Thesis for the Degree of Master of Agriculture

Resistance Induction against Plant Diseases in Cucumber and Pepper Plants by Colonization with *Glomus intraradices*

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오이와 고추에서 *Glomus intraradices*의 근권정착에 따른 식물병에 대한 유도저항성

濟州大學校 大學院

農 學 科

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ABSTRACT

Symbiotic effect on the growth and resistance inductions mediated by a mycorrhiza *Glomus intraradices* on the leaves of pepper and cucumber plant were 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 severity of anthracnose disease caused by *Collectotirchum orbiculare* was significantly decreased on the leaves of cucumber plant colonized with *G. intraradices* compared with those of non-treated control plants. As a positive control, pretreatment with DL-3-aminobutyric acid (BABA) caused a remarkable reduction of the disease severity on the pathogen-inoculated leaves. To clarify the cause of the disease severity reduction, the infection structure were observed at the penetration sites on the leaves of plant inoculated with *Collectorichum orbiculare* using a fluorescence microscope. There were no significant differences in the frequency of either germination or appressorium formation of the plant pathogen between mycorrhiza colonized and non-treated plants. It was also the same on the BABA pre-treated plants. However, the frequency of callose formation was significantly high on the leaves of *G. intraradices* colonized plants compared to those of non-treated control plants 5 days after challenge inoculation. On the leaves of BABA treated plants callose formation was not significantly high than those of non-treated, although the disease severity was more strongly

suppressed. It was suggested that the resistance induced by colonization with *G. intraradices* might be related to the enhancement of callose formation at the penetration sites on the leaves invaded by the pathogen, whereas resistance BABA did not. The length of pepper plants colonized with *G, intraradices* was higher compared with those of non-treated control plants. Similarly the fresh weight of pepper plants was increased by the colonization with the mycorrhiza in root system. The resistance against late blight pathogen *Phytophthora capsici* was expressed by the colonization with *G. intraradices*. But resistance induction mediated by *G. intraradices* may be changed by the plants environment.

Ⅰ. INTRODUCTION

Plant can be resistance against plant disease when the plants exposed to the exogenous stimuli such as an invasion of plant pathogens, chemicals, or microorganisms (Somssich and Hahlbrock, 1998). Although the mechanisms of the resistance are not clearly explained yet, in some plant-pathogen interactions the signal pathways of the induced resistance were evidenced (Somssich and Hahlbrock, 1998). One of the mostly known mechanisms is defined as systemic acquired resistance (SAR), which is mostly triggered by pre-inoculation with pathogen to certain parts of plants (Somssich and Hahlbrock, 1998). Usually, SAR is expressed after hypersensitive reaction (HR) (Press et al., 1997) or by necrosis on the treated parts of the plants (Van Driesche et al., 1996). In most cases, signaling of SAR is dependent on the 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; Vigo et al., 2000).

Another form of resistance termed as induced systemic resistance (ISR) has been reported, in which the resistance mostly induced by pre-inoculation with plant growth promoting rhizobacteria (PGPR) (Van Loon, 1997). ISR is distinguished from the classical SAR by different signal pathway and resistance expression (Knoester et al., 1999; Pieterse et al., 1996; Pozo et al., 1999; Van Loon et al., 1997). In some

cases of plants expressing ISR, PR-proteins were not accumulated (Pieterse et al., 1996; Van Loon et al., 1998). Although neither HR nor necrosis is formed in the roots inoculated with PGPR, the aerial parts of plants become resistant against plant pathogen (Kloepper et al., 1980). Furthermore, the signaling of ISR is usually independent on the accumulation of SA (Van Loon, 1997). However, in the ethylene or jasmonic acid insensitive arabidopsis plants, ISR was not triggered of resistance by PGPR.

Some microorganism 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 resistance against pathogens (Azcón-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., 2002, 1996; Strömberg & Brishammar, 1993; Van Wees et al., 1997). The resistance mechanisms of symbiotic associations between plants and AMF has been reported in some of studies (Pozo et al., 2002). The pathogenesis-related proteins such as chitinases, chitosanase and β-1, 3-glucanases in roots of tomato plants colonized with mycorrizae were accumulated (Pozo et al., 2002, 1996, 1998), indicating the effective defense reaction by antifungal proteins. Furthermore, in bean and wheat plants colonized by *G. intraradices*, the catalase and peroxidase were

accumulated (Blee and Anderson, 2000). The increases of these enzymes are involved with defense mechanism against plant pathogen. However, the mechanisms of this type of resistance have not been clearly understood.

This study was to investigate, the protective effects by colonization with *G. intraradices* against cucumber anthracnose caused by *Collectotrichum orbiculare* and late blight caused by *Phytophthora capsici* and the beneficial effects of *G. intraradices* on growth of pepper plants. Furthermore, infection structures of the pathogen and defense response of plant were cytologically examined on the leaf surfaces of the cucumber plants colonized with *G. intraradices.* Additionally, the resistance mechanism by chemical inducer BABA was also discussed.

