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β-Aminobutyric Acid Induces the Accumulation of Pathogenesis-Related Proteins in Tomato (*Lycopersicon esculentum* L.) Plants and Resistance to Late Blight Infection Caused by *Phytophthora infestans*[1]

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Tomato (*Lycopersicon esculentum* L.) plants were sprayed with aqueous solutions of isomers of aminobutyric acid and were either analyzed for the accumulation of pathogenesis-related (PR) proteins or challenged with the late blight fungal agent *Phytophthora infestans*. The β isomer of aminobutyric acid induced the accumulation of high levels of three proteins: P14a, β-1,3 glucanase, and chitinase. These proteins either did not accumulate or accumulated to a much lower level in α- or γ-aminobutyric acid-treated plants. Plants pretreated with α-, β-, and γ-aminobutyric acid were protected up to 11 d to an extent of 35, 92, and 6%, respectively, against a challenge infection with *P. infestans*. Protection by β-aminobutyric acid was afforded against the blight even when the chemical was applied 1 d postinoculation. Examination of ethylene evolution showed that α-aminobutyric acid induced the production of 3-fold higher levels of ethylene compared with β-aminobutyric acid, whereas γ-aminobutyric acid induced no ethylene production. In addition, silver thiosulfate, a potent inhibitor of ethylene action, did not abolish the resistance induced by β-aminobutyric acid. The results are consistent with the possibility that β-aminobutyric acid protects tomato foliage against the late blight disease by a mechanism that is not mediated by ethylene and that PR proteins can be involved in induced resistance.

Acquired resistance can be induced in plants as a result of the onset of necrosis during plant-pathogen interactions (Kuc, 1982). Alternatively, it can be induced by the application of salicylic acid (White, 1979) or certain chemicals such as 2,6-dichloroisonicotinic acid (Metraux et al., 1990; Ward et al., 1991). As a consequence of acquired resistance, plants show enhanced tolerance to a wide variety of pathogens. In many instances, the resistance was correlated with the accumulation of PR proteins. Thus, 2,6-dichloroisonicotinic acid was recently reported to induce general resistance in tobacco against pathogens (Metraux et al., 1990; Ward et al., 1991) and also to elicit PR-protein accumulation and resistance against a bacterial and a fungal pathogen in *Arabidopsis* (Uknes et al., 1992).

Christ and Mösinger (1989) studied PR proteins in tomato plants and showed a local and/or systemic accumulation of 11 PR proteins in plants infected with *Phytophthora infestans* or *Fulvia fulva*, as well as in plants treated with UV light, IAA, or ethephon. PR-protein accumulation was accompanied by induced resistance against *P. infestans* and *F. fulva* (Christ and Mösinger, 1989; Enkerli, 1990). In the case of *F. fulva*, a temporal difference correlation was observed between PR-protein accumulation and resistance. Cultivars of tomato (*Lycopersicon esculentum* L.) that are resistant and sensitive to *F. fulva* showed early and late accumulation, respectively, of PR proteins (De Wit and van der Meer, 1986). Chitinase and β-1,3-glucanase were reported to increase in tomato after inoculation with *P. infestans* (Enkerli, 1990). In that case chitinase was increased both locally and systemically, whereas β-1,3-glucanase increased only locally (Enkerli, 1990). Other PR proteins, such as the NP24 PR protein isolated from tomato plants infected with *P. infestans*, were reported to exhibit fungicidal activity against *P. infestans* in vitro (Woloshuk et al., 1991).

An interesting group of elicitors of the pathogenesis response in plants are chemical analogs of amino acids. They have been shown to elicit the accumulation of an array of PR proteins in tobacco leaf discs (Asselin et al., 1985). AABA (1–5 mM) induced the accumulation of ethylene and the PR proteins PR-1, β-1,3-glucanase (PR-2), and acidic chitinase (PR-3) in intact tobacco leaves in a light-dependent manner (Lotan and Fluhr, 1990). BABA and DL-β-methylaspartic acid applied to the soil were effective in controlling the pea pathogen *Aphanomyces euteiches*. It was postulated that these compounds did not act directly on the pathogen but rather prevented expression of disease symptoms (Papavizas, 1964).

The ability of certain chemicals to induce a wide array of plant defense responses is of interest when studying their mode of action and assessing their effectiveness in antipath-

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Abbreviations: AABA, DL-α-aminobutyric acid (DL-2-aminobutyric acid); BABA, DL-β-aminobutyric acid (DL-3-aminobutyric acid); GABA, γ-aminobutyric acid (4-aminobutyric, piperidinic acid); PR, pathogenesis related; STS, silver thiosulfate.

ogen measures. The purpose of this work was to study the effects of aminobutyric acid isomers on late blight development and PR-protein production in tomato plants. We report here the isomer-specific induction of resistance against late blight disease in tomato and show that it is correlated with accumulation of PR proteins.

