

Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water

Ghazi N. Al-Karaki*

Faculty of Agriculture, Jordan University of Science & Technology, P.O. Box 3030, Irbid, Jordan

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Abstract

Protected horticultural crops as well as those planted in open fields particularly in the Mediterranean region have to cope with increasing salinization of irrigation water. High salinity of the supply water has detrimental effects on soil fertility and plant nutrition and reduces crop growth and yield. This study was conducted to determine if pre-inoculation of transplants with arbuscular mycorrhizal (AM) fungi alleviates salt effects on growth and yield of tomato (*Lycopersicon esculentum* Mill. Cv. Marriha) when irrigated with saline water. Tomato seeds were sown in polystyrene trays with 20 cm³ cells and treated with AM fungi (AM) or without (nonAM) *Glomus mosseae*. Once the seedlings were reached appropriate size, they were transplanted into nonsterile soil in concrete blocks (1.6 m × 3 m × 0.75 m) under greenhouse conditions. The soil electrical conductivity (EC_e) was 1.4 dS m⁻¹. Plants were irrigated with nonsaline water (EC_w = 0.5 dS m⁻¹) or saline water (EC_w = 2.4 dS m⁻¹) until harvest. These treatments resulted with soil EC at harvest 1.7 and 4.4 dS m⁻¹ for nonsaline and saline water treatments, respectively. Root colonization with AM fungi at flowering was lower under saline than nonsaline conditions. Pre-inoculated tomato plants with AM fungi irrigated with both saline and nonsaline water had greater shoot and root dry matter (DM) yield and fruit fresh yield than nonAM plants. The enhancement in fruit fresh yield due to AM fungi inoculation was 29% under nonsaline and 60% under saline water conditions. Shoot contents of P, K, Zn, Cu, and Fe were higher in AM compared with nonAM plants grown under nonsaline and saline water conditions. Shoot Na concentrations were lower in AM than nonAM plants grown under saline water conditions. Results indicate that pre-inoculation of tomato transplants with AM fungi improved yield and can help alleviate deleterious effects of salt stress on crop yield.

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1. Introduction

Tomato (*Lycopersicon esculentum* L.) is considered a major vegetable crop in many parts of the world and mostly grown under irrigation under both protected (plastic house) and open field conditions. Tomato as well as other important vegetable, fruit tree and floriculture crops, and forestry species are commonly produced in nurseries. Plant macropropagation in nursery systems is usually started from seedlings, cuttings or graftings produced or developed in soil or sterile or partially sterile growth medium to minimize the risk of pathogenic organisms. In the case of micropropagation, sterilization of growth medium is an obvious requirement (Lovato et al., 1996). Sterilization of growth medium in which seedlings are produced usually includes application of

chemicals such as methyl bromide and others, which eliminate beneficial microorganisms such as arbuscular mycorrhizal (AM) fungi in addition to killing soil-borne pathogens. AM fungi form symbiotic associations with the roots of most plant species, and they aid those plants in uptake of nutrients especially those immobile in soil like phosphorus (P) (Al-Karaki and Al-Raddad, 1997; Marschner and Dell, 1994). Elimination of these fungi via sterilization of seedling growth medium can lead to plant nutrient deficiencies, stunting and crop loss, particularly on low-P and/or P-fixing soils as alkaline soils (Wood et al., 1989). In addition to enhancing plant mineral nutrition, AM fungi can benefit plants by stimulating growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought stress, increasing resistance to pests and tolerance to environmental stresses (e.g., drought, salinity), and improving soil properties (Bethlenfalvy and Linderman, 1992; Bethlenfalvy et al., 1988; Al-Karaki, 2000b; Copeman et al., 1996; Cordier et al., 1996).

* Tel.: +962 2 7201000; fax: +962 2 7095069.

E-mail address: gkaraki@just.edu.jo.

Mycorrhizal hyphae extend into soil past the zone of nutrient depletion and can increase the effectiveness of absorption of immobile elements. This can be attained by increasing the surface area of soil explored via fungal hyphae, and the much smaller hyphae can penetrate fissures in soil particles too small for roots (Marschner and Dell, 1994), AM fungi can excrete enzymes which allow to dissolve soil nutrients otherwise unavailable to the roots (Muchovej, 2001). AM fungi also enhance soil aggregation and water-holding capacity both by producing hyphae external to the host plant root tissues and by exuding glomalin, a glycoprotein, from extraradical hyphae (Wright and Upadhyaya, 1998).

