



Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity

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Abstract

The hypothesis that inoculation of transplants with vesicular-arbuscular mycorrhizal (VAM) fungi before planting into saline soils alleviates salt effects on growth and yield was tested on lettuce (*Lactuca sativa* L.) and onion (*Allium cepa* L.). A second hypothesis was that fungi isolated from saline soil are more effective in counteracting salt effects than those from nonsaline soil. VAM fungi from high- and low-salt soils were trap-cultured, their propagules quantified and adjusted to a like number, and added to a pasteurized soil mix in which seedlings were grown for 3–4 weeks. Once the seedlings were colonized by VAM fungi, they were transplanted into salinized (NaCl) soil. Preinoculated lettuce transplants grown for 11 weeks in the saline soils had greater shoot mass compared with nonVAM plants at all salt levels [2 (control), 4, 8 and 12 dS m⁻¹] tested. Leaves of VAM lettuce at the highest salt level were significantly greener (more chlorophyll) than those of the nonVAM lettuce. NonVAM onions were stunted due to P deficiency in the soil, but inoculation with VAM fungi alleviated P deficiency and salinity effects; VAM onions were significantly larger at all salt levels than nonVAM onions. In a separate experiment, addition of P to salinized soil reduced the salt stress effect on nonVAM onions but to a lesser extent than by VAM inoculation. VAM fungi from the saline soil were not more effective in reducing growth inhibition by salt than those from the nonsaline site. Colonization of roots and length of soil hyphae produced by the VAM fungi decreased with increasing soil salt concentration. Results indicate that preinoculation of transplants with VAM fungi can help alleviate deleterious effects of saline soils on crop yield.

Introduction

Increasing human populations will require increased agricultural production. Increases in agricultural production of 3 to 4% per year will be required over the next 20 years due to population growth alone, assuming that the availability of arable land remains unchanged (McDevitt, 1996; Toenniessen, 1984). About two-thirds of the projected increase in arable land is expected to come from the expansion of irrigation. Irrigation plays a pivotal role in this increase in agricultural production, but in general, it has failed to sustain increased productivity, transforming land, with time, into being unproductive, saline fallow.

Scientists have searched for new salt-tolerant crop plants (Aronson, 1985, 1989; Epstein, 1983; Gallagher, 1985; Glenn and O'Leary, 1985), developed salt-tolerant crops through breeding (Cuartero and Fernandez-Munoz, 1999; Ramage, 1980; Shannon, 1984), and continue to investigate the physiology of genetic alterations involved in salt tolerance (Apse et al., 1999). Other attempts to deal with saline soils have involved leaching excessive salts (Hamdy, 1990a, 1990b) or desalinizing seawater for use in irrigation (Lee, 1972; Muralev et al., 1997). Although these approaches have been successful, most are beyond the economic means of developing nations. Plant breeding may be available to those areas for some plant species, but they would not be available for all the crops being grown.

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Vesicular-arbuscular mycorrhizal (VAM) fungi are beneficial plant symbionts that form a mutualistic relationship with the roots of most crop plants. VAM fungi enhance the uptake of nutrients of low mobility in the soil solution such as P, Zn, and Cu, but they have many other impacts on crop productivity (Bethlenfalvay, 1992). VAM fungi can also reduce the impact of environmental stresses such as drought (Sylvia and Williams, 1992) and salinity (Ruiz-Lozano et al., 1996). Endomycorrhizal associations often result in greater yields of crop plants such as rice (Sharma et al., 1988), tomato and onion (Poss et al., 1985), and bell pepper (Hirrel and Gerdemann, 1980), even under saline conditions. Researchers proposed that increased P nutrition in VAM plants was responsible for the increased salt tolerance, and demonstrated that providing additional P fertilizer to the nonVAM plants could mimic VAM effects in saline soil (Hirrel and Gerdemann, 1980). Ojala et al. (1983) indicated that improved nutritional status of onion plants due to VAM fungi is at least partially responsible for increased plant growth under saline conditions.

Most studies concerning VAM-saline soil interactions have procedures in which salt was added progressively to the plant growth medium (usually soil), which does not simulate field conditions except where saline water is used for irrigation. To our knowledge, there are no reports of preinoculated VAM plants being transplanted into saline soils. Therefore, we tested the hypotheses that: (a) onion and lettuce plants preinoculated with VAM fungi have greater yield than noninoculated plants under saline conditions; and (b) plants colonized by VAM fungi from saline soil grow better than plants colonized by VAM fungi from non-saline soil when transplanted into saline soil. We also evaluated the potential of P fertilizer to reduce salt toxicity on nonVAM onions growing at the same salt levels as in the VAM inoculation experiments.

Materials and methods

Inoculum soils and trap cultures

The VAM inocula used in these experiments were isolated from a saline playa near Burns, Oregon (BU) and a nonsaline soil at the Vegetable Research Farm of Oregon State University, near Corvallis, Oregon (VF). Soils from both sites were analyzed for nutrient content and VAM fungal propagules. The BU soil was a sandy loam (Gee and Bauder, 1986) mapped in the

Alvodes series, classified as a fine, smectitic, mesic Sodic Aquicambids, with a pH of 8.8, and an organic matter content of 4.6%. It contained 11 mg kg⁻¹P (NaHCO₃-extractable); 6.9 mg kg⁻¹ N; 2691 mg kg⁻¹ K; and had a Sodium Adsorption Ratio (SAR) of 3.40. Electrical conductivity (EC) of the BU soil was 16 dS m⁻¹. The VF soil was mapped as part of the Chelalis series classified as a fine-silty, mixed, mesic Cumulic Ultic Haploxerolls with a pH of 6.3 and an organic matter content of 5.6%. It contained 67 mg kg⁻¹P (NH₄F-extractable); 9.4 mg kg⁻¹N; 382 mg kg⁻¹ K; and had a SAR of 0.038. The EC of the VF soil was 0.2 dS m⁻¹.

The initial search for spores of VAM fungi (assayed by wet sieving) yielded <2 spores g⁻¹ in the BU soil, and 6 spores g⁻¹ in the VF soil. Nevertheless, roots of plants at the BU (mostly annual grasses) and VF (corn, *Zea mays* L.) sites were highly colonized by VAM fungi. Onion (*Allium cepa* L. cv. White Bunching) and sudan grass (*Sorghum bicolor* L.) were planted in the two soils diluted with pasteurized sand to develop trap cultures of the VAM fungi and increase the relatively low spore numbers.

