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ORIGINAL PAPER

Suppression of the root-knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood] on tomato by dual inoculation with arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria

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Abstract Arbuscular mycorrhizal (AM) fungi and plant growth-promoting rhizobacteria (PGPR) have potential for the biocontrol of soil-borne diseases. The objectives of this study were to quantify the interactions between AM fungi [*Glomus versiforme* (Karsten) Berch and *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe] and PGPR [*Bacillus polymyxa* (Prazmowski) Mace and *Bacillus* sp.] during colonization of roots and rhizosphere of tomato (*Lycopersicon esculentum* Mill) plants (cultivar Jinguan), and to determine their combined effects on the root-knot nematode, *Meloidogyne incognita*, and on tomato growth. Three greenhouse experiments were conducted. PGPR increased colonization of roots by AM fungi, and AM fungi increased numbers of PGPR in the rhizosphere. Dual inoculations of AM fungi plus PGPR provided greater control of *M. incognita* and greater promotion of plant growth than single inoculations, and the best combination was *G. mosseae* plus *Bacillus* sp. The results indicate that specific AM fungi and PGPR can stimulate each other and that specific combinations of AM fungi and PGPR can interact to suppress *M. incognita* and disease development.

Keywords *Glomus mosseae* · *Glomus versiforme* · *Bacillus polymyxa* · *Bacillus* sp. · Disease control

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Introduction

That arbuscular mycorrhizal (AM) fungi contribute to the control of plant disease, and the mechanisms by which they do so have been well documented (Ahmed et al. 2009; Elsheikh and Mirghani 1997; Li and Liu 2007; Smith et al. 1986; Vierheilig et al. 2008; Whipps 2004). The presence of AM fungi in roots can reduce development of some soil-borne pathogenic bacteria, fungi, and nematodes and can also induce increased tolerance to plant diseases (Elsen et al. 2008; Liu and Chen 2007). In field experiments, Li et al. (2004) observed that the AM fungi *Glomus versiforme* (Karsten) Berch, *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, and *Gigaspora rosea* Nicolson & Schenck decreased the propagule density of the pathogen *Fusarium oxysporum* f. sp. *niveum* (E.F. Sm.) Snyder & Hans in the mycorrhizal roots and rhizosphere, and decreased disease incidence and severity on watermelon plants; pathogen propagule and disease reduction were greatest with *G. versiforme*. AM fungi have the ability to induce systemic resistance against plant-parasitic nematodes in a root system (Elsen et al. 2008). dos Anjos et al. (2010) demonstrated that the establishment of an AM fungus before nematode infection reduced reproduction of the root-knot nematode *Meloidogyne incognita* and reduced disease severity in infested soil. A consortium of AM fungi suppressed *Fusarium* wilt of cucumber and showed potential for biocontrol in greenhouse agroecosystems (Hu et al. 2010).

Like AM fungi, plant growth-promoting rhizobacteria (PGPR) can also suppress soil-borne pathogens. Becker et al. (1988) reported that the PGPR *Bacillus cereus* Frankland & Frankland, *Bacillus* sp., and two strains of *Pseudomonas* suppressed the plant-parasitic nematodes *M. incognita*, *Heterodera glycines* Ichinohe, *Heterodera zeae* Koshy, Swarup & Sethi, and *Heterodera avenae* Wollenweber.

PGPR also suppress take-all of wheat, potato soft rot, bacterial wilt, and damping off (Weller 1988). PGPR can antagonize soil-borne pathogens in many ways (Dai et al. 2008; Preston 2004; Padgham and Sikora 2007; Tian and Robert 2000; Zhang et al. 2010).

