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REGULAR ARTICLE

The plant growth-promoting fungus *Fusarium equiseti* and the arbuscular mycorrhizal fungus *Glomus mosseae* induce systemic resistance against *Cucumber mosaic virus* in cucumber plants

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Abstract
Aims The aim of this study was to elucidate the effects of the interactions between the arbuscular mycorrhizal fungus *Glomus mosseae* (Gm) and *Fusarium equiseti* GF18-3 on cucumber growth and the biocontrol of the yellow strain of *Cucumber mosaic virus* (CMV-Y).
Methods Cucumber plants were pre-inoculated with Gm and GF18-3 for 4 weeks before the leaves were inoculated with CMV. CMV accumulation in cucumber leaves was determined using an indirect enzyme-linked immunosorbent assay (ELISA) at 1, 2, and 3 weeks post-inoculation (WPI). An RT-PCR analysis was performed to evaluate the expression levels of defence-related genes.
Results The co-inoculation of cucumber plants with Gm and GF18-3 or GF18-3 alone resulted in effective control of CMV disease severity, though no significant reduction was observed in the Gm-alone treatment. CMV accumulation was significantly decreased in cucumber plants treated with combined inoculation or with GF18-3 alone at 1, 2, and 3 WPI. The RT-PCR results revealed higher expression levels of SA-inducible genes in all treatments, while only Gm treatment of plants induced JA-inducible genes.
Conclusion The dual inoculation treatment and inoculation with GF18-3 alone have the potential to reduce disease severity and increase plant growth. Moreover, modulation of plant defence responses in the shoots may contribute to this protection.

Keywords Cucumber mosaic virus · *Glomus mosseae* · Cucumber · Plant growth-promoting fungi · *Fusarium equiseti* GF18-3 · Induced systemic resistance

Abbreviations
CMV *Cucumber mosaic virus*
BGI barley grain inoculum
BTH benzothiadiazole
ISR induced systemic resistance

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JA	jasmonic acid
PDA	potato dextrose agar
PDB	potato dextrose broth
PGPF	plant growth-promoting fungi
PGPR	plant growth-promoting rhizobacteria
PR	pathogenesis related
DPI	days post inoculation
WAP	weeks after planting
RT-PCR	reverse transcription-PCR
SA	salicylic acid
SAR	systemic acquired resistance.

Introduction

Diseases caused by the *Cucumber mosaic virus* (CMV) are among the most important factors involved in reducing the production of crops, vegetables, fruits and ornamental plants (Roossinck 1999). Researchers have aimed to control incurable pathogens such as CMV. Because viruses cannot be eradicated chemically, alternative protection strategies need to be identified. All plants possess different inducible defence mechanisms to protect themselves against pathogen attack. Despite the existence of plant defence mechanisms, many pathogens can evade or suppress activated defences (DebRoy et al. 2004). If defence mechanisms are triggered by a stimulus prior to infection by a plant pathogen, the disease can be reduced (Van Loon et al. 1998). Induced resistance is a phenomenon wherein a plant exhibits enhanced resistance upon a challenge inoculation with a pathogen when it is appropriately stimulated by non-pathogens, certain chemicals, nonvirulent forms of the pathogen, incompatible races of pathogens or virulent pathogens under circumstances in which the infection is stalled due to environmental conditions (Görlach et al. 1996; Sticher et al. 1997). Thus, it is possible to control viral diseases by inducing resistance in plants without altering the plant genome (Ryu et al. 2007; Ipper et al. 2008; Wang et al. 2009).

The endotrophic arbuscular mycorrhizae (AMs) are the most prevalent type of mycorrhizae (Smith and Read 1997). Arbuscular mycorrhizal fungi (AMF) are a group of microorganisms that form symbiotic associations with a wide range of plant species. Mycorrhizal fungi enhance plants nutrition, growth (Jeffries et al. 2003; Duponnois et al. 2005; Meddad-Hamza et al. 2010) and tolerance to different types of biotic and abiotic stresses (Al-Karaki and McMichael 2004; Selosse et al. 2004; Liu et al. 2007). However, the use of AMF as inoculants to promote plant growth and health may help to reduce the inputs of pesticides and other environmentally harmful agrochemical products that are required for optimal plant growth and health (Jeffries and Barea 2001).

Plant growth-promoting fungi (PGPF) are a class of non-pathogenic, soil-borne, filamentous fungi that confer beneficial effects on plants (Hyakumachi 1994). *Fusarium equiseti* (Corda) Saccardo has been reported to be among the most effective PGPF isolates for the induction of systemic resistance against cucumber diseases (Horinouchi et al. 2007; Maciá-Vicente et al. 2009).

It is important to study the interactions between beneficial microorganisms associated with plant roots because these interactions could enhance or inhibit the beneficial effects of individual species. Application of different species of microorganisms has not been investigated regarding co-inoculations of AMF (endophytes) and PGPF (saprophytes), which could improve the obtained protection and widen the range of effectiveness by activating different defence mechanisms. Both AMF and *Fusarium equiseti* have been used independently to improve plant growth and/or to control pathogens (Punja et al. 2008; Whipps 2001). Many studies have reported co-inoculations with both microorganisms as mixed inoculants (McAllister et al. 1996, 1997; Garcia-Romera et al. 1998; Saldajeno and Hyakumachi 2011). Although several studies have shown interactions between fungal biocontrol agents and AMF (Green et al. 1999; Martínez et al. 2004), no such work has investigated the exploitation of these interactions in improving the efficacy of biocontrol against viral diseases.

The natural defence responses of plants include induction of pathogenesis-related (PR) proteins, accumulation of phytoalexins and deposition of structural polymers, such as callose and lignin (Benhamou and Nicole 1999). Phenylalanine ammonia lyase (PAL) catalyses the first step in the phenylpropanoid biosynthetic pathway and leads to the production of phenolic compounds, which exhibit effective biological functions (Hammerschmidt 1999). The accumulation of plant hydrolases, such as chitinases and β-1,3-glucanases, plays a key role in antimicrobial potential (Dalisay and Kuc 1995a; Van Loon 1997). Another important group of enzymes involved in plant resistance mechanisms is the peroxidases, which are involved in the synthesis of phenolic compounds and the formation of structural barriers (Dalisay and Kuc 1995b).

Some published data have demonstrated induction of ISR against CMV (Ryu et al. 2004); this study demonstrated that PGPR activated ISR in *Arabidopsis* plants that were exposed to CMV through a pathway that was independent of SA and NPR1, but dependent on jasmonic acid. Moreover, treatment of tobacco plants with *Bacillus* spp. enhanced the expression of the PR genes, *NPR1 and Coi1*, and led to increased resistance to CMV (Wang et al. 2009). Although many studies have investigated the interactions between AMF and PGPF, the mechanisms underlying these associations are generally poorly understood (Artursson et al. 2006).

Therefore, the aim of the present study was to test the ability of the interaction between AMF (*G. mosseae*) and PGPF (*F. equiseti* GF18-3) to induce systemic resistance in cucumber (*Cucumis sativus* L. cv. Tokiwa Jibai) and to improve plant growth and protection against CMV.

