Quantitative Changes of PR Proteins and Antioxidative Enzymes in Response to *Glomus intraradices* and *Phytophthora capsici* in Pepper (*Capsicum annuum* L.) Plants

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Abstract To investigate protective activity in pepper plants, which were pre-inoculated with arbuscular mycorrhizal (AM) fungi *Glomus intraradices* (Gi), against pathogenic strain *Phytophthora capsici* (Pc), pathogenesis-related (PR) proteins and antioxidant enzymes were examined. The growth of root and shoot was the highest in peppers inoculated with *G. intraradices,* compared with non-inoculated control plants and those challenged by the pathogen with and without mycorrhizae after nine days of infection. Mycorrhizal colonization rate was reduced by about 10% in pathogen-challenged plants, but disease pressure was reduced. The activities of PR proteins, β-1-3-glucanase and chitinase, were increased in Pc-treated plants compared to Gi+Pc-treated plants in leaves, but those in roots were suppressed. Superoxide dismutase activity and H_2O_2 content in Gi+Pc and Pc-treated plants were gradually increased in leaves. However, those in roots continuously increased up to 5 days, and then decreased dramatically. Peroxidase activity in leaves and roots increased after *P. capsici* infection both in plants inoculated with or without *G. intraradices.* These results suggest that AM fungi, *G. intraradices,* potentially act as one of the protective agents against plant pathogens. Changes of PR proteins and antioxidative enzymes in mycorrhizae-inoculated pepper appear to be regulated differently in leaves and roots by pathogen infection.

Key words: Biological control, arbuscular mycorrhizal fungi, *Glomus intraradices, Phytophthora capsici,* pathogenesis-related proteins

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Pepper (*Capsicum annuum* L.) is one of the most important crops in Korea, due to consumption, and nutritional and economical values. The continuous monocroping of pepper plant has resulted in various problems, including deterioration of soil geochemical properties, the accumulation of toxic compounds, and the increase of plant pathogens, which sometimes requires replanting of pepper. Late blight of pepper, caused by *Phytophthora capsici,* is one of the most devastating soilborne diseases in the world. Symptoms include root and crown rot, and aerial blight of leaves and stems, and fruits of pepper [30]. Pepper late blight disease is currently controlled by soil fumigation with methyl bromide. However, such practice has limitations, such as environmental pollution and increase in fumigant resistance strains of *Phytophthora* [30].

Biological control of plant pathogens has currently received great attention as a promising approach to increasing crop production by avoiding a number of problems related to chemical control, and a possibility to develop compatible practices in terms of sustainable and ecological agriculture has been proposed [2, 20, 27]. Colonization of the plant by certain nonpathogenic microbes can induce disease resistance. Wei *et al.* [40] reported that certain plant growth promoting microorganisms (PGPM) are able to induce plant disease resistance to soilborne pathogens and foliar pathogens. Koike *et al.* [24] found a *Trichoderma* strain which was stimulated to induce systemic resistance to a pathogenic strain of *Colletotrichum orbiculare* in cucumber, and Maurhofer *et al.* [32] suggested that *Pseudomonas fluorescens* strain CHA0 induced resistance in tobacco seedlings against Tobacco necrosis virus (TNV).

Among the PGPM, arbuscular mycorrhizal (AM) fungi are the most universally intimate and important symbiont in terrestrial ecosystems [5, 16]. This symbiont has been shown to assist plants to overcome biotic and abiotic stresses [10, 15, 16, 39]. Since root colonization of mycorrhizae induces disease resistance and/or constitutes a massive fungal invasion of plant tissues, general defense response in plants may be triggered by mycorrhizae during root colonization. General plant defense responses against plant pathogens include pathogenesis-related (PR) proteins and antioxidant enzymes [2, 7, 24, 25, 32, 35- 37].

PR proteins, such as β-1-3-glucanase and chitinase, degrade cell walls of fungal pathogens that contain glucan and/or chitin. Kim and Hwang [22, 23] reported that infection with a pathogenic strain of *P. capsici* induced these PR proteins in pepper stem, and Pozo *et al.* [37] showed that induced systemic resistance (ISR) in tomato was related to the rapid accumulation of specific isoform of β-1-3-glucanase, chitinase, and other PR proteins after pathogen infection. On the other hand, induction of PR in other cases did not lead to protection from pathogen challenge [3, 18, 32]. The induction of antioxidant enzymes in plants may protect themselves from the active oxygen damage due to pathogen invasion [7, 16, 24, 25, 33]. For instance, superoxide dismutase (SOD) and catalase are induced, when *Fusarium proliferatum* infects wheat [25], and peroxidase (POD) increases in alfalfa after *Rhizoctonia solani* infection [16].

The spatial and temporal expression of some defense related genes in mycorrhizae-colonized plants have been described [15]. However, little information is available on the changes in formation of reactive oxygen species, PR proteins, such as β-1-3-glucanase and chitinase, and antioxidant enzymes, such as superoxide dismutase and peroxidase, when pepper plants were inoculated with AM fungi and phytopathogen. This study investigated the changes of PR proteins and antioxidant enzymes in pepper after challenge by the phytopathogen, *P. capsici*. In addition, we examined biocontrol ability of mycorrhizae in pepper against *P. capsici*.

