Mycorrhiza (2000) 10:51–54 

ORIGINAL PAPER

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# Growth of mycorrhizal tomato and mineral acquisition under salt stress

Accepted: 21 February 2000

**Abstract** High salt levels in soil and water can limit agricultural production and land development in arid and semiarid regions. Arbuscular mycorrhizal fungi (AMF) have been shown to decrease plant yield losses in saline soils. The objective of this study was to examine the growth and mineral acquisition responses of greenhouse-grown tomato to colonization by the AMF *Glomus mosseae* [(Nicol. And Gerd.) Gerd. and Trappe] under varied levels of salt. NaCl was added to soil in the irrigation water to give an $EC_e$ of 1.4 (control), 4.7 (medium) and 7.4 dS $m^{-1}$ (high salt stress). Plants were grown in a sterilized, low P (silty clay) soil-sand mix. Mycorrhizal colonization was higher in the control than in saline soil conditions. Shoot and root dry matter yields and leaf area were higher in mycorrhizal than in nonmycorrhizal plants. Total accumulation of P, Zn, Cu, and Fe was higher in mycorrhizal than in nonmycorrhizal plants under both control and medium salt stress conditions. Shoot Na concentrations were lower in mycorrhizal than in nonmycorrhizal plants grown under saline soil conditions. The improved growth and nutrient acquisition in tomato demonstrate the potential of AMF colonization for protecting plants against salt stress in arid and semiarid areas.

**Key words** Growth · *Lycopersicon esculentum* · Mineral acquisition · Mycorrhiza · Salinity

## Introduction

Saline water is considered to be an alternative source of irrigation for agriculture in countries suffering from a shortage of fresh water, for example in the Middle East. Irrigation with saline water is often associated with enhanced salinization of the soil as water evaporates. A salinity problem exists when salt accumulates in the root zone to a concentration that causes crop damage and reduces the yield due to soil/plant osmotic imbalances (Wyn Jones and Gorham 1983). There is also a direct effect of salinity of high concentrations of Na, Cl, and B ions and the nutrient imbalance of soil solution (Ayers and Westcot 1985; Hasegawa et al. 1986). Thus, factors enabling plants to withstand salt stress would be helpful in improving crop production under saline conditions.

Arbuscular mycorrhizal fungi (AMF) have been shown to decrease yield losses of plants in saline soils (Hirrel and Gerdemann 1980; Pond et al. 1984; Poss et al. 1985). This may be due to increased uptake of nutrients with low mobility, such as P, Zn and Cu (George et al. 1994; Marschner and Dell 1994; Ruiz-Lozano et al. 1996; Al-Karaki and Al-Raddad 1997; Al-Karaki and Clark 1998), and to improved water relations (Bethlenfalvay et al. 1988; Sylvia et al. 1993; Ruiz-Lozano and Azcon 1995; Al-Karaki and Clark 1998). This may lead to increased growth and subsequent dilution of toxic ion effects (Juniper and Abbott 1993).

Salinity tolerance by tomato (*Lycopersicon esculentum* Mill) plants is a major concern in arid and semiarid regions with high salinity, due to the negative correlation between excess salinity and yield (Shalhevet and Hsiao 1986; Feigin et al. 1987). Study of the symbiotic interactions between AMF and host plants under saline conditions should help to optimize the beneficial effects of AMF. The objective of this study was to determine the effects of mycorrhizal infection on growth parameters and nutrient acquisition by tomato under different levels of soil salinity.

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## Materials and methods

Seeds of tomato (cv. Pello) were germinated in a moist mix of peat and sand in polystyrene trays. Three 21-day-old seedlings, uniform in size, were transplanted into 5 l plastic pots filled with a

silty clay soil (fine, mixed, thermic, Typic Xerochrept) mixed with washed cement grade sand (soil:sand, 1:1, v:v), fertilized with 0.35 g $NH_4NO_3$ $kg^{-1}$ soil. The soil mix was fumigated with methyl bromide under airtight plastic sheets for 3 days, and the fumigant allowed to dissipate for 10 days. The soil properties before mixture with sand and fertilizer were 6.5% sand, 45% silt, 48.5% clay, 1.2% organic matter, pH 8.1, electrical conductivity ($EC_e$) 1.4 dS $m^{-1}$, 0.26 P ($NaHCO_3$-extractable), 23.1 K, 6.2 Na, 0.2 Fe, 0.02 Zn and 0.03 Cu (5 mM DTPA-extractable) in mmol $kg^{-1}$ soil. P was not added to the soil mixes in order to stimulate mycorrhiza formation. Tomato plants were grown in a greenhouse with natural light at 27±5 °C during the spring of 1998. Photosynthetic photon flux density at plant height ranged between 700 and 1500 μmol $m^{-2}$ $s^{-1}$ throughout the growing period.

