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SHORT COMNUNICATION

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Interaction between arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth promoting fungus *Phoma* sp. on their root colonization and growth promotion of cucumber (*Cucumis sativus* L.)

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Abstract Interaction between arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth promoting fungus *Phoma* sp. was studied for its effect on their root colonization and plant growth of cucumber. Two isolates of *Phoma* sp. (GS8-2 and GS8-3) were tested with *G. mosseae*. The percent root length colonized by *G. mosseae* was not adversely affected by the presence of *Phoma* isolates. In contrast, the root colonization of both isolates GS8-2 and GS8-3 in 4-week-old plants was significantly reduced (80.7% and 84.3%, respectively) by added *G. mosseae*. Inoculating plants with each *Phoma* isolate significantly increased the shoot dry weight. However, dual inoculation of each *Phoma* isolate with *G. mosseae* had no significant effect on growth enhancement.

Key words Cucumber · *Glomus mosseae* · Interaction · *Phoma* sp. · Root colonization

Arbuscular mycorrhizal fungi (AMF) that form a symbiotic relationship with roots of most terrestrial plants are known to improve the nutritional status of their host (Smith and Read 1997) and protect plants against several soil-borne plant pathogens (Dehne 1982; Azcon-Aguilar and Barea 1996). Plant growth-promoting fungi (PGPF), which are non-pathogenic saprophytes, were also reported to suppress fungal and bacterial diseases of a number of crop plants (Hyakumachi 1994; Meera et al. 1994; Shivanna et al. 1996; Koike et al. 2001).

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Study of interactions between these beneficial organisms associated with plant roots is important because such interactions might either enhance or inhibit the beneficial effects of the individual species. These interactions must be identified and characterized for the successful use of these organisms as biological control agents. AMF are well known to provoke alterations in the host's physiology and root exudates, which influence the microbial community composition in the mycorrhizosphere (Linderman 1988). This phenomenon might affect the colonization of introduced PGPF to the plant rhizosphere. PGPF might also influence AMF establishment through competition or fungal exudates. Although arbuscular mycorrhiza–rhizobacteria interactions have been extensively studied (Meyer and Linderman 1986; Linderman and Paulitz 1990; Andrade et al. 1997), little is known about the interactions between arbuscular mycorrhizal fungi and beneficial saprophytic fungi (Vazquez et al. 2000; Green et al. 1999).

Observation of the colonization ability of AMF and PGPF that is influenced by their interactions is important because root colonization ability is one of the most important characteristics of beneficial fungi which helps to reduce the infection sites of root pathogens and/or increase nutrient availability to plants. The PGPF *Phoma* sp., which generally does not sporulate under natural conditions, has been found to improve plant growth, suppress plant pathogens, and induce systemic resistance (Meera et al. 1995; Hyakumachi and Kubota 2004). This study was planned to observe whether the interaction between the AMF *Glomus mosseae* and the PGPF *Phoma* sp. is synergistic or antagonistic on their colonization of cucumber plant roots. Its impact on cucumber plant growth was also evaluated.

A 1:1 mixture of brown loam soil (Gifu campus field, Yanagido, Gifu) and river sand that was sterilized by autoclaving for 1 h at 121°C two times, each on 2 consecutive days, was used. The soil contained 2% organic matter, 0.1% total N, and 55.8 mg/100 g available P. The following nutrients were mixed into the soil–sand mixture: NH_4NO_3 (86 mg/kg), KH_2PO_4 (44 mg/kg), K_2SO_4 (70 mg/kg), $CaCl_2$ (70 mg/kg), $CuSO_4 \cdot 5H_2O$ (22 mg/kg), $ZnSO_4 \cdot 7H_2O$ (5 mg/kg), $MnSO_4 \cdot 7H_2O$ (10 mg/kg), $CoSO_4 \cdot 7H_2O$ (0.33 mg/kg),

$NaMoO_4 \cdot 2H_2O$ (0.2 mg/kg), and $MgSO_4 \cdot 7H_2O$ (20 mg/kg) (Green et al. 1999). Cucumber (*Cucumis sativus* L. cv. Jibai) was used as the host plant.

An inoculum of *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe containing spores, colonized root fragments, and soil (Idemitsu Kosan, Japan), was used. Two isolates of *Phoma* sp., which colonize cucumber roots poorly (*Phoma* GS8-2) and highly (*Phoma* GS8-3), respectively (Meera et al. 1994, 1995), were used and their inocula were prepared using barley grains. Autoclaved (20 min, 121°C) barley grain (100 g in 100 ml distilled water) substrate was inoculated with 15–20 fungal disks (3 mm diameter) of a *Phoma* isolate from the actively growing margin of 7-day-old potato dextrose agar (PDA) cultures. After 10–12 days of incubation at 23–25°C, the completely colonized barley grains were air-dried at room temperature and ground to particles small enough to pass through a 1-mm sieve.

