Diversity of Arbuscular Mycorrhizal Fungi in Arable and Natural Soils in Korea

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ABSTRACT : The diversity of arbuscular mycorrhizal fungi (AM) was investigated in cultivated and natural field sites of Chungbuk, Korea. Soils were collected from rhizosphere of *Sorghum bicolor*, *Fagopyrum esculentum* and *Glycine max* in cultivated sites, and of *Miscanthus sinensis*, *Glycine soja* and *Lespedeza cuneata* in natural sites. Total 20 species of Glomalean fungi were found in this study. Species richness, species diversity and density of AM fungi were significantly lower in the arable sites. While only AM fungal spores belong to *Glomus* and *Acaulospora* were found in arable field sites, more diverse fungal spores including Gigasporaceae were found in natural grasslands. AM fungal spore composition did not significantly differ among crop plant species. Results suggest that the agricultural practices were significantly influenced on AM fungal community structures and mycorrhizal developments.

Key words : Arbuscular mycorrhizal fungi, Fungal spores, Glomales, Species diversity

INTRODUCTION

Arbuscular mycorrhizas (AM; also referred to as Vesicular-Arbuscular Mycorrhiza, VAM) are mutualistic symbiosis between Glomalean fungi and most terrestrial plants (Trappe 1987, Morton and Benny 1990, Smith and Read 1997). It has been well documented that the major benefits of plants from these relationships are improvement of uptakes in water and inorganic nutrients, especially phosphorus (Sanders and Koide 1994). Additional benefits include increased tolerance to the environmental stresses such as nutrient deficient soil, drought condition, salinity and pathogens (Newsham *et al.* 1995, Lapointe and Molard 1997).

AM fungi are ubiquitous in terrestrial ecosystem and it has been widely accepted that AM fungi play important roles in maintaining structure and functions of ecosystem (Smith and Read 1997). These fungi may influence plant fitness and inter- or intra competition, leading to relative shift of the plant community structure (Hartnett *et al.* 1993, Hetrick *et al.* 1994, van der Heijden *et al.* 1998, Hartnett and Wilson 1999, Urcelay and Diaz 2003). In return, as obligate symbionts, AM fungi entirely depend on host plants during their life history. Studies showed that sporulation of AM fungi varies with host species, suggesting that host plant species affect community composition and structure of AM fungal spores in soil and may be one of the most important factor regulating AM fungal community (Johnson *et al.* 1992, Bever *et al.* 1996, Eom *et al.* 2000). Likewise, AM fungal species have been shown differential effects across difference host plant species and importance roles of composition of AM fungi in productivity and diversity of plant community and ecosystem have been recognized (van der Heijden *et al.* 1998, Klironomos *et al.* 2000).

Modern agricultural practices such as fertilizers, fungicides application and tillage as well as monoculture affect community composition and diversity of AM fungi (Johnson 1993, Douds *et al.* 1995, Schreiner and Bethlenfalvay 1996, Helgason *et al.* 1998, Jansa *et al.* 2002). Application of fertilizer and fungicide in cultivated field influence AM fungal community directly or indirectly by changes in soil properties such as pH, nutrient concentration, moisture and organic matter (Johnson 1993, Schreiner and Bethlenfalvay 1996). The mycelium of AM fungi in soil extends of host plant roots and transport inorganic nutrients and water, also common hyphal networks connect between roots of plants (Graves *et al.* 1997, Simard *et al.* 1997). The tillage as a soil disturbance breaks up these networks and leads to a significant influence on community of AM fungi (Galvez *et al.* 2001). All of these processes including host species effect and changes in soil properties suggest that agricultural practices are important factors of regulating communty of AM fungi. In present study, species composition and distribution of AM fungal spores in soils of cultivated and natural field sites associated with various host plant species were compared to test whether agricultural practices and host plant species influence composition and abundance of AM fungal communities.