Because both kinds of organisms depend on or are associated with plant roots, AM fungi and PGPR are likely to interact in the rhizosphere. Some PGPR produce antifungal metabolites, and it is therefore possible that PGPR could suppress AM fungi. According to Dwivedi et al. (2009), however, PGPR that produce antifungal metabolites do not necessarily interfere with the AM symbiosis and may even promote it if specific species of PGPR and AM fungus are carefully chosen for soil infestation. Evidence that the two kinds of organisms do not necessarily interfere with each other is indicated by the disease control that has been reported when AM fungi and PGPR are used together. For example, the root-rot disease complex of chickpea may be controlled by the combined use of *Rhizobium*, *Pseudomonas striata* Chester, and *Glomus intraradices* Schenck & Sm. (Akhtar and Siddiqui 2008). Similarly, dual inoculation with both the AM fungus *G. intraradices* and *Pseudomonas* improved soil condition and vegetable yield more than inoculation with only one of the organisms (Srivastava et al. 2007).

M. incognita is widespread and important in tomato culture, as well as in many horticultural crops in China (Fan et al. 2009; Shi et al. 2010). The current study concerns the use of PGPR and AM fungi for the control of the root-knot nematode *M. incognita* on vegetable crops. In addition to determining how single and combined inoculations with PGPR and AM fungi affect plant growth and disease, this study also determined how the PGPR and AM fungi affect each other.

Materials and methods

Plants, mycorrhizal inocula, PGPR, nematode inoculum, and soil

Seeds of tomato (*Lycopersicon esculentum* Mill) cultivar Jinguan, produced by Jinan Sun Rise Seeds Company Lt., were soaked in a 0.10% mercury bichloride solution for 10 min, washed with tap water, soaked in water at 60°C for 15 min, soaked in water at room temperature for 12 h, and germinated at 25–28°C. Germinated seeds (those with emerging radicles) were used in the experiments described in the following section. Mycorrhizal inocula were obtained from sand cultures of *G. mosseae* and *G. versiforme* (both were originally provided by the University of Western Australia, Perth, Australia) with clover (*Trifolium repens* L.) as the host; the inocula contained sand, spores, hyphae, and root fragments. The tested PGPR were *Bacillus polymyxa* (Bp) (T79) and *Bacillus* sp. (Bsp) (T83), both isolated from the rhizosphere of Jinguan tomato plants grown in the field and able to kill *M. incognita* (Mi) juveniles in previous experiments (unpublished data). Nematode eggs and second-stage juveniles (J2) were obtained from the roots of tomato cv Jinguan. Whole root systems were macerated in a 0.12 to 0.15 NaOCl solution. Nematodes were collected in a 26-μm pore sieve (500 mesh), counted, and adjusted to inoculate 3,000 nematodes per pot. The plant growth medium was a sandy loam with pH 6.7, 0.8% organic matter, 0.3 $g kg^{-1}$ of total phosphorus, and 69, 43, and 38 mg kg^{-1} available nitrogen, phosphorus, and potassium, respectively. The soil was autoclaved at 121°C for 2 h.

Experimental design, inoculation, and plant culture

Three experiments were conducted. Experiment 1 did not include the nematode and had nine treatments: control (CK, no AM fungi or PGPR added), inoculation with *G. mosseae* (Gm), *G. versiforme* (Gv), *B. polymyxa* (Bp), *Bacillus* sp. (Bsp), Gm + Bp, Gm + Bsp, Gv + Bp, and Gv + Bsp. Plants were inoculated with AM fungi at sowing (see next paragraph) and were inoculated with PGPR immediately when seedlings at the 4-to-5 leaf stage were transplanted into pots. Experiment 2 had ten treatments: CK, inoculation with Mi, Gm + Mi, Gv + Mi, Bp + Mi, Bsp + Mi, Gm + Bp + Mi, Gm + Bsp + Mi, Gv + Bp + Mi, and Gv + Bsp + Mi. As in experiment 1, AM inocula were added when seeds were sown, while PGPR and Mi were added when seedlings at the 4-to-5 leaf stage were transplanted. Experiment 3 also had ten treatments: CK, inoculation with *G. mosseae* at sowing (Gm), inoculation with *Bacillus* sp. at transplanting (Bsp), inoculation with *G. mosseae* at sowing + inoculation with *Bacillus* sp. at transplanting (Gm + Bsp), inoculation with *M. incognita* at transplanting (Mi), inoculation with *G. mosseae* at sowing + inoculation with *M. incognita* at transplanting (Gm + Mi), inoculation with *Bacillus* sp. + *M. incognita* at transplanting (Bsp + Mi), inoculation with *G. mosseae* at sowing + inoculation with *Bacillus* sp. + *M. incognita* at transplanting (Gm + Bsp + Mi), inoculation with *G. mosseae* + *M. incognita* at transplanting (I: Gm + Mi), and inoculation with *G. mosseae* + *Bacillus* sp. + *M. incognita* at transplanting (II: Gm + Bsp + Mi).

