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Effectiveness of Arbuscular Mycorrhizal Fungi (AMF) Inoculation on the Growth of Perilla

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Abstract: To evaluate the effectiveness of AMF on the growth of horticultural crops, we compared mycorrhizal and non-mycorrhizal plants, perilla (*P. frutescens* Britt.), that were inoculated with AMF propagules. In the early stages of growth of perilla, compared to the AMF- perilla seedlings, in AMF+ perilla seedlings at 3 weeks after sowing, leaf length and width increased 17% and 29%, leaf area increased 28%, and shoot fresh weight increased 33%, root total length increased 1%, and chlorophyll content increased 3%. Further at 10 weeks after sowing, compared to the AMF- perilla plants, in perilla plants inoculated with AMF at the sowing and transplanting stages, leaf area increased 21% and 19%, shoot length increased 19% and 17%, root fresh weight increased 17% and 20%, and chlorophyll content increased 5.1% and 4.8%, respectively. Moreover, at 14 weeks after sowing, compared to the AMF- perilla plants, in perilla plants inoculated with AMF at the sowing and transplanting stages, the number of leaves increased 16% and 20%, root fresh weight increased 16% and 17% significantly. Further, leaf fresh weight increased 9% and 11%, shoot diameter increased 4.5% and 7.3%, and chlorophyll content increased 1.5% and 2.5%, respectively. The levels of many macronutrients and micronutrients were tended to be significantly higher in AMF+ plants than in AMF- plants, supporting the association between AMF and enhanced growth of plants grown from AMF+ seedlings.

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Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous components of most ecosystems worldwide. They are an important determinant of soil quality because they affect host plant physiology and soil ecology interactions and contribute to soil structure (Abbott and Robson, 1984; Koide and Mosse, 2004; Rillig, 2004; van der Heijden *et al*., 2006; Rillig and Mummey, 2006; Bouwmeester *et al*., 2007). Symbiotic AMF is the most widespread mycorrhizae associated with plants roots and is found with approximately 80–90% of land plants in both natural and agricultural ecosystems (Bouwmeester *et al*., 2007). In general, AMF forms mutualistic associations with the roots of the majority of higher plants, including major production crop species and pasture plant species (Sohn *et al*., 2003; Koide and Mosse, 2004; Bouwmeester *et al*., 2007).

AMF inoculation is also known to have a tremendously beneficial effect on plant growth by enhancing nutrient and water uptake (Davies *et al*., 1993), inducing changes in root morphology (Smith and Read, 1997), improving photosynthesis and transpiration (Yano-Melo *et al*., 1999), improving soil structure and soil aggregate stability (Rillig, 2004; Rillig and Mummey, 2006; van der Heijden *et al*., 2006; Cho *et al*., 2009), and providing

protection to colonized roots against pathogens (Abdalla and Abdel-Fattah, 2000).

Perilla frutescens (L.) Britt. (Lamiaceae), also known as ''wild coleus,'' ''Chinese basil,'' and ''Perilla mint'', is an annual short-day plant widely used in therapeutics as well as in food preparations in Asian countries, especially China, Japan, and Korea. It is classified as *Perilla frutescens* (L.) Britt. var. *acuta* Kudo forma*viridis* Makino (green perilla) and *Perilla frutescens* (L.) Britt. var. *acuta* Kudo (red perilla). Green- and red-leaved perilla are also broadly classified as smooth and crisp varieties based on leaf margin (Ravindran and Shylaja, 2004). Since perilla has many medical properties, it has been the subject of many studies. Perilla leaves and stems are reported to have anti-microbial (Yamamoto and Ogawa, 2002), anti-HIV (Kawahata *et al*., 2002; Yamasaki *et al*., 1998), anti-tumor (Banno *et al*., 2004; Ueda *et al*., 2003), and anti-allergic properties (Guo, Pittler and Ernst, 2007). It is traditionally used in Chinese medicine to treat various diseases, including depression, anxiety, and cough, and to promote intestinal propulsion.

The objectives of our study were to evaluate the growth of mycorrhizal and non-mycorrhizal plants, perilla (*P. frutescens* Britt.), in terms of differences in all growth responses, including chlorophyll content, and investigate AMF colonization rates and types, spore densities, uptake of macro- and micronutrients including phosphorus (P).