Horticultural crops and flowers have been used as host plants in several experimental tests as potential target plants for practical use of mycorrhizal inoculation (Chang, 1994; Lovato et al., 1995). The method of application of mycorrhiza in growing plant has been practiced by adding mycorrhizal fungi inoculum to planting hole at time of transplanting which require large amounts of inoculum. However, the necessity of AM inoculum production via a host plant is still an obstacle to ample utilization of AM fungi in agricultural crops. Nevertheless, progress is being made in this area and some commercial inoculum is currently marketed in the United States (Muchovej, 2001). The difficulty in producing a large amount of inoculum of AM fungi for agricultural practices might be a less problem in horticultural crops, where inoculation can take place in seedlings or cutting beds, over a relatively small surface area. Furthermore, the intensive use of artificial substrates where AM fungi are absent facilitate their introduction (Jeffries et al., 2003).

The most promising areas for practical use of AM fungi in horticulture are for nursery seedlings grown in trays, and for plantlets grown by micropropagation plantlets (Giananazzi et al., 2001). Nurseries can realize two main benefits from introducing mycorrhizal fungi to their plants: superior stronger growth in the nursery and improved performance after planting in field (Giananazzi et al., 2001). Inoculation with AM fungi at very early stages (e.g., at seed sowing) has been found to result in higher crop uniformity and reduce transplant mortality (Waterer and Coltman, 1988) and higher yields after transplantation to the field (Lovato et al., 1996; Vosalka, 1995). Since in most soils the indigenous populations of AM fungi are present, the pre-inoculation of seedlings in AM fungi free substrates in nurseries, gives the introduced fungal strain a special advantage over the indigenous fungi after transplanting in field (Dubsky et al., 2002).

In many regions, in particular in the Mediterranean region, protected horticultural crops (grown in green and plastic houses) as well as those planted in open field have to cope with increasing salinization of irrigation water. This is mainly due to low precipitation in these regions, in addition to over-exploitation of available water resources (e.g., ground water). Often soils become saline due to irrigation practices that bring salts to the soil surface where they can be toxic to crop plants. High salinity of the supply water has detrimental effects on soil fertility and reduces crop growth and yield (Al-Karaki, 2000a; Ashraf and Waheed, 1993; Feigin et al., 1987). Developing

proper cropping techniques to run sustainable horticulture in the presence of irrigation water of poor quality (e.g., saline) becoming essential especially with limiting availability of fresh water for irrigation use.

Plants with beneficial AM fungi are known to tolerate salinity better than nonAM plants (Al-Karaki, 2000b; Ruiz-Lozano et al., 1996; Cantrell and Linderman, 2001). However, most studies concerning AM fungi–saline soil interactions have not related to real field conditions where crops are seeded or transplanted directly into saline soils (Cantrell and Linderman, 2001) and plants are not usually grown to maturity which might limit AM fungi effectiveness. The hypothesis was that pre-inoculation of vegetable transplants would give an advantage to overcome salinity problems when grown under saline conditions. To the author knowledge, there are no reports of pre-inoculated AM fungi plants being grown in large plots where saline water is used for irrigation. This study was conducted to determine if inoculation of transplants with AM fungi before planting alleviates salt effects on growth and yield of tomato when irrigated with saline water.

2. Materials and methods

2.1. Inoculum production

Pot cultures of the AM fungi *Glomus mosseae* were initiated on corn (*Zea mays* L.), grown for approximately 10 weeks in a greenhouse, and harvested just prior to inoculation. Mycorrhizal inoculum was initially isolated from a wheat (*Triticum durum* Desf.) field from north of Jordan (Al-Karaki and Al-Raddad, 1997). Corn shoots were excised and discarded, mycorrhizal roots were removed from soil, cut into 1-cm lengths and then mixed back with the soil. A steam sterilized (two times on successive days) soil mix (soil:sand, 1:1) was used as the growth substrate for the inoculum. The inoculum potential was estimated by the infection-unit method of Francon and Bethlenfalvay (1989).

2.2. Production of seedlings

Polystyrene trays with 20-cm³ cells were filled with pasteurized (90 °C, 3 h twice at 24 h interval) soil amended with peatmoss (33% by volume) to increase moisture retention. Mycorrhizal inoculum was added in equal amounts to each cell to give similar propagule numbers (400–500 propagules/cell). The inoculum added consisted of AM fungi colonized root fragments and spores and hyphae mixed with soil. A noninoculated control (nonAM) treatment was prepared using only pasteurized soil plus peat. Tomato (*Lycopersicon esculentum* Mill. Cv. Marriha) seeds were sown and thinned to one plant per cell, and grown on a bench with mist irrigation in the greenhouse until plants reach appropriate size for planting (35 days). One day prior to transplant into soils, 10 seedlings from each treatment were subjected to a destructive measurement, where the following parameters were estimated: root AM fungi colonization, plant height and width, and the number of leaves.

