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Arbuscular-mycorrhizal Fungi Associated with Alfalfa Rhizosphere in Iran

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Abstract: 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 works have been done regarding their distribution in alfalfa fields in Iran. During the periods from July-September 2003-2004, 180 soil samples were collected from the main-alfalfa producing provinces in Iran. The population as well as identification and distribution of species were studied. All soil samples were classified into one of 13 major habitat groups according to soil type and crop rotation system. The number of (AM) spores varied between 95-427 and was recorded in 50-100% of the sites examined. Among the 19 species identified, 14 species were of *Glomus*, 3 species of *Gigaspora* and 1 species each of *Scutellospora* and *Sclerocystis* genera. The most abundant species recorded were *G. fasciculatum* and *G. mosseae* with a frequency of 38 and 15%, respectively.

Key words: Alfalfa · diversity · mycorrhiza

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) are obligately symbiotic soil fungi which colonize the roots of the majority of plants. These fungi are so named because they produce characteristic finely branched hyphal structures, termed arbuscules, inside cortical cells of plant roots. The AMF genera *Gigaspora* and *Scutellospora* produce only arbuscules and inter-and intracellular hyphae, whereas *Glomus*, *Entrophospora*, *Acaulospora* and *Sclerocystis* also produce vesicles (hence the frequently used term Vesicular-arbuscular Mycorrhizal (VAM) fungi), which are terminal, globose, lipid rich structures in intracellular areas of the root cortex [1]. The AM symbiosis is typically mutualistic. As obligate symbionts, AMF are believed to be dependent upon the host plant for fixed carbon. The plant receives a variety of benefits which may result in increased growth: improved water relations [2], pest and disease resistance [3], enhanced nutrient uptake over non-mycorrhizal controls [4] and modification of root morphology [5].

The most important of these benefits is increased nutrient uptake, notably of immobile nutrients such as P and Zn [6, 7]. Extra-radical hyphae of the AMF extend up to 8 cm beyond the root [8] and act, in effect, as extensions of the root system in acquiring nutrients from the soil. The below-ground ecosystem as a whole is affected by AMF. These fungi are important in maintaining and enhancing the stability of soil aggregates [9-11]. Soil aggregation is an important aspect of soil structure, which determines characteristics such as water inflow rate, pore space and resistance to erosion. Extra-radical AMF hyphae enmesh and entrap soil particles, stabilizing the aggregates. Members of the Gigasporineae may play a greater role in this than those of the Glomineae [11, 12]. Functioning extra-radical hyphae also secrete a glycoprotein termed glomalin [13]. This substance may be present at levels as high as 1.5% of the dry weight of soil [14] and there is evidence it plays a significant role in the production and maintenance of water stable aggregates in soil. In addition, AMF substantially affect nutrient cycling [15] and carbon flow from the autotrophic plant to the heterotrophic soil

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microbial community because of their effect on root exudation [16]. This regulation of carbon flow can be an important regulator of the soil microbial community [17]. For example, the presence of *Glomus mosseae* affected the relative abundance of rhizosphere bacteria species [18]. It is evident from their effects upon soil health and host plant growth that AMF are an important part of sustainable agricultural systems that have low inputs of chemical fertilizers and biocides [15, 19, 20]. Symbiosis efficiency depends on environmental factors as well as genetic determinants from both plant and AM fungi [21]. Moreover, increasing plant diversity in a field experiment can result in increased AM fungal sporulation and community composition [22].

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 alfalfa soybean rhizosphere in Iran
2. Determination of prevalent species found in alfalfa soybean rhizosphere
3. Study on species diversity in different alfalfa soybean cultivated regions in Iran

MATERIAL AND METHODS

During the periods from July-September 2003-2004, 180 soil samples were collected from main alfalfa-producing provinces in Iran, including Hamedan, Khorasan, Esfahan, Kerman, West Azaebaijan and East Azarbaijan. Also, additional data such as crop rotation status in previous years, host plant variety and soil texture were recorded. Collected soil samples were used directly for estimation of number of AM 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 [23, 24]. 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 [25]. Also, species diversity was recorded. In order to comparison of species similarities on different sampling areas, the Jaccard index [26] 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

Considerring to some additional data such as crop rotation status, host plant variety and soil texture and due to better facility of studies on species diversity, we classified the sampling areas to different several habitats. So the alfalfa-producing sampling regions were classified into one of 13 major habitat groups (Table1). The number of spores estimated on different alfalfa rhizosphere soils showed that the most number of spores belonged to A_2 habitat, i.e. Hamedan province with sandy loam soils without crop rotation. Also the least numbers observed in F habitat, i.e. East Azarbaijan province with clay loam soils with crop rotation by cereals (Table 1). The number of spores varied between 95-427 and was recorded in 50-100% of the sites examined, so the number of areas with spore might be varied in different habitats (Table 1).

