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N. Aliasgharzadeh · N. Saleh Rastin · H. Towfighi
A. Alizadeh

Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil

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Abstract The abundance and distribution of arbuscular mycorrhizal fungi (AMF) were evaluated in the Tabriz Plain, where soil salinity levels range from 7.3 to 92.0 dS/m. Soil and root samples were collected from the rhizosphere of several glycophytes (*Allium cepa* L., *Medicago sativa* L., *Triticum aestivum* L. and *Hordeum vulgare* L.) and halophytes (*Salicornia* sp. and *Salsola* sp.) and were analysed for spore number in soil, root colonization with AMF and some physical and chemical soil properties. The number of AMF spores was not correlated significantly with soil salinity but suffered adverse effects from the accumulation of some anions and cations. Cluster analysis of correlation matrices showed that root colonization, soil pH, sand and clay percent, and soil-available P, rather than soil salinity and ion concentrations, were closely related with spore number. The percentage of root length colonized in glycophytes significantly decreased with increasing soil salinity. Barley roots showed 5% mycorrhizal colonization in high soil salinity (~20 dS/m). Halophyte roots were not mycorrhizal but more spores were found in rhizosphere than in non-rhizosphere soil.

Keywords Arbuscular mycorrhizal fungi · Glycophytes · Halophytes · Salinity · Salt tolerance

N. Aliasgharzadeh (✉)
Department of Soil Science, Faculty of Agriculture,
University of Tabriz, Tabriz, 51664, Iran
e-mail: n-aliasghar@ark.tabrizu.ac.ir
Fax: +98-411-3345332

N.S. Rastin · H. Towfighi
Department of Soil Science, Faculty of Agriculture,
University of Tehran, Karaj, Iran

A. Alizadeh
Department of Plant Pathology, Faculty of Agriculture,
University of Tarbiat Modarres, Tehran, Iran

Introduction

The distribution of arbuscular mycorrhizal fungi (AMF) in different ecological regions and their relations to soil properties and native plants have been investigated by several researchers (Barrow et al. 1997; Bhardwaj et al. 1997; Cook et al. 1993; Khan 1974; Kilronomos et al. 1993; Kim and Weber 1985; Koske 1987; McGee 1989; Methew et al. 1990; Rozema et al. 1986; Stahl and Christensen 1991; Walker et al. 1982). Relatively large populations of these fungi have been reported in some saline soils (Bhaskaran and Selvaraj 1997; Khan 1974; Sengupta and Chaudhuri 1990), whereas in others, populations were small (Barrow et al. 1997; Hirrel 1981; Kim and Weber 1985).Variation in the populations of these fungi and their symbiosis with plant roots is related to both soil properties and host plants (Hayman 1982). In addition, species and isolates of AMF differ in their tolerance to adverse physical and chemical conditions in soil (Joshi and Singh 1995; Juniper and Abbott 1993; Mankarios et al. 1994; Sengupta and Chaudhuri 1990). For example, chenopod plants commonly are not mycorrhizal but may become mycorrhizal when salt-stressed (Hirrel 1981; Katembe et al. 1998; Khan 1974; Sengupta and Chaudhuri 1990). Recent investigations showed that salt tolerance of some plants increases under saline conditions when they are mycorrhizal with certain AM fungi (Aboulkhair and EL-Sokkary 1994; Jindal et al. 1993; Pond et al. 1984; Poss et al. 1985).

Saline soils occupy over 7% of the earth's land surface. In sustainable agriculture, solutions to salinity problems should include both plant breeding for salt tolerance and the application of biological processes such as mycorrhiza (Jurinak and Suarez 1989). In this respect, the study of population levels and effectiveness of AM fungi in saline soils and the impact of different soil factors on their activity could be very important. The objective of this present work was to study the distribution of AMF spores in the rhizosphere of different glycophyte and halophyte plants and to evaluate root colonization by AMF in the Tabriz Plain of Iran in relation to some phys-

ical and chemical properties of soil. Glycophytes are defined as plants which are affected by salt at concentrations lower than 50 mol/m^3; halophytes are plants that complete their life cycle at 500 mol/m^3 (Volkmar et al. 1998).