2.3. Transplanting and cultural practices

The experiment was conducted under greenhouse conditions. Concrete blocks were made and filled with nonsterilized silty clay soil amended with 15% peatmoss (by volume). Block dimensions were 1.6 m × 3 m × 0.75 m. The soil was collected from the top 30 cm of a nearby field. Plants were grown during March–June with four tomato rows in each block.

A composite soil samples were analyzed for major soil properties and indigenous AM fungi spores. Soil properties before mixing with peatmoss were 7% sand, 45% silt, and 48% clay; 1.2% organic matter, pH 8.1 (soil:water, 1:1), electrical conductivity (EC_e) 1.4 dS m⁻¹, 0.26 P (NaHCO₃-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. The initial search for indigenous AM fungi spores (assayed by wet sieving) yielded <2 spores g⁻¹ air-dried soil. Nitrogen was broadcast on all plots and incorporated below the soil surface at a rate of 50 kg N ha⁻¹ as urea and was added at 30 and 50 kg N ha⁻¹ after 30 and 60 days of seedlings transplantation, respectively. No phosphorus fertilization has been applied to avoid reducing colonization with AM fungi. Weeds were controlled by hand when needed.

Irrigation water management treatments were (i) nonsaline water (NSW)–tap water with $EC_w = 0.5$ dS m⁻¹ and (ii) saline water (SW)–irrigation with saline water with $EC_w = 2.4$ dS m⁻¹. This saline water was diluted from saline water which was brought from a well from Al-Khaldia Agricultural Research Station of National Center for Agricultural Research and Technology Transfer which had an EC_w value of 5.5 dS m⁻¹ (Table 1). The saline water was diluted to avoid osmotic shock. Soil EC_e was measured at the end of experiment and values of EC_e were 1.7 and 4.4 dS m⁻¹ for NSW and SW treatments, respectively. Water was supplied to individual blocks by a drip irrigation system.

Table 1
Salt components and conductivity of saline (before dilution) and nonsaline water used in this study

| Parameter | Nonsaline water | Saline |
|---|-----------------|--------|
| pH | 8.1 | 9.2 |
| EC (dS m ⁻¹) | 0.5 | 5.5 |
| TDS (ppm) ^a | 422 | 3770 |
| K (ppm) | 1.2 | 25.9 |
| Ca (meq L ⁻¹) | 3.5 | 14.0 |
| Mg (meq L ⁻¹) | 3.0 | 24.3 |
| Na (meq L ⁻¹) | 1.0 | 22.5 |
| Cl (meq L ⁻¹) | 1.8 | 48.0 |
| CO ₃ (meq L ⁻¹) | 0.5 | 0.8 |
| HCO ₃ (meq L ⁻¹) | 5.0 | 2.0 |
| SO ₄ (meq L ⁻¹) | 0.2 | 10.1 |
| Total cation (meq L ⁻¹) | 7.5 | 60.8 |
| SAR ^a | 0.5 | 5.1 |
| ESP ^a | – | 5.8 |

^a TDS, total dissolved salts; SAR, sodium adsorption ratio; ESP, exchangeable sodium percentage.

2.4. Plant growth responses

Plant samples with their roots (three plants) from each block were randomly sampled at flowering stage from one of outer rows. These samples were taken by a fork, fitted to excavate the soil volume under the area occupied by the plants. Roots were rinsed free from soil and weighed and subsamples were saved for assessment of AM fungi root colonization. Then shoots and roots were oven-dried (48 h, 60 °C) and weighed. Shoot samples from each replicate were saved for mineral analysis.

Tomato plants were grown until fruit maturity. Red and firm fruits were collected after they were mature for determination of fruit yield, fruit number, individual fruit fresh weight, and total soluble solids (TSS).

2.5. AM fungi root colonization

Assessment of roots for AM fungi colonization was made on those plants sampled for shoot and root growth at flowering stage in the same plants sampled for shoot and root measurement. Root samples for determination of root colonization with AM fungi were cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol as described by Phillips and Hayman (1970), and microscopically examined for AMF colonization by determining percentage of root segments containing arbuscules + vesicles using a gridline intercept method (Giovannetti and Mosse, 1980).