Estimation of VAM fungal inoculum potentials

The inoculum potential from each trap culture of the BU and the VF soils was estimated by the infection-unit method of Franson and Bethlenfalvay (1989). The fungi from each site consisted of several species. They were not completely identified taxonomically and were treated as a site-specific mixture of species. The VAM fungi from the BU soil were partially identified as *Glomus intraradices* (Schenck & Smith) and three other unidentified spore morphotypes. The VAM fungi from the VF soil (personal communication with R. P. Schreiner) were *Glomus mosseae* Gerd. & Trappe, *G. aggregatum/intraradices* Schenck and Smith, *Acaulospora trappei* Ames & Linderman, *Entrophospora infrequens* Ames & Schneider, an unknown clear *Glomus* sp., and an unknown yellow *Glomus* sp. Dilution of the site soils with a pasteurized coarse-loamy, mixed, mesic Fluventic Haploxerolls Newberg-series soil (pH 6.3, with a nutrient analysis of 14.6 mg kg⁻¹ NH₄-N; 1.4 mg kg⁻¹ NO₃-N; 27 mg kg⁻¹ NH₄F-extractable P; 587 mg kg⁻¹ total P; and a SAR of 0.051) was necessary to achieve similar number of propagules in the two soil mixes. After making the dilutions, another infection-unit assay was conducted that confirmed similar propagule numbers.

Seedling inoculation and growth conditions

Plug flats with 25-cc volume cells were filled with pasteurized (90 °C, 1 hr, twice at 24 hr interval) Newberg soil amended with Canadian peat moss (15% by volume) to increase moisture retention, and either BU or VF VAM trap-culture soil to give similar propagule numbers. The amount of BU or VF inoculum soil added to the plug soil was negligible relative to possible nutrient carry-over (VF=6.5%, BU=2.6%). A noninoculated control treatment was prepared using only the pasteurized Newberg soil plus peat. Lettuce (*Lactuca sativa* L. cv. Black-seeded Simpson) or onion (*Allium cepa* L. cv. White Bunching) were sown and thinned to one plant per cell, and grown on a bench with mist irrigation (19 d for lettuce, 30 d for onion). Prior to transplant into saline soils, roots of extra seedlings were assayed for VAM colonization (18 d for lettuce and 29 d for onion after seeding). Mean VAM colonization percentages of 10 seedlings per treatment were significantly different at 18% (BU) and 13% (VF) for lettuce roots (one-way ANOVA $p=0.046$), but not significantly different at 22% (BU) and 30% (VF) for onion roots (one-way ANOVA $p=0.112$). No VAM colonization was observed in the roots of noninoculated seedlings.

The Newberg soil was pasteurized, air-dried, potted (500 g/pot), and fertilized with 180 mL of ammonium nitrate solution ($1.56 \text{ g L}^{-1} \text{NH}_4\text{NO}_3$) per pot. Sodium chloride was added to the N-fertilizer solution to raise the EC of the remaining three solutions to 4, 8, and 12 dS m^{-1} , respectively. These salinity levels were selected to be at or above the level of 4 dS m^{-1} that is the toxic threshold for many plants. These solutions were applied only once at the beginning of the experiment to 13 replicate pots for each salt treatment for each plant species, and pots were equilibrated for 3 days before transplanting seedlings that had been preinoculated or not with VAM fungi.

Plants were grown for 11 weeks during the spring of 1999 in a greenhouse in Corvallis, Oregon. Deionized water was added to keep soil at 80% field capacity to prevent leaching. Long-Ashton nutrient solution (Hewitt, 1966) without P was added (25 mL pot^{-1}) once per week from week 3 through 11.

Plant growth responses

Leaf color. Seven weeks after transplant, the color of lettuce leaves was measured with a meter (Minolta SPAD 502 meter) to quantify chlorophyll content nondestructively. Five measurements were made on

the distal halves of recently fully-expanded leaves from 10 randomly chosen, replicate plants.

Shoot and root mass. Ten randomly chosen plants from the 13 in each treatment were harvested at the end of the experiments. Shoots were removed after 58 (lettuce) or 76 (onion) days of growth in saline soils. Onion bulbs were included in the shoot weights. Roots were washed to remove soil and weighed. Then shoots and roots were oven-dried (48 h, 60 °C) and ground in a Wiley mill (20 mesh).

Tissue elemental composition. The dried and ground shoot tissues of both plant species and root tissue of onion were analyzed for mineral nutrients by the Oregon State University Central Analytical Laboratory. Statistical analysis of mineral concentrations of onion plants was not possible because treatment samples had to be combined due to the limited plant biomass.

VAM root colonization. Assessment of roots for VAM colonization was made at the end of the experiment on a random sampling of the root system. All VAM fungal structures (hyphae, arbuscules, and vesicles) found in the roots were counted. Stained root pieces were examined under a dissecting scope at 40X magnification and extent of colonization was assessed by the grid-line intercept method (Giovannetti and Mosse, 1980).

Soil and hyphal length assays

Electrical conductivity and pH at harvest. After retrieving roots of the harvested plants, soil (~ 15 g) was bagged and refrigerated (4 °C) until hyphal length assays were done. The remaining soils were allowed to air dry for 4 d in the greenhouse before determining EC of the soil in each replicate pot at the end of the experiment. Soil pastes of each sample were prepared and the liquid extracted with a vacuum pump prior to making measurements using an Orion electrical conductivity meter. Soil pH was determined using 1:2 w:w soil:deionized water suspensions.

Extraradical hyphal length. Soil hyphae were extracted using the membrane-filter technique described by Hanssen et al. (1974) and modified as follows. At the end of the experiment, three pooled samples of 4 g (soil from two replicate pots from each treatment combined into one sample) were used to assess VAM fungal hyphal length by suspending soil in saline

buffer and mixing aliquots in glycerol:lactic acid (1:1 v:v). Hyphal fragments in aliquots were transferred onto a filter membrane (47-mm diameter, 3-mm grid) and stained with trypan blue. Intercepts were observed and counted under a stereo microscope at 40 \times magnification. Hyphal length was calculated by the grid-line intersect method (Giovannetti and Mosse, 1980) adjusting for soil moisture to report hyphal length on a dry soil basis (Gardner, 1986).