The inoculum of *G. mosseae* and/or barley grain inoculum of each *Phoma* isolate were hand-mixed completely with potting medium (2% w/w each) according to the treatments of (1) *G. mosseae* and *Phoma* GS8-2 (Gm + GS8-2); (2) *G. mosseae* and *Phoma* GS8-3 (Gm + GS8-3); (3) *Phoma* GS8-2 only (GS8-2); (4) *Phoma* GS8-3 only (GS8-3); (5) *G. mosseae* only (Gm); and (6) uninoculated control (Cont). Nonmycorrhizal treatments received the same amount of autoclaved *G. mosseae* inoculum, and non-*Phoma* sp. treatments received same amount of autoclaved ground barley grains.

Cucumber seeds were surface sterilized in 2% NaOCl for 3 min and pregerminated on autoclaved filter papers soaked with sterile distilled water. After 48 h incubation at 25°C, one germinated cucumber seed was planted per pot (150 ml) that was filled with potting medium and inoculum mixture. At planting, filtrate of AMF inoculum was added to the planted pots (10 ml/pot) to establish similar initial microflora communities in all nonmycorrhizal treatments to mycorrhizal treatments (Calvet et al. 1993; Green et al. 1999). The filtrate was prepared by filtering a suspension AMF inoculum (100 g AMF inoculum in 1 l distilled water for 30 min) through a 38-µm sieve to remove all mycorrhizal propagules (Calvet et al. 1993). Plants were grown at 25°C and 75% RH in growth chambers. The plants received 12/12 h light/darkness cycle. Cool fluorescent lamps were operated for 12 h per day (300 $\mu E m^{-2} s^{-1}$).

After 4 weeks of growth, plants were harvested. Shoots were separated from the root systems, dried for 24 h at 80°C, and shoot dry weight was measured. Root systems were washed clearly free of soil with running tap water and divided into two portions for the observation of root colonization by *G. mosseae* and *Phoma* sp.

Subsamples of roots collected from the washed root portion were cut into about 1-cm segments, cleared in 10% KOH (w/v) for 1 h at 90°C, and stained with 0.05% w/v chlorazol black E in a lacto glycerol solution (Brundrett et al. 1984) for 1.5 h at 90°C. The percentage of root length colonized by hyphae, arbuscules, and vesicles of *G. mosseae* was determined using the magnified intersections method (McGonigle et al. 1990).

The remaining root portion was washed with sterile distilled water, surface disinfected with 0.5% NaOCl for 3 min, rinsed four times with sterile distilled water, and blotted dry. Roots cut into about 1-cm-long segments were plated on PDA amended with cloramphenicol (250 mg/l) and incubated for 2 days at 25°C. After incubation, the colonies of *Phoma* isolate growing from root segments were identified by the color and growth pattern of mycelia and the pigments produced in comparison with those of the original cultures (Meera et al. 1995; Shivanna et al. 1996). The isolation frequency was determined by counting the number of colonized root segments of 100 root segments tested per replicate. Root colonization ability was evaluated based on the isolation frequency.

The experiment was set up in a completely randomized design consisting of five treatments and one control each with four replicates and repeated three times. All percent-

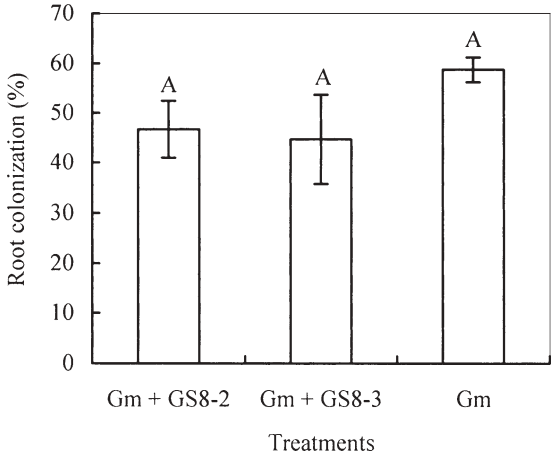

Fig. 1. Percentage root length colonized by *Glomus mosseae* (*Gm*) in 4-week-old cucumber plants treated with or without each *Phoma* isolate (*GS8-2* or *GS8-3*). Values are the means of three trials each with four replicates per treatment. *Bars with same letters* represent no significant difference between treatments according to Fisher's least significant difference test ($P = 0.05$)

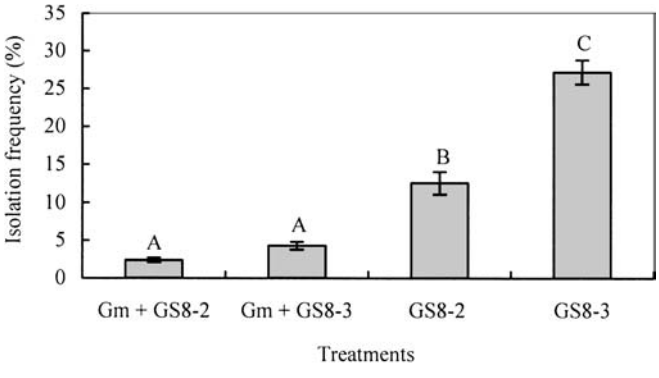

Fig. 2. Isolation frequency of *Phoma* isolates GS8-2 and GS8-3 from root segments of 4-week-old cucumber plants treated with or without *G. mosseae* (*Gm*). Values are the means of three trials each with four replicates per treatment. *Bars with different letters* represent the significant difference between treatments according to Fisher's least significant difference test ($P = 0.01$)