2.6. Tissue elemental composition

Dried shoots were ground to pass a 0.5 mm sieve in a cyclone laboratory mill, and prepared 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 (K) and Na concentrations in shoots were determined using flame photometer. Mineral contents were calculated by multiplying of mineral concentration by dry weight of shoots.

2.7. Experimental design and statistical analysis

The experiment was randomized in complete blocks with two water treatments (nonsaline and saline water) and two AM fungi inoculum treatments (AM and nonAM) to give a 2 × 2 factorial with six replications. Data were statistically analyzed using analyses of variance in the MSTATC program (Michigan State University, East Lansing, MI). Probabilities of significance among treatments and interactions and L.S.D.s ($P \leq 0.05$) were used to compare means within and among treatments.

3. Results

The AM fungi inoculation did not influence all measured growth parameters (plant height and width and the number of leaves) on seedlings before planting in comparison to

Table 2
Root AMF colonization, shoot, and root dry matter (DM) at flowering by AM and nonAM tomato grown under nonsaline (NSW) and saline (SW) water conditions

| Water treatment | AM status | Root colonization (%) | Dry matter (g plant ⁻¹) | |
|-----------------|-----------|-----------------------|-------------------------------------|---------|
| | | | Shoot | Root |
| NSW | NonAM | 18.2 b | 35.1 b | 4.19 ab |
| | AM | 48.7 a | 44.1 a | 5.97 a |
| SW | NonAM | 6.0 c | 12.5 d | 1.03 c |
| | AM | 23.6 b | 25.8 c | 3.26 b |

Values in each column followed by same letter(s) are not significantly different ($P \leq 0.05$) according to L.S.D.

noninoculated seedlings (data not shown). Mean AM fungi colonization of roots of 10 plants per treatment was $13 \pm 2\%$. No AM fungi colonization was observed in the roots of nonAM seedlings.

The AM fungi root colonization was noted in roots of both AM and nonAM plants, even though the AM plants had higher root AM fungi colonization than nonAM plants at flowering (Table 2). The AM fungi root colonization in tomato was reduced by salinity stress regardless of AM fungi inoculation status.

The AM plants generally had higher shoot and root DM yields than nonAM plants grown under both nonsaline and saline conditions (Table 2). However, shoot and root DM decreased in plants grown under saline compared to nonsaline conditions, regardless of AM fungi inoculation status.

Fruit fresh yield of AM plants was higher than that of nonAM plants grown under both saline and nonsaline conditions (Table 3). Fruit fresh yield decreased in both AM and nonAM plants grown under saline compared to nonsaline conditions, but the extent of decrease due to salinity was higher in nonAM than AM plants.

Fruit weight of AM plants was higher than nonAM plants grown under saline conditions, but not under nonsaline conditions (Table 3). Although the fruit number per plant in AM is higher than nonAM plants, the differences were only significant under nonsaline conditions (Table 3).

Total soluble solids in tomato fruits were higher for AM than nonAM plants grown under both saline and nonsaline conditions (Table 3). Salt treatment resulted in higher TSS in both nonAM and AM plants.

The AM plants had generally higher shoot P contents, but not concentrations, than shoots of nonAM plants grown under

Table 3
Fruit fresh yield, fruit number, fruit weight, and total soluble solids (TSS) by AM and nonAM tomato grown under nonsaline (NSW) and saline (SW) water conditions

| Water treatment | AM status | Fruit yield (kg m ⁻²) | Fruit number (m ⁻²) | Fruit weight (g) | TSS (%) |
|-----------------|-----------|-----------------------------------|---------------------------------|------------------|---------|
| NSW | NonAM | 5.77 b | 231 b | 25 a | 4.6 c |
| | AM | 7.42 a | 275 a | 27 a | 6.1 b |
| SW | NonAM | 3.28 c | 219 b | 15 b | 6.5 b |
| | AM | 5.26 b | 229 b | 23 a | 7.6 a |

Values in each column followed by same letter are not significantly different ($P \leq 0.05$) according to L.S.D.

