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Effects of Interspecific Interactions of Arbuscular Mycorrhizal Fungi on Growth of Soybean and Corn

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Growth responses of *Zea mays* and *Glycine max* to colonization by mixture of combination of three species of arbuscular mycorrhizal (AM) fungi, two species of *Glomus* and a species of *Scutellospora* were compared. In *Zea mays*, plants inoculated with single species of AM fungi showed significantly higher in dry weight than non-mycorrhizal plant for all three AM fungal species. Also, growth of plants inoculated with spores of two species of AM fungi was significantly higher than non-mycorrhizal control except for plants inoculated with two *Glomus* species. When three species of AM fungi were inoculated, the plants showed the highest growth. In *Glycine max*, plants with single AM fungal species inoculation were not significantly different in plant growth from nonmycorrhizal plants. When the plants were inoculated with combination of two or more AM fungal species, their growth significantly increased compared to nonmycorrhizal plants. In both plant species, mycorrhizal root colonization by *Scutellospora* species was significantly lower than by *Glomus* species.

KEYWORDS: Arbuscular mycorrhiza, *Glomus*, *Glycine max*, *Scutellospora*, *Zea mays*

Arbuscular mycorrhizal (AM) fungi have mutualistic symbiosis with most vascular plant and these fungi are ubiquitous on terrestrial ecosystem (Allen, 1991; Smith and Read, 1997). There are increasing evidences that AM fungi promote plant growth by improving plant uptake of water and inorganic nutrients and protect against various types of stress. Potential use of AM fungi in agriculture has received much attention to reduce the use of chemical fertilizers and pesticide in the past decades (Sharma *et al.*, 1997; Harrier and Watson, 2004). Several studies that carbon from plants transfers to belowground ecosystem through AM fungi and the fungi play important roles in plant ecology increase ecological interest of AM fungi.

It has been assumed that AM fungi are not host specific. However, recent studies reported that plant species differed in their dependency on AM fungi. Also, specific fungal species had different effect on its host plant growth (Streitwolf-Engel *et al.*, 1997; van der Heijden *et al.*, 1998). Smith *et al.* (2000) found that two fungal species in different genus showed different spatial abilities to acquire phosphorus. The mechanism underlying this differential effect of specific AM fungi on plants may involve the transfer of phosphorus from the fungi to plants as well as the transfer of carbon from the plant to the fungi.

In nature, several species of AM fungi colonize in a single plant. Studies have been demonstrated that increasing numbers of AM fungal species will increase both plant diversity and productivity, because of the added beneficial effect of each single AM fungal species (van der Heijden *et al.*, 1998; Klironomos *et al.*, 2000). However, the effects of interaction among AM fungal species on the host plant are not well understood although AM fungal species have been shown to vary in their response to the presence of another species of fungus (Hepper *et al.*, 1988; Pearson *et al.*, 1994). The detection of effective AM fungal species composition for a host species would be important in use of these fungi in agriculture. In this study, effects of plant inoculation with combination of three AM fungal species and their interaction on the growth of two crop species, *Zea mays* (corn) and *Glycine max* (soybean) were investigated under the greenhouse condition.

Materials and Methods

Seeds of two host plants species, corn (*Zea mays* cv. Golden Cross Bandam 70) and soybean (*Glycine max* cv. Hwangkeum) were sowed and germinated on autoclaved sand. The seedlings were transplanted to pots (21.5 cm dia.) after two weeks. Each pot contained mixture of a 100 *ml* of autoclaved soil and 1,900 *ml* of autoclaved sands. Before the transplants, the spores of each AM fungi were inoculated to the roots of the seedlings. The spores of three AM fungal species, *Glomus* sp.1 (G1), *Glomus* sp.2 (G2) and *Scutellospora* sp. (S), were isolated from pure cultures maintained with *Sorghum bicolor* as a host plant species. These fungi were originated from trap cultures of soil collected from field sites in Korea. Each plant in a pot was inoculated with a total of 150 spores of AM fungi. Single species treatment (G1, G2,

