Effects of Organic Farming on Communities of Arbuscular Mycorrhizal Fungi

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Red pepper (*Capsicum annum* L.) roots and soils representing different agricultural management practices such as conventional (CON), no-chemical (NOC), and organic farming systems (ORG) were collected from 32 farm field sites in Kyunggi, Korea to investigate the effects of these agricultural practices on arbuscular mycorrhizal (AM) symbiosis. ORG inoculum significantly increased plant growth compared to inoculum from CON and NOC. A community analysis of AM fungi (AMF) using morphological features of spores revealed that AMF spore abundance and species diversity were significantly higher in ORG than in CON. Additionally, a community analysis of AMF colonizing roots using a molecular technique revealed higher AMF diversity in ORG than in CON. These results suggest that agricultural practices significantly influence AM fungal community structure and mycorrhizal inoculum potential.

KEYWORDS : Arbuscular mycorrhizas, Organic farming, RFLP, Species diversity

Arbuscular mycorrhizas (AM) exhibit mutualistic symbiosis between fungi in phylum glomeromycota and most terrestrial plant roots (Schuessler *et al.*, 2001; Smith and Read, 1997). It has been well-documented that the major benefit to plants from this relationship is improvement of water and inorganic nutrient, especially phosphorus, uptake. Additional benefits include increased tolerance of environmental stresses such as nutrient deficient soil, drought conditions, salinity, and pathogens (Kurle and Pfleger, 1996).

Modern agricultural practices such as fertilization, biocide application, and monoculture affect the community composition and diversity of AM fungi (Douds and Millner, 1999; Johnson, 1993; Oehl *et al.*, 2004). In general, these agricultural practices have negative impacts on AM association. Soils in the conventional agricultural system are AM fungi-impoverished, particularly with regards to numbers of species (Eom *et al.*, 2004; Helgason *et al.*, 1998). Management practices typical of conventional high input systems, particularly P fertilizer application and the use of biocides, are known to be deleterious to AM fungal symbiosis (Bagyaraj *et al.*, 1989; Kabir *et al.*, 1998; Miller and Jackson, 1998; Thingstrup *et al.*, 1998).

Low-input organic farming systems have increasingly garnered interest due to their focus on natural resource conservation and reduction of environmental degradation. The general principals of organic farming include: (1) exclusion of synthetic biocides; (2) addition of organic fertilizers to the soil, including farmyard manure, compost and crop residue, and slow release mineral fertilizers such as rock phosphate; and (3) use of crop rotation (IFOAM, 1998). Organic fertilizers do not appear to suppress AM fungi and may even stimulate them (Douds *et*

al., 1997; Joner, 2000; Miller and Jackson, 1998). Additionally, although the effect of biocides on AM symbiosis is complex and not easily predictable, overuse of most biocides reduces AM fungi colonization rates and spore production (Kurle and Pfleger, 1994; Schreiner and Bethlenfalvay, 1997). Organic farming relies heavily on active soil microbial communities and AM fungi play an important role in agroecosystem function. It has been reported that compared to conventional systems organic farming increases AM colonization, propagule numbers, and species diversity (Eason *et al.*, 1999; Oehl *et al.*, 2004; Ryan *et al.*, 2000); however, the actual importance of AM symbiosis to particular crop species in organic farming remains to be determined. It is also well-known that different species of AM fungi have varying effects on plant growth, and high input conventional farming may select particular AM fungal species (Johnson, 1993; van der Heijden *et al.*, 1998). Therefore, the purpose of this study was to determine the relative impact of organic farming of red pepper (*Capsicum annum* L.), one of the most economically important crop plants in Korea, on the mycorrhizal inoculum potential and community structure of AM fungi based on morphological and molecular characteristics.

