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Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-ß-amino-n-butyric acid

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Abstract

Treatment of pepper plants with the nonprotein amino acid, DL-ß-amino-n-butyric acid (BABA) induced resistance to subsequent infection by *Phytophthora capsici*. In contrast, the α-, and γ-isomers of aminobutyric acid were ineffective as inducers of resistance. A relatively high concentration of BABA at 1,000 μg ml^{-1}, which had no antifungal activity *in vitro* against *P. capsici*, was required to induce resistance against Phytophthora blight with a foliar and stem spray, thus leading to complete control of the disease. About 1 day interval between BABA-treatment and challenge inoculation was sufficient to induce resistance in pepper plants. High inoculum levels of *P. capsici* caused Phytophthora development slowly in pepper stems treated with BABA, especially at early plant growth stage, which suggests that the induced resistance in pepper plants may be more quantitative rather than qualitative. BABA applied to the root system also protected pepper stems from *P. capsici* infection.

Introduction

Localized infection of certain plants by pathogens can induce resistance to subsequent pathogen attack either locally in infected area or systemically in noninfected area of plants. This resistance is directed against not only the same pathogen but also other unrelated fungal, bacterial, and viral pathogens. Induced resistance in a variety of host-pathogen systems is a well-documented phenomenon (Kuć, 1982). In our previous study, we demonstrated that resistance to a virulent isolate of *Phytophthora capsici* was induced in pepper plants by inoculation earlier or simultaneously with an avirulent isolate (Hwang and Kim, 1992).

Induction of resistance can also be attained by abiotic inducers. The chemicals exogenously applied as abiotic inducers have been known to be polyacrylic acid (Gianinazzi and Kassanis, 1974), acetylsalicylic acid (White, 1979), salicylic acid (White, 1979), and 2,6-dichloroisonicotinic acid (Métraux et al., 1991; Uknes et al., 1992). DL-ß-amino-n-butyric acid has also been implicated as an inducer of resistance in tomato against *Phytophthora infestans* (Cohen, 1993; Cohen et al., 1994) and in tobacco against *Peronospora tabacina* (Cohen, 1994). Tomato plants sprayed with DL-ß-amino-n-butyric acid were protected against a challenge infection with *P. infestans*. DL-α-amino-n-butyric acid was half as effective compared with DL-ß-amino-n-butyric acid, whereas DL-γ-amino-n-butyric acid was ineffective against the blight. In earlier study, Papavizas (1964) showed that DL-ß-amino-n-butyric acid applied to the soil controlled *Aphanomyces* root rot in pea plants. More recently, Cohen and Gisi (1994) provided evidence that the systemic resistance induced by DL-ß-amino-n-butyric acid against late blight in tomato is associated with the systemic acropetal translocation of the compound in the plant. Since this compound exhibits no fungicidal activity *in vitro* against pathogens (Cohen, 1993, 1994), it represents a new group of chemicals capable of inducing resistance against disease.

As far as we know, no studies have been reported on the induction of resistance against *P. capsici* infection in pepper plants by DL-ß-amino-n-butyric acid. In the

present study, therefore, we examined whether or not pepper plants can be protected against Phytophthora blight by treatment with aminobutyric acid isomers.

Materials and methods

Plant and fungus

Pepper (*Capsicum annuum* L.) cv. Hanbyul was used in the studies. Pepper seeds were sown in a plastic tray (55×35×15 cm) containing steam-sterilized soil mix (peat moss, perlite, and vermiculite, 5:3:2. v/v/v), sand, and loam soil (1:1:1, v/v/v). Four pepper seedlings at four-leaf stage were transplanted to a small plastic pot (5×15×10 cm) containing soil mix, sand, and loam soil (1:1:1.2, v/v/v). Pepper plants were raised in a growth room at 25 ± 2 °C with 5,000 lux illumination for 16 h a day.

All experiments were done with the virulent isolate S197 of *Phytophthora capsici*. The fungus was grown on oatmeal agar plates at 28 °C in the dark for 7 days and then induced to sporulate under fluorescent light at 28 °C. After incubation of culture plates in sterile water for 40 min at 4 °C and then 30 min at room temperature, zoospores released from the sporangia of *P. capsici* were collected by filtering through two layers of cheesecloth. The concentration of zoospores was adjusted to 10^5 zoospores per milliliter using a hemacytometer, unless stated otherwise.