Materials and Methods

Preparation of planting material and growth conditions

Seeds of perilla (*P. frutescens* Britt.) were obtained from Syngenta Seed Company, Seoul, Korea, and sown in a tray (66 mW × 0.25 mH) filled with a pasteurized medium of cocopeat : peatmoss : zeolite : perlite : vermiculite (65–70:8–12:6–8:4–6:8–10, w/w). Plantlets were grown under greenhouse conditions and natural illumination for 6 weeks and watered with tap water as needed in Sunchon National University, Shucheon city, Chonnam Province, South Korea.

Next, the perilla seedlings inoculated with AMF were transplanted in Suncheon city, Chonnam Province, South Korea, and grown under the field condition without any additional application of fertilizer or spraying of pesticides during experiments.

The soil was a sandy loam with the following chemical properties: pH of approximately 5.55–5.59, 0.20-0.22 ds/m EC, 161–180 mg/kg available P_2O_5, 2.22–2.88 g/kg O.M., 5.73–6.19 cmol/kg CEC, 1.62–2.29 cmol/kg K, 6.79–7.48 cmol/kg Ca, 2.34–2.61 cmol/kg Mg, and 1.02–1.13 cmol/kg Na.

Inoculation with AMF

To obtain the AMF inoculum, Sudan grass inoculated with isolated *Glomus* sp. was grown in pots for 6 months. The AMF propagules collected were a mixture of colonized Sudangrass roots, hyphae, soil, and spores. Spore densities in the propagules were determined using the wet-sieving method (An *et al*., 1990), and the propagules contained approximately 30 spores per gram of mixture. To examine the effects of AMF on the growth of perilla seedlings in the field, perilla seedling roots were inoculated with approximately 25 and 49 AMF spores per 1 seed and seedling at the sowing stage and transplanting stage, respectively.

Growth responses of perilla

Perilla was inoculated with AMF propagules at the time of sowing and transplanting, and growth characteristics, including fresh weight of shoots, number of leaves, leaf area, leaf length, leaf width, root fresh weight, root length, root total length, and chlorophyll content, were determined at 6, 10 and 14 weeks for 15 plants, using the method previously described by Sohn *et al*. (2003). The leaf area and root total length of individual leaves was measured using a digital WinRhizo® assay system (Regent Instruments Inc.) after scanning the plant. The chlorophyll content of fully expanded perilla leaves was measured using an in situ SPAD-502 chlorophyll meter (Minolta Co. Ltd.). Actual chlorophyll content Y (mg/100 cm^2) was calculated by substituting the SPAD reading for X in the standard formula Y = 0.0996X - 0.152 (Watanabe *et al*., 1994).

Plant mineral nutrient analysis

For chemical analysis, plant samples were finely ground after drying at 65℃ for 48 h. A 0.5 g sample was placed in a 100 mL flask with 10 mL concentrated H_2SO_4. Further, 0.5 ml H_2O_2 was added to the sample every 10 min for 90 min (total : 4.5 mL). After cooling, the solution was filtered through a Whatman No. 6 filter into 100 mL flasks. The concentrations of K, Mg, Ca, Na, Fe, Mn, Cu, and Zn were analyzed by using an ICP-Spectrometer (SHIMADZU ICPE-9000, Japan) (Sohn *et al*., 2003). All analyses were performed in triplicate.

AMF spore population

AMF spores were collected from the rhizosphere soil of each potted host plant, using the wet-sieving method (An *et al*., 1990). The rhizosphere soil was placed in sieves with 45–500 um pores and washed vigorously with cold tap water. The spores remained on the sieve along with larger soil particles. These large soil particles were removed by placing the sample in 50% glycerol and centrifuging at 5000 rpm for 5 min. The cleaned spore preparation was counted and examined under a microscope (Olympus SZX12, Japan).

