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Arbuscular mycorrhizal status of wild plants in saline-alkaline soils of the Yellow River Delta

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Abstract A survey was made of the arbuscular mycorrhizal (AM) status of five dominant wild plants *Tamarix chinensis*, *Phragmites communis*, *Suaeda glauca*, *Aeluropus littoralis* var. *sinensis* and *Cirsium setosum* in saline-alkaline soils of the Yellow River Delta that show low plant diversity. All of the species were colonized and showed typical AM structures (arbuscules, vesicles). The colonization percentage ranged from 0.2% to 9.5%, where *C. setosum* was the highest. The species richness of AMF at the different sites ranged from 2.00 to 2.40 per 50 ml soil, with an average of 2.16. Species diversity ranged from 1.99 to 2.22 per 50 ml soil, with an average of 2.13. Spore density ranged from 3 to 30 per 50 ml soil, with an average of 12. *Glomus* was the dominant genus, with a frequency and relative abundance of 88.1% and 68.4%, respectively. *G. caledonium*, with a frequency and relative abundance of 15.0% and 4.6%, respectively, was the dominant species. Differences were also observed in the distribution of AMF in different soil layers. Although there were still AM fungal spores in the layer 40 cm below the surface, most spores were found at a depth of 0–40 cm.

Keywords Arbuscular mycorrhizal fungi · Yellow River Delta · Saline-alkaline soils · Spore density · Relative abundance

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Introduction

Arbuscular mycorrhizal fungi (AMF) occur in a wide variety of ecosystems, such as farmland and forestland, as well as many stressful environments. AMF are known to be present in saline and acid-saline soils despite the stressful conditions of these ecosystems. The distribution of AMF in different soil regions and their relations to soil properties and native plants have been investigated by several researchers (Barrow et al. 1997; Cook et al. 1993; Hildebrandt et al. 2001; Hoefnagels et al. 1993; Johnson-Green et al. 1995; Kim and Weber 1985; Rozema et al. 1986; Udaiyan et al. 1996). Populations of AMF in saline-alkaline soils were variable and affected by many factors. For example, Hildebrandt et al. (2001) reported mycorrhizal colonization as well as a high spore density in Central European salt marshes. Landwehr et al. (2002) found the number of spores in samples in European saline, sodic and gypsum soils to be rather variable but high on average, with *G. geosporum* dominant. Cooke et al. (1993) observed the vertical distribution of vesicular-arbuscular mycorrhizae (VAM) in roots of salt marsh grasses growing in saturated soils. Aliasgharzadeh et al. (2001) reported that the spore number of AMF in saline soils of the Tabriz Plain was not correlated with soil salinity but suffered adverse effects of the accumulation of some anions and cations. Levy et al. (1983) demonstrated effects of irrigation, water salinity and rootstock on the vertical distribution of VAM in citrus roots.

Saline-alkaline soils occupy over 7% of land surface. In sustainable agriculture, solutions to salinity-alkalinity problems should include both plant breeding for salt tolerance and the application of biological factors such as mycorrhiza. In this respect, it is very important to study the diversity and effectiveness of AMF in saline-alkaline soils and their effects on host plants.

The objective of our present work was to investigate the distribution of AMF in the rhizosphere of different wild plants and to evaluate root colonization by AMF in the Yellow River Delta.

Methods and materials

Study area and sample collection

The Yellow River Delta, one of the largest deltas in China, is situated in the northeast of Shandong Province, on the southern bank of the Bohai Sea. It includes Dongying and Binzhou cities and parts of Zibo, Dezhou and Weifang cities. The delta at longitude 117°48'E–119°45'E and latitude 36°52'N–8°12'N covers an area of 787,000 ha, including 670,000 ha of alkaline wasteland still to be developed, with a population of 5.4 million. Unfortunately, the fragile ecological environment of saline-alkali soils is inhabited by only a few species of wild plants, such as *Tamarix chinensis*, *Phragmites communis*, *Suaeda glauca*, *Aeluropus littoralis* var. *sinensis* and *Cirsium setosum*. The total vegetation cover is quite low.