Inducer treatment, challenge inoculation, and disease assessment

Three isomers of aminobutyric acids such as DL-α-amino-n-butyric acid (AABA), DL-β-amino-n-butyric acid (BABA), and γ-amino-n-butyric acid (GABA) (Sigma) were used as chemical inducers. These aminobutyric acids were dissolved in water. Pepper plants were uniformly sprayed at various time intervals with aqueous solution of the chemical using a glass atomizer. Control plants were sprayed with water. Soil drench with 30 ml of chemical solution was done in a plastic pot (5×15×10 cm) containing four pepper plants. In the most experiments, eight plants were used per treatment. The plants treated with chemical solution were placed in a growth room until they were challenge-inoculated.

To evaluate systemic protection of pepper plants, BABA was applied to the bare roots of 8-leaf and first-branch plants. Plants were removed from pots with soil intact and their roots gently washed in large volumes of water. The soil-free roots only were then dipped in BABA solutions for 6 h. The plants treated with BABA via the root system were transplanted into the pots and challenged 4 days later.

The challenge inoculation with *P. capsici* was conducted at various time intervals after spray, a soil drench or root dipping with the chemical. A small piece of sterile cotton was soaked in a zoospore suspension and placed about the base of each stem at 1.5 cm from the soil surface. The inoculated sites were then covered with plastic tape to maintain the moist condition needed for penetration of the fungus into the stem tissue. The challenge-inoculated plants were placed in a growth room at 25 ± 2 °C.

Disease severity (DS) of Phytophthora blight in pepper plants was rated daily after inoculation of *P. capsici* based on a 0–5 scale: where 0 = no visible disease symptoms; 1 = leaves slightly wilted with brownish lesions beginning to appear on stems; 2 = 30–50 % of entire plant diseased; 3 = 50–70 % of entire plant diseased; 4 = 70–90% of entired plant diseased; 5 = plant dead. Time course changes in DS and differences in DS of control-inoculated plants and of treated-inoculated plants were examined. Percentage protection was calculated as % protection = 100(1–x/y), where x and y are disease severity ratings in treated-challenged and control (untreated-challenged) plants, respectively.

Results

DL-α-Amino-n-butyric acid (AABA), DL-ß-amino-n-butyric acid (BABA), and γ-amino-n-butyric acid (GABA) were not phytotoxic at 1,000 μg ml^{-1} when treated as a foliar spray to pepper plants (no data presented). However, a foliar spray of BABA produced a slight chlorosis in the upper young leaves at 2,000 μg ml^{-1}, whereas the other aminobutyric acids, AABA and GABA were not phytotoxic at this concentration. The three isomers of aminobutyric acid did not inhibit mycelial growth and zoospore germination of *P. capsici in vitro* at concentrations up to 1,000 μg ml^{-1} (no data presented).

Effect of the three isomers of aminobutyric acid on pepper-*P. capsici* interactions was evaluated in these experiments. Figure 1 shows the magnitudes of disease severity symptoms and degree of protection achieved in pepper plants (cv. Hanbyul) challenge-inoculated with *P. capsici* 4 days after treatment with each isomer of aminobutyric acid. In GABA-treated plants, disease developed similarly to or more quickly than untreated

control plants, indicating that there was no effect of protection. Less disease developed in AABA-treated than in the control plants. The disease development declined slightly at 1,000 and 2,000 μg ml^{-1}, so that about 30 and 40% protection was achieved, respectively. In BABA-treated plants, however, disease development declined remarkably, so that BABA provided about 26 and 30% protection at 100 and 500 μg ml^{-1}, respectively and 100% protection at 1,000 and 2,000 μg ml^{-1}. However, the leaves of pepper plants at first-branch stage became yellow with brown spots, when treated with 2,000 μg ml^{-1} BABA. In general, treatments with BABA at 1,000 μg ml^{-1} completely protected pepper plants at first or second-branch stage from *P. capsici* infection, as against AABA and GABA which induced no protection against the disease at this concentration (Figure 2).

Protection of pepper plants against *P. capsici* infection by treatment with BABA was markedly affected by growth stages of pepper plants (Figure 3). When BABA was applied to the pepper plants at four-leaf stage, *P. capsici* infection developed slowly as compared to the untreated-, AABA-, or GABA-treated plants. BABA also induced the strongest protection (90%) at eight-leaf stage, but provided complete protection from disease at second-branch stage. In contrast, other isomers of aminobutyric acid, AABA and GABA, did not reduce *P. capsici* infection on pepper plants at all growth stages tested.

