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Induction of resistance against powdery mildew by Beta aminobutyric acid in squash

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Summary

The non-protein amino acid beta-aminobutyric acid (BABA) enhances *squash* resistance to microbial pathogens through potentiation of the *squash* defense responses. In the present study, we investigated the effects of BABA pretreatment on cucurbit powdery mildew disease. Three concentrations of BABA (0.5, 2 and 4 mM) as priming media and distilled water as control plants were used. The results showed that the 4 mM BABA was the most effective concentration to control the disease symptoms. The expression of *PR-1* gene in relation to this pathogen was investigated and confirmed. Guaiacol peroxidase and Phenylalanine amonia-lyase enzymes activities increased in pretreated-inoculated squash plants. Bases on our results and regarding the adverse effects of fungicide on the environment, use of BABA as an environmentally safe agent is recommended as a valuable contribution to disease management.

Introduction

Powdery mildew, caused by *Podosphaera xanthii* [syn. *P. fusca* (Castagne) U. Braun & Shiskoff, Pollacci], is a serious disease affecting the leaves, stems and fruits of squash and zucchini squash grown in greenhouses and in the field. The disease is controlled in commercial cucurbit crops by applying a high rate of fungicides (Kimati et al., 1997). The extensive usage of fungicides has resulted in the development of resistant *P. xanthii* populations to fungicides and public concern over contamination of the environment and foods. The identification of biocompatible products for managing cucurbit powdery mildew with low animal toxicity and low potential risk to the environment would be a valuable contribution to disease management (Mc Grath and Staniszewska, 1996). Resistance can be systemically induced in plants by inoculation with non-pathogens, restricted inoculation with pathogens, or by application of chemical treatments including β-aminobutyric acid (Cohen, 2002; Jakab et al., 2001; Sharifi-Sirchi et al., 2011) salicylic acid, 2,6-dichloroisonicotinic acid (INA), benzothiadiazole (BTH), probenazole and microelements (Bai et al., 2010; Zimmerli et al., 2001). Such synthetic or natural compounds are named inducers and can induce resistance in plants (Durrant and Dong, 2004; Ton and Mauch-Mani, 2004; Shi et al., 2007; Von Rad et al., 2005).

Physiological conditions in which plants are able to better or more rapidly mount defense responses to biotic or abiotic stresses are called the "primed state" of the plant (Conrath et al., 2002). For example, cucumber plants that had been attacked by the fungus *Colletotrichum lagenarium* combated secondary penetration attempts by rapidly depositing effective papillae at the points of attempted pathogen ingress (Kuc, 1982). The non protein amino acid β-aminobutyric acid (BABA) is known as a potent inducer of resistance in plants against microbial pathogens (Cohen, 2002; Jakab et al., 2001), nematodes (Oka et al., 1999), insects (Hodge et al., 2005), and abiotic stresses (Jakab et al., 2005). Research on the mechanisms of BABA-induced resistance (BABA-IR) in Arabidopsis has shown that this form of induced resistance, like SAR, is mostly based on priming for different pathogen-inducible defense mechanisms. In Arabidopsis, BABA-enhanced resistance against *Botrytis cinerea* and *Pseudomonas syringae* were found to have correlations with primed transcription of the SA inducible *PR*-1 gene, whereas induced resistance towards *Hyaloperonospora arabidopsidis* depends on an earlier and stronger formation of callose rich papillae around the growing hyphae (Yu et al., 2006; Zimmerli et al., 2000, 2001). Pathogenesis-related proteins consist of enzymes including chitinase, peroxidase, phenylalanine amonia-lyase and certain other proteins which accumulate in high levels following pathogen attacks. Their induction has been correlated with greater resistance to subsequent pathogen attack (Tuzun et al., 1989). Phenylalanine ammonia lyase (PAL) catalyzes the deamination of L-phenylalanine to t-cinnamic acid, which is the first step in the phenylpropanoid pathway which supplies the precursors for phenolics, lignin, furanocoumarin, phytoalexins and other downstream metabolites (Tsuge et al., 2004). The activities of PAL and POD may rapidly be enhanced under the influence of elicitors or pathogen attack. Enhancement of PAL and POD activities was reported in response to *Rhizoctonia solani* inoculation in cowpea pretreated with SA (Chandra et al., 2007). PAL exhibits high reactivity to stress factors and plays a key role in the synthesis of compounds involved in plant-immunity. The purpose of this study was to evaluate the effects of the BABA priming *P. xanthii* on cucurbit plants (*Cucurbita pepo* L.cv. Peto Seed) and to analyze the expression of the *PR*1 gene, and GPX and PAL enzyme activities during resistance performance.