MATERIALS AND METHODS

Plant Materials

Most experiments were done with the tomato (*Lycopersicon esculentum* L.) F_1 hybrid cv Baby. Some experiments were conducted with the following F_1 hybrids: Florida Basket, Rheinland Rhum, Berner Rose, Mignon, Montfavet, and Ricello. All seeds were supplied by Mauser (Dubendorf, Switzerland). Plants were greenhouse grown in 12-cm pots filled with sandy loam, peat, and perlite mixed in equal volumes at 15 to 28°C. Plants were fertilized twice a week with chemical fertilizer containing 0.5% nitrogen:phosphate:potassium (20:20:20). They were used about 5 weeks after the seeding stage when they had six or seven compound leaves (unless otherwise stated).

Fungal Cultures

Most experiments were done with the metalaxyl-resistant field isolate MR1 of *Phytophthora infestans* (Mont.) DBy collected at Gevuloth, Israel, in 1984 (Kadish et al., 1990). Where indicated, experiments were conducted with the Israeli isolates MS1, MS2, MS3, MR2, and MR3 (Kadish et al., 1990). All isolates (except MS1) carry the virulence factors 1, 3, 4, 7, 8, and 10 and belong to the A_2 mating type. MS1 belongs to the A_1 mating type and does not carry the virulence factors 8 and 10 (Kadish et al., 1990). Some experiments were conducted with the metalaxyl-sensitive isolate S49 (Sandoz collection, A_1 mating type, virulence factors not determined). The fungus was grown on potato tuber slices (cv Bintje or Alpha) at 15°C in the dark. Fresh sporangia were harvested at 6 d postinoculation after the slices were immersed into double-distilled water (4°C), and their concentration was adjusted to 10,000 sporangia mL^{-1} (unless stated otherwise) before they were used for challenge inoculations.

Chemicals and Treatments

AABA [CH_3-CH_2-$CH(NH_2)COOH$], BABA [CH_3-$CH(NH_2)$-CH_2-$COOH$], and GABA [NH_2-CH_2-CH_2-CH_2-$COOH$] were purchased from Sigma. The compounds were dissolved in water and sprayed on either the abaxial (lower) or adaxial (upper) leaf surfaces of tomato plants with a fine glass atomizer (about 3 mL per plant). Plants were left on the bench until droplets dried out and were then placed in a growth cabinet calibrated to 20°C and 14 h of light (120 μE m^{-2} s^{-1}) d^{-1}. In some experiments BABA was also applied curatively 24 h after inoculation. Plants were challenged with *P. infestans* at various intervals after elicitor application, from 1 to 11 d, by spraying a sporangial suspension on the adaxial leaf surfaces (about 5 mL per plant) using a glass atomizer. Inoculated plants were kept at 100% RH in the dark for 20 h at 18°C and then returned to a growth cabinet maintained at 20°C with 12 h of light (100 μE m^{-2} s^{-1}) d^{-1}.

Disease Estimation

Disease severity was monitored by visually estimating the proportion of a leaf area occupied by blight lesions (Dowley et al., 1991). Proportion coverage was estimated using a 0 to 4 scale adapted from Dowley et al. (1991): 0.1 = a few circular 1- to 2-mm lesions, 0.5 = up to 5%, 0.75 = above 5 but below 10%, 1 = above 10 but below 25%, 2 = above 25 but below 50%, 3 = above 50 but below 75%, 3.5 = between 76 and 85%, 3.75 = 86 to 95%, and 4 = 96 to 100% of the leaf area blighted. Mean disease severity for a plant was calculated as the mean of disease severity values of all leaves of that plant. In some experiments lesion number and lesion size were monitored. Percentage protection was calculated as % protection = $100\ (1 - x/y)$, when x and y are disease severity values in treated- and control-challenged plants, respectively. In most experiments six plants were used per treatment. Experiments were repeated at least twice.

Sporangial Germination and Mycelial Growth in Vitro

Fresh sporangial suspensions were mixed with aminobutyric acids, and 20 μL were applied to depression glass slides. Slides were incubated at 15°C in the dark for 20 h. The numbers of germinating sporangia and cystospores were counted. Mycelial growth was examined in liquid rye seed medium (water extract of 60 g of rye seed, 20 g of Suc, and 2 g of yeast extract L^{-1} of water) amended with autoclaved aminobutyric acids. Autoclaving had no effect on the activity of the α and β isomers on tomato plants. Dry weight of mycelial mats was determined 2 weeks after the plates were inoculated. Sporangial germination in vivo was tested using calcofluor staining and epifluorescence as described previously (Cohen et al., 1987).

Ethylene Measurements

Plants (cv Baby) were sprayed with aminobutyric acids (2000 μg mL^{-1}), incubated in a growth chamber at 20°C, and placed in sealed transparent plastic bottles (0.5 L) 2 h later. An air sample (1 mL) was withdrawn 2 to 48 h after sealing, and the quantity of ethylene was measured using GC as described previously (Lotan and Fluhr, 1990).