The effect of time interval between chemical treatment and challenge inoculation on the protection against *P. capsici* infection was examined in pepper plants challenged at different days after treatment with three isomers of aminobutyric acid (Table 1). The results showed that BABA was very effective in protecting pepper plants against *P. capsici* infection in all different time intervals. In particular, its protection effects gradually increased with the elapse of time after BABA treatment. In contrast, the other aminobutyric acids, AABA and GABA, were not effective at the same treatments. As shown in Figure 4, treatments with BABA at 1,000 μg ml^{-1} 4 and 7 days before challenge inoculation induced high levels of protection (85–100%) against the disease, but the treatment 1 day before challenge inoculation provided moderate protection of about 47%. BABA provided no significant protection by applying 10 and 100 μg ml^{-1}, irrespective of inducing time before challenge inoculation.

A soil drench technique was employed to introduce the resistance-inducing BABA into the tissues of pepper plants (Table 2). In general, soil drench with BABA at different concentrations did not induce resistance to Phytophthora blight in pepper plants at first-branch stage. Early after challenge inoculation, the Phytophthora disease occurred uniformly in pepper plants independently of doses of BABA. Six days after challenge inoculation, disease development in BABA-treated plants became somewhat slower than in untreated plants. Disease development in BABA-treated plants was found to be similar to untreated plants at different concentrations except 2,000 μg ml^{-1}, 8 days after challenge inoculation.

The ability of root applications of BABA to protect pepper plants against *P. capsici* infection is shown in Figure 5. BABA applied to the bare roots protected pepper plants at first-branch stage from *P. capsici* infection, but not at eight-leaf stage. The level of protection at first-branch stage increased with increasing the concentration of BABA. Application of pepper roots with 2,000 μg ml^{-1} BABA induced some phytotoxicity on pepper leaves.

Concentration of zoospores of *P. capsici* distinctly affected development of Phytophthora blight in young plants of four-leaf stage treated with various doses of BABA (Figure 6). After challenge inoculation with relatively low inoculums of 10^2, 10^3, and 10^4 ml^{-1} zoospores, high levels of protection against the disease were achieved in young pepper plants treated with various doses of BABA. However, challenge inoculation with higher inoculums of 10^5 and 10^6 zoospores showed low levels of protection (about 10–25%) in BABA-treated plants, as compared to the low inoculum challenge.

Discussion

Induced resistance can protect plants against pathogen infection, as well demonstrated in a variety of host-pathogen systems (Ouchi, 1983; Kessman et al., 1994). Such resistance may be elicited in plants by virus, bacteria, fungi, or abiotic chemical agents. In pepper-*P. capsici* system, our earlier studies revealed that a previous or simultaneous inoculation with an avirulent isolate of *P. capsici* could induce resistance against subsequent inoculation with a virulent isolate (Hwang and Kim, 1992). These findings suggested that the induced resistance mechanism may act effectively in controlling Phytophthora blight in pepper plants.

Little information is available on the abiotic agents that induce resistance in pepper against *P. capsici*. We report here that immunization of pepper plants