Materials and methods

Biological material, growth conditions and chemicals

Cucurbit plants (*Cucurbita. pepo*) were grown in plastic pots (150 mm diameter * 150 mm deep) filled with a mixture of soft mold leaves and loamy sand (5: 2, v/v) under normal greenhouse conditions (18 °C and 16 h-photoperiod). Complex fertilizer was applied twice per week. All chemicals were obtained from Sigma (Deisendorf, Germany). Experiments were carried out when seedlings were in the four-leaf stage. In this stage plants were sprayed with different concentrations of BABA (0.5, 2 and 4 mM).

Pathogen and inoculation

P. xanthii obtained from "eld-grown" plants were maintained on squash plants grown in greenhouse conditions (18 °C and 16 h photoperiod). Inoculum was obtained from freshly sporulation infected leaves 9-12 days after inoculation. Conidia were gently brushed into 100 ml distilled water containing two drops of Tween-20 and counted with the aid of a haemocytometer to give a suspension of 4×10^6 conidia ml^{-1}. After 24 h from treatment, upper surfaces of all

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the leaves were sprayed with a conidial suspension delivered by a hand sprayer. After inoculation, plants were kept in the same green house (18 °C and 16 h photoperiod) until disease development. Control plants were sprayed with water and kept under similar conditions.

The infection degree was assessed visually using a 0-5 scale seven days after inuculation: where 0 = no disease symptoms, 1 = less than 1/5 of the host surface covered by mycelia, 2 = 1/5 -1/3 of the surface covered by mycelia, 3 = 1/3 -1/2 of the surface covered by mycelia, 4 = 1/2 -2/3 of the surface covered by mycelia, 5 = more than 2/3 of the surface covered by mycelia. Disease index of every plant was determined, according to the mathematical formula:

$$\text{Disease Index} = \frac{\sum a \times b}{N \times K} \times 100\,\%$$

Where a is the number of leaves with a corresponding infection degree, b is the infection degree of leaves (scale differences from 0-5), N is the total number of leaves counted in a plant, and K is the maximal value of lesion intensity (= 5 on the chosen scale) (SHTERNSHIS et al., 2002).

Isolation of partial *PR*1 gene

Primers were designed based on the conserved sequence of the *PR*1 gene in *Cucurbitaceae* family plants using the FPCR and DNAMAN package softwares (Microsoft visual studio 6.0, visual Basic 6.0 SP6 Company). The primers were synthesized by the Isogene Company, Netherlands. PCR reactions were performed in a Veriti thermal cycler (Applied Biosystems) with a total volume of 20 µL containing 60 ng of genomic DNA, 1 × PCR reaction buffer, 1.5 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTPs, 0.5 mmol L^{-1} of each primer, and 1 U of *Taq* polymerase. PCR condition was set at 94 °C for 5 min, following 35 cycles of 94 °C for 1 min, 55 °C for 45 s, 72 °C for 1 min, and followed by a final extension of 5 min at 72 °C. Samples were run in 1% agarose gel in 0.5 × Tris-borate-EDTA (TBE, pH 8.3) buffer. Gels were run at voltage 100 for 35 min and visualized with ultraviolet light after ethidium bromide staining as described by SAMBROOK and RUSSELL (2001).