Extraction of Acid-Soluble Proteins and Enzymic Assays

The method was adapted from Christ and Mösinger (1989). Fresh or liquid nitrogen-frozen tomato leaves (3 g) were homogenized in 3 mL of extraction medium consisting of 5% acetic acid and 0.1% 2-mercaptoethanol at pH 2.8. The resulting mixture was centrifuged for 30 min at 10,000g and 10°C. The protein content of the plant extract was determined by the method of Bradford (1976) using the Bio-Rad protein assay solution and BSA as a standard. For chitinase activity the radiochemical method of Molano et al. (1986) was used. The resulting GlcNAc was determined using the method of Boller et al. (1983) and Métraux and Boller (1986). To stay in the linear part of the reaction (2,000 dpm) 1 μg of protein per assay was used. Each 1000 dpm in the supernatant represented 7.2 nM GlcNAc equivalents. The amount of enzyme producing 1 nM s^{-1} GlcNAc equivalent was defined as

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1 nkatal. For β-1,3-glucanase activity a colorimetric assay of Waffenschmidt and Jaenicke (1987) with laminarin (Sigma) as substrate was used. One nkatal was defined as the enzyme activity catalyzing the formation of 1 nM Glc equivalent s^{-1}. The standard curve was linear from 4 to 25 nM per assay.

Immunoassays

ELISA tests were performed in polystyrene microtiter plates (Immulon IV; Dynatech) using standard procedures. P14a used in the ELISAs was prepared from purified P14a (Niderman et al., 1992). P14a level in extracts was determined by comparison with a P14a standard curve (linearity region = 0–1 μg mL^{-1}). Gel fractionation and immunoblots were carried out as described by Lotan and Fluhr (1990). P14a (Joosten et al., 1990) and β-1,3-glucanase antisera used in the immunoblots were a gift from P.J.G.M. De Wit (Agricultural University, Wageningen, The Netherlands).

RESULTS

Effect of Aminobutyric Acids on Sporangial Germination and Mycelial Growth and Phytotoxicity

When treated with 19.4 mM (2000 μg mL^{-1}) BABA, Florida Basket plants showed no visible symptoms, whereas Baby plants produced about 10 necrotic microlesions (0.5 mm in diameter) per leaflet on lower leaves and occasional chlorotic microlesions on upper leaves. General growth of the plants was not affected, nor were any symptoms produced at 9.7 mM (1000 μg mL^{-1}) or less. AABA and GABA produced no symptoms on tomato plants at 20 mM or less.

Sporangial germination in vitro of fungal isolates MR1, MR3, or S49 was unaffected by either the α, β, or γ isomer of aminobutyric acid up to a concentration of 2000 μg mL^{-1}. The number of germinating cytospores and the length of germ tubes were similar to those observed in water. None of the aminobutyric acids adversely affected growth of *P. infestans* (isolates MS3 and S49) in liquid cultures when amended at a final concentration of up to 1000 μg mL^{-1}. Calcofluor staining of inoculated leaf discs (20 h) revealed abundant cystospore germination on the surfaces of control and BABA-treated (2000 μg mL^{-1}, 5 d after spraying) leaves. Additional observations showed that up to 2000 μg mL^{-1} of BABA did not inhibit penetration of *P. infestans* into tomato leaves (A. Raviv and Y. Cohen, unpublished data).

Induced Resistance with Aminobutyric Acids

We examined whether isomers of aminobutyric acid could influence plant-pathogen interactions. Figure 1 exemplifies, in a qualitative manner, the magnitude of disease severity symptoms and degree of protection achieved in plants, 7 d after challenge with *P. infestans*, with or without BABA pretreatment. Pooled data from several experiments made with seven cultivars of tomato (see "Materials and Methods") revealed that BABA applied (at 2000 μg mL^{-1}) as a foliar spray 2 d before challenge inoculation provided a significantly higher protection (90%) against late blight compared to AABA (38%) and GABA (16%). Thus, the induced resistance to leaf blight exhibited isomer specificity. As shown in

Figure 1. Protection of tomato plants (cv Baby) against late blight by treatment with BABA. Control (CK) plant on left was sprayed with water; plant on right was sprayed with 2000 μg mL^{-1} of BABA. Plants were inoculated 1 d later with *P. infestans* (isolate MR1) and photographed 7 d after inoculation.

Figure 2, resistance increased with increasing elicitor concentration. Maximal protection (97%) was seen in plants sprayed with 2000 μg mL^{-1}. At 125 μg mL^{-1}, 30% protection was afforded. The effective dose for 50% protection was calculated as 175 μg mL^{-1}. BABA was effective in controlling leaf blight induced by seven isolates of *P. infestans*. Plants (cv

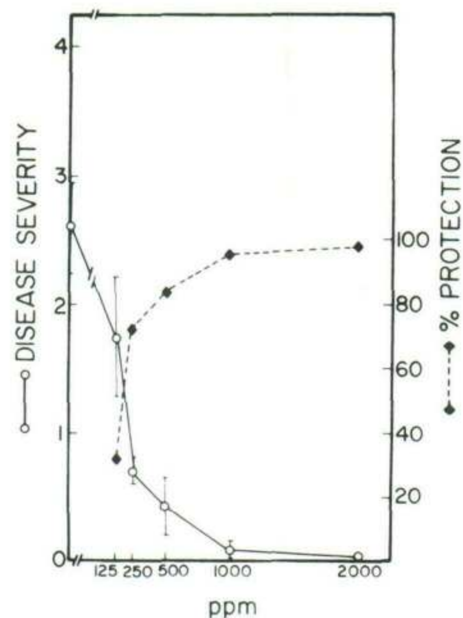

Figure 2. Disease severity and protection afforded by BABA. Plants were sprayed with various doses (125–2000 μg mL^{-1}) of BABA and challenged with *P. infestans* (isolate MR1) 1 d later. Mean disease severity was estimated 4 d after challenge inoculation using the index of severity described in "Materials and Methods." Bars represent SD of the mean.

