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Symbiotic Effect on the Growth and Resistance against *Phytophthora capsici* in the Pepper Plants by Colonization with *Glomus intraradices* in Root System

Chung-Sun Lee and Yong-Chull Jeun*

Department of Plant Resource Science, the Research Institute for Subtropical Agriculture and Biotechnology, Cheju National University, 690-756, Jeju, Korea

내생근균 *Glomus intraradices* 접종 고추에서 성장촉진효과와 고추역병균 *Phytophthora capsici* 침입에 대한 저항성 발현

이충선, 전용철*

제주대학교 생명자원과학대학 식물자원과학과

Abstract : Symbiotic effect on the growth and resistance inductions against *Phytophthora capsici* mediated by a mycorrhiza *Glomus intraradices* on the leaves of pepper plant was investigated. *G. intraradices* (BEG110) has been known as one of arbuscular mycorrhiza and it's colonization with host plant causes promotion of not only nutrient uptake but also plant growth. The length of pepper plants colonized with *G. intraradices* was higher compared with those of non-treated control plants. Similarly the weight of pepper plants was increased by the colonization with the mycorrhiza in root system. However, the resistance against late blight pathogen *Phytophthora capsici* was not always expressed by the colonization with *G. intraradices*. Among the 4 experiments only in one experiment the disease severity in the plants colonized with *G. intraradices* was dramatically reduced. Interestingly, the disease severity of non-treated control plants was not high as those of the plants in the other experiments. Based on the results the resistance mediated by *G. intraradices* might be related with the disease severity caused by against *P. capsici*.

key words : arbuscular mycorrhiza, induced systemic resstance(ISR) systemic acquired resistance(SAR)

INTRODUCTION

Systemic induced resistance is one of the resistances, which is mostly triggered by pre-inoculation with pathogen to certain parts of plants (Sticher et al., 1997). Local acquired resistance (LAR) is expressed in the part of the plants exposed to the exogenous stimuli such as pathogen or chemicals. Systemic acquired resistance (SAR) is a resistance expressed in the distance parts of the plants from the parts exposed to the stimuli. Usually, SAR is expressed after hypersensitive reaction (HR) (Siegrist et al., 2000) or by necrosis on the treated parts of the plants (Van Loon, 1997). In most cases, signaling of SAR is dependent on the

Corresponding author : Tel : 064-754-3319, E-mail : ycjeun@cheju.ac.kr

accumulation of salicylic acid (SA) (Gaffney et al., 1993). Moreover, pathogenesis-related proteins (PR-proteins), which showed antifungal activity against some plant pathogens, are accumulated (Niderman et al., 1995; Woloshuk et al., 1991).

Induced systemic resistance (ISR) has been reported, in which the resistance mostly induced by pre-inoculation with plant growth promoting rhizobacteria (PGPR) (Van Loon et al., 1998). ISR is distinguished from the classical SAR by different signal pathway and resistance expression (Knoester et al., 1999; Pieterse et al., 1996; Press et al., 1997; Van Wees et al., 1997). In some cases of plants expressing ISR, PR-proteins were not accumulated (Pieterse et al., 1996; Van Wees et al., 1997). Although neither HR nor necrosis is formed in the roots inoculated with PGPR, the aerial parts of plants become resistance against plant pathogen (Kloepper et al., 1980). Furthermore, the signaling of ISR is usually independent on the accumulation of SA (Van Loon et al., 1998). However, in the ethylene or jasmonic acid insensitive Arabidopsis plants, ISR was not triggered after pre-inoculation with PGPR (Pieterse et al., 1998), indicating an important role of ethylene or jasmonic acid for triggering of resistance by PGPR.