Ten grams of AM fungal inocula, which contained about 500 spores, was put into a seedling-culture tray (225 cm^3) and mixed with the soil; autoclaved inoculum plus 10 ml of the filter solution (free of the fungi) of the inocula were added in the control. Two germinated tomato seeds were then planted in each tray and kept in a greenhouse at 800± 100 μmol m^{-2} s^{-1} light intensity, light/dark 15:9 h, 28/18± 3°C (day/night), and 75±5% relative humidity. When seedlings had four to five leaves, they were transplanted into the 3-L pots (one seedling per pot). According to the

treatment, the soil in each pot was inoculated with 1.0×10^9 colony forming units (CFU) of PGPR and/or 3,000 eggs of Mi. Plants were kept in a greenhouse under the same growing conditions described above. Plants were irrigated weekly with P-free Hoagland solution or monthly with regular 1/3-strength Hoagland solution for mycorrhizal development. Plants were harvested 60 days after transplanting in experiments 1 and 2 and 0, 2, 4, 6, 9, 13, 17, 21, and 25 days after transplanting in experiment 3.

Assessment of plant growth, mycorrhizal colonization, and PGPR population

Plant height, stem diameter, number of nodes, and dry mass of shoots and roots were measured after the tomato plants were harvested. A sample of fine roots (5 g fresh mass per plant) was cut from the harvested plant, washed, cleared, and stained with acid fuchsine. AM fungal entry points, hyphae in roots, cells with arbuscules, and vesicles per unit root length were determined with a BX50 Olympus microscope equipped with a PM-30 Automatic Photomicrographic System. Mycorrhizal colonization was determined as described by Biermann and Linderman (1981). For PGPR population measurement, 1.0 g of dry soil was added with sterilized water to prepare a soil solution. PGPR numbers in the rhizosphere soil were counted with a dilution plate method 3 days after incubation under 30°C (Bashan et al. 1993; Zhao and He 2002).

Assessment of root-knot nematode damage

Another sample of fine roots (5 g fresh mass per plant) was boiled in 0.1% acid fuchsine–lactophenol for 3 min and cleared in lactophenol for 2 days (Byrd et al. 1983). The numbers of nematodes in the roots (juveniles and females/gram root) were then determined by examining the roots with a light microscope (Liu 1995). Penetration rate was calculated as the number of *M. incognita* juveniles and females in the root system/3,000 (the number of eggs added per plant)×100 (Caroli et al. 1996). After the egg masses were counted, up to ten egg masses per plant (egg masses were not always present depending on the treatment and harvest date) were soaked in a 5% sodium hypochlorite solution for 2–5 min, and the number of eggs per egg mass was determined.

Nematode galling was assessed according to a 0–6 root galling index: 0=no galls formed; 0.5=less than 10% of the roots with galls, galls are small, fine roots appear normal; 1=less than 10% of the roots with galls; 2=10% to 20% of the roots with galls; 3=20% to 50% of the roots with galls and severe root swelling affecting less than 30% of the root system; 4=50% to 70% of the roots with galls and severe root swelling affecting 30% to 70% of the root system; 5=70% to 90% of the roots with galls, severe root swelling affecting greater than 70% of the root system, and fine roots are rare; 6=100% of the roots with galls, severe swelling affecting the entire root system, fine roots are absent, and root rot is evident. Disease indexes (percent)=∑ (numbers of plants in each grade × the representative value of each grade/(total numbers of plants × the representative value of the highest grade) × 100; incidence (percent) = numbers of diseased plants/total plants and relative control (percent)=1−(disease index of treatment/disease index with Mi alone)×100, as described by Fang (1998).