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and S) was inoculated with 150 spores of each species, and two species treatment (G1 + G2, G1 + S and G2 + S) was inoculated with mixture of 75 spores of each species. A treatment for all three AM species (G1 + G2 + S) was inoculated with mixture of 50 spores of each species. All treatments including non-mycorrhizal control were replicated five times. The AM fungi treated pots were maintained in a greenhouse and watered as needed. After transplant, they were fertilized every 2 weeks after transplant with 100 *ml* of low P (1/20 phosphate) Hoagland nutrient solution (2.8 g H_3BO_3, 3.4 g $MnSO_4{\cdot}H_2O$, 0.1 g $CuSO_4{\cdot}5H_2O$, 16.22 g $ZnSO_4{\cdot}7H_2O$, 0.1 g $(NH_4)6MO_7O_2{\cdot}4H_2O$, 5 *ml* H_2SO_4, 6.72 g Na_2EDTA, 5.58 g $FeSO_4$, 0.94 g $Ca(NO_3)_2{\cdot}4H_2O$, 0.52 g $MgSO_4{\cdot}7H_2O$, 0.66 g KNO_3, 0.06 g $HN_4H_2PO_4$) (Hoagland and Arnon, 1950).

The roots and shoots were harvested 16 weeks after transplanting. Plant dry weight was measured after dehydration in a drying oven at 80°C for 48 hours. Roots were washed with tap water and cut into 1~3 cm in length. The roots samples were cleared in 10% KOH and stained with 0.05% trypan blue by a modified method of Phillips and Hayman (Koske and Gemma, 1989) and examined under dissect and light microscopes. The percent mycorrhizal root colonization rates were determined using gridline intersection method (Giovannetti and Mosse, 1980). Data were analyzed by analysis of variance using a statistical package SPSS. When the Fisher's values were significant, mean values were compared by Fisher's LSD test ($P < 0.05$).

Results

Mycorrhizal colonization was not observed in the root of the two crop species which was not inoculated with AM fungi, indicating no contamination with AM fungi during the experiment (Fig. 1). The roots of both plant species of *Z. mays* and *G. max* were colonized with AM fungi at the rates between 6.4~50.5% in *Z. mays* and 12.9%~44.2% in *G. max*. The root colonization rates of both plant species showed similar trends in colonization rate with different AM fungal treatments. The treatments of inoculum sources significantly affected percent root colonization rates of *Z. mays* ($F = 4.03$ $P < 0.015$, Fig. 1A). The mycorrhizal colonization rates in plants inoculated with spores of several AM fungal species showed no significant difference with other treatments, except for the inoculation of *Scutellospora* species (S1 in Fig. 1). The root colonization rates of *G. max* were not significantly affected by fungal treatments ($F = 1.942$ $P = 0.144$; Fig. 1B). However, plants inoculated with *Scutellospora* sp. and the mixture of *Glomus* sp.1 and *Scutellospora* sp. were significantly lower in root colonization rates than others. In both plant species, root colonization of plants inoculated with *Scutellospora* sp. alone (in *Z. mays*) or mixture with other AM fungal species (in *G. max*) was lower than other treatments species.