Experimental design and statistical analysis

The experiment was a factorial design with VAM fungi (VF and BU VAM fungal source sites, and nonVAM) and salt levels [EC 2.66 (control, hereafter called EC 2), 4.0, 8.0, and 12.0 dS m⁻¹] as factors. There were 12 treatments for each plant species with 13 replicate plants per treatment, but only 10 randomly chosen replicates were measured or harvested. Lettuce dry-mass data were analyzed as for a Complete Randomized Design. Onion dry-mass data were analyzed by Kruskal-Wallis one-way ANOVAs within each salt level, first including data of three fungal treatments and then including only those of the two VAM fungal treatments. ANOVA tools were used to find treatment differences when the data did not violate ANOVA's assumptions. Logarithmic transformations were applied when useful in correcting uneven variances, and results were back-transformed for interpretation purposes. Kruskal-Wallis nonparametric analysis was used when uneven variances were not corrected by this transformation (e.g., onion dry mass).

Orthogonal contrasts were used to compare plant responses of VAM against those of nonVAM plants (e.g., lettuce mass and leaf color). To analyze tissue elemental composition of lettuce plants, elements were divided as follows: (a) those for which two-way ANOVA resulted in a significant VAM versus salt interaction, and (b) those for which such interaction was not significant ($p > 0.05$). When this interaction in the full model ($n=36$ data points) was significant, the VAM main effect was used as the one-way ANOVA factor ($n=9$ data points), and differences between nonVAM and VAM responses and between BU- and VF-fungal responses were tested by orthogonal contrasts within each salt level. When the VAM versus salt interaction was not significant, differences between elemental composition of nonVAM and VAM plants were analyzed with orthogonal contrasts on the full model ($n=36$).

P fertilization experiment

This greenhouse experiment was conducted to determine to what extent P fertilization would ameliorate soil salinity effects on onions grown in highly saline and low-P soil. Pasteurized Newberg soil, amended with peat moss (15% v:v) for moisture retention, was used to grow nonVAM onion seedlings in plug flats. Air-dried Newberg soil was potted (500 g/pot) and all pots received 100 mg L⁻¹ N (as NH₄NO₃). Additional solutions were prepared using this solution as base to provide P at 0, 7.5, 15, and 22.5 mg L⁻¹ soil (as P₂O₅) initially, and sufficient NaCl to increase the EC of the solutions to 8 or 12 dS m⁻¹. Because plants did not grow well, P then was added to the nutrient solution (at 0, 15, 30, and 45 mg L⁻¹) to the corresponding pots after week 7 and once a week for the next 9 weeks. All plants were watered to maintain 80% of field capacity to avoid leaching. Biomass data were analyzed by Kruskal-Wallis tests.

Results

VAM inoculation experiment

Shoot dry mass. Dry shoot masses of VAM lettuce and onion plants were significantly greater than those of nonVAM plants (Figures 1, 2). Mean dry masses of nonVAM lettuce plants (Figures 1, 3) were smaller than the mean of the VAM plants (combined from both BU and VF inoculum sources) by increasing amounts with increasing salt level: 3.4% at EC 2 ($p=0.047$); 8.2% at EC 4 ($p=0.001$); 11.7% at EC 8 ($p=0.006$); and 29.3% at EC 12 ($p=0.001$). The mean shoot dry mass of the VAM onions (combined from both BU and VF inoculum sources) was 6 to 18-fold greater than the nonVAM onions (Figures 2, 4). At the highest salt level (12 dS m⁻¹), shoot dry weight of nonVAM onion plants was 88% less than that of the mean of the combined VAM plants (Kruskal-Wallis $p=0.0002$). Shoot masses of plants inoculated with BU and VF VAM fungi did not differ.

VAM colonization and extraradical hyphal length.

In lettuce, percent colonization by VAM fungi from either source within the same salt level was not significantly different at harvest ($p > 0.05$, Kruskal-Wallis test) (Table 1). Onion roots were more extensively colonized by BU than by VF VAM fungi (Table 1), but dry shoot mass did not differ between plants inoculated with either group of fungi. VAM colonization in

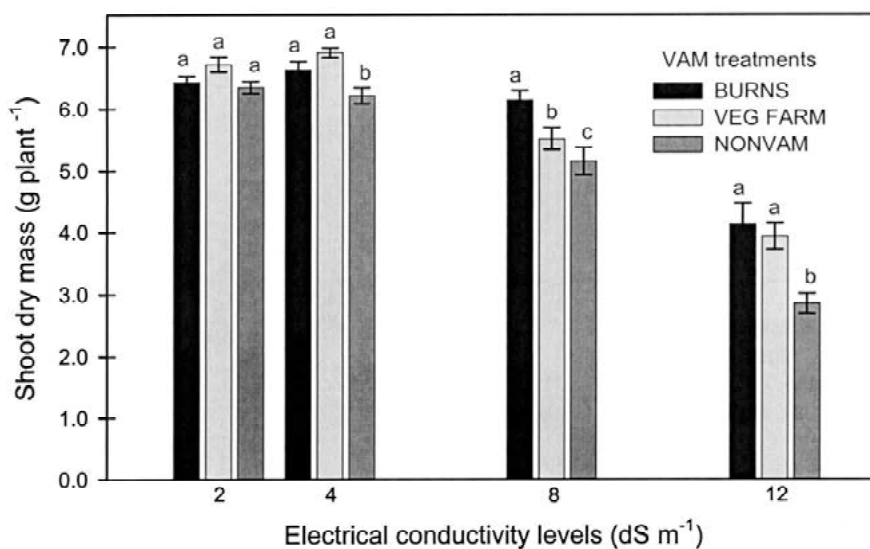


Figure 1. Dry mass of lettuce shoots inoculated with VAM fungal mixtures or not inoculated before transplant and grown for 58 days in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS m⁻¹). Fungal treatments were: Burns (VAM fungi from a saline soil), Veg Farm (VAM fungi from a nonsaline soil), or not inoculated (nonVAM). Means with the same letter within the same salt level are not different at $p \leq 0.05$ as indicated by one-way ANOVAs. Bars represent \pm SE of the means of 10 replicate plants.