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Table I. *Curative activity of BABA (2000 μg mL^{-1}) on late blight in tomato (cv Florida Basket)*

Plants were sprayed with 2000 μg mL^{-1} of BABA 24 h after inoculation. Disease symptoms were recorded 4 d after inoculation as indicated in "Materials and Methods."

	Leaf Position from Stem Base						
	1	2	3	4	5	6	7
Disease severity ± SD (0–4 scale)							
No treatment	4.0 ± 0	3.8 ± 0.5	3.8 ± 0.5	3.7 ± 0.5	3.1 ± 0.2	1.9 ± 0.6	1.1 ± 0.2
BABA treated	0.6 ± 0.4	0.3 ± 0.4	0.4 ± 0.4	0.2 ± 0.2	0	0	0
% protection	85	92	89	95	100	100	100

Baby and cv Florida Basket) treated with 2000 μg mL^{-1} of the compound and challenged 2 d later were protected 4 d after challenge to an extent of 88, 94, 96, 85, 96, 94, and 99% against MS1, MS2, MS3, MR1, MR2, MR3, and S49, respectively.

At the maximal protection level, a large difference in lesion development was detected between untreated and treated plants throughout the plant. Four days after inoculation control plants produced lesions with mean diameters between 4 and 12 mm, whereas treated plants produced lesions of 0 to 2 mm in diameter. Seven days after inoculation the mean diameters of the lesions developed in control and treated plants were 6 to 25 and 0 to 5 mm, respectively. Younger leaves showed complete disease containment because no lesions were detected. Additional experiments performed at the maximal protection level during a period of 12 d revealed 97, 90, and 80% protection on d 4, 7, and 12, respectively. In contrast, leaves of control water-treated challenged plants were fully desiccated from blight at d 12. We investigated whether application of BABA would be effective if applied after infection. As shown in Table I, when plants were treated with this compound 24 h after inoculation, a high rate of protection against *P. infestans* was obtained. The protection in older leaves was 85%, whereas the protection in younger leaves was 100%. Thus, application of BABA had a strong curative activity.

The persistence of resistance induced by BABA was examined by inoculating plants with fungus 11 d after treatment (longer periods were not tested). As shown in Table II, a high level of resistance was obtained (92%). We examined the isomer specificity of the persistence of resistance. Under the same conditions the resistance afforded by the isomers α and γ was considerably less dramatic, with 35 and 9% protection, respectively (Table II).

Table II. *Activity of different isomers of aminobutyric acid in protecting tomato plants (cv Baby) against late blight caused by P. infestans 11 d after spraying*

Five-leaf plants (*n* = 12) were sprayed with approximately 3 mL/plant of the test compounds and challenged 11 d after spraying with *P. infestans* (MR1, at 2500 sporangia mL^{-1}). Disease symptoms were recorded 4 d after challenge.

Compound	Concentration	Disease Severity (0–4 scale ± SD)	Percentage Protection
	μg mL^{-1}		
H_2O		2.81 ± 0.26	
AABA	2,000	1.83 ± 0.43	35
BABA	2,000	0.23 ± 0.21	92
GABA	2,000	2.56 ± 0.56	9

Ethylene Evolution

Ethylene production has been shown to be either a necessary or concomitant event in the pathogenesis response. Therefore, we monitored ethylene produced by leaves after the application of the three aminobutyric acid isomers. As shown in Table III, plants treated with AABA showed 3-fold more accumulation of ethylene compared with BABA-treated plants, whereas in GABA- or water-treated plants no evolution of ethylene was detected. STS is a potent inhibitor of ethylene action (Yang, 1985). If ethylene serves as an important intermediate in the defense response, the application of STS may attenuate the protection. However, as shown in Figure 3, for individual leaves and for the whole plant, daily application of 120 μM STS to plants previously sprayed with water or BABA (2000 μg mL^{-1}) did not abolish BABA-induced resistance against *P. infestans*. Only a slight decrease in disease severity was detected in plants treated with STS alone.

Induction of Accumulation of PR Proteins in Treated Leaves

The accumulation of PR polypeptides P14a and β-1,3-glucanase was used as molecular markers of the plant-path-

Table III. *Ethylene evolution, expressed as μL of ethylene per plant ± SD, from tomato plants (cv Baby) sprayed with aminobutyric acids and kept sealed under two light regimes at 20°C*

Plants at the six-leaf stage were sprayed with water or with 3 mL of 2000 μg mL^{-1} solutions (0.06 mM) of aminobutyric acids. Leaves were sealed in 0.5-L plastic bottles covered with a rubber stopper and incubated in two growth chambers. One-milliliter air samples were withdrawn and injected to a GC column at the indicated time intervals.

