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Distribution and abundance of arbuscular mycorrhizal fungi from soybean rhizosphere in Iran

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Arbuscular mycorrhizal (AM) fungi are known to be well distributed throughout both hemispheres. These fungi can be isolated from a wide variety of natural habitats and are particularly abundant in cultivated lands. Little work has been carried out regarding their distribution in soybean fields in Iran. During the periods from July-September 2003-2004, 120 soil samples were collected from the main soybean producing provinces in Iran. The population as well as identification and distribution of species were studied. All the 120 soil samples were classified into one of eight major habitat groups according to soil type, crop rotation and irrigiation system. The number of spores varied between 280-520 and was recorded in 75%-100% of the sites examined. Among the 21 species identified, 15 species were of *Glomus*, 2 species each of *Gigaspora* and *Acaulospora* and 1 species each of *Scutellospora* and *Sclerocystis* genera. The most abundant species recorded were *G. fasciculatum* and *G. mosseae* with a frequency of 35.3% and 15%, respectively.

Keywords: diversity, Iran, mycorrhiza, soybean

Introduction

Arbuscular mycorrhizal (AM) fungi are known to be well-distributed throughout both hemispheres. These fungi can be isolated from a wide variety of natural habitats and are particularly abundant in cultivated lands. The AM symbiosis, which appeared with the first land plants more than 400 million years ago, is still formed by the large majority of extent plant species with no host specificity (Redecker *et al*., 2000). Glomalean fungi provide plants with mineral nutrients in exchange for carbon compounds and protect them against diverse abiotic and biotic stresses (Smith and Read, 1997). It is, therefore,

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thought that AM fungi play an important role in most terrestrial ecosystems. Nontheless, symbiosis efficiency depends on environmental factors as well as genetic determinants from both plant and AM fungi (Giovannetti and Gianinazzi-Pearson, 1994). Plant species vary in their responsiveness to AM fungi with respect to growth, reproduction and resistance against stresses and, in turn, AM fungi can differ in their effects on plant health. The elimination of AM fungal propagules using fungicides in diverse field situations has led to either an increase or decrease in plant diversity. Moreover, increasing plant diversity in a field experiment can result in increased AM fungal sporulation and community composition (Burrows and Pfleger, 2002). Considering the ecological importance of AM fungi, especially in low-rate phosphorous fields, it is of interest to determine the fungal species as well as their distribution status in fields. The purposes of this study were:

1. Identification of AM fungal species in soybean rhizosphere in Iran
2. Determination of prevalent species found in soybean rhizosphere
3. Study on species diversity in different soybean cultivated regions in Iran

Materials and methods

During the periods from July-September 2003-2004, 120 soil samples were collected from main soybean-producing provinces in Iran, including Golestan, Mazandaran, Lorestan and Ardabil (Moghan). Also, additional data such as crop rotation status in previous years, host plant variety, soil texture and irrigiation system (with or without irrigiation) were recorded. Collected soil samples were used directly for estimation of number of spores. For this purpose, three replicates each 10g were selected for each soil sample and spores were separated using wet sieving and centrifugation by sucrose gradient method (Jenkins, 1964; Gerdemann and Nicolson, 1963). The number of spores was measured as the mean for each three replicates. Trap cultures with sorghum and maize established in order to propagation of spores for slide preparation and fungal morphological identification as well as species diversity. In this case, spores also were separated using the above mentioned standard method from 100g soil of each trap culture sample and 10 spores (with morphological similarities) were fixed on each slide. Five slides prepared for each trap soil sample, so totally 50 spores were studied for each trap sample. Fungal species identification carried out using valid and standard keys (Schenck and Perez, 1990). Also, species diversity was recorded. In order to comparison of species similarities on different sampling areas, the Jaccard index (Jaccard, 1912) was calculated as follow:

$$IS_J = C/(A+B+C)$$

IS_J: Similarity index of species population between two examined sites (a, b)
A: Number of species only on site a
B: Number of species only on site b
C: Number of species common on sites a and b

