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Effects of Soils Containing Arbuscular Mycorrhizas on Plant Growth and Their Colonization

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Four arbuscular mycorrhizal fungal (AMF) inocula collected from three arable sites in Korea were used to determine plant growth, mycorrhizal root colonization rate and spore production in three different host plant species; *Sorghum bicolor*, *Allium fistulosum*, *Tagetes patula*. Growth of plant treated with AMF differed from those without AMF. Different AMF inocula showed significantly different root colonization rates and spore production of AMF on the wild plants, *A. fistulosum* and *T. patula*, but did not on the cultivated plant, *S. bicolor*. Results suggested that indigenous mycorrhizal fungal community would be important factors in mycorrhizal symbiosis, and play important roles in the plant succession.

KEYWORDS: ***Allium fistulosum*, Arbuscular mycorrhiza, *Sorghum bicolor*, *Tagetes patula***

Microbial communities in soil are important factors in plant growth. Arbuscular mycorrhizal fungi (AMF) are the member of soil microorganisms, having mutualistic relationship with most of vascular plant species including ferns (Trappe, 1987). Improvement of water and some inorganic nutrient uptake such as phosphorus by AMF colonization, resulting in enhancing plant growth, has been well documented in many plant host species (Harley and Smith, 1983). Mycorrhizal plants have also been shown to better tolerate to the environmental stresses such as nutrient deficient soils, drought conditions, salinity and pathogens than non-mycorrhizal plants (McArthur and Knowles, 1993; Sylvia *et al.*, 1993; West, 1995). It has been reported that indigenous AMF in field soils affected infectivity and effectiveness of AMF inocula (Abbott and Robson, 1981; Kim *et al.*, 2000). Inoculation of non in- digenous AMF might reduce plant growth, as compared to inoculation with indigenous AMF (Dhillion, 1992). The manipulation of indigenous AMF in the field has received much attention to improve plant growth and reduce use of chemical fertilizers. Although mycorrhizal interaction has been thought to have little host specificity (Harley and Smith, 1983), several studies have shown that individual species of AMF differ in their ability to promote plant growth (van der Heijden *et al.*, 1998) and also differ in its growth response to host plant species (Bever *et al.*, 1996; Eom *et al.*, 2000). The aim of the present study was to determine the degree of variability among the agricultural plant species in their abilities to become colonized by mycorrhizal fungi with various AMF soil inocula collected from different fields.

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Materials and Methods

Three different arable sites, Danyang, Jecheon and Cheongwon were selected for this work in Chungbuk, Korea. Soils were collected from four different host plant species, *Glycine max* (GM), *Fagopyrum esculentum* (FE), *Sorghum bicolor* (SB) and *A. monathum* (AM) within each site. Three soil samples collected from the field growth with the same host plant were mixed together and used as an AMF inoculum. Seeds of three host plants species, *Sorghum bicolor*, *Allium fistulosum* and *Tagetes patula*, were sowed on autoclaved sands and the seedlings were transplanted to 21.5×21.5 cm pots after two weeks. Each pot contained a 100 m*l* of AMF soil inoculum and a 2 *l* of equal parts of autoclaved sand/soil mixture by volume. The plants were maintained in a greenhouse and watered as needed. They were fertilized every 10 days from 8 weeks after transplanting with 500 m*l* of 1/4 strength of Hoagland's solution (Hoagland and Arnon, 1950) per pot.

At 16 weeks after transplanting, the roots and shoots were harvested. Plant dry weight was measured after dehydration in a drying oven at 70°C for 48 hours. Roots were stained by 0.05% trypan blue (Koske and Gemma, 1989) and examined under dissect and light microscopes. The percent mycorrhizal colonization rate within roots was determined using gridline intersection method (Giovannetti and Mosse, 1980). To determine the population of AMF spores, soils were collected and homogenized manually. The AMF spores were extracted from 10 g dried weight soil using wet-sieving and sucrose density gradient centrifugation (Daniels and Skipper, 1982). The extracted spores were observed and counted under a light microscope (40×). Only the AMF spores which appeared

to be fresh (based on color, shape, surface conditions, and examination of spore contents) were counted. Data were analyzed by analysis of variance (ANOVA) using a statistical package SPSS-WIN. When the Fisher's values were significant, mean values were compared by Fisher's least square difference test ($P < 0.05$).