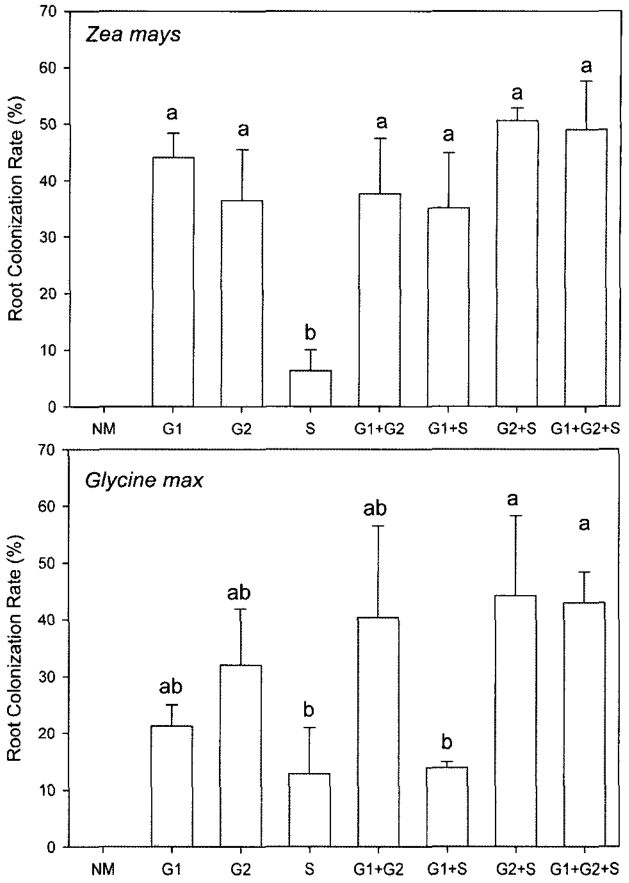

Fig. 1. Root colonization rates of *Glycine max* and *Zea mays* after 16 weeks growth with different combination of AM fungal species. NM, no AM fungi; G1, *Glomus* sp.1; G2, *Glomus* sp.2; S, *Scutellospora* sp. Different letters on each bar indicate significant difference at $P < 0.05$ ($LSD = 10.5$ in *Z. mays* and 13.8 in *G. max*).

Plant biomass inoculated with AM fungi was not correlated with root colonization rates (Fig. 2). The dry weights of both plants were significantly different with different treatments of AM fungal inoculum sources ($F = 14.833$, $P < 0.001$ in *Z. mays* and $F = 12.823$, $P < 0.001$ in *G. max*). The dry weight of *Z. mays* inoculated with single species of AM fungi showed significantly higher than non-mycorrhizal plant for all three AM fungal species (Figs. 2 & 3). Also, dry weight of plants inoculated with mixture of two species of AM fungi was significantly higher than that of non-mycorrhizal control plants except for the plant inoculated with combination of two *Glomus* species. The plants inoculated with the mixture of two *Glomus* species, *G.* sp.1 and *G.* sp.2, showed no significant difference with plants without AM fungi in dry weight after 16 weeks growth. Plants grown with mixture of spores of three different AM fungal species (G1 + G2 + S1) showed the highest increase in dry weight. The inoculation of all single species as well as the other combination of AM fungal species promoted the growth of *Z.*

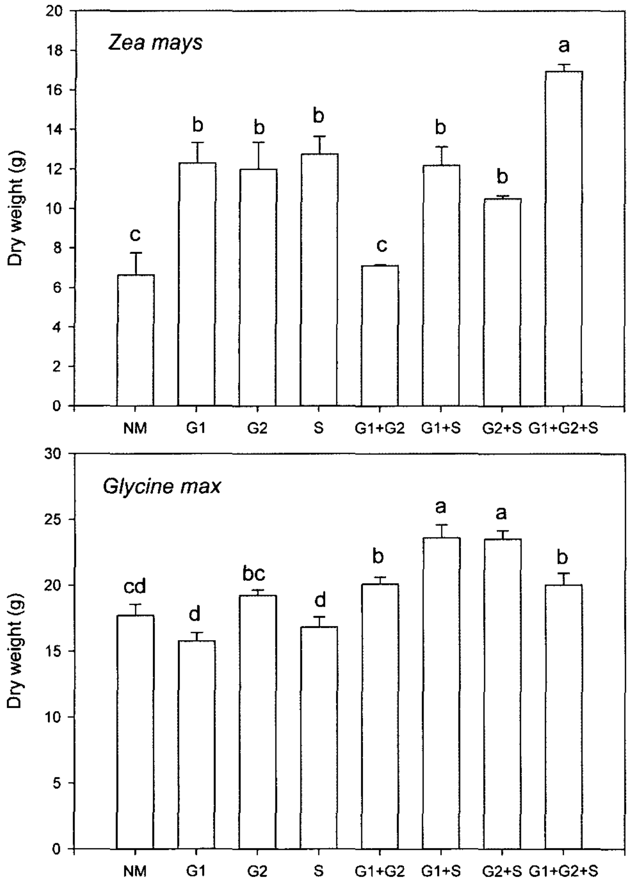

Fig. 2. Dry mass of *Glycine max* and *Zea mays* after 12 weeks growth with different combination of AM fungal species. NM, no AM fungi; G1, *Glomus* sp.1; G2, *Glomus* sp.2; S, *Scutellospora* sp. Different letters on each bar indicate significant difference at $P < 0.05$ (LSD = 1.2 in *Z. mays* and 1.0 in *G. max*).