Half of the pots received the AMF *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe by placing 30 g (moist weight) of inoculum in soil below the tomato seedlings prior to planting. The AMF inoculum (consisting of soil and root fragments and spores: ~600 chlamydospores $kg^{-1}$ soil mix) was placed directly adjacent to each seedling root to facilitate fungal colonization of plant roots. *G. mosseae* was initially isolated from a wheat (*Triticum durum* desf.) field in northern Jordan (Al-Raddad 1993) and multiplied in pot cultures using chickpea (*Cicer aritinum* L.) as a host. Control treatments received no AMF inoculum.

Plants were established for 2 weeks and then subjected to three salt levels by adding NaCl to the irrigation water. This resulted in ECe values of 1.4 (control without salt stress), 4.7 (medium salt stress), and 7.4 (high salt stress) dS $m^{-1}$. The soil was salinized step-wise to avoid osmotic shock. Plants were watered with tap water (EC = 0.4 dS $m^{-1}$) in such a way as to avoid leaching. If leaching occurred, the leachate was collected and added to the soil to maintain salinity treatments near the target levels. The plants were harvested after they had been grown under salt stress conditions for 6 weeks and shoots and roots were separated. Leaf area was determined using an LI-3000 leaf area meter. Shoots were then oven-dried at 70 °C for 48 h, weighed and saved for mineral analysis.

Roots were rinsed free of soil and cut into 1-cm fragments. These were thoroughly mixed and representative fresh samples (1 g) were removed for determination of root AMF colonization. Root samples were cleared with 10% (w/v) KOH, stained with 0.05% (v/v) trypan blue in lactophenol as described by Phillips and Hayman (1970) and examined microscopically. Colonization was expressed as the percentage of root segments containing arbuscules and vesicles using a gridline intercept method (Bierman and Linderman 1981).

Dried shoots were ground to pass through a 0.5-mm sieve in a cyclone laboratory mill and saved for determination of mineral nutrients. Shoot P concentration was determined colorimetrically (Watanabe and Olsen 1965) and Zn, Fe and Cu concentrations were determined by atomic absorption spectroscopy. Potassium and Na concentrations in plant shoots were determined by flame photometry.

A factorial arrangement of treatments in a randomized complete block design was used with four replications. Data was analyzed statistically by analyses of variance using MSTATC (Michigan State University, East Lansing, Mich.). Probabilities of significance among treatments, interactions and LSDs ($P<0.05$) were used to compare means within and among treatments. Mean percentages of AMF colonization were calculated from arcsine transformed data.

## Results

Nearly all salinity and AMF treatments had significant effects on the growth and nutrient acquisition traits (Table 1), but the only significant salinity × AMF interactions were for AMF colonization, P concentration and total accumulation of P, Fe and Zn.

**Table 1** Probabilities of significance for analyses of variance of growth, root colonization with arbuscular mycorrhizal fungi (AMF) and shoot mineral (P, Na, K, Fe, Cu, and Zn) concentrations and total accumulation in tomato grown under different salinity levels. Nutrient accumulation is the product of shoot dry matter and nutrient concentration

| Trait | Salinity level | AMF | Salinity × AMF |
|---|---|---|---|
| Shoot dry matter | ** | ** | NS |
| Root dry matter | ** | * | NS |
| Leaf area | ** | ** | NS |
| AMF colonization | ** | ** | ** |
| P concentration | ** | ** | ** |
| P accumulation | ** | ** | * |
| Na concentration | ** | ** | NS |
| Na accumulation | ** | NS | NS |
| K concentration | ** | NS | NS |
| K accumulation | ** | NS | NS |
| Fe concentration | * | ** | NS |
| Fe accumulation | ** | ** | * |
| Cu concentration | ** | ** | NS |
| Cu accumulation | ** | ** | NS |
| Zn concentration | ** | ** | NS |
| Zn accumulation | ** | ** | * |

*, ** Significant at $P<0.05$ and $P<0.01$, respectively. (*NS* not significant)

Uninoculated plants showed no AMF colonization. Relatively high AMF root colonization occurred after inoculation and plants grown in control soil had higher AMF colonization than plants grown in saline soil (Table 2).