Statistical analysis

Treatments were replicated five times in experiments 1 and 2. In experiment 3, each combination of treatment and nine harvest times was replicated three times. Any data in percent were transformed before statistical analysis. Data were analyzed by ANOVA. Differences in means were compared with Duncan's new multiple range test and considered significant at $P\leq0.05$. Costat (CoHort Software, Berkeley, CA, USA) and 2003 Microsoft® Excel were used for statistical analyses.

Results

Experiment 1: colonization of roots and rhizosphere by AM fungi and PGPR, and plant growth

Colonization of roots by AM fungi was greater with *G. mosseae* + *B. polymyxa* and *G. mosseae* + *Bacillus* sp. than with *G. mosseae* or *G. versiforme* alone, and colonization

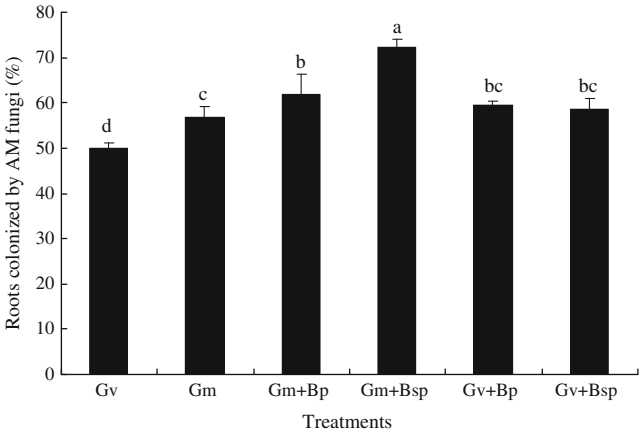

Fig. 1 Mycorrhizal percentage of tomato (*L. esculentum* Mill) seedlings inoculated with arbuscular mycorrhizal fungi in experiment 1. *Gv G. versiforme* (Karsten) Berch, *Gm G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *Bp B. polymyxa* (Prazmowski) Mace (T79), *Bsp Bacillus* sp.(T83). Tomato seeds were planted in soil inoculated with the arbuscular mycorrhizal fungi (500 spores), and the seedlings were inoculated with the bacteria (1.0×10^9 CFU/ml, 10 ml per plant) immediately when they were transplanted into pots. Values are means of five replicates

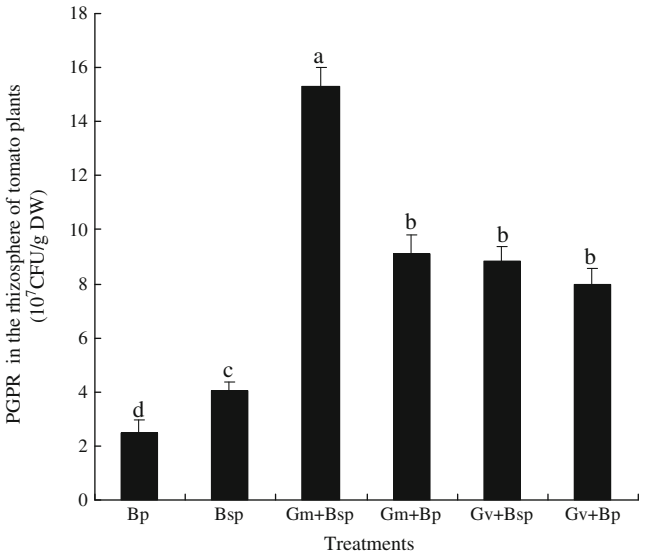

Fig. 2 Population density of PGPR in the rhizosphere of tomato seedlings in experiment 1. *Gv G. versiforme* (Karsten) Berch, *Gm G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *Bp B. polymyxa* (Prazmowski) Mace(T79), *Bsp Bacillus* sp.(T83). Tomato seeds were planted in soil without or with the arbuscular mycorrhizal fungi (500 spores) and were inoculated with the bacteria (1.0×10^9 CFU/ml, 10 ml per plant) as indicated when the seedlings were transplanted into pots. Values are means of five replicates

was greatest with *G. mosseae* + *Bacillus* sp. (Fig. 1). There was no mycorrhizal colonization in the noninoculated plants.