Materials and Methods

Sample collection. Soil and red pepper (*Capsicum annum* L) root samples were collected from 15 organic farm (ORG) sites, 8 no chemical farming (NOC) sites, and 9 conventional field (CON) sites in Kyeongi, Korea. ORG sites were maintained with organic fertilizers and synthetic biocides. NOC sites were treated with mineral fertilizers rather than synthetic biocides. All study sites were maintained using their respective farming system for

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Table 1. Average Chemical properties of soils in this study

	Conventional	No Chemicals	Organic
pH	7.9	8.1	8.1
Organic matter (g/kg)	40.5	41.0	36.0
P_2O_5 (mg/kg)	1521.0	1338.0	1113.0
K ($cmol^+$/kg)	1.42	0.75	0.73
Ca ($cmol^+$/kg)	13.95	10.9	62.5
Mg ($cmol^+$/kg)	2.2	3.4	1.3
Cation Exchange Capacity ($cmol^+$/kg)	22.0	25.3	18.9

more than three years. 1000 g of soil from around the plant roots were collected from the center of each field site, sealed in plastic bags, and stored at 4°C until analysis. From each treatment, two soil samples were randomly selected and analyzed for total C, total P, extractable Mg, and extractable K (Table 1).

Assessment of mycorrhizal inoculum potential. Mycorrhizal inoculum potential was determined using a *C. annum* bioassay. 50 g of collected soil sample were mixed with 100 g of sterilized sands in a 3.8-cm-diameter, 21-cm-deep Cone-tainer Super Cells (Stuewe & Sons, Inc., Corvallis, OR, USA) under greenhouse conditions. Plants were fertilized every other week from 8 wk with 100 ml of 1/4 strength Hoagland solution (Hoagland and Arnon, 1950). After 5 months of growth, above- and below-ground biomass was harvested and dried at 60°C for 48 h to determine dry weight.

Root colonization rates. Roots were stained with Trypan blue (Koske and Gemma, 1989) and observed under stereomicroscopes. Percent root mycorrhizal colonization rates within were measured using the gridline intersect method (Giovannetti and Mosse, 1980).

Spore extraction. AM fungi spores were extracted from 10 g of soil using wet-sieving and sucrose density gradient centrifugation methods (Daniels and Skipper, 1982). Extracted spores were observed under a light microscope and identified morphologically based on shape, surface ornamentation, spore color, spore contents, and wall structure (Schenck and Perez, 1990).

DNA extraction and PCR. Total DNA was extracted from the plant roots and ground with liquid nitrogen using a DNeasy Plant mini kit (Qiagen Science, USA). Partial fungal small-subunit ribosomal DNA fragments were amplified by nested PCRs (van Tuinen *et al.*, 1998). The first PCR was performed using AM fungal specific Primer AML1 (5'-AAC TTT CGA TGG TAG GAT AGA-3') and universal primer NS4 (5'-TTC CAT CAA TTC CTT TAA G-3') for 30 cycles (1 cycle at 95°C for 3 min, at 45°C for 1 min, and at 72°C for 1 min 30 sec; 28 cycles at 95°C for 30 sec, at 45°C for 1 min, and at 72°C for 1 min 30 sec;

1 cycle at 95°C for 30 sec, at 45°C for 1 min, and at 72°C for 10 min). DNA amplified by PCR was separated on 1% agarose gel, stained with ethidium bromide (EtBr), and checked under a UV transilluminator. The PCR product was diluted to 1 : 100 and used as a template for the second amplification with AM fungal-specific primers AML1 and AML2 (5'-CCA AAC ACT TTG GTT TCC-3') for 30 cycles (1 cycle at 95°C for 3 min, at 47°C for 1 min, and at 72°C for 1 min; 1 cycle at 95°C for 30 sec, at 47°C for 1 min, and at 72°C for 10 min). Second PCR products were separated by electrophoresis on a 1% agarose gel and stained with EtBr. Expected fragments for cloning were removed from the gel and purified with a gel purification kit (Bioneer, Korea).

