: +90 414 3146958; fax: +90 414 2474480.
E-mail address: c_kaya70@yahoo.com (C. Kaya).

0304-4238/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2009.01.001

Kaya et al., 2003). However, to our knowledge, the ecophysiological plasticities of AMF under saline stress are still poorly understood. A strategy for management of salinity through improvement of nutrient uptake would be to inoculate soils with appropriate AMF immediately prior to transplanting a horticultural crop or to adopt cultural practices that encourage native populations of AMF in the field soil. In this paper, we report the effects of mycorrhizal inoculation on pepper plants subjected to saline conditions.

2. Materials and methods

2.1. Plant culture, mycorrhizal inoculation and treatments

Experiments were conducted in a glasshouse at the Research Station of the Agriculture Faculty, University of Harran (Turkey) from the beginning of May to the end of July 2006 with pepper (*Capsicum annum* cv. 11B 14). Environmental conditions were typical of those for a small-scale pepper crop grown under glasshouse conditions. Temperature was controlled using fans from mid-season with the aim of keeping daytime temperature in the 25–30 °C range and night temperature above 10 °C. The relative humidity in the glasshouse during the experiment was 60–75%. The nutrient solution used in this experiment was a modified Hoagland and Arnon (1940) formulation. All chemicals used were of analytical reagent grade, and pH of the nutrient solution was adjusted to 5.6 ± 0.2 immediately prior to use with a minimum volume of 0.1 M KOH. Hoagland's solution contained the following mineral elements (in molarity): 0.002 Ca and 0.006 N as $Ca(NO_3)_2$, 0.002 P as $NH_4H_2PO_4$, 0.002 Mg as $MgSO_4{\cdot}7H_2O$, and in mg liter: 0.5 B, 0.5 Mn, 0.05 Zn, 0.08 Cu, 0.02 Mo and 5.6 Fe, as Fe-sequestrene 330 (10% Fe).

Seeds were germinated in fine sand moistened with deionized water. Sand was sterilized by autoclaving at 105 °C for one day. One week after germination (at first true leaf stage). Seedlings were transplanted into a small pots (one seedling per pot) containing a mixture of perlite and sand. Each pot received 50 ml Hoagland's nutrient solution and the plants were grown for 2 weeks before transplanting to 15 l black polypropylene pots. The tops of these pots were covered with black plastic sheet minimize the evaporation of the nutrient solution. The first 5 cm of each container was filled with pebbles to provide good drainage. The rest of the container was filled with a 1:1 mixture of autoclaved perlite and sand. The experiment was a 3×2 complete factorial, which comprised three salinity levels {(0, 50 and 100 mM NaCl), electrical conductivity (EC), 2.15, 7.15 and 12.15 dS/m respectively)} and two mycorrhizal inoculations (with and without) treatments. Each treatment was replicated three times in a randomized block design and each replicate comprised six plants (i.e., 18 plants per treatment). The volume of the nutrient solution applied to the root zone of plants ranged from 50 ml to 1000 ml per container each day depending on age of plants. Excess nutrient solution was drained through the holes in the bottom of each container.

Plants were inoculated with *Glomus clarum* Nicolson & Schenck obtained from the Soil Science Department of Cukurova University, Turkey. The isolate was originally supplied by Nutri-Link, USA (Ortas et al., 2002). The isolate was propagated on maize grown in a greenhouse for 8 weeks on a perlite/vermiculite medium. The colonized maize roots were used as an inoculum (30 g fresh weight per pot containing approximately 1200 spores) placed at 15 cm pot depth immediately prior to transplanting of pepper seedlings into the pots to facilitate fungal colonization of plant roots. Non-AM treatments received the same weight of autoclaved growth mixture.

