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Induction of resistance to *Penicillium digitatum* in grapefruit by β-aminobutyric acid

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Abstract

β-Aminobutyric acid (BABA), an inducer of pathogen resistance in plants, induced disease resistance in reproductive parts of the plant, such as grapefruit peel tissue. Application of BABA to specific wound sites on the fruit peel surface induced resistance to *Penicillium digitatum*, the main postharvest pathogen of citrus fruit, in a concentration-dependent manner, being most effective at 20 mM, and rather less effective at either higher or lower concentrations. The effect of BABA in inducing resistance to *P. digitatum* in the fruit peel surface was local and limited to the vicinity (within 1–2 cm) of the BABA-treated site. In addition to inducing pathogen resistance, increasing concentrations of BABA (from 1 to 100 mM) also exhibited direct antifungal activity and inhibited *P. digitatum* spore germination and germ tube elongation *in vitro*. The induction of resistance to *P. digitatum* by BABA was accompanied by the activation of various pathogen defense responses in grapefruit peel tissue, including activation of chitinase gene expression and protein accumulation after 48 h, and an increase in phenylalanine ammonia lyase (PAL) activity after 72 h.

Introduction

β-Aminobutyric acid (BABA) is a non-protein amino acid, which induces resistance against a broad range of disease-causing organisms, including fungi, bacteria, viruses and nematodes (Cohen, 2001; 2002; Jakab et al., 2001). Most of the studies describing the phenomenon of BABA-induced disease resistance were done in annual weedy plant species, especially those belonging to the *Solanaceae* family, such as tomato and potato, and to the *Cucurbitaceae* family, such as tobacco, pepper, cucumber and melon (Cohen, 1994; Cohen et al., 1994; Hong et al., 1999; Siegrist et al., 2000). In addition, it was reported that BABA-induced disease resistance in other weedy species such as *Arabidopsis*, cotton, cauliflower and sunflower (Tosi et al., 1998; Zimmerli et al., 2000; 2001; Silue et al., 2002). More recently, it was demonstrated that BABA may also induce disease resistance in woody plants, such as grapevines (Cohen et al., 1999; Reuveni et al., 2001). However, as far as we know, no report is yet available regarding the effects of BABA on the induction of disease resistance in fruit tissues.

The reduction in disease incidence that follows the application of BABA must result from its effects on the induction of pathogen defense responses in the host, since the compound itself did not seem to have any direct antifungal activity and did not affect the growth of various pathogens *in vitro* (Cohen, 1994; 2001; Cohen et al., 1994; Sunwoo et al., 1996; Tosi et al., 1998). Moreover, in many cases, BABA induced

systemic resistance: application to the root system or to the lower leaves of the plant induced pathogen resistance in other distant non-treated portions of the plant (Cohen, 1994; Hong et al., 1999; Oka et al., 1999; Silue et al., 2002). It was concluded that BABA protects plants from infection by potentiating pathogen-specific resistance mechanisms (Zimmerli et al., 2000; 2001).

The mode of action of BABA in inducing plant pathogen resistance is not yet fully understood. However, several reports indicated that BABA activated pathogenesis-related (PR) protein accumulation. For example, BABA induced PR-1a, chitinase, and β-1,3-glucanase protein accumulation in pepper, tomato and tobacco (Cohen, 1994; Cohen et al., 1994; Hwang et al., 1997; Siegrist et al., 2000). On the other hand, although BABA increased disease resistance it did not induce PR-protein accumulation in cauliflower, *Arabidopsis* or tobacco, suggesting that activation of PR-proteins cannot be its only mode of action (Cohen, 1994; Jakab et al., 2001; Silue et al., 2002). Other host pathogen defense responses that were reported to be induced by BABA include induction of the hypersensitivity response, callose deposition and lignin accumulation (Cohen et al., 1999; Siegrist et al., 2000; Zimmerli et al., 2000). In any case, BABA may induce pathogen resistance in plants either through the activation of a signaling pathway that is dependent on salicylic acid (SA) or through the activation of a novel signaling cascade that is not dependent on the SA, jasmonic acid (JA) or ethylene signaling pathways (Zimmerli et al., 2000; 2001).