Cloning and RFLP. Purified DNAs were inserted into a pGEM-T EASY Vector and transformed to *Escherichia coli* strain JM109, and 30 putative positive *E. coli* transformants were subsequently selected. Recombinant plasmid DNAs were extracted from the bacterial transformants and used for PCR reaction using primers AML1 and AML2. The PCR products were digested with restriction enzymes *Hinf*I and *Hsp*92II. Restriction fragments were separated on a 3% agarose gel.

Sequence analysis and molecular identification. The nucleotide sequence of 1 clone from each RFLP type was determined using an automatic sequencer (ABIPRISM, USA). DNA sequence analyses were performed with BLAST software available through the National Center for Biotechnology Information (NCBI).

Statistical snalysis. Data from root colonization rates were analyzed using analysis of variance with least significant differences. Comparisons of plant growth between treatments were conducted using Mann-Whitney U-tests. Species diversity of AM fungal spores and RFLP fragment patterns were calculated using the Shannon-Wiener diversity index (H'; Magurran, 1988).

Results and Discussion

Inoculum potentials. Organic management revealed no consistent effect on soil phosphorus and organic matter

Table 2. Dry weights and mycorrhizal colonization rates of *Capsicum annum* L. inoculated with soils collected from different agricultural practices*

Agricultural practices	Root weights (mg)	Shoot weights (mg)	Colonization (%)
Conventional	325.6 ± 115.7a	763.6 ± 97.6a	69.8 ± 12.8a
No chemicals	411.4 ± 33.4ab	939.1 ± 36.8a	83.4 ± 1.2a
Organic	476.0 ± 132.4b	1206.7 ± 82.2b	76.6 ± 5.6a

*Values are means ± 1 standard error; Different letters within columns indicate significant differences (< 0.05).

Table 3. Species diversity of the spores of arbuscular mycorrhizal fungi

AM fungal species	Relative abundance of AM fungal Spores (%)		
	Conventional	No-chemical	Organic
Glomus sp1	–	4	5
Acaulospora scrobiculata	6	7	13
Glomus sp2	–	–	3
Glomus sp3	4	5	14
Glomus sp4	4	3	10
Total Number of Spores	14	19	45
Species diversity Index (H')	1.079	1.339	1.480

compared to conventional management (P = 0.10 and 0.62, respectively; Table 1). Mycorrhizal root colonization showed no significant difference among the agricultural practices (Table 2). However, both root and shoot dry weights were significantly higher in ORG soils than in CON and NOC soils. There was no significant difference in both *C. annum* root and shoot dry weights between inoculums from CON and NOC. These results are consistent with a previous report that AMF inoculum isolated from organic farms was more effective in plant growth promotion under conditions of low nutrient availability than inoculum from conventional farms (Scullion *et al.*, 1998), suggesting that mineral fertilizers used in both CON and NOC influenced mycorrhizal inoculum potential and this might be due to selection of AMF strains that are inferior mutualists in low nutrient soils (Johnson, 1993). AM fungi promote plant growth by providing soil mineral nutrients, particularly phosphorus, to host plants (Smith and Read, 1997); however, high concentrations of mineral phosphorus in soil inhibit AM fungal growth and colonization in the roots of host plants.

AMF spore communities. In three soil samples from plots representing the three different farming systems, five AMF species in two genera (one species in the genus *Acaulospora* and four in the genus *Glomus*) were distinguished based on morphological features of spores. The spores of *Acaulospora scrobiculata* could be readily identified at species level due to the specific ornamentations

of the wall surface; however, the four spore types belonging to the genus *Glomus* could not be indentified to the species. As shown in Table 3, all five species were found in ORG, and three and four species were found in CON and NOC, respectively. Both AMF spore numbers and species diversity were significantly higher under organic rather than conventional management (Table 3).