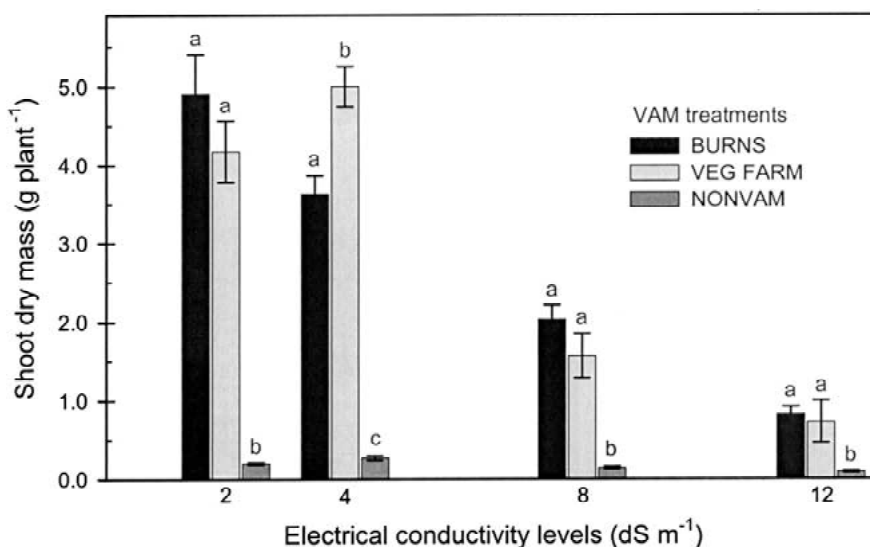


Figure 2. Dry mass of onion shoots inoculated with VAM fungal mixtures or not inoculated before transplant and grown for 76 days in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS m⁻¹). Fungal treatments were: Burns (VAM fungi from a saline soil), Veg Farm (VAM fungi from a nonsaline soil), or not inoculated (nonVAM). Means with the same letter within the same salt level are not different at $p \leq 0.05$ as indicated by Kruskal-Wallis nonparametric test. Bars represent \pm SE of the means of 10 replicate plants.

both inoculation treatments significantly decreased as salinity increased for both plant species (Table 1). No VAM colonization was observed in nonVAM control plants.

Length of soil hyphae in each of the VAM fungal treatments was not significantly different at varying soil salt levels (Table 1), although there was a trend

to decreasing soil hyphal lengths as salt concentration increased.

Leaf color of lettuce. When measured at week 7, chlorophyll content of recently expanded leaves was influenced by salt and by VAM fungi (ANOVA $p=0.008$ and $p<0.001$, respectively). In nonVAM

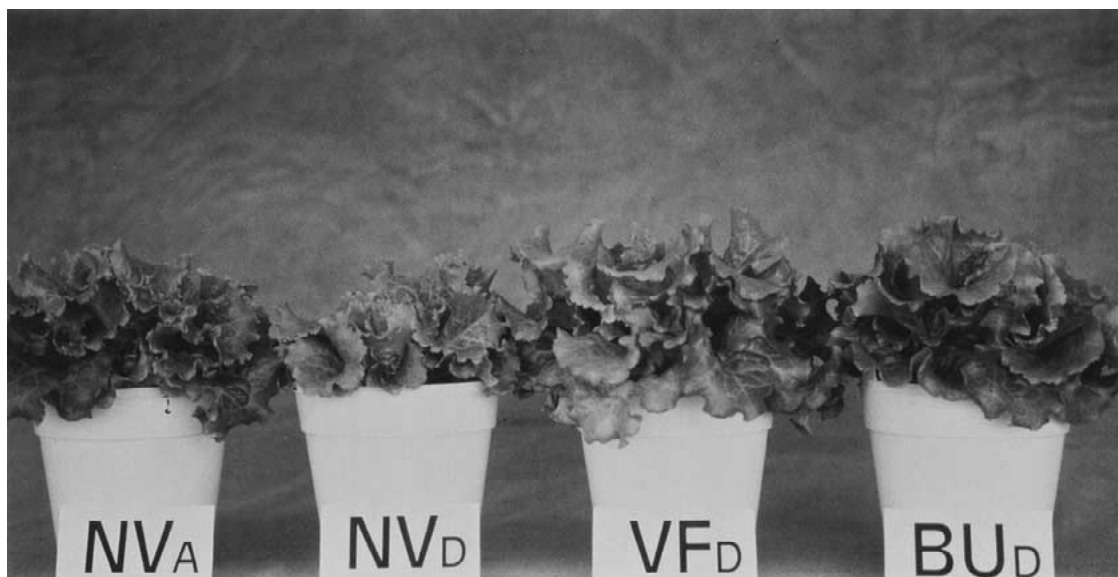


Figure 3. Photograph of representative lettuce plants grown in soil treated with the highest level of NaCl solutions D ($EC=12 \text{ dS m}^{-1}$) compared to the untreated control level A ($EC=2 \text{ dS m}^{-1}$). Fungal treatments were: NV= nonVAM, VF= Veg Farm VAM fungi, or BU= Burns VAM fungi.

Table 1. Colonization of roots and production of extraradical hyphae by VA mycorrhizal (VAM) fungal mixtures from two sites inoculated to lettuce or onion transplants grown in soil salinized to four levels with NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS m^{-1}). Fungal mixtures were from Burns (BU, a saline soil) and the Vegetable Research Farm (VF, a nonsaline soil); controls were not inoculated (NV). Values are the means of 10 replicate plants (\pm SE). Within each parameter, each plant species, and at each salt level, VAM fungal treatment means followed by the same letter are not significantly different ($p < 0.05$) as determined by Kruskal-Wallis nonparametric test for root colonization and by orthogonal contrasts for hyphal length. $EC(t)$ is the electrical conductivity of treatment saline solutions

Lettuce				
$EC(t) (\text{dS m}^{-1})$	Colonization (%)		Hyphal length (m g^{-1} soil)	
	BU	VF	BU	VF
2	43.0 (5.2) a	34.8 (4.0) a	11.31 (1.83) a	14.48 (2.03) a
4	32.7 (3.8) a	32.6 (1.5) a	10.47 (1.12) a	9.87 (0.93) a
8	29.8 (3.5) a	27.8 (1.1) a	9.13 (0.66) a	10.02 (0.55) a
12	26.2 (3.1) a	29.9 (1.4) a	7.43 (1.49) a	8.54 (1.06) a

Onion				
$EC(t) (\text{dS m}^{-1})$	Colonization (%)		Hyphal length (m g^{-1} soil)	
	BU	VF	BU	VF
2	61.7 (3.0) a	28.0 (3.4) b	7.10 (1.01) a	8.72 (0.75) a
4	59.4 (2.4) a	28.8 (4.5) b	6.96 (0.86) a	7.09 (0.75) a
8	49.0 (6.2) a	20.3 (1.7) b	8.54 (0.98) a	6.00 (1.76) a
12	38.8 (5.2) a	18.3 (1.8) b	4.97 (0.75) a	5.04 (0.79) a

plant leaves, chlorophyll content was linearly and more negatively affected by increasing salt levels than leaves of VAM plants (orthogonal contrast, $p=0.002$). Leaves of VAM lettuce plants in the highest salt treatment had significantly more chlorophyll than the non-VAM plants (orthogonal contrast, $p < 0.001$) (Figure 5). No significant differences were observed between plants inoculated with the VAM fungi from the two sources.

