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Effect of Organic Farming on Spore Diversity of Arbuscular Mycorrhizal Fungi and Glomalin in Soil

Ji-Eun Lee and Ahn-Heum Eom*

Department of Biology Education, Korea National University of Education, Chungbuk 363-791, Korea

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In this study, eight soil samples were collected from organic and conventional farms in a central area of South Korea. Spore communities of arbuscular mycorrhizal fungi (AMF) and glomalin, a glycoprotein produced by AMF, were analyzed. Spores of *Glomus clarum*, *G. etunicatum*, *G. mosseae*, *G.* sp., *Acaulospora longula*, *A. spinosa*, *Gigaspora margarita*, and *Paraglomus occultum* were identified at the study sites, based on morphological and molecular characteristics. While *Acaulospora longula* was the most dominant species in soils at organic farms, *Paraglomus occultum* was the most dominant species in soils at conventional farms. Species diversity and species number in AMF communities found in soils from organic farms were significantly higher than in soils from conventional farms. Glomalin was also extracted from soil samples collected at organic and conventional farms and was analyzed using both Bradford and enzyme-linked immunosorbent assays. The glomalin content in soils from organic farms was significantly higher than in soils from conventional farms. These results indicate that agricultural practices significantly affect AMF abundance and community structure.

KEYWORDS : Arbuscular mycorrhizas, Fungal community, Glomalin, Organic farming

Arbuscular mycorrhizas (AM) is the mutualistic symbiosis that exists between plant roots and fungi belonging to phylum Glomeromycota (Schuessler *et al.*, 2001). AM fungi (AMF) are obligate symbionts, which form a symbiotic association with over 80% of the terrestrial plants. The fungal hyphae spread into the soil from host plant roots and improve the efficiency of nutrient uptake, such as immobile phosphate ions, as the fungal extraradical mycelium develop over the phosphate depletion zone that rapidly grows around the root of the plant (Sanders and Tinker, 1971; Smith and Read, 2008). Moreover, AMF provides mineral forms of nitrate, potassium, and other nutrients to the host plant. In addition, AMF provides enhanced drought resistance (Auge *et al.*, 1994), improved resistance to foliar-feeding insects (Gange and West, 1994), increased tolerance to heavy metals and soil salts (Feng *et al.*, 2002; Khan *et al.*, 2000), and improved protection from soil pathogens (Smith and Read, 2008). AMF hyphae abundantly produce glomalin, an insoluble glue-like substance (Rillig, 2004). Glomalin is a major component of organic matter in soil and contributes to better soil aggregate formation, which is important for soil structure and stability against erosion (Wright *et al.*, 1996; Wright and Upadhyaya, 1998).

Intensive agricultural systems that use large quantities of inorganic fertilizers and pesticides were developed long ago as a method to alleviate food shortages. However, these high-input (conventional) agricultural systems cause serious problems, such as disturbance of agricultural ecosystems, and have high environmental costs. These disadvantages have led to increased demands to make agricultural systems more sustainable by reforming agricultural practices. As a result, interest in organic farming as an alternative agricultural strategy has increased in Korea.

AMF are important for plant physiology, plant health, nutrient cycling, and soil aggregation, particularly for low-input, sustainable agriculture systems (Bethlenfalvay and Barea, 1994). A recent study on the effects of conventional versus organic agriculture reported that different management practices affected AMF communities (Douds and Millner, 1999; Lee *et al.*, 2008b; Oehl *et al.*, 2004). Oehl *et al.* (2004) examined AMF diversity in long-term conventional and organic farming. Their results showed that AMF diversity was higher in organic farming than in conventional farming. On the other hand, Purin *et al.* (2006) reported that AMF sporulation and diversity were higher in conventional farming than in organic farming.

