Tomato Growth in Response to Salinity and Mycorrhizal Fungi from Saline or Nonsaline Soils

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Abstract. Tomato (*Lycopersicon esculentum* Mill. 'Heinz 1350 VF 402') seedlings were inoculated with populations of vesicular–arbuscular mycorrhizal (VAM) fungi collected from saline or nonsaline soil or remained nonmycorrhizal as a control. Plants then were salinated for 8 weeks at 1.0, 2.0, 5.0, or 10.0 dS·m⁻¹ produced by dilutions of 1 M NaCl : 1 M CaCl₂ in deionized water. Inoculation with VAM fungi from nonsaline soil enhanced shoot growth, while VAM fungi from saline soil suppressed shoot and root growth. Plants inoculated with VAM fungi from nonsaline soil and non-VAM control plants showed a quadratic increase in leaf Cl⁻ concentration in response to an increased salinity level, whereas plants inoculated with VAM fungi from saline soil showed a linear increase in leaf Cl⁻ concentration. Mycorrhizal-induced growth responses and changes in leaf Cl⁻ concentration were not associated with any apparent alterations in tomato plant P status. Although VAM fungi originating from saline soil did not promote plant growth, reduction in leaf Cl⁻ concentration mediated by these VAM fungi at moderate salinity levels may have beneficial implications for plant survival in saline soil.

Field production soils in arid and semiarid regions are often high in salinity from soluble salts in irrigation water and fertilizers. Vesicular–arbuscular mycorrhizal (VAM) fungi have increased and suppressed herbaceous plant growth in saline conditions compared with nonmycorrhizal control plants (Hirrel and Gerdemann, 1980; Pfeiffer and Bloss, 1988; Pond et al., 1984; Poss et al., 1985). These differential effects might be due to the origin of the VAM fungi since mycorrhizal fungi adapt to edaphic conditions (Brundrett, 1991).

Our objective was to investigate effects of VAM fungi populations originating from saline or nonsaline soils on the growth response of young tomato plants to levels of salinity. Although many studies of host plant responses to mycorrhizal infection have been performed using a single VAM fungal isolate, we chose to inoculate plants with populations of VAM fungi because in soils they normally exist as an assemblage of organisms (Brundrett, 1991).

Materials and Methods

VAM fungal populations were collected from the rhizosphere of mesquite (*Prosopis* sp.) trees found in two soils of disparate salin-

ity. The saline soil [electrical conductivity (EC) = 12.2 dS·m⁻¹] was from an arid desert scrub community near Tornillo, Texas (lat. 31°24'N; long. 106°2'W), and the nonsaline soil (EC = 0.6 dS·m⁻¹) was from a semiarid grassland community in San Rafael, Ariz. (lat. 31°21'N; long. 110°33'W). VAM fungal populations from each soil type were grown for three generations in pot cultures in a greenhouse with Sudangrass [*Sorghum sudanense* (Staph.) Piper] as a host plant. The original rhizosphere soil and each subsequent pot culture were diluted as follows: 2 rhizosphere soil : 1 coarse silica sand (particle diameter ≤4.1 mm) : 1 fine silica sand (particle diameter ≤1.3 mm) (by volume). Fungal inoculum consisted of pot culture soil, VAM fungal spores, hyphae, and colonized root fragments. The VAM fungal species present in each inoculum were determined by collecting spores using wet sieving and sucrose gradient centrifugation (Daniels and Skipper, 1982). Species compo-

sition of the two populations consisted mostly of small-spore *Glomus* sp. (Table 1). Fungal inoculum potential was determined by the most probable numbers method (Alexander, 1982), and each treatment contained ≈9 × 10³ propagules per pot.

Fourteen-day-old, greenhouse-grown tomato ('Heinz 1350 VF 402') seedlings were transplanted into 3-liter, black, polyethylene containers (0.16 m top diameter × 0.14 m bottom diameter × 0.18 m height). Rooting substrate consisted of 3 autoclaved river sand : 2 coarse silica sand (particle diameter ≤4.1 mm) : 1 Gilman clay loam (by volume) (pH = 7.3, EC = 0.25 dS·m⁻¹, and P at 11.26 μg·g⁻¹ soil). Plants were grown in a glasshouse (26C day/15C night, 30% photosynthetically active radiation exclusion). At transplanting, two-thirds of all seedlings were inoculated with either one of two VAM fungi populations. The remaining one-third of tomato seedlings were treated with nonmycorrhizal grass roots, sand, and background microorganisms as a non-VAM control. Inoculum was placed in an inverted cone-like pattern directly adjacent and subjacent to the tomato seedling transplants to facilitate fungal colonization of plant roots. Non-VAM control plants remained nonmycorrhizal.