MATERIALS AND METHODS

Microorganisms

AM fungi species, *Glomus intraradices*, was purchased from International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (West Virginia University, U.S.A.). The AM fungi inoculum was obtained from pot cultures of sudan grass as the host plant. *Phytophthora capsici* KACC 40483 was purchased from KACC (Korea Agriculture Culture Collection).

Plant Growth and Fungi Treatment

Soil was mixed with quartz sand and vermiculate in a ratio of 1:2:1 (v/v/v), and rock phosphate was added to 0.2%

concentration. Chemical composition of the mixture consisted of 0.35% of organic matter (OM), 0.15% of nitrogen, 56 ppm P_2O_5, 0.34 cmol of K, 3.42 cmol of Ca, 1.15 cmol of Mg, 4.8 cmol of cation exchangeable capacity (CEC), and 0.65 ds m^{-1} of electronic conductivity (EC).

Four treatments of pepper (*Capsicum annuum* L. Chungok) were used: *G. intraradices* added at planting (Gi); *P. capsici* added at 5 weeks after inoculation without (Pc) and with *G. intraradices* (Gi+Pc) and controls lacking *G. intraradices* and *P. capsici* (Con). Pepper seeds were surface-sterilized with 3% NaOCl for 2 min and thoroughly washed with sterile distilled water. Three seeds were planted in each pot. The AM fungus was introduced by adding 150- 200 spores to the site at which the seeds were added. Pepper plants in each pot were thinned to one at 2 weeks after planting.

P. capsici was grown on V8 juice agar medium for 4 days and then induced to sporulate under fluorescent light for 2 days at 28°C. The mycelia and sporangia were incubated in sterile water for 1 h at 4°C and then 30 min at room temperature. Zoospores released from the sporangia were collected by filtering through two layers of cheesecloth [22]. Ten ml of collected zoospores (5×10^4 zoospore ml^{-1}) were infected to each plant after five weeks of growth. Such a delayed infection time with *P. capsici* was chosen, because bioprotection by AM fungi occurs mainly when symbiosis was well established before the pathogen attack [2].

Plants were watered with a nutrient solution [KNO_3, 2.57 mM; $MgSO_4 \cdot 7H_2O$, 1.01 mM; $Ca(NO_3)_2 \cdot 4H_2O$, 3.6 mM; KH_2PO_4, 0.51 mM; $ZnSO_4 \cdot 7H_2O$, 0.11 μM; H_3BO_3, 4.58 μM; $MnCl_2 \cdot 4H_2O$, 1.01 μM; $CuSO_4 \cdot 5H_2O$, 0.08 μM; $Na_2MoO_4 \cdot 2H_2O$, 0.05 μM; $C_{10}H_{12}FeN_2NaO_8$, 62.7 μM] throughout the growth period [21]. Plants were grown in greenhouse conditions at 25- 30°C and 60- 70% relative humidity. Samples were harvested at 0, 1, 3, 5, 7, and 9 days after *P. capsici* infection. The samples were washed with tap water, rinsed in deionized water, and weighed. Plant tissues were immediately frozen in liquid nitrogen and lyophilized.

Determination of Chlorophyll Concentration

Chlorophyll was extracted by soaking 100 mg of leaves in 10 ml dimethyl sulfoxide (DMSO) at 60°C for 60 min, and its concentration [31] was measured at 663 and 645 nm using a spectrophotometer (U-1100; Hitachi Ltd., Tokyo, Japan).

Mycorrhizal Colonization

Mycorrhizal colonization was assayed by a modified method of Brundrett *et al.* [8]. Roots of pepper plant were gently collected and rinsed free of soil with distilled water. Roots were immerged in FAA solution (the mixture of formalin/ acetic acid/ethanol, 13/5/200, v/v/v) for 24 h. After washing

and rinsing several times with distilled water, the mycorrhizal roots were placed into 20-ml vials containing 10% KOH, and then incubated at 90°C for 45 min. After incubation, mycorrhizal roots were washed with distilled water and stained overnight with stain solution (400 ml of 85% lactic acid, 1.2 g of chlorazole black E, 400 ml of glycerine, 400 ml of distilled water) at 50°C. The root samples were rinsed with water until they were clear and put into destaining solution (50% glycerol) overnight. Mycorrhizal colonization was visualized by a microscope (Olympus Model HB2-RFC). Total mycorrhizal colonization rate (%) was expressed as the percentage of number of colonized roots versus total number of roots examined as described by Read et al. [38].