Semi-RT-PCR

For each sample, 3-week old squash leaves from three pots were harvested at the indicated time points, flash frozen in liquid nitrogen and kept at -80 °C. Total RNA was extracted from 0.1 g of frozen leaves purified using the RNeasy Plant Mini Kit (Qiagen, Germany) with additional DNA clean-up using the RNase-Free DNase Set (Fermentas, Lithuania). Complementary DNA was synthesized from 2 µg of total RNA using a First Strand cDNA Synthesis Kit (Fermentas, Lithuania). The resulting first strand cDNA was used for PCR. Specific primers were used to amplify cDNA fragments, including: F 5'-ACTCACCTCAAGACT-3 (forward) and 5'-GGATGTGCCAAAG-3' (reverse) for *PR*1 (*EMBL: JF332040*) and 5'-AAGACGAACAACTGCG-3' (forward) and 5'- CGACCATACTCCCCCC-3' (reverse) for 18S rRNA. 18S rRNA was used as the reference gene for normalization of gene expression levels in all samples.

Purification and cloning of cDNA fragment

PCR products were purified with an AccuPrep PCR Purification Kit according to the manufacturer's instruction (Bioneer, South Korea). The purified PCR products were ligated into the pTZ57R/T vector using InsT/A Clone™ PCR product Cloning Kit (Fermentas, Lithuania). Then, recombinant plasmids were transformed into competent *E. coli* XL1Blue cells. Screening was performed based on blue-white selection method as described in Sambrook and Russell (SAMBROOK and RUSSELL, 2001). Transformed white colonies were selected on X-Gal/IPTG LB ampicilin Agar Plates, after 24 h of growth and confirmed by colony PCR and enzyme digestion (*Eco*R1 and *Bam*H1). The recombinant plasmids were extracted by AccuPrep® Plasmid extraction Kit and AccuPrep PCR Purification Kit according to the manufacturer's instructions (Fermentas, Lithuania). Recombinant plasmids were sequenced in both directions by extending M13 reverse and forward primers, using the Automatic DNA Sequencer 3730Xl (Macrogene, Korea). The sequencing data were edited using Chromas software Version 1.41.

Comparison of homologues sequences for query and drawing phylogenetic tree

Homology of Obtained *PR*1 gene sequence (as query) was compared with database sequences in NCBI by nucleotide blast program (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). Results of seeding were used for calculating the triangular matrix of similarity and drawing a phylogenetic tree by DNAMAN software version 4.02 (Lynnon Biosoft. 1994-98).

Enzyme extraction and activity determination

500 mg of leaves were homogenized in cool 50 mM potassium phosphate buffer (pH = 7.0) containing 1% soluble polyvinilpyrolidone[1] (W/V), 1 mM ethylene diamine tetra acetic acid[2] and 1 mM phenylmethylsulfonyl fluoride[3]. All processes were carried out in ice. The homogenate was centrifuged at 20,000 × g for 20 min and the supernatant used for assay of the enzyme activity.

Catalase (CAT) activity (EC 1.11.1.6)

CAT activity was determined spectrophotometrically following the decrease in absorbance of H_2O_2 within 30 s at 240 nm (DHINDSA et al., 1981). The 3 ml reaction solution consisted of 50 mM potassium phosphate buffer (pH = 7.0), 15 mM H_2O_2, and 100 ml of enzyme extract. Addition of H_2O_2 started the reaction and the decrease in absorbance was recorded after 30 s. Unit of activity was taken as the amount of enzyme, which decomposes one µmol of H_2O_2 in one minute (ε = 40 mM^{-1} cm^{-1} for H_2O_2).