2.2. 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).

$$\text{Chl.a}\,(\text{mg ml}^{-1}) = 11.64 \times (\text{A663}) - 2.16 \times (\text{A645})$$
$$\text{Chl.b}\,(\text{mg ml}^{-1}) = 20.97 \times (\text{A645}) - 3.94 \times (\text{A663})$$

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

2.3. Electrolyte leakage

Electrolyte leakage was determined at fruit harvest stage (50 days after germination). To determine electrolyte leakage, fresh leaf samples (200 mg) were cut into 5 mm length and placed in test tubes containing 10 ml distilled deionized water. The tubes covered with plastic caps were placed in a water bath at a constant temperature of 32 °C. After 2 h the initial electrical conductivity of the medium (EC1) was measured using an electrical conductivity meter. The samples were autoclaved afterwards at 121 °C for 20 min to completely kill the tissues and release all electrolytes. The samples were then cooled to 25 °C and the final electrical conductivity (EC2) measured. The electrolyte leakage (EL) was estimated using the following formula: EL = EC1/EC2 × 100 (Dionisio-Sese and Tobita, 1998).

2.4. Proline determination

Proline in fresh leaves was determined at fruit harvest stage (50 days after germination). Proline was determined following Bates et al. (1973). Fresh leaf material (0.5 g) was homogenized in 10 ml of 3% aqueous sulfosalicyclic acid and then this aqueous solution was filtered through Whatman's No. 2 filter paper and finally two ml of the filtrate solution mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was read at 520 nm using Shimadzu UV 1601 spectrophotometer. Appropriate proline standards were used for the calculation of proline in the sample.

2.5. Mycorrhizal colonisation

Root samples were washed well with 10% KOH solution and stained with 0.1% Trypan blue before estimation of mycorrhizal colonisation (Rufykiri et al., 2000). Arbuscular mycorrhizal (AM) colonisation was estimated using a modified line intersect method (McGonigle et al., 1990), where a minimum of 100 line intersections per root sample, replicated three times, were scored for the presence of AM structures. These observations were made using the light microscopy to rate the degree of root infection by AMF in one plant per replicate (three plants per treatment). The percentage of AM infection was calculated from the following equation:

$$\text{Percentage of AM infection} = \left(\frac{\text{Root length infected}}{\text{Root length observed}}\right) \times 100$$

2.6. Fruit harvest, dry weight and nutrient analysis

Fruits were harvested weekly from the mid July to end of July for two weeks. The values for the fruit yields are the means of the fruit yield of six plants per replicate and given in grams per plant.

Three randomly selected plants per replicate were divided into leaves, stems, and roots, and dried in an oven at 70 °C for two days to determine dry weights and elemental concentrations. Nutrient analyses were carried out on dry weight basis. Total N was determined in samples of 0.1 g dry weight using the Kjeldahl method. Ground samples were dry-ashed at 550 °C for 6 h, mixed with 2 M hot HCl, filtered, and then brought to a final volume of 50 ml with distilled water. Sodium and K were determined in these sample solutions. Phosphorous was analysed by a vanadate-molybdate method using a UV/visible spectrophotometer (Shimadzu UV 1601) and other elements were analyzed using an ICP (inductive coupled plasma) following Chapman and Pratt (1982).

Differences among treatments were analysed for main effects (salinity and mycorrhizae) and their interaction by a two-way ANOVA using the Minitab statistical software package (Minitab Inc., State College, PA). Treatment effects were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Colonization rate, plant growth and fruit yield

AM colonization rates are shown in Table 1. Microscopie assessment confirmed that plants of the non-inoculation treatment were not colonized by AM. The plants inoculated with isolate of mycorrhizae had colonization percentages ranging from 46 to 15% in the roots of non-stressed and salt stressed plants, respectively. As 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 inhibiting the germination of spores (Hirrel, 1981), inhibiting growth of hyphae in soil and hyphal spreading after initial infection had occurred (McMillen et al., 1998), and reducing the number of arbuscules (Pfeiffer and Bloss, 1988).