Root Mortality

Root mortality was measured by the modified method of Liu and Huang [29]. Five-hundred mg of fresh roots were incubated with 10 ml of 0.6% 2,3,5-triphenyltetrazolium chloride in 0.05 M phosphate buffer (pH 7.4) for 24 h in dark at 30°C. Roots were then rinsed twice with sterilized water. Formazan was extracted from the roots twice with 95% ethanol at 70°C for 4 h. Combined extracts from the two extractions were adjusted to a final volume of 50 ml with 95% ethanol, and absorbance was measured at 490 nm. A standard curve was prepared by using different proportions of living roots and roots killed in an autoclave to calculate root mortality. Root mortality was expressed as the percentage of dead root dry weight in the total root dry weight.

Disease Severity Assay

Disease severity was calculated by using the disease index, which was related to the symptom ranging between 0 through 5, where 0=no visible symptoms; 1=leaves slightly wilted with brownish lesions beginning to appear on stems; 2=stem lesion extending for 1–3 cm from inoculation point, and 30–50% of entire plant diseased; 3=some upper leaves defoliated, stem lesion progressed to a half of plant height, and 50–70% of entire plant diseased; 4=stem lesion progressed toward the shoot apex and 70–90% of entire plant diseased; 5=plant dead [22].

Enzyme Extraction

Two grams of frozen sample were ground at 4°C in an ice-chilled mortar with liquid nitrogen and the resulting powder was suspended in 2.5 ml of 50 mM extracting Tris buffer (pH 6.7). The homogenates were centrifuged at 12,000 ×g for 15 min at 4°C and the supernatants were kept frozen at -20°C until enzyme analysis.

PR Protein Activities

β-1-3-Glucanase (EC 3.2.1.6) activity was assayed by measuring the amount of reducing end group, glucose, produced from laminarin. The assay mixture consisted of 30 μl of enzyme extract, 25 μl of 1% laminarin, and 445 μl of 50 mM sodium acetate buffer (pH 5.0). After incubation for 1 h at 37°C, 1.5 ml of dinitrosalysilic acid (DNS) were added, and the reaction was then stopped by heating in boiling water for 5 min. Absorbance was immediately read at 550 nm using a spectrophotometer. The reducing sugar was calculated from a standard curve obtained from known concentrations of glucose. One unit of β-1-3-glucanase activity was defined as the amount of enzyme to liberate 1 μmol of glucose per hour at 37°C [41].

Chitinase (EC 3.2.1.14) activity was assayed by measuring the amount of reducing end group, N-acetylglucosamine (NAG), produced from colloidal chitin. The assay mixture consisted of 15 μl of enzyme extract, 235 μl of distilled water, 500 μl of 0.5% colloidal chitin, and 250 μl of 50 mM sodium acetate buffer (pH 5.0). After incubation at 37°C for 1 h, 200 μl of 1 N NaOH were added to stop the reaction. After centrifugation at 12,000 ×g for 10 min, 750 μl of supernatant was mixed with 1 ml of Schales' reagent (0.5 M sodium carbonate+1.5 mM potassium ferricyanide), and the mixture was then heated in boiling water for 15 min. Absorbance at 420 nm was immediately read with a spectrophotometer. The reducing sugar was calculated from a standard curve obtained from known concentrations of NAG. One unit of chitinase activity was defined as the amount of enzyme to liberate 1 μmol of NAG per hour at 37°C [39].

Antioxidant Enzyme Activities

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to the method of Fu and Huang [14]. A 3 ml reaction mixture contained 63 mM nitroblue tetrazolium (NBT), 1.3 μM riboflavin, 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), and 20 μl of enzyme extract in a test tube. The tube containing the mixture was placed under light at 78 μmol photons s^{-1} m^{-2} for 20 min, and absorbance at 560 nm was recorded. A non-irradiated reaction mixture that did not develop color served as the control. One unit of SOD activity was defined as the amount of enzyme to inhibit 50% of the NBT reduction rate.

Peroxidase (POD; EC 1.11.1.7) activity was determined according to the method of Lin and Kao [28]. The reaction mixture contained 50 μl of 20 mM guaiacol, 2.8 ml of 10 mM phosphate buffer (pH 7.0), and 0.1 ml of enzyme extract. The reaction was initiated by the addition of 20 μl of 40 mM H_2O_2, and the change in absorbance at 470 nm was measured. The POD activity was calculated using an absorption coefficient (26.6 mM^{-1} cm^{-1} at 470 nm) for the tetraguaiacol. One unit of POD activity was defined as the amount of enzyme to form 1 μmol of tetraguaiacol per minute at room temperature.

Hydrogen Peroxide Content

Hydrogen peroxide (H_2O_2) content was colorimetrically measured as described by Lin and Kao [28]. H_2O_2 was extracted by homogenizing 200 mg of sample with 3 ml of 50 mM phosphate buffer (pH 6.8). The homogenate was centrifuged at 12,000 ×g for 20 min. The supernatant was mixed with 1 ml of 0.1% titanium chloride in 20% H_2SO_4, and the mixture was then centrifuged at 12,000 ×g for 10 min. The intensity of the yellow color of the supernatant was measured at 410 nm. H_2O_2 content was calculated using the extinction coefficient of 0.28 μM^{-1} cm^{-1}.