Guaiacol peroxidase (GPX) (EC1.11.1.7)

GPX activity was measured using Guaiacol as a substrate. Reaction mixture (3 ml) contained 25 ml of enzyme extract, 2.77 ml of 50 mM phosphate buffer (pH = 7.0), 0.1 ml of 1% H_2O_2 (V/V), and 0.1 ml of 4% guaiacol (V/V). The increase in absorbance at 470 nm due to guaiacol oxidation was recorded for 3 min using an extinction coefficient of 25.5 mM^{-1} cm^{-1} (PLEWA et al., 1991).

Phenylalanine amonia-lyase (EC 4.3.1.5)

Phenylalanine amonia-lyase (PAL) activity was determined by the modified method of CHENG and BREEN (1991). The reaction mixture contained leaf extract (0.3 ml), 0.2 M phenylalanine solution (1 ml) and 0.05 M borate buffer (pH = 8.8, 2.7 mL). The reaction was quenched with 6 N HCl (0.1 mL). The production of Cinnamic acid during 1 h at 30 ºC was measured by the absorbance change at 290 nm. One unit of enzyme represents the conversion of 1 µmol substrate to cinammic acid per min.

[1] PVP
[2] EDTA
[3] PMSF

Total soluble proteins
Protein content was determined according to the method of BRADFORD (1976) using Bovine serum albumin as standard.

Measurement of Chlorophyll and Carotenoid Contents
Chlorophyll a, b and carotenoid were extracted using 80% acetone based on Lichtenthaler (1987) method. The Chlorophyll a, b and carotenoid contents of leaves were measured at the wave lengths 646.8, 663.2 and 470 nm by using spectrophotometry and the formulae.

$$Chl.a = (12.25A_{663.2} - 2.79A_{646.8})$$
$$Chl.b = (21.21A_{646.8} - 5.1\ A_{663.2})$$
$$Car = [(1000A_{470} - 1.8\ Chl.a - 85.02\ Chl.b)/198]$$

Statistical analysis
All determinations were carried out in triplicate and the data subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses were performed according to the MSTATC software. Significant differences between means were determined by Duncan's multiple range tests. *P* values less than 0.01 were considered statistically significant (DUNCAN, 1955).

Result

BABA-treated cucurbit plants show resistance against *Podosphaera xanthii*
In primary experiments, two approaches (root drench and foliar spray) with three doses of BABA (0.5, 2 and 4 mM) were analyzed. The results showed that application of BABA through root drench was not effective on *P. xanthii* while foliar application of BABA was very effective. However, foliar spray with 0.5 mM BABA was not appropriate to control *P. xanthii*. Therefore, in other research we applied a concentration of 2 and 4 mM BABA.
Seven days after inoculation, the powdery mildew disease index was estimated on every plant. Disease index for water control plants (60) was significantly higher than those of 4 and 2 mM for BABA treatment (21 and 43, respectively) (Fig. 1 and 2). The concentration of 4 mM of BABA showed the best control after seven days of inoculation.

Isolation of Partial *PR*1 gene and its homology analysis
The amplified PCR fragment of 317 bp was obtained by using forward and reverse *PR*1 gene primers (Fig. 3). It was submitted to

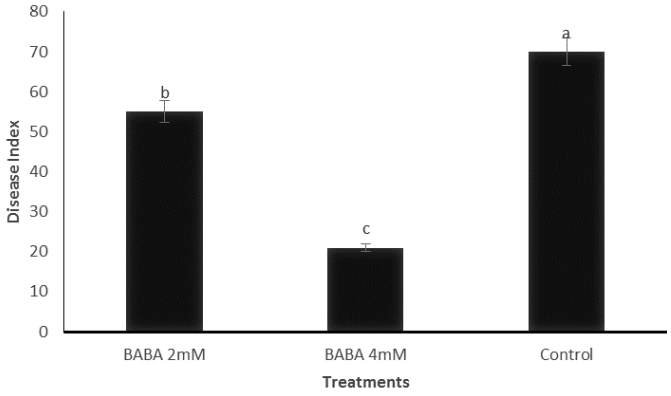

Fig. 1: Mean comparison of treatments in induced resistance of squash to powdery mildew (Duncan test, P < 1%). Different letters show significant differences. Standard error (SE) bars are presented for each sample.