Three weeks after transplanting, tomato seedlings were salinated with concentrations of 1.0, 2.0, 5.0, or 10.0 dS·m⁻¹ from dilutions of 1 M NaCl : 1 M CaCl₂ in deionized water to container capacity during each irrigation. The salt treatments at 5.0 and 10.0 dS·m⁻¹ were salinized step-wise to avoid osmotic shock. Plants receiving 5.0 dS·m⁻¹ first were salinated at 2.0 dS·m⁻¹ for 1 week before receiving the full amount. Plants receiving 10.0 dS·m⁻¹ were salinated at 2.0 dS·m⁻¹ for 1 week and then at 5.0 dS·m⁻¹ for 1 week before receiving the full amount. Salinity treatments were repeated when rooting substrate water potential fell to between -0.4 and -0.8 MPa as measured with a tensiometer (Soilmoisture Equipment, Santa Barbara, Calif.). Leachate from the container substrate was collected regularly and had EC values nearly identical to salinity treatments.

At transplanting, all tomato seedlings were fertilized with isobutylidene diurea slow-release fertilizer (20N–0P–16.6K–2Fe–1.4Mn) at 8.5 g/pot. At the start of the salinization treatments and after 4 weeks, mycor-

Table 1. Species composition of vesicular–arbuscular mycorrhizal (VAM) fungal inoculum originating from nonsaline or saline soil.

VAM fungal species	Relative inoculum abundance in soil (%) ²	
	Saline	Nonsaline
<i>Acaulospora mellea</i> Spain & Schneck	---	10
<i>Glomus etunicatum</i> Daniels & Trappe	12	15
<i>Glomus intraradices</i> Smith & Schneck	5	---
<i>Glomus macrocarpum</i> Tulasne & Tulasne	20	24
<i>Glomus microaggregatum</i> Koske, Gemma & Olexia	5	7
<i>Glomus mosseae</i> (Nicol. & Gerd.) Gerd. & Trappe	19	7
<i>Glomus occultum</i> Walker	---	37
<i>Glomus</i> AZ 201 ³	23	---
<i>Glomus</i> AZ 241	16	---

²Number of VAM fungal spores for each species as a percentage of the total number of spores in a 100-cm³ sample.

³Undescribed species number refers to accession number at International Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi, West Virginia Univ., Morgantown.

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rhizal-treated plants received 200 ml of P at 22 $\mu\text{L}\cdot\text{liter}^{-1}$, applied as a dilute phosphoric acid solution. At the same time, non-VAM control plants received 200 ml P at 44 $\mu\text{L}\cdot\text{liter}^{-1}$ to equalize plant size and tissue P concentrations for mycorrhizal and nonmycorrhizal plants (Davies et al., 1992).

Total shoot length was measured weekly after starting salinity treatments. After 8 weeks of salinization, root systems were severed at the soil line and washed free of rooting substrate. Roots were visually examined and appeared healthy. Total root length of each root system was measured with a digital image analysis system (model DIAS; Decagon Devices, Pullman, Wash.). A subsample of each root system was stained with acid-fuchsin (Kormanik and McGraw, 1982), and percent VAM root infection was determined using the grid-line-intercept method (Giovannetti and Mosse, 1980). Shoots and roots then were oven-dried at 65°C for 48 h, and dry weights were determined.

Phosphorus concentration in dry, pulverized leaf tissue was determined by an ascorbic method (Watanabe and Olson, 1965). Chloride concentrations in leaf tissue were determined after soaking 5 g ground tissue samples in 250 ml deionized water for 24 h on an agitating shaker and measuring bathing solution Cl^- concentration with a Cl^- -specific electrode (model 9617B; Orion Research, Boston).

Plants were arranged in a randomized complete-block design with a 3 mycorrhizal fungi $\times$ 4 salinity factorial arrangement and four plant replications. An analysis of variance for treatment main effects and interactions was calculated for all data using general linear models procedure (SAS version 6.03; SAS Inst., Cary, N.C.). Duncan's multiple range test was used to test for VAM treatment effects; regression analysis was used to test for significant response of dependent variables to increased salinity level. Percent VAM root infection data were arcsin-transformed to approximate a normal distribution for analysis of treatment effects. Actual percentage means are reported. Repeated measures analysis (Littell, 1989) was used to test if temporal changes in tomato shoot extension were affected by VAM and salinity treatment combinations.