These five plant species were surveyed at four representative sites, Wudi, Dongying, Changyi and Shouguang. Samples were collected from the rhizospheres of five plants of the same species randomly distributed at each site in May 2001, according to the procedures described by Liu and Li (2000). In addition, samples at Wudi were also collected from soil layers at 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, and 40 cm below the surface. 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, which had a sandy texture, were then air-dried in the shade at laboratory temperature for spore counting and identification.

Physical and chemical analysis of soil samples

Soil properties were measured using methods described by Lu (2000). Values in brackets represent standard errors of means. Soil characteristics were: pH (H_2O) 8 (0.41), organic matter content 0.32% (0.06), electrical conductivity 40.2 dS/m (8.2), available P 10.4 mg/kg (3.2), available N 56.6 mg/kg (11), available K 4.4 mg/kg (0.6), Mg 25 mM (4.8), Na 156.8 mM (54.6), Cl 211.2 mM (84.5), Ca 64.5 mM (14.8), SO_4 15.4 mM (3.2).

Recovery and counting of AMF spores

Spores or sporocarps were extracted from 50 ml dried 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 patterned (4×4 mm) filter paper, washed with distilled water to spread them evenly over the entire grid and counted using a dissecting microscope at ×30 magnification. A sporocarp was counted as one spore. The number of spores was expressed as the mean of three replicates.

For observation and identification of spore characters, spores were mounted on glass slides in polyvinyl-lacto-glycerol (PVLG) and PVLG+Melzer's reagent and then identified to species using current taxonomic criteria (Schenck and Perez 1990) and internet information from INVAM (http//:invam.caf.wvu.edu).

Root colonization

Fresh roots were rinsed with distilled water, cleared in 10%(w/v) KOH for 15 min at 90°C, bleached in hydrogen peroxide for 20 min, acidified in 1% HCl, and stained for 15 min with acid fuchsin (0.05% in lactoglycerol) (Kormanik and McGraw 1982). For quantification of AMF colonization, 60 0.5- to 1.0-cm root sections were mounted on slides (30 per slide) and colonized root tissue was evaluated as the proportion of total length of observed roots (percent root length colonized).

Numbers and distribution of AMF spores

Species richness, spore density, frequency, and relative abundance of AMF were expressed as follows: spore density = number of AMF spores in 50 ml soil (spore density in different soil layers = number of spores in 200 ml soil); species richness = number of AMF species in 50 ml soil; relative abundance = (number of spores of a species or a genus/total spores) × 100%; frequency = (number of the samples in which the species or genus was observed/ total samples) × 100%. Species diversity was measured by the Shannon-Weiner index:

$$\text{Diversity} = -\sum_{i=1}^{S} (\text{Pi ln Pi})$$

where s = number of total species in the sample site and Pi = number of spores of a species/ total number of spores in the sample site.

Statistical analysis

The data were subjected to two-way ANOVA using SPSS software version 10.0. Means and standard errors were calculated for the replicate values.

Results

Mycorrhizal colonization

The AM statuses of the five dominant wild plants *T. chinensis*, *P. communis*, *S. glauca*, *A. littoralis* var. *sinensis* and *C. setosum* are shown in Table 1. All of the species were colonized by AMF at the four surveyed sites. The colonization percentage was low, ranging from 0.2% to 9.5%. There were significant differences in mycorrhizal colonization between plants but no significant differences between different sites; however, there was no correlation in mycorrhizal colonization between plants and sites. The mean colonization of *C. setosum* was the highest of the five plant species.

Table 1 Colonization percentage of different plants at four different sites. Each value represents the mean of five replicates. The ANOVA results were Site: F value 0.67*, P value 0.58; Plant: F value 76.2**, P value 1.76×10^{-26}; Plant×Site: F value 0.80*, P value 0.65 (where **P 0.05, *not significant)

Host plant	Changyi	Wudi	Shouguang	Dongying
Tamarix chinensis	3.1	2.7	2.8	2.5
Phragmites communis	0.5	0.6	0.3	0.3
Suaeda glauca	1.0	0.8	0.8	0.9
*Aeluropus littoralis*var. *sinensis*	3.0	3.5	2.8	3.0
Cirsium setosum	9.2	8.0	7.8	9.0

