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ORIGINAL ARTICLE

Molecular defense response of mycorrhizal bean plants infected with *Rhizoctonia solani*

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Abstract A time course study was conducted to investigate disease development and molecular defense response in common bean (*Phaseolus vulgaris* L.) plants colonized by a mixture of five arbuscular mycorrhizal (AM) fungi, namely, *Glomus mosseae*, *G. intraradices*, *G. clarum*, *Gigaspora gigantea*, and *Gigaspora margarita*, and post-infected with the soil-borne pathogen *Rhizoctonia solani*. Results showed that pre-colonization of bean plants by AM fungi significantly reduced disease severity and disease incidence. DNA fingerprinting using the differential display technique revealed a genetic polymorphism (86.8 %) in bean plants that resulted from the colonization by AM fungi. Two genetic mechanisms were recorded: (1) switching on of new genes and (2) induction of other active genes, including the defense genes chitinase and β-1,3-glucanase, to a highly expressed state.

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Introduction

Rhizoctonia solani Kühn is a common necrotrophic soil-borne fungus which can cause seed decay, damping-off, stem canker, root rot, fruit decay, and foliage diseases in a wide range of plant species over a large part of the world (Tu et al. 1996). This unlimited host range, combined with competitive saprophytic ability and lethal pathogenic potential, earns *R. solani* its status as formidable pathogen.

During recent years research has focused on identifying potential biocontrol agents to reduce the severity of root-rot disease caused by *R. solani*. A limited number of fungal antagonists against *R. solani* have been reported, among which antagonistic and plant growth-promoting yeasts (El-Tarabily 2004), *Penicillium* spp. (Ciavatta et al. 2006), *Trichoderma koningii*, *T. pseudokoningii*, *T. viride*, *T. polysporum*, *T. aureoviride* (Shalini and Kotasthane 2007), and *Gliocladium roseum* (Tarantino et al. 2007) are known to be very efficient. Bacterial antagonism has also been reported against *R. solani* (Kai et al. 2007). Among the potential biocontrol agents, the arbuscular mycorrhizal (AM) fungi have received special attention (Aly and Manal 2009; Abdel-Fattah et al. 2011). Biological control using AM fungi is unique, being an eco-friendly and cost-effective strategy for disease management that provides greater levels of protection and sustains plant yields, in addition to the positive effects on the plant growth and its nutrition.

Common bean (*Phaseolus vulgaris* L.) is susceptible to *Rhizoctonia* root-rot disease in most of the tropical, subtropical, and temperate areas of the world where it is grown. Yield losses of 5–10 % are common, but 60 % yield losses have been reported in Brazil (Tu et al. 1996).

Mechanisms that can account for the disease control ability of AM fungi may include competition for infection sites and host photosynthates, root damage compensation, enhancement of plant resistance through various physical and physiological mechanisms, such as increasing the cell-wall thickness of the host or the accumulation of some antimicrobial substances (Abdel-Fattah et al. 2011), or changes in the composition of the microbial communities in the mycorrhizosphere (Singh et al. 2000). Several inducible defense-related genes, including those encoding isoflavonoid phytoalexins, such as phenylalanine ammonia lyase, chalcone synthase, and chalcone isomerase, have been reported to be induced during mycorrhizal establishment (Guillon et al. 2002). The expression of genes encoding enzymes that synthesize phenolpropanoid compounds has also been detected in mycorrhizal roots (Garcia-Garrido and Ocampo 2002). Other defense-related genes shown to be up-regulated in mycorrhizal symbioses include genes involved in the metabolism of reactive oxygen species, chitinase, and β-1,3-glucanase, and genes involved in senescence, including glutathione-S-transferase (Zeng 2006).

The role of AM colonization in sustaining bean plants against *R. solani* is unclear. Depending on the time after infection with *R. solani* and the tissue examined, responses varying from stimulation to suppression to no change in transcript levels have been detected (Guillon et al. 2002). Therefore, the aim of our study was to investigate the molecular aspects of defense responses in mycorrhizal bean plants infected with *R. solani*.

Materials and methods

Causal organism and bean cultivar

Rhizoctonia solani (AG-2-2 IIIB) was isolated originally from naturally diseased common bean plants. Fungal identification was based on cultural properties and morphological and microscopical characteristics, as described by Sneh et al. (1991). The isolate was then assigned an AG designation according to hyphal anastomosis with tester isolates from AG 1 to AG 10 using the slide technique of Kronland and Stanghellini (1988). The inoculum was prepared by growing the pathogen in bottles containing sterilized sorghum grains for 15 days at 25±2 °C. The most common bean cultivar in Egypt (Giza 3) was used.