Salinity stress significantly reduced shoot and root dry matter compared with the control treatment. However, AM colonization significantly improved these parameters in the salt-stressed plants but they remained lower than the values for control plants in all cases. AM colonization also significantly improved shoot and total dry matter, but it did not significantly affect root dry matter in control plants (Table 1). Interactions between salinity and mycorrhizal colonization were significant for both shoot and whole plant dry matter, but not for root dry matter ($P < 0.05$). It has been widely accepted that AM are able to adapt to edaphic conditions (Stahl and Williams, 1986; Brundrett, 1991; Copeman et al., 1996; del Val et al., 1999; Giri et al., 2003). It might be expected that an isolate from saline soil would have a higher capacity to promote plant growth under saline stress. Enhancement of growth in mycorrhizal plants in saline conditions has been related partially to mycorrhizal-mediated enhancement of host plant P nutrition (Hirrel and Gerdemann, 1980; Pond et al., 1984; Poss et al., 1985). In the present study, mycorrhizal pepper plants had higher leaf P than non-mycorrhizal plants at both salinity treatments (Table 3).

Fruit yield was significantly reduced by increasing salt concentration of the growth medium compared to the control plants (Table 1). A number of other workers have reported similar effects of salinity in reducing fruit yield for a range of other agricultural and horticultural crops including corn (Bar-Tal et al., 1991), tomato (Adams, 1988; Satti and Al-Yahyai, 1995), and cotton (Leidi and Saiz, 1997). Mycorrhizal colonization significantly improved fruit yield of salt-stressed tomato plants, but not that of non-stressed plants. A substantial root colonization of pepper plants by *G. clarum* resulted at the inoculation of the soil in the root before transplant. Thus, differences in growth, fruit yield and other measured characteristics between S − M and S + M treatments are reasonably linked to the presence and function of mycorrhizae. A variety of mechanisms have been proposed to determine how mycorrhizae ameliorate the effects of salinity stress on plants. For example, mycorrhizal colonisation may increase nutrient acquisition of plants grown at high salinity (Al-Karaki, 2000; Poss et al., 1985).

3.2. Some key cellular parameters

Chlorophyll concentrations were significantly reduced by salinity treatments (Table 2). The adverse effects of high NaCl on chlorophyll concentration have previously been shown in rice (Yeo et al., 1990), barley (Belkhodja et al., 1994), and 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 (Feigin et al., 1991; Grattan and Grieve, 1994), a nutrient which is an essential component of the structure of chlorophyll molecule.

In the present investigation, a higher concentration of N was observed for pepper plants as a result of AM colonization, which suggests that mycorrhizal fungi can reduce the antagonistic effect of NaCl on N uptake (Table 3).

Proline concentration was significantly higher in the salt treated plants than that in the non-treated plants (Table 2). Proline was significantly lower in mycorrhizal than in non-mycorrhizal plants at both salinity treatments except for non-stressed plants. In a previous

Table 1
Growth, fruit yield and root colonization response of arbuscular mycorrhizal (+AM) and non-arbuscular mycorrhizal (−AM) pepper plants grown under control (C) or salinity-stressed (S) conditions. Two-way ANOVA test: within each column, same letters indicate no significant difference for the different treatments at the $P < 0.05$ level.

Treatments	Shoot DW (g plant^{-1})	Root DW (g plant^{-1})	Whole plant DW (g plant^{-1})	Fruit yield (kg plant^{-1})	Root colonization (%)
C − AM	45.6b	6.7a	52.3b	2.25a	0d
C + AM	50.4a	6.9a	57.3a	2.35a	46a
S1 − AM	33.4d	5.5b	38.9d	1.44c	0d
S1 + AM	39.7c	5.9b	45.6c	1.79b	24b
S2 − AM	23.4e	3.3c	26.7e	1.01d	0d
S2 + AM	32.7d	3.8c	36.5d	1.55c	15c
Interaction S × AM	**	ns	**	*	**

ns: Not significant. C: Control; S1 and S2: addition of 50 and 100 mM NaCl to nutrient solution respectively.
* $P < 0.05$.
** $P < 0.01$.