The population density of *B. polymyxa* or *Bacillus* sp. in the rhizosphere was two to five times greater in the treatment with the bacteria added with *G. mosseae* or *G. versiforme* than in the treatment with the bacteria alone (Fig. 2). No PGPR were detected in the rhizosphere of the noninoculated plants.

Plant height, stem diameter, number of nodes, and dry weight of the tops and roots were significantly higher with *G. mosseae* + *Bacillus* sp. than with the noninoculated plants or with other single inoculations. The dry weight of the tops in single inoculations was greater than that of the noninoculated plants (Table 1).

Experiment 2: effects of AM fungi and PGPR on the root-knot nematode and galling of roots

Plant growth was lower in the treatment with *M. incognita* than in the noninoculated plants but was greater in the treatment with *M. incognita* added with AM fungi, PGPR, or AM fungi + PGPR than in the treatment with *M. incognita* (Table 2), while disease incidences and disease indexes were all significantly lower in the treatment with *M. incognita* added with AM fungi, PGPR, and AM fungi + PGPR than in the treatment with *M. incognita* (Table 3). Relative control was greatest in Gm + Bsp, Gv + Bsp, and Gm + Bp treatments (Table 3). The numbers of J2, females, total nematodes, egg masses, and eggs per egg mass were lower in the treatment with *M. incognita* added with AM fungi or PGPR than in the treatment with *M. incognita* (Table 4). The numbers of J2 and eggs per egg mass were lower in Gm + Bsp + Mi treatment than in any other treatment (Table 4).

Experiment 3: *M. incognita* colonization as affected by AM fungi and PGPR

When *M. incognita* was added alone, the number of *M. incognita* in roots increased rapidly between days 0 and 2 and slowly thereafter (Fig. 3). By day 25, the number of nematodes in roots was in the following order by treatment (from highest to lowest): Mi, I Gm + Mi, Bsp + Mi, II Gm + Bsp + Mi, Gm + Mi, and Gm + Bsp + Mi (Fig. 3). In other words, penetration of roots by *M. incognita* was

Table 1 Growth of tomato plants inoculated with arbuscular mycorrhizal fungi and PGPR 60 days after establishment in experiment 1

Treatments	Plant height (cm)	Stem diameter (cm)	No. of nodes/plant	Dry mass of shoots (g)	Dry mass of roots (g)
Gm + Bsp	84.6a	0.91a	14.5a	6.75a	5.16a
Gm + Bp	74.2abc	0.88ab	14.0ab	6.61ab	4.35bc
Gv + Bsp	84.1a	0.82abc	13.5abc	5.38de	4.54b
Gv + Bp	80.7ab	0.88ab	13.0abc	6.15abc	4.08bcd
Gm	68.9abc	0.87ab	12.8abc	6.02bcd	4.15bcd
Gv	64.9bc	0.84abc	12.5abc	5.56cde	4.00bcd
Bsp	64.6bc	0.80bc	12.5abc	5.29e	4.03bcd
Bp	66.5bc	0.80bc	12.0bc	4.97e	3.82cd
CK	61.9c	0.76c	11.5c	4.23f	3.67d

Tomato seeds were planted in soil without or with the arbuscular mycorrhizal fungi (500 spores) and were inoculated with the bacteria (1.0×10^9 CFU/ml, 10 ml per plant) as indicated by treatments when the seedlings were transplanted into pots. Values are means of five replicates. Means in a column with different letters are significantly different at $P=0.05$