Colonization rates and types

Soil cores (25 mm in diameter) were taken from the soil surface (0–200 mm depth). Sampling from replicate pots was randomized. Fine roots (<1 mm in diameter) from these cores were fixed in formalin acetic acid (FAA) solution (13 mL formalin + 5 mL acetic acid + ethyl alcohol) and cut into 1-cm-thick segments. Mycorrhizal colonization was assessed according to the method of Phillips and Hayman (1970). The colonization of host plant roots was assayed by using a modified method originally described by Brundrett *et al*. (1984). The mycorrhizal root segments were washed with water and placed in 20 mL vials containing 10% KOH solution. The vials with root samples were incubated for 30 min at 90℃. After incubation, mycorrhizal roots were washed with water and dyed with 0.05% trypan blue (lactic acid : grycerol : distilled water = 1 : 2 : 2) and maintained at 50℃ overnight. Next, the stained roots were examined for mycorrhizal infection under an Olympus BX50 transmitted-light bright field microscope (Olympus, Japan). The percentage of root colonization was determined by dividing the number of colonized roots by the total number of roots examined.

Statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA) in the SAS software program, version 6.08 (SAS Institute, 1990). The average of 3 independent experiments using 15 plants was used in our calculations. Probabilities of significance were used to test significance of data, and the least significant difference (LSD) was calculated at a significance level of $P<0.05$ to compare means.

Results and Discussion

Growth responses of perilla

The effects of AMF inoculation on the growth responses of perilla at 6, 10, and 14 weeks after sowing are presented in Tables 1, 2, and 3 and Figs. 1, 2, and 3. Including number of leaves, leaf fresh weight, leaf area, leaf length and width, root length,

Table 1. Growth characteristics of AMF+ and AMF- perilla seedlings at 6 weeks after sowing

Treatmentz	No. of leaves (ea/plant)	Leaf length (mm)	Leaf width (mm)	Leaf area (cm^2/plant)	Shoot fresh weight (g/plant)	Root total length (cm)	Chlorophyll content (mg/100 cm)
AMF-	10	44.08	37.70	50.94	2.130	569.32	1.79
AMF+(IAS)	10^{ns}	51.37**	48.63**	65.45**	2.843**	573.48^{ns}	1.84^{ns}

Means were presented. And each value was compared with Student's *t*-test, and determined from 3 independent replicates (n = 15). For each parameter, the data presented in each line are followed by; the significance level was set at $^{**}P<0.01$ and *ns*, non-significant (IAS=inoculation at sowing).

Table 2. Growth characteristics of AMF+ and AMF- perilla plants at 10 weeks after sowing

Treatment	No. of leaves (ea/plant)	Leaf area (cm^2/plant)	Shoot length (cm)	Leaf fresh weight (g/plant)	Shoot diameter (mm)	Root fresh weight (g/plant)	Chlorophyll content (mg/100 cm)
AMF-	64.50	2531.0	22.30	89.03	11.42	24.08	3.420
AMF+(IAS)	69.67	3065.9*	26.50*	100.13	12.25	28.25	3.595
AMF+(IAT)	72.50	3008.3*	26.12*	108.87*	12.79*	28.92	3.583
$LSD^{0.05}$	14.821	422.43	2.200	15.608	1.045	4.922	0.227

Means were presented. And each value was compared with least significant difference (LSD), and determined from 3 independent replicates (n = 15). For each parameter, the data presented in each line are followed by; the significance level was set at $^{*}P<0.05$, (IAS=inoculation at sowing, IAT=inoculation at transplanting).

Table 3. Growth characteristics of the soil used to cultivate AMF+ and AMF- perilla plants at 14 weeks after sowing

Treatment	No. of leaves (ea/plant)	Shoot length (cm)	Leaf fresh weight (g/plant)	Shoot diameter (mm)	Root fresh weight (g/plant)	Chlorophyll content (mg/100 cm)
AMF-	141.83	63.83	207.41	20.84	44.42	3.175
AMF+(IAS)	164.00*	70.08*	225.30	21.78	51.42*	3.223
AMF+(IAT)	170.17*	66.33	230.82	22.36	51.83*	3.254
$LSD_{0.05}$	15.591	2.529	41.326	2.029	7.836	0.213

Means were presented. And each value was compared with least significant difference (LSD), and determined from 3 independent replicates (n = 15). For each parameter, the data presented in each line are followed by; the significance level was set at *P<0.05, (IAS=inoculation at sowing, IAT=inoculation at transplanting).