In Korea, there have been only a few studies assessing the impact of agricultural systems on AMF diversity and glomalin concentrations. Therefore, the purpose of this study was to determine the impact of organic farming on AMF spore diversity and glomalin concentrations in soils growing the red pepper, *Capsicum annum* L., which is one of Korea's most important vegetables.

Materials and Methods

Soil sample collection. In April 2009, soil samples at a depth of 0~10 cm were collected from 4 organic farming sites (O1~O4) and 4 conventional farming sites (C1~C4)

*Corresponding author <E-mail : eomah@knue.ac.kr>

Table 1. Chemical characteristics of soils used in the study sites*

Soil chemical attributes	Conventional	Organic
pH (1 : 5)	5.68 ± 0.40	6.02 ± 0.30
Organic matter (g/kg)	15.50 ± 1.19	18.20 ± 1.56
P_2O_5 (mg/kg)	241.75 ± 61.66	560.80 ± 111.90
K ($cmol^+$/kg)	0.47 ± 0.11	0.55 ± 0.06
Na ($cmol^+$/kg)	0.17 ± 0.03	0.06 ± 0.00
Ca ($cmol^+$/kg)	8.20 ± 2.17	5.70 ± 0.69
Mg ($cmol^+$/kg)	2.78 ± 0.54	2.32 ± 0.28
Cation exchange capacity ($cmol^+$/kg)	15.15 ± 2.16	11.62 ± 0.85
EC (ds/m)	1.12 ± 0.19	0.65 ± 0.15
NO^3 ¯N (mg/kg)	37.91 ± 28.06	26.44 ± 13.32

*Values are means ± standard deviation (n = 4).

in Goesan, Korea (C1; 36° 49' 38" N 127° 51' 41" E, C2; 36° 49' 38" N 127° 51' 43" E, C3; 36° 49' 31" N 127° 50' 40" E, C4; 36° 48' 52" N 127° 48' 59" E, O1; 36° 48' 28" N 127° 52' 7" E, O2; 36° 48' 27" N 127° 52' 8" E, O3; 36° 50' 16" N 127° 53' 34" E, O4; 36° 49' 41" N 127° 51' 55" E). Three replicates per farm were collected, sealed in plastic bags, and kept at 4°C until analyzed. The soil chemical properties were analyzed in Chungbuk Agricultural Research and Extension Service (Table 1).

Spore extraction and morphological identification. AM fungal spores were extracted from 10 g of soil using wet sieving and sucrose density gradient centrifugation methods (Daniels and Skipper, 1982) and were then observed under a light microscope. The AMF spores were identified on the basis of morphology (i.e., shape, surface ornamentation, color, contents, and wall structure) (Schenck and Perez, 1990) and were compared to the morphological descriptions of species presented on the International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) webpage (http://invam.caf.wvu.edu).

Molecular identification of AMF spores. Morphologically identified spores were separated to a single spore that was used for molecular identification. The spore was washed 3 times with distilled water, crushed in a 0.2-*ml* polymerase chain reaction (PCR) tube to extract the DNA, and then 2 μl of sterilized water was added to the PCR tube. Partial fungal small-subunit ribosomal DNA fragments were amplified by nested PCR (van Tuinen *et al.*, 1998). The first PCR was performed using the universal primers NS1 and NS4. PCR amplified DNA (about 1100 bp) was separated on 1.2% agarose gel, stained with ethidium bromide (EtBr), and examined under an ultraviolet (UV) transilluminator. The first PCR product was diluted 1 : 100 and used as a template for the second amplification with AM fungal-specific primers, AML1 and AML2 (Lee *et al.*, 2008a). The second PCR product was sequenced using the AMIPRISM 377 automated sequencer (Perkin-Elmer, USA). A sequence-similarity search of the National Center for Biotechnology Information (NCBI) database was conducted using the Basic Local Alignment Search Tool (BLAST) algorithm. The sequences were aligned, and a phylogenetic bootstrap consensus tree (1000 replicates) was obtained using MEGA 4 (Tamura *et al.*, 2007).