Some microorganisms maintain endosymbiotic interaction with plant, such as the mutualistic symbiosis between plant roots and arbuscular mycorrhizal fungi (AMF). The plants colonized with AMF benefit not only to improve plant health but also to be resistant against plant pathogens (Azcn-Aguilar and Barea, 1996). Like the case of PGPR, the AMF can be potential biocontrol agents for agricultural crop species (Pozo et al., 2002). Indeed, in the tomato plants colonized with *Glomus mosseae* in root system the disease severity caused by *Phytophthora parasitica* was reduced (Cordier et al., 1996, 1998; Pozo et al., 1996,1999; Trotta et al., 1996; Vigo et al., 2000). In some of studies the resistance mechanisms of symbiotic associations between plants and AMF has been reported (Pozo et al., 2002). The pathogenesis-related proteins such as chitinases, chitosanases and -1, 3-glucanses in the mycorrhizal colonized roots of tomato plants were accumulated (Pozo et al. 1996, 1998, 1999) indicating the effective defense reaction by antifungal proteins. Furthermore, in bean and wheat plants colonized by *G. interaradices* the catalase and peroxidase were accumulated (Blee and Anderson, 2000). The increases of these enzymes are involved with defense mechanism. However, the mechanisms of this type of resistance have not been clearly understood.

In the present study, the beneficial effects of *G. interaradices* on growth of pepper plants were determined. Furthermore, the protection effects against late blight caused by *Phytophthora capsici* were investigated on the stem of pepper plants colonized with *G. intraradices*.

MATERIALS and METHODS

Plant and pathogen

Pepper seeds (*Capsicum annuum* cv. Manitja) were sown in plastic pots (10-cm in diameter) filled with commercial soil (Choroc Nala, Bokyung Nongsang, Korea) containing 10 % of perlite (Parat, Sam Son, Korea). Pepper seedlings were grown in the greenhouse maintaining 28 C at daytime and 25 C at night.

Phytophthora capsici was grown on V8 agar medium for 7-10 days at 25 C. To induce sporangium formation the mycelia were harvested with a spacula and exposed under fluorescent light at 28 C for 7 days. For the initiation of zoospore release from sporangia 10 ml H_2O $_{bidest}$ was added to the agar plate and then the plates were immediately placed in a refrigerator at 4 C until zoospores were released. The suspension containing zoospores was filtered through three times folded cheesecloth and the concentration of the zoospores was adjusted to 1.0 x 10^4 zoospores / ml for the inoculation of pepper plants.

Propagation of the mycorrhizal fungus

Glomus intraradices (BEG 110) were propagated several times on white clover grown on autoclaved substrate (sand : vermiculite = 1 : 1) at 121 C for 30 min. For propagation, the inoculum always comprised 10 % (v / v) of the pot volume, where usually approximately 0.5 g seeds were used per 500 g of substrate. Pots were placed on a balance one or two times per day and watered with nutrient solution to maintain a soil water content equivalent to 65 % of field capacity. Nutrient solution was consisted of following macronutrient (mM): $Ca(NO_3)_2 \cdot 4H_2O$ (2), $KH_2PO_4 \cdot H_2O$ (0.0752), $K_2HPO_4 \cdot 2H_2O$ (0.0048), K_2SO_4 (0.7), KCl (0.1), $MgSO_4 \cdot 7H_2O$ (0.5) and micronutrients (μM): H_3BO_3 (10), $MnSO_4 \cdot H_2O$ (3), $ZnSO_4 \cdot 7H_2O$ (0.5), $CuSO_4 \cdot 5H_2O$ (0.2), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.01), FeEDTA (10). Plants were grown in a greenhouse for ten weeks. Thereafter, the substrate was left to dry out (until plants wilted), reirrigated and left to dry for a further two weeks, both drying periods serving to promote spore production. Roots and substrate were sieved (1 mm) and the percentage colonization of roots was determined (usually around 50 %). The air-dried substrate with spores and colonized root pieces were stored at 4 C as inoculum.

Colonization with *Glomus intraradices*

The percentage of root length colonized by mycorrhizal fungi was determined on roots stained in trypan blue (Koske and Gemma 1989) using the gridline-intersect method (Giovannetti and Mosse 1980).

Ten % (V/V) of the soil containing mycelium of *G. intraradices* was mixed with the commercial soil, which was already sterilized at 100 C for 1 h. The cucumber seeds were sown in the soil mixture in which *G. intraradices* was colonized. As a negative control, water treated plants were used.