In previous studies, we showed that by applying various treatments, such as UV irradiation (Droby et al., 1993), hot water sprays (Porat et al., 2000a), JA (Droby et al., 1999) or yeast antagonists (Droby et al., 2001), it was possible to increase grapefruit resistance against the green mould pathogen *Penicillium digitatum*. In the present study, the effects of BABA on the induction of resistance to *P. digitatum* were evaluated in grapefruit and on fungal growth *in vitro*, and characterized its effects in inducing defense mechanisms against pathogens.

Materials and methods

Plant material

Grapefruits (*Citrus paradisi* cv. 'Marsh Seedless') were obtained from a local orchard and used shortly after harvest. Prior to use, fruits were thoroughly washed with tap water and surface sterilized by wiping with technical grade (75%) ethanol.

Fungal cultures

Cultures of *P. digitatum* were isolated from decayed citrus fruits, stored on potato dextrose agar (PDA) slants at 4 °C, and grown on PDA plates for 1 week at 25 °C. Spore suspensions were prepared by removing the spores from the sporulating edges of a 2–3-week-old culture with a bacteriological loop, and suspending them in sterile distilled water. The spore concentration was adjusted to 5×10^4 spores ml^{-1} with a haemocytometer.

Effects of BABA on the induction of resistance to P. digitatum

Fruits were wounded (1–2 mm deep and width) with a dissecting needle at three different sites around their blossom end. Thirty microliter of BABA at the desired concentration were pipetted into each wound site. Wounds treated with the same amount of distilled sterilized water served as a control. After 24 h incubation at 20 °C, either the same wounds or fresh wounds that were made at various distances from the original BABA-treated wounds or the water control wounds, were inoculated with 20 µl of *P. digitatum* (5×10^4 spores ml^{-1}). Fruits were incubated at 20 °C in plastic trays under humid conditions and the percentage of infected wounds was determined 4 days after inoculation. Eighteen fruits were used for each treatment (total of 54 wounds per treatment) and each experiment was repeated at least three times with similar results. BABA (Sigma) was dissolved at the indicated concentrations in distilled sterilized water.

Effect of BABA on P. digitatum *growth* in vitro

Spores of *P. digitatum* were suspended in Potato Dextrose Broth (Difco) at a final concentration of 2×10^4 spores ml^{-1}. Aliquots (450 µl) of spore suspensions were transferred to wells of tissue culture clusters (Corning Costar Corporation, Cambridge, Mass.), and 50-µl aliquots of the various BABA stock solutions were added to give final concentrations of 1–1000 mM. Samples of the various test solutions (30-µl drops), were placed on ethanol-washed microscope slides (three drops per slide) kept in Petri dishes padded with moistened filter paper, and incubated for 24 h at 25 °C

in darkness. Spore germination and germ tube elongation were measured in three microscope fields, each containing 40–50 spores, under a light microscope.

PAL activity

PAL activity was evaluated according to Lisker et al. (1983). Intact fruit were slightly wounded (100 punctures with a needle per fruit) and dipped in water or in 20 mM BABA. After different intervals, 10 g of flavedo tissue were removed from the peel and homogenized in 100 ml of acetone at −15 °C, and the homogenate was filtered through Whatman No. 1 filter paper. This step was repeated three times, and the acetone powder was then air-dried for 3 h and stored at −15 °C until use. For the PAL assay, 500 mg of the acetone powder were added to 10 ml of cold 0.1 M borate buffer, pH 8.8, at 4 °C and stirred for 1 h. The suspension was centrifuged for 10 min at 12,000*g*, and the supernatant was dialyzed for 48 h at 4 °C against 0.2 M borate buffer, pH 8.8. PAL activity was determined by incubating 1.5 ml of enzyme preparation, 2.5 ml of 0.1 M borate buffer, pH 8.8 and 1 ml of 0.05 M L-phenylalanine as a substrate at 40 °C for 1 h. The reaction was stopped with 0.5 ml of 5 N HCL and 7 ml of diethyl ether. The mixture was centrifuged at 12,000*g* for 5 min, and 3.5 ml of the clear ether supernatant were taken for spectrophotometric measurements of cinnamic acid at $\lambda = 269$. The results were expressed as μmol cinnamic acid per gram dry weight of acetone powder per hour.