AM inoculum

A mixture of Egyptian formulated AM fungi (Multi-VAM) kindly provided by Dr. Safwat El-Haddad (Mycological Research and Disease Survey Department, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt) was used. This mixture consists of spores (in equal proportions) of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. intraradices* Schenck & Smith, *G. clarum* Nicol. & Schenck, *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe, and *Gigaspora margarita* (Becker & Hall) in suspension at a concentration of 1×10^6 spores L^{-1} (El-Haddad et al. 2004).

Planting and growth conditions

Pots (diameter 25 cm) were each filled with 2.5 kg sterilized soil (autoclaving at 121 °C for 2× 1 h). A sandy-loam soil (sand:silt:clay, 70:20:10 %) was used; soil characteristics were pH 8, electrical conductivity 270 μS cm^{-1}, total nitrogen 1.05 %, total organic matter 1.64 %, and phosphorus content 4.43 μg g^{-1}. Five healthy seeds of common bean *Phaseolus vulgaris* L. (cv. Giza 3) were surface-disinfected with 2 % sodium hypochlorite for 2 min, washed with sterile distilled water, and sown in each pot. Half of the pots received AM inoculum as a suspension at two time points: in the bean seed bed at the beginning of the experiment and as a soil drench 14 days after sowing) at a concentration of 5 mL L^{-1} water (El-Haddad et al. 2004). Plants were inoculated with *Rhizobium leguminosarum* one time with 1 mL of culture suspension at a concentration of 5×10^7 CFU mL^{-1} and watered regularly to near field capacity with tap water. Pots did not receive any fertilizers in this study. All pots were kept outdoors under natural conditions [day temperature 25 °C, night temperature 20 °C, 16/8-h (light/dark) photoperiod].

At 4 weeks after inoculation with AM, the pathogen inoculum was mixed with the upper layer of the pot soil at a rate of 2 % (w/w) potential inoculum. Five pots were treated with tap water to serve as a negative control. Five pots were used as replicates for each treatment. The treatments applied in this study can be summarized as follows: untreated control (no mycorrhiza, no pathogen; CNM); mycorrhiza only (CM); pathogen only (no mycorrhiza; PNM); mycorrhiza + pathogen (PM). All pots were arranged in a completely randomized design. Three plants from each treatment were harvested at 1, 3, 7, and 28 days after pathogen infection for molecular analysis (Guillon et al. 2002; Mohr et al. 1998). Five plants of each treatment were carefully harvested with their entire roots (2 weeks after inoculation with the pathogen), washed under running water to remove soil particles, and evaluated for shoot length, root length, leaf area, and shoot and root dry weights. Dry weights (in grams) were recorded after the samples had been dried at 80 °C for at least 48 h in a hot air oven until a constant weight was reached. Disease severity (DS) and disease incidence (DI) of the *Rhizoctonia* root rot were assessed (2 weeks after inoculation with the pathogen) for each treatment. Disease severity was estimated as the degree of root damage according to the scale of Carling et al. (1999).

Staining and estimation of mycorrhizal root colonization

Five plants of each treatment were carefully harvested with their entire roots and washed under running water to remove soil particles. The roots were separated and fixed in FAA (formalin–acetic acid–alcohol) for evaluation of mycorrhizal root colonization. Roots fixed in FAA were rinsed repeatedly in tap water, cut into small segments (0.5–1 cm), and stained with 0.05 % trypan blue (Sigma, St. Louis, MO) according to Phillips and Hayman (1970). Fifty randomly selected stained root segments of each treatment were mounted on slides in lactoglycerol and examined microscopically for estimation of mycorrhizal root colonization according to Trouvelot et al. (1986).

DNA fingerprinting using the differential display technique

Total RNA was extracted from treated common bean roots using a GStract^{TM} RNA Isolation kit II (Maxim Biomedical, Rockville, MD). About 0.5 g of root sample was subjected to RNA extraction, and the extracted RNA was dissolved in DEPC-treated water, quantified spectrophotometrically, and analyzed on 1.2 % agarose gel.

Reverse-transcription (RT)-PCR analyses were performed in a total reaction volume of 25 μL. The reaction mixture contained 2.5 μL of 5× buffer with $MgCl_2$, 2.5 μL (2.5 mM) dNTPs, 1 μL (10 pmol) oligo dT primer (Promega, Madison, WI), 2.5 μL RNA, and 0.2 μLM-MuLV reverse transcriptase (New England Biolabs, Ipswich, MA). RT-PCR amplification was performed in a thermal cycler (Eppendorf, Thermo Fisher Scientific, Dublin, Ireland) programmed at 95 °C for 5 min, 42 °C for 1 h, and 72 °C for 10 min; the cDNA was then stored at −20 °C (Chen et al. 2005).