Results

Additional data such as crop rotation status, host plant variety, soil texture and the irrigation system and also facilities for studies on species diversity was available and therefore we classified the soybean-producing sampling areas into eight major habitat groups (Table 1). The number of spores estimated on different soybean rhizosphere soils showed that the highest number of spores belonged to A_2 habitat, i.e. Golestan Province with silt clay soils without crop rotation and irrigiation. The lowest numbers were observed in the C_2 habitat, i.e. Lorestan Province with coarse sandy soils without crop rotation and irrigiation (Table 1). The number of spores varied between 280-520 and was recorded in 75-100% of the sites examined, so the number of areas with spore might vary in different habitats (Table 1). Fields with more or less heavy soil texture, without crop rotation as well as no irrigation generally had the highest number of spores, but this was not observed in all habitats. However, it should be mentioned that different biological and physico-chemical factors effect the number of spores estimated as well as their diversity. Totally 21 species of AM fungi were identified. These belonged to 5 genera including *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora* and *Sclerocystis*, respectively. Among the species identified, 15 species were of *Glomus*, 2 species each of *Gigaspora* and *Acaulospora* and one species each of *Scutellospora* and *Sclerocystis*, respectively. The list of all species identified as well as their frequencies are shown in Table 2. All of these species are recorded for the first time from soybean rhizosphere in Iran. Among the species, 12 including *Glomus microcarpum*, *G. albidum*, *G. dimorphicum*, *G.reticulatum*, *G. botryoides*, *G. boreale*, *G. multisubstensum*, *Gigaspora candida*, *G. albida*, *Scutellospora coralloidea*, *Acaulospora mellea* and *Acaulospora dilatata* are new for the mycota of Iran.

The results of species diversity showed that there is no similar pattern in the species diversity and distribution among examined sites and seems to be patchy. So, may be one species can be found in one province while not in others. Also, this pattern may be observed in different habitats of one province. The most abundant species recorded were *Glomus fasciculatum* and *G. mosseae* with a frequency of 35.3% and 15%, respectively (Table 2). Soybean habitat groups classified to three different subgroups according to all species

diversity. Subgroup I including the habitas A_1, A_2, A_3 each with 16.7%, subgroup II including the habitats C_1 and D each with 12.5% and subgroup III including habitats B, C_2 and C_3 each with 8.3% species diversity. There is no specific correlation between the number of spores and species diversity in one habitat. The results of Jaccard similarity index (Table 3) showed that the most similarity was observed between Lorestan and Golestan provinces. May be this is due to no crop rotation in these two provinces. Most of the fields in these Provinces have been under soybean cultivation for more than 5 years. However, as mentioned before, there are so many different environmental and physico-chemical factors which effect on number of spores and species diversity among different provinces as well as the habitats of one province.

Table 1. Soybean rhizosphere habitat groups and total number of spores in each habitat

Province	Habitat Code	Soil Texture	Crop Rotation	Irrigation	No. Sampling Area	No. Area with Spores	% Area with Spores	Number of Spores
Golestan	A_1	Silt Clay	> 5 years	+	20	19	95	451
	A_2	Silt Clay	> 5 years	-	20	20	100	520
	A_3	Sandy Loam	< 5 years	+	20	15	75	400
Mazandaran	B	Sandy Clay Loam	< 5 years	+	10	8	80	302
Lorestan	C_1	Sandy Loam	> 5 years	+	15	15	100	370
	C_2	Coarse Sandy	> 5 years	-	10	8	80	280
	C_3	Sandy Clay Loam	> 5 years	-	10	9	90	370
Ardabil (Moghan)	D	Sandy Loam	> 5 years	+	15	13	86.7	410

Discussion

A diverse AM fungal population is a key factor to improve the sustainability of low input and organic agricultural systems (Madre *et al*., 2002; Oehl *et al*., 2003). To increase our ability to optimize management of AM fungi in field situation, there is a need for more information on how agricultural practices influence the variation in AM fungal community