Results and Discussion

Symbiosis was reported not only to be important in plant growth but also to contribute to adaptation of plant under the given environments (Abbott and Robson, 1981). In agricultural practices, the presence of AMF was considered to have more advantages of tolerance for both plant diseases and environmental stress in growth of plants than the absence of AMF. Physiological researches, related to productions of secondary metabolites, have been progressed with symbiotic relations with AMF. In this respect, AMF have been speculated to induce different kinds of plant metabolism under laboratory condition (Harley and Smith, 1983). Thus, in this study, plant growth responses were investigated whether they are dependent on AMF community composition in soil.

Control plants in sterilized soil showed no AMF infections in roots and spore productions in all three host plant species. Internal, intercellular and intracellular hyphae, arbuscules and vesicles were observed from roots of all the host plants (Fig. 1). The hyphal coils were observed frequently in the roots of *A. fistulosum*. High proportions of external and internal hyphae were observed in the roots of two host species, *S. bicolor* and *T. patula*, compared to the roots of other species. Typical arbuscular mycorrhizal structures such as vesicles, arbuscules and internal hyphae were observed in all three host plant species inoculated with four different inoculum sources. There are two different morphological types (ie 'Arum' and 'Paris') of arbuscular mycorrhizal colonization in roots as described by Gallaud (cited in Smith and Smith, 1997). In this

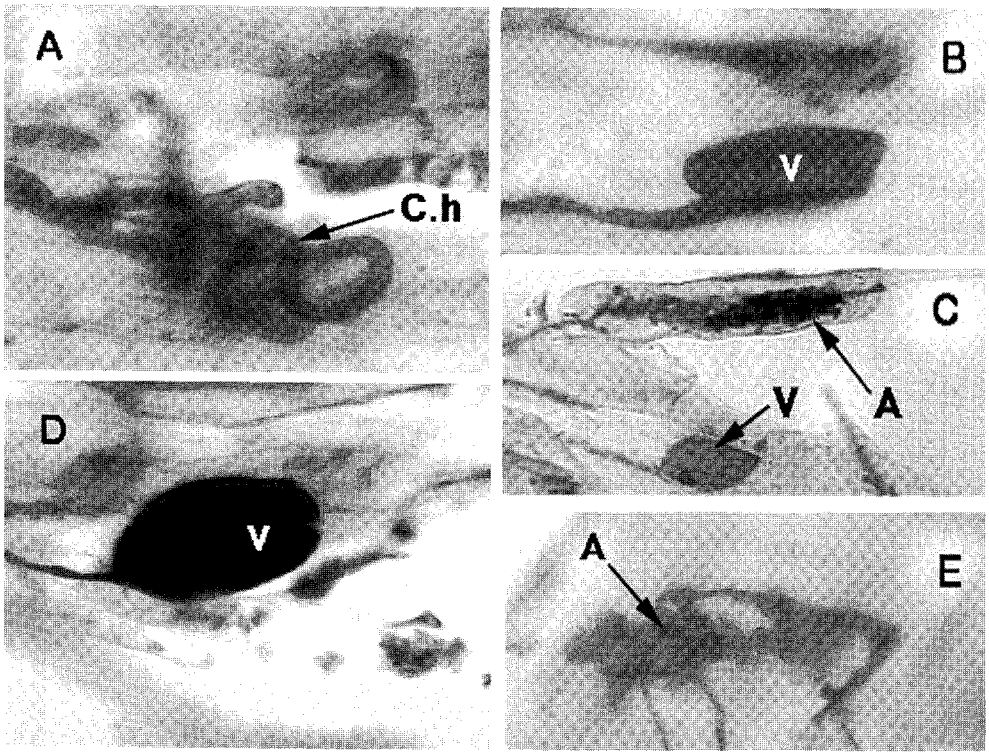

Fig. 1. The morphological characteristics of Arbuscular Mycorrhizae colonized in roots of three host species,*Allium fistulosum* (A-C), *Sorghum bicolor* (D), *Tagets patula* (E). A, arbuscules; V, vesicles; C.h, coiled hyphae.

Table 1. One-way analysis of variance showing total dry weight, percent root colonization rate and AM fungal spore density in 10 g of dry soil from three host plant species on the main effects of different AM inoculum sources.