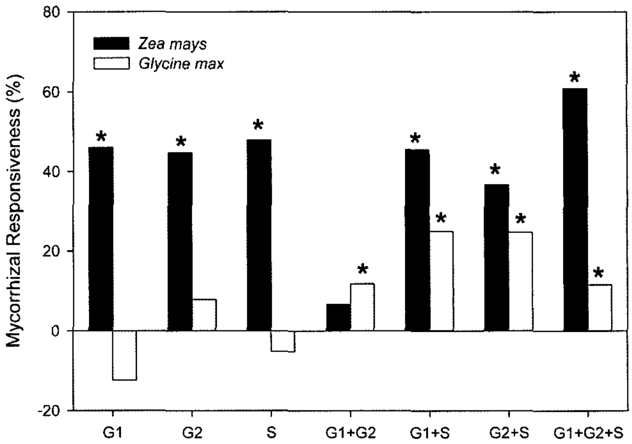

Fig. 3. Plant growth responsiveness to different inoculations of AM fungal spores. G1, *Glomus* sp.1; G2, *Glomus* sp.2; S, *Scutellospora* sp. Mycorrhizal responsivenss (%) = [(mean biomass of mycorrhizal plant − mean biomass nonmycorrhizal control)/mean biomass mycorrhizal plant] × 100. Asterisks on bars represent significant responses to mycorrhizal inoculation determined by LSD test at $P < 0.05$).

mays, but inoculation of *Glomus* species showed no significant increase of plant growth.

In contrast, *G. max* inoculated with mixture of two or tree species of AM fungi showed significantly better growth than inoculation of single species (Fig. 2). When spores of each fungal species were inoculated to the plants, dry weight was not significantly different from nonmycorrhizal plants (Figs. 2 & 3). However, combination of two (G1 + G2, G1 + S and G2 + S) or tree fungal species (G1 + G2 + S) showed significant difference with non mycorrhizal plants. The growth of *G. max* was improved with 2 or more species of AM fungi in soil rather than with single species.

Discussion

Most vascular plants have mutualistic symbiosis with AM fungi and these relations improve plant growth. It leads to study on effectiveness of agricultural and environmental application of AM fungi. However, the possibility of ecological, agricultural, and environmental use of AM fungi would be low because specificity of AM fungi to host plants and interspecific relationships among AM fungal species were not clearly understood yet.

In this study, dry weight of plants and root colonization rate were measured to study the effect of interactions among AM fungal species on plant growth and mycorrhizal root colonization. Our results suggest that mycorrhiza promote the growth of *Z. mays* but combination of specific AM fungi would reduce the mycorrhizal benefit to plant growth although root colonization rate did not show significant reduction. The results of this study indicate that the interactions between the two species of *Glomus* might reduce the benefit of host plants from the mycorrhizas.

The formation and function of mycorrhizas can be quite variable among fungal species. They improved nutrition and growth of the host plant in comparison with nonmycorrhizal controls was not always the case as *G. max* in this study. Absence of growth response and even growth decrease can occur, depending on individual fungus-plant combination and environmental conditions. Recently Smith *et al.* (2000) found that AM fungi, *S. calospora* and *G. caledonium*, have different strategies for living in symbiosis with their host plants. The functional differences of each AM fungal species used in this study were not clear. However, *Scutellospora* and *Glomus* species might have different function while two *Glomus* species having similar function in nutrient uptake from soil might affect negatively each other, but how the interspecific interactions influence on the host plant growth is not clear in this study.

Both the plant species used in this study showed better growth when they were inoculated with more than two AM fungal species although certain combination of AM fungal species reduced host plant growth. The result indicates that colonization with more number of AM fungal species increases growth of host plants, as van der Heijden *et al.* (1998) showed that increasing AM fungal diversity increase productivity through more efficient exploitation of soil phosphorus and better use of the resources. It has been reported that *Scutellospora* and *Glomus* had different function in nutrient uptake in soil (Smith *et al.*, 2000). Under natural field condition, a single plant species was colonized with several AM fungal species. However, several studies also showed that only a few fungal species dominantly colonized in the roots of crop species in agricultural fields (Helgason *et al.*, 1998). Agricultural practices such as applying fungicide and chemical fertilizers and tillage significantly might reduce species diversity of AM fungal community in the agricultural field soil (Jansa *et al.*, 2002; Eom *et al.*, 2004). This study suggests that more number of AM fungal species would increase plant productivity of corn and soybean and also selection of AM fungal species could be an important factor for the two species of plant growth when AM fungi apply to agriculture.

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