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Effects of Endomycorrhizal Fungi and Salt Stress on Nutrient Acquisition and Growth of *Pistacia vera* L.

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Abstract: The effects of Arbuscular Mycorrhizal Fungi (AMF) *Glomus etunicatum* inoculation on growth and mineral acquisition of *Pistacia vera* L. grown under salinity condition was studied. Different concentration of NaCl as 0 (control), 50 (low), 100 (medium) and 200 mM (high) were employed for salinity stress. Plants were grown in a sterilized, low-P sandy soil in a greenhouse. It was seemed that mycorrhizal colonization was higher in the control than in saline soil condition. Dry weight of shoots, roots and also leaf area of Mycorrhizal (M) plants were higher than Non Mycorrhizal (NM) ones in both control and salinity conditions. The contents of P, K, Cu and Zn were higher in M than NM plants in control, low and medium salinity conditions. Concentration of Na in shoots of M plants was lower than NM grown under salinity condition. Generally, it can be said that M plants of *Pistacia vera* showed higher tolerance toward salinity than NM plants and their growth improved by AMF colonization.

Key words: Arbuscular mycorrhizal fungi, growth, *Pistacia vera,* salinity

INTRODUCTION

In nature, plants are frequently exposed to adverse environmental conditions that have negative effects on plant survival, development and productivity. Salinity is considered as the most important abiotic factors limiting plant growth and yield in many areas[1]. High salinity causes both hyperionic and hyperosmotic stress effects and the consequence of these can be plant demise[2-4]. Most commonly, the stress is caused by high Na^+ and Cl^- concentrations in the soil solution. Altered water status most likely bring about initial growth reduction; however, the precise contribution of subsequent processes to inhibition of cell division and expansion and acceleration of cell death has not been well elucidated[4,5]. Membrane disorganization, reactive oxygen species, metabolic toxicity, inhibition of photosynthesis and attenuated nutrient acquisition are factors that initiate more catastrophic events[4,6-8]. Plants can respond to salt stress at morphological, anatomical and cellular levels with modifications that allow the plant to avoid the stress or to increase its tolerance[9]. The morphological and anatomical adaptations can be of vital importance for some plant species, but they are not a general response of all plants species. In contrast, the cellular response to salt stress seem to be conserved in the plant kingdom.

In addition to the intrinsic protective systems of plants against stress, plants grow in association with a number of soil microorganisms that can alleviate the stress symptoms. Arbuscular Mycorrhizal (AM) fungi are widespread microorganisms able to establish a symbiotic association with the roots of most terrestrial plants. AM plants have an improved ability for growth and tolerance to salt stress[10-12].The improved productivity of AM plants has been attributed especially to enhanced acquisition of low mobility nutrients such as P, Zn and Cu[13-16] and improved water relations[17,18]. Neverthless, the physiological role of the AM symbiosis is not limited to uptake and transfer of nutrients to the host plant. Many other beneficial effects for the host plant and for ecosystems have been described[12], including enhancement of tolerance to salinity[10,19,20]. Improved salt tolerance following mycorrhizal colonization may be caused by more efficient P uptake by mycorrhizal plants in P-deficient soils[21], Leading to increased growth and subsequent dilution of toxic ion effects[22]. In some cases, however, salt tolerance of AM plants appears to be independent from plant P concentration[23,24].

Salinity tolerance by *Pistacia vera* plants is a major concern in arid and semiarid regions with high salinity, due to the negative correlation between excess salinity and yield[25-27]. Wide variation in plant responses to AM

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fungi inoculation has been reported for different plant species under environmental stresses[13,19,21]. It has been suggested that mycorrhizal colonization is a host-dependent and heritable trait[28,29]. Study of the symbiotic interactions between AM fungi and host plants under saline conditions should help to optimize the beneficial effects of AM fungi. The objectives of this study were to determine the effects of mycorrhizal infection on growth parameters and nutrient acquisition of *Pistacia vera* under different levels of soil salinity.

MATERIALS AND METHODS

A greenhouse experiment was conducted at 24±5°C under artificial illumination during the spring of 2004. The day length was 18 h and the illumination intensity was 6500-800 lux from fluorescent tubes. Pistacia plants were grown in a silty clay soil (fine, mixed) mixed with washed sand (clay:sand, 1:5). Soil properties after mixture with sand were 2.7% silt, 13.7% clay, 83.6% sand, pH 6.8, Electrical Conductivity (EC) 1.3 dS m^{-1}. This soil mixed with peat (soil:peat, 9:1). P was not added to the soil mixes in order to stimulate mycorrhiza formation. Seeds of *Pistcia vera* (sort of Akbari) collected from city of rafsanjan in the southeasterly of iran were surface sterilized with 20% solution of sodium hypochlorite in distilled water[30] and aseptically germinated on a moist mix of peat and sand in a polystyrene trays. 20-day-old seed lings, uniform in size, were transplanted into plastic pots for plant growth.