Table 2
Chlorophyll contents (mg kg^{-1} fresh weight), proline (μmol g^{-1} FW) and electrolyte leakage (EL) of pepper plants grown at salinity conditions with or without arbuscular mycorrhizal (AM) inoculation. Two-way ANOVA test: within each column, same letters indicate no significant difference for the different treatments at the $P < 0.05$ level.

Treatments	Chl a	Chl b	Chl a + b	Proline	EL[a]
C − AM	1345a	845a	2190a	1.45	18.12d
C + AM	1354a	868a	2222a	1.38	18.24d
S1 − AM	1025d	745c	1770d	2.35	31.66b
S1 + AM	1236b	808b	2044b	2.03	26.87c
S2 − AM	945e	625d	1570e	3.07	42.45a
S2 + AM	1154c	758c	1912c	2.67	30.98b
Interaction S × AM	*	*	*	*	*

C: Control; S1 and S2: addition of 50 and 100 mM NaCl to nutrient solution respectively.
[a] Percentage value converted into arcsin for EL before ANOVA test.
* $P < 0.05$.

study, it was also reported that shoot proline was significantly lower in mycorhizal than in non-mycorrhizal soybean plants subjected to saline conditions (Sharifi et al., 2007). Proline accumulation is thought to be an adaptive feature under salinity stress in AM (Jindal et al., 1993) and non-AM (Ashraf, 1989; Sharma et al., 1990) legumes. The high level of proline enables the plants to maintain osmotic balance when growing under low water potentials (Stewart and Lee, 1974). Proline acts as a major reservoir of energy and nitrogen for utilization by plants subjected to salinity stress (Goas et al., 1982; Ashraf and Foolad, 2007).

Salt stressed caused a significant increase in electrolyte leakage compared to that in the non-stressed plants (Table 2). Similar results were obtained by Lutts et al. (1996) for NaCl-sensitive rice varieties wherein high salt concentration increased membrane permeability. However, mycorrhizal inoculation significantly reduced the electrolyte leakage in the salt-stressed plants of pepper. 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 non-inoculated plants (Zhongoun et al., 2007). Mycorrhizal pepper 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 pepper plants may have accumulated some potential antioxidants to counteract ROS. Thus, this phenomenon needs to be further elucidated.

Table 3
Concentrations (%) of Na, P, K and N in leaves of arbuscular mycorrhizal (+AM) and non-arbuscular mycorrhizal (−AM) pepper plants grown under control (C) or salinity-stressed (S) conditions. Two-way ANOVA test: within each column, same letter indicates no significant difference for the different treatments at the $P < 0.05$ level.

Treatments	Na	P	K	N
C − AM	0.27c	0.24b	1.87a	2.56a
C + AM	0.32c	0.35a	1.98a	2.55a
S1 − AM	1.08b	0.19c	1.02c	1.74c
S1 + AM	1.02b	0.27b	1.34b	1.99b
S2 − AM	1.43a	0.15d	0.65d	1.05e
S2 + AM	1.18b	0.22c	1.04c	1.56d
Interaction S × AM	**	**	*	*

C: Control; S1 and S2: addition of 50 and 100 mM NaCl to nutrient solution respectively.
* $P < 0.05$.
** $P < 0.01$.

3.3. Concentration of mineral elements

Concentration of Na^+ was significantly increased in the leaves and roots of pepper plants in the presence of NaCl stress. These data closely match those reported by other workers for other crop species, e.g., tomato (Kaya et al., 2001), and in rice (Asch et al., 1999). Mycorrhizal plants had significantly lower Na^+ concentrations compared to the non-mycorrhizal plants at both salinity treatments, except for those in non-salt stressed plants (Table 3). The decrease in leaf Na^+ may partially be explained by a "dilution effect" due to an increase in dry matter accumulation. Alternatively, it appears that the role of mycorrhizal inoculation in alleviating salt stress is partly to prevent Na^+ absorption by the root and translocation to shoot tissues. Mycorrhizal inoculation had a relatively modest effect on reducing these elevations, but apparently this reduction is sufficient to significantly restore the key growth processes to the levels approaching those for unstressed plants. Some earlier studies have indicated that AM can increase plant growth and uptake of nutrients, decrease yield losses of tomato under saline conditions, and hence improve salt tolerance of tomato (Ruiz-Lozano et al., 1996; Al-Karaki, 2000).