Gv G. versiforme, *Gm G. mosseae*, *Bp B. polymyxa*, *Bsp Bacillus* sp., *CK* noninoculated control

Table 2 Growth of tomato plants inoculated with *M. incognita*, arbuscular mycorrhizal fungi, and PGPR 60 days after establishment in experiment 2

Treatments	No. of nodes/plant	Plant height (cm)	Shoot dry mass (g)	Root dry mass (g)
Gm + Bsp + Mi	14.2a	84.1a	5.76a	4.84a
Gv + Bsp + Mi	14.2a	80.6ab	4.76bc	4.12bc
Gv + Bp + Mi	14.2a	81.7ab	5.14ab	4.56ab
Gm + Bp + Mi	14a	80.5ab	4.62bc	4.05bc
Gm + Mi	13.6a	78.8ab	4.5bcd	3.97bc
Bp + Mi	12.8ab	62.3bc	3.86d	3.56cd
Bsp + Mi	14.2a	72.4ab	4.08cd	3.61cd
Gv + Mi	13.4a	78.2ab	4.34cd	3.83c
Mi	11.2b	49.5c	3.05e	3.14d
CK	11.2b	62bc	4.23cd	3.67cd

Tomato seeds were planted in soil without or with the arbuscular mycorrhizal fungi (500 spores) and were inoculated with the bacteria (1.0×10^9 CFU/ml, 10 ml per plant) and Mi (3,000 eggs per plant) as indicated by treatments when the seedlings were transplanted into pots. Values are means of five replicates. Means in a column with different letters are significantly different at $P=0.05$

Mi M. incognita, *Gv G. versiforme*, *Gm G. mosseae*, *Bp B. polymyxa*, *Bsp Bacillus sp.*, *CK* noninoculated control

lowest with the combination of *G. mosseae* added at sowing and *Bacillus* sp. and *M. incognita* added at transplanting.

Discussion

Multimicrobial inoculation has been proposed as a way of protecting plants against environmental stress and increasing the sustainability of plant production. AM fungi and PGPR are well recognized as microorganisms that can improve plant nutrition and growth and also reduce plant disease, and they are usually more effective when added together than alone (Miroslav and Milan 2000; Attia and Awad 2003; Akhtar and Siddiqui 2008; Siddiqui and Akhtar 2009). For instance, dual inoculation with the AM fungus *Glomus fasciculatum* and the bacterium *Azospirillum brasilense* enhanced nitrogen acquisition and growth of *Medicago sativa* L. (Biró et al. 2000). In the present study, tomato plant growth was improved more by dual inoculation with AM fungi and PGPR than by single inoculations, and enhancement was greater with the dual inoculation of *G. mosseae* and *Bacillus* sp. than with other dual or single inoculations. Single inoculations were consistently effective at increasing shoot growth but did not improve root size. Meyer and Linderman (1986) found that plant growth and

Table 3 Disease status and control in tomato plants inoculated with *M. incognita* as affected by arbuscular mycorrhizal fungi and PGPR 60 days after establishment in experiment 2

Treatments	Disease incidence (%)	Disease index (%)	Relative control (%)
Gm + Bsp + Mi	37.8ef	12.5g	77.9a
Gv + Bsp + Mi	32.3f	12.5g	77.9a
Gv + Bp + Mi	43.7cd	24.3e	57c
Gm + Bp + Mi	42cde	16.3f	71.2b
Gm + Mi	41.5de	22.5e	60.2c
Bp + Mi	54.9b	41.8b	26f
Bsp + Mi	58.6b	33.3c	41.1e
Gv + Mi	47.4c	28.8d	49d
Mi	86.3a	56.5a	–
CK	0	0	–

Tomato seeds were planted in soil without or with the arbuscular mycorrhizal fungi (500 spores) and were inoculated with the bacteria (1.0×10^9 CFU/ml, 10 ml per plant) and Mi (3,000 eggs per plant) as indicated by treatments when the seedlings were transplanted into pots. Relative control was calculated as: 1−(disease index of treatment/disease index with Mi alone)×100. Values are means of five replicates. Means in a column with different letters are significantly different at $P=0.05$