Materials and methods

Study area and sample collection

The Tabriz Plain consists of 53,000 ha along the eastern coastal plain of Lake Uromieh, which extends from Tabriz to Azarshar in Azarbyjan Province of Iran at latitude 37 53′N and longitude 46 50′E. At present, approximately 20,000 ha of this Plain are arable but the rest is too saline for producing crops and is occupied by halophytes such as *Salsola* sp., *Salicomia* sp. and *Atriplex* sp. The salinity of the agricultural area increases as it nears the coastal lands, and the plant cover differs with increasing soil salinity from onion (*Allium cepa* L.), alfalfa (*Medicago sativa* L.), wheat (*Triticum aestivum* L.) to barley (*Hordeum vulgare* L.).

Samples were collected along four parallel equidistant transects approaching the Lake. In each transect, a total of 9–14 complex samples were collected from the rhizospheres of different plants between 22 and 30 July 1998. A complex sample was prepared by mixing five samples at each site for the same plants. A "site" was a complex sample area of less than 1 ha for a plant species. Sites in each transect were spaced 1–3 km apart, depending upon both plant species diversity and soil salinity levels. Samples were returned to the laboratory and the fine roots in each sample were removed, rinsed with tap water and fixed in formalin, acetic acid, alcohol for determination of root colonization. The soil samples were then air dried in the shade at laboratory temperature for spore counting.

Recovery and counting of AMF spores

Spores were extracted from 10 g soil in triplicate for each sample by wet-sieving followed by flotation-centrifugation in 50% sucrose (Dalpe 1993). The finest sieve was 53 µm. The spores were collected on a grid pattern (4×4 mm) filter paper and washed with distilled water to spread spores evenly over the entire grid. They were counted using a dissecting microscope at ×30 magnification. The number of spores was expressed as the mean of three replicates.

Generally, *Salsola* sp., *Salicornia* sp. and *Atriplex* sp. plants are not mycorrhizal (Hirrel et al. 1978; Juniper and Abbott 1993; Khan 1974). Therefore, some non-rhizosphere soils were sampled in addition to rhizosphere soils for determination of possible effects of these plants on spore populations.

For character observation and identification, spores were mounted on glass slides in PVLG and PVLG+Melzer's reagent (Schenck and Perez 1988) and were sent to INVAM for confirmation.

Root colonization

Roots were rinsed with distilled water, cleared by 10% KOH for 15 min at 90°C, bleached in alkaline hydrogen peroxide for 20 min, acidified in 1% HCl, and stained using lacto-glycerol acid fuchsin (Kormanik and McGraw 1982).

For quantification of AMF colonization, 60 1-cm sections were mounted on slides (30 per slide) and colonized root tissue was evaluated as a proportion of total length of observed roots (percent root length colonized).

Physical and chemical analysis of soil

The soil properties measured were pH (water), electrical conductivity of soil saturated extract (ECe), concentrations of Na, Ca, Mg, Cl, SO_4, the sodium absorption ratio (SAR) in saturated soil extract, percent of calcium carbonate equivalent (%CCE), available phosphorus (Olsen P) and soil texture, using methods described by Klute (1986) and Page et al. (1982).

Statistical analysis

Correlation analysis was performed for evaluation of the relationships between different soil properties and number of spores or percentage root length colonized. Percent values for root colonization were subjected to arcsine transformation before correlation and cluster analysis to obtain normal distribution of data. To represent the complex multivariate relationships among the variables, agglomerative hierarchical cluster analysis was performed on the correlation matrices and results were expressed as a dendrogram (Chatfield and Collins 1980; Romesburg 1984). SPSS software version 7.5 was used for statistical analysis.