Statistical Analysis

Treatment effects were determined by analysis of variance according to the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, U.S.A.). Differences among various treatment means were separated by least significant difference (LSD). Significance of between-treatment means was tested at the 0.05 level of probability.

RESULTS

Mycorrhizal Colonization, Root Mortality, and Disease Severity

The AM fungus was established in pepper roots at a level of 55% at five weeks after planting, with an increase to about 88% at further nine days of growth (Fig. 1). Infection of the mycorrhizal roots by *P. capsici* decreased the rate of increase in *G. intraradices* colonization to a level of about 70% after nine days of challenge by the pathogen. Increasing root mortality and disease severity were observed after *P. capsici* challenge of nonmycorrhizal plants. Pepper plants inoculated with the pathogen had root
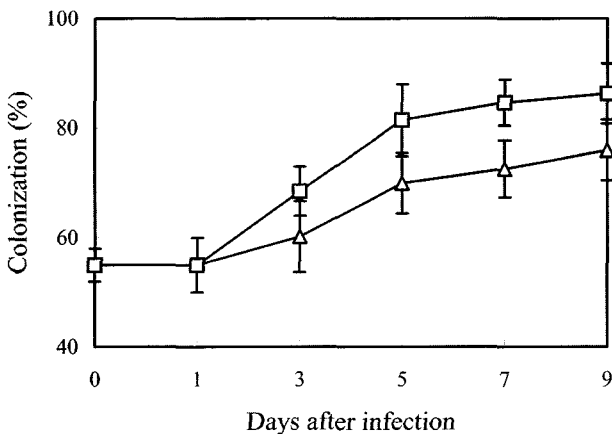

Fig. 2. Change of root mortality in pepper roots as influenced by Control (■), *G. intraradices* (□), *G. intraradices* +*P. capsici* (△), and *P. capsici* (▲).
Mean values are from 6 replicates. Bars represent standard error.

decay, and the leaves were chlorotic and wilted at nine days after pathogen infection. Pathogen inoculation on mycorrhizae-colonized roots showed little disease, as evidenced by root mortality and disease severity (Figs. 2 and 3).

Measurement of plant growth, root and shoot mass, and leaf chlorophyll concentration confirmed that the mycorrhizal plants were protected from the *P. capsici* challenge. By five days after pathogen infection, the pathogen inhibited the growth of pepper and lowered the chlorophyll content of the pepper plants (Table 1) in comparison to the control noninfected plants. Growth and chlorophyll concentrations in mycorrhizal plants inoculated with *P. capsici* were maintained at the levels of noninfected control plant. Mycorrhizal plants showed the fastest growth and the highest leaf chlorophyll content (Table 1).

Fig. 1. Effect of *P. capsici* infection on *G. intraradices* colonization in pepper roots.
P. capsici was infected 5 weeks after *G. intraradices* inoculation. *G. intraradices* (□), *G. intraradices*+*P. capsici* (△). Mean values are from 6 replicates. Bars represent standard error.
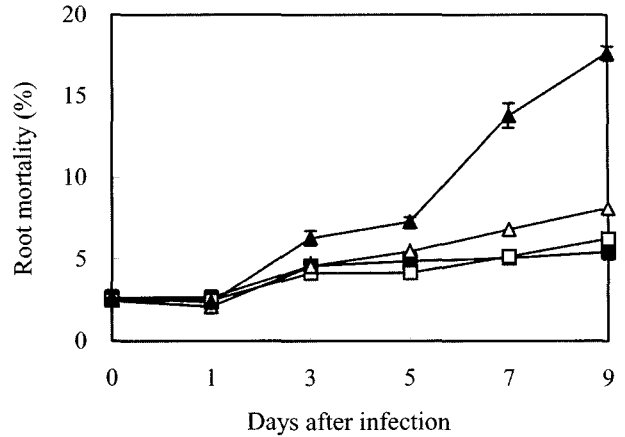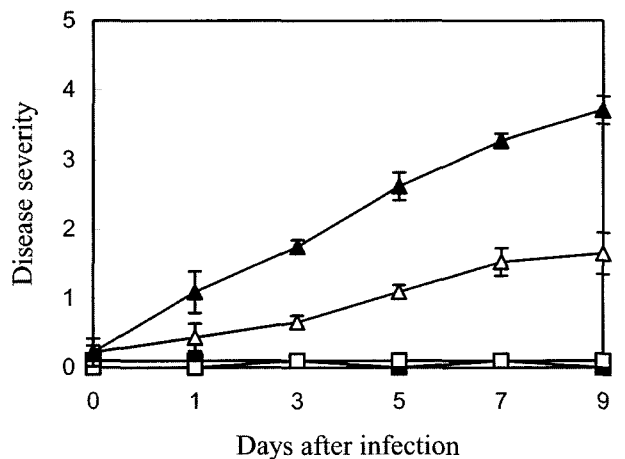

Fig. 3. Change of disease severity in pepper plants as influenced by Control (■), *G. intraradices* (□), *G. intraradices* +*P. capsici* (△), and *P. capsici* (▲).
Mean values are from 6 replicates. Bars represent standard error.

