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Effects of Mycorrhizal and Endophytic Fungi on Plant Community: a Microcosm Study

Sang-Hyun Park and Ahn-Heum Eom*

Department of Biology Education, Korea National University of Education, Chungbuk 363-791, Korea
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This study was conducted to investigate the effects of foliar endophytic fungi and arbuscular mycorrhizal fungi (AMF) on plant community structure in experimental microcosms containing an assemblage of five species of plants (*Oenothera odorata*, *Plantago asiatica*, *Trifolium repens*, *Isodon japonicas* and *Aster yomena*). Leaves of *Sasa borealis*, *Potentilla fragarioides*, and *Viola mandshurica* were collected in Chungbuk, Korea. Endophytic fungi were isolated from the surface sterilized leaves and identified to species level using molecular and morphological techniques. Four isolates of the endophytic fungi were inoculated to the leaves of host plants in the microcosms. Also, three species of AMF spores were extracted from pure cultures and the mixture of the three species inoculated to the roots of the plants. After four months of growth in a green house, effects of both symbiotic fungi on plant species diversity, community composition and productivity were examined. The plant species diversity showed significant differences with inoculation of the symbiotic fungi. Results indicate that AMF significantly affect plant productivity and plant community structure.

KEYWORDS: Community structure, Endophytes, Mycorrhizas, Microcosm

Biological and non-biological factors affect the variety of the plant community and evolution of the species. Intra- and interspecific competition and mutualistic symbiosis such as mycorrhizas and endophytes are included in biological factors. The symbiotic relationships between fungi and plants are widely distributed and these relationships affect the structure and function of ecosystems (Grime *et al.*, 1987; Kranz, 1990). Mycorrhizae and endophytes are representative symbiotic association of the plants and fungi (Clay, 1990).

Mycorrhizae provide benefits to both the fungi and the host plants involved (Smith and Read, 1997). It appears that most groups of higher plants are actually mycorrhizal and grow best under natural conditions when specific fungal partners are present in the soil. Elongation of hyphae of arbuscular mycorrhizal fungi (AMF) from roots to soil greatly increases a potential for the uptake of phosphorus, other nutrients and the absorption of water by the plants. AMF not only benefit host plants with increased growth, but also increase plant ability to withstand or overcome many harsh environmental conditions such as mineral toxicities and deficiencies, water stress, eroded and disturbed soils (Busses and Ellis, 1985; Gildon and Tinker 1981).

The endophytes are defined as a group of fungi which colonize in leaves and stems of plants but cause no symptoms of disease (Petrini, 1991). Endophytes restricted to grasses and sedges were found in groups of *Hypocreales*, *Xylariales* and other ascomycetous fungi (Siegel *et al.*, 1987). Presence of the fungi is frequently often associated with the enhanced fitness of their host plants. These endophytes produce a variety of secondary products, notably alkaloid, which are believed to act as anti-feedants and metabolic inhibitors of insects, to increase drought resistance for their host, and to retard fungal infections (Carroll, 1992). The herbaceous plants infected with endophytes had a greater resistance to high temperature (Bouton *et al.*, 1993; Cunningham *et al.*, 1993; Marks and Clay, 1996). Some endophytes produce poisonous secondary metabolic products, and the products protect the plants from pathogens (Burpee and Bouton, 1993; Christensen, 1996).

The purpose of this study was to investigate effects of foliar endophytes and AMF on growth of host plants and community structure of plants.

Materials and Methods

Isolation and identification of endophytes. Healthy leaves of *Capsicum annuum*, *Sasa borealis*, *Potentilla fragarioides* and *Viola mandshurica* collected from an arable site and its surrounding field areas in Goesan-gun, Chungbuk, Korea (128° 1'E, 36° 46'N). Only green leaves without signs of insect or microbial injury were used for fungal isolation. Collected leaves were gently washed with ddH_2O and cut with a sterile scalpel into a small piece (1 × 1 cm). The pieces were surface-sterilized with 5% sodium hypochlorite (5 min) and 70% ethyl alcohol

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

(1 min). After surface sterilization, four pieces on 0.5% malt extract agar (MEA) medium and the medium was incubated in dark at 25°C for four weeks. Fungal hyphae and structures from slide cultures were observed under a light microscope. The size of condia was measured and the shape was carefully described.