Glomalin analysis. Glomalin was extracted from soil samples as described previously (Wright *et al.*, 1996; Wright and Upadhyaya, 1998). Total glomalin (TG) was extracted with 50 mM sodium citrate, pH 8.0, at 121°C in 1-h cycles until the supernatant was nearly colorless. All the supernatants from a sample were combined, and the volume was measured. An aliquot was centrifuged at 10,000 ×*g* to remove soil particles, and the protein concentration was determined by the Bradford assay using bovine serum albumin (BSA) as the standard. An enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody MAb32B11 against glomalin was used to perform immunoassays on TG.

Statistical analysis. We calculated relative abundance on the basis of morphological and molecular identification data. Relative abundance was calculated as the number of spores of each species divided by the total number of spores. Species diversity of the AM fungal spores was calculated using the Shannon-Wiener diversity index (Magurran, 1988). Differences in the number of species, Shannon diversity indices, and glomalin concentrations between sites were analyzed using a Student's *t*-test and SPSS 12.0 software (SPSS Inc., USA). The AMF community composition was analyzed on the basis of AMF spores using principle component analysis.

Results

AM fungal spore identification. Spores isolated from study sites were separated and identified using morphological characters and molecular identification. A total of 8 species in 4 genera were identified: 4 species of *Glo-*

Table 2. Small ribosomal subunit DNA sequence comparison between arbuscular mycorrhizal fungal spores

Species	Accession number	Sequence similarity (%)
Glomus sp.	EF136891	754/758 (99%)
Glomus mosseae	AY635833	743/749 (99%)
Glomus etunicatum	AJ852598	762/766 (99%)
Glomus clarum	AJ852597	742/745 (99%)
Gigaspora margarita	AJ852605	726/727 (99%)
Acaulospora longula	AJ306439	735/741 (99%)
Acaulospora spinosa	Z14004	735/743 (98%)
Paraglomus occultum	DQ322629	740/748 (98%)