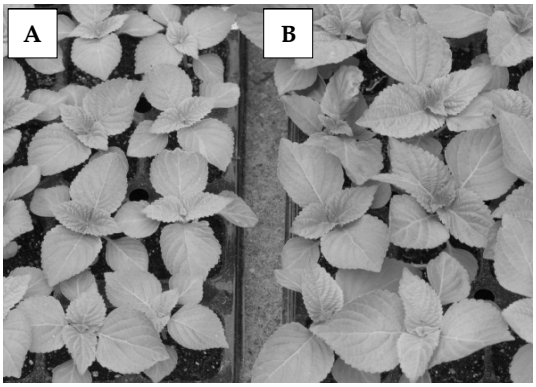

Fig. 1. Comparison of AMF+ (B) and AMF- perilla seedlings (A) at 6 weeks after sowing.

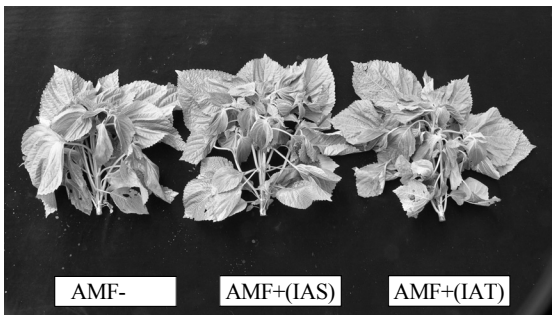

Fig. 2. Comparison of AMF+ and AMF- perilla plants at 10 weeks after sowing (IAS=inoculation at sowing, IAT=inoculation at transplanting).

and chlorophyll content, all growth characteristics were significantly enhanced in AMF+ seedlings compared with in AMF- seedlings.

In the early stages of growth, compared to the AMF-perilla seedlings, in AMF+perilla seedlings at 3 weeks after sowing, the number of leaves were the same, but leaf length and width increased 17% and 29%, respectively, leaf area increased 28%, and shoot fresh weight increased 33% (significant), and root total length and chlorophyll content increased 1% and 3%, respectively (not significant).

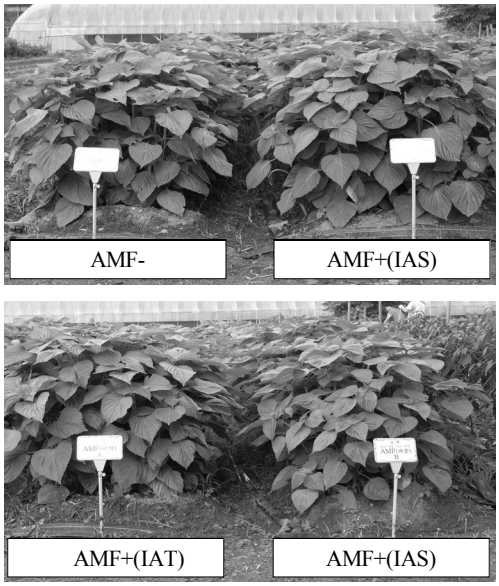

Fig. 3. Comparison of AMF+ and AMF- perilla plants at 14 weeks after sowing (IAS=inoculation at sowing, IAT=inoculation at transplanting).

As shown in Fig. 1, in this experiment, in the early stages of growth, the perilla seedlings inoculated with AMF propagules exhibited enhanced growth and development, including leaf length, width, and fresh weight. These results imply that early infection with AMF is important for the growth and yield of mycorrhizal plants, especially short-duration crops (Koide and Mosse, 2004; Brouwmemeester *et al*., 2007; Cho *et al*., 2009).

At 10 weeks after sowing, compared to the AMF-plants, in perilla plants inoculated with AMF at the sowing and transplanting stages, leaf area increased 21% and 19%, and shoot length increased 19% and 17%, respectively. In perilla plants inoculated with AMF at the transplanting stage, leaf fresh weight increased 22% and shoot diameter increased 12% (significant), while in perilla plants inoculated with

AMF at the sowing and transplanting stages, root fresh weight increased 17% and 20% and chlorophyll content increased 5.1% and 4.8% respectively, although the differences were not significant.