For molecular identification, total DNA of the fungi was extracted from cultures isolated from each plant using DNeasy Plant Mini kit (Qiagen Science, USA) and was amplified using PCR. The partial internal transcribed spacer (ITS) region including 5.8 s ribosomal DNA (rDNA) was amplified with a universal primer set, ITS1F and ITS4, for fungi (Gardes and Bruns, 1993). The thermal cycler was programmed for 1 cycle of 3 min at 94°C, and 30 cycles of 1 min at 94°C denaturing, 1 min at 55°C annealing and 1 min at 72°C extension, and finally 1 cycle of 5 min at 72°C for hold. Nucleotide sequences were determined using ABIPRISM 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. Four species of endophytes, *Glomerella* sp., *Xylaria* sp. and two species of *Pestalotiopsis* (*P.* sp1 and *P.* sp2) were identified using morphological and molecular characteristics (Table 1).

Extraction of AMF spores. Spores of four species of AMF, *Acaulospora longula*, *Glomus mosseae* and *Archeospora leptotica* were extracted from pure cultures using wet-sieving and sucrose density gradient centrifugation methods (Daniels and Skipper, 1982). The extracted spores were observed under light microscopes and identified based on their morphological characteristics such as color, shape, surface ornamentation, contents and wall structures of spores (Schenck and Perez, 1990). AM fungal spores were surface-sterilized for inoculation (Hildebrandt *et al.*, 2002).

Composition of microcosm. Three treatments by types of fungal inoculum were designed in this study; AMF only, endophytes only, and combination AMF and endophytes. No inoculum on plants was included as control. For root inoculation with AMF, plastic pots (50(W) × 38(L) × 8(H) cm) were filled with the sterilized vermiculites and sands (1 : 1, v/v). Seeds of five plant species (*Oenothera odorata*, *Plantago asiatica*, *Trifolium repens*, *Isodon japonicas* and *Aster yomena*) were obtained from a commercial seed source (Seedkorea Co., LTD., Korea) and surface-sterilized with 20% sodium hypochlorite and 96% ethyl alcohol. Thirty seeds of each plant species were sawed together into a pot. A potting medium were mixed with four species of AMF spores, and 400 spores of each AMF species were contained in a pot. For leaf inoculation, newly emerged leaves of healthy plants were inoculated with mycelium of four species of endophytes by brushing without wounds. Root and leaf inoculation in a plant were done as described above. Each treatment was replicated four times at the same time. After four months of growth in a greenhouse, effects of both symbiotic fungi on species diversity, species composition, and productivity of plant community within the microcosm were examined. Dry weights of plants were measured after dehydration in a drying oven at 70°C for 48 hours. Shannon-Wiener index of species diversity (H') was calculated for plant community analysis (Magurran, 1988). All data was analyzed with one-way analysis of variance (ANOVA) using statistical package SPSS-WIN. The mean values were compared by Fisher's least significant difference test (LSD, $P < 0.05$).

Results

Experimental microcosms contained an assemblage of five species of plants treated with AMF and endophytes were set up in a greenhouse. After four months of growth, effects of the fungi on plant productivity were examined. The responses of plant growth to each treatment showed variation among plant species (Table 2). Growth of *O. odorata*, *P. asiatica*, *I. japonicus* and *A. yomena* in the community inoculated with AMF was significantly increased compared to controls, while no significant growth effect of AMF was detected in *T. repens*. Also, microsms inoculated with both symbiotic fungi, the growth of *O. odorata*, *P. asiatica*, *I. japonicus* and *A. yomena* was significantly increased compared to controls. However, in the communities inoculated with endophytes, a significant increase of dry weights was observed only in *P. asiatica* and *I. japonicas*.

In plants with AMF, mean biomass of all 5 plant species were significantly higher than biomass of control for

Table 1. Identification of endophytic fungi isolated from plants by sequences of rDNA ITS region for inoculums in this study

Fungal isolates	Host plant speceis	Results of BLAST search on NCBI		
		Fungal species	Accession No.	Similarity (%)
Gl1	*Viola mandshurica*	*Glomerella acutata*	AF489565	568/569 (99%)
Xy	*Capsicum annuum*	*Xylaria* sp.	AF153741	406/425 (95%)
Pe1	*Potentilla fragarioides*	*Pestalotiopsis microspora*	AF377292	538/540 (99%)
Pe2	*Sasa borealis*	*Pestalotiopsis neglecta*	DQ000992	576/577 (99%)