Treatment	12-h Photoperiod (120 μE m^{-2} s^{-1})		Continuous light (250 μE m^{-2} s^{-1})	
	16 h	40 h	4 h	24 h
H_2O	0	0	0	0
AABA	0	46 ± 15	0	73 ± 28
BABA	0	17 ± 9	0	26 ± 4
GABA	0	0	0	0

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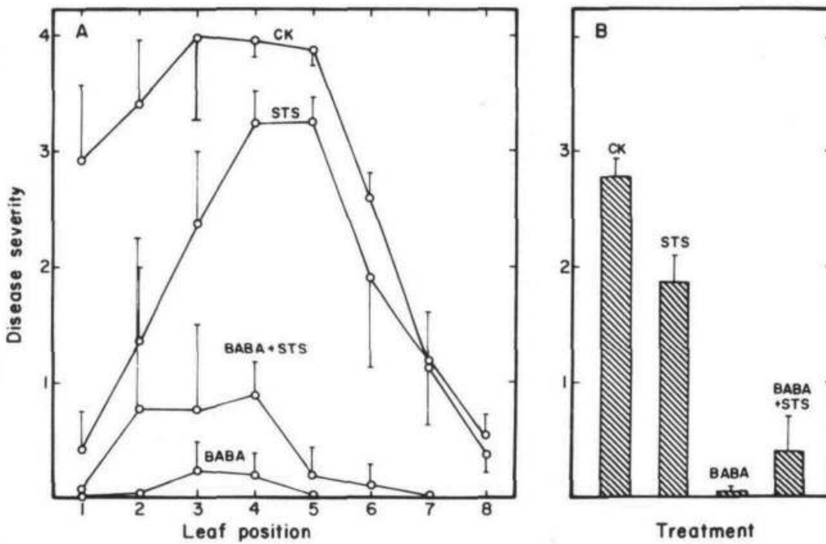

Figure 3. The effect of STS on disease severity symptoms in nontreated and BABA-treated tomato leaves. Plants (cv Baby) were sprayed with either water (CK), STS (120 μM), BABA (2000 μg mL^{-1}), or BABA (2000 μg mL^{-1}) and STS (120 μM) together. BABA was applied once at time zero, and STS was applied three times (0, 1, and 2 d). Plants were challenged with *P. infestans* (isolate MR1) 3 d after the first spraying, and disease symptoms were recorded 4 d later. A, Mean disease severity (±SD) on individual leaves in leaf position 1 to 8. B, Mean disease severity (±SD) on individual plants. Averages are from six plants.

ogenesis response. Leaf extracts were examined by the immunoblot technique for the presence of PR proteins. Figure 4 shows that P14a and β-1,3-glucanase increased markedly at 1 to 2 d in leaves treated with BABA but not in leaves treated with water. Both PR proteins remained at high levels in BABA-treated plants up to 4 d after treatment. The α and γ isomers induced weaker and more delayed signals of P14a and β-1,3-glucanase.

Plants were treated with isomers of aminobutyric acid and were evaluated quantitatively, on a leaf-by-leaf basis, for the accumulation of P14a by the ELISA technique (Fig. 5A). P14a content in water-treated plants was approximately 35 ng mL^{-1} throughout the plant. AABA- and GABA-treated plants showed similar quantities. Corresponding values for plants sprayed with BABA were 10-fold higher (350 ng mL^{-1}). The largest increment appeared in the older, treated leaves.

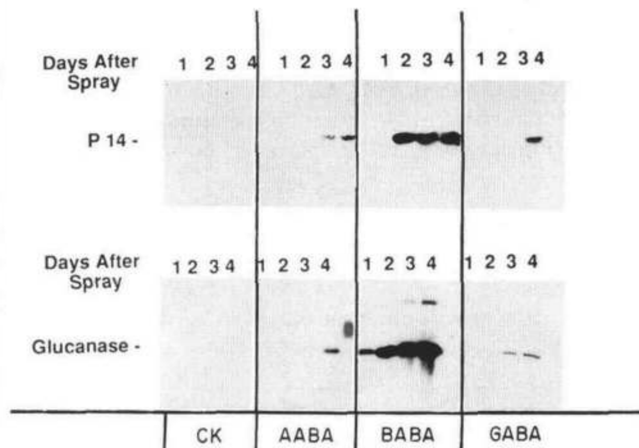

Figure 4. Immunoblot of total protein extracts of tomato plants developed with P14a and β-1,3-glucanase antisera. Tomato plants were sprayed with water (CK), AABA, BABA, and GABA (2000 μg mL^{-1}) and sampled at 1, 2, 3, and 4 d after spraying. Extracts were fractionated in 10% SDS-polyacrylamide denaturing gels and immunoblotted as described in "Materials and Methods."

Total chitinase activity increased from a mean value (for leaves 2–6) of 7.4 nkatal mg^{-1} of protein in water-treated plants to 9.0, 15.2, and 9.5 nkatal mg^{-1} of protein in plants treated with AABA, BABA, and GABA, respectively. This is depicted in Figure 5B as the percentage increments in chitinase activity relative to leaves of water-treated control plants. The α and γ isomers induced up to 50 and 40% increments, respectively, whereas the β isomer induced a 70 to 200% increase in the enzyme activity.