Host plant species	Total dry weight		% root colonization rate		Spore density/10g soil	
	F	P	F	P	F	P
Sorghum bicolor	3.239	0.082	1.625	0.259	1.810	0.223
Allium fistulosum	0.322	0.809	2.340	0.150	4.502	0.039
Tagetes patula	1.348	0.334	10.267	0.009	4.983	0.046

study, most mycorrhizal structures in the roots of *A. fistulosum* was a typical 'Paris' type, being distinguished by the absence of intercellular hyphae within colonized roots. However, 'Arum type' colonization of AMF, which has been observed more frequently in cultivated plants than 'Paris type', were observed in the two plant species, both *T. patula* and *S. bicolor*. These observations indicated that AMF were successfully infected in the roots of plant employed in this work.

Analysis of variance for the plant dry weight of three plant species did not show significant main effect of different soil inocula (Table 1). None of mycorrhizal treatment showed significant difference in plant growth, as compared to non mycorrhizal control (Table 2). In *T. patula* different mycorrhizal inoculum sources significantly influenced both on AMF growth in roots and spore production and in *A. fistulosum* only spore productions. *T. patula* showed higher plant dry weight in the soils grown with GM and SB (Table 2).

Mycorrhizal colonization of *S. bicolor, A. fistulosum* and *T. patula* ranged at the 12~29%, 89~92% and 8~43%, respectively. It showed significant difference among host plant species (Table 3). Mycorrhizal colonization was the highest in *A. fistulosum*, but *T. patula* showed high variation of mycorrhizal root colonization rate with soil inoculum source. AMF spore production was significantly influenced by inoculum treatment in both *A. fistulosum* and *T. patula*, however, *Sorghum bicolor* was not different in AMF growth or root or spore production in the soil, as compared with the different inoculum sources (Table 4).

Table 2. Total plant dry weight of different host plant species inoculated by different mycorrhizal inoculum sources (soils)

Treatments (Soils)	Total dry weight of different host plant species (g/individual)		
	Sorghum bicolor	*Allium fistulosum*	*Tagetes patula*
Control	1.13±0.33[a]	0.11±0.02	0.65±0.18
Glycine max	0.85±0.06	0.09±0.07	0.90±0.54
Fagopyrum esculentum	0.78±0.14	0.10±0.04	0.53±0.39
Sorghum bicolor	0.88±0.21	0.17±0.10	0.93±0.48
Allium monathum	0.78±0.25	0.29±0.12	0.48±0.09

[a]Mean±standard error.

Table 3. Percent root colonization rates of different host plant species inoculated by different mycorrhizal inoculum sources (soils)

Treatments (Soils)	% root colonization rates of different host plant species		
	Sorghum bicolor	*Allium fistulosum*	*Tagetes patula*
Glycine max	29.11[a]*	79.89[a]	42.67[a]
Fagopyrum esculentum	12.89[a]	91.67[a]	24.83[ab]
Sorghum bicolor	23.44[a]	89.33[a]	8.44[bc]
Allium monathum	21.22[a]	91.78[a]	12.84[bc]

*Different letters within each column indicate significant differences ($P < 0.05$) according to Fishers least significant difference (LSD).

Table 4. Arbuscular mycorrhizal fungal spore density in 10 g dry soils of different host plant species inoculated by different mycorrhizal inoculum sources (soils) after 16 weeks growth

Treatments (Soils)	Number of AM fungal spores of different host plant species		
	Sorghum bicolor	*Allium fistulosum*	*Tagetes patula*
Glycine max	216.6[a]*	263.7[a]	157.0[a]
Fagopyrum esculentum	243.0[a]	122.7[b]	87.5[ab]
Sorghum bicolor	81.3[a]	166.7[ab]	37.0[b]
Allium monathum	193.0[a]	229.3[a]	113.0[ab]

*Different letters within each column indicate significant differences ($P < 0.05$) according to Fishers least significant difference (LSD).

The host plant species played more important role than inoculum on mycorrhizal colonization rate and host growth. It has been reported that colonizing arbuscular mycorrhizal fungi into plant root did not show any specificity with host plant (Harley and Smith, 1983). However, there are several reports demonstrating biological specificity between AMF and host plant species (Bever *et al.*, 1996; Dhillion, 1992; McGonigle and Fitter, 1990). While several field studies showed differences between host plants, studies in greenhouse condition did not show clear result. This study using soils collected from several fields showed that *A. fistulosum* and *T. patula* showed specificity with AMF but *S. bicolor* showed no significant difference with AMF. These results from this study indicate differences flora of AMF in rhizosphere and are similar to the specificity between AMF and Legume plants. This result demonstrated host specificity within certain range and that succession of plant communities in natural field sites would induce succession of host plants.

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