Materials and methods

Potting medium and plant material

Cucumber (*Cucumis sativus* L. cv. Tokiwa Jibai) was used as the test plant in this experiment. Clay loam soil collected from the campus field in Gifu City, Japan, containing 2.01 % organic matter, 0.10 % total N and 18.8 mg/100 g of available P. with a pH of 6.5, was sieved through a 2-mm mesh, mixed with river sand (1:1) and sterilised via autoclaving twice for 1 h at 121 °C; the autoclaving was performed on two consecutive days. Mineral nutrients were added to the soil-sand mixture as described by Chandanie et al. (2006).

Fungal and viral inoculum

The PGPF used in this study was *Fusarium equiseti* GF18-3, which was isolated from a turf grass rhizosphere (Hyakumachi 1994) and stored in a PGPF collection in the Laboratory of Plant Pathology, Gifu University. GF18-3 was grown in 2 % potato dextrose agar (PDA) in petri plates for 7 days at 25 °C. Twenty mycelial disks (5 mm) were obtained from GF18-3 cultures from the growing margin of a colony on PDA and were transferred to a 500-ml Erlenmeyer flask containing autoclaved barley grains (100 g of barley grains and 100 ml of distilled water). After 10 days of incubation at 25 °C in the dark with shaking every 2 days, the completely colonised barley grains were air-dried at room temperature (23–25 °C). The dried BGI was ground to a 1–2-mm particle size and stored at 4 °C until it was used.

Inocula of the AMF *G. mosseae* (Gm) (Nicol. and Gerd.) Gerd.- and Trappe-containing spores, hyphae, colonised root fragments and potting soil were obtained from commercial inocula (Idemitsu Kosan Co. Ltd., Japan). The yellow strain of *Cucumber mosaic virus* (CMV-Y) that was used in this study was obtained from the Laboratory of Plant Pathology, Tohoku University, Japan. The virus was maintained in tobacco plants (*Nicotiana tabacum* cv. Xanthi-nc).

Experimental design and biological treatments

Two experiments were designed to test the interaction between GF18-3 and Gm. The first experiment investigated the effects of their interaction on fungal colonisation and the promotion of plant growth, and the second experiment evaluated the suppression of disease severity and gene expression using RT-PCR analyses. Both of the experiments included the same treatments, as follows: (a) *F. equiseti* GF18-3 alone (GF18-3); (b) *F. equiseti* GF18-3 with *G. mosseae* (GF18-3+Gm); (c) *G. mosseae* alone (Gm); (d) the synthetic SAR inducer benzothiadiazole (BTH) as a positive control treatment (Lawton et al. 1996); and (e) a noninoculated control (Cont). Three replicates were performed for each treatment, with 5 plants per replicate. The inocula of *F. equiseti* GF18-3 and *G. mosseae* were hand-mixed completely with the potting medium at rates of 1 and 2 % w/w, respectively. The non-mycorrhizal treatments were mixed with equal amount of autoclaved Gm inocula, while the non-Fusarium treatments were blended with equal amount of autoclaved barley grain. Cucumber plants grown in soil were treated with 0.3 mM BTH (Novartis Agro, Tokyo, Japan) via soil drenching 1 day prior to the challenge inoculation.

Evaluation of plant growth and fungal colonisation

The cucumber seeds were surface sterilised (2 % NaOCl for 3 min) and then pre-germinated on autoclaved filter paper that was soaked with sterile, distilled water and incubated for 48 h at 25 °C. Sterilised plastic pots (150 ml) were filled with 150 g of inoculated potting medium. Two pre-germinated cucumber

seeds were planted per pot. The medium–inoculum mixture and filtrate of the Gm inoculum were prepared according to Calvet et al. (1993). The suspension was filtered through a 38-μm sieve to remove mycorrhizal propagules and was added to non-mycorrhizal pots (10 ml/pot) to establish similar initial microflora communities in all of the treatments (Calvet et al. 1993; Green et al. 1999). Equal amount of distilled water was added to the mycorrhizal pots. The plants were grown in growth chambers at 25 °C under 75 % relative humidity and a 12/12 h light/dark cycle (cool fluorescent lamps at 300 $\mu Em^{-2} s^{-1}$). The pots were watered at 48-h intervals, and nitrogen (40 mg/plant) was supplied weekly in the form of a NH_4NO_3 solution.

The colonisation of GF18-3 in the rhizosphere and roots was measured 4 and 7 weeks after planting (WAP). To estimate the degree of rhizosphere colonisation, the plants were uprooted, and the shoots were separated from the roots. The shoot fresh weight, dry weight (80 °C for 36 h) and number of leaves were measured. Soil that adhered to the roots was collected as rhizosphere of this soil, and 10-fold dilutions were made and plated on PDA supplemented with chloramphenicol (250 mgl^{-1}) and then incubated for 3 days at 25 °C. After incubation, the colonies were counted, and the population was quantified as CFU per gram of dry soil.

To evaluate root colonisation, the collected roots were washed with tap water to remove any adherent soil particles, and the root samples were divided into two portions for root colonisation by GF18-3 and Gm. For colonisation by GF18-3, the roots were cut into 1-cm segments, surface disinfected with 0.5 % NaOCl, rinsed with sterilised distilled water (SDW), dried on sterilised filter paper and plated on PDA supplemented with chloramphenicol (250 mgl^{-1}) followed by incubation for 4 days at 25 °C. Following incubation, any GF18-3 colonies growing-from the root segments were identified, and the isolation frequency was determined by counting the number of colonised segments per 100 segments plated per replicate. To verify the root colonisation of GF18-3, the root fragments were homogenised. The homogenised root suspension was filtered through two layers of sterilised cheesecloth. Then, 10-fold dilutions were made and plated on PDA supplemented with chloramphenicol (250 mgl^{-1}). GF18-3 colonies were counted, and the population was quantified as colony-forming units (CFUs) per gram of fresh roots after 4 days of incubation at 25 °C.

To estimate the degree of root colonisation by mycorrhizae, 1-cm root segments were cleared in 10 % (w/v) KOH for 1 h at 90 °C and stained with 0.05 % (w/v) chlorazol black E (CBE) for 1.5 h at 90 °C (Brundrett et al. 1984). The stained root segments were stored in 50 % glycerol until being subjected to microscopic observation. The percentage of the root length that was colonised by hyphae, arbuscules or vesicles of Gm was quantified according to McGonigle et al. (1990).

Cucumber mosaic virus inoculations

The CMV inoculum that was used throughout the experiments consisted of infected tobacco leaf tissue that was ground in 0.01 M sodium phosphate buffer, pH 7.0, containing 0.002 M EDTA (1 g of tissue:50 ml of sodium phosphate buffer). All of the inoculation materials were chilled at 4 °C prior to inoculation and were maintained on ice during the inoculation. The plants were inoculated 4 weeks after planting via rub inoculation on the oldest leaf (Ipper et al. 2008). Disease severity was assessed according to the following scale: 0=no symptoms; 2=vein clearing; 4=50 % of leaves on a plant exhibiting mosaic symptoms; 6=100 % of leaves on a plant exhibiting mosaic symptoms; 8=50 % of leaves showing severe mosaic symptoms and malformation; and 10=100 % of leaves showing severe mosaic symptoms and malformation. The disease severity experiment was performed three times with 5 plants per treatment.