RNA extraction and gel blot analysis

Total RNA was isolated from grapefruit flavedo by phenol/chloroform extraction, followed by precipitation with LiCl. RNA (10 μg per lane) was separated on a 1% formaldehyde gel and blotted onto a Hybond-N^+ membrane (Amersham). Blots were hybridized with a citrus-specific chitinase cDNA probe (Porat et al., 2000b) labeled to high specific activity by random priming with [^{32}P]dCTP (Biological Industries, Israel). Following hybridization and stringency washes the blots were autoradiographed with a Fuji *RX* film.

Protein extraction and immunoblotting analysis

Protein extraction and immunoblotting were performed according to Lers et al. (1998). Flavedo tissue (1 g) was homogenized with a mortar and pestle in 2 ml of ice-cold 20 mM Tris–HCl, pH 7.5, and centrifuged at 10,000*g* for 20 min to remove cell debris. The supernatant was filtered through four layers of Miracloth, and the soluble protein concentration was determined according to Bradford (1976). Ten micrograms of proteins were separated on 12% SDS-PAGE, and transferred to nitrocellulose membranes (Bio-Rad). Immunodetection was performed with citrus-specific chitinase antibodies (McCollum et al. 1997). The primary antibody reacting bands were visualized by using commercial secondary antibodies conjugated to alkaline phosphatase (Bio-Rad) according to the manufacturer's instructions.

Results

Effects of BABA on induction of resistance to P. digitatum *in grapefruit*

To examine the effects of BABA on the induction of resistance to *P. digitatum* in grapefruit, artificial wound sites were pretreated with different concentrations of the compound and inoculated 24 h later. The results (Figure 1) show that treatments with BABA effectively reduced the incidence of green mould decay development in the infected wounds in a concentration-dependent manner, with a peak of efficacy at 20 mM. At the optimal concentration (20 mM) BABA reduced decay development by 65% as compared with that in control untreated wounds, whereas at concentrations of

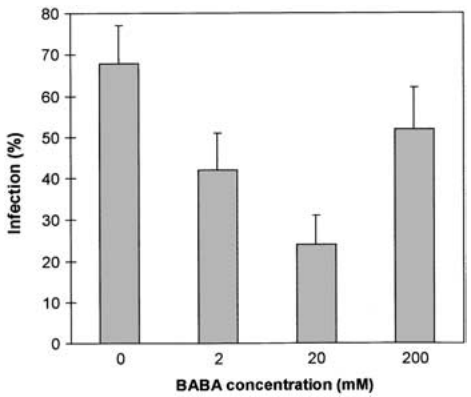

Figure 1. Effects of BABA concentrations on green mould decay development following artificial inoculation of grapefruit with *P. digitatum*. Surface wounds were treated with 30 μl of various concentrations of BABA, and after 24 h were inoculated with *P. digitatum*. Decay was evaluated after 4 days of incubation at 20 °C. Data are means ± SE of 54 wounds per treatment.

200 and 2 mM, BABA reduced infection by just 23% and 38%, respectively (Figure 1).

To determine whether the effects of BABA on the induction of grapefruit resistance to *P. digitatum* were systemic or limited to the peel tissue in the vicinity of its application site, specific wounds were pretreated with 20 mM BABA and after 24 h fresh wounds were made at increasing distances from the BABA-treated sites and inoculated with the pathogen. The results (Figure 2) show that induction of resistance to *P. digitatum* was most apparent in the vicinity (1–2 cm) of the BABA-treated site and was less pronounced at greater distances. When the fruit were inoculated at a distance of 1 or 2 cm from the BABA-treated site, the percentage of infected wounds was reduced by 52% as compared with that in control fruits that were treated with water alone, whereas at greater distances of 4 and 8 cm, infection was reduced by only 35% and 15%, respectively (Figure 2).

Effects of BABA on germination and growth of P. digitatum

To examine the effects of BABA on *P. digitatum* spore germination and germ-tube elongation *in vitro*, fungal spores were incubated in growth medium containing various concentrations of BABA. The results (Table 1) show that at increasing concentrations of BABA from 1 to 100 mM, a marked reduction in percentage of germination and germ-tube elongation was observed. At concentrations of 100 and 1000 mM BABA there was complete inhibition of *P. digitatum* spore germination and growth (Table 1).