Tissue elemental composition. VAM lettuce plant tissues had significantly greater concentrations ($p < 0.05$) of Ca, P, Zn, B, Cu, Mg and Na than nonVAM plants (Table 2). Concentrations of other elements were not consistently different between the VAM and nonVAM plants at all salt levels. VAM and nonVAM lettuce plant shoots had comparable amounts of Na in their tissues at each salt level. Chloride levels in the VAM and nonVAM treatments varied, being higher in VAM than nonVAM plants at the EC 4 and EC 8 levels of salinity, high in the VF and NV treatments at the EC 12 level, but very low in the BU treatment.

VAM onion roots generally had greater P, Fe, Cu, and Na concentrations (except at EC 12 in BU) than nonVAM onion roots, but shoot concentrations were greater only for P and Cu (Table 3). Concentrations of other elements varied considerably among VAM treatments and EC levels. Although statistical ana-

Table 2. Concentrations of mineral elements in shoots of lettuce plants inoculated or not with VA mycorrhizal fungal mixtures from two sites and transplanted into soil with different levels of salinity (EC 2 control, EC 4, EC 8, and EC 12 dS m⁻¹). Fungal mixtures were from Burns (BU, a saline soil) and the Vegetable Research Farm (VF, a nonsaline soil); controls were not inoculated (NV). Values are means of three replicate plants (converting from % to g kg⁻¹).

VAM source	EC(dS m ⁻¹)	P	Ca	Mg	K	Mn	Fe	Cu	B	Zn	Na	Cl	C	N
		(----- g kg ⁻¹ × 0.1 -----)					(----- mg kg ⁻¹ -----)						(g kg ⁻¹ × 0.1)	
BU	2	0.19	0.86	0.35	2.03	305	213	6.33	26.67	47.33	1135	261	43	1.60
VF	2	0.20	0.79	0.33	1.73	191	298	5.67	25.67	34.67	1297	95	43	1.50
NV	2	0.13	0.76	0.29	2.01	273	434	4.00	24.00	33.67	779	277	43	1.75
BU	4	0.17	0.78	0.33	2.17	233	258	6.00	26.67	47.33	2924	9608	42	1.66
VF	4	0.18	0.79	0.34	1.99	159	239	6.00	25.00	37.33	3726	4221	43	1.48
NV	4	0.12	0.76	0.30	2.35	199	474	4.00	24.67	34.00	2529	1804	42	1.67
BU	8	0.17	1.02	0.43	3.05	210	345	7.67	31.00	56.33	6609	17449	42	2.12
VF	8	0.19	1.60	0.75	2.79	318	666	8.67	29.00	65.67	8826	8643	41	1.90
NV	8	0.14	0.95	0.37	3.14	198	467	7.33	29.00	48.33	6074	6766	41	2.03
BU	12	0.17	1.31	0.47	3.94	199	244	8.33	30.00	56.33	10524	3720	40	2.65
VF	12	0.20	2.11	0.93	3.57	294	747	11.00	31.00	88.00	14264	23243	39	2.51
NV	12	0.12	1.36	0.44	3.72	200	416	5.00	27.33	39.67	11575	26084	40	2.63

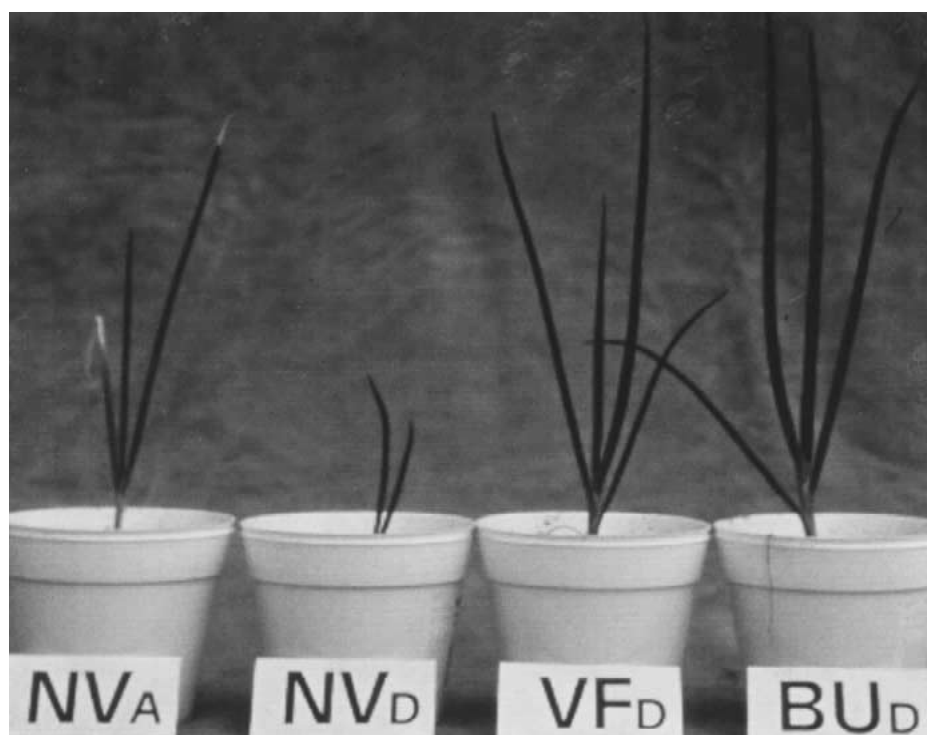


Figure 4. Photograph of representative onion plants grown in soil treated with the highest level of NaCl solution D (EC=12 dS m⁻¹) compared to the untreated control level A (EC=2 dS m⁻¹). Fungal treatments were: NV= nonVAM, VF= Veg Farm VAM fungi, or BU= Burns VAM fungi.