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MATERIALS AND METHODS

This study was conducted in an area of Goesan, Chungbuk, Korea. Total nine sampling sites for both arable and natural fields were located on the road from Goesan to Danyang in the study area and sites were all approximately more than 1 km and less than 6 km apart. Three plant species which were abundant in the study area were selected: *Miscanthus sinensis* (Gramineae), *Glycine soja* (Fabaceae) and *Lespedeza cuneata* (Fabaceae). Three sites were randomly selected in the study area, where all three species were growing within approximately 20 meters. Also, six arable sites growing *Sorghum bicolor* (Gramineae), *Fagopyrum esculentum* (Polygonaceae) and *Glycine max* (Fabaceae) were selected within study area, two sites for each plant species and three soil samples were taken from each arable site (total 18 samples).

In September of 2002, soil samples were collected from each plot using a soil corer (5 cm diam.×15 cm depth) and homogenized manually. The AM fungal spores were extracted from 100 g dry weight soil sample using wet-sieving and sucrose density gradient centrifugation (Daniels and Skipper 1982). The extracted spores

Table 1. Mean density, percent relative abundance and frequency of AM fungal spores (in 100 g dry soil) found in soils collected from cultivated (n=18) and natural (n=9) fields with ANOVA results for effect of cultivation on each species of AM fungi

	Mean Density[a] (spore # / 100 g soil)		RA[b] (%)		Frequency[c]	
	Cultivated	Natural	Cultivated	Natural	Cultivated	Natural
Acaulosporaceae						
Acaulospora denticulate	1.2	–	0.9	–	2	–
A. scrobiculata	–	184.4	–	20.6	–	6
A. sp1	2.7		2.1		3	–
A. morrowiae	–	11.1	–	1.2	–	3
Glomaceae						
Glomus fecundisporum	12.6	36.7	9.7	4.1	5	1
G. geosporum	1.9	–	1.5	–	2	–
G. intraradices	1.2	–	0.9	–	1	–
G. mosseae	23.3	111.1	18.0	12.4	9	9
G. rubiformis	–	2.2	–	0.2	–	1
G. etunicatum	45.9	114.4	35.5	12.8	11	3
G. aggregaturm	6.3	234.4	4.9	26.2	4	8
G. fasciculatum	24.9	1.1	19.3	0.1	10	1
G. sp1	2.4	–	1.8	–	2	–
G. sp2	1.2	–	0.9	–	1	–
G. sp3	–	87.8	–	9.8	–	8
G. sp4	–	4.4	–	0.5	–	3
G. sp5	5.7	–	4.4	–	4	–
Gigasporaceae						
Gigaspora margarita	–	55.6	–	6.2	–	4
Scutellospora erythropa	–	45.6	–	5.1	–	5
S. gregaria	–	6.7	–	0.7	–	3

[a] Mean of the total spore counts from the subplots in which the species was observed.

[b] Relative abundance = (number of spores of a species / total number of spores) × 100.

[c] The number of samples in which the species was observed (cultivated, n=18; natural, n=9).

were observed and identified under a light microscope using current taxonomic criteria. Only the AM fungal spores which appeared to be viable (based on color, shape, surface conditions, and spore contents) were counted. Voucher specimens of the species sampled are maintained at Korea National University of Education. Approximately 100 g of dry weight root samples were isolated from each soil sample, washed free of soil and stained in 0.05% trypan blue (Koske and Gemma 1989). Roots were observed under dissect and light microscopes to assess the percentage of root length colonized by AM fungi using gridline intersection method (Giovannetti and Mosse 1980).

Abundances of each fungal species were estimated based on spore counts. Brillouin species diversity (Eom *et al.* 2000) and species richness index were calculated for AM fungal community analysis (Magurran 1988). Sorenson's similarity index was calculated based on presence-absence data. This index was calculated for all pairwise comparisons of the cultivated and natural sites to calculate within-similarities of the AM fungal communities in both sites. Mixed model univariate analysis of variance (ANOVA) was used to evaluate the fixed effect of cultivation and the random effect of site location on species diversity, richness and spore density of AM fungal communities. Multivariate analysis of variance (MANOVA) was used to assess the effect of cultivation and host on AM fungal species composition using a statistical package SPSS-WIN.

RESULTS

AM fungal structures, i.e. arbuscules, vesicles, hyphal coils and intercellular non-septate hyphae were present. AM fungal hyphal development in roots, measured as percent root colonization tended to be higher in natural sites (mean 46.2%) than in cultivated sites (mean 25.3%). However, it was not significantly different between cultivated and natural sites ($F=2.89$, $P=0.13$).