Table 1: Alfalfa rhizosphere habitat groups and total number of spores in each habitat

Province	Code	Soil Texture	Crop rotation	Regions with Spores (%)	Number of Spores
Hamedan	A_1	Sandy	-	100	251
	A_2	Sandy loam	-	80	427
	A_3	Clay loam	-	100	200
	A_4	Coarse sandy	-	90	185
Khorasan	B_1	Coarse sandy	-	86.7	300
	B_2	Clay loam	-	66.7	280
	B_3	Silty clay loam	-	73.3	385
Esfahan	C_1	Sandy loam	-	85	250
	C_2	Sandy loam	+	75	145
Kerman	D_1	Sandy clay loam	-	70	160
	D_2	Sandy clay loam	+	50	100
West Azarbaijan	E	Sandy loam	+	55	115
East Azarbaijan	F	Clay loam	+	66.7	95

Table 2: Mycorrhizal species and their relative abundance in alfalfa rhizosphere

Species	% of Frequency	Species	% of Frequency
Glomus fasciculatum	38	*Glomus fulvum*	0.5
Glomus mosseae	15	*Glomus microcarpum*	0.5
Glomus etunicatum	11	*Glomus geosporums*	0.5
Glomus intraradices	9.5	*Glomus caledonium*	0.5
Glomus albidum	8	*Glomus reticulatum*	0.3
Glomus constrictum	6	*Gigaspora margarita*	0.3
Gigaspora albida	6	*Gigaspora gigantea*	0.3
Glomus macrocarpum	1	*Glomus diaphanum*	0.3
Scutellospora coralloidea	1	*Glomus dimorphicum*	0.3
Sclerocystis coremioides	1		

It seems generally that fields with more or less heavy soil texture and without crop rotation have the higher number of spores but it is not observed in all habitats. However, it should be mentioned that different biological and physico-chemical factors influence the number of spores as well as their diversity. Totally 19 species of AM fungi were identified. These belonged to 4 genera including *Glomus*, *Gigaspora*, *Scutellospora* and *Sclerocystis*, respectively. Among the species identified, 14 species were of *Glomus*, 3 species of *Gigaspora* and one species each of *Scutellospora* and *Sclerocystis*, respectively. The list of all species identified as well as their frequencies is shown in Table 2. All of these species are recorded for the first time from alfalfa rhizosphere in Iran. Among the species, 4 including *Glomus dimorphicum*, *Glomus reticulatum*, *Gigaspora albida* and *Scutellospora coralloidea* are new record for the mycoflora 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 found in one province while not observed in others. Also, this pattern may be observed in different habitats of one province. The most abundant species recorded were *Glomus fasciculatum* and *Glomus mosseae* with a frequency of 38% and 15%, respectively (Table 2). Alfalfa habitat groups classified to six different subgroups according to all species diversity (Table 3). Subgroup I including the habitas C_1, $C_{,2}$ E each with 11.11%, subgroup II including the habitat B_3 with 8.24%, subgroup III including habitats F and B_1 each with 8.05%, subgroup IV including habitat B_2 with 7.84%, subgroup V including habitats A_2, A_3, A_4, D_1, D_2 each with 5.56% and subgroup VI including habitat A_1 with 5.46% species diversity. Species number in each sample from alfalfa habitat groups varied from minimum 7 (habitat A3) to maximum 18 (habitat B2) with mean 12 species. There is no specific correlation

Table 3: Numbers and diversity of AM fungal species in alfalfa rhizosphere habitat groups