Mi M. incognita, *Gv G. versiforme*, *Gm G. mosseae*, *Bp B. polymyxa*, *Bsp Bacillus sp.*, *CK* noninoculated control

Table 4 Population of *M. incognita* on tomato plants as affected by arbuscular mycorrhizal fungi and PGPR 60 days after establishment in experiment 2

Treatments[a]	J2 per g root	Females per g root	All stages per g root	Number of egg masses per g root	Number of eggs per egg mass
Mi	129a	492a	621a	142a	497a
Bp + Mi	38.7b	236d	275c	108b	429c
Gv + Mi	25.8cd	357b	383b	95.6bc	451b
Gv + Bp + Mi	8.4e	295c	304c	79.1cd	392d
Gm + Mi	18.7d	138ef	156de	93.2bc	392d
Bsp + Mi	32.3bc	151e	183 d	109b	420c
Gv + Bsp + Mi	38b	305c	310c	65.3de	355e
Gm + Bp + Mi	36.5b	112g	145de	58.8ef	362e
Gm + Bsp + Mi	6e	116fg	122e	44.6f	333f
CK	0	0	0	0	0

Tomato seeds were planted in soil without or with the arbuscular mycorrhizal fungi (500 spores) and were inoculated with the bacteria (1.0×10^9 CFU/ml, 10 ml per plant) and Mi (3,000 eggs per plant) as indicated by treatments when the seedlings were transplanted into pots. Values are means of five replicates. Means in a column with different letters are significantly different at $P=0.05$

Mi M. incognita, *Gv G. versiforme*, *Gm G. mosseae*, *Bp B. polymyxa*, *Bsp Bacillus sp.*, *CK* noninoculated control, *J2* the second-stage juveniles of *M. incognita*

nodulation of subterranean clover were enhanced by indigenous AM fungi and *Pseudomonas putida*, and the PGPR increased colonization by AM fungi, while the latter did not increase populations of the former. The results of the present study not only indicate that the tested PGPR, *B. polymyxa* and *Bacillus* sp., increased AM fungal colonization of roots, but also that the AM fungi increased population of PGPR in the rhizosphere. So the AM fungi and PGPR can stimulate each other and play a synergistic activity and function in improving plant growth and in reducing plant disease.

Although the effects of single inoculations with AM fungi or PGPR on pathogens and disease have often been studied (Barea et al. 2002; Cooper and Grandisons 1986; Padgham and Sikora 2007; Preston 2004; Shreenivasa et al. 2007a; Wang and Hu 2000), the effects of dual inoculations on plant pathogens and plant diseases have seldom been studied. In one study, dual inoculation with *Bacillus subtilis* Ehrenberg M3 and *G. mosseae* BEG29 decreased crown rot shoot symptoms as well as the numbers of *Phytophthora cactorum* (Lebert & Cohn) Schröt oospores in the roots of strawberry in autumn, while in the summer, root necrosis was slightly decreased only by *B. subtilis* and *G. mosseae* + *Gliocladium catenulatum* Gilman & Abbott (Vestberg et al. 2004). Serfoji et al. (2010) observed that application of vermicompost + *Glomus aggregatum* + *Bacillus coagulans* increased plant growth characters and reduced root-knot index, nematode reproduction rate, number of galls, and egg masses on tomato cv Pusa Ruby in sandy loam acidic soils. In the present experiments, nematode penetration in roots was similar for most of the treatments as recorded in the second day (Fig. 3). However, root colonization by AM fungi and PGPR, individually or in combination, had a negative effect on the development of *M. incognita* following the second day of inoculation and successive harvest dates. Nematode population build up decreased with time as indicated by the differences in nematode development in the roots for the different treatments. So we