β-1,3-Glucanase activity did not increase in plants treated with AABA or GABA relative to water-treated plants. It ranged between 0.23 and 0.24 nkatal mg^{-1} of protein for all leaves (leaves 2–6) tested. BABA, however, induced an approximately 5-fold elevation in the enzyme activity to values ranging from 0.3 (leaf 6) to 1.1 (leaves 3 and 4) nkatal mg^{-1} of protein. The relative increments in β-1,3-glucanase activity in aminobutyric acid-treated plants relative to water-treated control plants are shown in Figure 5C. The most prominent increases of 360 and 330% occurred in leaves 3 and 4, respectively, of BABA-treated plants.

We examined whether fungal challenge of pretreated plants with the three aminobutyric isomers influenced the accumulation of PR proteins. As shown in Table IV, infection of untreated plants with *P. infestans* resulted in a 2- to 3-fold increase in the measured amounts of P14a, chitinase, and β-1,3-glucanase. The largest increase in accumulation of P14a and enzymic activities was detected in plants treated with BABA. In the case of P14a, particularly high increases of 10- and 20-fold were detected with AABA and BABA, respectively.

DISCUSSION

Of the three isomers of aminobutyric acid used, the β isomer caused the strongest induction of PR-protein accumulation in tomato foliage. The α and γ isomers induced little accumulation. The β isomer rendered excellent protection in tomato plants against the late blight fungus (97%), whereas partial (38%) or negligible protection (16%) was

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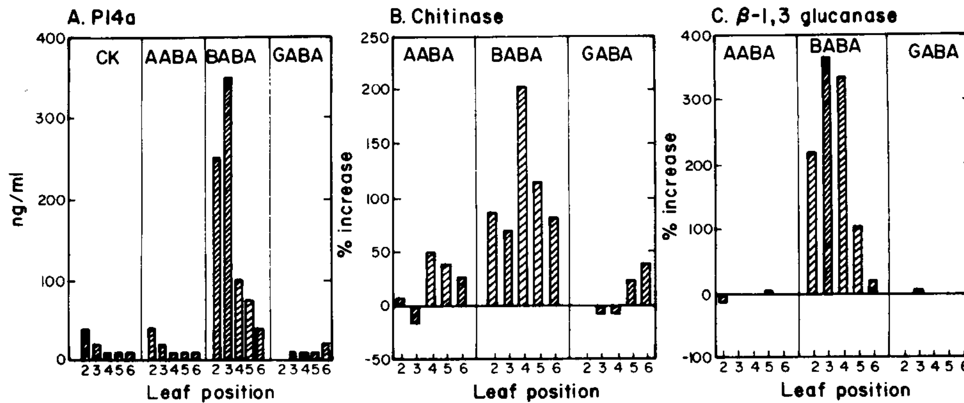

Figure 5. Quantitative examination of P14a polypeptide accumulation and chitinase and β-1,3-glucanase enzymic activities after treatment with isomers of aminobutyric acid. A, P14a polypeptide content as measured by ELISA. B, Percentage increase in chitinase activity. C, Percentge increase in β-1,3-glucanase activity. Extracts were taken from six-leaf tomato plants 5 d after spray treatment with either water (CK) or 2000 μg mL^{-1} of AABA, BABA, or GABA. Bars show values for leaves 2, 3, 4, 5, and 6 (from stem base). A, Values are in ng of polypeptide mL^{-1} of extract. B and C, Values represent the percentage difference in specific activity between the respective aminobutyric acid and the water-treated control plants. Water-treated plants exhibited activities of 7.4 and 0.23 nkatal mg^{-1} for chitinase and glucanase measurements, respectively.

induced by the α or γ isomers. Thus, the accumulation of PR proteins induced in tomato by aminobutyric acids and the protection against the late blight fungus *P. infestans* induced by these acids are positively correlated. Although PR proteins may play a direct role in plant defense, it is possible that additional fungitoxic or fungistatic elements are induced as well. Indeed, the rapid curative effect of BABA that precedes the peak of PR-protein accumulation may indicate the existence of accessory components.

Relatively high doses (mM range) of the BABA elicitor were necessary to achieve protection. At such high dosages, nonspecific perturbations in cellular metabolism or cellular pH caused by uptake of weak acids may be suspected. However, the inability of other isomers of aminobutyric acid to elicit a response shows that this is not the case. Our findings (Y. Cohen and U. Gisi, unpublished data) that only 5% of radioactive BABA applied as a spray was taken up by tomato leaves may explain why a relatively high dosage of the compound is required to elicit the biological responses described here. Protection afforded by BABA was not limited to tomato. BABA was found to be effective in inducing resistance to *Perenospora tabacina* in tobacco, *Perenospora parasitica* in broccoli, and *Pseudoperonospora cubensis* in cucurbits (Y. Cohen, unpublished data).

