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Research Article

Effects of Arbuscular Mycorrhizal Fungi and Soil Conditions on Crop Plant Growth

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Abstract We investigated the effects on various crops of inoculation with species of arbuscular mycorrhizal fungi (AMF) in soils from different sources and selected AMF species suitable for domestic environment-friendly farming. Effects on plants varied with the AMF species used. In carrot, *Scutellospora heterogama*, *Acaulospora longula*, and *Funneliformis mosseae* had a positive effect on growth of the host, whereas AMF had only weak effects on the growth of red pepper and leek. AMF inoculation had positive effects on the growth of carrot and sorghum. The results of this study indicate the nature of the relationship between soil, plants, and AMF; this study therefore has important implications for the future use of AMF in environment-friendly agriculture.

Keywords Arbuscular mycorrhizal fungi, Environment-friendly agriculture, Organic farming, Soil

Arbuscular mycorrhizal fungi (AMF) have symbiotic associations with plants, and are found in more than 80% of terrestrial plants [1, 2]. More than 240 AMF species have been reported worldwide, and the genetic and functional diversities of these species are thought to be much higher than the morphological diversity [3]. AMF generally promote plant growth by providing plants with inorganic nutrients from the soil instead of obtaining carbon from the host [4]. In addition, resistance to environmental stresses and pathogens can be enhanced in the rhizosphere of host plants, and several attempts have been made to utilize the effects of AMF on plant growth in agriculture.

Environment-friendly agriculture is a sustainable agricultural practice based on the harmonization of agricultural and environmental aspects, and it uses organic matter or other organisms to increase crop yield instead of relying on pesticides and chemical fertilizers [5]. Studies indicated that plants with AMF exhibited better growth than do plants without AMF [6-9]. Therefore, AMF are potentially valuable in environment-friendly agriculture, and AMF usage could reduce the use of chemical fertilizers and pesticides. AMF have been used as bio-fertilizers, which have been actively studied and produced in other countries. However, many limitations exist regarding the application of environment-friendly agriculture in Korea, because of the effects of AMF on ecosystems. In addition, the results of previous studies indicated that that AMF exhibit species-dependent host specificity, and AMF distributions vary depending on the soil conditions [10, 11]. However, most studies of AMF in Korea were conducted with controlled soil compositions and nutrients, and few studies have examined the effects of AMF on crop growth in arable fields. Therefore, in order to examine the use of native Korean AMF species for environment-friendly agriculture, we investigated the growth effects of various crops associated with AMF in organic soils, and we selected AMF species that are suitable for domestic environment-friendly farming.

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MATERIALS AND METHODS

Preparation of AMF inocula. AMF samples were trap-cultured for 6 mon using red pepper (*Capsicum annuum*) as a host species. Field soil samples were collected from various regions in Korea, and were mixed with sterilized soil. Spores were isolated and their morphological characteristics were determined using wet sieving and sucrose density gradient centrifugation methods and observation under an optical microscope, respectively [12, 13]. In order to identify the spores, DNA was extracted from a single spore, and nested PCR methods were used to amplify the 18S rDNA region using NS1/NS4 and AML1/AML2 primers [14, 15].