Ⅱ. MATERIALS AND METHODS

1. Plant and pathogen

Cucumber seeds (*Cucumis sativus* L, cv Eun Sung) were sown in a plastic tray (72 holes, diameter 4 ㎝) and pepper seeds (*Capsicum annuum* cv. *Manitja*) were sown in plastic pots (7-㎝ in diameter) filled with commercial soil (Choroc Nala®, Bokyung Nongsang, Korea) containing 10% of Perlite (Parat®, Sam Son, Korea). Cucumber and pepper seedlings were grown in a growth chamber with a day/night temperature of 28/25℃. Only in the case of pepper plants, 10 ㎖ of the complex fertilizer (Chamzonne®, Youngkwang, Korea) was drenched per plants according to the commercial usage every two weeks after the first leaf was appeared.

Colletotrichum orbiculare which cause anthracnose on cucumber plants were grown in green beans agar (GBA) medium for 5 days. GREEN BEANS® (SAHA PRACHINBURI FOODS INDUSTRY, THAILAND) was shattered by a electric mixer, added 20 g agar powder and then the volume was adjusted to 1 L with distilled water. To prepare the challenge inoculation, 10 ㎖ distilled water was poured in the GBA plate on which the anthracnose pathogen was grown and then the fungal conidia were harvested by using a brush. This conidial suspension (2.5×10^5 conidia / ㎖) with 100 $\mu\ell L^{-1}$ of Tween 20, which enhances the adhesion of conidia on leaf surface, was used as inoculum for challenge inoculation on cucumber leaves.

Phytophthora capsici which cause late blight on pepper plants was grown on V8 agar medium for 7-10 days at 25℃. To induce sporangium formation, the mycelia on plate were harvested with a spacula and exposed under fluorescent light at 28℃ for 3 days. And then 10 ㎖ H_2O $_{bidest}$ was added to the plate to initiate the zoospore release from sporangia and the plates were immediately placed in a refrigerator approximately at 4℃ until zoospores were released. The suspension containing zoospores was adjusted to 1.0×10^4 zoospores / ㎖ for the inoculation of pepper plants.

2. Propagation of the mycorrhiza fungus

The mycorrhiza fungus *Glomus intraradices* (BEG 110) were distributed by National Institute of Agricultural Science and Technology, RDA. *G. intraradices* was propagated several times on white clover grown on sterilized sbustrate (sand : vermiculite = 1 : 1) at 121℃ for 30 min. For propagation, the inoculum always comprised 10% (v/v) of the pot volume, where 50 g inoculum was mixed with 450 g substrate. Usually approximately 0.5 g clover seeds were sowed in the pot containing with the 10% inoculum. Pots were placed on a balance one or two times per day and watered with distilled water to maintain a soil water content equivalent to 65% of field capacity. After 6 weeks, a nutrient solution containing 2 mM $Ca(NO_3)_2$ was daily watered to compensate the nitrogen deficiency for the plants. Plants were grown in a greenhouse for ten weeks. Thereafter, the substrate was left to dry out (until plants wilted), re-irrigated and left to dry for the further two

weeks, both drying periods served to promote spore production. Roots and substrate were sieved to harvest the spores (1mm in diameter) and the air-dried substrate with spores and colonized root pieces were stored at 4℃ and used as inoculum. The length of roots colonized by mycorrhizal fungi was determined by staining with trypan blue Koske and Gemma (1989) using the gridline-intersect method (Giovannetti and Mosse, 1980).

3. Treatment with *Glomus intraradices* and DL-3-aminobutryic acid (BABA)

Ten% (v/v) of the soil containing mycelium of *G. intraradices* was mixed with the commercial soil, which was already sterilized at 100℃ for 1 h. The cucumber and pepper seeds were sown in the soil mixture in which *G. intraradices* was colonized.

Thirty mℓ of 10 mM BABA solution per plant was drenched in soil 5 days before challenge incoulation with C. *orbiculare*. As a negative control, water treated plants were used.

4. Challenge inoculation and assessment of anthracnose disease and late blight

The conidial suspension of *C. orbiculare* (2.5×10^5 conidia / mℓ) was sprayed on the aerial leaves of cucumber plants, 5 days after treatment with *G. intraradices* and BABA. The inoculated cucumber plants were kept in a humid chamber maintaining 100% RH for 24 h and then transferred into a growth chamber with a day/night temperature of

28/25℃ and a relative humidity of 60%.

The development of lesions on the inoculated leaves was determined 5 days after challenge inoculation by visual estimation of the leaf area occupied by anthracnose lesions. Protection rate was calculated as described by Cohen (1994), that is the rate (%) = 100 (1 - x / y) in which x and y are number of lesions on the leaves treated and non-treated plants, respectively.

The peppers plants of 4-leaf stage were inoculated with 70 ㎖ zoospor suspension of the concentration of *P. capsici* (1.0×10^4 zoospores / ㎖). The inoculated plants were kept at 100 RH in the dark for 24 h at 16℃ and then placed in a growth chamber at 80% RH and 18-20℃.

Disease development was recorded every day after challenge inoculated by visually estimating the stem length with late blight lesions. In principle, disease was evaluated according to the method described by Kim (1994). The disease severity on pepper plants was calculated based on the following indicates : 0 = no visible lesion, 1 = leaves slightly wilted with brownish lesions beginning to appear on stems, 2 = stem lesion extending for 1-3 ㎝ from the surface of the soil, 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, and 5 = deathly whole plant.