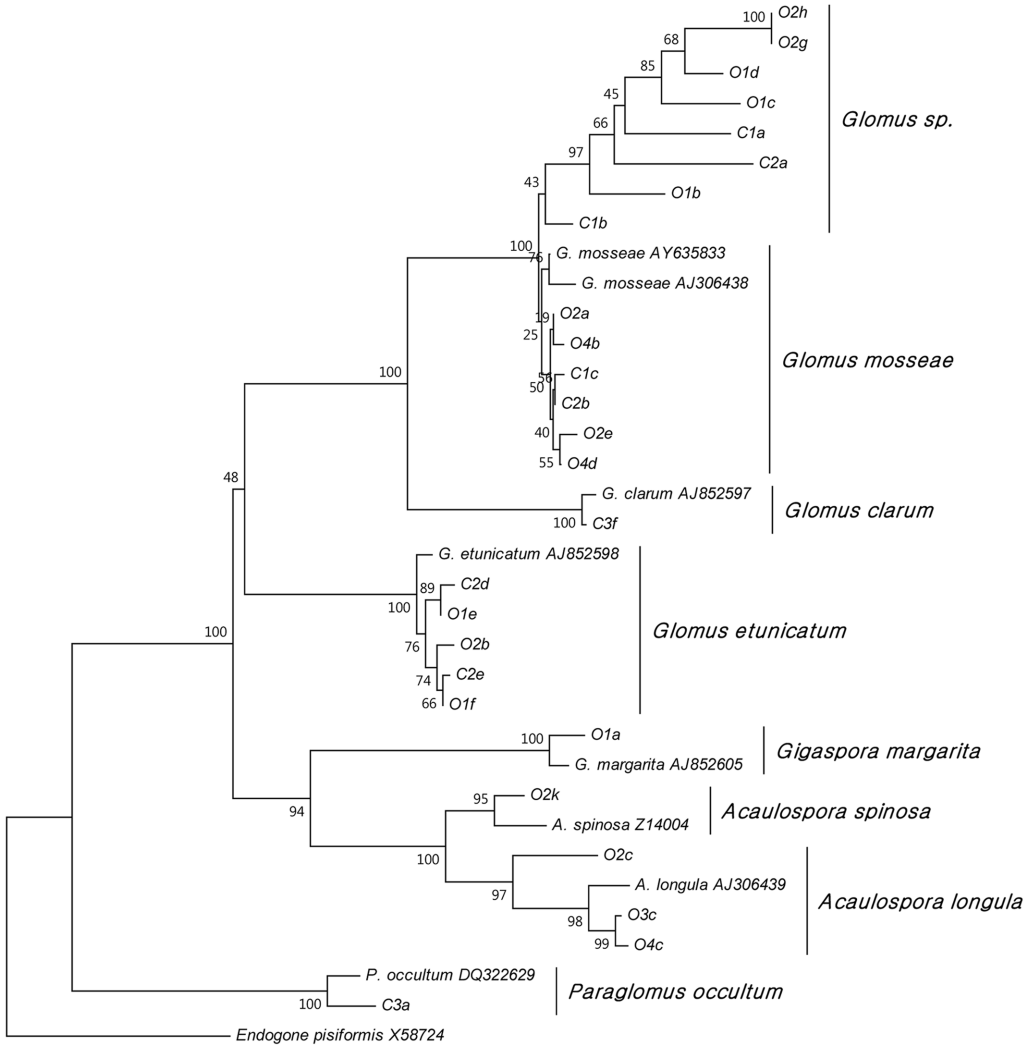

Fig. 1. Neighbor-joining phylogram for partial 18S rDNA sequence of AMF spores. Numbers at nodes indicate the percentage of bootstrap support with 1000 replicates.

mus, 2 species of *Acaulospora*, 1 species of *Gigaspora*, and 1 species of *Paraglomus* (Table 2; Fig. 1).

Relative abundance and community structure of AMF. As shown in Table 3, 6 of the 8 AMF species identified were found in organic farming soils and only 5 were found in conventional farming soils (Table 3). *Glomus clarum* and *Paraglomus occultum* were observed only in conventional farming soils. On the other hand, *Acaulospora longula*, *A. spinosa*, and *Gigaspora margarita* were observed only in organic farming soils. Spores of *Glomus mosseae*, *Glomus* sp., and *P. occultum* accounted for a relatively large proportion of spores identified in conventional farming soils, whereas spores of *A. longula* were found mostly in organic farming soils. The number of AM fungal spore species was significantly higher in organic farming soils in comparison to conventional farming soils (Table 3). Species diversity of AMF spores was

Table 3. Relative spore abundance of AMF species in conventional and organic farming soils

AM fungal species	Relative abundance of AM fungal spores (%)	
	Conventional	Organic
G. mosseae	27.46	21.73
G. etunicatum	0.77	3.99
G. clarum	2.08	–
G. sp.	33.86	22.68
*A. longula**	-	44.01
A. spinosa	-	2.99
*Gi. margarita**	-	4.60
*P. occultum**	35.83	-
Total	100.0	100.0
Number of species*	3.25	5.25
Species diversity index (H')*	0.92	1.29

*The asterisks indicate a significant difference ($P < 0.05$) according to LSD test of one-way ANOVA.