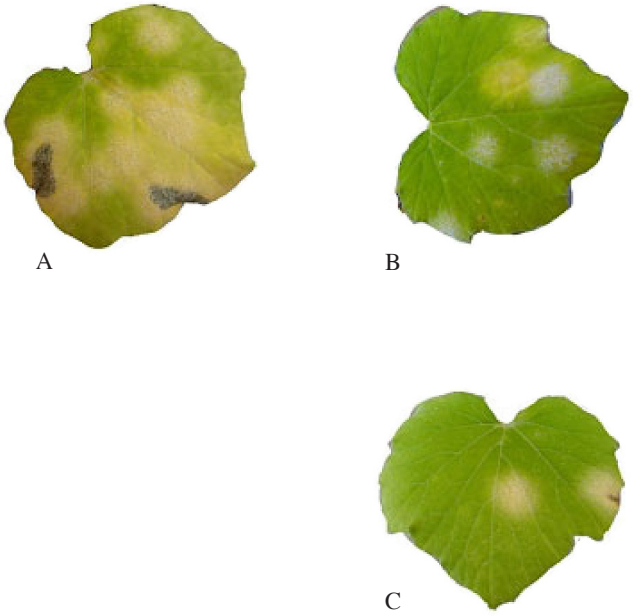

Fig. 2: Comparison of infected squash leaves exposed to powdery mildew seven days post inoculation. A; control, B; primed squash leave with 2 mM BABA, C; primed squash leave exposed to 4 mM BABA.

GenBank (JF332040.1 mRNA accession number - *Cucurbita pepo* pathogenesis-related protein 1 (PR1) mRNA, partial cds). Its deduced amino acid sequence (AEA11234.1) was 114 amino acids long (Fig. 3). The partial sequence of the *PR*1 gene in seedings of *Cucurbita pepo* showed highest homology with the *Cucumis melo* *PR*1 gene (68%). *C. melo* belongs to the family of Cucurbitaceae (Fig. 4). Cluster analysis of partial sequence of *PR*1 in *Cucurbia pepo* with seven *PR*1 genes from different plants which had homology with the *PR*1 gene from *Cucumis melo* in one class (Fig. 5).

a)
ACTCACCTCAAGACTATGTTGATGCTCACAATATTGCTCGTTCTCAAGTGAGCACTGTCCAAGTTGGC
ATCAAACCCATCCAGTGGGATGAAGAACTTGCAAACTACGCTACCCAATATGCCAATGAACGTAGCA
ATGACTGTCAACTGTTGCACTCTAATGGGCCTTATGGCGAGAATCTTGCAATGCATTCTAGTGAAATG
ACAGGCATTGAAGCAGTTCAAATGTGGGTGGATGAGCAACAATTCTACGATTATGCCTCCAACACCT
GCACTGAAGGAAAAATGTGCGGTCACTACACTCAAGTGGTGTGGCAAAACACAACAAAAGTTGGAT
GTGCCAAAG

b)
SPQDYVDAHNIARSQVSTVQVGIKPIQWDEELANYATQYANERSNDCQLLHSNGPYGENLAMHSSEMTG
IEAVQMWVDEQQFYDYASNTCTEGKMCGHYTQVVWQNTTKVGCAK

Fig. 3: *Cucurbita pepo* pathogenesis-related protein 1 (PR1) mRNA, partial cds (a) and partial protein (b).

Cucurbita_pepo	100%
Arabidopsis_thalian	33.1% 100%
Cucumis_sativus	65.2% 48.4% 100%
Cucumis_melo	68.0% 42.9% 74.1% 100%
Brassica_rapa	24.9% 63.4% 30.8% 29.7% 100%
Brassica_carinata	23.2% 57.3% 26.8% 24.8% 91.1% 100%
Eutrema_wasabi	34.8% 84.8% 49.6% 43.9% 66.3% 59.9% 100%
Vitis_vinifera	22.2% 30.9% 27.8% 28.5% 35.1% 37.4% 33.5% 100%

Fig. 4: Homology matrix of *Cucurbita pepo* Partial PR1 cds and seven other plants.