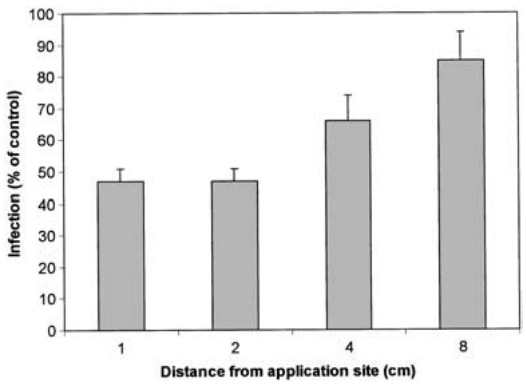

Figure 2. Effects of the distance from the BABA-treated wound sites on fruit resistance to *P. digitatum*. Fruit were wounded and treated with 30 µl of sterile water or 20 mM BABA, and after 24 h were wounded again at different distances from the original BABA application site, and inoculated with *P. digitatum*. Decay was evaluated after 4 days of incubation at 20 °C. Data are means ± SE of 54 wounds per treatment.

Effects of BABA on PAL activity

PAL is a key enzyme in the phenylpropanoid pathway leading to the biosynthesis of lignin, phytoalexins and other antifungal secondary metabolites (Dixon and Paiva, 1995). A basal increase in PAL activity was detected in the slightly wounded control fruit dipped in water alone (Figure 3). Nevertheless, in BABA-treated

Table 1. Effects of different BABA concentrations on *P. digitatum* spore germination and germ-tube elongation *in vitro*

BABA (mM)	Spore germination (%)	Germ tube length (µm)
0	100 a	42 a
0.1	100 a	40 a
1	88 b	21 b
10	44 c	10 c
100	0 d	0 d
1000	0 d	0 d

Spores of *P. digitatum* were grown in Potato Dextrose Broth containing various concentrations of BABA. After 24 h, the spore germination percentages and germ-tube elongations were measured in three light-microscope fields, each containing 40–50 spores. Data are from one out of two different experiments with similar results. Columns marked with different letters are significantly different at $P < 0.05$ according to a Student–Newman–Keuls one-way ANOVA test on ranks.

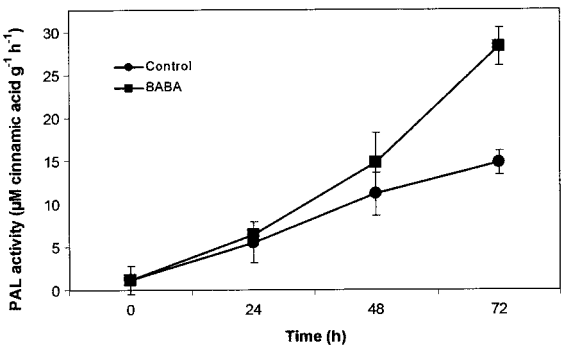

Figure 3. Effects of BABA on PAL activity in grapefruit peel tissue. Fruits were slightly wounded and dipped in water or in 20 mM BABA, and after various intervals peel samples were taken for enzyme extractions. Data of PAL activity are means ± SE of three different experiments.