Results

The ionic analysis of the soils indicated that sodium and chloride ions dominate as soil salinity increases, although Mg and SO_4 concentrations were considerable under severe soil salinity (Table 1). Soil texture analysis indicated that the Tabriz Plain has mostly fine textured, clay soils. The number of spores in rhizosphere soils differed between plant species. The highest number was found in onion rhizosphere soil and the lowest in *Salsola* sp. and *Salicornia* sp. rhizosphere soil (Table 1). In addition to the plant species, the number of spores showed significant correlations with extent of root colonization and soil properties. With the exception of the halophytes *Salsola* sp. and *Salicornia* sp. (Tables 1, 2), the percentage root length colonized increased with increasing spore number (r=0.455, P<0.01). There was no significant correlation between spore number and ECe (soil salinity), SAR, pH or %CCE. Spore numbers positively correlated with sand percent (r=0.384, P<0.05) but clay percent showed a negative correlation (r=–0.360, P<0.05). As shown in Table 2, spore abundance was negatively correlated with concentrations of Mg^{2+}, Ca^{2+}, Na^+, Cl^- in soil (P<0.05) and with soil available phosphorus (P<0.01). The numbers of spores in *Salsola* sp. and *Salicornia* sp. rhizosphere soils were about twofold higher than in non-rhizosphere soils of these plants (~100 and 55 spores per 10 g dry wt. soil at about 120 dS/m, respectively). The most predominant species of AMF in the Tabriz Plain were *Glomus intraradices* Schenck & Smith, *G. versiform* (Karsten) Berch, *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe and *G. etunicatum* Becker & Gerdemann. The latter two were observed at high salinity levels (>40 dS/m). The percentage of root length colonized was affected by plant species and soil properties. As shown in Table 2, the different soil factors, except clay percent and soil pH, had considerable effects on root colonization. The percentage of root length colonized in glycophytes significantly decreased with increasing soil salinity. However, barley roots showed about 5% mycorrhizal colonization at high soil salinity(~20 dS/m). Root colonization negatively

Table 1 Mean spore number in soil, percentage of root length colonized and soil properties in rhizosphere soils of different plants in the Tabriz Plain. Values in parentheses represent standard errors of means (*CCE* percent calcium carbonate equivalent in soil, *Clay* percent clay, *EC* electrical conductivity in saturated soil extract (dS/m), *Mg*, *Ca*, *Na*, *Cl*, SO_4 concentrations of magnesium, calcium, sodium, chloride and sulfate ions in saturated soil extract (mmolc/l), *Pava* Olsen soil available phosphorus (mg/kg), *pH* pH in saturated soil extracts, *RLC* percent root length colonized, *sand* percent sand, *SAR* sodium absorption ratio (mmolc/l), *Sp* number of spores in 10 g dry wt. soil)

Parameter	Onion	Alfalfa	Wheat	Barley	*Salsola* sp. and *Salicornia* sp.
Sp	144.8(23.8)	129.8(16.9)	119.5(11.4)	115.3(37.9)	100.0(13.2)
RLC	32.8(3.6)	30.8(4.2)	11.2(2.4)	4.5(0.8)	0.0
pH	7.71(0.56)	7.61(0.09)	7.45(0.10)	7.50(0.09)	7.47(0.06)
EC	7.3(1.7)	12.3(3.4)	12.1(2.6)	21.1(5.6)	92.0(16.7)
CCE	11.5(1.7)	14.3(1.9)	14.5(1.6)	17.7(1.1)	18.6(0.4)
Sand	50.6(4.8)	37.6(6.7)	32.4(7.5)	28.0(8.0)	19.8(2.8)
Clay	25.8(2.9)	31.7(3.6)	38.4(5.6)	35.0(6.7)	32.9(4.2)
Mg	13.7(3.4)	20.2(4.3)	18.8(2.8)	27.5(4.8)	131.2(38.8)
Ca	23.0(7.1)	32.9(6.2)	38.5(6.5)	47.2(5.4)	69.6(12.0)
Na	39.5(9.3)	84.0 (31.7)	78.4(21.9)	153.2(50.3)	1102.5(238.5)
Cl	51.6(16.0)	83.8(27.2)	85.3(23.6)	162.7(53.8)	1022.5(228.2)
SO_4	20.1(5.2)	48.0(14.7)	46.1(11.8)	61.7(15.1)	276.7(72.2)
SAR	9.6(1.8)	15.2(4.1)	13.7(3.0)	23.7(7.1)	108.0(17.4)
Pava	7.3(2.2)	17.7(4.9)	17.2(3.2)	14.6(2.6)	14.1(2.5)

Table 2 Correlation coefficients (*r*) between spore number or percentage of root length colonized and soil properties in the Tabriz plain. Abbreviations as in Table 1