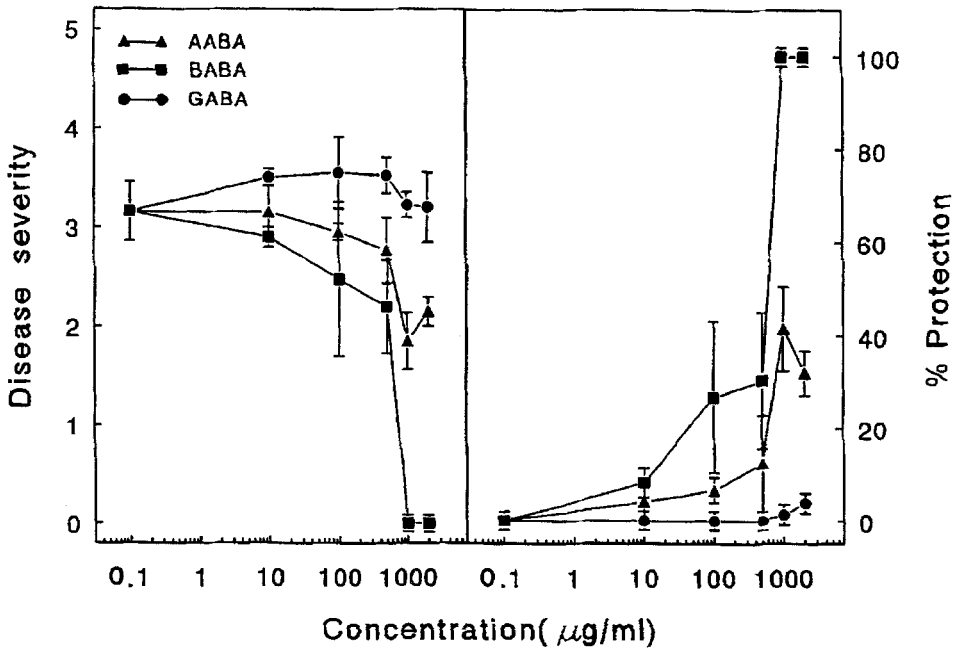

Figure 1. Protection of pepper plants (cv. Hanbyul) against *Phytophthora capsici* infection by various doses of DL-α-amino-n-butyric acid (AABA), DL-ß-amino-n-butyric acid (BABA), and γ-amino-n-butyric acid (GABA). Plants at first-branch stage were uniformly sprayed with various doses of compounds and challenged 4 days later. Disease severities were recorded 8 days after challenge inoculation. Vertical bars represent standard deviations.

Table 1. Effect of time of chemical treatment on protection of pepper plants (cv. Hanbyul)[a] against *Phytophthora capsici* infection by DL-α-amino-n-butyric acid (AABA), DL-β-amino-n-butyric acid (BABA), and γ-amino-n-butyric acid (GABA)

Days after chemical treatment	Disease severity[b] (mean ± SD)			
	Water	AABA	BABA	GABA
1	4.7 ± 0.18 *a*	4.9 ± 0.19 *a*	2.5 ± 0.87 *b*	4.9 ± 0.28 *a*
4	4.7 ± 0.19 *a*	4.8 ± 0.25 *a*	0.7 ± 0.80 *ab*	4.7 ± 0.34 *a*
7	4.7 ± 0.18 *a*	4.8 ± 0.25 *a*	0.0 ± 0.00 *b*	4.7 ± 0.29 *a*
10	4.7 ± 0.18 *a*	4.6 ± 0.29 *a*	0.0 ± 0.00 *b*	4.9 ± 0.09 *a*
15	4.7 ± 0.17 *a*	4.7 ± 0.29 *a*	0.0 ± 0.00 *b*	4.9 ± 0.10 *a*

[a] Pepper plants at eight-leaf stage were uniformly sprayed with 1,000 μg ml^{-1} of aminobutyric acid at different time intervals before challenge inoculation with *P. capsici*.

[b] Disease severities were recorded 12 days after challenge inoculation. Values for disease severity followed by the same letter in the same column are not significantly different at the 5% level according to Duncan's multiple range test.

by treating with DL-ß-amino-n-butyric acid induces resistance to subsequent challenge with normally virulent *P. capsici* isolate. The nonprotein DL-ß-amino-n-butyric acid had no inhibitory activity against zoospore germination and mycelial growth of *P. capsici in vitro*, but effectively inhibited Phytophthora blight in pepper plants. A comparison made among the three isomers of aminobutyric acid revealed that only the ß-isomer was highly effective as an inducer of resistance against *P. capsici* in pepper whereas the α- and the γ-isomers were ineffective. These results were in agreement with the previous findings of Cohen (1993, 1994), who showed that DL-ß-amino-n-butyric acid, as an inducer of local and systemic resistance, strongly protected tomato and tobacco plants against the oomycetes fungi *Phytophthora infestans* and *Peronospora tabacina*, respectively.

Figure 2. Effect of spray treatments (1,000 μg ml^{-1}) with water (CK), DL-α-amino-n-butyric acid (AABA), DL-ß-amino-n-butyric acid (BABA), and γ-amino-n-butyric acid (GABA) on *Phytophthora capsici* infection in pepper plants (cv. Hanbyul) at second-branch stage. Photograph was taken 6 days after challenge inoculation.