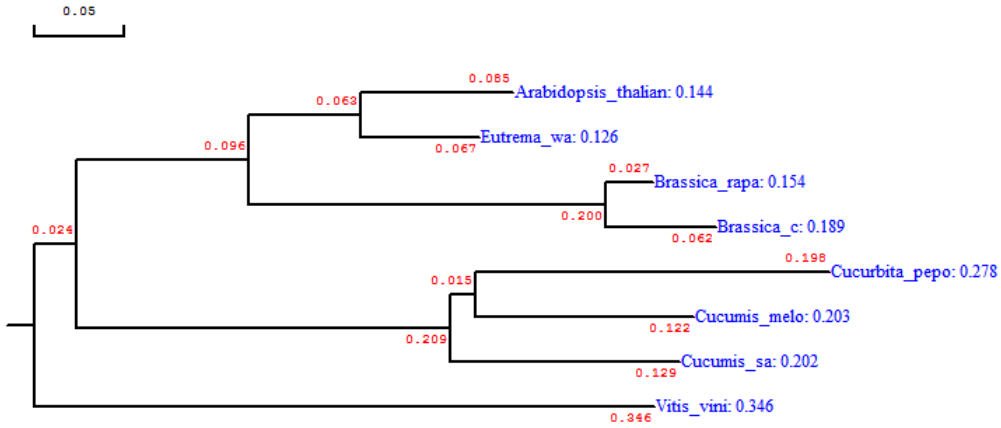

Fig. 5: Phylogeny tree base on distance matrix of *Cucurbita pepo* Partial PR1 cds and seven other plants.

The effect of BABA pretreatment on the expression of *RP*1 gene
The expression level of *PR1* was evaluated by semi RT-PCR at post *P. xanthii* inoculation (Fig. 6). *PR*1 gene was detected in both control and BABA treated cucurbit for the six investigated time points (Fig. 6-a and 6-b). There was a slight increase in *PR*1 expression in infected-BABA-primed (4 mM) cucurbit plants compared to the infected and water-treated cucurbit plants at 12 h post infection (Fig. 6-a and 6-b).

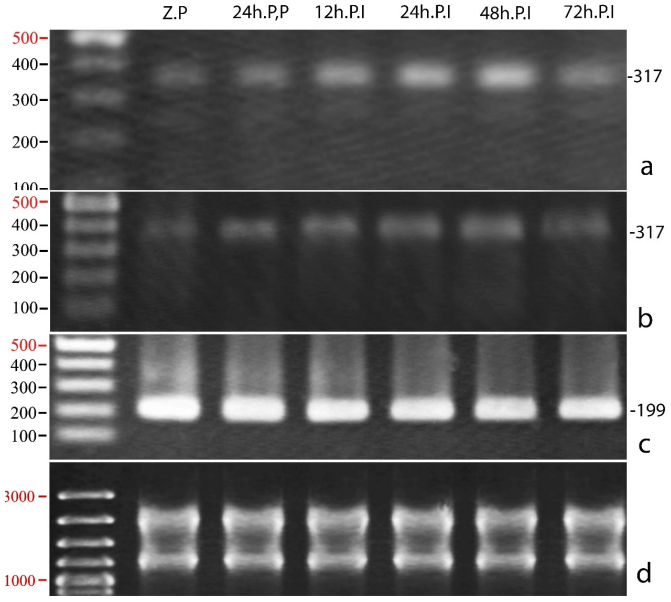

Fig. 6: *PR*1 gene expression after inoculation. Effect of BABA treatment on the expression of *PR*1 during of disease development by *P. xanthii*. (a); *PR*1 gene expression pattern on infected and BABA-primed (4 mM) cucurbit plants. (b); *PR*1 gene expression pattern on not infected control cucurbit plants. (c); Balanced 18SrRNA expression after 35 cycle of PCR. (d); Balanced total RNA. Z.P; time point 0. 24 P.P; 24 h post priming. 12hpI, 24hpI, 48hpI and 72hpI; hours post inoculation.