Table 4
Shoot concentrations and contents of P, K, and Na by AM and nonAM tomato grown under nonsaline (NSW) and saline (SW) water conditions

| Water treatment | AM status | Concentration (mg g ⁻¹ DM) | | | Content (mg plant ⁻¹) | | |
|-----------------|-----------|---------------------------------------|------|--------|-----------------------------------|--------|------|
| | | P | K | Na | P | K | Na |
| | | | | | | | |
| | AM | 3.52 a | 55 a | 1.15 c | 155 a | 2425 a | 52 a |
| SW | NonAM | 1.88 b | 39 a | 4.48 a | 24 d | 487 d | 56 a |
| | AM | 2.72 ab | 45 a | 2.86 b | 70 c | 1161 c | 60 a |

Values in each column followed by same letter(s) are not significantly different ($P \leq 0.05$) according to L.S.D.

both saline and nonsaline conditions (Table 4). Shoot P contents were lower in AM and nonAM plants grown under saline compared to nonsaline conditions.

Shoot K concentrations of AM and nonAM tomato plants were similar for plants grown under nonsaline and saline conditions (Table 4). Shoots of AM plants had generally higher K contents than shoots of nonAM plants. Shoot K contents decreased for plants grown under saline conditions compared to the plants grown under nonsaline conditions.

Shoot Na concentrations, but not contents, were lower in AM than nonAM plants grown under saline conditions only (Table 4). No significant differences were noted for shoot Na contents regardless of water treatment or AM fungi inoculation status.

Shoot concentrations of Cu, Fe, and Zn were generally higher for AM than nonAM plants regardless of water treatment, although the differences for Cu and Zn concentrations were only significant under nonsaline conditions (Table 5). The AM plants had significantly higher shoot contents of Cu, Fe, and Zn than nonAM plants grown under both saline and nonsaline conditions. Shoot contents of Cu, Fe, and Zn were lower for plants grown under saline compared to nonsaline conditions (Table 5).

The overall effects of AM fungi inoculation on the tomato yield and mineral contents (percentage-wise) of plants grown under nonsaline and saline conditions are summarized in Table 6. The enhancement in fruit fresh yield due to AM fungi inoculation was 29 and 60% for tomato grown under nonsaline and saline water conditions, respectively. Shoot P contents were enhanced by 44 and 192% for plants inoculated with AM fungi and grown under nonsaline and saline water conditions, respectively.

Table 5
Shoot concentrations and contents of Cu, Fe, and Zn AM and nonAM tomato grown under nonsaline (NSW) and saline (SW) water conditions

| Water treatment | AM status | Concentration ($\mu\text{g g}^{-1}$ DM) | | | Content ($\mu\text{g plant}^{-1}$) | | |
|-----------------|-----------|--|--------|------|--------------------------------------|--------|--------|
| | | Cu | Fe | Zn | Cu | Fe | Zn |
| NSW | NonAM | 8.1 b | 150 ab | 44 b | 284 b | 5265 b | 1544 b |
| | AM | 11.7 a | 159 a | 53 a | 516 a | 7012 a | 2337 a |
| SW | NonAM | 5.1 c | 112 b | 30 c | 64 d | 1400 d | 375 d |
| | AM | 7.3 bc | 144 ab | 32 c | 188 c | 3715 c | 826 c |

Values in each column followed by same letter(s) are not significantly different ($P \leq 0.05$) according to L.S.D.

Table 6
Percentage change in fruit yield and nutrient contents due to AM and nonAM of tomato grown under nonsaline (NSW) and saline (SW) water conditions

| Water treatment | Fruit yield ^a | Nutrient content ^b (%) | | | | | |
|-----------------|--------------------------|-----------------------------------|-----|----|-----|-----|-----|
| | | P | K | Na | Cu | Fe | Zn |
| NSW | 29 | 44 | 33 | 21 | 93 | 33 | 51 |
| SW | 60 | 192 | 138 | 7 | 193 | 165 | 120 |

^a Fruit yield (FY) change = $((\text{FY}_{\text{AM}} - \text{FY}_{\text{nonAM}}) \times 100) / \text{FY}_{\text{nonAM}}$.

^b Nutrient content (NC) change = $((\text{NC}_{\text{AM}} - \text{NC}_{\text{nonAM}}) \times 100) / \text{NC}_{\text{nonAM}}$.

4. Discussion

Inoculation with AM fungi did not affect seedling growth in comparison to nonAM seedlings. The primary purpose of nursery inoculation is not to promote plant growth at this stage of production, but to establish AM fungi on plant roots so that mycorrhizae will be efficiently transferred to the field (Sylvia, 1989). The minimum level of colonization necessary for successful transfer of mycorrhizal plants to the field is not known; however, Bierman and Linderman (1983) reported that low levels of colonization (<10%) spread rapidly to new roots after transplanting. Results of this study indicated that the level of colonization with AM fungi before planting was 13% which might be considered adequate for successful establishment of mycorrhizal plants after transplanting.