AM are probably the most ancient type of symbiosis between plants and microorganisms. AM symbiosis can frequently increase host resistance to salinity stress, although salinity attenuates AM growth to a varying degree according to salt level (Giri and Mukerji, 2004).

Increasing NaCl levels reduced P concentration in non-inoculated plants (Table 3). It is known that salt-stress induces P deficiency in plants by reducing P uptake or translocation (Martinez and Lauchli, 1991; Munns, 1993). In the present study, mycorrhizal inoculation promoted the growth of pepper plants under saline stress by increasing P accumulation in plant tissues. AMF increased P uptake, thereby alleviating the adverse effects of salt stress on pepper plants. This is consistent with some previous findings that the main mechanism for enhanced salinity tolerance in mycorrhizal plants was the improvement of P nutrition (Copeman et al., 1996; Al-Karaki et al., 2001; Giri et al., 2007), although in some cases, however, salt-tolerance of mycorrhizal plants appeared to be independent of plant P concentration (Ruiz-Lozano et al., 1996; Feng et al., 2002). Jakobsen et al. (1992) reported that the efficiency of P uptake by an AMF was strongly affected by the spatial distribution of its hyphae in the soil and possibly also by the differences in the capacity for uptake per unit length of hyphae. Mycorrhizal inoculation improves P nutrition of plants under salinity stress and reduces the negative effects of Na^+ by maintaining vacuolar membrane integrity, which prevents this ion from interfering in growth metabolic pathways (Rinaldelli and Mancuso, 1996).

Concentrations of K^+ and N were decreased in the leaves of pepper in the presence of NaCl stress (Table 3). It has been earlier reported that leaf K^+ concentration was lowered in plants by increasing NaCl concentration in nutrient solution or in soil, e.g., spinach (Chow et al., 1990), artichoke (Graifenberg et al., 1995), tomato (Pérez-Alfocea et al., 1996) and maize (Botella et al., 1997). However, mycorrhizal inoculation increased the concentration of K^+ and N in pepper plants. It is well known that mycorrhizal formation commonly increases plant nutrient acquisition, particularly that of P and N (Smith and Read, 1997; Giri et al., 2007).

Taken together, although salinity reduced mycorrhizal colonization, the dependency of pepper plants on mycorrhizal fungi was increased. This may be a sign showing the ecological importance of AM association for plant survival and growth under salinity stress. In the present study, mycorrhizal inoculation enhanced the growth and fruit yield in pepper plants by reducing leaf Na^+ and increasing membrane stability and concentrations of essential inorganic nutrients such as P, K and N. Our results show that, under saline

conditions, pepper plants need mycorrhizae not only for acclimatization but also for continued nutrient uptake during the progressive growth stages. In view of these results, it is possible to recommend mycorrhizal inoculation to attain reasonable growth and fruit yield of pepper under saline conditions.

Acknowledgement

Authors wish to thank University of Harran (Turkey) for supporting this study.