Antioxidant enzyme activities
Activities of GPX, CAT and PAL in infected and primed cucurbit plant leaves with *P. xanthii* and control plants assayed.

CAT activity: Results showed that CAT activity was significantly decreased after inoculation and the minimum activity of CAT was observed 240 h after inoculation. However, no significant differences were observed in CAT activity between the treatments given at any one time point except 24 h after inoculation (Fig. 7).

GPX activity: As shown in Fig. 7, pretreatment of plants with BABA had no significant effect on GPX before inoculation, while infection of plants with *P. xanthii* promoted the activity of GPX in water and BABA pretreated plants in comparison with non-inoculation plants. GPX showed very high activity after 168 and 240 h post inoculation. Application of BABA pretreatment increased the activity of 4 mM GPX after 48h post inoculation. Pretreatment of plants with 2 mM BABA had no significant effects on GPX activity in comparison with water treated plants (Fig. 7).

PAL activity: Result showed that Pal activity was significantly increased at 24 hours after priming with both BABA concentrations (2 and 4 mM). Also PAL activity was significantly increased in all sampling time points after inoculation in both primed and control cucurbit plants. However, PAL activity was the highest at 240 hours after inoculation sampling time points in Primed plants with 4 mM BABA (Fig. 8).

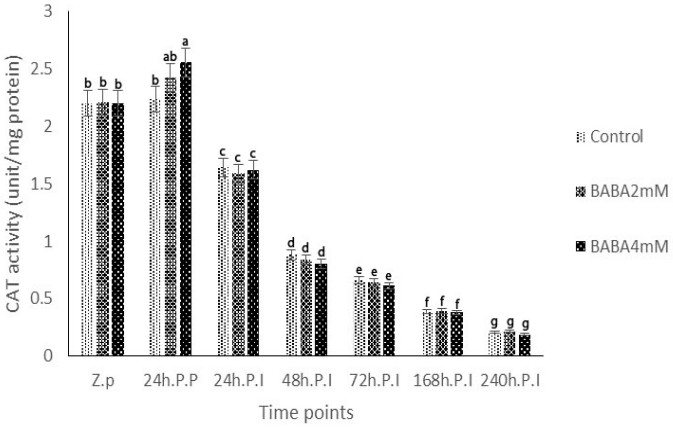

Fig. 7: Effect BABA pretreatments on catalase activity. The mean comparisons of CAT activity of treated squash plants compared using the Duncan method at a $P < 0.01$ significance level. Different letters show a significant difference. Z.P; time point 0. 24 PP; 24 h post priming. hpI; hours post inoculation.

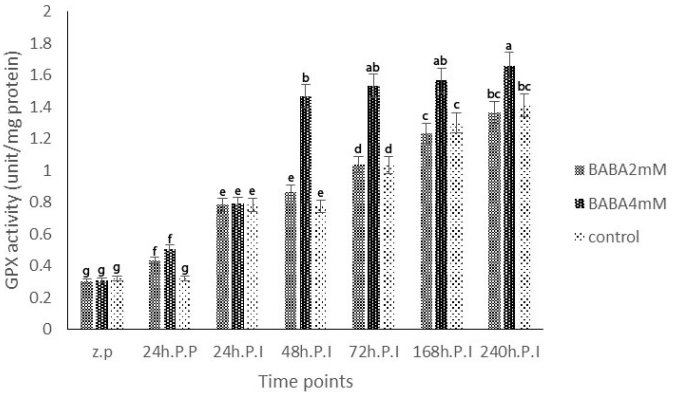

Fig. 7: Effect BABA pretreatments on the Guaiacol peroxidase (GPX) activity. Mean comparison of GPX activity of treated squash plants using Duncan method at a $P < 0.01$ significance level. Different letters show a significant difference. Z.P; time point 0. 24 PP; 24 h post priming. hpI; hours post inoculation. Standard bar showed differences at $\alpha = 0.5\%$.