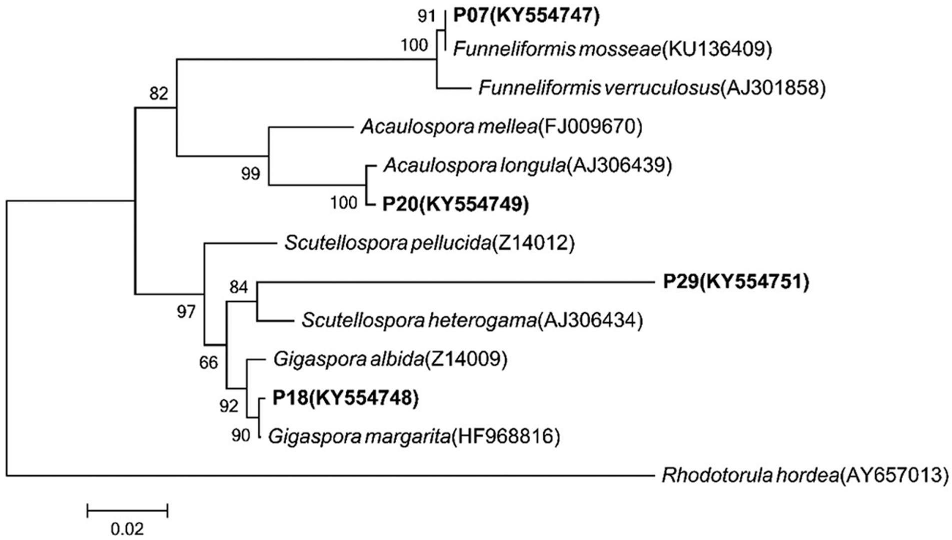

Fig. 1. Neighbor-joining phylogenetic tree for the partial 18S rDNA regions of arbuscular mycorrhizal fungi. Fungal strains used in the present study are in bold text. *Rhodoturula hordea* was used as an outgroup; numbers on branches indicate bootstrap values with 1,000 replicates.

PCR products were sequenced by SolGent (Daejeon, Korea), and phylogenetic analyses were performed using MEGA 6 [16]. Based on morphological characteristics and sequence analyses (Fig. 1), the following four AMF species were selected as inoculants: *Gigaspora margarita* P18 (KY554748, GM), *Scutellospora heterogama* P29 (KY554750, SH), *Acaulospora longula* P20 (KY554749, AL), and *Funneliformis mosseae* P07 (KY554747, FM). For mass production of the four selected AMF inoculant species, five spores from each species were inoculated into the roots following the germination and growth of red pepper seedlings (2–3 cm tall) in sterilized vermiculite [17]. The seedlings were transplanted to pots containing sterilized sand, and were then cultured for 4–6 mon in a culture room to obtain adequate spore amounts for each AMF species; spores of four AMF species were used as fungal inoculants.

Preparation of culture soil. Three types of soils were used in this study. Two soil samples (A and B) were collected from organic farms at Nayangju, Gyeonggi-do of Korea, on which red peppers had been cultivated for over 5 yr, and soil C was collected from arable fields at Miwon, Chungbuk of Korea that had been abandoned for over 10 yr. Soil analyses were conducted to determine the physical and chemical composition of soils used in the experiment (Pan Korea Co., Seoul, Korea) (Table 1). Soil samples were sterilized at 121°C for 60 min before being used as culture soils.

Table 1. Chemical analysis of the soil used in the present study

Content	Soil type		
	A	B	C
pH (1 : 5, w/w)	6.48	6.81	6.51
Organic matter (%)	3.23	7.74	0.81
Total nitrogen (%)	0.290	0.260	0.074
Total phosphate (%)	0.16	0.12	0.02
Available phosphate (mg/kg)	1239	503	91
Exchangeable cations potassium ($cmol^+$/kg)	3.86	2.33	0.22

Plant cultivation and data analysis. Seeds of sorghum (*Sorghum bicolor*), leek (*Allium tuberosum*), red pepper (*C. annuum*), and carrot (*Daucus carota*) were germinated in sterilized vermiculite, and seedlings were transplanted to individual pots containing 170 g of soil after the plant roots were inoculated with 50 spores of the designated AMF species. There were five replicates for each treatment, including the control group. Water was supplied twice per day during plant cultivation, and no additional inorganic nutrients were supplied. After 10 wk of growth, each plant was harvested and dried at 70°C for 72 hr. The dry weights of the shoots and roots were measured, and the roots were stained with trypan blue to determine the mycorrhizal colonization rates [18, 19]. The effects of soil type and AMF species on plant growth and mycorrhizal colonization were tested using SPSS ver. 18 (SPSS Inc., Chicago, IL, USA).