References

Adams, P., 1988. Some responses of tomatoes grown in NFT to sodium chloride. Proc. 7th Int. Congr. Soilless Cult. 7, 59–70.
Al-Karaki, G.N., 2000. Growth and mineral acquisition by mycorrhizal tomato grown under salt stress. Mycorrhiza 10, 51–54.
Al-Karaki, G.N., Hammad, R., Rusan, M., 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. Mycorrhiza 11, 43–47.
Asch, F., Dingkuhn, M., Wittstock, C., Doerffling, K., 1999. Sodium and potassium uptake of rice panicles as affected by salinity and season in relation to yield and yield components. Plant Soil 207, 133–145.
Ashraf, M., 1989. The effect of NaCl on water relations, chlorophyll and protein and proline contents of two cultivars of blackgram (*Vigna mungo* L.). Plant Soil 129, 205–210.
Ashraf, M., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59, 206–216.
Ashraf, M., Harris, P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166, 3–16.
Ashraf, M.Y., Hussain, F., Akhter, J., Gul, A., Ross, M., Ebert, G., 2008. Effect of different sources and rates of nitrogen and supra optimal level of potassium fertilization on growth, yield and nutrient uptake by sugarcane grown under saline conditions. Pak. J. Bot. 40, 1521–1531.
Azcon, R., Gomez, M., Tobar, R., 1996. Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions. Biol. Fertil. Soils 22, 155–161.
Azcon-Aguilar, C., Barea, J.M., 1997. Applying mycorrhiza biotechnology to horticulture: significance and potentials. Sci. Hortic. 68, 1–24.
Bar-Tal, A., Fergenbaun, S., Sparks, D.L., 1991. Potassium–salinity interaction in irrigated corn. Irrig. Sci. 12, 27–35.
Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. Plant Soil 39, 205–207.
Belkhodja, R., Morales, F., Abadia, A., Gomez-Aparisi, J., Abadia, J., 1994. Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). Plant Physiol. 104, 667–673.
Botella, M.A., Martinez, V., Pardines, J., Cerda, A., 1997. Salinity induced potassium deficiency in maize plants. J. Plant Physiol. 150, 200–205.
Brundrett, M., 1991. Mycorrhizas in natural ecosystems. Adv. Ecol. Res. 21, 300–313.
Cerda, A., Martinez, V., 1988. Nitrogen fertilization under saline conditions in tomato and cucumber plants. J. Hortic. Sci. 63, 451–458.
Chapman, H.D., Pratt, P.F., 1982. Methods of Analysis for Soils, Plants and Water. Chapman Pub., Riverside, CA, pp. 60–193.
Chartzoulakis, K., Klapaki, G., 2000. Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci. Hortic. 86, 247–260.
Chow, W.S., Ball, M.C., Anderson, J.M., 1990. Growth and photosynthetic responses of spinach to salinity: implication of K nutrition for salt tolerance. Aus. J. Plant Physiol. 17, 563–578.
Copeman, R.H., Martin, C.A., Stutz, J.C., 1996. Tomato growth in response to salinity and mycorrhizal fungi from saline or nonsaline soil. HortScience 31, 341–344.
del Val, C., Barea, J.M., Azcon-Aguilar, C., 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy-metal contaminated soils. Appl. Environ. Microbiol. 65, 718–723.
Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135, 1–9.
Feigin, A., Pressman, E., Imas, P., Milta, O., 1991. Combined effects of KNO_3 and salinity on yield and chemical composition of lettuce and chinese cabbage. Irrig. Sci. 12, 223–230.
Feng, G., Zhang, F.S., Li, X.L., Tian, C.Y., Tang, C., Rengel, Z., 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 12, 185–190.
Giri, B., Mukerji, K.G., 2004. Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 14, 307–312.
Giri, B., Kapoor, R., Mukerji, K.G., 2003. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. Biol. Fertil. Soils 38, 170–175.
Giri, B., Kapoor, R., Mukerji, K.G., 2007. Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K:Na ratios in root and shoot tissues. Microb. Ecol. 54, 753–760.
Goas, G., Goas, M., Larher, F., 1982. Accumulation of free proline and glycine betaine in *Aster tripolium* subjected to a saline shock: a kinetic study related to light period. Physiol. Plant 55, 383–388.
Graifenberg, A., Giustiniani, L., Temperini, O., Lipucci di Paola, M., 1995. Allocation of Na, Cl, K and Ca within plant tissues in globe artichoke (*Cynara scolimus* L.) under saline-sodic conditions. Sci. Hortic. 63, 1–10.
Grattan, S.R., Grieve, C.M., 1994. Mineral nutrient acquisition and response by plants grown in saline environments. In: Pessarakli, M. (Ed.), Handbook of Plant and Crop Stress. Marcel Dekker, New York, pp. 203–226.
Hirrel, M.C., 1981. The effect of sodium and chloride salts on the germination of *Gigaspora margaria*. Mycology 43, 610–617.