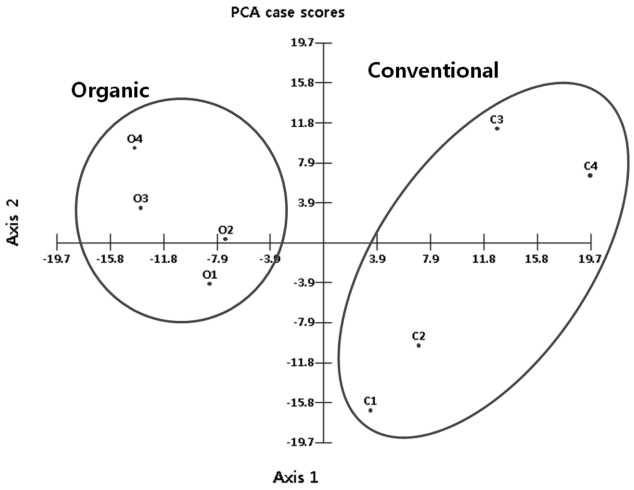

Fig. 2. PCA oridination of AMF community composition based on AMF spores in organic and conventional farming soils.

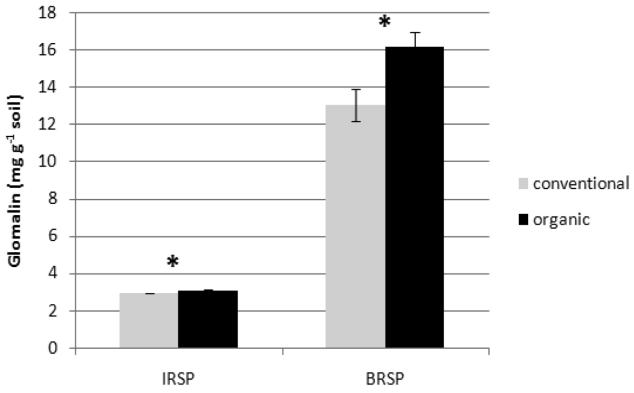

Fig. 3. Mean concentrations of glomalin in soils of conventional and organic farms. BRSP, Bradford reactive soil protein; IRSP, Immunoreactive soil protein. Error bars indicate standard error. Asterisks indicate that the mean is significantly different between agricultural practices ($P < 0.05$).

also significantly higher in organic farming soils in comparison to conventional farming soils (Table 3). Ordination analysis of AMF spore communities using PCA shows that the communities are distinctly separated into two types of agricultural practices along with PCA1 and PCA2 (Fig. 2), It suggests significant differece in species composition AMF spore communitiy between agricultural practices.

Soil glomalin concentrations. Concentrations of Bradford-reactive soil protein (BRSP) and immunoreactive soil protein (IRSP) were significantly different between organic farming soils and conventional farming soils (Fig. 3). Organic farming soils had significantly greater BRSP and IRSP than conventional farming soils ($P < 0.05$).

Discussion

Our study represents the first attempt in Korea to compare conventional and organic red pepper farming on the basis of AMF community structure and glomalin concentrations. We used morphological and molecular characteristics to identify species of AMF spores in conventional and organic farming soils. A total of 8 species in 4 genera were identified. *G. clarum* and *P. occultum* were observed only in conventional farming soils, whereas *A. longula*, *A. spinosa*, and *G. margarita* were observed only in organic farming soils. The result indicate the effect of agricultural practices in community composition of AMF spores. Moreover, both AMF spore numbers and species diversity were significantly higher in soils from farms with organic agricultural practices than they were in soils from farms with conventional farming practices. These results are consistent with previous reports by Oehl *et al.* (2004), which showed that long-term conventional farming systems reduced the number of AMF spores, species diversity, and AMF species belonging to *Acaulospora* and *Scutellospora*. In addition, glomalin content was greater in organic farming soils than in conventional farming soils. These results suggest that agricultural practices significantly influence AM fungal community structure and glomalin contents, and that organic farming increases AMF species diversity. The use of environmentally friendly agricultural practices has been recognized as an important method of sustainable agriculture. Therefore, studies on AMF and agricultural practices are critical.

The results of our study clearly indicate that agricultural practices severely affect AMF abundance and community structure. Remarkably, the AMF communities differed not only in diversity but also in functional aspects (concentrations of glomalin). These findings suggest that agricultural practices should be converted from high-input agricultural systems into more sustainable organic agricultural systems.

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