Half of the pots received the AMF *Glomus etunicatum* and trappe by placing 70 g (moist weight) of inoculum in soil below the pistacia seedling prior to planting. The AMF inoculum consisting of soil and root fragments and spores, placed directly adjacent to each seedling root to facilitate fungal colonization of plant roots. *G. etunicatum* was initially isolated from maize (*Zea mays*). Control treatments received no AMF inoculum.

Plants were established for 4 weeks and then subjected to four salt levels by adding NaCl to the irrigation water. Different concentration of NaCl as, 0 (control without salt stress), 50 (low), 100 (medium) and 200 mM (high salt stress) were employed for salinity stress. The soil was salinized step-wise to avoid subjecting plants to an osmotic shock. Plants were watered with tape water until harvest. When leaching occurred, the leachate was collected and added back to soil to maintain salinity treatments near target levels.

The plants were harvested after they had been grown under salt stress conditions for 8 weeks and shoots and roots were separated. Leaf area was determined using an AM-200 leaf-area meter. Shoots were then oven-dried at 70°C for 48 h, weighed and saved for mineral analysis. Roots were rinsed free from soil and cut into 1 cm fragments. The fragments were thoroughly mixed and representative fresh samples (1 g) were removed for determination of root AMF colonization. The remaining roots were dried and weighed. Root samples for determination of root colonization with AMF were cleared with 10% KOH and stained with 0.05% trypanblue in lactophenol as described by Phillips and Hayman[31]. AMF colonization in terms of percentage root segments containing arbuscules and vesicles was determined using a gridline intercept method Bierman and linderman[32]. P of shoots was determined colorimetrically[33] and Zn, Cu were determined by atomic absorption. K and Na in plant shoots were determined using flame photometry[34].

The experiment was randomized in complete blocks with four salt stress levels, two AMF inoculum treatments to give a 4×2 factorial with four replications. Data were analyzed statistically using analyses of variance with SPSS. Probabilities of Significance among treatments and interactions and Tukey ($p<0.05$) were used to compare means within and among treatments.

RESULTS

Characteristic morphological and physiological structures of AM were observed in the roots of pistacia after inoculation with *G. etunicatum*. All salinity and AMF treatments had significant effects on the growth and nutrient acquisition traits. Salt × AMF interactions were significant for AMF colonization, Shoot Dry Matter (DM) yields, leaf area and P, K contents in plants (Table 1).

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

Pistacia shoot and root DM yields and leaf area were generally higher in mycorrhizal than nonmycorrhizal

Table 1: Probabilities of significance for analyses of variance of growth, root colonization with arbuscular mycorrhizal fungi and shoot mineral contents in pistacia under different salinity levels

Trait	AMF	Salinity	Salinity×AMF
AMF colonization	**	**	**
Shoot dry matter	**	**	**
Root dry matter	**	**	Ns
Leaf area	**	**	**
P content	**	**	*
K content	**	**	**
Na content	**	**	Ns
Zn content	**	**	Ns
Cu content	**	**	Ns

*, ** Significant at $p<0.05$ and $p<0.01$, respectively. Ns: Not significance

Table 2: Root AMF colonization (%), shoot and root dry matter yields (g/plant), leaf area (mm^2/plant) of Mycorrhizal (M) and Nonmycorrhizal (NM) pistacia grown at different salinity levels

Salinity level (mM)	AMF status	Root colonization	Shoot DM	Root DM	Leaf area
0	M	49.6a	1.97a	0.4a	928a
	NM	0.0e	1.46b	0.29bc	752c
50	M	41.33b	1.49b	0.38ab	817b
	NM	0.0e	0.93c	0.27cd	648d
100	M	29.0c	1.01c	0.25cde	683d
	NM	0.0e	0.713d	0.18de	493e
200	M	8.66d	0.61d	0.19de	445ef
	NM	0.0e	0.63d	0.16ef	422f

Different letter(s) in each column indicate significant differences at $p<0.05$ according to Tukey

Table 3: Shoot content of P, K and Na (mg g^{-1} dw), Zn and Cu (µg g^{-1} dw) in Mycorrhizal (M) and Nonmycorrhizal (NM) pistacia grown at different salinity levels

Salinity level (mM)	AMF status	P	K	Na	Zn	Cu
0	M	20.3a	1272a	152a	732a	68a
	NM	15.2c	1075b	160a	623b	52bc
50	M	18.4ab	1182ac	186a	533bc	55ac
	NM	12.1de	901de	284bc	431d	46ce
100	M	14.2ce	890ef	234acd	450cd	45ce
	NM	11.1df	798f	361be	373d	41ce
200	M	10.4df	606g	352be	248e	39de
	NM	8.4f	576g	415e	214e	38de

Different letter(s) in each column indicate significant differences at $p<0.05$ according to Tukey

plants (Table 2). However, AMF inoculation had no significant effects on either shoot DM or leaf area at the highest salinity (Table 2). Moreover, similar root DM yield were noted at medium and high salinity treatment for both mycorrhizal and nonmycorrhizal plants (Table 2). Shoot and root DM yields and leaf area declined in both mycorrhizal and nonmycorrhizal plants as soil salinity increased (Table 2).