Hirrel, M.C., Gerdemann, J.W., 1980. Improved growth of onion and bell pepper in saline soils by two vesicular–arbuscular mycorrhizal fungi. Soil Sci. Soc. Am. J. 44, 654–655.
Hoagland, D.R., Arnon, D.I., 1940. Crop production in artificial culture solutions and in soils with special reference to factors influencing yield absorption of inorganic nutrients. Soil Sci. 50, 463–483.
Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. New Phytol. 120, 373–379.
Jindal, V., Atwal, A., Sekhon, B.S., Singh, R., 1993. Effect of vesicular–arbuscular mycorrhizae on metabolism of moong plants under NaCl salinity. Plant Physiol. Biochem. 3, 475–481.
Juniper, S., Abbott, L., 1993. Vesicular–arbuscular mycorrhizas and soil salinity. Mycorrhiza 4, 45–57.
Kaya, C., Kirnak, H., Higgs, D., 2001. Enhancement of growth and normal growth parameters by foliar application of potassium and phosphorus in tomato cultivars grown at high (NaCl) salinity. J. Plant Nutr. 24, 357–367.
Kaya, C., Higgs, D., Kirnak, H., Tas, I., 2003. Mycorrhizal colonisation improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions. Plant Soil 253, 287–292.
Leidi, E.O., Saiz, J.F., 1997. Is salinity tolerance related to Na accumulation in upland cotton seedlings? Plant Soil 190, 67–75.
Lutts, S., Kinet, J.M., Bouharmont, J., 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. 78, 389–398.
Martinez, V., Lauchli, A., 1991. Phosphorus translocation in salt-stressed cotton. Physiol. Plant 83, 627–632.
McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, D.L., Swan, G.A., 1990. A new method which gives an objective measure of colonisation of roots by vesicular–arbuscular mycorrhizal fungi. New Phytol. 115, 495–501.
McMillen, B.G., Juniper, S., Abbott, L.K., 1998. Inhibition of hyphal growth of a vesicular–arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. Soil Biol. Biochem. 30, 1639–1646.
Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.
Munns, R., 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16, 15–24.
Navarro, J.M., Garrido, C., Carvajal, M., Martinez, V., 2002. Yield and fruit quality of pepper plants under sulphate and chloride salinity. J. Hortic. Sci. Biotechnol. 77, 52–57.
Ortas, I., Ortakci, D., Kaya, Z., 2002. Mycorrhizal dependency of sour orange in relation to phosphorus and zinc nutrition. J. Plant Nutr. 25, 1263–1279.
Pérez-Alfocea, F., Balibrea, M.E., Santa Cruz, A., Estan, M.T., 1996. Agronomical and physiological characterization of salinity tolerance in a commercial tomato hybrid. Plant Soil 180, 251–257.
Pfeiffer, C.M., Bloss, H.E., 1988. Growth and nutition of guayule (*Parthenium argentatum*) in a saline soil as influenced by vesicular–arbuscular mycorrhiza and phosphorus fertilization. New Phytol. 108, 315–321.
Pond, E.C., Merge, J.A., Jarrell, W.M., 1984. Improved growth of tomato in salinized soil by vesicular–arbuscular mycorrhizal fungi collected from saline soils. Mycologia 76, 74–84.
Poss, J.A., Pond, E., Menge, J.A., Harrell, W.M., 1985. Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. Plant Soil 88, 307–319.
Rabie, G.H., 2005. Influence of arbuscular mycorrhizal fungi and kinetin on the response of mungbean plants to irrigation with seawater. Mycorrhiza 15, 225–230.
Rao, D.L.N., 1998. Biological amelioration of salt-affected soils. Microbial Interactions in Agriculture and Forestry, vol. 1. Science Publishers, Enfield, USA, pp. 21–238.
Rinaldelli, E., Mancuso, S., 1996. Response of young mycorrhizal and non-mycorrhizal plants of olive tree (*Olea europaea* L.) to saline conditions. I. Short-term electrophysiological and longterm vegetative salt effects. Adv. Hortic. Sci. 10, 126–134.
Rufykiri, G., Declerck, S., Dufey, J.E., Delvaux, B., 2000. Arbuscular mycorrhizal fungi might alleviate aluminium toxicity in banana plants. New Phytol. 148, 343–352.
Ruiz-Lozano, J.M., Azcon, R., Gomez, M., 1996. Alleviation of salt stress by arbuscular mycorrhizal *Glomus* species in *Lactuca sativa* plants. Physiol. Plant 98, 767–772.
Sabir, P., Ashraf, M., 2008. Inter-cultivar variation for salt tolerance in proso millet (*Panicum miliaceum* L.) at the germination stage. Pak. J. Bot. 40, 677–682.
Satti, S.M.E., Al-Yahyai, R.A., 1995. Salinity tolerance in tomato: implications of potassium, calcium and phosphorus. Commun. Soil Sci. Plant Anal. 26, 2749–2760.
Sharifi, M., Ghorbanli, M., Ebrahimzadeh, H., 2007. Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi. J. Plant Physiol. 164, 1144–1151.