Spores of twenty different AM fungal species in four genera were found from the soils collected both in the natural and cultivated sites including six unidentified species: *Acaulospora denticulate*, *A. scrobiculata*, *Glomus aggregatum*, *G. etunicatum*, *G. fasciculatum*, *G. fecundisporum*, *G. mosseae*, *G. geosporum*, *G. intraradices*, *G. rubiformis*, *Gigaspora margarita*, *Scutellospora erythropa* and *S. gregaria* (Table 1). Across all the sites, the most common spores were *Glomus mosseae* and the most dominant spores were *G. aggregatum* (Table 1). Total twelve species in two genera were found in the cultivated soils and thirteen species in four genera were in the natural soils. In the natural soils, *G. mosseae* was the most common spore type of AM fungi while *Glomus etunicatum* was the most common in cultivated sites. Only five species out of 20 species were found in the both sites. Seven species were found only in the cultivated sites and eight species including 3 species in Gigasporaceae were found only in the natural sites. No spore belong to Gigasporaceae was found in the natural sites.

Relative abundance of *Glomus aggregatum* was significantly higher in natural sites than cultivated sites ($F=11.5$, $P<0.01$), but relative abundance of *Glomus fasciculatum* in cultivated site were higher than in natural sites ($F=7.3$, $P<0.05$). The number of spores of most AM fungi was equal or more numerous in the natural soils than in the arable soil (Table 1). Spore count of *G. aggregatum* and *G. mosseae* were significantly higher in natural sites while *G. fasciculatum* were higher in cultivated sites.

Univariate analysis of variance showed no significant effect of host plant species on AM fungal community composition, in terms of species diversity, evenness, richness and spore density (Table 2). However, cultivation affected species diversity and richness of AM fungal communities (Fig. 1). Also, significantly more AM fungal spores were observed in soil from natural field sites. Analysis of Sorenson's similarity index showed significantly higher similarity

Table 2. Means (with standard error in parentheses) for total number of AM fungal spores, species richness, Brillouin diversity and evenness on the different host plant species from the natural and arable field soils

Species	Diversity	Evenness	Richness	Total no. spores
Natural				
Miscanthus sinensis	1.31 (0.10)	0.71 (0.03)	6.67 (0.88)	1,123.3 (531.7)
Glycine soja	1.39 (0.22)	0.76 (0.10)	6.33 (0.88)	646.7 (161.7)
Lespedeza cuneata	1.05 (0.20)	0.65 (0.02)	5.33 (1.20)	916.7 (449.0)
Arable				
Sorghum bicolor	1.00 (0.19)	0.91 (0.03)	3.67 (0.76)	130.2 (48.1)
Fagopyrum esculentum	0.62 (0.22)	0.54 (0.17)	2.50 (0.96)	141.5 (74.0)
Glycine max	0.95 (0.15)	0.88 (0.05)	3.50 (0.67)	116.8 (31.2)

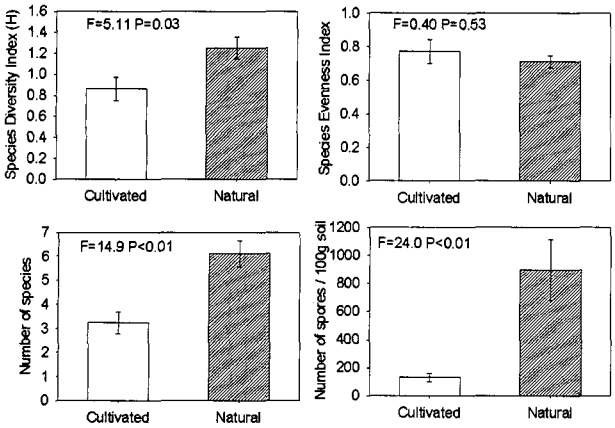

Fig. 1. Effects of cultivation on species diversity, evenness, richness and spore density of AM fungi. Mean values ± standard error with F statistics and P value are presented (n=18 in cultivated, 9 in natural soils).