Enzyme-linked immunosorbent assay (ELISA)

Determination of the CMV concentration was conducted as described previously by Zehnder et al. (2000) with some modifications. Leaf samples were collected 7, 14, and 21 days post-inoculation (DPI). The samples were ground in 50 mM carbonate buffer (pH 9.6) and added to microtitre plates at a final dilution of 1:10 (g leaf tissue:ml buffer). The plates were incubated overnight at 4 °C, and they were then washed 3 times with phosphate buffered saline containing Tween (PBS-T). Anti-CMV (primary antibody) was added to the plates at a concentration of 1 Fg/ml in PBS-T. The plates were incubated overnight at 4 °C again and then washed 3 times with PBS-T. Goat anti-rabbit immunoglobulin conjugated to alkaline phosphatase was diluted 1:7500 in PBS-T and

added to the plates. The plates were incubated at 37 °C for 1 h and then triple-washed with PBST, and the substrate (p-nitrophenylphosphate at 1 mg/ml in 10 % diethanolamine, pH 9.8) was added. The reactions were allowed to develop at room temperature. The absorbance values were read at 405 nm on a BIORAD model 550 microplate reader. The ELISA experiment was performed three times with 5 plants per treatment.

Examination of defence-related genes using RT-PCR analysis

RNA isolation

For the RNA analysis, leaves and roots were harvested 1, 2, 4, and 6 DPI and stored at −80 °C until being used. Each treatment included three replications with two plants per replicate. Total RNA was extracted following Suzuki et al. (2004), with some modifications. Leaves and roots were ground in liquid nitrogen using a sterilised mortar and pestle and homogenised with the following extraction buffer: 100 mM Tris–HCl (pH 9.5), 10 mM EDTA (pH 8.0), 2 % lithium dodecyl sulphate, 0.6 M NaCl, 0.4 M trisodium citrate and 5 % 2-mercaptoethanol. The aqueous phase obtained following centrifugation at room temperature was re-extracted with a chloroform:isoamyl alcohol (24:1) mixture. The collected supernatant was then extracted with water-saturated phenol, guanidium thiocyanate, sodium acetate (pH 4.0) and chloroform. The upper aqueous phase was precipitated with isopropanol. The precipitated RNA was collected, washed, briefly air-dried and dissolved in RNase-free water. After treatment with RNase-free water and DNase and inactivation of the DNase according to the instructions of the supplier (Takara Bio Inc., Shiga, Japan), approximately 1 μg of total RNA was reverse transcribed into single-stranded cDNA. An aliquot of the obtained cDNA was amplified via RT-PCR as described by Suzuki et al. (2004) to monitor the expression of a set of well-characterised defence-related genes, including *PR-1a* (Cools and Ishii 2002), *peroxidases* (*POX*), *lipoxygenase* (*LOX1*), *phenylalanine ammonia lyase* (*PAL1*), *chitinase* (*Chit1*) and *β-1, 3-glucanase* (*Glu*) (Shoresh et al. 2005). *Actin* was used as internal standard (Cools and Ishii 2002). The gene-specific primers used in these experiments are listed in Table 1.

Data analysis

The obtained data were subjected to one-way ANOVA, with the exception of the PGPF and AMF colonisation data, which were compared using Student's *t*-test at $P \leq 0.05$. When ANOVA indicated a significant F value, the treatment means were separated using Fisher's least significant difference (LSD) test at $P \leq 0.05$. All of the analyses were performed using the XLSTAT Pro statistical analysis software package (Addinsoft, New York, NY, USA).

Results

Effect of inoculation with *F. equiseti* and/or *G. mosseae* on the colonisation of the rhizosphere and roots of cucumber plants by the fungi

The proportion of root colonisation by Gm in cucumber plants inoculated with Gm alone was significantly different from that in the co-inoculation treatment with both *F. equiseti* GF18-3 and Gm at 7 WAP. Mycorrhizal root colonisation was not observed in the control plants (Fig. 1).

Additionally, root colonisation by GF18-3 was affected by treatment with Gm in the co-inoculated cucumber plants. The frequency of re-isolation of GF18-3 at 4 and 7 WAP decreased significantly in the co-inoculated cucumber plants compared to plants inoculated with GF18-3 alone (Fig. 2a). Similar results were obtained regarding the population density (CFU) of GF18-3 in the roots of the co-inoculated plants, which was significantly lower than that in the plants inoculated with GF18-3 alone (Fig. 2b). In contrast, the CFU of GF18-3 in the rhizosphere soil of co-inoculated plants was similar to that in plants inoculated with GF18-3 alone (data not shown).

Effects of inoculation with *F. equiseti* and/or *G. mosseae* on the growth of cucumber plants

The cucumber subjected to both the dual inoculation treatment and inoculation with GF18-3 alone exhibited a significantly higher shoot fresh weight, shoot dry weight, and number of leaves than the non-inoculated controls at both 4 and 7 WAP. The results of the co-inoculation treatment with GF18-3 and Gm were not significantly different compared to

Table 1 Gene-specific primers used in RT-PCR analysis in this study

Gene name	Forward primers (5′-3′)	Reverse primers (5′-3′)
PR-1a	GGCAGCCCAGACTTCTCAGC	GCATCTCACTTTGGCACATCCTA
Chitinase 1	TGGTCACTGCAACCCTGACA	AGTGGCCTGGAATCCGACT
β-1,3-glucanase	TCAATTATCAAAACTTGTTCGATGC	AACCGGTCTCGGATACAACAAC
POX	AGAGCAACAAGGTCGGTTTCA	GTGCCGACATCCTAGCTCAAG
LOX1	AAGGTTTGCCTGTCCCAAGA	TGAGTACTGGATTAACTCCAGCCAA
PAL1	ATGGAGGCAACTTCCAAGGA	CCATGGCAATCTCAGCACCT
Actin	GGCCGTTCTGTCCCTCTAC	CAGCTCCGATGGTGATGAC

the plants inoculated with GF18-3 alone. The plants treated with Gm alone displayed a significantly increased shoot fresh weight, shoot dry weight, and number of leaves at 4 WAP; however, at 7 WAP, no significant differences were found relative to the non-inoculated cucumber plants. The BTH treatment caused a significant reduction in plant growth compared to the control plants (Table 2).

Inoculation with *F. equiseti* and/or *G. mosseae* protects cucumber plants against *Cucumber mosaic virus*

The CMV disease severity was rated at 14 DPI. The dual inoculation, GF18-3 alone, and BTH treatments significantly reduced the disease severity rating compared to the untreated control plants. However, no significant differences were observed between the cucumber plants treated with Gm alone and the control plants (Fig. 3).