5. Observation of infection structures using fluorescent microscope

Leaves of the inoculated cucumber plants were detached 3 and 5 days after the challenge inoculation. The leaf tissues were stained according to the method described by Jeun et al. (2000). The leaves were cut with a cork borer (5-mm in diameter) and fixed with 2% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 2 h. After the fixation, the sections of leaves were washed in phosphate buffer for 10 min 3 times. In order to observe the plant defense response such as callose formation, the section were soaked in 0.005% aniline blue (w/v) for 20 min fallowing each wash with the phosphate buffer for 10 min 3 times each. For observation of the fungal structure, the sections were soaked in 0.02% Uvitex 2B (w/v) (Diethanol) for 20 min. After washing with phosphate buffer for 10 min 3 times the leaves were laid on a slide glass, mounted with the 50% glycerin and then covered with cover glass. The infection structures of the anthracnose fungus at the penetration sites were observed using fluorescent microscopy (Olympus) equipped with filter set 05 (BP 400-440, FT 460, LP 470). Numbers of conidia, germinated conidia and appressoria of fungi and autofluorescent plant cells under the appressorium were counted on the leaf surfaces of the plants non-treated, colonized with mycorrhizal and treated with BABA. The rate of appressorium formation and autofluorescent cells at the penetration sites were calculated from the data counted on the 4 leaf discs detached from each 4 plants in the 3 separated experiments.

6. Determination of symbiotic effects on pepper plants growth

To determine the symbiotic effect of *G. intraradices*, the length and the fresh weight of pepper plants were measured 60 days after sowing. The length was measured from the stem below the soil line to top of the plant. The weight was measured based on the whole plants except roots. These experiments were replicated three times separately and each contained 30 plants.

7. Data analysis

The lesion areas of the plant leaves, the germination rate and frequency of appressorium formation of the fungus, and fluorescent cells in the inoculated leaves were compared using a two sample *t*-test ($p = 0.001$) between untreated control and BABA or mycorrhiza treated. Also, The enhancement of length and fresh weight of pepper plants were analysed using a two sample *t*-test ($p = 0.05$) as the same manner above.

Ⅲ. RESULTS

1. Effects of colonization with arbuscular mycorrhiza on anthracnose severity

There was no visible difference on leaves between plants treated with *G. intraradices* and the untreated control (data not shown). Five days after challenge-inoculation, visible lesion spots were formed on the leaves of the control as well as. However, lesion area was significantly reduced in the leaves of the treated with the mycorrhiza compared with the control plants (Fig. 1 and 2). Furthermore, the size of lesions on the leaves of untreated plants was rapidly increased at 7 days after challenge inoculation, indicating rapid development of fungal growth. In contrast to the control plants, the development of anthracnose fungus was distinctly restricted on the leaves mycorrhizal plants (Fig. 2)

There were not visual difference in cucumber plant treated with BABA compared to that of control plants (data not shown). BABA was effective on mediating resistance against anthracnose in the aerial parts of the cucumber plants (Fig. 1 and 2). The protection rate by BABA treatment against anthracnose reached to approximately 95%, indicating that BABA could be an effective inducer of systemic acquired resistance (Table 1). In particular, the reduction of lesion area by BABA treatment was much higher than those by *G. intraradices* colonization (Fig. 1), indicating that defense reaction by chemicals may be much more effective than those induced by mycorrhizal colonization.

2. Microscopical observation on the leaf surface

Using fluorescence microscope the resistance expression was examined both on the leaf surface and in the epidermal cell layer of cucumber colonized with *G. intraradices* as well as treated with BABA. On the leaf surfaces of non-treated plants about 36.4% of total conidia were germinated and only 29.3% of total conidia formed appressoria 3 days after inoculation. Some conidia were germinated but failed to form appressoria. Most of appressoria formed melanin, which was identified as black spot under the microscope (Fig. 3). Most of the penetration sites were not intensively fluorescent, indicating no active defense reaction of the host cells (Fig. 3A).

On the leaf of plants colonized with *G. intraradices* some penetration sites became fluorescent 3 days after challenge inoculation, indicating the plant response to the fungal invasion of pathogen (Fig. 3C). However, there was no significant difference in callose formation, although by mycorrhizal colonization was slightly increased callose formation compare to those of control one (Fig. 4C). Similarly, there were no difference in germiantion rate of in appressorium formation between non-treated and both BABA and mycorrhizal treated plants (Fig. 4A and 4B).

Two days later, the conidia germination on the leaf surface of non-treated plants increased slightly while the conidia germination of both mycorrhizal and BABA treated plants were not increased (Fig. 4A and 5A). There was no significant difference in germination rate and appressorium formation between non-treated and both treated plants 5

days after inoculation (Fig. 5A and 5B). However, callose was more frequently formed at the penetration sites on the BABA treated as well as the *G. intraradices* colonized plants 5 days after challenge inoculation compare to those of 3 days, whereas no difference was found on the leaves of control leaves (Fig. 4C and 5C). In the BABA pre-treated plants the autofluorescence was very strong at the penetration site (Fig. 3B). Remarkably, the callose formation on the mycorrhizal-colonized plants was significantly increased compared to those of control plants (Fig. 5C), indicating an enhancement of defense reaction of the plants, The autofluorescence intensity was not stronger than that of at 3 days (Fig. 3C and 3D).