Table 2 Genera and species of arbuscular mycorrhizal fungi (AMF) in saline-alkaline soils of the Yellow River Delta

Genus	Species
Acaulospora	*A. bireticulata, A. denticulata, A. elegans, A. foveata, A. laevis, A. scrobiculata, A. tuberculata*
Archaeospora	*Ar. gerdemannii, Ar. leptoticha*
Glomus	*G. aggregatum, G. albidum, G. ambisporum, G. caledonium, G. claroideum, G. clarum, G. constrictum, G. delhiense, G. deserticola, G. dimorphicum, G. etunicatum, G. geosporum, G. glomerulatum, G. hoi, G. intraradices, G. manihotis, G. melanosporum, G. microaggregatum, G. mosseae, G. pansihalos, G. pustulatum, G. reticulatum, G. tenebrosum, G. versiforme*

Table 3 Frequency (F%) and relative abundance (RA%)of genera and several dominant species of AMF in saline-alkaline soils of the Yellow River Delta

AMF	F	RA
Glomus	88.1	68.4
Acaulospora	52.7	34.7
Archaeospora	13.3	5.2
G. caledonium	15.0	4.6
G. mosseae	10.5	0.8
A. denticulata	8.2	0.4
Ar. leptoticha	5.0	0.5

Genera and species of AMF

A total of 33 species representing three genera of AMF, including two species in *Archaeospora*, seven in *Acaulospora* and 24 in *Glomus*, were isolated and identified in the rhizosphere of the five wild plants in saline-alkaline soils of the Yellow River Delta (Table 2).

Species richness and species diversity of AMF

The species richness of AMF at the four different sites ranged from 2.00 at Wudi to 2.40 at Changyi, with an average of 2.16. Species diversity ranged from 1.99 at Wudi to 2.22 at Changyi, with an average of 2.13.

Frequency and relative abundance of AMF

Frequencies (F) and relative abundances (RA) of the genera and some dominant species of AMF are listed in Table 3. Spores of the genus *Glomus* were the most numerous, both in frequency and relative abundance. The most frequent and most abundant species present was *G. caledonium*.

AMF spore density in soils

The spore density in saline-alkaline soils of the Yellow River Delta ranged from 3 to 30 per 50 ml soil, with an average of 12. Significant differences were observed between rhizospheres of the different plant species. Spore densities of AMF in the rhizospheres of *C. setosum*, *T. chinensis* and *P. communis* were higher than in the rhizosphere of *S. glauca* and *A. littoralis* var. *sinensis*.

Table 4 Spore density of AMF in the rhizosphere of different plants at four different sites. Each value in the table represents the mean of five replicates. The ANOVA results were Site: F value 19.2**, P value 1.83×10^{-9}; Plant: F value 42.1**, P value 6.1×10^{-19}; Plant×Site: F value 18.4*, P value 0.11 (where **P 0.05, *not significant)

Host plants	Changyi	Wudi	Shouguang	Dongying
T. chinensis	15.5	13.2	12.4	19.2
P. communis	12.0	10.5	9.5	17.8
S. glauca	8.1	8.7	5.6	10.2
A. littoralis var. *sinensis*	7.5	4.7	4.8	7.2
C. setosum	16.8	15.5	14.2	26.3

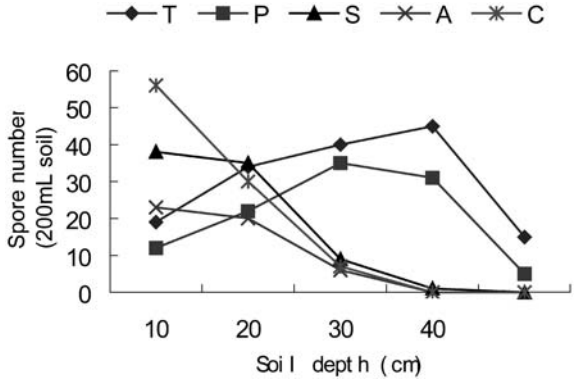

Fig. 1 The spore numbers of AMF in different layers of soil (*T Tamarix chinensis*, *P Phragmites communis*, *S Suaeda glauca*, *A Aeluropus littoralis* var. *sinensis, C Cirsium setosum*)

Sample site also had a significant effect on spore density, while the interaction between host plant and site was not significant (Table 4).