P14a and β-1,3-glucanase exhibited large increments in BABA-treated plants as compared with control plants. However, in comparison, the measurement of enzyme activities showed that β-1,3-glucanase was stimulated to a lesser extent (to 4.7-fold) and chitinase was stimulated the least (to 2.5-fold). The differences in fold induction between the immunoassay and the enzymic assay can be the result of a high background of constitutive enzymic activities in the nontreated plants. Indeed, both glucanase and chitinase activities have been implicated in plant growth and development (Fincher and Stone, 1981; De Jong et al., 1992). The results emphasize the importance of measuring PR-specific polypeptides directly by an immunotechnique.

In tobacco leaf discs, both AABA and BABA were effective in stimulating PR-protein accumulation in intercellular fluid extract (Asselin et al., 1985). However, our preliminary ob-

Table IV. *Accumulation of PR proteins in acidic extracts of tomato plants sprayed with isomers of aminobutyric acid with or without subsequent challenge with P. infestans*

Unchallenged plants were inoculated 3 d after spraying (60,000 sporangia/plant) and harvested 2 d later. The figures represent mean values for leaves 2 to 6. The figures in parentheses are percentages relative to the nonchallenged control.

Aminobutyric Acid	Challenge	P14a	Chitinase	β-1,3-Glucanase
		ng mL^{-1} (%)	*nkatal mg^{-1} of protein (%)*	*nkatal mg^{-1} of protein (%)*
None	None	21 (100)	7.4 (100)	0.24 (100)
None	*P. infestans*	60 (285)	13.9 (188)	0.45 (128)
AABA	*P. infestans*	250 (1190)	16.4 (222)	0.78 (325)
BABA	*P. infestans*	450 (2142)	19.5 (263)	1.20 (500)
GABA	*P. infestans*	90 (429)	15.1 (204)	0.50 (208)

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servations show that in intact tobacco plants only BABA, but not AABA or GABA, protects against the blue mold fungus (Y. Cohen, unpublished data). It may well be that temporal and quantitative differences in the eliciting capabilities exist or that the β isomer stimulates additional components in the pathogenesis response leading to resistance.

Lotan and Fluhr (1990) demonstrated that 1 mM (about 100 μg mL^{-1}) AABA induced the production of PR proteins and ethylene in tobacco. In that case concomitant PR-protein accumulation was abrogated by inhibiting ethylene formation or action. However, the response to another type of PR-protein elicitor, xylanase, was found to be independent of ethylene.

The present results show that tomato plants respond differently than tobacco. Whereas AABA induced the evolution of ethylene in tomato, it was a poor elicitor of tomato PR-protein accumulation. These results and the fact that STS did not abolish the resistance induced by BABA lead us to conclude that ethylene is not involved in the induction of resistance or PR-protein accumulation in tomato plants by this chemical.

We have no explanation for the specificity of activity of the different aminobutyric acid isomers on PR-protein induction. BABA could interfere with general amino acid metabolism and, thus, indirectly induce a state of stress in the plant that resembles a response to pathogenesis. Alternatively, specificity could result from binding of the β isomer to components of the cell wall matrix (Fry, 1986; Y. Cohen and U. Gisi, unpublished data) or binding to a cytoplasmic protein receptor that recognizes specifically the β isomer in the manner described for salicylic acid (Chen and Klessig, 1991) or to a membrane-bound receptor as described for heptaglucan elicitor in soybean (Ebel, 1989; Cheong and Hahn, 1991). The presence of specificity is not unexpected since nonprotein amino and imino acids are widespread secondary plant metabolites with extremely diversified chemical and physiological properties. Although some are biologically toxic, they can also serve as a nitrogen source (Rosenthal, 1982). GABA appears to be ubiquitous in the plant kingdom (Chung et al., 1992), but the physiological role of GABA in plants has not been clearly established. GABA synthesis results predominantly from the decarboxylation of carbon-1 of L-glutamate in a reaction catalyzed by glutamate decarboxylase (Satya Narayan and Nair, 1990). Increases in GABA content and glutamate decarboxylase activity have been observed in many plants under a variety of stress conditions (Satya Narayan and Nair, 1990). AABA and BABA, isomers of GABA, have been detected in plants as well (Rosenthal, 1982; Gamliel and Katan, 1992).

Aminobutyric acids exhibited no fungicidal activity against *P. infestans* in vitro. Therefore, they represent a new group of chemicals capable of inducing both resistance against disease and the accumulation of PR proteins.

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LITERATURE CITED

Asselin A, Grenier J, Cote F (1985) Light-induced extracellular accumulation of **b** (pathogenesis-related) proteins in *Nicotiana* green tissue induced by various chemicals or prolonged floating on water. Can J Bot **63:** 1276–1283

Boller T, Gehri A, March F, Vögeli U (1983) Chitinase in bean leaves: induction by ethylene, purification properties and possible function. Planta **157:** 22–31

Bradford M (1976) A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem **72:** 248–254

Chen Z, Klessig DF (1991) Identification of a soluble salicylic acid-binding protein that may function in signal transduction in the plant disease resistance response. Proc Natl Acad Sci USA **88:** 8179–8183

Cheong JJ, Hahn MG (1991) A specific, high-affinity binding site for the hepta-α-β-glucoside elicitor exists in soybean membranes. Plant Cell **3:** 137–147