Tomato shoot and root dry matter (DM) yields and leaf area were higher for mycorrhizal than for nonmycorrhizal plants regardless of salinity level (Table 2). However, no significant differences were recorded between mycorrhizal and nonmycorrhizal plants either for shoot and root DM at the high salinity (7.4 dS $m^{-1}$) treatment or for leaf area in the control treatment. Shoot and root DM and leaf area decreased as soil salinity increased (Table 2).

Shoot P concentration and total accumulation were higher in mycorrhizal than nonmycorrhizal plants in the control and with medium soil salinity but not at high salinity (Tables 3, 4). However, shoot P concentrations and total accumulation in both mycorrhizal and nonmy-

**Table 2** Root AMF colonization, shoot and root dry matter yield (DM), and leaf area by nonAMF and AMF tomato grown at different salinity levels

| Salinity level dS $m^{-1}$ | AMF status | AMF colonization % root length | DM g $plant^{-1}$ | | Leaf area $cm^2$ $plant^{-1}$ |
|---|---|---|---|---|---|
| | | | Shoot | Root | |
| 1.4 | NonAMF | 0 | 1.62 | 0.20 | 256 |
| | AMF | 49 | 2.33 | 0.28 | 277 |
| 4.7 | NonAMF | 0 | 1.05 | 0.10 | 119 |
| | AMF | 42 | 1.57 | 0.18 | 186 |
| 7.4 | NonAMF | 0 | 0.44 | 0.08 | 43 |
| | AMF | 36 | 0.60 | 0.09 | 87 |
| LSD (0.05) | | 4 | 0.48 | 0.08 | 37 |

**Table 3** Shoot concentration of P, Na and K (mmol $kg^{-1}$), Fe, Cu, and Zn ($\mu$mol $kg^{-1}$)in nonAMF and AMF tomato grown at different salinity levels

| Salinity level dS $m^{-1}$ | AMF status | P | Na | K | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|
| 1.4 | NonAMF | 44 | 169 | 1080 | 2529 | 164 | 622 |
| | AMF | 63 | 123 | 1137 | 3416 | 230 | 713 |
| 4.7 | NonAMF | 40 | 1155 | 710 | 2534 | 137 | 407 |
| | AMF | 60 | 773 | 909 | 2860 | 222 | 431 |
| 7.4 | NonAMF | 39 | 1739 | 624 | 1930 | 114 | 394 |
| | AMF | 43 | 1229 | 654 | 2747 | 163 | 428 |
| LSD (0.05) | | 5 | 352 | 343 | 666 | 41 | 51 |

**Table 4** Shoot total accumulation of P, Na and K (mmol $plant^{-1}$), Fe, Cu, and Zn ($\mu$mol $plant^{-1}$) by nonAMF and AMF tomato grown at different salinity levels

| Salinity level dS $m^{-1}$ | AMF status | P | Na | K | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|
| 1.4 | NonAMF | 0.07 | 0.28 | 1.78 | 4.13 | 0.27 | 1.00 |
| | AMF | 0.15 | 0.29 | 2.71 | 7.91 | 0.54 | 1.67 |
| 4.7 | NonAMF | 0.04 | 1.20 | 0.75 | 2.66 | 0.14 | 0.43 |
| | AMF | 0.09 | 1.19 | 1.44 | 4.45 | 0.35 | 0.68 |
| 7.4 | NonAMF | 0.03 | 0.77 | 0.27 | 0.85 | 0.05 | 0.17 |
| | AMF | 0.02 | 0.74 | 0.38 | 1.65 | 0.10 | 0.26 |
| LSD (0.05) | | 0.02 | 0.30 | 1.15 | 1.37 | 0.15 | 0.23 |

**Table 5** Percentage change in shoot dry matter (DM) yield and total nutrient accumulation due to AMF colonization of tomato grown at different salinity levels [*Shoot DM* = $DM_{AMF} - DM_{nonAMF} \times 100/DM_{nonAMF}$, *Total nutrient accumulation (TNA)* = $TNA_{AMF} - TNA_{nonAMF} \times 100/TNA_{nonAMF}$]

| Salinity level DS $m^{-1}$ | Shoot DM | Total nutrient accumulation (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | P | Na | K | Fe | Cu | Zn |
| 1.4 | 44 | 114 | 4 | 52 | 92 | 100 | 67 |
| 4.7 | 50 | 125 | −1 | 92 | 67 | 150 | 58 |
| 7.4 | 36 | 50 | −4 | 41 | 94 | 100 | 53 |

corrhizal plants decreased with increasing soil salinity (Tables 3, 4).