	Sp	RLC	pH	EC	CCE	Sand	Clay
Sp	1.000	0.455**	0.186	–0.293	–0.095	0.384*	-0.360*
RLC	–	–	0.266	–0.521***	–0.345*	0.386**	–0.208
	Mg	Ca	Na	Cl	SO_4	SAR	Pava
Sp	–0.330*	–0.331*	–0.309*	–0.327*	–0.244	–0.136	–0.405*
RLC	–0.407**	–0.464**	–0.484***	–0.487***	–0.409**	–0.526***	–0.415**

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

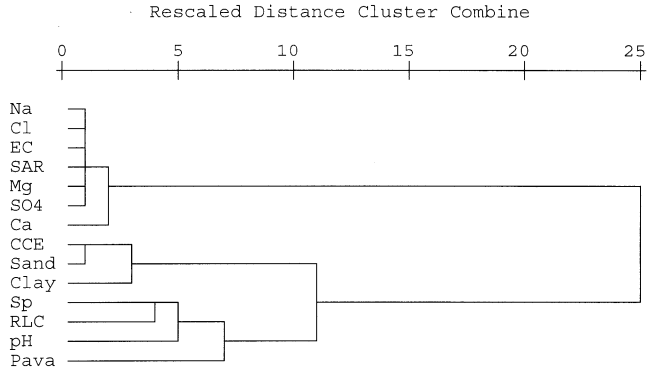

Fig. 1 Dendrogram based on the ward method to represent cluster analysis of spore number, percent root length colonized and soil properties according to the correlation matrices. Abbreviations as in Table 1.

correlated with SAR and concentration of Cl^- and Na^+ ($P<0.001$). In contrast, the percent sand and spore numbers were positively correlated ($P<0.01$). Reduced root colonization was related to the presence of other ions such as Ca^{2+}, Mg^{2+} and SO_4 and soil available phosphorus ($P<0.01$) and %CCE ($P<0.05$). The roots of *Salsola* sp. and *Salicornia* sp. were not mycorrhizal but, in some cases, aseptate hyphae similar to AMF hyphae were observed in the cortex and at the root surface.

Cluster analysis on correlation matrices (Fig. 1) indicated that the number of spores, percent root length colonized, soil pH, percent sand and clay, and soil available P were closely related. In this diagram, other soil factors such as ECe and ion concentrations are relatively distant from spore number and root colonization.

Discussion

The number of AMF spores in soils did not significantly decrease with increasing soil salinity. It has been suggested that sporulation by AMF is stimulated under salt-stress conditions (Hirrel 1981; Tressner and Hayes 1971), which means that the fungi may produce more spores at lower root colonization levels in severe saline conditions. McMillen et al. (1998) reported that spore germination and hyphal growth of AMF were inhibited by 150 mM NaCl. This may again cause accumulation of spores in saline soil. However, salinity induced by NaCl may differ from natural salinity, due to the presence of different salts in naturally saline soils (Bowen 1987). The occurrence of relatively high spore numbers (mean of 100 per 10 g soil) in the severely saline soils (ECe ~162 dS/m) of the Tabriz Plain contrasts with other studies of saline soils where low or even zero spore populations were found in soils with ECe higher than 45 dS/m (Barrow et al. 1997; Hirrel et al. 1978; Kim and Weber 1985).

Although, no typical mycorrhizal invasion was seen in roots of *Salsola* sp. and *Salicornia* sp., the number of spores was higher in rhizosphere than in non-rhizosphere

soils of these plants. Schmidt and Reeves (1984) noted that non-mycorrhizal *Salsola* enhances growth of AMF in rhizosphere soil. They suggested that the fungal hyphae benefit from root exudates and sporulation thus increases (Schmidt and Reeves 1984). Chenopod plants such as *Atriplex* sp. and *Salsola* sp., which are regarded as non-mycorrhizal, have been reported to be mycorrhizal under drought and/or salt-stress conditions (Barrow et al. 1997; Hirrel et al. 1978; Katembe et al. 1998; Kim and Weber 1985; Rozema et al. 1986). Percentage of viable spores in soil was not determined in this study and, thus, the ecophysiological implications of the results are uncertain. It is almost impossible to distinguish between biotic and abiotic factors affecting spore abundance and percent root colonization of AMF in natural conditions. Furthermore, it is extremely difficult to distinguish between direct and plant-mediated effects of salinity on their biology. Presumably, any environmental factor affecting the physiology of the host plant is likely to affect its fungal symbiont.

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