Fungal species	A_1	A_2	A_3	A_4	B_1	B_2	B_3	C_1	C_2	D_1	D_2	E	F
G. fasciculatum	410	375	400	150	100	150	300	290	300	230	155	350	210
G. mosseae	50	43	90	80	230	90	60	165	140	75	90	135	102
G. etunicatum	0	15	0	70	50	40	10	100	125	80	100	300	100
G. intraradices	10	10	5	75	100	100	50	150	85	50	40	80	100
G. albidum	2	30	0	48	60	50	70	60	100	10	40	100	150
G. constrictum	0	0	0	0	50	70	80	100	50	20	20	25	10
Gi. albida	0	0	0	20	13	20	115	120	180	20	30	7	15
G. macrocarpum	0	0	0	0	5	20	30	0	0	5	10	0	20
S. coralloidea	3	10	0	20	25	20	0	0	10	2	0	0	0
S. coremioides	0	5	1	10	20	30	10	10	4	0	0	0	0
G. fulvum	0	0	1	9	10	20	0	0	0	0	5	0	0
G. microcarpum	5	5	0	3	15	15	2	0	0	0	0	0	0
G. geosporum	5	2	2	5	12	16	3	0	0	0	0	0	0
G. caledonium	0	0	0	0	0	10	10	0	0	5	5	2	13
G. reticulatum	0	2	1	3	0	20	0	0	1	0	0	0	0
Gi. margarita	5	3	0	2	15	0	2	0	0	0	0	0	0
Gi. gigantea	1	0	0	0	5	20	0	0	0	0	0	1	0
G. diaphanum	0	0	0	5	10	10	0	0	0	2	0	0	0
G. dimorphicum	0	0	0	0	1	5	0	5	5	1	5	0	5
Number of species	9	11	7	14	17	18	13	9	11	12	11	9	10
Species diversity (%)	5.46	5.56	5.56	5.56	8.05	7.84	8.24	11.11	11.11	5.56	5.56	11.11	8.05

Table 4: Jaccard similarity index between alfalfa-producing provinces

Compared provinces	Jaccard index
Hamedan/Khorasan	0.79
Hamedan/Esfahan	0.53
Hamedan/Kerman	0.47
Hamedan/West Azarbaijan	0.40
Hamedan/East Azarbaijan	0.32
Khorasan/Esfahan	0.58
Khorasan/Kerman	0.68
Khorasan/West Azarbaijan	0.47
Khorasan/East Azarbaijan	0.53
Esfahan/Kerman	0.67
Esfahan/West Azarbaijan	0.54
Esfahan/East Azarbaijan	0.61
Kerman/West Azarbaijan	0.61
Kerman/East Azarbaijan	0.83
West Azarbaijan/East Azarbaijan	0.73

between the number of spores and species diversity in one habitat. The results of Jaccard similarity index (Table 4) showed that the most similarity was observed between Kerman and East Azarbaijan provinces. May be this is due to soil texture similarity in these two provinces. Most of the fields in these Provinces have soils with clay loam contents. 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.

DISCUSSION

A diverse AM fungal population is a key factor to improve the sustainability of low input and organic agricultural systems [27, 28]. 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 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 [29]. 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 [29, 30]. 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 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 [29]. 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 [31]. Colonization rates and spore densities also differed among the plant families. 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 [32, 33]. The colonization pattern varied among the plant species and even among the ecotypes of individual species. Mycorrhizal fungi and their hosts occupy different positions on the "mutualism-parasitism continuum" under different environmental conditions [34]. Also, It has been shown that AM fungal diversity has a strong effect on the plant community, on plant productivity and on succession [29, 35].

On other hand, 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. The presence and infectivity of mycorrhizal fungi in the soil may be greatly reduced by soil disturbance [36, 37]. We also found that species richness and diversity were somewhat lower in disturbed soils than in undisturbed grassland soils and decreased with increasing severity of disturbance. For instance, 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 [38, 39]. These symptoms are related to a decline in mycorrhizal propagules in the soil and the consequent decrease in colonization and nutrient uptake [39]. A strong negative correlation was found between soil organic matter content and root colonization rate. We can speculate that the relative paucity of AMF communities in the studied field was due to heavy texture of the soil. Small size of pores in clay soil may hamper the growth of AMF hyphae in soil [40] either mechanically by imposing a penetration barrier to AMF hyphae [41] or by affecting oxygen concentration in the soil [42]. Such conditions may exert strong selective pressure on AMF, resulting in changes in their community composition. The mechanisms by which soil properties and other factors influence mycorrhizal associations still remain unclear and require more detailed research. Generally, there is no specific correlation between number of spores and species diversity in one province or one habitat. The relationship between spore number and percentage of root length colonized by AM fungi is complicated and may be influenced by many environmental and biological factors [43]. Interpretation of diversity indices with respect to AMF should be carried out with caution because spore densities are one component of these indices. In such studies, where a large percentage of AMF species were not sporulating at the time of sampling, indices only give some measure of diversity, evenness and dominance of the sporulators in a mycorrhizal community [45].

Management of inherent biological and ecological cycles to preserve soil resources and maintain economic productivity is the central tenant of organic farming [46]. 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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