Table 1. Changes of root and shoot fresh weight (FW), and chlorophyll concentration as influenced by *G. intraradices*, *G. intraradices+P. capsici*, and *P. capsici*.

DAI[1]	Treatment	Root FW (g plant^{-1})	Shoot FW (g plant^{-1})	Chlorophyll Con. (g^{-1} FW)
1	Con[2]	4.16±0.31[3)a]	4.85±0.40[bc]	1.90±0.03[ab]
	Gi	4.46±0.15[a]	5.64±0.30[a]	1.94±0.07[a]
	Gi+Pc	4.58±0.35[a]	5.38±0.36[ab]	1.80±0.06[b]
	Pc	4.01±0.35[a]	4.41±0.20[c]	1.82±0.05[bc]
	LSD(0.05)	0.69	0.75	0.12
5	Con	7.76±0.39[b]	7.01±0.7[b]	1.91±0.05[b]
	Gi	8.55±0.13[a]	9.57±0.82[a]	1.97±0.01[a]
	Gi+Pc	7.12±0.09[b]	7.32±0.09[b]	1.73±0.03[c]
	Pc	5.97±0.38[c]	6.30±0.67[c]	1.46±0.01[d]
	LSD(0.05)	0.65	0.40	0.06
9	Con	9.21±0.31[ab]	9.46±0.93[b]	1.69±0.05[a]
	Gi	9.90±0.31[a]	11.09±0.96[a]	1.73±0.02[a]
	Gi+Pc	8.65±0.53[b]	8.96±0.41[b]	1.59±0.02[b]
	Pc	5.07±0.70[c]	7.00±1.16[c]	1.02±0.01[c]
	LSD(0.05)	1.13	0.79	0.07

[1]Days after infection with *P. capsici*.

[2]Control (Con), *G. intraradices* (Gi), *G. intraradices+P. capsici* (Gi+Pc), and *P. capsici* (Pc).

[3]Each value is mean of 6 replicates ±SE. Means within column followed by the same letter(s) were not significantly different, based on a LSD test at P=0.05.

β-1-3-Glucanase and Chitinase Activities

β-1-3-Glucanase and chitinase activities in roots challenged by *P. capsici* decreased, correlating with damage to these

tissues, whereas the activities in the leaves increased (Figs. 4 and 5). Less change was observed in *P. capsici*-challenged peppers that were colonized by *G. intraradices*.
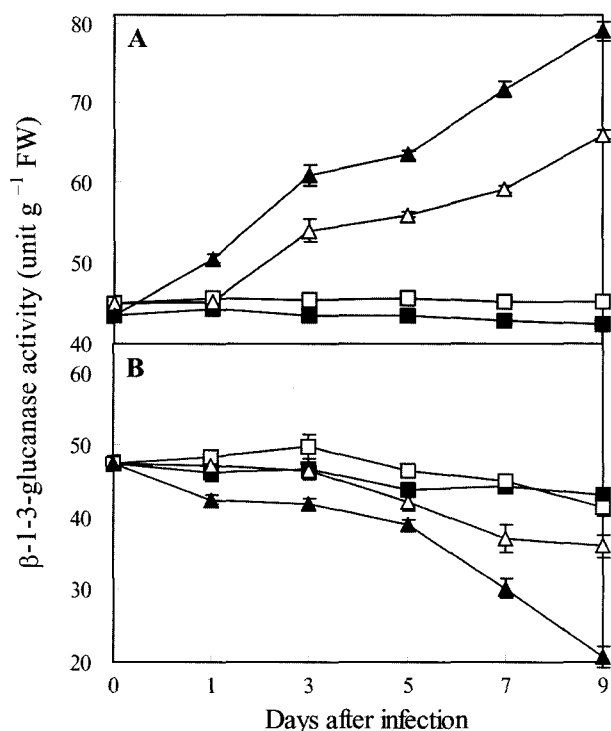

Fig. 4. Change of β-1-3-glucanase activity in pepper leaves (A) and roots (B) as influenced by Control (■), *G. intraradices* (□), *G. intraradices+P. capsici* (△), and *P. capsici* (▲).
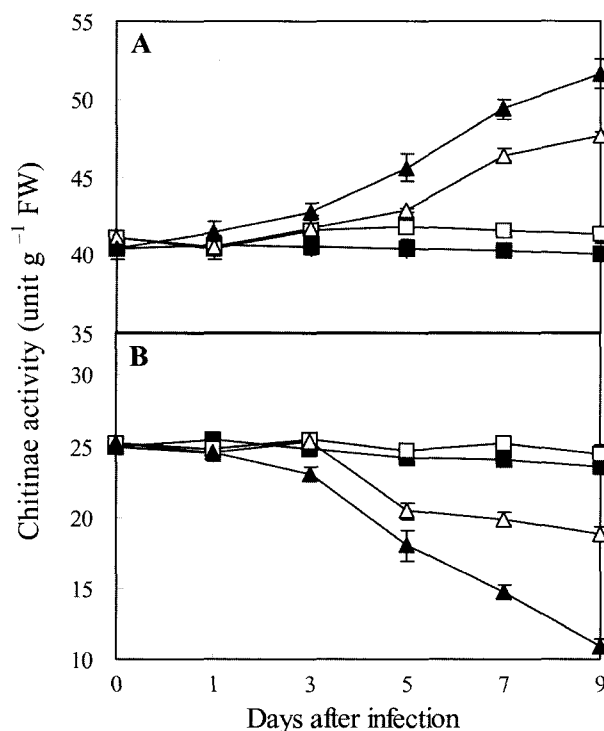Mean values are from 6 replicates. Bars represent standard error.