Table 2. Mycorrhizal species and their relative abundance in soybean rhizosphere

Species	% of Frequency	Species	% of Frequency
Glomus fasciculatum	35.3	*Glomus constrictum*	0.5
Glomus mosseae	15	*Glomus dimorphicum*	0.5
Glomus macrocarpum	12	*Glomus intraradices*	0.35
Glomus microcarpum	10	*Glomus reticulatum*	0.35
Glomus albidum	10	*Scutellospora coralloidea*	0.35
Glomus geosporum	7	*Sclerocystis coremioides*	0.35
Gigaspora candida	3	*Glomus botryoides*	0.2
Glomus etunicatum	3	*Glomus boreale*	0.2
Glomus caledonium	0.65	*Glomus multisubstensum*	0.2
Gigaspora albida	0.65	*Acaulospora mellea*	0.2
		Acaulospora dilatata	0.2

Table 3. Jaccard similarity index between soybean-producing provinces

Compared Provinces	Jaccard Index
Golestan/Mazandaran	0.7
Golestan/Lorestan	0.73
Golestan/Moghan	0.48
Mazandaran/Lorestan	0.6
Mazandaran/Moghan	0.56
Lorestan/Moghan	0.69

development and function in different crop species. The first step is to fully characterize the AM fungi community composition. Evidence of the ecological importance of AM fungi is abundant, but an understanding of the distinct roles of individual fungal species is limited. Spore morphology and enumeration are the traditional methods for taxonomic identification and AMF diversity studies. In field samples, low spore number, parasitization of spores, and age and environmental alteration of spores (e.g., discoloration) will hinder accurate identification (Bever *et al.*, 2001). Hence, trap cultivation in greenhouse, i.e., propagation of field AMF on a host plant in a controlled environment, is often practical to increase spore numbers. In this approach, the spores of some species detected in the original inoculum may not be detected or some species undetected in the original inoculum may be detected because of unknown stimulatory or inhibitory cultivation conditions (Talukdar, 1993; Bever *et al.*, 2001). For example, root exudates of host plant are important regulators of microbial community composition and activity, and these compounds are a

source of reduced C and amino acids for microbial consumption. So it seems that a complex environmental as well as physico-chemical factors effect on AM fungal diversity in rhizosphere. For instance, AM fungi are considered to have low specificities of association with host species, but this conclusion is based mostly on experiments in which individual isolates of fungal species are grown separately, apart from competitive interactions (Bever *et al*., 2001). When the fungi are examined as a community, evidence suggests fungal growth rates are highly host specific. In an experiment in which AMF were trapped on different plant hosts, isolates of different fungal species sporulated differentially, with the relative dominance of fungal species being reversed, depending on the plant species with which they were associated (Bever *et al*., 1996).

Fungal spore diversity differs seasonally, with some fungi sporulating in late spring and others sporulating at the end of summer. As the spores represent the dormant state of the fungus, the physiologically active state is most likely the mirror image of the seasonal spore counts. This factor also can effect on spore number estimation as well as species diversity. Crop rotation with periods of bare fallow and non-mycorrhizal plants have been known to cause stunting and P and Zn deficiencies in subsequent planting with species highly dependent on mycorrhizal fungi for mineral nutrition (Thompson, 1987; Thompson, 1994). These symptoms are related to a decline in mycorrhizal propagules in the soil and the consequent decrease in colonization and nutrient uptake (Thompson, 1994). Generally, there is no specific correlation between number of spores and species diversity in one province or one habitat. Management of inherent biological and ecological cycles to preserve soil resources and maintain economic productivity is the central tenant of organic farming (Atkinson *et al*., 2002). However, non-standardized organic practices may result in the use of some modern agricultural methods such as continous monoculture, fallow and non-host crop in rotation and tillage that have adverse effects on the diversity and activity of AM fungi. Therefore, describing the community of AMF at a site becomes an important first step in determining the effects of agricultural treatments uppon AMF and the eventual development of management regimes for these fungi.

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