The differential display PCR reaction was carried out in a total reaction volume of 25 μL containing 2.5 μL 5× Colorless GoTaq® Flexi Buffer, 2.5 μL 5× Green GoTaq® Flexi Buffer, 2.5 μL $MgCl_2$, 2.5 mM dNTPs, 5 μL (10 pmol) primer, 1.5 μL cDNA, and 0.2 μL (5 U μL^{-1}) GoTaq® Flexi DNA Polymerase (Promega). PCR amplification was performed in a thermal cycler (Eppendorf, Thermo Fisher Scientific) programmed for one cycle at 95 °C for 5 min, 40 cycles of 1 min at 95 °C, 1 min at 30 °C, and 1 min at 72 °C, followed by one cycle at 72 °C for 10 min. The following primers were used: A1A13 (5′-CAGGCCCTTCCAGCACCCAC-3′), Chi15 (5′-GGYGGYTGGAATGARGG-3′), F1 (5′-CCSCSCCGGATCAAYAAGTWYTAYATC-3′), and P2 (5′-CGCTGTCGCC-3′). Loading dye (2 μL) was added prior to loading of 10 μL per gel slot. Electrophoresis was performed at 80 V with 0.5× TBE as running buffer in 1.5 % agarose/0.5× TBE gels. To visualize the electrophoresis products, we stained the gel in 0.5 μg mL^{-1} (w/v) ethidium bromide solution and then destained it in deionized water. The gel was visualized and photographed using a gel documentation system.

DNA fingerprints obtained using the differential display technique were first analyzed visually and a positive response (a score ‘1’) was defined as the presence of a visible band of a given size, while a negative response (a score ‘0’) was defined as the absence of any band of the same size. These scores were then merged in a Microsoft Excel spreadsheet and then inserted in the SPSS ver. 13.0 computer software (SPSS 2004) for the construction of the dendrogram using the genetic distance method.

Gene expression of two defense-related genes: chitinase and β-1,3-endoglucanase

The expression of two defensin genes [chitinase (Chi) and endoglucanase (EGase)] was examined using a gene-specific RT-PCR (Bishop et al. 2005):

Chitinase: primer CHI15 (5′-GGYGGYTGGAATG ATGG-3′) and anti-sense primer CHI25 (5′-GAYTT AGATTGGGAATAYCC-3′); the amplified fragment is 560 bp.

β-1,3-Endoglucanase: primer EGase forward (5′-TCCGGGGTATGTTATGGAAGA-3′) and EGase reverse (5′-GCCATCCACTCTCAGACACA-3′); the amplified fragment is 681 bp.

The PCR for each gene was performed in a total reaction volume of 25 μL containing 2.5 μL 5× Colorless GoTaq® Flexi Buffer, 2.5 μL 5× Green GoTaq® Flexi Buffer, with 2.5 μL $MgCl_2$, 3 μL dNTPs, 2 μL (10 pmol) primer (forward), 2 μL (10 pmol) primer (reverse), 1.5 μL cDNA, and 0.2 μL (5 U μL^{-1}) GoTaq® Flexi DNA Polymerase (Promega). PCR amplification was performed in a thermal cycler (Eppendorf, Thermo Fisher Scientific) programmed for one cycle at 95 °C for 5 min, followed by 34 cycles of 1 min at 95 °C, 1 min at 41 °C for Chi and at 60 °C for Egase, and 1 min at 72 °C. The reaction was then incubated at 72 °C for 10 min for a final extension. The gel was visualized and photographed using a gel documentation system, and the gene expression was analyzed using PCR Analyzer ver. 1.0 software (Smith et al. 2007). The reaction was repeated many times, and the concentration was calculated as the mean of the replicates.

Statistical analysis

Data were analyzed with the statistical analysis system (CoStat 2005). All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using Duncan’s multiple range test (Duncan 1955).

Results

Effect of mycorrhizal colonization on plant growth and disease incidence

The growth parameters of mycorrhizal plants infected with *R. solani* were significantly enhanced when compared to those of the non-mycorrhizal plants infected with *R. solani* (Table 1). However, AM colonization of pathogen-free plants significantly increased shoot and root length, shoot and root dry weight, and leaf area when compared with the non-mycorrhizal control (CNM) (Table 1).

Disease assessment

Mycorrhizal plants infected with *R. solani* (PM) showed a significant decrease in both disease severity and disease incidence when compared with the non-mycorrhizal plants (PNM) (Table 2).

Mycorrhizal root colonization

The level of mycorrhizal root colonization continued to increase with increasing plant age in all treatments (Table 3). At the same time, the level of mycorrhizal root colonization (frequency and intensity of root colonization and frequency of arbuscules) in the PM treatment at 1 or 4 weeks after inoculation with the pathogen was significantly lower than that of the mycorrhyzal control (CM). No mycorrhizal colonization was observed in the CNM and PNM treatments. Mycorrhizal colonization in the roots of bean plants is shown in Fig. 1.