Fig. 5. Change of chitinase activity in pepper leaves (A) and roots (B) as influenced by Control (■), *G. intraradices* (□), *G. intraradices+P. capsici* (△), and *P. capsici* (▲).
Mean values are from 6 replicates. Bars represent standard error.

No change of glucanases and chitinase activities were observed over the nine-day course of sampling in the control plants or the mycorrhizal plants (Figs. 4 and 5).

Antioxidant Enzyme Activities and Hydrogen Peroxide Content

The activities of the antioxidant enzymes were changed the most in the plants that were inoculated with *P. capsici*, and this change was reduced when the pathogen was challenged to mycorrhizal plants. SOD and peroxidase activities were increased after the pathogen challenge. SOD activity of pepper leaves in all treatments showed almost constant values till 3 days, slightly increasing thereafter in Pc and Gi+Pc-treated plants (Fig. 6A). In roots, SOD activity increased during the early stage of the infection (Fig. 6B). SOD activity in Pc-treated plants increased to a maximum value of 18.6 unit g^{-1} fresh weight 5 days after the infection and decreased thereafter. Changes in SOD occurred earlier than those of peroxidase in the leaf and root tissues. However, SOD activity in roots declined after serious damage was sustained by the pathogen (Fig. 6).

POD activity of pepper leaves in all treatments was constant till 5 days, and then the activity in Pc-treated plants rapidly increased (Fig. 7A). In roots, POD activity in Pc-treated plants gradually increased till 7 days and
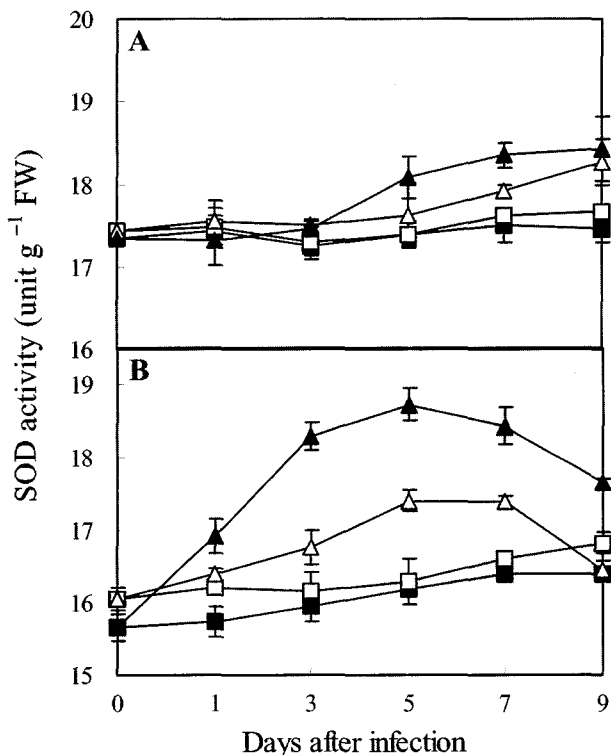

Fig. 7. Change of POD activity in pepper leaves (A) and roots (B) as influenced by Control (■), *G. intraradices* (□), *G. intraradices+P. capsici* (△), and *P. capsici* (▲).
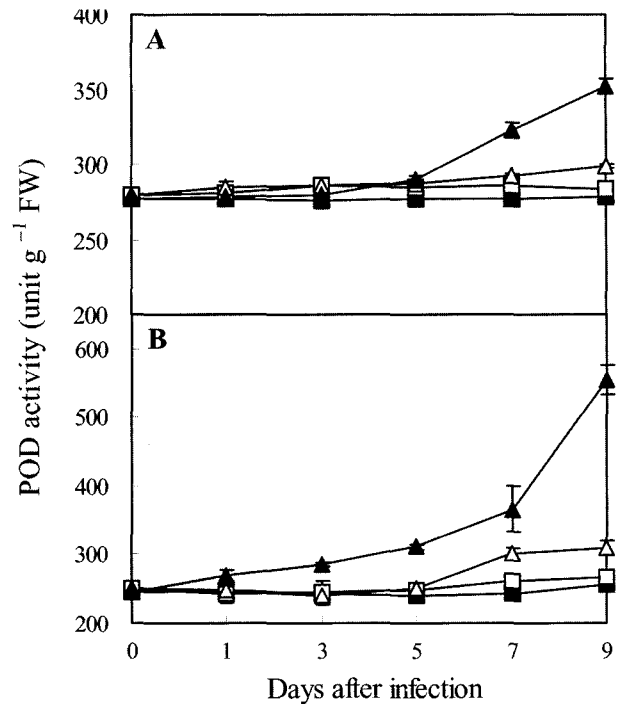Mean values are from 6 replicates. Bars represent standard error.

markedly increased thereafter, whereas the activity in Gi+Pc-treated plants was unchanged till five days, slightly increasing thereafter (Fig. 7B).