At 14 weeks after sowing, compared to the AMF-plants, in perilla plants inoculated with AMF at the sowing and transplanting stages, the number of leaves increased 16% and 20%, root fresh weight increased 16% and 17% (significant), respectively. Further, leaf fresh weight increased 9% and 11%, shoot diameter increased 4.5% and 7.3%, and chlorophyll content increased 1.5% and 2.5% respectively, although these increases were not significant.

Perilla (*P. frutescens* Britt.) is an annual short-day crop. To determine the effect of infection timepoint on the growth responses of perilla, we carried out AMF inoculation at the sowing and transplanting stages. This study indicates that the growth responses of perilla plants inoculated with AMF at the transplanting stage were similar to those of plant inoculated at the sowing stage. Moreover, the results imply that the AMF infection timepoint is not important based on the growth responses of lettuce inoculated with AMF.

Plant mineral nutrient analysis

The levels of macronutrients (K_2O, CaO, MgO, and Na_2O), micronutrients (Fe, Cu, Mn, and Zn), and P_2O_5 in perilla shoots, including the stems and leaves, of AMF+ and AMF-plants at 10 weeks after sowing are presented in Table 4 and that in perilla stems and leaves at 14 weeks after sowing are presented in Tables 5 and 6, respectively.

At 10 weeks after sowing, the macronutrient (P_2O_5 and Ca) and micronutrient (Cu, Zn, Fe, and Mn) levels were higher in AMF+ perilla plants than in AMF-perilla plants. In particular, the contents of Zn, Fe, and Mn in shoots were significantly higher (11–15%, 25–38%, and 11–25%) in AMF+ plants than AMF- plants. At 14 weeks after sowing, the contents of K, Ca, Zn, Fe, and Mn in the stems and Ca and Mn in leaves were also higher in AMF+ plants than in AMF- plants. Moreover, the levels of macronutrients such as K in stems and Ca in leaves were 24–28% and 24–27% higher, respectively, in AMF+ plants than in AMF- plants. Further, the levels of micronutrient such as Zn, Fe, and Mn in the stems and Fe in the leaves of AMF+ plants were significantly higher (201–231%, 191–249%, 124–138%, and 187–223%, respectively) than in AMF- plants, significantly.

AMF inoculation is known to enhance the macronutrient and micronutrient contents of roots, which may, in turn, enhance plant growth (Davies *et al*., 1993; Koide and Mosse, 2004; van der Heijden ea al., 2006; Cho *et al*., 2009). As shown in Tables 4, 5, and 6, the levels of many macronutrients and micro-

Table 4. Macronutrient, micronutrient, and P_2O_5 levels in the shoots of AMF+ and AMF- perilla plants at 10 weeks after sowing

Treatment	Macronutrient (%)					Micronutrient (mg/kg)			
	P_2O_5	K_2O	CaO	MgO	Na_2O	Cu	Zn	Fe	Mn
AMF-	0.220	5.64	1.86	0.47	nd	11.80	63.10	972.73	205.05
AMF+(IAS)	0.274	5.52	1.86	0.49	nd	12.00	72.58*	1343.53*	256.62*
AMF+(IAT)	0.226	6.12	2.20*	0.48	nd	12.13	69.88*	1217.33*	228.27*
LSD $_{0.05}$	0.170	0.589	0.177	0.043	-	1.174	5.940	273.885	18.670

Means were presented. And each value was compared with least significant difference (LSD), and determined from 3 independent replicates. For each parameter, the data presented in each line are followed by; the significance level was set at *P<0.05, (IAS=inoculation at sowing, IAT=inoculation at transplanting).