Results and Discussion

Shoot growth was promoted by inoculation with VAM fungi from nonsaline soil and was inhibited by inoculation with VAM fungi from saline soil, irrespective of salinity treatments (Fig. 1, Tables 2 and 3). In addition, VAM fungi from saline soil inhibited root growth more than shoot growth as shown by reduced total root length and root dry weight and a higher shoot : root ratio compared with plants inoculated with VAM fungi from nonsaline soil or non-VAM control plants (Table 3). Growth-promoting effects of VAM fungi when host plants were grown in saline environments have been related to mycorrhizal-mediated enhancements of host plant P

nutrition (Hirrel and Gerdemann, 1980; Pond et al. 1984; Poss et al., 1985). In our study, tomato leaf tissue P was similar for VAM fungal treatments and the non-VAM control (mean range: 2.8 to 3.3 $\text{mg}\cdot\text{g}^{-1}$ tissue) because control plants were given supplemental P. Therefore, mycorrhizal treatment effects on tomato growth were apparently not a result of VAM-mediated changes in tomato plant P status, but could have been due to mycorrhizal-mediated uptake of other nutrients (Graham and Syvertsen, 1989).

Percent root infection by VAM fungi was similar for both VAM treatments ($\text{Pr} > F =$

0.173). Mycorrhizal colonization increased quadratically in response to a linear increase in salinity level (Table 4), suggesting that the effect of increased salinity on mycorrhizal infection was most evident between 2 and 5 $\text{dS}\cdot\text{m}^{-1}$. Graham and Syvertsen (1989) reported citrus root infection by *G. intraradices* was unchanged by salinity level, while Hirrel and Gerdemann (1980), Duke et al. (1986), and Pfeiffer and Bloss (1988) reported VAM colonization decreased as salinity increased.

Shoot (Table 2) and root (Table 4) growth were not affected by salinity treatments. Salinity stress may be separated into short-term,

Fig. 1. Shoot length of young tomato plants as affected by vesicular–arbuscular mycorrhizal (VAM) fungi populations originating from saline (+S) or nonsaline (–S) soil or grown with non-VAM control inoculum (N). Values are treatment means obtained by pooling four replications of four salinity levels, $n = 16$.

Table 2. Analysis of variance of polynomial time trends and treatment contrasts for shoot length of young tomato plants in response to salinity level (1.0, 2.0, 5.0, or 10.0 $\text{dS}\cdot\text{m}^{-1}$) and populations of vesicular–arbuscular mycorrhizal (VAM) fungi originating from saline (+S) or nonsaline (–S) soil or grown with non-VAM control inoculum (N).

Source of variation	df	Mean square	F	P value ^a
Time	1	26712.8	454.1	0.0001 L
Mycorrhizae $\times$ time				
–S vs. +S and N ^b	1	1208.5	20.5	0.0001 L
+S vs. N	1	608.9	10.4	0.0027 L
Salinity $\times$ time	3	12.5	1.1	0.3915
Mycorrhizae $\times$ salinity $\times$ time	6	7.9	0.7	0.7070

^aLinear (L) fitted line responses across time.

^bTreatment contrasts are populations of VAM mycorrhizal fungi from +S soil, populations of VAM fungi from –S soil, or N.

Table 3. Shoot and root dry weight, shoot : root ratio, and total root length of young tomato plants as affected by populations of vesicular–arbuscular mycorrhizal (VAM) fungi originating from saline or nonsaline soil or non-VAM control.

VAM fungus source	Dry wt (g)		Shoot : root ratio	Root length (m)
	Shoot	Root		
Saline soil	3.5 c ^a	0.5 b	7.2 a	0.9 b
Nonsaline soil	7.0 a	1.3 a	5.4 b	2.1 a
None (control)	5.3 b	1.1 a	5.0 b	2.0 a

^aValues are treatment means obtained by pooling four replications of four salinity levels, $n = 16$. Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$.

Table 4. Effect of irrigation salinity on percent infection of tomato roots by vesicular–arbuscular mycorrhizal (VAM) fungi from saline or nonsaline soils and tomato shoot and root dry weights.