Increases of H_2O_2 almost duplicated the pattern of change of SOD activity. During the early infection, H_2O_2 in leaves remained constant until five days after pathogen challenge, thereafter, rapidly increased in Pc and Gi+Pc-treated plants; and the highest value was observed in Pc-treated plants (Fig. 8A). In roots, H_2O_2 content in Pc-treated plants increased till five days after pathogen challenge with a maximum value of 26 μmol g^{-1} fresh weight, and then dramatically decreased at nine days after the infection (Fig. 8B). Activities continued to increase in the *P. capsici* challenged tissues, with lower levels in the mycorrhizal than in nonmycorrhizal leaves.

DISCUSSION

P. capsici infection causes severe symptoms in pepper, resulting in reduced biomass and chlorophyll content. Plants were protected from the root rot symptoms by colonization of the roots by *G. intraradices*. Similar protective effects have been described in other mycorrhizal plants [10- 12, 16, 37, 39].

Several possibilities have been suggested to account for plant protection by AM formation. Dassi *et al.* [12]
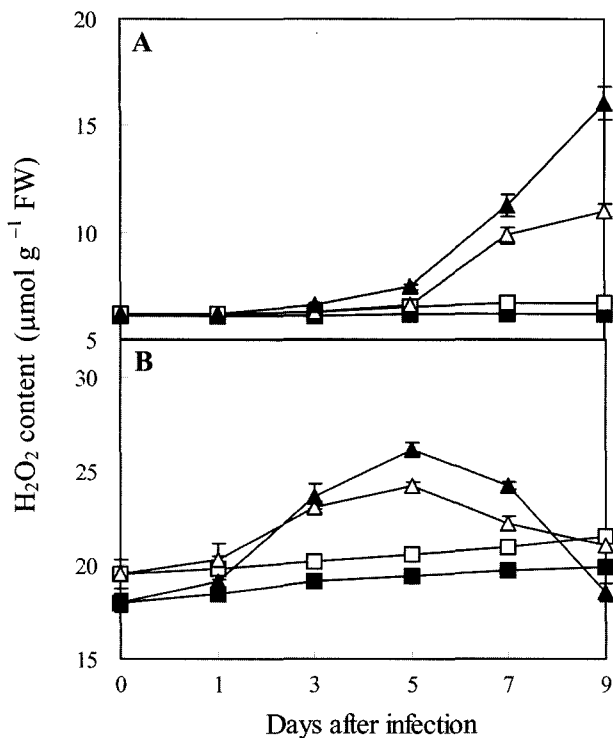

Fig. 6. Change of SOD activity in pepper leaves (A) and roots (B) as influenced by Control (■), *G. intraradices* (□), *G. intraradices+P. capsici* (△), and *P. capsici* (▲).
Mean values are from 6 replicates. Bars represent standard error.

Fig. 8. Change of H_2O_2 content in pepper leaves (A) and roots (B) as influenced by Control (■), *G. intraradices* (□), *G. intraradices+P. capsici* (△), and *P. capsici* (▲).
Mean values are from 6 replicates. Bars represent standard error.

suggested that the presence of mycorrhizal hyphae drastically reduced the percentage of *Phytopthora* zoospore germination, resulting in reduction of penetration to root via epidermal grooves; Pozo *et al.* [37] reported that *Phytophthora* infected nonmycorrhizal roots, but failed to infect pre-inoculated mycorrhizal roots, suggesting an induced defense mechanism in place; Trotta *et al.* [39] showed that *Phytophthora* development was reduced in the colonized and adjacent uncolonized regions of the mycorrhizal root system, suggesting a systemic effect. On the other hand, Pozo *et al.* [37] showed that plants colonized by *G. intraradices* responded to *P. capsici* attack by producing larger roots in the parts of the root system that were not infected by *P. capsici*. These roots would help sustain growth by absorbing nutrients that the damaged roots could not.

Other mechanisms for protection could include induction of PR proteins [37]. Pozo *et al.* [35, 36] reported that AM fungus *Glomus mosseae* induced new β-1-3-glucanase and chitinase isoforms upon *Phytophthora parasitica* infection of tomato root. Our findings showed that (1) the damage due to *P. capsici* roots infection did not produce PR protein in the root, but had a systemic effect by increasing glucanase, chitinase, and peroxidase in leaves, and (2) inhibited growth of the pathogen in the mycorrhizal

roots was not correlated with glucanase or chitinase induction.