Table 3 shows the CMV accumulation detected by ELISA at 3 different time points: 7, 14, and 21 DPI. The CMV accumulation decreased significantly in all of the treatments compared to the control plants at 7 DPI. At 14 and 21 DPI, the cucumber plants that were treated with the dual inoculation, GF18-3 alone and BTH displayed significantly reduced CMV accumulation compared to the control plants, while the CMV accumulation was not reduced in the cucumber plants treated with Gm alone at 14 and 21 DPI. Additionally, the co-inoculation treatment significantly reduced CMV accumulation compared to treatment with GF18-3 alone at 7 and 14 DPI.

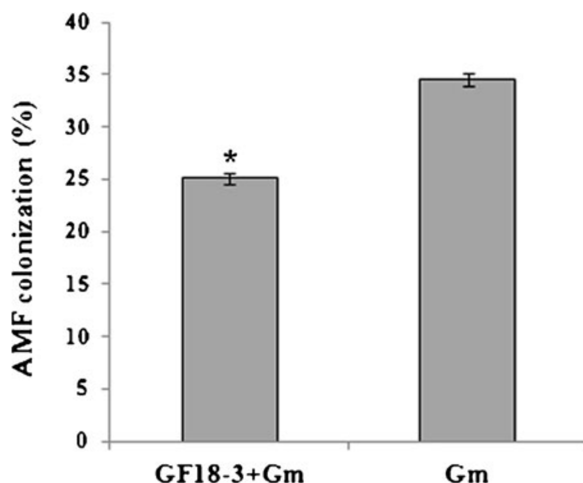

Fig. 1 Percentage of root length colonized by *G. mosseae* (Gm) in cucumber plants either co-inoculated with *Fusarium equiseti* GF18-3 or not at 7 weeks after planting (WAP). Columns represent mean values (n=15). Bars indicate standard errors. Asterisks indicate significant difference based on Fisher's LSD test at $P \leq 0.05$

Induction of defence-related genes through inoculation with *F. equiseti* and/or *G. mosseae* in cucumber plants

To investigate the possibility that combined or individual inoculation of Gm and GF18-3 can stimulate defence-related genes, we determined the expression of SA-inducible genes (*PR1-a, chitinase* and *β-1,3-glucanase*), JA-inducible genes (*LOX1* and *PAL1*) and antioxidant enzyme-inducible *peroxidase* (*POX*) locally in roots and systemically in leaves at 0, 1, 2, 4, and 6 days after the CMV challenge inoculation.

Increased expression of *PR1-a*, *chitinase (Chit 1)*, *β-1, 3-glucanase* and *peroxidase* (*POX)* was observed in the leaves and roots in all of the treatments. In contrast, expression of the JA-inducible genes (*LOX1* and *PAL1*) was initiated quickly in the leaves of only the plants that were either co-inoculated or inoculated with Gm alone, and these elevated levels were maintained in all of the treatments in both the roots and leaves. The BTH treatment resulted in no stimulation

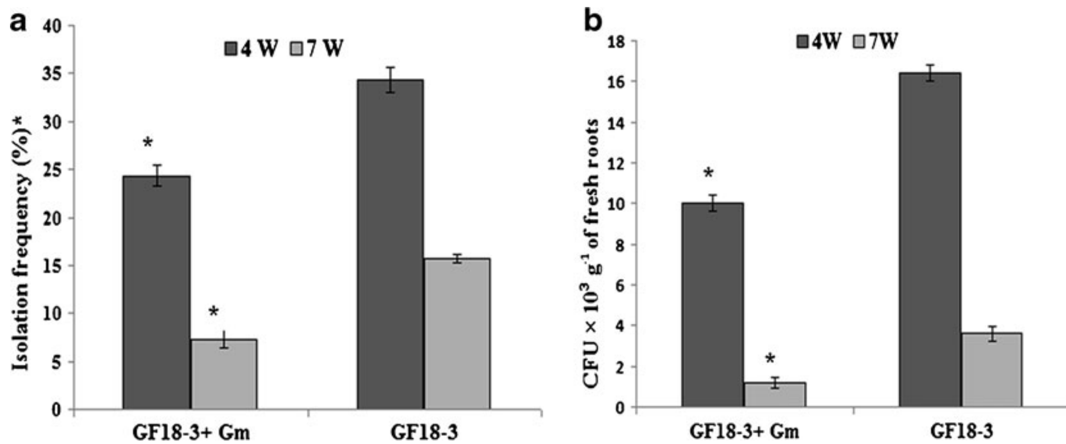

Fig. 2 Population of *Fusarium equiseti* GF18-3 in cucumber roots either co-inoculated with *G. mosseae* (Gm) or not at 4 and 7 weeks after planting (WAP). **a** Isolation frequencies from the roots of cucumber plants. **b** Number of colony-forming units (CFU) obtained from the roots of cucumber. Columns represent mean values ($n=15$). Bars indicate standard errors. Asterisks indicate significant difference based on Fisher's LSD test at $P \leq 0.05$. *Isolation frequency=(Number of colony-forming root segments/ total number of root segments plated) 100 %

of JA-inducible genes compared to control plants, while GF18-3 treatment led to only weak induction of these genes compared to control plants (Fig 4a and b).

Discussion

Pathogenic microorganisms that affect plants represent a major chronic threat to food production and ecosystem stability worldwide. Food producers have become increasingly dependent on agrochemicals as a relatively reliable method of crop protection and fertilisation (Compant et al. 2005). Viral diseases are fastidious diseases, and there are few chemical solutions available to combat them which are often ineffective, or cannot be used based on the increasing demand for pesticide-free food (Gerhardson 2002). Biological control and fertilisation are therefore being considered as alternative or supplemental methods of reducing the use of chemicals in agriculture (Postma et al. 2003; Whipps 2001).

We studied the effect of *G. mosseae* (Gm) on *F. equiseti* GF18-3 populations and the effect of GF18-3 inoculations on a Gm population. Our data showed that the GF18-3 population was significantly suppressed in the co-inoculation treatment at 4 and 7 WAP. This result indicates an antagonistic effect of Gm toward GF18-3. Similarly, Saldajeno and Hyakumachi

Table 2 Shoot fresh weight (g), shoot dry weight (g) and number of leaves of cucumber plants inoculated with *Fusarium equiseti* GF18-3 and/or *Glomus mosseae* (Gm) at 4 and 7 weeks after planting (WAP)

Treatment	Fresh weight		Dry weight		No. of leaves	
	4 WAP	7 WAP	4 WAP	7 WAP	4 WAP	7 WAP
GF18-3+Gm	12.07±0.27^{a}	23.00±0.31^{a}	0.55±0.01^{a}	1.45±0.03^{a}	7.20±0.11^{a}	8.47±0.13^{a}
GF18-3	11.67±0.29^{ab}	22.93±0.33^{a}	0.54±0.01^{ab}	1.43±0.03^{a}	7.27±0.15^{ab}	8.40±0.13^{a}
Gm	11.40±0.25^{b}	17.33±0.45^{b}	0.53±0.01^{b}	0.98±0.03^{b}	6.93±0.12^{b}	6.80±0.20^{b}
BTH	5.60±0.13^{c}	9.13±0.22^{c}	0.18±0.00^{c}	0.61±0.02^{c}	5.53±0.13^{c}	5.67±0.13^{c}
Control	5.60±0.13^{c}	16.80±0.43^{b}	0.18±0.00^{c}	0.91±0.02^{b}	5.47±0.13^{c}	6.73±0.12^{b}