The phosphorus status of shoot showed that P uptake was stimulated by AM inoculation. Shoot P contents were higher in mycorrhizal than nonmycorrhizal pistacia plants in the control, low and medium soil salinity but not at high salinity (Table 3). Salt stress reduced the P content of shoot in both mycorrhizal and nonmycorrhizal plants.

Shoot Cu content was higher in mycorrhizal than nonmycorrhizal plants only in the control treatment. Shoot Cu content decreased as soil salinity increased (Table 3). K and Zn content of shoot were apparently higher for mycorrhizal than nonmycorrhizal plants, but the differences were not significant in high and medium salinity (Table 3). However, shoot contents of K and Zn in both mycorrhizal and nonmycorrhizal plants decreased with increasing soil salinity (Table 3).

Shoot sodium content of both mycorrhizal and nonmycorrhizal plants increased by salinity (Table 3) and were lower in mycorrhizal than in nonmycorrhizal plants at low, moderate and high salinity levels but not in the control treatment (Table 3).

DISCUSSION

Inoculation of *Pistacia vera* with AM fungus *G. etunicatum* caused significantly higher shoot and root DM yields and leaf area than nonmycorrhizal plants under low and medium salinity. This was also seen in shoot DM and leaf area and for root DM under nonsaline conditions. This increase in mycorrhizal plants grown in saline environment was probably indirectly due to mycorrhizal enhancement of P uptake[10,19-21], since mycorrhizal plants also had higher P contents in shoot than nonmycorrhizal ones at control, low and medium salinity. Growth increases may be attributed directly to enhanced photosynthesis associated with increased P uptake in plants[35]. Plant growth enhancements attributed to AMF root colonization decreased at high salinity (200 mM). This may have been due to reduced hyphal P transport into roots and uptake by the plant under these conditions.

While much of the P in the soil is inorganic, a large fraction may also be found in organic compounds. We have known for quit some times that roots of many plant species secrete phosphates to help hydrolyze phosphate from such compounds, but evidence that AMF could do the same was obtained only recently[36,37]. Sanders and Tinker[38] reasoned that the hyphae took up and transferred P to the host because P inflow into mycorrhizal root was substantially higher than in nonmycorrhizal roots, which was limited by diffusion. Plants grown under high salinity may have lower $H_2PO_4^-$ activity (preferred phosphate ion for plant uptake) than under low salinity conditions[39,40]. Reduced uptake of P by mycorrhizal plants grown at high salinity levels has been reported by other researchers[10,19-21].

Many studies have indicated that AMF contributes to plant growth via enhancement of mineral nutrient uptake, especially of immobile soil nutrients (P, Cu and Zn)[13-17]. In this study, colonized pistacia plants had considerably higher above ground mineral nutrient contents (P, K and Zn) than nonmycorrhizal plants in control, low and medium salinity. This probably resulted from a greater absorption surface area provided by extensive fungal hyphae[41] and enhanced root growth as resulted, increased availability or increased transport (absorption and/or translocation) by AMF hyphae. Similar results for enhanced acquisition of P, Zn and K by mycorrhizal plants have been reported[13,14,21,42]. However, AMF root colonization had little effect on shoot Cu content in plants grown at the low and medium salinity. Ghazi *et al.*[43,44] reported that Cu uptake was affected by AMF root colonization under saline condition.

Shoot sodium content was lower in mycorrhizal than nonmycorrhizal plants regardless of salinity level. The lack of response of sodium content to AMF treatment may be explained by dilution effects of plant growth enhancement caused by AMF colonization. Similar results were reported by other researchers[10,43-46].

In summary, from present results, it appears that plant tolerance to salt stress is improved by AMF colonization. AM pistacia plants had more effective in nutrient acquisition especially P than NM plants at all salinity levels. Increased nutrient uptake in response to AMF infection was suggested to be a plant strategy for salt stress tolerance[19-21]. The improved growth and nutrient acquisition in AM pistacia plants demonstrate the potential of mycorrhizal inoculation for the protection from salt stress of plants grown in arid and semiarid areas of the world. However, several AMF isolates should be investigated in order to optimize the effects of this AMF symbiosis.

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