Christ U, Mösinger E (1989) Pathogenesis-related proteins of tomato: 1. Induction by *Phytophthora infestans* and other biotic and abiotic inducers and correlation with resistance. Physiol Mol Plant Pathol **35:** 53–65

Chung I, Bown A, Shelp BJ (1992) The production and efflux of 4-aminobutyrate in isolated mesophyll cells. Plant Physiol **99:** 659–664

Cohen Y, Peer S, Balass O, Coffey M (1987) A fluorescent technique for studying growth of *Peronospora tabacina* on leaf surfaces. Phytopathology **77:** 201–204

De Jong AJ, Cordewener J, Schiavo FL, Terzi M, Vandekerckhove J, Van Kammen Am De Vries SC (1992) A carrot somatic embryo mutant is rescued by chitinase. Plant Cell **4:** 425–433

De Wit PJGM, van der Meer FE (1986) Accumulation of pathogenesis-related tomato leaf protein P14 as an early indicator of incompatibility in the interaction between *Cladosporium fulvum* (syn. *Fulvia fulva*) and tomato. Physiol Mol Plant Pathol **28:** 203–214

Dowley LJ, O'Sullivan EK, Kehoe HW (1991) Development and evaluation of blight resistance in potato cultivars. *In* JA Lucas, RC Shattock, DS Shaw, LR Cooke, eds, Phytophthora. Cambridge University Press, Cambridge, UK, pp 373–382

Ebel J (1989) Elicitor-binding proteins and signal transduction in elicitor action. J Cell Biochem **13D** (Suppl): 248

Enkerli J (1990) Durch *Phytophthora infestans* induzierte Resistenz sowie lokale und systemische Induktion von Chitinasen und β-1,3 glucanasen in Tomaten. Botanisches Institut Universitat Basel (Diplomarbeit), Basel, Switzerland

Fincher GB, Stone BA (1981) Metabolism of noncellulosic polysaccharides. Enycl Plant Physol New Series **13B:** 68–132

Fry SC (1986) Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annu Rev Plant Physiol **37:** 165–186

Gamliel A, Katan J (1992) Influence of seed and root exudates on fluorescent pseudomonads and fungi in solarized soil. Phytopathology **82:** 320–327

Joosten MHAJ, Bergmans CJB, Meulenhoff EJS, Cornelissen BJC, De Wit PJGM (1990) Purification and serological characterization of three basic 15-kilodalton pathogenesis-related proteins from tomato. Plant Physiol **94:** 585–591

Kadish D, Grinberger M, Cohen Y (1990) Fitness of metalaxyl-sensitive and metalaxyl resistant isolates of *Phytophthora infestans* in susceptible and resistant potato cultivers. Phytopathology **80:** 200–206

Kuc J (1982) Induced immunity to plant disease. Bioscience **32:** 854–860

Lotan T, Fluhr R (1990) Xylanase, a novel elicitor of pathogenesis-related proteins in tobacco, uses a non-ethylene pathway of induction. Plant Physiol **93:** 811–817

Métraux JP, Boller T (1986) Local and systemic induction of chitinase in cucumber plants in response to viral, bacterial and fungal

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infections. Physiol Mol Plant Pathol **28:** 161–169

Métraux JP, Signer H, Ryals J, Ward E, Wyss-Benz M, Gaudin J, Raschdorf K, Schmid E, Blum W, Inverardi B (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science **250:** 1004–1006

Molano J, Duran A, Cabib E (1986) A rapid and sensitive assay for chitinase using tritiated chitin. Anal Biochem **83:** 648–656

Niderman T, Bruyère T, Guegler K, Mösinger E (1992) Antifungal activity of native and recombinant tomato P14a protein. *In* B Fritig, M Legrand, eds, Mechanisms of Plant Defense Responses. Kluwer Academic, Dordrecht, The Netherlands, p 450

Papavizas GC (1964) Greenhouse control of *Aphanomyces* root rot of peas with aminobutyric acid and methylaspartic acid. Plant Dis Rep **48:** 537–541

Rosenthal GA (1982) Plant Non-Protein Amino and Imino Acids: Biological, Biochemical and Toxological Properties. Academic Press, New York

Satya Narayan V, Nair PM (1990) Metabolism, enzymology, and possible roles of 4-aminobutyrate in higher plants. Phytochemistry **29:** 367–375

Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E, Ryals J (1992) Acquired resistance in *Arabidopsis*. Plant Cell **4:** 645–656

Waffenschmidt S, Jaenicke L (1987) Assay of reducing sugars in the nanomole range with 2,2′ bicinchoninate. Anal Biochem **165:** 337–340

Ward ER, Uknes SJ, Williams SC, Dincher, SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Métraux J-P, Ryals J A (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell **3:** 1085–1094

White RF (1979) Acetyl salicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology **99:** 410–422

Woloshuk CP, Meulenhoff JS, Sela-Buurlage M, van den Elzen PJM, Cornelissen BJC (1991) Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. Plant Cell **3:** 619–628

Yang SF (1985) Biosynthesis and action of ethylene. Hortscience **20:** 41–45

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