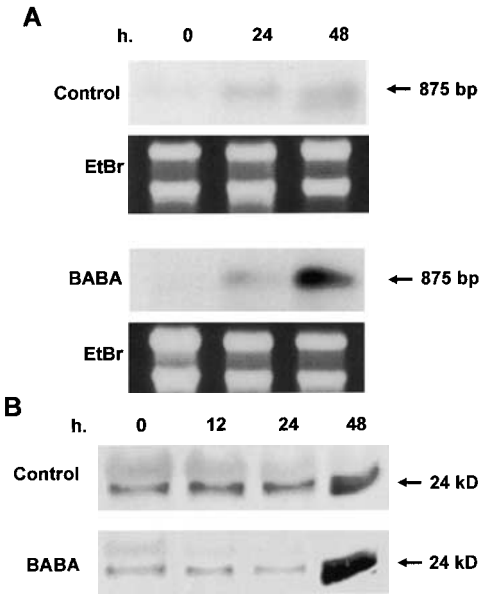

Figure 4. Effects of BABA on chitinase gene expression (A) and protein accumulation (B) in grapefruit peel tissue. Peel strips were dipped in water or in 20 mM BABA, and after various intervals samples were taken for RNA and protein extractions. For Northern blot analysis, 10 μg of total RNA were loaded in each lane, and the blots were probed with the *chi1* cDNA. For Western blot analysis, 10 μg of proteins were separated on a 12% polyacrylamide gel, blotted onto nitrocellulose, and then reacted with citrus chitinase antibodies. The arrows and values at the right indicate the approximate molecular mass of the grapefruit chitinase cDNA and polypeptide cross-reacting bands.

fruit there was a further increase in PAL activity as compared with that observed in control fruit already after 48 h, and this difference became significant after 72 h (Figure 3). After 72 h, PAL activity in BABA-treated fruit was twice as great as that observed in control fruit treated with water alone (Figure 3).

Effects of BABA on chitinase gene expression and protein accumulation

Chitinases are well characterized antifungal proteins that hydrolyze the chitin present in various fungal cell walls (Collinge et al., 1993). Dipping grapefruit peel strips in 20 mM BABA induced chitinase gene expression (Figure 4A). A slight increase in chitinase mRNA abundance was detected 24 h after the treatment and this increase became much more evident after 48 h (Figure 4A). A subsequent increase in chitinase protein accumulation was evident after 48 h (Figure 4B). A slight increase in chitinase mRNA levels and protein accumulation was also detected in control peel strips dipped in water alone, and this was probably due to the wounding of the tissue, which occurred during the preparation of the peel strips (Figure 4).

Discussion

BABA induces disease resistance in a wide variety of plants (Zimmerli et al., 2000; Cohen, 2001; 2002; Jakab et al., 2001). However, all of the studies that have been done hitherto addressed the induction by BABA on pathogen resistance in the vegetative portions of the plant, whereas in the present study we showed that BABA may increase disease resistance in reproductive tissues, such as mature fruit tissues of grapefruit.

This study reports that BABA induced resistance in grapefruit peel tissue against infection of *P. digitatum* in a concentration-dependent manner, being most effective at a concentration of 20 mM but less so at either higher or lower concentrations (Figure 1). A similar phenomenon of maximal induction of resistance to *P. digitatum* in grapefruit at an optimal concentration was also observed following application of other elicitors, such as JA (Droby et al., 1999). Therefore, these data suggest that BABA, like JA probably acts as a plant growth regulator, which is most effective when applied at an optimal concentration, and not as a fungicide which usually becomes continually more effective as the dose increases. Furthermore, application of fungicides, such as imazalil and thiabendazole, to specific wound sites on the fruits peel surface were unable to reduce decay development in new infected wounds made at a distance of 1–2 cm from their original application site as BABA did (data not shown). Thus, the main mode of action of BABA in reducing decay development on the fruit surface *in vivo* was through the activation of pathogen resistance mechanisms and not via any possible direct antifungal effects.

The optimal concentration of BABA (20 mM) required to induce disease resistance in grapefruit was the same as that previously reported for other plant species (Cohen et al., 1993; 1994; Oka et al., 1999; Silue et al., 2002). A possible explanation that such a high concentration of BABA is needed to elicit disease resistance is probably that only a small portion of the compound is actually absorbed by the tissue, and also that, of the entire BABA compound mixture only a small portion of its R-anantiomer isoform was found to be active in inducing pathogen resistance (Cohen, 2001; Cohen and Gisi, 1994).

In most previous studies (mainly in annual weedy plants) BABA induced both local and systemic resistance, and its application to one part of the plant increased resistance in other, distant portions of the plant (Cohen, 1993; Tosi et al., 1998; Hong et al., 1999; Oka et al., 1999). Our results, however, show that in grapefruit peel tissue BABA induced only local disease resistance, with effects limited to the vicinity (within 1–4 cm) of its application site (Figure 2). The reason for this difference in the response of vegetative *versus* the fruit to BABA is probably that the transportation system in the fruit peel tissue is not as effective as that through the xylem, and therefore the compound was effective only in the nearby tissue, which it could have reached by slow diffusion.