In contrast to the case of mycorrhizal plants, the BABA pre-treated plants had no significant difference in the frequency of the fluorescent cells compared with that of untreated plants (Fig. 4C and 5C), although fluorescent cells were slightly increased (Fig. 5C).

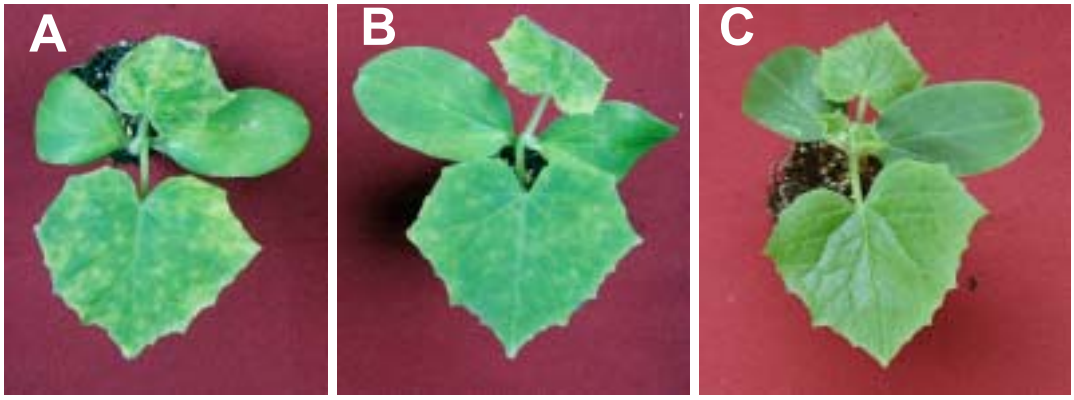

Fig. 1. Leaf symptom caused 7 days after inoculation with *C. orbiculare* (2.5×10^5 conidia / mℓ). A : untreated control plant, B : colonized with *G. intraradices*, C : treated with 30 mℓ of 10 mM BABA

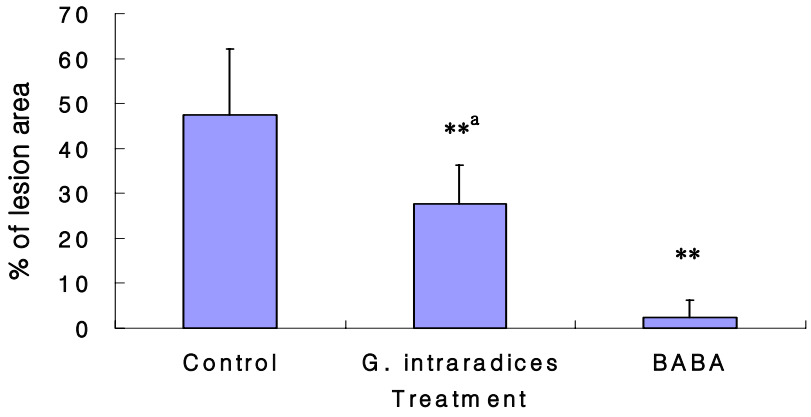

Fig. 2. Protective effects by colonization with *G. intraradices* and pre-treatment with BABA against anthracnose on cucumber plants. The lesion areas were measured 7 days after inoculation with *C. orbiculare* (2.5×10^5 conidia / ㎖). The vertical bars indicate the standard deviation of the 5 separated experiments each containing 12 plants per treatment.

[a] Two sample *t*-test comparing the untreated control with *G. intraradices* or BABA treatment (**, $P < 0.01$)