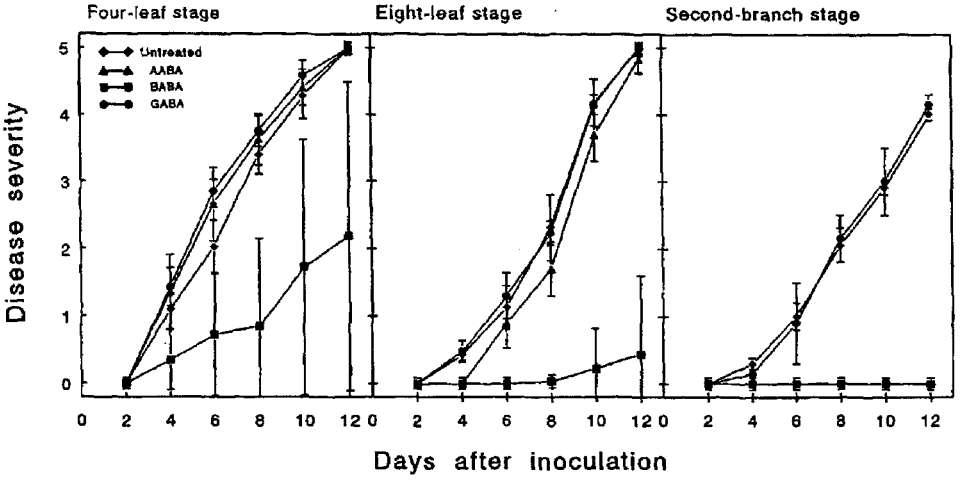

Figure 3. Effect of plant growth stages on protection of pepper plants (cv. Hanbyul) against *Phytophthora capsici* infection by DL-α-amino-n-butyric acid (AABA), DL-ß-amino-n-butyric acid (BABA), and γ-amino-n-butyric acid (GABA). Plants at different growth stages were uniformly sprayed with 1,000 μg ml^{-1} of compounds and challenged 4 days later. Vertical bars represent standard deviations.

A relatively high concentration of DL-ß-amino-n-butyric acid was required to induce resistance against Phytophthora blight with a foliar and stem spray, thus leading to complete control of the disease. However, a much lower concentration of the amino acid induced only a weak to moderate resistance against Phytoph-

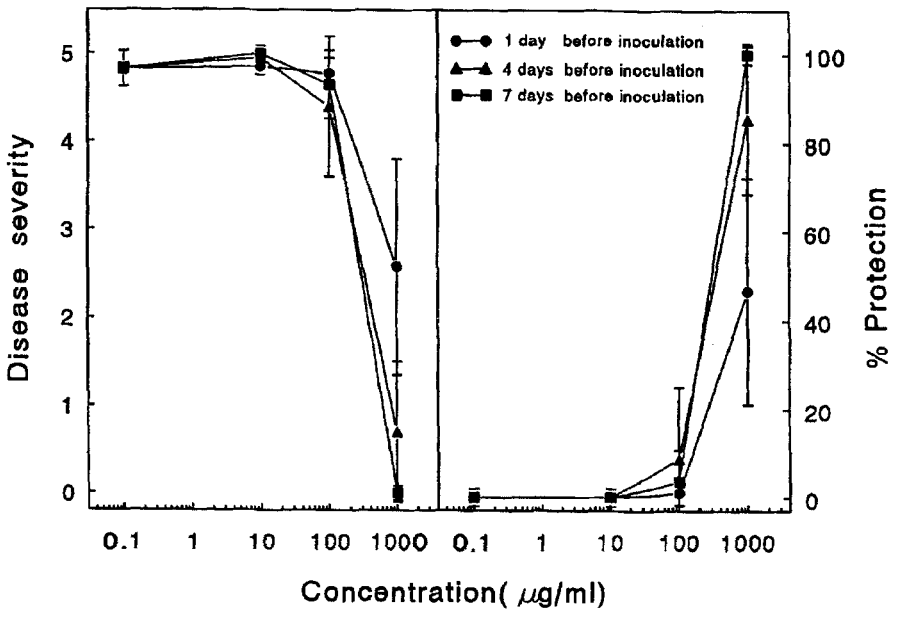

Figure 4. Effects of chemical treatment before challenge-inoculation on protection of pepper plants (cv. Hanbyul) against *Phytophthora capsici* infection by various doses of DL-ß-amino-n-butyric acid. Plants at eight-leaf stage were uniformly sprayed with various doses of the compound 1, 4, and 7 days before inoculation and then challenged. Disease severities were recorded 12 days after challenge-inoculation. Vertical bars represent standard deviations.