LSD least significant difference

*Values are means±SEM ($n=15$). Data within the same column followed by different letters are significantly different ($P \leq 0.05$)

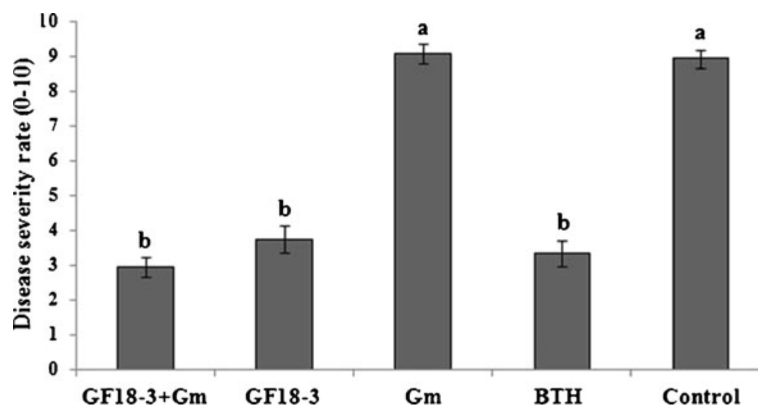

Fig. 3 Disease severity of *Cucumber mosaic virus*-inoculated cucumber plants treated with *Fusarium equiseti* GF18-3 and/or *G. mosseae* (Gm) or BTH compared to non-treated control plants at 14 days post-inoculation. Disease severity was rated on the following scale: 0=no symptoms; 2=vein clearing; 4=50 % of leaves on a plant exhibiting mosaic symptoms, 6=100 % of leaves on a plant exhibiting mosaic symptoms; 8=50 % of leaves showing severe mosaic symptoms and malformation and 10=100 % of leaves showing severe mosaic symptoms and malformation. Columns represent mean values (n=15). Bars indicate standard errors. Different letters indicate significant difference based on Fisher's LSD test at $P \leq 0.05$

(2011) reported an antagonistic effect of Gm toward GF18-3 in cucumber plants at 4 and 6 WAP. Additionally, McAllister et al. (1994) observed an antagonistic interaction between Gm and *T. koningii* in the rhizosphere of maize plants. Furthermore, Chandanie et al. (2005, 2006, 2009) demonstrated that the growth of *T. harzianum* in and/or around cucumber roots was suppressed by the presence of Gm, while the growth of *Penicillium simplicissimum* was unaffected. Soybean plants have been shown to exhibit antagonistic, synergistic and neutral interactions between Gm and associated saprophytic fungi (Fracchia et al. 1998).

AMF have also been widely used as microbial inocula for improving plant growth and/or as biocontrol agents in trials with various plant species. Mycorrhizal colonisation triggers a variety of physiological and biochemical responses in host plants, which include altered root exudation, increased phytohormone production, and production of inhibitory or stimulatory compounds (reviewed by Bi et al. 2007). Altered root exudates can modify the microbial population around mycorrhizal roots. Additionally, the extraradical mycelium of AMF may also have impact on the microbial population around the mycorrhizal roots (Filion et al. 2003).

In addition to the previously described effect of Gm on GF18-3, GF18-3 has been shown to impact the composition of mycorrhizal communities. In the current investigation, the presence of GF18-3 significantly

Table 3 *Cucumber mosaic virus* accumulation in leaves of cucumber plants treated with *Fusarium equiseti* GF18-3 and/or *G. mosseae* (Gm) or BTH relative to non-treated control plants

Treatment	CMV concentration by ELISA		
	7 DPI	14 DPI	21 DPI
GF18-3+Gm	0.27±0.01^{d}	0.39±0.01^{c}	0.54±0.01^{c}
GF18-3	0.36±0.01^{c}	0.44±0.01^{b}	0.53±0.01^{c}
Gm	0.54±0.02^{b}	0.97±0.02^{a}	0.95±0.02^{a}
BTH	0.29±0.01^{d}	0.42±0.01^{bc}	0.51±0.01^{c}
Control	0.87±0.01^{a}	0.98±0.02^{a}	0.85±0.01^{b}

LSD least significant difference

*Values are means±SEM (n=15). Data within the same column followed by different letters are significantly different ($P \leq 0.05$)

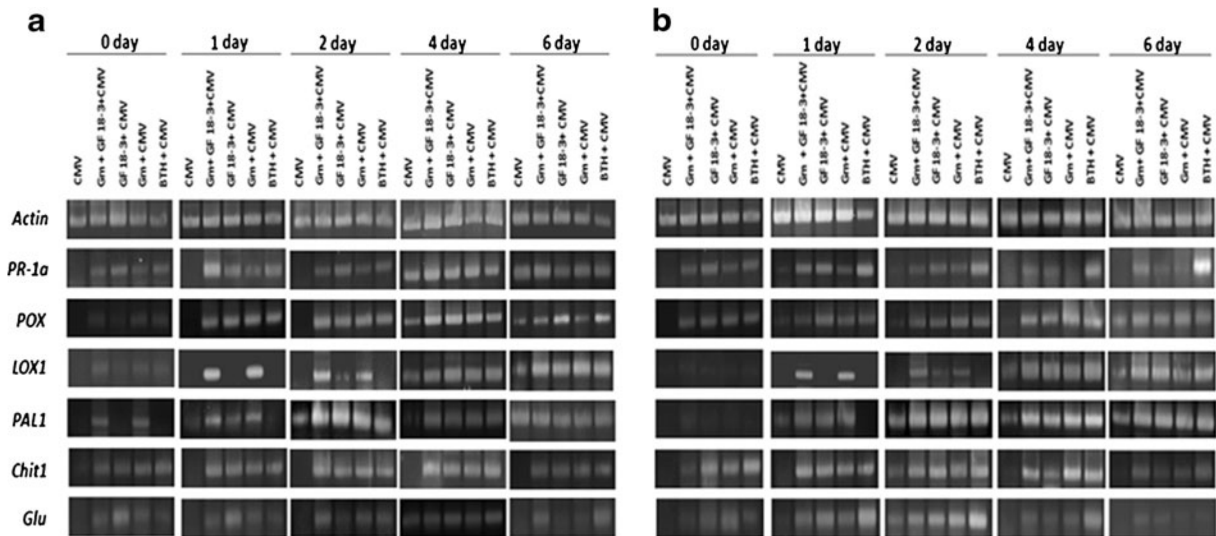

Fig. 4 Expression of defence-related genes in (**a**) leaves and (**b**) roots of cucumber plants treated with *Fusarium equiseti* GF18-3 and/or *G. mosseae* (Gm) or BTH and inoculated with *Cucumber mosaic virus* at the indicated time point. SA-inducible (*PR-1a*, *Peroxidases (POX), Chitinase (Chit1) β-1, 3-glucanase (Glu))* and JA-inducible (*Lipoxygenase (LOX1)* and *Phenylalanine ammonia lyase (PAL1))* marker genes were analysed via RT-PCR using specific primers for each gene. Constitutively expressed *Actin* was used as a control in the RT-PCR analysis

decreased root colonisation by Gm compared to when Gm was inoculated alone. Thus, an antagonistic interaction regarding Gm colonisation was observed between the saprophytic fungus GF18-3 and Gm. Similar interactions have been reported for other saprophytic fungi (McAllister et al. 1996, 1997; Martínez et al. 2004). The presence of fungal exudates, such as volatile substances produced by saprophytic fungi, could explain the reduction of root colonisation by Gm (McAllister et al. 1996). However, this result differs from neutral effects that have been observed in other studies (Camprubí et al. 1995; Vázquez et al. 2000). It also contrasts with the positive effect of *Fusarium oxysporum* on AMF colonisation observed by Fracchia et al. (2000).