In all of the studies that we are presently aware of, BABA did not exhibit any direct antifungal activity and did not affect spore germination and germ-tube elongation of *Phytophthora infestans, Phytophthora capsici, Peronospora tabacina*, and *Plasmopara helianthi* (Cohen, 1994; Cohen et al., 1994; Sunwoo et al., 1996; Tosi et al., 1998). In those studies, however, the maximum BABA concentrations tested were 10–20 mM. In the present study, the antifungal effects of much higher concentrations of BABA were tested. Application at concentrations of 1–100 mM increasingly inhibited *P. digitatum* spore germination and growth *in vitro* (Table 1). Nevertheless, it should be noted that although increasing concentrations of BABA in the fungus growth medium exhibited progressively increasing antifungal properties, its optimal concentration for the induction of disease resistance was at a lower level (only 20 mM), suggesting that its major effect was probably through the induction of pathogen resistance rather than as a pathogen growth inhibitor. A similar contradictive effect was also detected in other cases. For example, JA was most effective in enhancing resistance against *Botrytis cinerea* in cut rose flowers when it was sprayed at an optimal concentration of 200 μM, but showed direct antifungal activity *in vitro* at rather higher concentrations of up to 400 μM (Meir et al., 1998).

In the present study, BABA has potentiated the fruit peel tissue to activate its pathogen defense mechanisms in response to wounding, e.g., by increasing PAL enzyme activity or inducing chitinase gene expression and protein accumulation (Figures 3 and 4). The increases in chitinase gene expression and protein accumulation were markedly apparent after 48 h, whereas the increase in PAL activity was most pronounced after 72 h (Figures 3 and 4). Thus, there is a lag of at least 48 h between the application of BABA and the induction of the fruits' defense responses. Indeed, it was previously reported that BABA was most effective in protecting the plants from infection when applied a few days before inoculation rather than in the same time or after inoculation (Sunwoo et al., 1996; Tosi et al., 1998).

Overall, in the present study, BABA increased pathogen resistance in grapefruit peel tissue, and it also appeared to be effective when applied to the commodity after harvest. Collectively, our findings highlight the possibility of inducing resistance to postharvest pathogens in fruits, and may open new directions of research aiming at exploring the use of elicitors in harvested commodities.

References

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 341–374

Cohen Y (1993) Local and systemic control of *Phytophthora infestans* in tomato plants by DL-3-amino-*n*-butanoic acid. Phytopathology 84: 55–59

Cohen Y (1994) 3-Aminobutyric acid induces systemic resistance against *Peronospore tabacina*. Physiological and Molecular Plant Pathology 44: 273–288

Cohen Y (2001) The BABA story of induced resistance. Phytoparasitica 29: 375–378

Cohen Y (2002) β-Aminobutyric acid-induced resistance against plant pathogens. Plant Disease 86: 448–457

Cohen Y and Gisi U (1994) Systemic translocation of ^{14}C-DL-3-aminobutyric acid in tomato plants in relation to induced resistance against *Phytophthora infestans*. Physiological and Molecular Plant Pathology 45: 441–456

Cohen Y, Gisi U and Niderman T (1993) Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. Phytopathology 83: 1054–1062

Cohen Y, Niderman T, Mosinger E and Fluhr R (1994) 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*. Plant Physiology 104: 59–66

Cohen Y, Reuveni M and Baider A (1999) Local and systemic activity of BABA (DL-3-aminobutyric acid) against *Plasmopara viticola* in grapevines. European Journal of Plant Pathology 105: 351–361

Collinge B, Kragh KM, Mikkelsen JD, Nielsen KK, Rasmussen U and Vad K (1993) Plant chitinases. Plant Journal 3: 31–40

Dixon RA and Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7: 1085–1097

Droby S, Chalutz E, Horev B, Cohen L, Gaba V, Wilson CL and Wisniewski M (1993) Factors affecting UV-induced

resistance in grapefruit against the green mould decay caused by *Penicillium digitatum*. Plant Pathology 42: 418–424

Droby S, Porat R, Cohen L, Weiss B, Philosoph-Hadas S and Meir S (1999) Suppression of green mold decay in grapefruits by postharvest application of jasmonates. Journal of American Society of Horticultural Science 124: 184–188