Table 2. Dry weights (standard error) of plant species and relative of abundance (RA) in plant communities inoculated with AM fungi and endophytes

Plant species	Treatments *							
	AMF		Endophytes		AMF + Endophyte		Control	
	Dry weight (g)	RA (%)	Dry weight (g)	RA (%)	Dry weight (g)	RA (%)	Dry weight (g)	RA (%)
Oenothera odorata	1.21 (0.22) a	6.2	0.64 (0.12) ab	b4.9	1.61 (0.30) a	9.1	0.35 (0.03) b	2.8
Plantago asiatica	3.40 (0.35) a	17.4	1.63 (0.05) c	12.5	2.72 (0.17) b	15.4	0.97 (0.14) d	7.8
Trifolium repens	9.67 (0.15) a	49.4	7.82 (0.39) b	60.0	8.96 (0.71) ab	50.7	8.99 (0.34) ab	72.3
Isodon japonicus	2.66 (0.26) a	13.6	1.98 (0.21) b	15.2	2.30 (0.21) ab	13.0	1.29 (0.12) c	10.4
Aster yomena	2.63 (0.22) a	13.4	0.97 (0.08) b	b7.4	2.10 (0.26) a	11.9	0.84 (0.10) b	6.8

*Different letters indicate significant difference among fungal treatments within plant species at $P < 0.05$.

Table 3. The responses of plant growth inoculated with symbiotic fungi after four months

Plant species	MR*	P**
Oenothera odorata	29.87	$P < 0.001$
Plantago asiatica	46.13	$P < 0.001$
Trifolium repens	26.95	$P < 0.001$
Isodon japonicus	29.50	$P < 0.001$
Aster yomena	20.01	$P < 0.001$

*MR: Mycorrhizal response (%) = [(mean biomass mycorrhizal plant − mean biomass nonmycorrhizal plant)/mean biomass mycorrhizal plant] × 100.

**P values were determined by two-sample t-test using mean biomass of inoculated plants and uninoculated control.

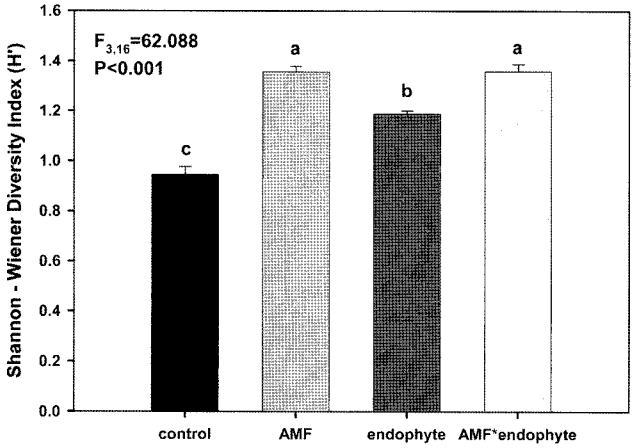

Fig. 1. The species diversity indices of plant communities inoculated with the symbiotic fungi. Different letters above each bar indicate significant difference at $P < 0.05$. AMF: arbuscular mycorrhizal fungi.

each plant species, indicating positive mycorrhizal responsive (MR, Table 3). MR showed significant variation among plant species; highest in *P. asiatica* and lowest in *A. yomena.*

The total biomass of plants in a microcosm was significantly higher in communities with AMF or with both symbiotic fungi than control (Table 2). However, a significant increase of growth was not observed in the microcosms inoculated with endophytes.

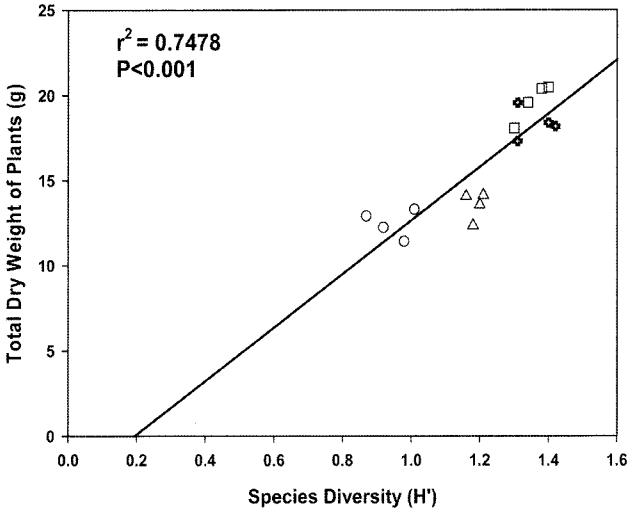

Fig. 2. Correlation between species diversity and total dry weight of plant community in a microcosm. Circle: control, Square: AM fungi, Triangle: endophytic fungi, Cross: AM fungi and endophytes.