In the present study, we examined three growth parameters to analyse growth promotion in cucumber plants: shoot fresh weight, shoot dry weight, and the number of leaves. Co-inoculation with Gm and GF18-3 significantly increased the shoot biomass and the number of leaves at both 4 and 7 WAP relative to the non-treated control plants. This result was also observed for the GF18-3 alone treatment, whereas the shoot biomass and number of leaves in the plants treated with Gm alone increased at 4 WAP, but not at 7 WAP when compared to the control plants. Similarly, Saldajeno and Hyakumachi (2011) reported a significant increase in the shoot dry weight of cucumber plants at 4 WAP, but not at 7 WAP. Additionally, Arpana and Bagyaraj (2007) showed that Gm and *T. harzianum*, both individually and in combination, significantly increased the leaf area, number of leaves, plant height and dry weight of Kalmegh plants (*Andrographi paniculata*). AMF and PGPF are capable of stimulating plant growth through a variety of mechanisms. Avis et al. (2008) classified plant growth-promoting microorganisms into two broad groups based on the primary mechanisms of actions by which they are known: (i) microorganisms that directly affect the promotion of plant growth (such as through phosphorus solubilisation and phytohormone production) and (ii) microorganisms that indirectly promote plant growth and productivity through the biocontrol of plant pathogens (such as via the production of siderophores, antibiotics, and HCN). However, both groups of microorganisms can simultaneously utilise secondary mechanisms. The AMF are obligate symbionts that supply a plant with mineral nutrients, especially phosphorus, and increase water uptake (Selosse et al. 2006). Shivanna et al. (2005) showed that cucumber plants displayed better growth and increased productivity of marketable fruits due to increased levels of soil nutrients made available by PGPF, and these plants accumulated more inorganic

minerals, such as Ca, Mg, and K, in their aerial shoots. Therefore, the effect of PGPF on plant growth may be due to the introduction of more available forms of soil nutrients and minerals to the plant.

Our results showed that prior treatments with the combined inoculation of GF18-3 and Gm or GF18-3 alone or BTH-induced SAR protected cucumber plants against *Cucumber mosaic virus* (CMV) for more than 21 DPI. The protection conferred on the treated plants was manifested in the reduction of disease severity and CMV accumulation in the leaves.

In our study system, the GF18-3 and/or Gm treatments were applied to the roots, while CMV was inoculated in the leaves, and the two treatments remained spatially separated on the plant. Furthermore, this protective effect is not based on direct parasitic or antibiotic activity of GF18-3 and/or Gm. Therefore, the observed disease suppression may operate through activation of induced systemic resistance. As a result of induced systemic resistance, a reduction of disease severity and increased plant growth have been observed following CMV infection in many crops (Ryu et al. 2004; Ryu et al. 2007; Ipper et al. 2008; Wang et al. 2009).

The nature of the interaction between PGPF and Gm depends on the inherent characteristics of the PGPF species tested (Chandanie et al. 2006). In some cases, a single inoculation has been found to result in the greatest improvement of resistance. For example, Bhromsiri (2009) demonstrated that following a single inoculation of PGPR or AMF, either group played a role as the predominant factor. Our data demonstrated that both the interaction between Gm and GF18-3 and treatment with GF18-3 alone were effective for disease suppression, although no effect of Gm alone was observed. This result was in accordance with another study by Chandanie et al. (2006), which showed that Gm had no effect in a combined inoculation with *P. simplicissimum* but exhibited an inhibitory effect against anthracnose disease in a combined inoculation with *Phoma* sp. Interestingly, Saldajeno and Hyakumachi (2011) reported a stimulatory effect of the combined inoculation of Gm and GF18-3 in controlling anthracnose disease.

A weak and transient plant defence response against *Cucumber mosaic virus* was identified in the Gm-alone treatment. Inoculation with Gm alone did not reduce the observed disease severity or viral accumulation when compared to control plants at 14 and 21 DPI. Many studies indicate that mycorrhizal host plants show an increased intensity of viral disease (Nemec and Myhre 1984; Shaul et al. 1999). This effect may be due to the enhanced nucleic acid and protein production in these plants fostering viral multiplication, which leads to an increased spread of a virus throught the whole plant (Dehne 1982), or due to the increased phosphate uptake associated with mycorrhizal plants (Daft and Okusanya 1973).

The current study aimed to elucidate the plant signals involved in the induction of systemic defence responses against CMV by Gm and/or GF18-3. In response to microbial changes in the rhizosphere, plant defence responses were enhanced through modulation of the host's signalling pathways through jasmonic acid (JA)- and salicylic acid (SA)-dependent pathways (Pozo and Azcón-Aguilar 2007).

AMF may enhance the induction of pathogenesis-related (PR) proteins and hydrolytic enzymes. The roles of PR proteins may be readily understood based on their activity as glucanases or chitinases or by their association with other antimicrobial properties. For example, the roots of plants that were inoculated with mycorrhizal fungi showed higher activities of chitinase, β-1,3-glucanase and superoxide dismutase (Kjoller and Rosendahl 1997; Pozo et al. 2002). Our data showed that the expression levels of SA-inducible *PR-1a* quickly increased in the co-inoculation and GF18-3-alone treatments compared to treatment with Gm alone at 1 day after inoculation. Alterations in the SA signalling pathway have been previously described in mycorrhizal plants (Blilou et al. 2000; Shaul et al. 1999).

PGPF isolates have been shown to induce a primed state in plants. For example, disease resistance against *Pseudomonas syringae* pv. *tomato* in *Arabidopsis* is regulated by multiple signal transduction pathways in which salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) were activated using *P. simplicissimum* GP17-2 (Hossain et al. 2007). Additionally, in the presence of *Trichoderma harzianum* and *Rhizoctonia. solani*, Gallou et al. (2009) observed induction of the expression of the *LOX* gene at 24 h post inoculation (hpi) and of the expression of the *GST1, PAL, PR1* and *PR2* genes at 72 hpi in the roots of potato plantlets.

Induction of *peroxidase* (*POX*) activity in plants occurs in response to many biotic and abiotic stimuli, including pathogen attacks and exposure to elicitor preparations, chemical oxidising agents, red light,

and mechanical stimuli (Casal et al. 1994; Donald and Cipollini 1998; Morkunas and Gmerek 2007). In the present study, all of the treatments increased the expression of *peroxidase* (*POX*) in the roots and leaves of cucumber plants following CMV inoculation.