DNA fingerprinting using the differential display technique

Total extracted RNA of 16 treated common bean plants was subjected to analysis using the differential display technique with four different arbitrary primers (Chi15, A1A13, F1, P2). About 38 bands were obtained using these four different arbitrary primers (Table 4), of which 33 bands showed polymorphism (86.8 %) and the other five bands were monomorphic. The molecular weights of the 38 bands ranged from 100 to 900 bp. Primers Chi15 and A1A13 were the most informative, allowing 100–91.7 % variation between the examined samples, respectively. With primers F1 and P2, the percentages of the genetic variation obtained were 88.9 and 62.5 %, respectively.

Most of bands obtained in the treated samples were novel bands compared with those of the control sample; this was especially evident with primers (Chi15 and A1A13) (Fig. 2). For primers F1 and P2, the most conspicuous findings was that of the up-regulated bands (high expression of these bands) in all treated samples compared with the control sample (Fig. 2).

Cluster analysis of the data

The cluster analysis was carried out using the SPSS program. The dendrogram illustrated in Fig. 3 shows that the data analysis based on the DNA band pattern separated the examined samples into two main groups, with each group containing two subgroups and eight samples. In the first group, subgroup I contained CM 28, PM 28, and PM 7, while subgroup II contained CNM 7, CNM 28, PNM 28, CNM 1, and CNM 3. In the second group, subgroup I contained PM 1, CM 7, and CM 1, while subgroup II contained PNM 1, PM 3, PNM 3, PNM 7, and CM 3.

Based on this analysis, in subgroup I of the first group, samples CM 28 and PM 28 were very closely related to each other, while the third sample (PM 7) was not closely related to the other two samples. Subgroup II of the first group was divided into two sub-subgroups. The first contained three samples (CNM 7, CNM 28, PNM 28) and the other contained two samples (CNM 1 and CNM 3). In the first sub-subgroup, the same observation was obtained as in subgroup I; samples CNM 7 and CNM 28 were very closely related to each other, while sample PNM 28 was poorly related to these two. The other sub-subgroup showed more

Table 1 Effect of mycorrhizal colonization on growth parameters of common bean (*Phaseolus vulgaris* L.) plants infected with *Rhizoctonia* root-rot disease

Treatment[a]	Shoot length (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	Leaf area (cm^2)
CNM	32.9 b	0.68 b	21.8 b	0.32 b	46.4 b
CM	37.8 a	0.85 a	29.8 a	0.42 a	63.0 a
PNM	26.5 d	0.41 c	18.1 c	0.23 d	34.9 d
PM	31.6 bc	0.58 b	21.3 b	0.29 c	43.2 bc

Data are presented as the mean of five replicates. Values in each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p=0.05$)

[a] CNM, Untreated control (not mycorrhiza, no pathogen); CM, mycorrhiza only; PNM, pathogen only (no mycorrhiza); PM, mycorrhiza + pathogen

Table 2 Effect of mycorrhizal colonization on disease incidence and disease severity of common bean *Rhizoctonia* root-rot disease

Treatment	Disease incidence (%)	Disease severity (%)[a]
CNM	0 c	0 c
CM	0 c	0 c
PNM	100 a	84.5 a
PM	73.9 b	67.4 b

Data are presented as the mean of five replicates. Values in each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05)

[a] Disease severity was estimated according to Carling et al. (1999)

difference, where this sub-subgroup contained only two samples which were poorly related to each other. In the second group, subgroup I showed the same behavior as subgroup I of the first group, where samples PM 1 and CM 7 were closely related to each other more than the third one (CM 1). Finally, subgroup II of the second group was divided into two sub-subgroups. One of these contained two samples (PNM 1 and PM 3), which were moderately related to each other. The other sub-subgroup included three samples (PNM 3, PNM 7, CM 3); samples PNM 3 and PNM 7 were very closely related to each other, while sample CM 3 was moderately related to the other two samples.

Gene expression of two defense-related genes

Chi gene

Expression of the Chi gene in common bean plants was presented in Fig. 4a. Band intensity for the different expression of the induced gene (1, 3, 7, and 28 days post-inoculation) was converted into DNA (gene) concentration (ng μL^{-1}) using two-dimensional gel documentation software (Fig. 4b). The results obtained on the first day of inoculation showed an increase in Chi gene expression with all treatments (PM, PNM and CM) versus the untreated control (CNM). The highest gene expression was recorded in the PM treatment (450 ng μL^{-1}). However, gene expression in the PNM treatment was more than that in the CM treatment when compared with the untreated control. In increase in gene expression was lower on post-inoculation day 3 than on day 1, but the variation in gene expression between the treatments remained the same as on the first. On post-inoculation day 7 gene expression of all treatments decreased compared with that on post-inoculation day 3, but the same variation pattern remained. The decrease in gene expression continued up to post-inoculation day 28 with the same variation pattern.