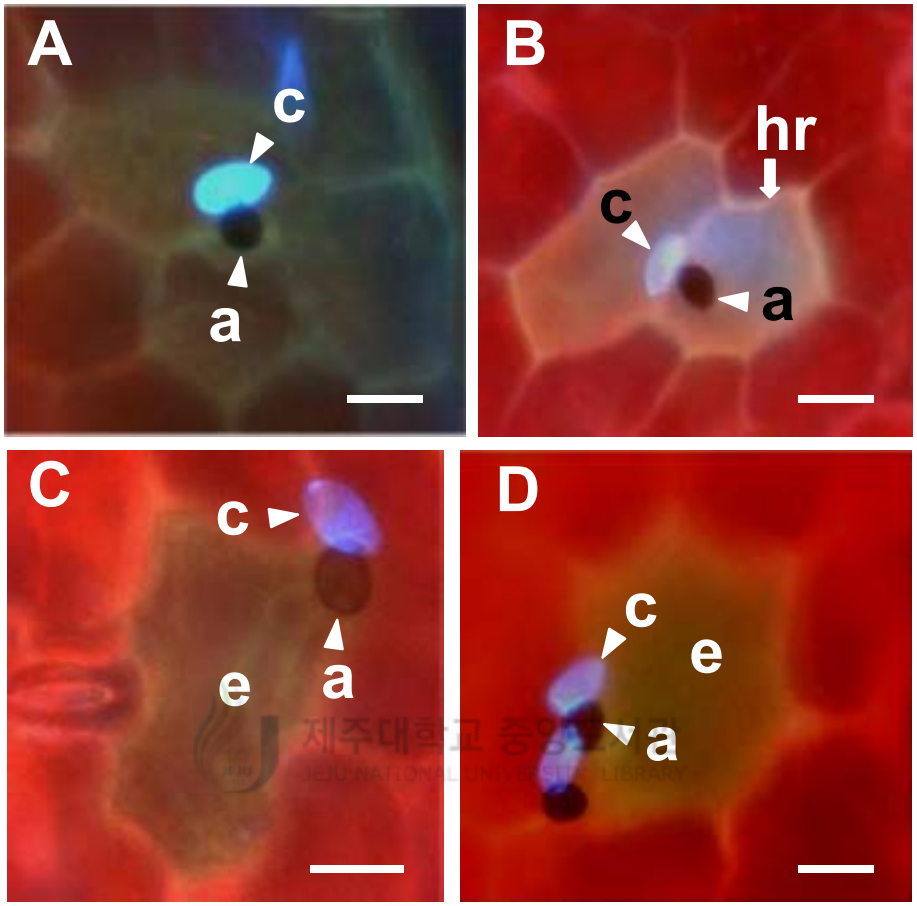

Fig. 3. Fluorescence microscopical observation of infection structures and resistance response on the leaves of the cucumber plants untreated (A), pre-treated with BABA (B) and colonized with *G. intraradices* at 3 days (C) and 5 days (D) after challenge-inoculation with *C. orbiculare*. The arrow shows plant cell fluorescent by staining with aniline blue and indicates a reaction of plant cell hypersensitively. The bars = 20 ㎛. Abbreviations: a, appressorium; c, conidium; e, epidermal cell; hr, hypersensitive reaction.

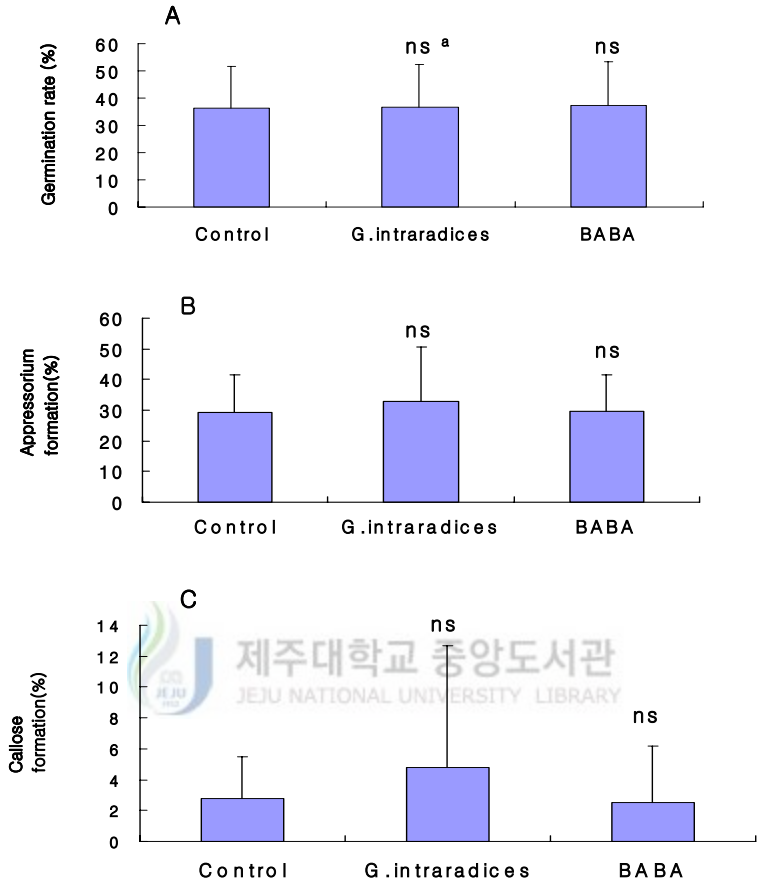

Fig. 4. Conidial germination, appressorium formation of *C. orbiculare* and callose formation of the plant cells on the leaves of cucumber plants untreated control, colonized with *G. intraradices* and pre-treated with BABA. The leaves were attached 3 days after challenge inoculation with *C. orbiculare* (2.5×10^5 conidia / ㎖). The vertical bars indicate the standard deviation of the 3 separated experiments each containing 4 leaf discs from 4 plants per treatment.

[a] Two sample *t*-test comparing the untreated control with *G. intraradices* or BABA treatment (ns, non significant)