In conclusion, the present study identified a beneficial effect of the co-inoculation of Gm and GF18-3 on growth parameters and CMV accumulation in cucumber. Pre-treatment with the co-inoculation of Gm and GF18-3 or inoculation of GF18-3 alone reduced the CMV disease severity and increased growth parameters; such as the fresh weight, dry weight, and number of leaves. Co-inoculation with Gm does not block the biocontrol potential of GF18-3 against CMV and some synergy can even be seen with regard to the reduction of CMV accumulation within cucumber leaves. All of the applied treatments, which were applied to the roots, were able to affect plant defence mechanisms not only in the roots but also in the shoots, which may contribute to disease resistance.

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References

Al-Karaki GB, McMichael JZ (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269

Arpana J, Bagyaraj DJ (2007) Response of kalmegh to an arbuscular mycorrhizae fungus and a growth promoting rhizomicroorganism at two levels of phosphorus fertilizer. Am-Euras J Agric Environ Sci 2:33–38

Artursson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8:1–10

Avis TJ, Gravel V, Antoun H, Tweddell RJ (2008) Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol Biochem 40:1733–1740

Benhamou N, Nicole M (1999) Cell biology of plant immunization against microbial infection: The potential of induced resistance in controlling plant diseases. Plant Physiol Biochem 37:703–719

Bhromsiri C (2009) Use of soil microbial inoculation for improving the effectiveness of vetiver grass and the effect on natural soil microbial ecology. Dissertation, Chiang Mai University

Bi HH, Song YY, Zeng RS (2007) Biochemical and molecular responses of host plants to mycorrhizal infection and their roles in plant defence. Allelopathy J 20:15–28

Blilou I, Ocampo JA, Garcia-Garrido JM (2000) Induction of *Ltp* (Lipid transfer protein) and *Pal* (Phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*. J Exp Bot 51:1969–1977

Brundrett MC, Piché Y, Peterson RL (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can J Bot 62:2128–2134

Calvet C, Pera J, Barea JM (1993) Growth response of marigold (*Tagetes erecta* L.) to inoculation with *Glomus mosseae*, *Trichoderma aureoviride* and *Pythium ultimum* in a peat–perlite mixture. Plant Soil 148:1–6

Camprubí A, Calvet C, Estaún V (1995) Growth enhancement of *Citrus reshni* after inoculation with *Glomus intraradices* and *Trichoderma aureoviride* and associated effects on microbial population and enzyme activity in potting mixes. Plant Soil 173:233–238

Casal JJ, Mella RA, Ballare CL, Maldonado S (1994) Phytochrome-mediated effects on extracellular peroxidase activity, lignin content and bending resistance in etiolated *Vicia faba* epicotyls. Physiol Plant 92:555–562

Chandanie WA, Kubota M, Hyakumachi M (2005) Interaction between arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth promoting fungus *Phoma* sp. on their root colonization and growth promotion of cucumber. Mycoscience 46:201–204

Chandanie WA, Kubota M, Hyakumachi M (2006) Interactions between plant growth promoting fungi and arbuscular mycorrhizal fungus *Glomus mosseae* and induction of systemic resistance to anthracnose disease in cucumber. Plant Soil 286:209–217

Chandanie WA, Kubota M, Hyakumachi M (2009) Interactions between the arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). Appl Soil Ecol 41:336–341

Compant S, Duffy B, Nowak J, Clement C, Ait Barka E (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Cools HJ, Ishii H (2002) Pre-treatment of cucumber plants with acibenzolar-S-methyl systemically primes a *phenylalanine ammonia lyase* gene (*PAL*) for enhanced expression upon attack with a pathogenic fungus. Physiol Mol Plant Pathol 61:273–280

Daft MJ, Okusanya BO (1973) Effect of Endogone mycorrhiza on plant growth. V1. Influence of infection on the anatomy and reproductive development in four hosts. New Phytol 72:1333–1339

Dalisay RF, Kuc JA (1995a) Persistence of induced resistance and enhanced peroxidase and chitinase activities in cucumber plants. Physiol Mol Plant Pathol 47:315–327

Dalisay RF, Kuc JA (1995b) Persistence of reduced penetration by *Colletotrichum lagenarium* into cucumber leaves with induced systemic resistance and its relation to enhanced peroxidase and chitinase activities. Physiol Mol Plant Pathol 47:329–338

DebRoy S, Thilmony R, Kwack YB, Nomura K, He SY (2004) A family of conserved bacterial effectors inhibits salicylic

acid-mediated basal immunity and promotes disease necrosis in plants. Proc Natl Acad Sci USA 101:9927–9932

Dehne HW (1982) Interaction between vesicular mycorrhizal fungi and plant pathogens. Phytopathology 72:1115–1119

Donald F, Cipollini JR (1998) The induction of soluble peroxidase activity in bean leaves by wind induced mechanical perturbation. Am J Bot 85:1586–1591

Duponnois R, Colombet A, Hien V, Thioulouse J (2005) The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. Soil Biol Biochem 37:1460–1468

Filion M, St-Arnaud M, Jabaji-Hare SH (2003) Quantification of *Fusarium solani* f. sp. *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using real-time polymerase chain reaction and direct isolation on selective media. Phytopathology 93:229–235

Fracchia S, Mujica MT, García-Romera I, García-Garrido JM, Martin J, Ocampo JA (1998) Interactions between *Glomus mosseae* and arbuscular mycorrhizal sporocarp-associated saprophytic fungi. Plant Soil 200:131–137

Fracchia S, Garcia-Romera I, Godeas A, Ocampo JA (2000) Effect of the saprophytic fungus *Fusarium oxysporum* on arbuscular mycorrhizal colonization and growth of plants in greenhouse and field trials. Plant Soil 223:175–184

Gallou A, Cranenbrouck S, Declerck S (2009) *Trichoderma harzianum* elicits defence response genes in roots of potato plantlets challenged by *Rhizoctonia solani*. Eur J Plant Pathol 124:219–230

Garcia-Romera I, Garcia-Garrido JM, Martin J, Fracchia S, Mujica MT, Godeas A, Ocampo JA (1998) Interactions between saprotrophic Fusarium strains and arbuscular mycorrhizas of soybean plants. Symbiosis 24:235–246

Gerhardson B (2002) Biological substitutes for pesticides. Trends Biotechnol 20:338–343

Görlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel KH, Oostendorp M, Staub T, Ward E, Kessmann H, Ryals J (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activated gene expression and disease resistance in wheat. Plant Cell 8:629–643

Green H, Larsen J, Olsson PA, Jensen DF, Jakobsen I (1999) Suppression of the biocontrol agent *Trichoderma harzianum* by mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* in root-free soil. Appl Environ Microbiol 65:1428–1434

Hammerschmidt R (1999) Phytoalexins: what have we learned after 60 years? Annu Rev Phytopathol 37:285–306