PR proteins involved in the general defense response of plants include glucanases and chitinases [9]. Generally, these enzymes increase in early plant-AM interactions and then diminish as root colonization proceeds [26], indicating that general defense responses upon mycorrhizal colonization are partially induced only in early plant-mycorrhizae interaction and repressed when root colonization of mycorrhizae is established. In addition, expressions of PR proteins are restricted in arbuscule-containing cells [5, 26]. This local expression of PR proteins is different from systemic expression of PR proteins in plant-pathogenic interactions, in which PR proteins accumulate in entire infected roots [4]. The AM fungi do not elicit the full cascade of nonspecific defense responses in host roots, but elicit rather transient, weak, localized expression of the responses.

We cannot understand the mechanisms of decrease of these enzymes in roots and increase in leaves. However, our findings of differential β-1-3-glucanase and chitinase activities in roots and leaves could well be explained by several possibilities. Our results may suggest that overall PR activities may not provide bioprotection of pepper roots by mycorrhizae, but some specific isoforms of PR proteins, which are induced by mycorrhizal root colonization, may play an important role in protecting pepper roots from *P. capsici*. This is consistent with the results obtained by Hoffland *et al.* [18] and Pieterse *et al.* [34], that PGPM *Pseudomonas fluorescens* strain WCS417 was able to induce resistance against pathogen *Fusarium oxysporum* without detectable accumulation of PR proteins in radish and *Arabidopsis*, respectively. In addition, Beffa *et al.* [3] suggested that the decrease of PR proteins expression in tobacco leaves may be related to bioprotection against Tobacco mosaic virus (TMV). All these results demonstrate that the absence of PR proteins did not lower the level of protection [18]. In general, PR proteins are known to play an important role in pathogen localization and/or in ISR. However, the accumulation of PR protein is not necessarily a prerequisite for the expression of ISR [13, 19, 34], although it still could be argued that PR proteins contribute to disease reduction in the case of pathogen-induced resistance when they are accumulated [19, 34].

There are two distinct signal pathways in plant disease resistance; salicylic-dependent and jasmonic-acid-dependent pathways [1]. These two pathways induce different sets of defense related genes. PR proteins, such as glucanase and chitinase, are generally induced by the salicylic-dependent pathway. Recent study indicates that jasmonate biosynthesis is induced in arbuscular mycorrhizal barley roots [17]. Therefore, the decrease of overall PR proteins level in mycorrhizae-colonized roots may be a consequence of

activation of the jasmonic acid pathway. More detailed studies on changes of PR proteins isozyme at the physiological and molecular levels are in need to elucidate the mechanism of PR proteins in bioprotection of mycorrhizae-colonized plants. Furthermore, more information are needed on the induction of defense-related genes or enzymes regulated by the jasmonic acid pathway in mycorrhizae-colonized plants inoculated with pathogen. One possibility of increased PR proteins in leaves of mycorrhizae-colonized or pathogen-inoculated peppers might have been resulted from activation of nonspecific defense related genes by translocation of mobile signal(s), as observed in systemic acquired resistance or induced systemic resistance elsewhere [1]. We conclude that induced systemic resistance by *G. intraradices* was not associated with accumulation of PR proteins in pepper plants, suggesting that unknown mechanisms are involved in bioprotection of plants.

Our results are in agreement with the suggestion that localized competition occurs between *G. intraradices* and *P. capsici* [10, 11, 37]. Cordier *et al.* [10] reported that the bioprotective effect in the mycorrhizal root system was directly linked to reduced *Phytophthora* development within AM fungi-colonized cortical tissues. They showed that hyphae-vesicles-containing cortical cells of mycorrhizal plants exhibited resistance against *P. capsici* infection.

In the present study, induction of SOD activity was found to be positively correlated to H_2O_2 content in response to *P. capsici* infection. These results are consistent with those of Borden and Higgins [6], who showed that SOD activity and H_2O_2 were induced in tomato plants when infected with *Cladosporium fulvum*. The level of H_2O_2 was related to the degree of root damage caused by the pathogen. SOD activity and H_2O_2 in Pc-treated plants increased more than in Gi+Pc-treated plants in both roots and leaves. These results can explain that Pc-treated plants generated more AOS than Gi+Pc-treated plants, resulting in more cellular damage in the former plants. Furthermore, the decrease of SOD activity and H_2O_2 content in roots after 5 days may indicate that the scavenging function of SOD was impaired with prolonged pathogenic stresses. However, disease severity in Gi+Pc-treated plants was lower than in Pc-treated plants. These results, thereafter, suggest that maintenance of physiological activities under pathogenic conditions, as manifested by chlorophyll levels, disease severity, and plant growth, could be related to *G. intraradices*, which alleviated active oxygen stresses.

Our findings illustrate that establishment of a mycorrhizal state in pepper is a promising means to enhance the resistance of field-grown pepper against root rot disease, such as *P. capsici*. Understanding the synergistic contribution of the complex sequence leading to plant protection may help improve the efficacy of AM fungi in achieving more sustainable healthy plant production systems. However,

the extent by which the other unknown defense mechanisms are involved is an open question.

Acknowledgments

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