Sodium concentrations increased with soil salinity in both mycorrhizal and nonmycorrhizal plants (Table 3), and were lower in mycorrhizal than in nonmycorrhizal plants at moderate and high salinity levels but not in the control treatment (Table 3). Total accumulation of Na in shoots was similar for mycorrhizal and nonmycorrhizal plants grown at all salinity levels (Table 4). Shoot K concentration and total accumulation were similar for mycorrhizal and nonmycorrhizal plants grown at all salinity levels, but decreased as soil salinity increased (Tables 3, 4).

Shoot concentrations of Fe and Cu were generally higher for mycorrhizal than for nonmycorrhizal plants at all salinity levels, although the differences for Fe was not significant at the medium salinity level (Table 3). Shoot concentration of Zn was higher for mycorrhizal than for nonmycorrhizal plants only in the control treatment. Total accumulations of Fe, Cu, and Zn were generally higher for mycorrhizal than for nonmycorrhizal plants, but these differences were not significant for plants grown at the high salinity level (Table 4). Shoot concentrations and total accumulation of Fe, Cu and Zn decreased as soil salinity increased (Tables 3, 4).

The overall effects of AMF colonization on shoot DM yield and mineral nutrient acquisition of saline and control plants are summarized in Table 5.

## Discussion

Plants inoculated with mycorrhiza had apparently higher shoot and root DM yields and leaf area than nonmycorrhizal plants at all salinities, but the responses were only significant at medium salinity (4.7 dS $m^{-1}$) and in the control conditions. Enhanced growth of mycorrhizal plants in saline environments has been related partly to mycorrhizal-mediated enhancement of host plant P nutrition (Hirrel and Gerdemann 1980; Pond et al. 1984; Poss et al. 1985). In this study, mycorrhizal plants had apparently higher shoot P concentrations and total P accumulation than nonmycorrhizal plants at all salinity levels, but the differences were not significant at the highest level. Plant growth enhancements attributed to AMF root colonization decreased at high salinity (7.4 dS $m^{-1}$). This may have been due to reduced hyphal P transport into roots and uptake by the plant under these conditions. Plants grown under high salinity may have a lower $H_2PO_4^-$ affinity (the preferred phosphate ion for plant uptake) than under low salinity conditions (Sentenac and Grignon 1985). Reduced uptake of P by mycorrhizal plants grown under high salinity levels has been reported by other workers (Hirrel and Gerdemann 1980; Pond et al. 1984; Poss et al. 1985).

Many studies have indicated that AMF contributes to plant growth via enhancement of mineral nutrient uptake, especially immobile soil nutrients (P, Cu, Zn) (Bethlenfalvay et al. 1988; Marshner and Dell 1994; Al-Karaki and Al-Raddad 1997; Al-Karaki and Clark 1998). In this study, higher Fe, Cu, and Zn concentrations and total accumulation occurred in mycorrhizal than in nonmycorrhizal plants. This may have been the result of increased availability or transport (absorption and/or translocation) by AMF hyphae. Enhanced acquisition of P, Zn, Cu, and Fe by mycorrhizal plants has been reported (Marshner and Dell 1994; Trimble and Knowles 1995; Al-Karaki and Al-Raddad 1997; Al-Karaki and Clark 1998). However, AMF root colonization did not significantly affect shoot K concentration and total accumulation in plants grown at all salinity levels. Poss et al. (1985) reported that K uptake was little affected by AMF root colonization in tomato grown under saline conditions.

Shoot Na concentrations but not total accumulation were lower in mycorrhizal than nonmycorrhizal plants regardless of salinity level. The lack of response of total

Na accumulation to AMF treatment may be explained by dilution effects of plant growth enhancement caused by AMF colonization. Similar results were reported by other researchers (Bernstein et al. 1974; Jarrell and Beverly 1981).

In summary, the results of this study indicate that plant tolerance to salt stress is improved by AMF colonization. Mycorrhizal tomato plants had greater nutrient acquisition (especially P) than nonmycorrhizal plants at all salinity levels. Greater nutrient acquisition in response to AMF colonization was suggested to be a plant strategy for salt stress tolerance (Hirrel and Gerdemann 1980; Pond et al. 1984; Poss et al. 1985). The improved growth and nutrient acquisition in mycorrhizal tomato demonstrate the potential of AMF colonization for the protection from salt stress of plants grown in arid and semiarid regions. However, several AMF isolates should be investigated in order to optimize the effects of this AMF symbiosis.

**Acknowledgement** This work was supported by a grant from the Deanship of Scientific Research, Jordan University of Science and Technology.

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