Table 3. Concentrations of mineral elements in roots and shoots of onion plants inoculated or not with VA mycorrhizal fungal mixtures from two sites and transplanted into soil with different levels of salinity (EC 2 control, EC 4, EC 8, and EC 12 dS m⁻¹). Fungal mixtures were from Burns (BU, a saline soil) and the Vegetable Research Farm (VF, a nonsaline soil); controls were not inoculated (NV). Samples analyzed were the combined 10 plants from each treatment. * indicates not enough tissue to analyze

Roots														
VAM source	EC(dS m ⁻¹)	P	Ca	Mg	K	Mn	Fe	Cu	B	Zn	Na	Cl	C	N
		(- - - - - g kg ⁻¹ × 0.1 - - - - -)					(- - - - - mg kg ⁻¹ - - - - -)					(g kg ⁻¹ × 0.1)		
BU	2	0.31	0.80	0.57	1.60	233	3057	71	42	82	6206	1896	42	2.66
VF	2	0.30	0.59	0.46	1.44	164	2523	50	31	34	5575	1514	43	2.32
NV	2	0.11	1.05	0.69	3.34	178	2306	14	55	26	1204	6341	41	3.82
BU	4	0.32	0.82	0.65	1.79	183	2467	56	34	89	12707	15147	41	3.43
VF	4	0.27	0.67	0.50	1.62	185	3032	45	33	45	11901	17155	41	2.38
NV	4	0.11	0.99	0.57	3.46	146	2004	13	51	31	2449	12412	41	4.00
BU	8	0.33	0.67	0.63	2.66	173	2304	47	45	95	16093	25430	40	3.89
VF	8	0.33	0.62	0.58	2.93	202	2511	62	50	50	15834	24164	39	3.94
NV	8	0.10	0.87	0.62	3.05	155	2012	11	70	33	6752	16288	41	3.78
BU	12	0.31	0.58	0.61	3.12	181	1913	40	38	86	13895	28525	41	4.13
VF	12	0.29	0.61	0.57	2.88	196	2771	47	43	48	16296	24360	39	3.78
NV	12	0.12	0.77	0.55	2.70	203	2590	12	91	35	10058	—*	41	3.78
Shoots														
VAM source	EC(dS m ⁻¹)	P	Ca	Mg	K	Mn	Fe	Cu	B	Zn	Na	Cl	C	N
		(- - - - - g kg ⁻¹ × 0.1 - - - - -)					(- - - - - mg kg ⁻¹ - - - - -)					(g kg ⁻¹ × 0.1)		
BU	2	0.23	1.59	0.33	1.82	133	147	6	23	40	145	1612	44	2.85
VF	2	0.22	1.53	0.36	1.44	119	124	6	23	24	109	1535	45	2.45
NV	2	0.10	1.34	0.27	2.75	198	349	4	28	42	196	3942	43	4.59
BU	4	0.21	1.60	0.32	1.95	114	119	7	20	41	240	11122	44	2.74
VF	4	0.22	1.18	0.26	1.55	97	104	6	19	23	247	8721	44	2.28
NV	4	0.09	1.37	0.27	2.75	182	300	4	27	46	282	7210	43	4.52
BU	8	0.20	1.49	0.30	2.50	125	153	7	21	43	747	20376	43	3.38
VF	8	0.25	1.76	0.35	2.64	147	153	9	21	35	818	19736	43	3.40
NV	8	0.08	1.76	0.31	2.20	145	433	4	29	43	1390	17389	43	4.31
BU	12	0.19	1.72	0.31	2.74	136	227	7	22	45	1651	27063	42	3.72
VF	12	0.20	1.94	0.36	2.58	210	181	8	22	35	1205	24280	43	3.44
NV	12	0.08	2.00	0.38	2.12	275	640	5	30	40	3153	29369	41	4.34

lysis was not possible, it appears that Na and Fe in VAM onion roots were not readily translocated to the shoots. For example, VAM onion root Na concentrations were greater than those of nonVAM plants, yet

VAM onion shoot Na concentrations were lower than those of nonVAM plants (Table 3).

Soil EC and pH at harvest. Soil in which nonVAM onion plants were grown had higher EC at the end

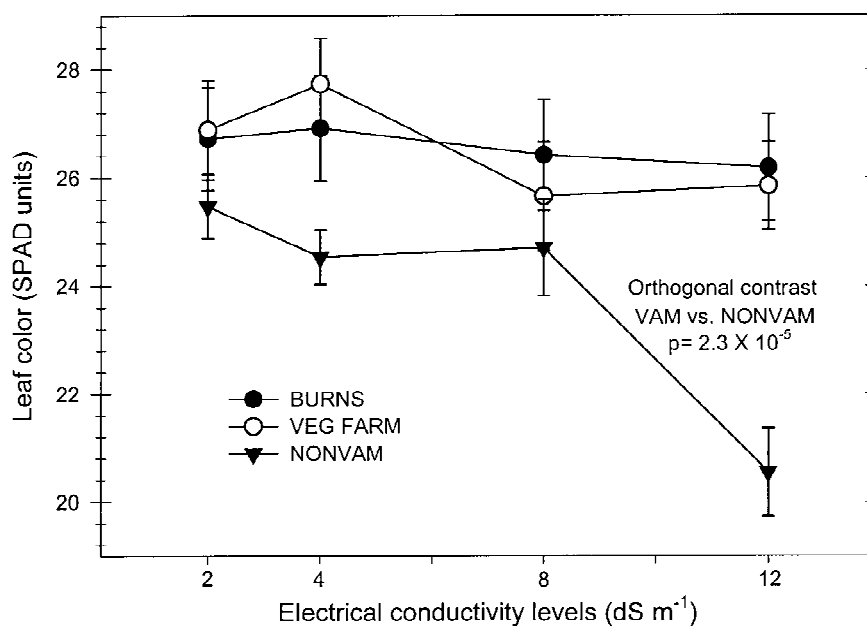


Figure 5. Leaf color of lettuce shoots inoculated with VAM fungal mixtures from two sites: Burns (high salt) or Veg Farm (low salt) or not inoculated (nonVAM) prior to transplant in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS m⁻¹). Values are means of five measurements on 10 replicate plants. Bars represent \pm SE of the means.

of the experiment within each salt level than the soil in which VAM onion plants were grown (Table 4). This difference was more apparent at low salt levels in the onion experiment than in the lettuce experiment. Tissue analysis and soil EC measured at the end of the experiments confirmed that VAM plants absorbed greater quantities of Na than the nonVAM plants.