Table 5. Macronutrient, micronutrient and P_2O_5 levels in the stems of AMF+ and AMF- perilla plants at 14 weeks after sowing

Treatment	Macronutrient (%)					Micronutrient (mg/kg)			
	P_2O_5	K_2O	CaO	MgO	Na_2O	Cu	Zn	Fe	Mn
AMF-	0.199	3.03	1.14	0.30	nd	10.40	22.67	266.93	276.27
AMF+(IAS)	0.143	3.89*	1.16	0.32	nd	10.73	45.53*	509.80*	341.73*
AMF+(IAT)	0.192	3.77*	1.42*	0.32	nd	10.07	52.40*	664.53*	382.60*
LSD $^{0.05}$	0.089	0.245	0.050	0.011	-	1.375	4.500	153.100	53.960

Means were presented. And each value was compared with least significant difference (LSD), and determined from 3 independent replicates. For each parameter, the data presented in each line are followed by; the significance level was set at *P<0.05, (IAS=inoculation at sowing, IAT=inoculation at transplanting).

Table 6. Macronutrient, micronutrient, and P_2O_5 levels in the leaves of AMF+ and AMF- perilla plants at 14 weeks after sowing

Treatment	Macronutrient (%)					Micronutrient (mg/kg)			
	P_2O_5	K_2O	CaO	MgO	Na_2O	Cu	Zn	Fe	Mn
AMF-	0.152	4.39	1.26	0.34	nd	13.65	85.98	102.73	370.43
AMF+(IAS)	0.135	3.78	1.56*	0.34	nd	13.67	81.32	192.20*	324.58
AMF+(IAT)	0.187*	4.21	1.60*	0.37	nd	14.03	84.20	229.13*	198.97
LSD $^{0.05}$	0.056	0.213	0.064	0.017	-	1.700	3.825	83.320	15.520

Means were presented. And each value was compared with least significant difference (LSD), and determined from 3 independent replicates. For each parameter, the data presented in each line are followed by; the significance level was set at *P<0.05, (IAS=inoculation at sowing, IAT=inoculation at transplanting).

nutrients tended to be significantly higher in AMF+ plants than in AMF-plants, supporting the association of AMF with enhanced growth of plants grown from AMF+seedlings.

AMF spore population

An objective of this experiment was to determine if perilla plants were colonized after AMF inoculation. Therefore, we investigated the spore densities in perilla rhizosphere soil and colonization rates of perilla roots. Table 7 presents the AMF spore densities in the mycorrhizosphere of AMF+ and AMF-perilla plants at 6, 10, and 14 weeks after sowing. Fig. 4 presents the morphological characteristics of the AMF spores in the rhizosphere of AMF+ and AMF- perilla seedlings at 6 weeks after sowing.

At 6 weeks after sowing of perilla, the AMF spore density per 30 g mycorrhizosphere for seedlings inoculated with AMF was 47.3 and that for seedlings not inoculated with AMF was 10.7, and at 10 weeks after sowing, that of plants inoculated with AMF at the sowing and transplanting stages was 275.3 and 159.0,while that of non-mycorrhizal plants was 116.0, and at 14 weeks after sowing that of AMF+(IAS), AMF+(IAT), and AMF- plants was approximately 196.7, 134.0, and 84.0, respectively. These results imply that spore densities in the AMF+ mycorrhizosphere was 4 times that in the AMF-rhizosphere in the early stages and this increase was significant. The spore densities in the rhizosphere soil of all treatments decreased in later stages.

Fig. 4 presents the morphological characteristics of AMF spores in the rhizosphere of AMF+ and AMF-perilla seedlings at 6 weeks after sowing. The mycorrhizal fungus was identified by using a diagnostic key (Schenck and Perez, 1990) which was used to distinguish between spores of *Glomus* sp. and those not related to AMF inoculation.

Table 7. AMF spore densities in the mycorrhizosphere of AMF+ and AMF- perilla plants at 3 and 9 weeks after sowing

Treatment		Spore density (spores/30 g fresh soil)
6 WAS	AMF-	10.7 ± 8.1
	AMF+(IAS)	47.3 ± 20.7
10 WAS	AMF-	116.0 ± 43.5
	AMF+(IAS)	275.3 ± 44.9
	AMF+(IAT)	159.0 ± 52.9
14 WAS	AMF-	84.0 ± 5.3
	AMF+(IAS)	196.7 ± 64.1
	AMF+(IAT)	134.0 ± 19.1

Means were ±SE presented. And each value was compared with least significant difference (LSD), and determined from 3 independent replicates. The data presented in each line are followed by; the significance level was set at *P<0.05 (IAS=inoculation at sowing, IAT=inoculation at transplanting, WAS=weeks after sowing).