Droby S, Vinokur V, Weiss B, Cohen L, Daus A, Goldschmidt EE and Porat R (2001) Induction of resistance to *Penicillium digitatum* by the yeast biocontrol agent *Candida oleophila*. Phytopathology 92: 393–399

Hong JK, Hwang BK and Kim CH (1999) Induction of local and systemic resistance to *Colletotrichum coccodes* in pepper plants by DL-β-amino-*n*-butyric acid. Journal of Phytopathology 147: 193–198

Hwang BK, Sunwoo JY, Kim YJ and Kim BS (1997) Accumulation of β-1,3-glucanase and chitinase isoforms, and salicylic acid in the DL-β-amino-*n*-butyric acid-induced resistance response of pepper stems to *Phytophthora capsici*. Physiological and Molecular Plant Pathology 51: 305–322

Jakab G, Cottier V, Toquin V, Rigoli G, Zimmerli L, Metraux JP and Mauch-Mani B (2001) β-Aminobutyric acid-induced resistance in plants. European Journal of Plant Pathology 107: 29–37

Lers A, Jiang WB, Lomaniec E and Aharoni N (1998) Proteins functionally and immunologically related to pathogenesis-related proteins are induced during parsley leaf senescence. Physiologia Plantarum 103: 497–502

Lisker N, Cohen L, Chalutz E and Fuchs Y (1983) Fungal infections suppress ethylene-induced phenylalanine ammonia-lyase activity in grapefruit. Physiological Plant Pathology 22: 331–338

McCollum TG, Doostdar H, Mayer RT and McDonald RE (1997) Characterization of chitinases and β-1,3-glucanases in grapefruit flavedo during fruit development. Physiologia Plantarum 99: 486–494

Meir S, Droby S, Davidson H, Alsevia S, Cohen L, Horev B and Philosoph-Hadas S (1998) Suppression of Botrytis rot in cut rose flowers by postharvest application of methyl jasmonate. Postharvest Biology and Technology 13: 235–243

Oka Y, Cohen Y and Spiegel Y (1999) Local and systemic induced resistance to the root-knot nematode in tomato by DL-β-amino-*n*-butyric acid. Phytopathology 89: 1138–1143

Porat R, Pavoncello D, Peretz J, Weiss B, Daus A, Cohen L, Ben-Yehoshua S, Droby S and Lurie S (2000a) Induction of resistance to *Penicillium digitatum* and chilling injury in Star Ruby grapefruit by a short hot water rinse and brushing treatment. Journal of Horticultural Science and Biotechnology 75: 428–432

Porat R, Weiss B, Cohen L, Droby S, Jacob-Wilk D and Holland D (2000b) Identification of chitinase and β-1,3-glucanase cDNAs from citrus fruit. Acta Horticulture 535: 133–137

Reuveni M, Zahavi T and Cohen Y (2001) Controlling downy mildew (*Plasmopara viticola*) in field-grown grapevine with β-aminobutyric acid. Phytoparasitica 29: 125–133

Siegrist J, Orober M and Buchenauer H (2000) β-Aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. Physiological and Molecular Plant Pathology 56: 95–106

Silue D, Pajot E and Cohen Y (2002) Induction of resistance to downy mildew (*Peronospora parasitica*) in cauliflower by DL-β-amino-*n*-butyric acid (BABA). Plant Pathology 51: 97–102

Sunwoo JY, Lee YK and Hwang BK (1996) Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-β-amino-*n*-butyric acid. European Journal of Plant Pathology 102: 663–670

Tosi L, Luigetti R and Zazzerini A (1998) Induced resistance against *Plasmopara helianthi* in sunflower plants by DL-β-amino-*n*-butyric acid. Journal of Phytopathology 146: 295–299

Zimmerli L, Jakab G, Metraux JP and Mauch-Mani B (2000) Potentiation of pathogen-specific defense mechanisms in *Arabidopsis* by β-aminobutyric acid. Proceedings of the National Academy of Science USA 97: 12920–12925

Zimmerli L, Metraux JP and Mauch-Mani B (2001) β-Aminobutyric acid-induced protection of Arabidopsis against the necrotrophic fimgus *Botrytis cinerea*. Plant Physiology 126: 517–523