Table 3 Effect of infection with *Rhizoctonia* root-rot disease on the levels of mycorrhizal colonization in common bean

Treatment	Weeks after inoculation with *R. solani*	F (%)[a]	M (%)[b]	A (%)[c]
CNM	1	0 d	0 e	0 e
CM		92.5 b	43.7 b	15.0 bc
PNM		0 d	0 e	0 e
PM		73.8 c	12.4 d	3.3 d
CNM	4	0 d	0 e	0 e
CM		100.0 a	71.5 a	59.3 a
PNM		0 d	0 e	0 e
PM		93.3 b	34.8 c	15.9 bc

Data are presented as the mean of three replicates. Values in each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05)

[a] F (%), Frequency of root colonization,

[b] M(%), Intensity of cortical colonization

[c] A (%), Frequency of arbuscules

EGase gene

Expression of the EGase gene in common bean plants is shown in Fig. 5a. The band intensity for the differential expression of the induced gene was converted into digital DNA (gene) concentration (ng μL^{-1}) and is illustrated in Fig. 5b. The results on the first day showed higher expression levels in the pathogen-incorporated treatments (PNM and PM) than in the pathogen-free ones (CNM and CM). However, the gene expression in the treatment (PM) was higher than that in PNM) (400 vs. 200 ng μL^{-1}, respectively). On post-inoculation day 3, gene expression increased in treatments PM and PNM (420 and 250 ng μL^{-1}, respectively), while no variation was recorded in the CNM and CM treatments when compared with the first day. Gene expression decreased for all treatments on post-inoculation day 7 in comparison with the third day, but the same variation pattern remained. On post-inoculation day 28, gene expression continued to decrease compared with that on the seventh day, with gene expression remaining the highest in the PM treatment.

Discussion

Results of DNA fingerprinting using the differential display technique demonstrated a markedly genetic polymorphism (86.8 %) in bean plants as a result of AM colonization. Most of the bands obtained in the mycorrhizal samples were novel bands compared with the control sample, especially those obtained with primers Chi15 and A1A13. In contrast, with primers F1 and P2, the most conspicuous observation was that of up-regulated bands (high expression of these bands) in all treated samples compared with the control sample. The results obtained here reveal that the colonized cell made