Determination of symbiotic effect on plant growth

To determine the symbiotic effect of *G. intraradices*, the length and the weight of pepper plants were measured at two month after sowing of the plants in the G. intraradices colonized soil. The length was measured form the stem below the soil line to top of the plant. The weight was measured the whole plants except roots. These experiments were replicated three times separately each contained 30 plants.

Challenge inoculation and evaluation of late blight disease severity

The soil of pepper plants of 4-leaf growth stage was detached by pouring water. The root of the plant was dipped in a 150 ml erlenmeyer flask containing 100 ml zoospore suspension of *P. capsici* (1.0 x 10^4 zoospores /

ml). The inoculated plants were kept at 100 % RH in the dark for 24 h at 16 C and then placed in a growth cabinet at 80 % RH and 18 - 20 C.

Disease development was recorded every day after fungal inoculation by visual estimating the stem length with late blight lesions. In principle, disease was evaluated according to the method described by Kim (1994). The disease severity of pepper plants was calculated using the disease index between 0 to 5, in which 0 = no visible lesion; 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.

Data analysis

The length and weight of pepper plants non-treated as well as colonized with *G. intraradices* were statistically analyzed using a paired t-test. Significance levels at P = 0.05 were used for all statistical tests.

RESULTS

Symbiotic effect on growth of pepper plants

There was no visible difference in phenotype between the inoculated with mychorrhiza and the untreated control plants after colonization with *G. intraradices* in the root system of cucumber plants (Fig. 1). However, more number of plants was sprung in the mycorrhiza-colonized soil compared with those in non-treated soil (Fig. 1). The growth of pepper plants colonized with *G. intraradices* was slightly enhanced compared to those of control plants (Table 1 and 2) indicating the beneficial interaction between the mycorrhiza and plant. The length of plants was significantly increased in two experiments and in one experiment also slightly increased thought no significant (Table 1). Similarly, the weight of the colonized plants with mycorrhiza was also slightly increased in all of experiments, although they were not significant (Table 2).

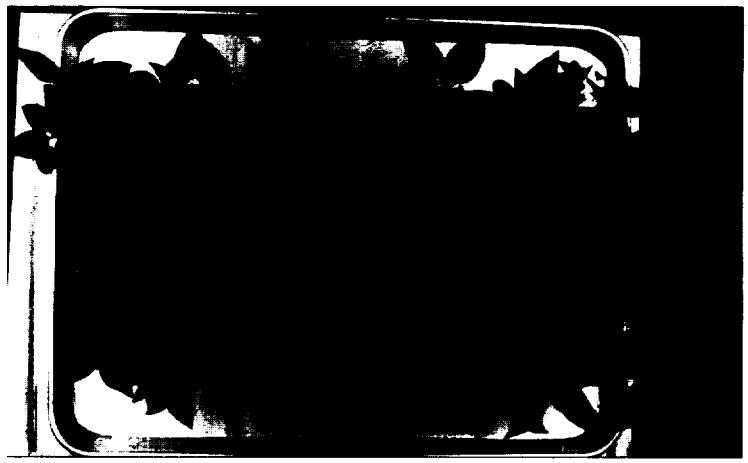

Fig 1. Budding and growth promating effect by colonization with *G. intrardices* in root system of pepper plant. The plants presented in the right were grown in the soil colonized with *G. intrardices*. In the left showed non-treated control plant. The photo was taken at two month after sowing of the plant.