RESULTS

Mycorrhizal root colonization rates. Available P and K as well as soil organic matter were higher in soils A and B than in soil C, and soil pH values ranged from 6.48 to 6.81 (Table 1). Soil type was significantly related to root colonization (Table 2), and the root colonization rates of three plant species in soil C (36.8–78.2%) were significantly higher than in soils A (0.6–13.6%) and B (2.6–38.0%). These results indicated that plants grown in soils with lower

Table 2. Average rates of percent mycorrhizal root colonization

Soil type	Host plant	AMF inoculant				
		GM	SH	AL	FM	NM
A	Sorghum	4.2 a	5.8 a	3.2 a	5.0 a	0.0 a
	Leek	4.0 a	3.2 a	4.6 a	4.4 a	0.0 a
	Red pepper	0.6 a	2.0 a	2.6 a	2.0 a	0.0 a
	Carrot	13.6 a	12.0 a	10.8 a	10.2 a	0.0 a
B	Sorghum	12.4 ab	14.0 ab	24.8 a	17.2 ab	0.0 b
	Leek	19.2 a	14.2 a	20.0 a	18.6 a	0.0 b
	Red pepper	6.4 b	17.2 a	8.6 abc	13.2 ab	0.0 c
	Carrot	34.8 a	33.0 a	2.6 b	38.0 a	0.0 b
C	Sorghum	65.0 a	70.2 a	69.2 a	60.4 a	0.0 b
	Leek	67.4 ab	57.6 b	76.4 a	66.8 ab	0.0 c
	Red pepper	39.8 a	36.8 a	44.8 a	53.2 a	0.0 b
	Carrot	78.2 a	62.0 b	61.6 b	65.6 ab	0.0 c

Different letters on the numerical values indicate significant difference among different fungal inoculants at $p < 0.05$ according to least significant difference.
AMF, arbuscular mycorrhizal fungi; GM, *Gigaspora margarita*; SH, *Scutellospora heterogama*; AL, *Acaulospora longula*; FM, *Funneliformis moseae;* NM, nonmycorrhizal control.

available P had higher AMF colonization rates. Mycorrhizal colonization rates differed depending on the host plant and the AMF species. In soil A, inoculation with different AMF species did not have significant effects on the colonization rates of any of the host plants. However, different AMF species significantly affected the colonization rates of host plants in soils B and C. In soil B, AL resulted in the highest colonization rates in sorghum and leeks, and SH and FM had the highest colonization rates in red peppers and carrots, respectively. When cultivated in soil C, SH, AL, FM, and GM exhibited the highest colonization rates in sorghum, leeks, red peppers, and carrots, respectively.

Plant growth. Soil type significantly influenced the dry weights of plants (Table 3). Sorghum, red peppers, and carrots showed higher growth in organic soils A and B than in soil C, indicating that plants grew better in soils collected from organic farms than in non-cultivated soils. However, the dry weight of leeks in soil C (mean = 4.4 mg) was higher than that in soil B (mean = 2.25 mg). In soil A, inoculation with different AMF species did not result in significant increase in sorghum growth ($p = 0.15$), but leeks, red peppers, and carrots responded differently to the various AMF species. The greatest growth effects were observed in leeks that were inoculated with AL (7.2 mg), but dry weights in plants inoculated with FM were 3.14 mg, significantly lower than in the control plants. In addition, red pepper growth was highest in plants inoculated with AL (39.56 mg), but AMF inoculation with FM resulted in significantly lower growth rates (18.62 mg) than those in the control plants. In carrots, SH and FM, showed the highest inoculation rates (14.46 mg) and smallest growth effects (2.20 mg), respectively. In soil B, sorghum, leeks, and red peppers responded differently to inoculation with various AMF species. However, the growth of AMF-inoculated carrots did not differ significantly with differing AMF inoculants, but differed significantly from that of control. In sorghum, AL inoculation had the greatest growth effect (35.50 mg), and the other AMF species led to no significant growth effect or to lower than that of the control. In leeks, GM inoculation had the highest growth effect (3.08 mg), but plants inoculated with AMF species did not exhibit significantly higher growth than that of the control. In soil C, different AMF species had significant effects on the growth of sorghum and carrots. In leeks and red peppers, AMF inoculation did not significantly affect plant growth. Sorghum growth was highest in plants inoculated with SH (14.24 mg), and inoculation with AL (11.06 mg) and FM (9.24 mg) resulted in lower plant growth