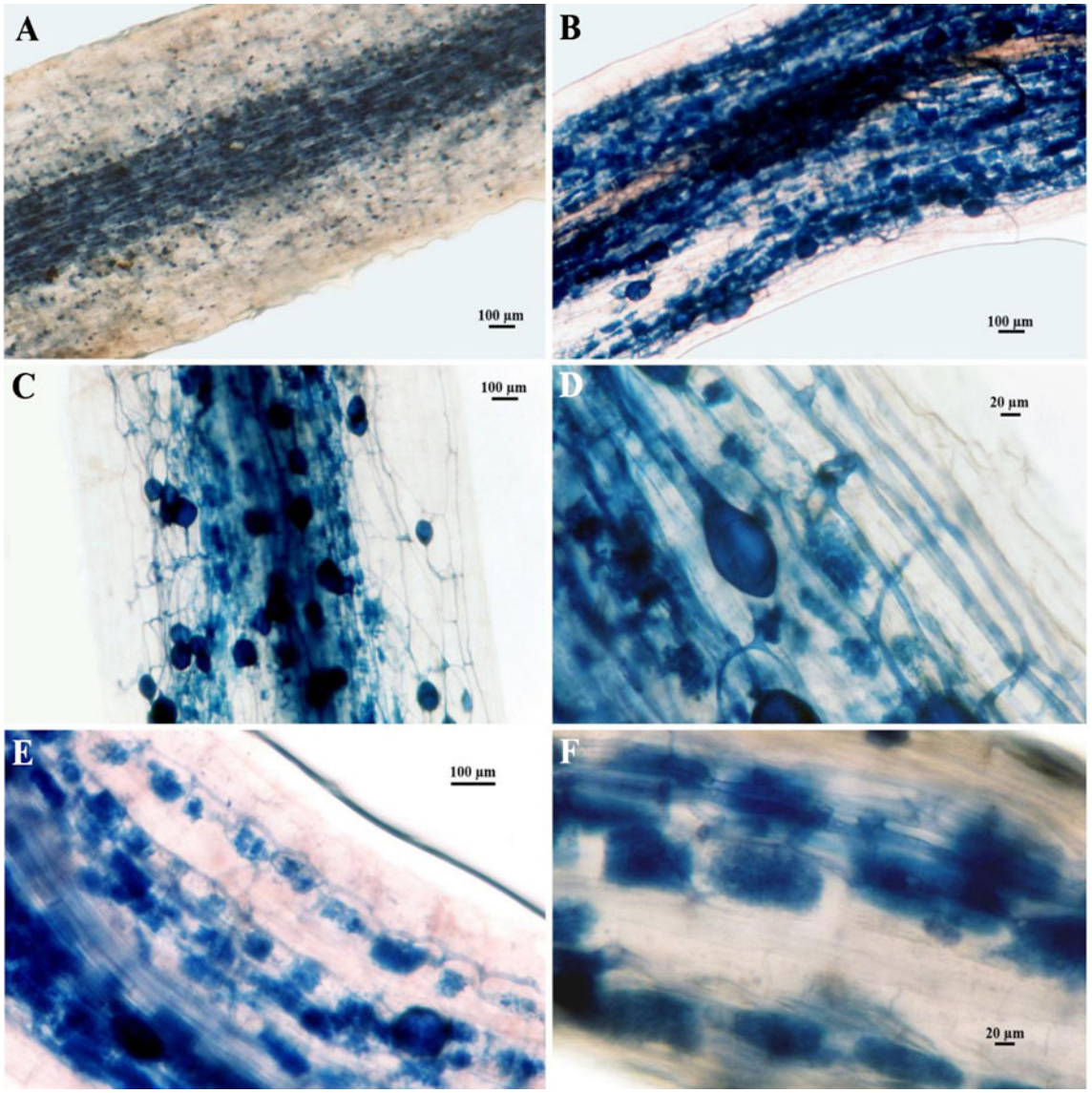

Fig. 1 Arbuscular mycorrhizal (AM) colonization in the roots of common bean (*Phaseolus vulgaris* L.) plants. **a** Non-mycorrhizal root, **b** heavily colonized root, **c** low magnification image of colonized root showing many vesicles, **d** high magnification image of colonized root showing a vesicle, **e** low magnification image of colonized root showing many arbuscules, **f** high magnification image of colonized root showing arbuscules

use of two mechanisms: (1) switching on of new genes; (2) induction of other active genes to a higher expression level. This genetic variation was also supported by the cluster analysis of the data.

Table 4 Polymorphism among the bands obtained using the differential display technique

No.	Primer	Sequence (5′–3′)	length (bp)	Total no. of bands	No. of polymorphic bands	Polymorphism (%)
1	Chi15	GGYGGYTGGAATG-ARGG	100–500	9	9	100
2	A1A13	CAGGCCCTTCCAG-CACCCAC	100–900	12	11	91.7
3	F1	CCSCSCCGGATCAA-YAAGTWYTAYATC	200–700	9	8	88.9
4	P2	CGCTGTCGCC	300–700	8	5	62.5
Total				38	33	86.8