The finding that AM tomato plants irrigated with saline water had greater fruit fresh yield, fruit weight, and shoot DM than nonAM plants supports the hypothesis that pre-inoculated AM plants grow better than nonAM plants under saline conditions. Inoculation of transplants prior to salt exposure bypasses the potential inhibitory effects that salt could have on AM fungal spore germination. Such inhibitory effects have been reported (Juniper and Abbott, 1993; Koske et al., 1996; McMillen et al., 1998). In the past, researchers have studied AM fungi effects on plants grown under saline conditions, but mostly these researchers added AM fungi inocula at the time of plants transplanting into soil and they usually allow sometime for AM fungi establishment before they add saline solutions to soil (Al-Karaki, 2000b; Duke et al., 1986; Mancuso and Rinaldelli, 1996; Rinaldelli and Mancuso, 1996). Under field conditions, salinity level is not an adjustable variable. The procedure used in this study of pre-inoculating transplant

seedlings with AM fungi can be of practical importance in the cultivation of many horticultural crops grown under saline conditions, especially high-value crops such as fruit and vegetables.

Many studies have indicated that AM fungi contributes to plant growth via enhancement of mineral nutrient uptake especially immobile soil nutrients (P, Cu, Zn) (Bethlenfalvay et al., 1988; Marschner and Dell, 1994). In this study, AM tomato plants had higher shoot P contents than nonAM plants. Higher shoot Cu, Fe, and Zn contents in AM compared to nonAM plants were also noted for plants grown under nonsaline conditions. The higher mineral nutrient acquisition in AM compared to nonAM plants likely occurred because of increased availabilities or transport (absorption and/or translocation) by AM fungi hyphae. Enhanced acquisition of P, Cu, Fe, and Zn by AM plants has been reported (Al-Karaki, 2000b; Marschner and Dell, 1994; Trimble and Knowles, 1995). However, AM fungi root colonization did not significantly enhanced shoot K concentrations. Poss et al. (1985) reported that K uptake was affected little by AM fungi root colonization in tomato grown under saline conditions. It is possible that improved plant nutrition by AM fungi allows cells to more effectively regulate and separate flowing ions. Ion pumps in the plasma membrane and tonoplast of root cells that bring about and maintain salt compartmentalization must be efficient if the nutrition in the cell remains balanced (Zhu, 2003). Cantrell and Linderman (2001) suggested that improved P nutrition by AM fungi in plants grown under saline conditions might reduce the negative effects of Na and Cl by maintaining vacuolar membrane integrity, which prevented these ions from interfering in metabolic pathways of growth.

Although AM fungi mitigate growth reduction caused by salinity (Gupta and Krishnamurthy, 1996; Jindal et al., 1993; Pfeiffer and Bloss, 1988; Tsang and Maum, 1999), the mechanism involved remains unresolved. Poss et al. (1985) concluded that the salt tolerance mechanism in onion is primarily related to P nutrition. Similarly, Pfeiffer and Bloss (1988) stated that “the major effect of the mycorrhiza on sodium uptake is through mediation of P accumulation”. Duke et al. (1986) concluded that improved P uptake by AM versus nonAM citrus plants did not totally account for the improved salt tolerance of AM plants. Other mechanisms that improve salt tolerance may include maintaining membrane integrity (Mancuso and Rinaldelli, 1996; Rinaldelli and Mancuso, 1996) that would facilitate compartmentalization within vacuoles, and selective ion intake. Induction of osmotica could lead to osmotic adjustment (Duke et al., 1986), and improved and balanced nutrition in plants could also increase salt tolerance (Marschner, 1995). Such mechanisms could involve all effects of AM fungi.

Data of this study show that Na concentrations in tomato shoots was lower in AM than in nonAM plants grown under saline conditions. This might indicate that Na was retained in roots without being translocated to the shoots and suggest that it might be retained in intraradical AM fungal hyphae or was compartmentalized in the root cell vacuoles (Cantrell and Linderman, 2001). However, some other researchers suggested

that lower Na concentrations in AM plant tissues compared to nonAM plants might be explained by dilution effects due to growth enhancement by AM fungi colonization (Al-Karaki, 2000b; Jarrell and Beverly, 1981).