Table 3. Average dry weight (mg) of plants inoculated with different AMF in different soil types

Soil type	Host plants	AMF inoculant					F	*p*-value
		GM	SH	AL	FM	NM		
A	Sorghum	22.08 ab	21.68 ab	24.66 ab	13.38 b	29.56 a	1.866	0.156
	Leek	6.28 ab	5.26 ab	7.20 a	3.14 b	6.92 a	4.416	0.010
	Red pepper	38.70 a	31.56 ab	39.56 a	18.62 b	39.80 a	4.022	0.015
	Carrot	7.32 b	14.46 a	7.04 b	2.20 b	2.68 b	6.609	0.001
B	Sorghum	19.42 b	10.26 c	35.50 a	14.88 bc	20.02 b	10.071	0.000
	Leek	3.08 a	1.36 b	2.04 ab	2.38 a	2.38 a	4.385	0.010
	Red pepper	36.92 a	35.88 a	41.18 a	11.58 b	37.74 a	10.473	0.000
	Carrot	7.90 a	8.52 a	4.08 ab	7.98 a	2.54 b	2.275	0.097
C	Sorghum	13.34 a	14.24 a	11.06 ab	9.24 b	12.14 ab	2.863	0.050
	Leek	4.92 a	4.26 a	4.72 a	4.30 a	4.02 a	1.087	0.390
	Red pepper	7.24 a	5.56 ab	6.04 ab	4.56 b	5.48 ab	2.063	0.124
	Carrot	2.00 ab	2.48 a	1.46 bc	1.10 c	1.00 c	4.710	0.008

Different letters on the numerical values indicate significant difference among different fungal inoculants at $p < 0.05$ according to least significant difference.
AMF, arbuscular mycorrhizal fungi; GM, *Gigaspora margarita*; SH, *Scutellospora heterogama*; AL, *Acaulospora longula*; FM, *Funneliformis moseae;* NM, nonmycorrhizal control.

than that in control (12.14 mg). The greatest growth effects in carrots were associated with SH inoculation (2.48 mg), and inoculation with the other three AMF species led to higher growth than that of the control.

DISCUSSION

Previous studies showed that AMF colonization decreased in soils with high phosphate concentrations [20]. Therefore, the available phosphate contents in soil could be a major factor influencing the colonization rates in the different soil types utilized in this study. When AMF are applied in organic farming, it is necessary to consider the amount of available phosphorus in the soil.

Previous studies found that differences in growth depended on the AMF inoculant species when the same host species was examined [21], and the results of this study corroborated those of previous studies. In this study, the effects of AMF inoculation on plant growth in soils from organic farms varied depending on the plant species and the nutrient status of the soil. Specifically, the effects of the same AMF species on plants may have differed depending on the soil type. In general, AMF colonization is known to have a positive effect on plant growth, but the physicochemical state of the soil could directly impact the symbiotic relationship between the plant and fungus [22]. Therefore, soil characteristics are important factors for plant growth when environment-friendly farming using AMF is introduced.

The results indicated that SH enhanced the growth of carrots in both soils A and B, and AL was effective for sorghum growth. Therefore, these fungi are recommended for use in organic soils. However, red peppers inoculated with AMF in organic soil exhibited nearly the same growth as the control plants, or growth was inhibited by the AMF species. Since the growth of red peppers in the organic soils was significantly higher than that in non-cultivated soil C, the nutrient condition of the soil likely has a greater effect on red peppers than AMF inoculation. However, this study focused on the early growth of crops, so the results may differ in crops that are cultivated for longer periods.

This study examined 10 wk of crop growth, but growth over a longer period could lead to different results. Additional studies that assess the influence of various environmental factors in fields are needed. This study was conducted to select AMF species that are useful for the cultivation of crops in organic soil, and two species were effective for all of the crop plants that were grown in organic soil. These results can be used as basic data for the development of AMF-based products such as farming methods that utilize AMF species or biological fertilizers.

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