AMF spore density in different soil layers

Because the populations of AMF spores in the surveyed saline-alkaline soils were small, 200-ml soil samples were used for the measurement of spore density in different soil layers. Most spores were found at a depth of 0–40 cm. There were significant differences between different depths. Spores decreased with increasing soil depth in the rhizosphere of *S. glauca*, *A. littoralis* var. *sinensis* and *C. setosum*. However, in the rhizosphere of *T. chinensis* and *P. communis*, most spores occurred in layers 10–

40 cm deep and only a few were present 40 cm below the surface (Fig. 1).

Discussion

Variation in spore density and colonization of AMF associated with different host plant species may be generated by a variety of mechanisms, including variation in host species and their phenology, mycorrhizal dependency, host plant-mediated alteration of the soil microenvironment, or other unknown host plant traits (Eom et al. 2000; Lorgio et al. 1999). In this study, the host species apparently had direct effects on spore density and colonization of AMF. For example, these were both higher in the rhizosphere of *C. setosum* than of other plants. The results support the conclusion of Carvalho et al. (2001) that the distribution of arbuscular mycorrhizas in salt marshes depends more on host plant species than on environmental stresses.

All the surveyed plants in saline-alkaline soils of the Yellow River Delta were colonized by AMF, but colonization percentages of all plants were significantly lower than those of other plants in saline soils reported by Brown and Bledsoe (1996) and Udaiyan et al. (1996). However, our results support the conclusion that AMF populations are small in saline soils reached by some researchers (Barrow et al. 1997; Carvalho et al. 2001) but not others (Aliasgharzadeh et al. 2001; Bhaskaran and Selvaraj 1997; Khan 1974; Landwehr et al. 2002). Spore density and root colonization showed significant correlation with plant species and soil properties (Aliasgharzadeh et al. 2001), and the high soil salinity, poor plant diversity and low vegetation cover in saline-alkaline soils of the Yellow River Delta may severely restrict colonization and diversity of AMF.

AMF spores were found in soils 40 cm below the surface in the rhizosphere of *T. chinensis* and *P. communis* but not *S. glauca*, *A. littoralis* var. *sinensis* or *C. setosum*. This suggests that the distribution of AMF is related not only to the physiological characteristics of hosts but also to morphological characteristics of the roots. It has been reported that AMF seldom occur in soils below about 40 cm in the rhizosphere of soybeans (An et al. 1990). However, since the roots of some mycorrhizal woody plants extend deep into the soil, such AMF may be also found at such depths.

AMF may not only benefit plant growth and development, but also increase the resistance of plants to stresses such as extremes of pH and salinity (Feng et al. 2000, 2002; Rosendahl and Rosendahl 1991). For example, AMF can alleviate salt stress on *Lactuca sativa* (Ruiz-Lozano et al. 1996). Mycorrhizal inoculation protected plants from salt stress more efficiently than any amount of plant-available P in the soil, particularly at the highest salinity level examined (43.5 dS/m) (Azcón and El-Atrash 1996). Feng et al. (2002) reported that improved tolerance of maize plants to salt stress endowed by AM is related to higher accumulation of soluble sugars in roots. Wang et al. (1994) selected a salt-tolerant isolate of AMF, *G. mosseae* 93. Since *G. caledonium* was dominant in saline-alkaline soils of the Yellow River Delta and distributed widely in the rhizosphere of most of the plant species, the possibility that *G. caledonium* can improve tolerance to salt stress should be tested to select useful isolates.

The application of molecular approaches such as PCR for studying the biodiversity of AMF has been reported by many researchers (Kjøller and Rosendahl 2001; Prosser 2002; Tuinen et al. 1998). PCR with taxon-specific primers could be used to identify AMF within colonized roots, as *G. caledonium* is dominant in saline-alkaline soils but may not be the main root colonizer. Our results suggest that there may be many unknown but useful AMF in saline-alkaline soils, with potential for use as a type of biofertilizer and biocontrol microbe. The application of PCR to find promising AMF for revitalizing saline-alkaline soils will be the subject of our future work.

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