In conclusion, pre-inoculation of transplants with AM fungi reduced the detrimental effects of salt on tomato growth and productivity. Pre-inoculating transplants could be an economically feasible means of growing crops in agrosystems affected by salt. The mechanism(s) by which AM fungi alleviate salt stress remains unresolved, but appears to involve several possible metabolic processes that could be mediated by P nutrition or other element balance, and possibly compartmentalization of sodium within some plant tissues. However, several AM fungi isolates should be investigated in order to maximize efficiencies of AM fungi symbiosis under saline conditions.

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References

- Al-Karaki, G.N., 2000a. Growth, water use efficiency, and mineral acquisition by tomato cultivars grown under salt stress. *J. Plant Nutr.* 23, 1–8.
- Al-Karaki, G.N., 2000b. Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza* 10, 51–54.
- Al-Karaki, G.N., Al-Raddad, A., 1997. Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance. *Mycorrhiza* 7, 83–88.
- Ashraf, M., Waheed, A., 1993. Responses of some genetically diverse lines of chickpea (*Cicer arietinum* L.) to salt. *Plant Soil* 154, 257–266.
- Bethlenfalvay, G.J., Linderman, R.G., 1992. Mycorrhizae and crop productivity. In: Bethlenfalvay, G.J., Linderman, R.G. (Eds.), *Mycorrhizae in Sustainable Agriculture*. American Society of Agronomy, Special Publication No. 54, Madison, WI, pp. 1–27.
- Bethlenfalvay, G.J., Brown, M.S., Ames, R.N., Thomas, R.S., 1988. Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake. *Physiol. Plant* 72, 565–571.
- Bierman, B.J., Linderman, R.G., 1983. Increased geranium growth using pretransplant inoculation with a mycorrhizal fungus. *J. Am. Soc. Hortic. Sci.* 108, 972–976.
- Cantrell, I.C., Linderman, R.G., 2001. Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant Soil* 233, 269–281.
- Chang, D.C., 1994. What is the potential for management of vesicular–arbuscular mycorrhiza in horticulture? In: Robson, A.D., Abbott, L.K., Malajczuk, N. (Eds.), *Management of Mycorrhiza in Agriculture, Horticulture and Forestry*. Kluwer Academic Publishers, The Netherlands, pp. 187–190.
- Copeman, R.H., Martin, C.A., Stutz, J.C., 1996. Tomato growth in response to salinity and mycorrhizal fungi from saline and nonsaline soils. *Hortscience* 31, 341–344.
- Cordier, A.T., Gianinazzi, S., Gianinazzi-Pearson, V., 1996. Arbuscular mycorrhiza technology applied to micropropagated *Prunus avium* and to protection against *Phytophthora cinnamomi*. *Agronomie* 16, 676–688.
- Dubsky, M., Sramck, F., Vosatka, M., 2002. Inoculation of cyclamen (*Cyclamen persicum*) and poinsettia (*Euphorbia pulcherrima*) with arbuscular mycorrhizal fungi and *Trichoderma harzianum*. *Rost. Vyroba* 48, 63–68.
- Duke, E.R., Johnson, C.R., Koch, K.E., 1986. Accumulation of phosphorus, dry matter and betaine during NaCl stress of split-root citrus seedlings colonized with vesicular–arbuscular mycorrhizal fungi on zero, one or two halves. *New Phytol.* 104, 583–590.
- Feigin, A., Rylski, I., Meriri, A., Shalhevet, J., 1987. Response of melon and tomato plants to chloride–nitrate ratio in saline nutrient solution. *J. Plant Nutr.* 10, 1787–1794.
- Francon, R.L., Bethlenfalvay, G.J., 1989. Infection unit method of vesicular–arbuscular mycorrhizal propagule determination. *Soil Sci. Soc. Am. J.* 53, 754–756.
- Gianinazzi, S., Schuepp, H., Barea, J.M., Haselwandter, K., 2001. *Mycorrhizal Technology in Agriculture: From Genes to Bioproducts*. Birkhauser, Basel, Switzerland.
- Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* 84, 489–500.
- Gupta, R., Krishnamurthy, K.V., 1996. Response of mycorrhizal and nonmycorrhizal *Arachis hypogea* to NaCl and acid stress. *Mycorrhiza* 6, 145–149.
- Jarrell, W.M., Beverly, R.B., 1981. The dilution effect in plant nutrition studies. *Adv. Agron.* 34, 197–224.
- Jeffries, P., Gininazzi, S., Perotto, S., Turnau, K., Barea, J., 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol. Fertil. Soils* 37, 1–16.
- Jindal, V., Atwal, A., Seckhon, B.S., Singh, R., 1993. Effect of vesicular–arbuscular mycorrhizae on metabolism of moong plant under NaCl salinity. *Plant Physiol. Biochem.* 31, 475–481.
- Juniper, S., Abbott, L.K., 1993. Vesicular–arbuscular mycorrhizas and soil salinity. *Mycorrhiza* 4, 45–57.
- Koske, R., Bonin, C., Kelly, J., Martinez, C., 1996. Effect of sea water on spore germination of a sand-dune-inhabiting arbuscular mycorrhizal fungus. *Mycorrhiza* 8, 947–950.
- Lovato, P.E., Gininazzi-Pearson, V., Trouvelot, A., Gininazzi, S., 1996. The state of art of mycorrhizas and micropropagation. *Adv. Hortic. Sci.* 10, 46–52.
- Lovato, P.E., Schuepp, H., Trouvelot, A., Gianinazzi, S., 1995. Application of arbuscular mycorrhizal fungi in orchard and ornamental plants. In: Varma, A., Hock, B. (Eds.), *Mycorrhiza Structure, Function, Molecular Biology and Biotechnology*. Springer, Heidelberg, pp. 521–559.
- Mancuso, S., Rinaldelli, E., 1996. Response of young mycorrhizal and non-mycorrhizal plant of olive tree (*Olea europaea* L.) to saline condition. II. Dynamics of electrical impedance parameters of shoots and leaves. *Adv. Hortic. Sci.* 10, 135–145.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plant*, second ed. Academic Press, New York, 889 pp.
- Marschner, H., Dell, B., 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* 159, 89–102.
- McMillen, B.G., Juniper, S., Abbott, L.K., 1998. Inhibition of hyphal growth of a vesicular–arbuscular mycorrhizal fungus in soil containing sodiumchloride limits the spread of infection from spores. *Soil Biol. Biochem.* 30, 1639–1646.
- Muchovej, R.M., 2001. *Importance of Mycorrhizae for Agriculture Crops*. University of Florida Extension Service. Pamphlet SS-AGR-170, 5 pp.
- Pfeiffer, C.M., Bloss, H.E., 1988. Growth and nutrition of guayule (*Parthenium argentatum*) in a saline soil as influenced by vesicular–arbuscular mycorrhiza and phosphorus fertilization. *New Phytol.* 108, 315–321.
- Phillips, J., Hayman, D., 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55, 158–161.
- 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.
- Rinaldelli, E., Mancuso, S., 1996. Response of young mycorrhizal and non-mycorrhizal plant of olive tree (*Olea europaea* L.) to saline condition. I. Short-term electrophysiological and long-term vegetative salt effect. *Adv. Hortic. Sci.* 10, 126–134.
- 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.
- Sylvia, D.M., 1989. Nursery inoculation of sea oats with vesicular–arbuscular mycorrhizal fungi and outplanting performance on Florida beaches. *J. Coastal Res.* 5, 747–754.