Horinouchi H, Muslim A, Suzuki T, Hyakumachi M (2007) *Fusarium equiseti* GF19-1 as an effective biocontrol agent against Fusarium crown and root rot of tomato in rock wool systems. Crop Prot 26:1514–1523

Hossain MM, Sultana F, Kubota M, Koyama H, Hyakumachi M (2007) The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in *Arabidopsis thaliana* by activation of multiple defence signals. Plant Cell Physiol 48:1724–1736

Hyakumachi M (1994) Plant-growth-promoting fungi from turf-grass rhizosphere with potential for disease suppression. Soil Microorganisms 44:53–68

Ipper NS, Lee SH, Suk JK, Shrestha A, Seo DU, Park DH, Cho JM, Park DS, Hur JH, Lim CK (2008) Antiviral activity of the expolysaccharaide produced by *Serratia* sp. strain Gsm01 against *Cucumber mosaic virus*. J Microbiol Biotechnol 18:67–73

Jeffries P, Barea JM (2001) Arbuscular mycorrhiza – a key component of sustainable plant–soil ecosystems. In: Hock B. Springer (ed) Fungal Associations, Vol. IX of The Mycota, Berlin, pp 95–113

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fert Soils 37:1–16

Kjoller R, Rosendahl S (1997) Polyacrylamide gel electrophoresis (PAGE) and densiometric measurement of enzyme activity of the pea root pathogen *Aphanomyces euteiches* in pea roots. J Phytopathol 145:253–256

Lawton KA, Friedrich L, Hunt M, Weymann K, Delaney T, Kessmann H, Staub T, Ryals J (1996) Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. Plant J 10:71–82

Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant J 50:529–544

Maciá-Vicente JG, Jansson HB, Talbot NJ, Lopez-Llorca LV (2009) Real-time PCR quantification and live-cell imaging of endophytic colonization of barley (*Hordeum vulgare*) roots by *Fusarium equiseti* and *Pochonia chlamydosporia*. New Phytol 182:213–228

Martínez A, Obertello M, Pardo A, Ocampo JA, Godeas A (2004) Interactions between *Trichoderma pseudokoningii* strains and the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea*. Mycorrhiza 14:79–84

McAllister CB, García-Romera I, Godeas A, Ocampo JA (1994) Interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*: effects on plant growth, arbuscular mycorrhizas and the saprophyte inoculants. Soil Biol Biochem 26:1363–1367

McAllister CB, García-Garrido JM, García-Romera I, Godeas A, Ocampo JA (1996) In vitro interactions between *Alternaria alternata*, *Fusarium equiseti* and *Glomus mosseae*. Symbiosis 20:163–174

McAllister CB, Garcia-Garrido JM, García-Romera I, Godeas A, Ocampo JA (1997) Interaction between *Alternaria alternata* or *Fusarium equiseti* and *Glomus mosseae* and its effects on plant growth. Biol Fertil Soils 24:301–305

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115:495–501

Meddad-Hamza A, Beddiar A, Gollotte A, Lemoine MC, Kuszala C, Gianinazzi S (2010) Arbuscular mycorrhizal fungi improve the growth of olive trees and their resistance to transplantation stress. Afr J Biotechnol 9:1159–1167

Morkunas I, Gmerek J (2007) The possible involvement of peroxidase in defence of yellow lupine embryo axes against *Fusarium oxysporum*. J Plant Physiol 164:185–94

Nemec S, Myhre D (1984) Virus-glomus etunicatum interactions in citrus root stocks. Plant Dis 68:311–314

Postma J, Montanari V, Van den Boogert PHJF (2003) Microbial enrichment to enhance the disease suppressive activity of compost. Eur J Soil Biol 39:157–163

Pozo MJ, Azcón-Aguilar C (2007) Unravelling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398

Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcón-Aguilar C (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to Phytophthora infection in tomato plants. J Exp Bot 53:525–534

Punja ZK, Wan A, Rahman M (2008) Growth, population dynamics, and diversity of *Fusarium equiseti* in ginseng fields. Eur J Plant Pathol 121:173–184

Roossinck MJ (1999) Cucumoviruses (Bromoviridae)—general features. In: Granoof L, Webster RG (eds) Cucumoviruses (Bromoviridae)—general features. Encyclopedia of Virology, 2nd edn. Academic, San Diego, pp 315–320

Ryu C-M, Murphy JF, Mysore KS, Kloepper JW (2004) Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and NPR1- independent and jasmonic acid-dependent signaling pathway. Plant J 39:381–392

Ryu C-M, Kang BR, Han SH, Cho SM, Kloepper JW, Anderson AJ, Kim YC (2007) Tobacco cultivars vary in induction of systemic resistance against *Cucumber mosaic virus* and growth promotion by *Pseudomonas chlororaphis* O6 and its gacS mutant. Eur J Plant Pathol 119:383–390

Saldajeno MGB, Hyakumachi M (2011) The plant growth-promoting fungus *Fusarium equiseti* and the arbuscular mycorrhizal fungus *Glomus mosseae* stimulate plant growth and reduce severity of anthracnose and damping-off diseases in cucumber (*Cucumis sativus*) seedlings. Ann Appl Biol 159:28–40

Selosse MA, Baudoin E, Vandenkoornhuyse P (2004) Symbiotic microorganisms, a key for ecological success and protection of plants. C R Biol 327:639–648

Selosse MA, Richard F, He X, Simard SW (2006) Mycorrhizal networks: des liaisons dangereuses? Trends Ecol Evol 21:621–628

Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, Kapulnik Y (1999) Mycorrhiza-Induced Changes in Disease Severity and PR Protein Expression in Tobacco Leaves. Mol Plant-Microbe Interact 12:1000–1007

Shivanna MB, Meera MS, Kubota M, Hyakumachi M (2005) Promotion of growth and yield in cucumber by zoysiagrass rhizosphere fungi. Microb Environ 20:34–40

Shoresh M, Yedidia I, Chet I (2005) Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. Phytopathology 95:76–84

Smith SE, Read DJ (1997) Mycorrhizal Symbiosis. Academic, San Diego

Sticher L, Mauch-Mani B, Métraux JP (1997) Systemic acquired resistance. Ann Rev Phytopathol 35:235–270

Suzuki Y, Kawazu T, Koyama H (2004) RNA isolation from siliques, dry seeds and other tissues of *Arabidopsis thaliana*. Biotechniques 37:542–544

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis related proteins. Eur J Plant Pathol 103:753–765

Van Loon LC, Bakker RPAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Ann Rev Phytopathol 36:453–483

Vázquez MM, César S, Azcón R, Barea JM (2000) Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rizosphere of maize plants. Appl Soil Ecol 15:261–272

Wang S, Wu H, Qiao J, Ma L, Liu J, Xia Y, Gao X (2009) Molecular mechanism of plant growth promotion and induced systemic resistance to *Tobacco mosaic virus* by *Bacillus* spp. J Microbiol Biotechnol 19:1250–1258

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Zehnder GW, Yao C, Murphy JF, Sikora ER, Kloepper JW (2000) Induction of resistance in tomato against *Cucumber mosaic cucumovirus* by plant growth promoting rhizobacteria. BioControl 45:127–137