Sharma, K.D., Datta, K.S., Verma, S.K., 1990. Effect of chloride and sulphate type of salinity on some metabolic drifts in chickpea (*Cicer arietinum* L.). Indian J. Exp. Biol. 28, 890–892.

Singh, R.P., Choudhary, A., Gulati, A., Dahiya, H.C., Jaiwal, P.K., Sengar, R.S., 1997. Response of plants to salinity in interaction with other abiotic and factors. In: Jaiwal, P.K., Singh, R.P., Gulati, A. (Eds.), Strategies for Improving Salt Tolerance in Higher Plants. Science Publishers, Enfield, USA, pp. 25–39.

Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis. Academic Press, San Diego.

Sonneveld, C., 1988. The salt tolerance of greenhouse crops. Neth. J. Agric. Sci. 36, 63–73.

Stahl, P.D., Williams, S.E., 1986. Oil shale process water affects activity of vesicular–arbuscular fungi and *Rhizobium* 4 years after application to soil. Soil Biol. Biochem. 18, 451–455.

Steppuhn, H., Curtin, D., 1992. Salinity and Sustainable Agriculture. Prairie Salinity Publication No. 1. Research Branch, Agriculture Canada. Swift Current Research Station, Swift Current, Sask., pp. 192.

Stewart, C.R., Lee, J.A., 1974. The rate of proline accumulation in halophytes. Planta 120, 279–289.

Strain, H.H., Svec, W.A., 1966. Extraction, separation, estimation and isolation of chlorophylls. In: Vernon, L.P., Seely, G.R. (Eds.), The Chlorophylls. Academic Press, pp. 21–66.

Tian, C.Y., Feng, G., Li, X.L., Zhang, F.S., 2004. Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. Appl. Soil Ecol. 26, 143–148.

Weissenhorn, I., Leyval, C., Berthelin, J., 1993. Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. Plant Soil 158, 250–256.

Yeo, A.R., Yeo, M.E., Flowers, S.A., Flowers, T.J., 1990. Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. Theor. Appl. Genet. 79, 377–384.

Zhongoun, H., Chaoxing, H., Zhibin, Z., Zhirong, Z., Huaisong, W., 2007. Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhizae under NaCl stress. Colloids Surf. B: Biointerfaces 59, 128–133.