Colonization rates and types

The percentage of colonization in perilla roots with AMF inoculation at 6, 10, and 14 weeks after sowing and the morphological characteristics of the mycorrhizal association in the roots of perilla seedlings grown in trays and a field are presented in Table 8 and Figs. 5, 6, and 7.

Perilla roots were rapidly colonized by AMF in the early stages of growth after AMF inoculation; the colonization rate of AMF+(IAS) plants at 6 and 10 weeks after sowing and that of AMF+(IAT) plants at 4 weeks after transplanting were investigated and found to be 16.82%, 28.31%, and 23.71%, respectively. However, in the subsequent stages of growth, mycorrhizae

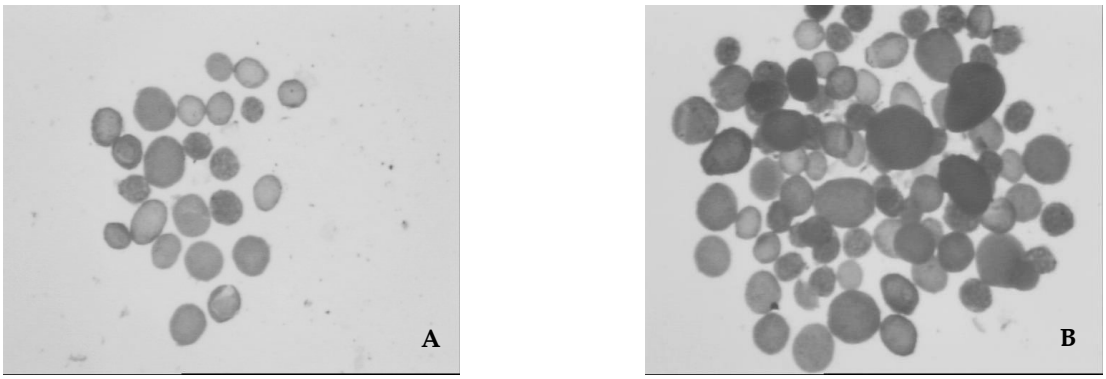

Fig. 4. Morphological characteristics of the AMF spores in the rhizosphere of AMF+ (B) and AMF- perilla seedlings (A) at 6 weeks after sowing.

Table 8. AMF colonization rates in roots of AMF+ and AMF- perilla plants at 6, 10, and 14 weeks after sowing

Treatment		Colonization rate (%)		
		Hyphae	Spore	Total
6 WAS	AMF-	0	6.15	6.15
	AMF+(IAS)	9.30	7.52	16.82
10 WAS	AMF-	2.47	9.29	11.76
	AMF+(IAS)	13.66	14.65	28.31
	AMF+(IAT)	11.20	12.51	23.71
14 WAS	AMF-	3.65	13.69	17.37
	AMF+(IAS)	21.23	13.85	35.08
	AMF+(IAT)	16.07	13.24	29.31

(IAS=inoculation at sowing, IAT=inoculation at transplanting, WAS=weeks after sowing).

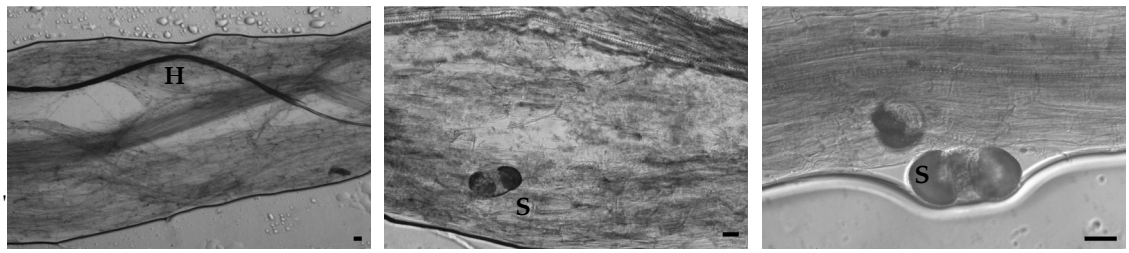

Fig. 5. Morphological characteristics of the mycorrhizal association in the roots of perilla seedlings cultivated in trays at 6 weeks after sowing (Scale bar=10 um. S=spore, H=hyphae).