Table 3. MANOVA results for host effect on AM fungal communities

Source of variation	Wilk's lambda	F	Num df	Den df	P
Host					
Within natural	0.005	2.231	12	2	0.351
Within arable	0.062	1.369	22	10	0.311
Cultivation	0.000	11643	19	7	0.000

among communities in natural sites than communities in cultivated sites (0.60 and 0.32 respectively; F=42.9 P<0.01). Overall mean of similarities was 0.31 and similarity between arable and natural sites was 0.24. MANOVA results confirmed significant differences in abundance of some AM fungi with respect to cultivation with no significant effect of host species on AM spore communities within both cultivated and natural sites (Table 3).

DISCUSSION

No significant difference showed in root colonization rates between cultivated and natural sites in this study. It could be due to different host plant species used in both sites and showed high variation among different host species. Only 3~6 species of AM fungi were found in a sample (100 g of dry weight soil) examined in present study. However, across all the sites, total 20 species of AM fungi were found and this is quite high number of species in a small scale of the study area. This indicates high heterogeneity of composition of AM fungal communities among sites within this study area (overall similarity among sites= 0.31).

It has been known that different host species produce differential patterns of sporulation of AM fungi in both the greenhouse and in the field study (Johnson *et al.* 1992, Bever *et al.* 1996, Eom *et al.* 2000). However, in this study, host species did not significantly affect any factor of mycorrhizal community structure including species richness, diversity, spore density and spore composition, within treatment sites nor whole sites (Tables 2 and 3). Within arable or natural sites, no effect of host species may be due to small size of samples (n=6 in cultivated or 3 in natural). Eom *et al.* (2000) used total 50 soil samples for 5 species collected from natural grassland and showed significant differences in fungal diversity and community composition. Also, many *Glomus* species could not be identified accurately due to the morphological modification as field collected material. The spore counts might not necessarily give representative values for the actual abundance and functional roles of AM fungi in the soil (Clapp *et al.* 1995). The molecular technique has been developed to assess diversity of AM fungi colonizing roots (Helgason 1998) and future work would be use of these techniques to assess activities and functional roles of AM fungi.

Although any significant effect of host plant species on community structure of AM fungal spores was not detected, this study described how AM fungal spore communities were present in soils of cultivated and natural field sites. Natural field sites have AM fungal communities with higher diversity, richness and spore density than cultivated sites as reported in other studies. These results suggest that agricultural practices affected diversity and abundance of AM fungal community. The continuing monoculture may be one of the factors reducing diversity of the AM fungi in arable fields (Boddington and Dodd 2000). Other aspects of the agricultural practices such as tillage, fertilization or fungicide application may also reduce the diversity and abundance of the fungal spores (Johnson 1993, Schreiner and Bethlenfalvay 1996, Eom *et al.* 1999). In this study, AM fungal spores of *Glomus* were dominated in both cultivated and natural sites, but relative abundance of spores of *Glomus* was higher in cultivated sites than in natural sites. In all the arable fields, regardless of host plant or location, any spore belong to Gigasporaceae was not found. The previous studies showed absence or lower abundance of species of *Gigaspora* and *Scutellospora* in arable soils (Helgason *et al.* 1998, Daniell *et al.* 2001, Jansa *et al.* 2002). Giovannetti *et al.* (1999) observed anastomoses between hyphae from *Glomus* species, but not from *Gigaspora* or *Scutellospora*, indicating that species of *Glomus* have the ability to re-establish interconnected network after disturbance between tillage and it could explain abundance of *Glomus* over species of *Gigaspora* or *Scutellospora*. The fungal species belong to *Glomus* may fit better in the disturbed soil in cultivated sites (Boddington

and Dodd 2000). However, it has been reported that species of *Gigaspora* or *Scutellospora* may play a greater role in soil aggregation than *Glomus*, which is important aspects of soil structure such as water inflow rate, resistance to erosion and soil microbial communities (Miller and Jastrow 1990, Schreiner and Bethlenfalvay 1996).

This study and others have reported lower diversity and abundance of AM fungal spores in cultivated field sites than in natural field sites. The losses in the diversity of organisms have been reported around the world and human activities threat the biodiversity, including soil microorganisms in agroecosystem. Because low diversity may be related to the loss of functional role in ecosystem and low stability to environmental changes, this study suggests that agricultural practices should be maintained in minimum level.

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