Table 1. Enhancement of length of pepper plants by colonized with *G. interaradices* in root system

Experiment	Length (cm)		P	t-test
	Control	*G. intraradices*		
Ex.1	14.6±3.5[a]	16.5±3.8	0.015	*[b]
Ex.2	12.0±2.7	13.5±2.5	0.021	*
Ex.3	16.9±3.1	17.1±3.1	0.403	ns

[a] Mean of values standard deviation of 30 plants.
[b]* = significant at the 5 % probability level; ns = non significant

Table 2. Enhancement of weight of pepper plants by colonized with *G. interaradices* in root system

Experiment	Weight (g)		P	t-test
	Control	*G. intraradices*		
Ex.1	2.8±0.8[a]	3.0±0.8	0.146	ns [b]
Ex.2	2.1±0.6	2.3±0.6	0.093	ns
Ex.3	2.8±0.5	3.1±0.9	0.077	ns

[a] Mean of values standard deviation of 30 plants.
[b] ns = non significant

Effects of *G. intraradices* on late blight severity

In the first experiment the symptom of non-treated control pepper plants was apparently visible at 10 days after inoculation with *Phytophthora capsici*, in which the stem lesion extended for 1~3 cm from inoculation point and 30-50 % of entire plant diseased (Fig. 3A). The length of diseased stem was gradually increased and half of the stem was diseased at 15 days after inoculation (Fig. 3A). At 20 days the lesion of stem occupied almost to the shoot apex and the plants totally die at 25 days (Fig. 3A). In the plant colonized with *G. intraradices* the leaves were slightly wilted and stems were infected with brownish lesions at 10 days after inoculation (Fig. 3A). Generally, the disease symptoms were developed similar with those of control plants (Fig. 3A).

In the second and fourth experiments the disease symptoms were visible in the stems at 10 days after inoculation both control and colonized with *G. intraradices* (Fig. 3B and 3D). Contrast to the first experiment the disease was not totally developed until at 25 days after inoculation (Fig. 3B and 3D). There was no differences in the disease progress between the control and mycorrhiza colonized plants (Fig. 3B and 3D).

In the third experiment the disease progresses were different with the other experiments. At 10 days the symptoms were appeared in the control plants (Fig. 2 and 3C). The severity of disease, however, was slowly increased

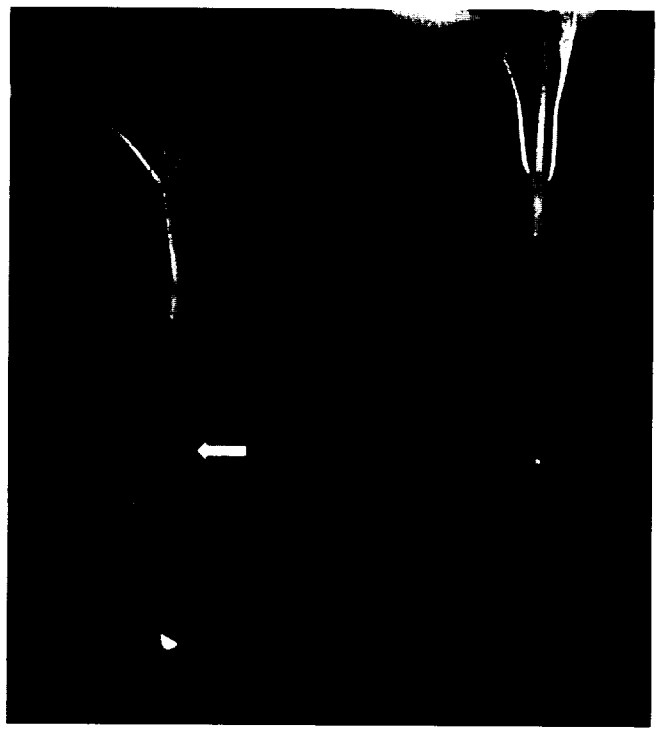

Fig 2. Induction of systemically induced resistance in pepper plants against late blight at 25 days after inoculation with P. capsici(1.0×10^4 zoospores/㎖). The left plant presented untreated control and the right plant colonized with *G. intrardices*. The arrow showed the lesion of the stem caused by the late bilhht pathogen in control plats.

compared with those in the other experiments and the plants were still not died at 25 days after inoculation (Fig. 3C). Remarkably, in the *G. intraradices* colonized plant the disease severity was more slowly developed compared with that of control plants (Fig. 2 and 3C). Eventually, the plants were stayed in healthy even until 25 days after inoculation (Fig. 3C) indicating resistance expression by colonization with *G. intraradices* against late blight.

