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Full Length Research Paper

Study on occurrence and diversity of *Arbuscular mycorrhizal* fungi associated with grapevine rhizosphere in West Azerbaijan Province 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 grapevine fields in Iran especially in west Azerbaijan as important producing area. During the periods from July to September 2008 to 2009, 40 composite soil samples were collected from 4 main vineyard growing sites in west Azerbaijan province of Iran. The spore numbers as well as identification and distribution of fungal species were studied. The number of spores varied between 32 to 695 spores with an average of 213 spores per 100 g soil sample. After root clearing and staining, fungal colonization as well as different fungal structures including fungal hyphae, vesicles and arbuscules could be observed. Cluster analysis of sampling habitats based on average spore numbers using UPGMA (unweighted pair group method with arithmetic mean) method showed that habitats put in 2 main clusters. Habitats A, B and C put in one cluster and habitat D put in separate one. Among the 12 fungal species identified, 11 species were of *Glomus* and 1 species belonged to *Scutellospora* genera. The most and least abundant species recorded were *Glomus fasciculatum* and *Glomus glomerulatum* with a frequency of 78 and 1%, respectively.**

Keywords: *Arbuscular mycorrhiza* fungi, identification, diversity, grapevine.

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, thought that AM fungi play an important role in most terrestrial ecosystems. Nonetheless, 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

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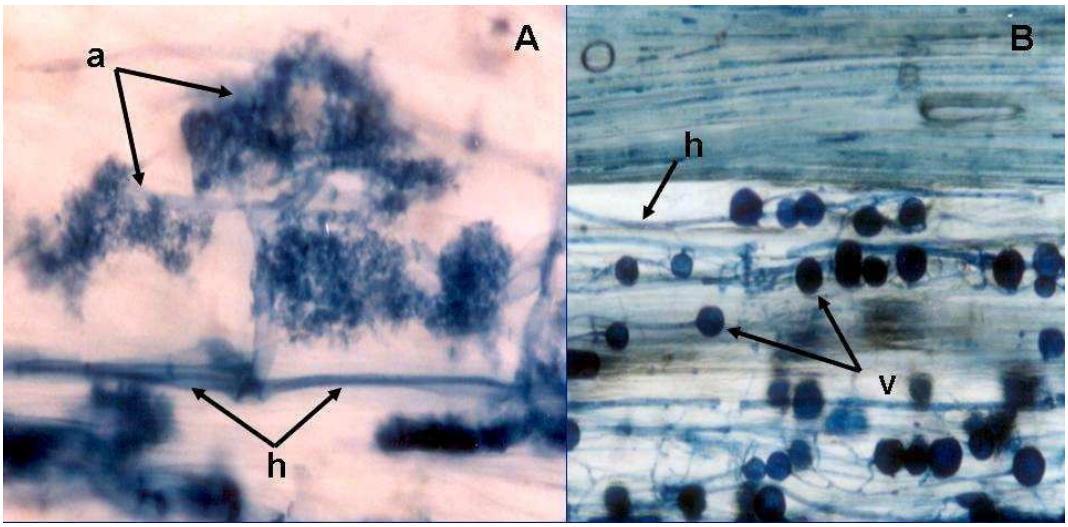

Figure 1. Different fungal structures in root samples after clearing and stainin A:Mycorrhizal hyphae (h) and arbuscules (a) (×400); B: fungal hyphae (h) and vesicles (v) (×100).

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 west Azerbaijan province; 2. Determination of prevalent species found in rhizosphere; 3. Study on species diversity in different grapevine orchards in west Azerbaijan Province.

MATERIALS AND METHODS

During the periods from July to September 2008 to 2009, 40 composite soil samples were collected from 4 main vineyard growing sites in west Azerbaijan province of Iran. These sites were Urmia (A), Miandoab (B), Sardasht (C) and Naghadeh (D). Collected soil samples were used directly for estimation of number of spores. For this purpose, three replicates each 100 g were selected for each soil sample and spores were separated using wet sieving and centrifugation by sucrose gradient method (Jenkins, 1964). The numbers of spores were measured as the mean for each three replicates. Then different sampling sites compared with each other based on average spore numbers using cluster analysis. Root samples were cleared and stained for observing mycorrhizal colonization as well as different fungal structures (Philips and Hayman, 1970). Trap cultures with 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

In all soil samples, mycorrhizal fungal spores were observed and extracted. The number of spores estimated on different sampling sites showed that the most and least number of spores belonged to Miandoab and Naghadeh sites with 223.77 and 200.6 spores per 100 g soil, respectively. The number of spores varied between 32 to 695 spores with an average of 213 spores per 100 g soil sample. 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 physicochemical factors affect the number of spores estimated as well as their diversity. After root clearing and staining, in all root samples fungal colonization could be observed. Also, different fungal structures including fungal hyphae, vesicles as well as arbuscules could be detected, which of them the vesicles were dominant (Figure 1). These findings were compatible with other researchers (Muthukumar et al., 2004). Cluster analysis of sampling habitats based on average spore numbers using UPGMA method showed that habitats put in 2 main clusters. Habitats A, B and C put in one cluster and habitat D put in separate one. Also, the similarity between A and C habitats were more than B based on average spore numbers (Figure 2).