In the lettuce experiment, soil pH decreased with the addition of NaCl ($p < 0.001$) (data not shown). VAM treatments also affected pH ($p = 0.002$). The pH of soils inoculated with the VF fungi at the two highest salt levels was lower than that of either of the other two fungal treatments. In soil in which plants inoculated with VF VAM fungi were grown, mean pH was 6.3 at EC 2 (not significantly different from other fungal treatments, $p = 0.252$) and 5.2 at EC 12 (significantly different from the soil in which plants inoculated with BU VAM fungi were grown, $p < 0.001$). In the onion experiment, pH also decreased with increasing salt concentration. The difference in pH between VAM and nonVAM soil was larger at lower than higher salt levels. At the highest salt level, pH in soil in which non-inoculated plants were grown differed the most (5.5 to 6.2); pH of soil in which plants inoculated with VF VAM fungi were grown ranged from 5.4 to 5.8, and 5.7 to 6.0 where plants were inoculated with BU VAM fungi. At the highest EC treatment in the

onion experiment, pH of soils where plants were inoculated with BU and VF VAM fungi was significantly different ($p = 0.046$).

P fertilization experiment

Increasing salt concentration in soil in which nonVAM onions were grown progressively reduced shoot and root dry mass (Figure 6). This effect was reduced somewhat by additional P. At the highest salt level (12 dS m⁻¹), many onion plants died when no additional P was provided. Mean shoot and root dry mass of onions treated with 45 mg L⁻¹ P were two-fold greater than onions that received 15 mg L⁻¹ P. The amount of P added did not stimulate onion plants to grow as large as in the experiment where plants were inoculated with VAM fungi, even in the absence of salt. Some of the added P may not have been available to the plant due to low pH, to the formation of insoluble precipitates, or to fixation by the colloidal soil clays.

Discussion

The finding that mycorrhizal lettuce and onion transplants grown in saline soil had greater fresh and dry

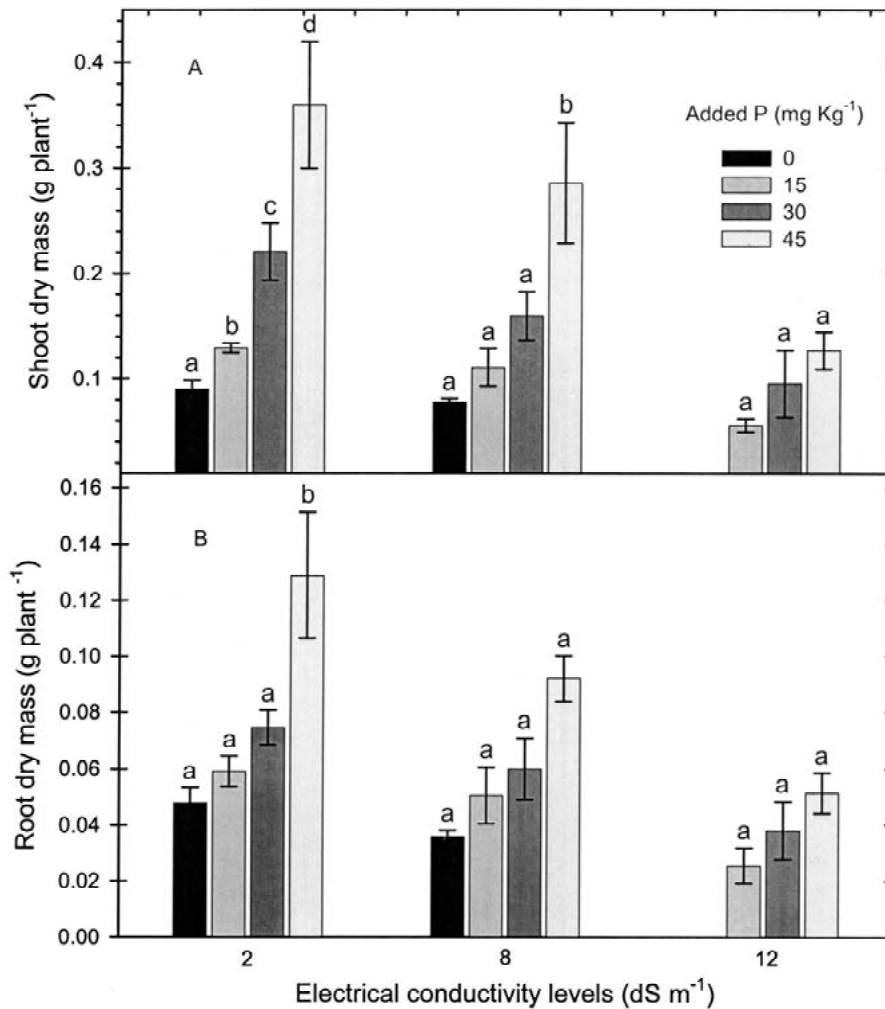


Figure 6. Dry mass shoots (A) or roots (B) of onions transplanted into Newberg soil treated with NaCl solutions (EC 2 control, EC 8, and EC 12 dS m⁻¹) and fertilized with various levels of P from 15 up to 45 mg L⁻¹. Means with the same letter within the same salt level are not different at $p=0.05$ as indicated by Kruskal-Wallis tests. Bars represent \pm SE of the means of 10 replicate plants.

shoot mass than noninoculated plants supports our first hypothesis that preinoculated VAM plants grow better than nonVAM plants under saline conditions. Onions were more responsive to VAM inoculation than lettuce plants. Inoculation of transplants prior to salt exposure bypasses the potential inhibitory effects that salt could have on VAM 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 VAM effects on plants under saline conditions, but only few adjusted the inocula used, as we did, to have similar levels of colonization, and in most previous studies, saline solutions were added progressively to soil after some time allowed for VAM establishment (Al-Karaki, 2000;

Duke et al., 1986; Estaun and Save, 1990; Mancuso and Rinaldelli, 1996; Rinaldelli and Mancuso, 1996). Under field conditions, salinity level is not an adjustable variable. The procedure we used of preinoculating transplant seedlings with VAM fungi can be of practical importance in the cultivation of many horticultural crops grown in saline soils as well as in revegetation of non-agricultural saline lands.