DISCUSSION

The arbuscular mycorrhizal fungi (AMF) can enhance resistance / tolerance against plant pathogen by the improving plant health. It seems to be that AMF play a role in the regulation of plant nutrient transporter genes, which regulated by a feed-back mechanism (Burleigh and Bechmann, 2002). The plant reaches an optimal level of nutrition by the fungal symbionts (Burleigh and Bechmann, 2002). In this study, the colonized with one of the effective AMF *Glomus intraradices* on root system enhanced significantly the length of pepper plants (Table 1). Although there was no significant data, the weight of plants was also increased by the mycorrhiza-colonized plants in all of the experiments (Table 2). Furthermore, more number of plants was budded in the *G. intraradices*-colonized soil compare to the non-treated control one (Fig. 1). These results indicated that the growth of pepper plants might be improved by the colonization with *G. intraradices* in root

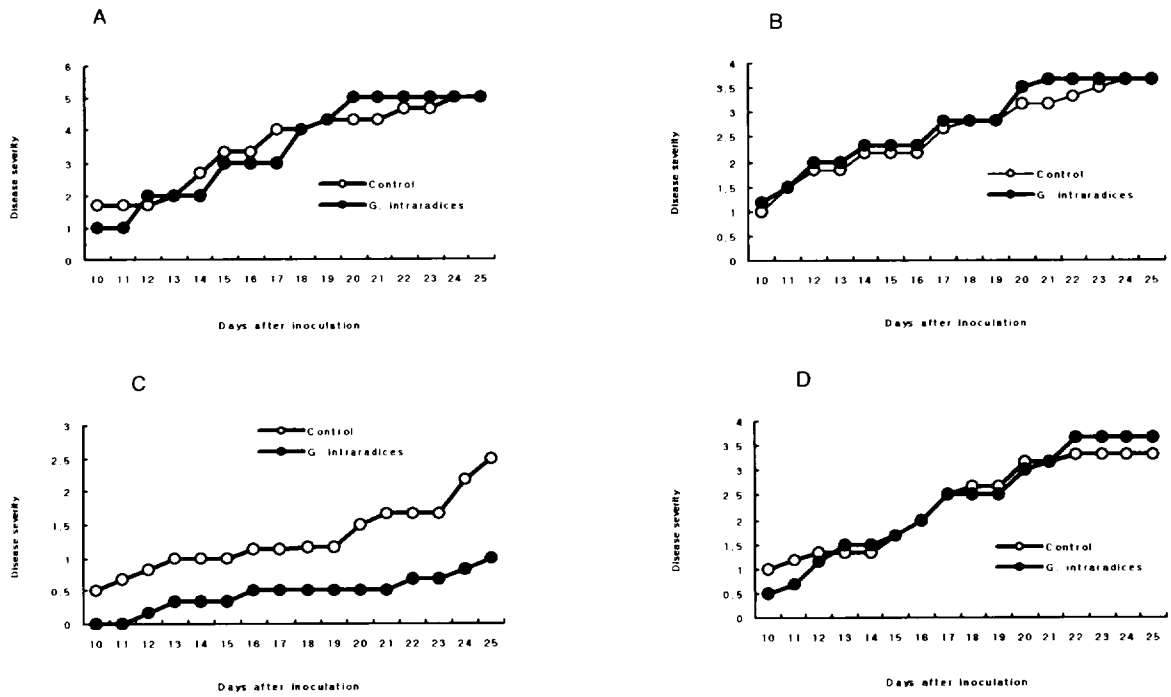

Fig 3. Disease progress caused by P. capsici(1.0×10^4 zoospores/㎖) in the pepper plants non-treated as well as colonized with *G. intrardices*. The level of disease severity was calculated from 0 to 5 by visible measure of lesion in the stem(see Material and methods). Each diagram presented the disease development of the experiment carried out separately.