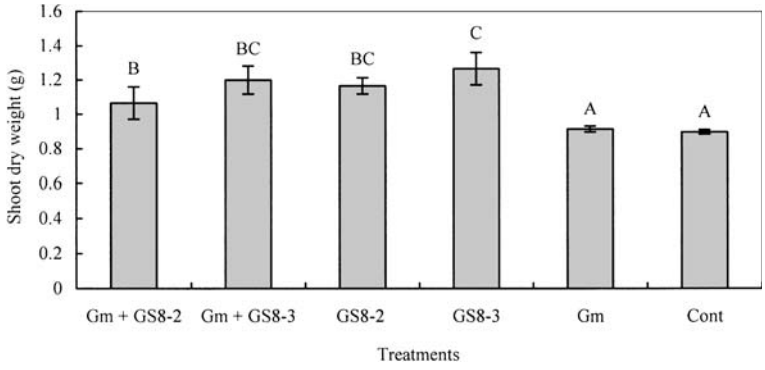

Fig. 3. Shoot dry weight of 4-week-old cucumber plants treated with each *Phoma* isolate (GS8-2 or GS8-3) and/or *G. mosseae* (*Gm*). Values are the means of three trials each with four replicates per treatment. *Bars with different letters* represent the significant difference between treatments according to Fisher's least significant difference test ($P = 0.05$)

age data were angular transformed before statistical analysis, and data from the repeated trials were pooled because variances among trials were homogeneous. The treatment means were compared using Fisher's least significant difference test (LSD) at $P = 0.05$ and $P = 0.01$.

The percent root length colonized by *G. mosseae* was not significantly reduced either by *Phoma* GS8-2 or GS8-3, although that was slightly lower in the treatments amended with GS8-2 or GS8-3 (47% and 45%, respectively) than that of in the plants inoculated with *G. mosseae* alone (59%) (Fig. 1). Although it has been reported that rhizosphere microorganisms enhance AM formation (Calvet et al. 1993; Fracchia et al. 2000), the fungal isolates tested in our experiment had no positive effect on AM formation.

Both isolates of *Phoma* GS8-2 and GS8-3 were isolated at significantly lower frequencies from the roots of 4-week-old cucumber plants treated in combination with *G. mosseae* (2.4% and 4.3%, respectively) than that of plants treated only with each *Phoma* isolate (12.6% and 27.2%, respectively) (Fig. 2). This result clearly showed that the root colonization of both *Phoma* isolates GS8-2 and GS8-3 was adversely affected by *Glomus mosseae*. Suppression of the rhizosphere population of *Trichoderma koningii* and *Fusarium solani* by *G. mosseae* has also been demonstrated by McAllister et al. (1994).

Plants treated with each *Phoma* isolate with or without *G. mosseae* significantly increased shoot dry weight (SDW) compared to that of plants treated with *G. mosseae* alone or uninoculated controls. However, plants treated in combination with each *Phoma* isolate and *G. mosseae* did not showed further growth stimulation. Similar SDW observed in cucumber plants treated with *G. mosseae* alone and uninoculated controls indicated that *G. mosseae* has no effect on plant growth enhancement (Fig. 3). The benefits (enhancing nutrient acquisition, producing plant growth hormones, etc.) offered by AMF could be overcome by its increased carbon requirement from their host (Smith and Smith 1996). In fact, some studies have shown no growth stimulation during plant interactions with AMF (Pozo et al. 2002; Shaul et al. 1999).

According to Linderman (1988), AM fungi are able to produce substances capable of interfering with microorganisms and strongly modify the microbial community composition around mycorrhizal roots compared with nonmycorrhizal controls. The root exudation is also modified both qualitatively and quantitatively by the AM symbiosis (Bansal and Mukerji 1994). Although releases of substances from AM fungi are not well documented, different substances released by the roots, such as soluble sugars, phenolics, organic acids, amino acids, antibiotics, and volatile compounds are usually responsible for these changes (Filion et al. 1999). The root colonization by *Phoma* sp. could have been affected by the substances released by the external mycelium of AMF and/or root exudates modified by AM symbiosis.

In conclusion, this study demonstrated that, combined inoculation of each *Phoma* isolate with *G. mosseae* had an inhibitory effect on *Phoma* colonization in, on, or around cucumber roots. Furthermore, a synergistic effect on *G. mosseae* colonization or plant growth promotion was not found. However, further investigations are necessary to confirm whether *Phoma* isolates behave in the same way with the existence of *G. mosseae* in the later stage of plant growth under greenhouse conditions. Additional research is also necessary to investigate how the observed inhibitory action of *G. mosseae* influences the biocontrol efficacy of *Phoma* isolates.

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