Contrary to our second hypothesis, VAM fungi from a saline soil did not alleviate salt effects better than VAM fungi from nonsaline soil. In fact, the fungi from the VF soil were more effective than the BU fungi at the high salt levels. This is also interesting because they did not colonize onion as well as the BU fungi. Root colonization in lettuce was not statistically

Table 4. Electrical conductivity (EC in dS m^{-1}) of soil salinized with NaCl solutions (EC_t) before transplanting lettuce or onion plants inoculated or not with VA mycorrhizal (VAM) isolated from a saline site (Burns, BU) or a nonsaline site (Vegetable Research Farm, VF); controls were not inoculated (NV). Values are means of soil extracts from 10 replicate pots (+/- SE) measured at the end of the experiment (EC_e)

VAM Source	EC_t	EC_e	
		Lettuce	Onion
BU	2	0.18 (0.05)	0.12 (0.02)
VF	2	0.15 (0.02)	0.24 (0.05)
NV	2	0.17 (0.02)	3.25 (0.10)
BU	4	0.32 (0.02)	0.99 (0.16)
VF	4	0.28 (0.02)	0.51 (0.09)
NV	4	0.45 (0.04)	4.63 (0.11)
BU	8	2.71 (0.08)	5.90 (0.33)
VF	8	2.47 (0.15)	7.44 (0.68)
NV	8	3.06 (0.15)	8.60 (0.17)
BU	12	6.66 (0.39)	11.49 (0.18)
VF	12	6.47 (0.23)	10.86 (0.68)
NV	12	8.32 (0.29)	12.95 (0.12)

different for the two VAM inocula within each salt level, but there was significantly lower colonization in onion by the VF fungi than the BU fungi. In spite of differences in VAM colonization, the increase in shoot mass brought about by the VAM fungi from the two sources (compared to the nonVAM plant response) was statistically significant in both plant species (Figures 1 and 2). This indicates that the beneficial effects of VAM fungi on plant growth were, to some degree, independent of percent root colonization and of hypothetical adaptation to salt by the BU fungal symbionts. Pond et al. (1984) and Copeman et al. (1996) investigated crop plant responses brought about by VAM fungi from saline soils as compared to VAM fungi from nonsaline soils. Results of Pond et al. (1984) were inconclusive since the same VAM fungus species either increased or decreased shoot dry weight (compared to noninoculated controls) of tomato plants grown in saline soil. Copeman et al. (1996) showed that tomato shoot growth was enhanced by inoculation with VAM fungi from a nonsaline soil and was inhibited by inoculation with VAM fungi from a saline soil. While our results did not show differential dry mass of plants treated with VAM fungi from either source, leaf color,

and concentrations of some elements were different in the tissues of plants treated with different VAM fungi. These differences may relate to mechanisms by which fungi with different adaptations affect the plant. We conclude that different capacity of different VAM fungi to influence salinity effects is unrelated to any hypothetical adaptation to salinity and thus simply reflects isolate or ecotype variation.

Addition of P is known to relieve salt stress in plants (Awad et al., 1990; Champagnol, 1979; Hirrel and Gerdemann, 1980; Poss et al. 1985; Ruiz-Lozano et al., 1996). In our Newberg soil, however, additions of P to nonVAM onion plants did not sufficiently increase available P for plant uptake. Each pot (containing 500 g dry soil at potting) treated weekly with 45 mg L^{-1} P received a total of 10.1 mg P during the last 9 weeks of the experiment. It is likely that this level of fertilization would exceed economical thresholds in onion crop production for this soil. P availability is reduced in saline soil not only because of ion competition that reduces the activity of P, but also because P concentrations in soil solution are tightly controlled by sorption processes and by the low solubility of Al-P or Fe-P precipitates (Grattan and Grieve, 1999). Therefore, P fertilization may be successful in alleviating salt stress if the rates applied overcome the P-fixing capacity of the soil.

Although VAM fungi mitigate growth reduction caused by soil salinity (Gupta and Krishnamurthy, 1996; Hirrel and Gerdemann, 1980; Jindal et al., 1993; Ojala et al., 1983; Pfeiffer and Bloss, 1988; Pond et al., 1984; Poss et al., 1985; Tsang and Maun, 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 phosphorus accumulation." Duke et al. (1986) concluded that improved P uptake by VAM vs. nonVAM citrus plants did not totally account for the improved salt tolerance of VAM plants. Other mechanisms that improve salt tolerance may include maintaining membrane integrity (Rinaldelli and Mancuso, 1996; Mancuso and Rinaldelli, 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 all involve effects of VAM fungi.

Content and concentrations of P in VAM lettuce and onion plants in our experiments were higher than those of nonVAM plants. Increased growth compared to the nonVAM plants is evidence of the beneficial effects of VAM fungi on plants growing in saline soil. The ability to protect the plants from salt stress corresponded to the growth-promoting effect of VAM fungi. Selective ion intake may be the reason why onion plants in our P fertilization experiment grew only after more P was available.

Our results generally showed greater concentrations of P, Zn, Cu, Na (except in onion shoots), and Fe (in onion roots) in VAM than in nonVAM plants. Opposite to our findings, Pfeiffer and Bloss (1988) showed that the addition of NaCl resulted in reduced concentrations of P, Cu, and Fe in plant tissue. Rosendahl and Rosendahl (1991) reported greater water uptake by VAM plants under saline conditions. It is possible that improved plant nutrition by VAM 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 (Larcher, 1980) must be more efficient if the nutrition in the cell remains balanced. Reducing cell-membrane permeability by providing P via VAM or inorganic fertilizer to plant cells (particularly root cells) enhances cell structural organization. As cells are able to maintain membrane integrity under saline conditions, it is possible to avoid interference of excessive ions with metabolic processes (e.g., photosynthesis). In our study, VAM lettuce plants had greener leaves than nonVAM plants at the highest salt level, suggesting that salt interfered in chlorophyll synthesis or turnover more in nonVAM than VAM plants. Tsang and Maum (1999) reported a similar response in a mycorrhizal foredune plant; Ezz et al. (1994) also reported that VAM inoculation ameliorated a decrease in chlorophyll for sour orange seedlings watered with saline water. Thus, we suggest that VAM fungi improved P nutrition of plants under saline conditions and reduced the negative effects of Na⁺ and Cl⁻ by maintaining vacuolar membrane integrity, which prevented these ions from interfering in growth metabolic pathways.

In the case of onion, our data showing that Na was retained in roots without being translocated to the shoots suggest some other mechanism whereby salt tolerance in VAM plants is increased. How or where Na was retained in the roots was not determined, but we suggest the possibility that it might have been retained in intracellular VAM fungal hyphae or was compartmentalized in the root cell vacuoles without

moving into root cell cytoplasm from which it could be translocated to the shoots.

In conclusion, preinoculation with VAM fungi reduced the detrimental effects of salt in the soil on lettuce or onion transplants. Fungi isolated from saline or nonsaline soils did not differentially affect the degree to which VAM fungi reduced plant response to salt stress. Preinoculating transplants could be an economically feasible means of growing crops in agrosystems or in the restoration/reclamation of sites affected by salt. The mechanism(s) by which VAM 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 tissues, including the VAM fungal hyphae in the roots.

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