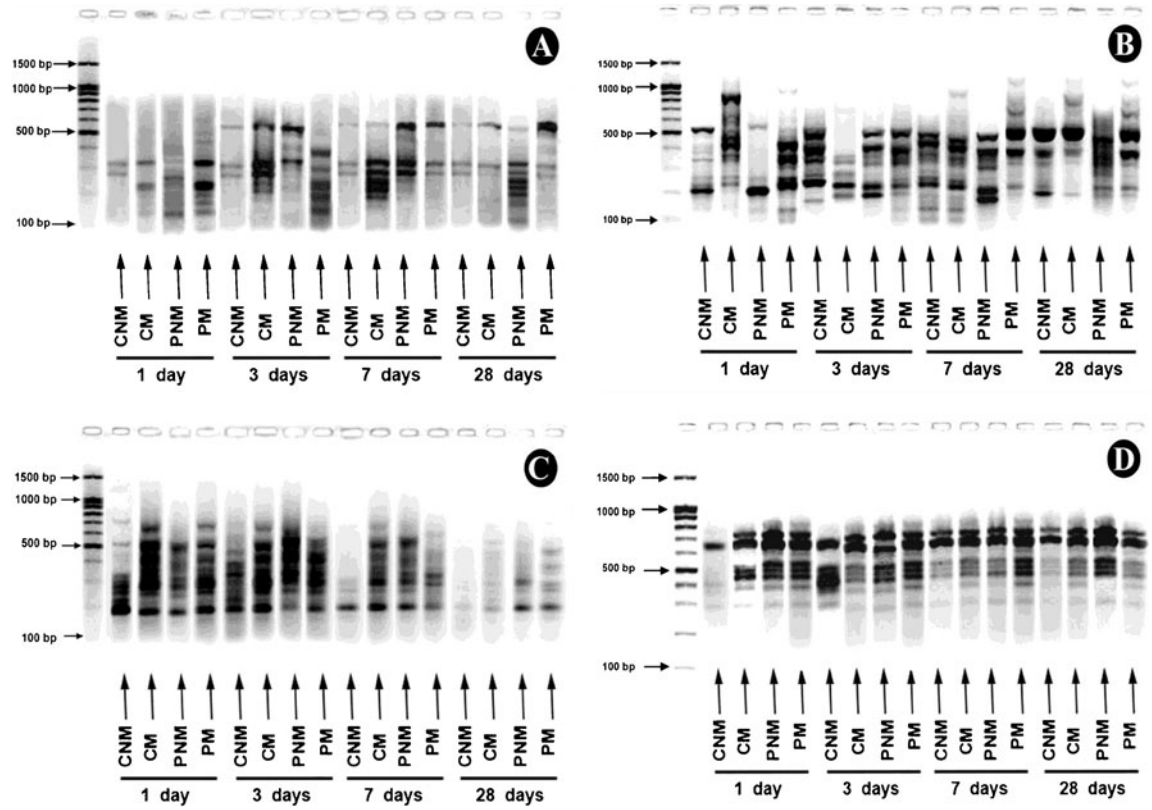

Fig. 2 DNA fingerprinting using the differential display technique. Four different arbitrary primers were used: Chi15 (**a**), A1A13 (**b**), F1 (**c**), P2 (**d**). Treatment: *CNM* Untreated control (not mycorrhiza, no pathogen), *CM* mycorrhiza only, *PNM* pathogen only (no mycorrhiza), *PM* mycorrhiza + pathogen

Our results show that infection with the pathogen singly led to a higher induction in the expression of the two defense genes (Chi and EGase) than that which occurred in the case of AM colonization alone (pathogen-free). Despite the negative effects of pathogen infection on AM colonization (frequency and intensity of root colonization and frequency of arbuscules), the expression of the two defense genes in mycorrhizal bean plants infected with the pathogen was higher than that recorded in plants treated with either one alone, indicating a synergistic effect when both AM and the pathogen were present. The work of Lambais and Mehdy (1998) lends support to our results. These authors described Chi and EGase coding mRNA accumulation in arbuscule-containing cells and adjacent ones and the repression of EGase mRNA accumulation some millimeters distant from the AM fungi colonized zone. In an in situ hybridization study of common bean colonized roots using probes for PAL and Chi, Blee and Anderson (1996) showed that the accumulation of both transcripts occurred only in arbusculated cells. In addition, the accumulation of both PAL and Chi mRNA was greater in cortical cells containing young arbuscules than in cells containing clumped arbuscules. These results may explain the decrease in gene expression of Chi and EGase over time in the mycorrhizal roots in our study. In another study, Pozo et al. (1999) studied β-1,3-glucanases in tomato roots after AM colonization by *Glomus mosseae* and *G. intraradices* and/or pathogenic infection by *Phytophthora parasitica* using polyacrylamide gel electrophoresis. In control roots, two

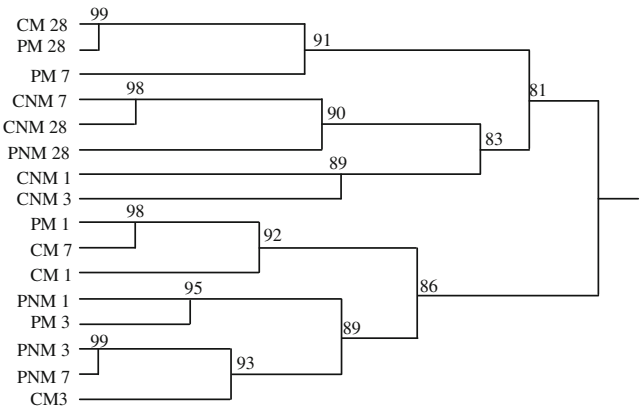

Fig. 3 Dendrogram using average linkage (between groups). *Number on bar* Genetic similarity between samples, with genetic similarity increasing with increasing value

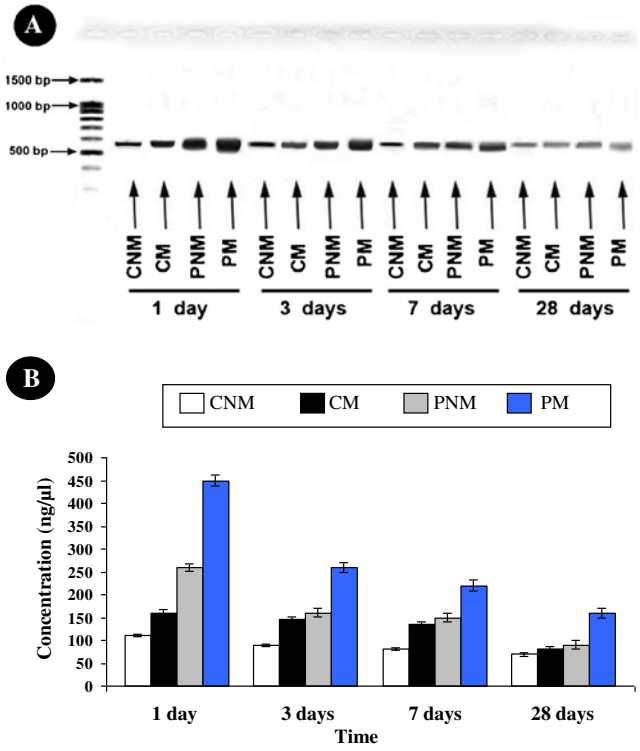

Fig. 4 Effect of AM colonization on gene expression of the chitinase gene (**a**) and DNA concentration (**b**). Results are presented as the mean of three independent experiments