The species diversity index (H') was significantly increased in the plant community inoculated with symbiotic fungi (Fig. 1). The number of species in the microcosm was not changed during the period of this study, but relative abundances of each species consisting of a community were changed (Table 2). There was a positive correlation between species diversity and total dry weight of plants per microcosm (Fig. 2).

Discussion

Many plants have symbiotic relationships with AMF in roots and with endophytes in shoots. These relationships affect plant growth and competitive ability of the host plants. However, influence of AMF and endophytes to host plants is not fully understood yet. In this study, the effects of foliar endophytes and AMF on plant community in experimental microcosms containing an assemblage of five species of plants were investigated. The

results in this study showed that AMF and endophytes affect growth of host plants and plant community structure.

AMF can influence structure of the plant community through various ways. For example, the nutrition and inter-specific relationships of plants were affected by microbes of root systems (Stanton, 1988) and therefore composition of the plant community was affected by mycorrhizas (Allen and Allen, 1984; Bever, 1994). Nutrients flowing through the hyphal connection plant to plant affect both growth and competition ability of plants resulted in influence of the species composition in plant community. Also, studies were reported that host plant species differed in their dependency on AMF (Hetrick *et al.*, 1996; Streitwolf-Engel *et al.*, 1997; van der Heijden *et al.*, 1998) as in this study (Table 3) showing variation among the plant species in mycorrhizal response of each plant species. The differences can affect to competitive abilities of the plant community.

AMF increased species diversity by increasing species evenness of the plant community. Although AMF increased the growth of all plants in the community, they could influence relative abundance of each species in the community due to the interspecific variation of response to the mycorrhizas. As a result, in this study, AMF would contribute to raise the species evenness of plant community. Mycorrhizal symbiosis can strongly influence the patterns and intensity of both intraspecific density effects and interspecific competition of plants (Grime *et al.*, 1987; Hartnett *et al.*, 1993). Mycorrhizas were played a vital role in the maintenance of biological diversity on the plant community.

A significant increase was not observed in the productivity of the community with endophytes in this study. However, relative abundance of plant species in the communities inoculated with endophytes was changed and the species evenness of plant community was increased with inoculation of endophytes, indicating endophytes increased species diversity of plant community through increasing species evenness. In this study, endophytes changed the relative abundance by increasing growth of two species, *P. asiatica* and *I. japonicus*, in the community and the endophytes infected to these plants could promote resistance of the host plants to various environmental stresses (West *et al.*, 1993). It has been observed that plants infected with the endophytic fungi had greater resistance to the drought and high temperatures (Marks and Clay, 1996). The increased resistance to the environmental stresses with infection of endophytes could raise inter-specific competitive ability of the plants and these factors could influence the composition of plant community (Hill *et al.*, 1991; Marks *et al.*, 1991).

The community treated with both symbiotic fungi showed significant differences in growth responses of plant in the community from the community with only AMF and endophytes, respectively. The biomass of *P. asiatica* in the community treated with both symbiotic fungi was lower than when treating only AMF, suggesting that the endophytes could negatively influence AMF of *P. asiatica*. If plants were infected with endophytes, the movement of photosynthetic products toward a root could be limited, and it would be possible to affect growth of AMF (Clay, 1992). Also, toxic metabolites produced by endophytes may inhibit colonization of mycorrhizal fungi.

In this study, the plant species diversity and the total dry weight of plants showed a significant positive correlation. These results were supported by preceding studies showing that the productivity in the plant community was increased with the increased plant diversity (van der Heijden *et al.*, 1998). Results of this study indicate that both symbiotic fungi may significantly affect the plant community structure. The interactions between the symbiotic fungi and host plants might increase the species diversity and plant productivity. The result of study would increase the scope of understanding with respect to the role of symbiotic fungi on the plant community.

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