system. However, although some studies have been performed on the interaction between the arbuscular mycorrhiza and root of plants (Garca-Garrido and Ocampo, 2002), the symbiotic mechanism of *G. intraradices* to pepper plants has been not clearly illustrated. On the other hand, in the AMF colonized plants resistance against pathogenic bacteria and fungi was enhanced (van Driesche and Bellows, 1996; Azcn-Aguilar and Barea, 1996). The efficacy of *G. intraradices* as well as *G. mosseae* on resistance to plant pathogen have been reported, which enhance plant resistance against *Phytophthora* disease in tomato plants (Pozo et al., 2002). In cucumber plants the colonization with *G. intraradices* in roots triggered resistance against anthracnose disease caused by *C. orbiculare* (Lee et al. submitted).

In this study the efficacy of *G. intraradices* to protect the late blight in pepper plants was investigated through 4 separate experiments. The disease severity was not reduced by the colonization with the mycorrhiza in three experiments (Fig. 3A, 3B and 3D). In all of dieses experiments the disease severity was reached to the level 4 at 25 days after inoculation in which the stem lesion progressed toward the shoot apex and 70-90 % of entire plants were diseased (Fig. 3A, 3B and 3D). Contrast to these results in one experiment the disease severity in the plants colonized with *G. intraradices* was dramatically reduced (Fig. 3C). Interestingly, the disease severity of non-treated control plants was not high as those of the plants in the other experiments (Fig. 3). From this results one can suggest that the resistance mediated by *G. intraradices* may be expressed

when the condition for disease development is not optimal. Also, the resistance may be not expressed consistently when the plants treated a biological resistance inducer or activator. Similar results have been already reported in our previous study, in which the resistance was not always expressed in the cucumber plants after treatment with a cell suspension of *Bacillus amylolquefaciens* whereas the resistance by amino salicylic acid was consistently expressed (Jeun et al. 2001).

In our cytological study it was attempt to illustrate the resistance mechanism mediated by colonized with *G. intraradices* against anthracnose caused by *Colletotrichum orbiculare* in cucumber plants (Lee et al. submitted). In cucumber plants colonized with *G. intraradices* more numerous autofluorescent cells at the penetration sites were found on the leaves of plants inoculated with *C. orbiculare* compared to those of untreated control plants. The autofluorescent cells indicate the active defense reaction such as callose formation of the host cells against fungal attack. Similar results were also observed in the leaves of cucumber plants pre-inoculated with plant growth promoting rhizobacteria (PGPR) in which the callose formation was enhanced (Jeun et al., 2004). The enhanced callose formation has been well known as a resistance mechanism in many host-parasite interactions (Strmberg and Brishammar, 1993; Kovats et al., 1991b). However, the resistance mechanism mediated by mycorrhiza has been not clearly explained and further research illustrating resistance mechanism mediated by mycorrhiza should be performed.

Summarily, the colonization with *G. intraradices* improved the growth of pepper plants and enhanced resistance against late blight disease when the environment condition was not optimal for disease development.

요 약

내생근균 *Glomus intraradices* 에 의한 고추식물의 성장촉진 효과 및 고추역병균에 대한 저항성 발현 여부를 조사하였다. 내생근균인*G. intraradices* (BEG110)의 근권정착에 의한 식물 성장 및 영양분 섭취 촉진 효과는 이미 알려져 있다. *G. intraradices* 이 정착된 고추는 무처리한 고추에서 보다 식물 길이가 높았으며 무게도 증가되어 성장 촉진효과가 뚜렷하게 나타났다. 그러나 *G. intraradices* 에 의한 고추역병균인 *Phytophthora capsici* 의 침입에 대해서는 저항성이 항상 발현되지는 않았다. 4회에 걸친 저항성 발현 실험에서 단 한번의 실험에서 고추역병균에 대한 저항성이 나타났고 3번은 무처리한 식물보다 역병억제가 나타나지 않았다. 특이하게 저항성이 발현된 실험에서는 다른 실험에서 보다 역병 발생 정도가 낮았다. 따라서 내생근균에 의한 저항성 발현은 병발생 정도와 상관관계가 있는 것으로 생각된다.

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