Totally, 12 fungal species belonged to 2 genera,

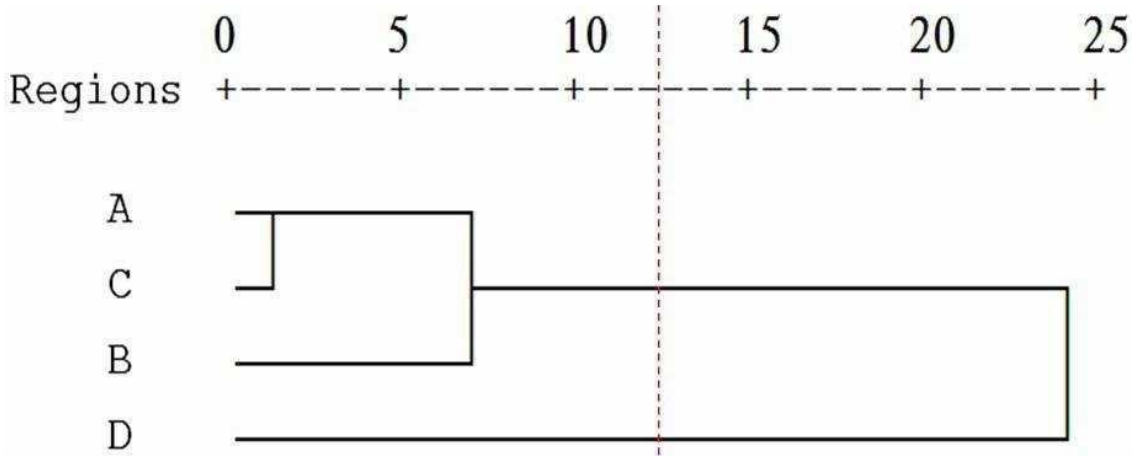

Figure 2. Cluster analysis of sampling regions based on average spore numbers using UPGMA method.

Table 1. Mycorrhizal species and their relative abundance in grapevine rhizosphere.

Fungal Species	Frequency (%)
Glomus fasciculatum	78
G. mosseae	46
G. aggregatum	40
G. macrocarpum	32
G. geosporum	25
G. constrictum	20
G. intraradices	14
G. caledonium	10
G. etunicatum	4
G. versiforme	4
*Scutellospora calospora**	
*G. glomerulatum**	

*New species for mycoflora of Iran.

Table 2. Jaccard similarity index between grapevine-producing regions.

Regions compared	Jaccard Index
Urmia/Miandoab	0.85
Urmia/Sadasht	0.67
Miandoab/Naghadeh	0.55
Urmia/Naghadeh	0.35
Miandoab/Sardasht	0.14
Sardasht/Naghadeh	0.1

Glomus and *Scutellospora*, 2 orders, *Glomerales* and *Diversisporales* and 2 familes, *Glomeraceae* and *Scutellosporaceae* were identified. Among the species, 11 were of *Glomus* and one belonged to *Scutellospora*, respectively. The lists of all species identified as well as their frequencies are shown in Table 1. Two species; *Glomus glomerulatum* and *Scutellospora callospora* were reported for the first time in Iran. Also, *Glomus versiforme, Glomus etunicatum* and *Glomus caledonium* were new for grapevine mycoflora in 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 site while not in others. The number of species in each sample varied between 3-7 species with average 4 species. The most and least abundant species recorded were *Glomus fasciculatum* and *G. glomerulatum* with a frequency of 78 and 1%, respectively (Table 1). Fungal species diversity was different among sampling regions as follow: Urmia (47%)> Sardasht (25%)> Miandoab (18%)> Naghadeh (10%).

There is no specific correlation between the number of spores and species diversity in one habitat. The results of Jaccard similarity index (Table 2) showed that the most similarity was observed between Urmia (A) and Miandoab (B) regions. May be this is due to no crop rotation in these two provinces. Most of the orchards in these regions have been under grapevine cultivation for more than 15 years. However, there are so many different environmental and physico-chemical factors which effect on number of spores and species diversity among different habitats.

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 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, that is, 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 (Bever et al., 2001; Talukdar, 1993). 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 affect 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, that is, 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 (Bever et al., 1996; Talukdar, 1993). 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, 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 continuous 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 upon AMF and the eventual development of management regimes for these fungi.

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