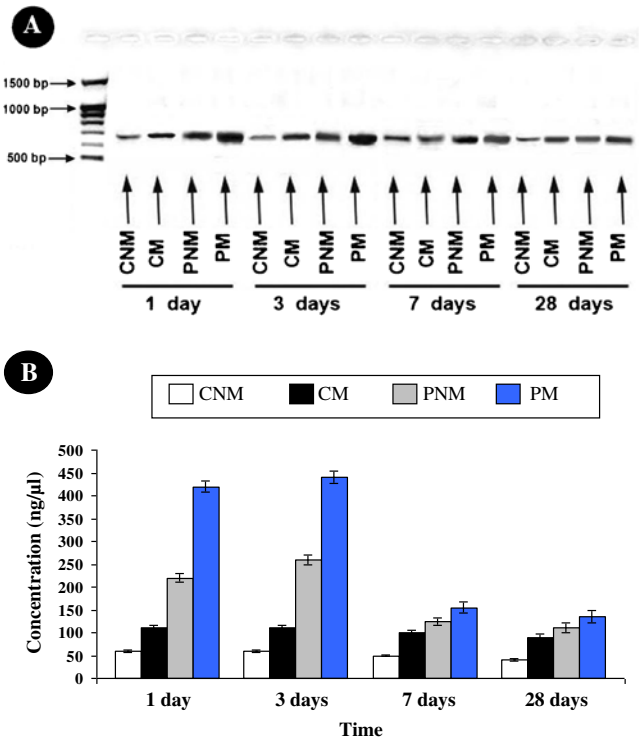

Fig. 5 Effect of AM colonization on gene expression of the β-1,3-endoglucanase gene (**a**) and DNA concentration (**b**). Results are presented as the mean of three independent experiments

acidic EGase isoforms were constitutively expressed, and their activity was higher in that in mycorrhizal roots. Two additional acidic isoforms were detected in extracts from *G. mosseae*-colonized tomato roots, but not in *G. intraradices*-colonized roots. In addition, when tomato plants were pre-inoculated with *G. mosseae* and post-infected with *P. parasitica*, two additional basic isoforms were clearly revealed. The findings of Garmendia et al. (2006) are in agreement with these results. These authors studied the role of AM colonization in the induction of defense-related enzymatic activities in pepper roots before and after infection with *Verticillium dahliae*. Their results show that the colonization of pepper roots by *Glomus deserticola* induced the appearance of new isoforms of acidic chitinases, superoxide dismutase and, at early stages, peroxidases, but only in mycorrhizal plants, and that the inoculation with *V. dahliae* slightly increased both phenylalanine ammonia-lyase and peroxidase activities 2 weeks later. In this same context, El-Khallal (2007) also recorded an induction in these enzymes in infected pepper and tomato plants, respectively, when the plants were pre-colonized by AM fungi. These enzymes were found at low levels in healthy plants; however, their expression was induced during pathogen attack. In contrast, Guenoune et al. (2001) found that defense responses of alfalfa roots to the pathogenic fungus *Rhizoctonia solani* were reduced significantly in roots simultaneously colonized by the AM fungus *G. intraradices*. The production of chitinases elicits other plant responses, including the synthesis of antifungal phytoalexins (Gooday 1999). In addition, the production of Chi and EGase are considered important in the biological control of soil-borne pathogens because of their ability to degrade major component of cell walls (chitin and β-1,3-glucan, respectively) (Cota et al. 2007).

No effects of inoculation with *Rhizobium leguminosarum* were found (i.e., no nodulation occurred) as the Egyptian soil conditions do not support the nodulation of the common bean. Generally, *P. vulgaris* is considered to be a poor fixer of atmospheric nitrogen compared with other legume crops (Graham 1981) and generally responds poorly to inoculation with *Rhizobium leguminosarum* bv. *phaseoli* strains (Buttery et al. 1987). The response depends on the ability of each strain to adapt to environmental stress factors, such as soil pH.

Our data shows that AM colonization of common bean plants infected with *R. solani* resulted in a significant enhancement in the plant growth parameters and also a significant reduction in both disease severity and disease incidence. These findings support our molecular findings. Based on these results, we conclude that the application of AM fungi played an important role in enhancing plant resistance molecularly against *R. solani* via the activation of a number of plant defense genes.

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