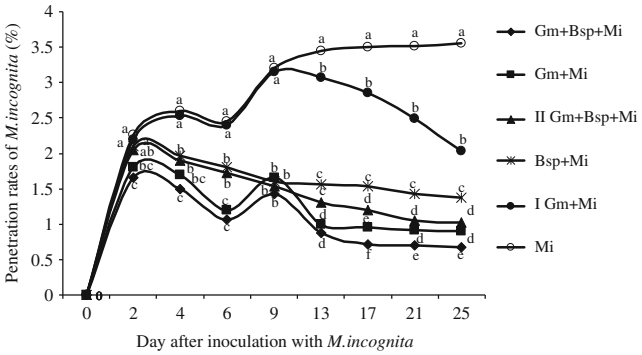

Fig. 3 Penetration rate of *M. incognita* on tomato roots as affected by time after inoculation with arbuscular mycorrhizal fungi and PGPR in experiment 3. *Mi* plants inoculated with 3,000 eggs of *M. incognita* at transplanting. *Bsp* + *Mi* plants inoculated with *Bacillus* sp. (1.0×10^9 CFU/ml×10 ml per plant) and *M. incognita* at transplanting. *Gm* + *Mi* seeds sowed in soil containing 500 spores of *G. mosseae* + *M. incognita* at transplanting. *Gm* + *Bsp* + *Mi* seeds sowed in soil containing *G. mosseae* + *Bacillus* sp. + *M. incognita* at transplanting. *I Gm* + *Mi* plants inoculated with *G. mosseae* + *M. incognita* at transplanting. *II Gm* + *Bsp* + *Mi* plants inoculated with *G. mosseae* + *Bacillus* sp. + *M. incognita* at transplanting. Penetration rate was calculated as the number of nematodes in a root system/3,000 (the number of eggs added per root system)×100. Values are means of three replicates

concluded that nematode penetration of roots, nematode reproduction, and nematode-incited disease were decreased more by dual inoculations with AM fungi and PGPR than by single inoculations, and dual inoculation with *G. mosseae* and *Bacillus* sp. gave the best results. This may be due to AM fungus's strong induction of systemic resistance in plants towards nematodes (Elsen et al. 2008), and PGPR can do this through their own metabolism (such as phosphate solubilization, hormone production, N_2-fixation, etc.), directly affect the plant metabolism (for instance, enhance water and mineral uptake, improve root development, enhance plant enzyme activity, etc.), or affect the plant by "helping" another beneficial microorganism to function better (e.g., *Azospirillum* increasing modulation of legumes by rhizobia, or enhancing mycorrhizal phosphate solubilization or mycorrhizal colonization) (Bashan and Holguin 1998; Gryndler 2000; Linderman 1988; Shreenivasa et al. 2007b; Fig. 1, Tables 1 and 2). However, the mechanisms of nematode suppression in the roots are unknown but would seem to be related to physiological changes in roots affecting nematode food source or feeding (unfavorable condition for nematode development) rather than a direct competition for space. de la Peña et al. (2006) even considered that nematode suppression by AM fungi did not occur through a systemic plant response but through local mechanisms. If the experiments were carried out in non-sterile soil simulating field conditions, what would be the results? Sometimes the addition of PGPR does not seem to improve the results of AM fungi single treatments in terms of nematode infection (Jaizme-Vega et al. 2006). So the mechanisms by which AM fungi and PGPR interact to decrease plant disease also require additional study.

It is important to evaluate and select the best combination of microorganisms to use in dual or tri-inoculations with AM fungi and PGPR. The “best combination” must be established on the basis of inoculation time and method, host plant, pathogen, and ecological conditions. Although the best combination was indicated for experimental conditions and organisms in the current study, further investigation is needed on a wider range of AM fungi (Tchabi et al. 2010), PGPR, plant species (Powell et al. 2009), and pathogens. Even though there were experiments which showed that many AM fungi and PGPR successfully improved growth and reduced root knot nematode infestation and they could be applied as a biofertilizer, as well as a biocontrol agent against the root-knot nematode (Ahmed et al. 2009; Elsheikh and Mirghani 1997), there will still be a long way to go before AMF + PGPB becomes a promoting technique for sustainable agricultural production.

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