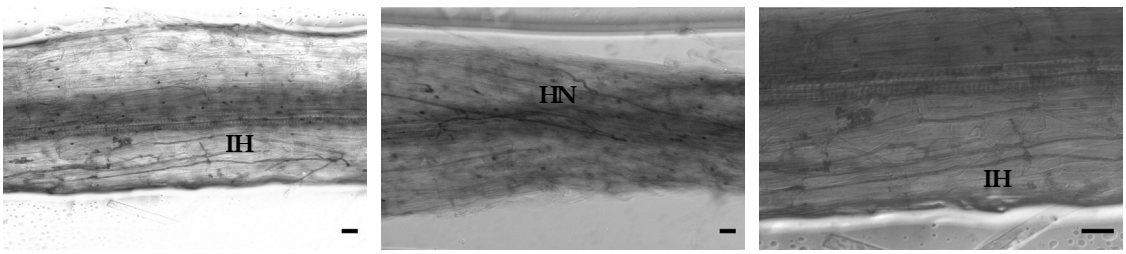

Fig. 6. Morphological characteristics of the mycorrhizal association in the roots of perilla plants cultivated in a field at 10 weeks after sowing (Scale bar=10 um. H=hyphae, IH=internal hyphae).

developed less rapidly, and the colonization rate of AMF+(IAS) and AMF+(IAT) plants at 14 weeks after sowing was 35.08% and 29.31%, respectively, while that of AMF-plants at 6, 10, and 14 weeks after

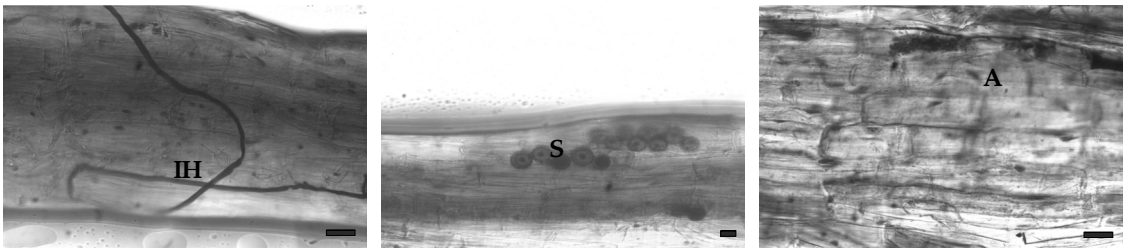

Fig. 7. Morphological characteristics of the mycorrhizal association in the roots of perilla plants grown in a field at 14 weeks after sowing (Scale bar=10 um. S=spore, A=arbuscule, IH=internal hyphae).

sowing was 6.15%, 11.76%, and 17.37%, respectively.

As shown in Figs. 5, 6, and 7, the AMF colonization in perilla roots grown in trays at 6 weeks after sowing showed morphological characteristics such as spores and hyphae, and those grown in the field at 9 and 14 weeks after sowing had well-developed arbuscules and internal hyphae the number of spores were also increased in the perilla roots.

The negative effects of non-mycorrhizal plants on the propagule density of AMF in soils and on root colonization of a succeeding crop have previously been reported for field soils low in plant-available P (Baltruschat and Dehne, 1988; Gavito and Miller, 1998; Fontenla *et al*., 1999; Arihara and Karasawa, 2000; Sorensen *et al*., 2005). Plants grown in P-rich soils are less dependent on AMF and become less colonized (Hayman *et al*., 1975; Hepper, 1983; Lu and Miller, 1989; Amijee *et al*., 1989; Sorensen *et al*., 2005). In the present experiment, perilla plants were grown in soils with moderate to high levels of plant-available P and could explain the observed decrease in spore population and low levels of perilla root colonization. However, root colonization of highly mycorrhizae-dependent crops appears to be only slightly influenced by high soil P levels (Hamel *et al*., 1997).

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