Salinity (dS·m ⁻¹)	Root infection (%)	Dry wt (g/plant)	
		Shoot	Root
1	26 ^c	5.2 ^y	0.9
2	23	6.2	1.2
5	56	5.1	1.0
10	48	4.9	0.7
Significance	**Q	NS	NS

^aValues for root infection represent treatment means obtained by pooling four replications of two VAM treatments, n = 8.

^bValues for dry weight are treatment means obtained by pooling four replications of three VAM treatments, n = 12.

NS, **Nonsignificant or significant at $P \leq 0.01$; Q = quadratic.

reversible, osmotic and longer-term, ion toxicity effects (Munns, 1993; Yeo et al., 1991). The short-term, osmotic effects of the 5- or 10-dS·m⁻¹ salinity levels apparently were alleviated by applying these treatments stepwise. Also, the presence of Ca ions in salinized rooting substrate has mitigated against the negative effects of Na⁺ on plant growth (Evlagon et al., 1990). Others (Al-Rawahy et al., 1990; Snapp and Shennan, 1992) found no growth inhibition of tomato plants by salt stress until plants attained full size. If salinity treatments had been extended beyond 8 weeks, it is possible that tomato growth would have been inhibited by increased salinity caused by the build-up of salt in old leaves (Munns, 1993). Maas (1986) reported that tomato growth under field condition was reduced 50% by a salinity level of 7.6 dS·m⁻¹, with a threshold for growth reduction at ≈ 2.5 dS·m⁻¹.

Leaf Cl⁻ concentration was affected by a

VAM $\times$ salinity levels interaction ($Pr > F = 0.045$). Plants inoculated with VAM fungi from nonsaline soil and non-VAM control plants showed a quadratic increase in leaf Cl⁻ concentration in response to increased salinity, while plants inoculated with VAM fungi from saline soil showed a linear increase in leaf Cl⁻ concentration (Fig. 2). These data suggest that mycorrhizae altered the pattern of Cl⁻ concentration in tomato leaves, especially at moderate salinization levels. Graham and Syversten (1989) also reported that leaf Cl⁻ increased in citrus infected by a VAM fungal isolate that originated from a nonsaline environment. In both cases, alterations in Cl⁻ concentrations were not related to P nutrition. In our experiment, the source of the VAM population appeared to be important in determining the pattern of Cl⁻ sequestration.

Based on our results, we conclude that a population of VAM fungi from nonsaline soil acted as a shoot growth promoter, but tended to increase leaf sequestration of Cl⁻ at moderate salinization levels. Conversely, VAM fungi originating from saline soil acted as a growth suppressant, particularly of roots, but tended to decrease leaf sequestration of Cl⁻ at moderate salinization levels. Graham and Syversten (1989) proposed that mycorrhizae indirectly mediated enhanced uptake of anions, such as Cl⁻, through a root carbon drain, which would decrease the pool of root organic acids. However, this explanation does not allow for mycorrhizal-associated decreases in Cl⁻ sequestration. One explanation for this phenomenon in plants inoculated with VAM fungi from saline soil at moderate salinization levels is that there was less root absorption surface area relative to shoot size, as indicated by the reduced root lengths and increased shoot : root ratio (Table 3).

Fig. 2. Effect of salinity level on leaf Cl⁻ concentration of young tomato plants inoculated with populations of vesicular–arbuscular mycorrhizal (VAM) fungi originating from saline (+S) or nonsaline (–S) soil or grown with non-VAM control inoculum (N). Values are treatment means, n = 4. Predictive model equations were as follows: +S, $y = 19.04 + 3.26x$, $r^2 = 0.84$; –S, $y = 13.85 + 12.58x - 0.967x^2$, $r^2 = 0.63$; and N, $y = 18.73 + 7.56 - 0.485x^2$, $r^2 = 0.76$.

Mycorrhizal-induced growth inhibition could be advantageous to plants in saline soil and may improve long-term plant survival under salinity stress by limiting the Cl⁻ concentration. Restricting Cl⁻ concentration in leaves has been associated with salt tolerance of wild tomato species and tolerant F₁ hybrids (Saranga et al., 1993). Because of the differences measured in leaf Cl⁻ concentration, future experiments might find that plants inoculated with VAM fungal populations from saline soils could be more tolerant of long-term, moderate salt stress than those inoculated with populations from nonsaline soils.

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