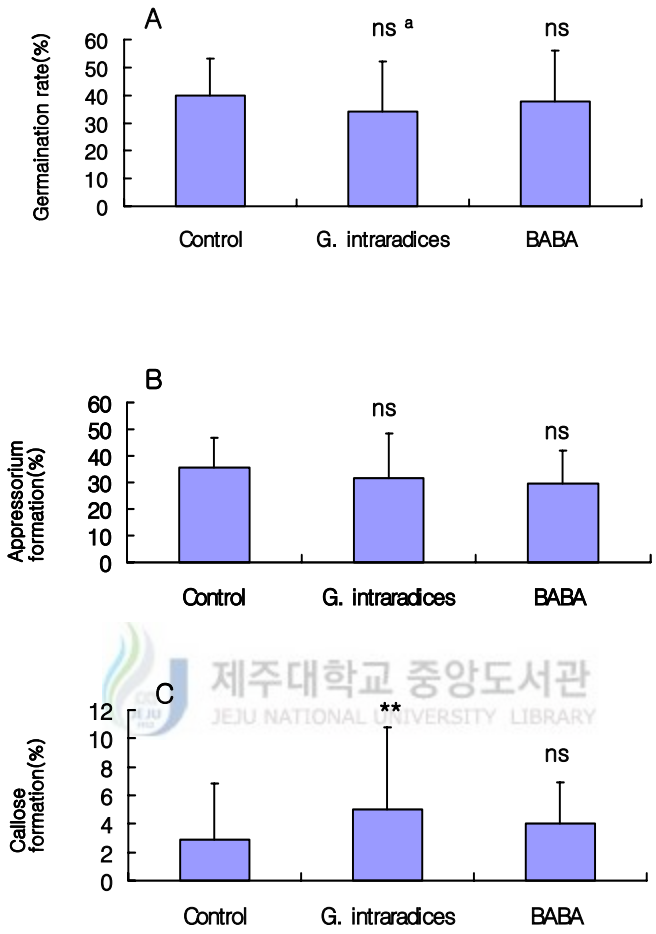

Fig. 5. Frequency of conidial germination, appressorium formation of *C. orbiculare* and callose formation of the plant cells on the leaves of cucumber plants untreated control, colonized with *G. intraradices* and pre-treated with BABA. The leaves were attached at 5 days after challenge inoculation with *C. orbiculare* (2.5×10^5 conidial / mℓ). The vertical bars indicated the standard deviation of the 3 separated experiments each containing 4 leaf discs from 4 plants per treatment.

[a] Two sample *t*-test comparing the untreated control with *G. intraradices* or BABA treatment (**, $P < 0.01$; ns, non significant)

Table 1. Protective rate on the leaves of cucumber plants treated with *G. intraradices* or DL-3-aminobutyric acid (BABA) 7 days after inoculation with *Colletotrichum orbiculare*

Treatment	Protection rate (%)[c]
G. intraradices[a]	41.6
BABA[b]	94.9

[a] The seeds of cucumber plant were sowed in the soil colonization with *G. intraradices* (see Materials and methods).

[b] Thirty mℓ of BABA solution (10 mM) were drenched on the soil 5 days before the challenge inoculation.

[c] Percentage were calculated by the formula, protection (%) = 100 (1 - x / y) in which x and y are % of lesion area on the leaves of treated and non-treated control plants, respectively. Values represent means of values ± standard deviation of 3 separated experiments each containing 12 plants per treatment.

3. Symbiotic effect of growth of pepper plants

There was no visible difference in form between the plants colonized with mycorrhiza and the control plants (Fig. 6). However, The seeds were more germinated in soil colonized with mycorrhiza than in non-treated soil. *G. intraradices* slightly enhanced pepper growth compared to those of control plants (Table 2 and 3), indicating the beneficial interaction between the mycorrhiza and plants. The length of plants was significantly increased in two experiments, and it in one experiment was also slightly increased although no significant (Table 2). Similarly, the fresh weight of plants the colonized with mycorrhiza was also slightly increased in all of experiments, although they were not significant (Table 3).

4. Inhibitive effect of *G. intraradices* against late blight of pepper plants

In this experiment the symptom of non-treated control pepper plants was apparently visible 10 days after inoculation with *Phytophthora cpasici*, in which the stem lesion extended for 1 ㎝ from the surface of soil. The length of diseased stem was gradually increased and 30-50% of entire plants were diseased 23 days after inoculation (Fig. 8). At 25 days the leaves were defoliated and 50-70% plants were died (Fig. 8). However, in the plants colonized with *G. intraradices* the leaves were slightly wilted and stems were healthy 10 days after inoculation (Fig. 8). Moreover the disease severity was more slowly developed compared with that of control plants (Fig. 8). Eventually, the plants were stayed in healthy even until 25 days after inoculation (Fig. 8) indicating resistance

expressed by colonization with *G. intraradices* against late blight.

Fig. 6. Effect on germination and growth of pepper plants by colonization with *G. intraradices* in root system. Right : The plants were grown in the soil colonized with *G. intraradices*, Left : non-treated control plants. The photo was taken at two month after sowing of the plants.

Fig. 7. The late blight symptom in pepper plants against late blight 25 days after inoculation with *P. capsici* (1.0 × 10^4 zoospores / mℓ). The left plant presented untreated control and the right plant colonized with *G. intraradices*. The arrow shows the late blight lesion caused by *P. capsici*.