Table 2. Effects of a soil drench with different doses of DL-β-amino-n-butyric acid on *Phytophthora capsici* infection in pepper plants (cv. Hanbyul) at first-branch stage[a]

Chemical concentration	Disease severity (mean ± SD, days after inoculation)		
	6 days	8 days	10 days
Water(control)	1.4 ± 0.47 *a*[b]	2.7 ± 0.45 *ab*	3.7 ± 0.28 *bc*
10 μg ml^{-1}	1.2 ± 0.34 *abc*	3.0 ± 0.69 *a*	4.4 ± 0.40 *a*
100 μg ml^{-1}	1.3 ± 0.34 *ab*	2.9 ± 0.50 *a*	4.0 ± 0.25 *ab*
1000 μg ml^{-1}	0.9 ± 0.15 *bc*	2.1 ± 0.60 *b*	3.4 ± 0.66 *c*
2000 μg ml^{-1}	0.8 ± 0.26 *c*	2.5 ± 0.50 *ab*	3.7 ± 0.30 *bc*

[a] Pepper plants at first-branch stage were treated by a soil drench with DL-ß-amino-n-butyric acid and 4 days later challenged with *P. capsici.*

[b] Figures in columns followed by the same letter are not significantly different at the 5 % level according to Duncan's multiple range test.

thora blight in pepper plants. In contrast, the treatment with 2,000 μg ml^{-1} of DL-ß-amino-n-butyric acid caused some phytotoxicity on pepper leaves with yellowish and brown spots, although completely controlling the Phytophthora disease.

Plant growth stages of pepper affected greatly the induction of resistance against *P. capsici* achieved by treatment with DL-ß-amino-n-butyric acid. The control efficacy of the disease on pepper plants at late growth stages was more pronounced than that at young plant stages, which suggest that increase in resistance to *P. capsici* at older pepper plants (Kim et al., 1989; Kim and Hwang, 1992) may contribute to the induction of resistance by DL-ß-amino-n-butyric acid. Stimulation of capsidiol production in pepper stems at late growth stages of plants (Hwang, 1995) may also be closely related to the induced resistance mechanism in mature pepper plants that leads to complete or almost complete control of Phytophthora blight.

About 4 day interval between DL-ß-amino-n-butyric acid treatment and challenge inoculation (Table 1) was sufficient to induce resistance in pepper plants with foliar and stem application, although some protection was found at day 1 after BABA treatment. Effect

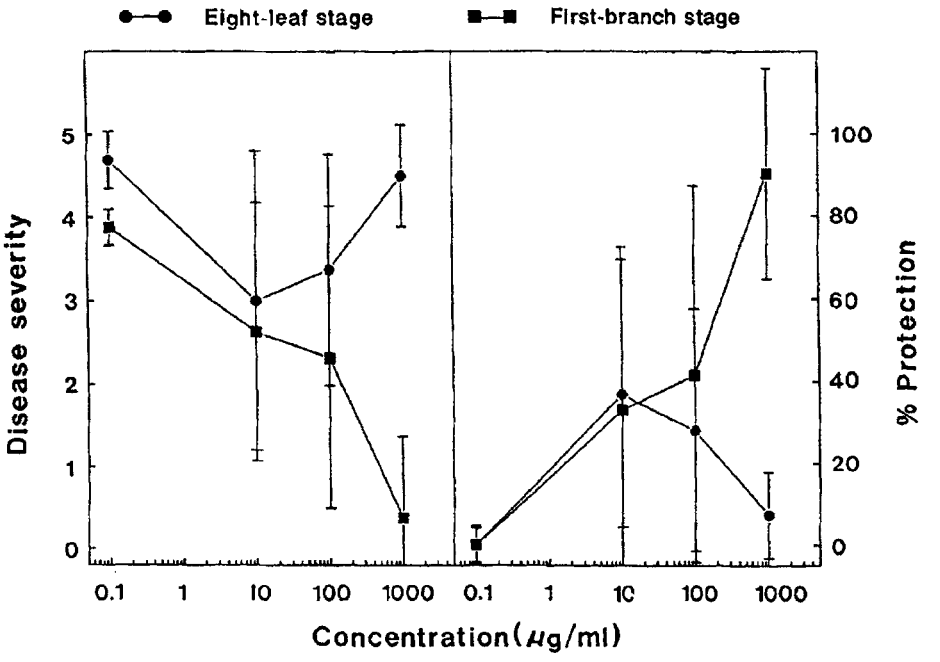

Figure 5. Protection of pepper plants (cv. Hanbyul) against *Phytophthora capsici* infection by DL-ß-amino-n-butyric acid applied to the root systems. The root systems of pepper plants at 8-leaf and first-branch stages were dipped in BABA solutions for 6 h. The plants treated with BABA were transplanted into the pots and challenged 4 days later. Disease severities were recorded 7 days after challenge inoculation. Vertical bars represent standard deviations.