- Trimble, M.R., Knowles, N.R., 1995. Influence of vesicular–arbuscular mycorrhizal fungi and phosphorus on growth, carbohydrate partitioning and mineral nutrition of greenhouse cucumber (*Cucumis sativus* L.) plants during establishment. *Can. J. Plant Sci.* 75, 239–250.
- Tsang, A., Maum, M.A., 1999. Mycorrhizal fungi increase salt tolerance of *Strophostyles helvola* in coastal foredunes. *Plant Ecol.* 144, 159–166.
- Vosalka, M., 1995. Influence of inoculation with arbuscular mycorrhizal fungi on the growth and mycorrhizal infection of transplanted onion. *Agric. Ecosyst. Environ.* 53, 151–159.
- Watanabe, F.S., Olsen, S., 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO_3 extract for soil. *Soil Sci.* 21, 677–678.
- Waterer, D.R., Coltman, R.R., 1988. Phosphorus concentration and application interval influence growth and mycorrhizal infection of tomato and onion transplants. *J. Am. Soc. Hortic. Sci.* 113, 704–798.
- Wood, T., Nance, L., Jedrzejek, S., Johnson, G., 1989. Use of VA mycorrhizal inoculum to improve growth of forest seedlings in fumigated soil. In: *Proceedings, Intermountain Forest Nursery Association, Bismark, ND, August 14–18*, pp. 58–59.
- Wright, S.F., Upadhyaya, A., 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by haphae of arbuscular mycorrhizal fungi. *Plant Soil* 198, 97–107.
- Zhu, J.K., 2003. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.* 6, 441–445.