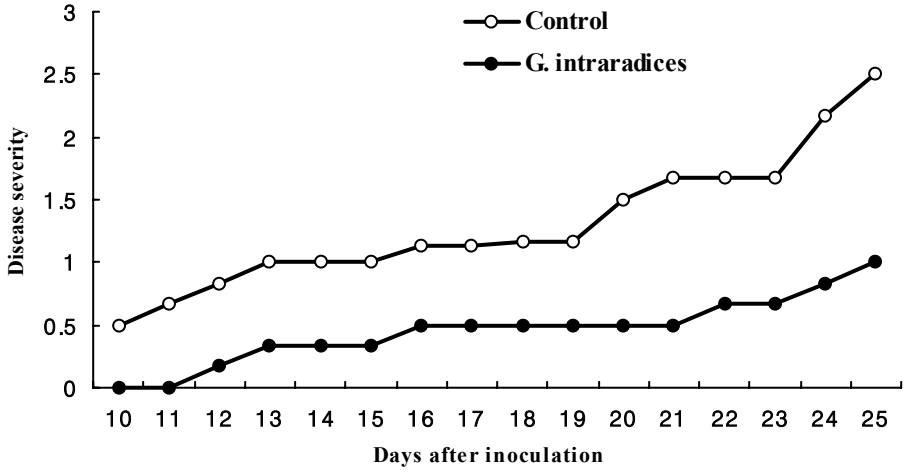

Fig. 8. Protection effects by colonization with *G. intraradices* against late blight diseases of pepper plants. Disease severity based on a 0 - 5 scale (see Material and methods).

Table 2. Enhancement of length of pepper plants by colonization with *G. intraradices* in root system

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

[a] Mean of values ± standard deviation of 30 plants.

[b] Two sample *t*-test (* = significant at the 5 % probability level; ns = non significant)

Table 3. Enhancement of the fresh weight of pepper plants by colonization with *G. intraradices* in root system.

Experiment	Fresh weight (g)	
	Control	*G. intraradices*
Ex. 1	2.8±0.8[a]	3.0±0.8 ns[b]
Ex. 2	2.1±0.6	2.3±0.6 ns
Ex. 3	2.8±0.5	3.1±0.9 ns

[a] Mean of values ± standard deviation of 30 plants.

[b] ns = non significant

Ⅳ. DISCUSSION

The AMF can improve not only the growth but also enhance resistance against the invasion of plant pathogens. That is, the AMF plays an important role in the regulation of plant nutrient transporter genes, which is regulated by a feedback mechanism (Burleigh and Bechmann, 2002), and enhancement of the plant resistance / tolerance against biotic stress including pathogenic bacteria and fungi in the AMF colonized plants (Trotta et al., 1996; Azcón-Aguilar and Barea, 1996). It has been reported that *G. intraradices* and *G. mosseae* enhanced plant resistance against *Phytophthora*-induced diseases in tomato plants (Pozo et al., 2002). In the present study *G. intraradices* triggered resistance against anthracnose disease of cucumber plant by colonization in root (Fig. 1 and 2), while *G. mosseae* did not (data not shown).

It has been reported that BABA had not antimicrobial activity against fungal pathogen such as *Phytophthora infestans* (Cohen, 1994) as well as bacterial pathogen *Pseudomonas paraitica* (Woloshuk et al., 1991). In our study, the BABA did not directly effect on the anthracnose pathogen, *C. orbiculare in vitro* test (data not shown). The BABA reduced the anthracnose on cucumber plants by soil drenching, indicating the induction of resistance (Fig. 1 and 2).

In this study, the lesion area was decreased by approximately 42% in the plants colonized with *G. intraradices*, whereas about 95% of lesion area was decreased by the application of BABA (Table 1). Based on

this result it can be suggested that an abiotic activator may induce resistance more effectively compare to those of a biotic inducer such as mycorrhiza. Similar results have been observed in cucumber plants e.g. the pre-treatment with plant with promoting rhizobacteria (PGPR). PGPR caused 40% reduction of lesion number of anthracnose in cucumber plants, while about 85% of lesion number was decreased by the application of BABA (Jeun et al., 2004).

We attempted cytological study to illustrate the resistance mechanism mediated by colonization with arbuscular mycorrhiza such as *G. intraradices*, and to compare to those of resistance by chemical BABA. The rate of conidial germination may have been a criterion of expressing resistance in much plant-fungal pathogen interactions (Kovats et al., 1991a). In this study, there was no differences in germination rate between resistance induced and non-treated susceptible plants (Fig. 4A and 5A), indicating no role of conidial germination in expressing resistance.

Like some other fungi, the anthracnose fungus forms an appressorium, which is structurally differentiated from a conidium, in order to penetrate the host cell walls. In some plant-pathogen interactions, the formation of appressorium may be enhanced by a certain compound excreted from the host (Hwang and Kolattukudy, 1995; Lee and Dean, 1993). Because anthracnose fungi cannot penetrate into the host cells without formation of appressorium, the plant may acquire resistance against anthracnose by the suppression of appressorium formation. Indeed, the reduction of appressorium formation has been demonstrated

in the resistance expressing leaves of cucumber plants (Kovats et al., 1991a). However, in this study, appressorium formation did not suppressed on the leaves of plants colonized with *G. intraradices* (Fig. 4B and 5B). Nevertheless, resistance against cucumber anthracnose was triggered by the colonization with the mycorrhiza (Fig. 2), and indicating that some resistance mechanisms other than the suppression of appressorium formation may be involved in the expression of resistance induced by *G. intraradices*.