AMF communities colonizing roots. Restriction fragment analysis using *Hinf*I and *Hsp*92II showed eight distinct patterns (Table 4). Of the eight patterns, four fragment patterns were from CON roots, one from NOC, and six from ORG. Sequence analysis revealed that eight sequences belong to Glomeromycota and were seven species in the genus *Glomus* (Table 5): *Glomus caledonium*, *G. fasciculatum*, *G. mosseae*, *G. proliferum*, *G.* sp1, *G.* sp2, and *G.* sp3. Six species of AMF species were identified in ORG roots, one species in NOC roots, and three in CON roots (Table 5). The species diversity index (H') in the AMF community was higher in ORG roots than those of NOC and CON (Table 5). AM fungi species diversity was higher both in ORG roots and ORG soils; however, there was no difference between CON and NOC in species diversity. These results suggest that mineral nutrients rather than synthetic biocides influence AMF communi-

Table 4. Restriction fragment patterns of DNA extracted from the 23 clones

RFLP Group	Clones	Fragment size (bp)					
		*Hinf*I			*Hsp*92II		
1	O1,O3, O5	426	259	130	481	322	12
2	M9	435	263	102	481	319	
3	C6, C17, C18, O2, O7, O11, O12, O15	721	94		481	322	12
4	C13, C15, C19	432	353		481	304	
5	C2, O8, O10	577	300		481	322	74
6	C1, C4, C6	416	539		481	322	152
7	O9	472	332	89	481	322	90
8	O17*						

*Restriction fragment patterns of DNA were not obtained.

Table 5. Arbuscular mycorrhizal fungal (AMF) species identified by DNA sequences extracted from roots of *Capsicum annum*

Clone number	Closest sequence in Genbank database		% of Clones		
	AMF Species (Accession number)	Identity (%)	Agricultural Practices		
			Conventilanal	No Chemical	Organic
O1	*Glomus* spB (EF393586)	632/738 (85%)			23.1
M9	*G* spC (DQ396695)	727/739 (98%)		100	
O11	*G. caledonium* (Y17635)	715/747 (95%)	33.3		38.5
C4, C19	*G. fasciculatum* (Y17640)	720/737 (97%)	55.6		7.7
O10	*G. mosseae* (AY635833)	724/757 (95%)	11.1		15.4
O9	*G. proliferum* (AF213462)	703/739 (95%)			7.7
O17	*G.* spA (DQ085240)	710/739 (96%)			7.7
	Species diversity (H')		0.94	0	1.59

ties. Overuse of mineral nutrients in CON or NOC farming systems increases concentrations of inorganic ions, which affects AMF diversity.

Spore composition in soils showed that four of the five species found in soils were in the genus *Glomus*. Additionally, molecular analysis of plant roots revealed that AM fungi colonizing the roots were species in the genus *Glomus*. These results are consistent with previous reports that roots in arable fields were dominantly colonized by AMF species belonging to *Glomus* (Helgason *et al.*, 1998). Previous studies also reported that long-term organic farming or reduced tillage management resulted in an increase in AMF species belonging to *Acaulospora*, *Entrophosphora*, and *Scutellospora* (Jansa *et al.*, 2002; Oehl *et al.*, 2004), indicating that these species might play important roles in natural ecosystems and low input sustainable agricultural management, and that conventional agricultural management practices such as high input and intensive tillage remove these species.

Interest in environmental conservation and food safety has recently increased and environmentally friendly agricultural practices have been recognized as an important farming system that could possibly solve the problem of environmental conservation and food safety by reducing the use of synthetic biocides and mineral fertilizers. The number of environmentally friendly agricultural products has increased rapidly. Therefore, studies on AM fungi which play important roles in soil ecosystems are critical. This study confirms that organic farming increases mycorrhizal inoculum potential and AMF species diversity. Losses in the diversity of organisms have been reported globally, including microorganisms in agroecosystems. Because low diversity might relate to the loss of a functional role in the ecosystem and low ability to adapt to environmental changes, this study suggests that agricultural practices should be maintained at minimum levels.

Acknowledgements

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