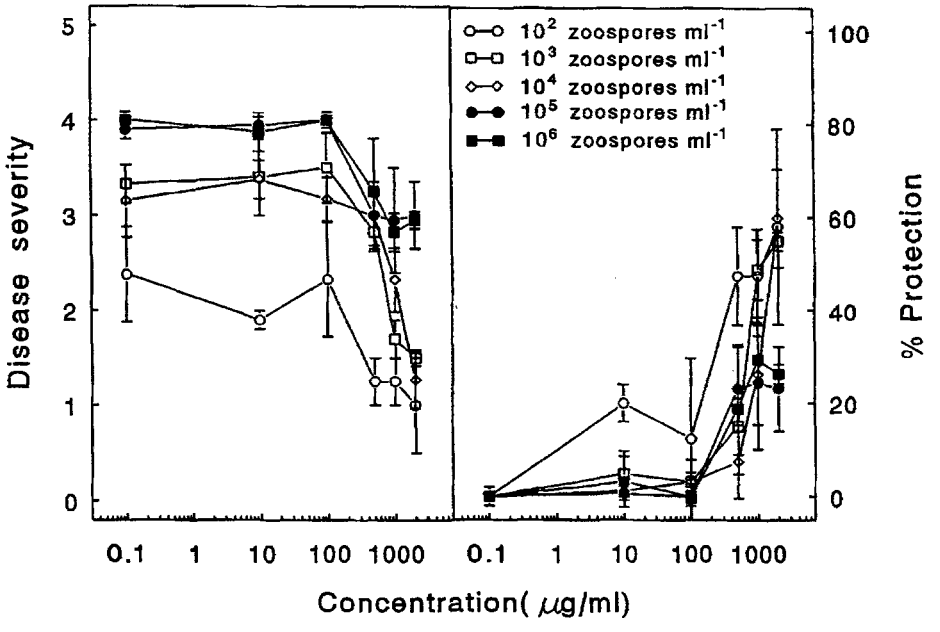

Figure 6. Effects of challenge inoculums on protection of pepper plants (cv. Hanbyul) against *Phytophthora capsici* infection by various doses of DL-ß-amino-n-butyric acid. Plants at four-leaf stage were uniformly sprayed with various doses of the compound and challenged 4 days later with different concentrations of zoospores. Disease severities were recorded 6 days after challenge inoculation. Vertical bars represent standard deviations.

of the induced resistance was retained in pepper plants at least over 15 days after treatment with DL-ß-amino-n-butyric acid, suggesting that the resistance may last for several weeks to protect pepper against *P. capsici* infection.

Treatment with DL-ß-amino-n-butyric acid as a soil drench was ineffective in protecting pepper stems from *P. capsici* infection (Table 2), which suggests that soil drench may not cause uptake through the root system due to a strong adhesion of BABA to the organic matter in soil. A simple root dipping experiment could clarify if this was the case (Figure 5). Our results showed that BABA applied to the root system protected pepper plants against *P. capsici* infection, indicating a transport of BABA into the stem tissues through the root system. A recent study of Cohen and Gisi (1994) demonstrated that when BABA was applied to the root system of tomato plants, it was preferentially translocated to the uppermost leaves and these leaves showed the greatest protection against *Phytophthora infestans*.

Experiments using different challenge-inocula of *P. capsici* demonstrated that high inoculum levels of *P. capsici* could cause Phytophthora development in the pepper stems treated with DL-ß-amino-n-butyric acid (Figure 6). These results suggest that the induced resistance in pepper plants may be more quantitative rather than qualitative, because Phytophthora blight developed more slowly on the chemically induced-resistant plants, especially at early growth stages.

In conclusion, DL-ß-amino-n-butyric acid is the nonprotein amino acid capable of inducing resistance in pepper plants against Phytophthora blight. However, more studies are required to elucidate the induced resistance mechanism in pepper-*P. capsici* system based on the detailed biochemical and molecular data.

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