Numerous autofluorescent cells were detected at the penetration sites on the leaves of the plants colonized with *G. intraradices* compared to those of untreated control plants at 5 days after challenge inoculation (Fig. 5C). Although it was not significant, the callose formation on plants colonized with *G. intraradices* was higher than that of control plants 3 days after challenge inoculation (Fig. 4C). The autofluorescent cells indicate the active defense reaction against fungal attack similar to the callose formation of the host cells. The enhanced callose formation has been well known as a resistance mechanism in many host-parasite interactions (Sticher et al., 1997; Kovats et al., 1991b). Similar results were observed in cucumber plants, in which the callose formation was enhanced on the leaves of cucumber plants pre-inoculation with PGPR (Jeun et al., 2004). In contrast to the mycorrhiza, there was no difference in callose formation between the BABA treated and non-treated plants (Fig. 4C and 5C), indicating the resistance expressing by BABA without the thickening of cell walls by callose formation.

On the bases of the results of cytological observations, it is

suggested that the callose deposits at the penetration site, one of the defense responses of the plant cells against pathogen, may be play an important role for expressing a resistance against anthracnose in the cucumber plants colonized with *G. intraradices*. In plants treated with BABA, however, callose deposits was not important for expressing resistance although the disease severity was more effectively suppressed by BABA. These different resistance expressions may be caused the different protection values by *G. intraradices* colonized plant and BABA treated plant. It could be also involved in the other defense responses such as the production of anti-fungal substance phytoalexin (Siegrist et al., 2000), the accumulation PR-proteins (Hwang et al.,1997; Jeun, 2000), and encoding of enzymes involved in the metabolism of reactive oxygen species (Lamb and Dixon, 1997). To confirm this hypothesis, further investigations are required at the biochemical level.

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 (Stroömberg 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.

In this study, *Glomus intraradices* enhanced significantly the length of pepper plants by colonization in root system (Table 2). Although there was no significant data, the fresh weight of plants was also increased by the mycorrhiza-colinized plants in all experiments (Table 3). Furthermore, germination was increased in the *G. intraradices*-colonized soil compare to the non-treated control one (Fig. 6). These results indicated that the growth of pepper plants might be improved by the colonization performed on the interaction between the arbuscular mycorrhiza and root of plants (García-Garrido and Ocampo, 2002). though the symbiotic mechanism of *G. intraradices* to pepper plants has been not clearly illustrated.

In this study the protection efficiency of *G. intraradices* against the late blight of pepper plants was investigated. The disease severity in the plants colonized with *G. intraradices* was reduced compared to that of non-treated plants (Fig. 8). However the plants colonized *G. intraradices* seems to be not always resistance. It has been well known that microorganism fails to induce resistance in certain environment such as field (Weller, 1988).

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 previous treatment with a cell suspension of *Bacillus amylolquefaciens* whereas the resistance by amino salicylic acid was consistently expressed (Jeun et al., 2001).

Ⅴ. 適 要

내생근균인 *Glomus intraradices*에 의한 고추에서의 성장촉진 효과 및 고추 역병과 오이탄저병균에 대한 저항성 발현 여부를 조사하였다. *Glomus intraradices* (BEG110)는 내생근균 중의 하나이며, *G. intraradices*의 근권정착에 의한 식물 성장 및 영양분 섭취 촉진 효과는 이미 알려져 있다. *C. orbicular*에 의하여 발생하는 탄저병 발생정도는 무처리 대조구와 비교하였을 때 *G. intraradices* 가 근권에 정착한 오의 잎에서 현저하게 감소되었다. 또한 DL-3-aminobutyric acid (BABA)를 전처리한 양성 대조구의 오이 잎에서는 병 발생 정도가 두드러지게 감소되었다. 병발생 감소의 원인을 알고자 *Collectotrichum orbiculare*를 접종한 오이 식물체 잎의 관통조직에서 병원균의 침입 기작을 형광현미경을 통하여 관찰한 결과 내생근균을 처리한 오이와 무처리 대조구 오이 사이에서의 탄저병균의 발아율과 부착기 형성율은 유의적 차이가 없었다. 또한 BABA를 전처리한 오이에서도 대조구와 비교 하였을 때 동일한 결과를 보였다. 그러나 *G. intraradices*가 근권에 정착된 오이 잎에서의 callose 형성율이 대조구의 callose 형성율과 비교하였을 때 유의적으로 높게 나타났다. BABA를 처리한 오이에서의 병발생 정도는 아주 강하게 억제 되지만, BABA 처리 오이에서의 callose 형성율은 무처리 대조구에 비하여 유의적 차이가 없었다. *G. intraradices*가 근권에 정착함으로써 유도된 저항성은 병원균에 의하여 침입을 받은 잎의 관통조직에서의 callose 형성율의 향상과 연관되어 있는 것으로 사료된다. 반면 BABA의 의한 저항성 유도는 callose 형성율 증가가 아닌 다른 기작에 의한 것으로 판단된다. *G. intraradices*가 근권에 정착한 고추의 초장은 무처리한 고추에서보다 길이가 길었으며 생체중도 증가하여 성장촉진 효과가 뚜렷하게 나타났다. 또한 *G. intraradices*에 의한 고추역병

균인 *Phytophthora capsici*의 침입에 대해서도 저항성이 발현되었다. 그러나 *G. intraradices*에 의한 저항성의 발현은 식물환경에 의해 달라질 수도 있으며 이에 대한 연구가 필요하다고 생각된다.

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