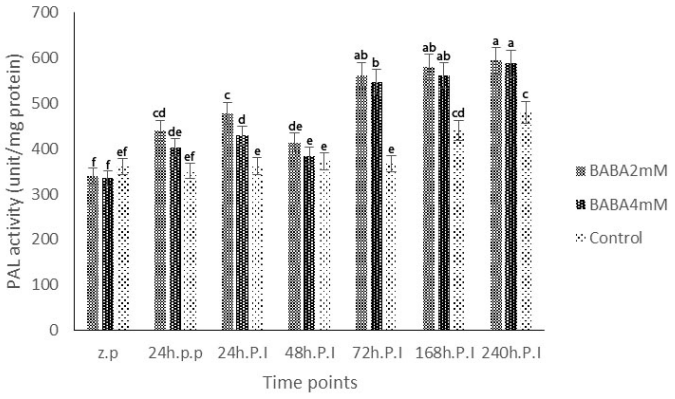

Fig. 8: Effect of BABA pretreatments on Phenylalanine ammonia lyase (PAL) activity. Mean comparison of PAL activity of treated squash plants using Duncan method at a $P < 0.01$ significance level. Different letters show significant difference. Z.P; time point 0. 24 PP; 24 h post priming. hpI; hours post inoculation. Standard bar showed differences at $\alpha = 0.5\%$.

Chlorophyll and Carotenoid contents

Our results showed that the amount of chlorophyll a and b pigments of cucurbit plants were significantly higher at 240 hours after inoculation compared to those of controls (Fig. 9). Carotenoid contents of primed inoculated leaves were significantly higher than inoculated control plants and Primed cucurbit plants with 4 mM BABA had a higher carotenoid amount than Primed cucurbit plants with 2 mM BABA (Fig. 9).

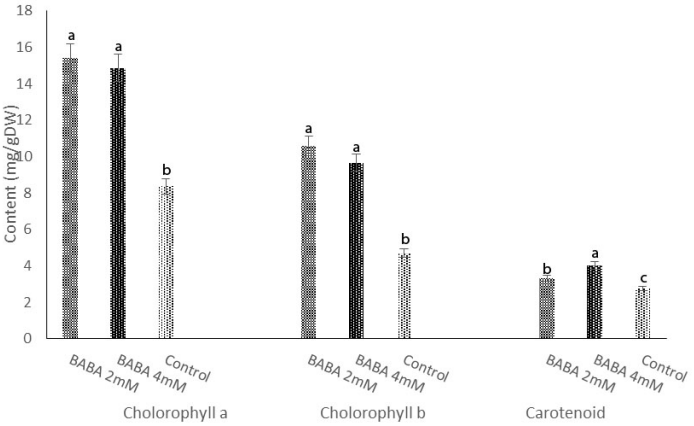

Fig. 9: The Mean comparisons of chlorophyll a and b, and carotenoid content of control and Primed inoculated cucurbit plants with 4 mM BABA at 240 hours post inoculation. Standard bar showed differences at $\alpha = 0.5\%$.

Discussion

The resistance inducer BABA has been shown to work mainly through priming of defense responses by sensitizing the plants to respond faster and more adequately to exposure against a given stress situation (Conrath et al., 2002; Conrath et al., 2006; Jakab et al., 2001). In this study, we investigated the influence of BABA pretreatment against *P. xanthii* infection and the activation of pathogenesis-related proteins. Our results confirmed that application of BABA to cucurbit leaves reduced the disease index compared to plants before inoculation. We provide the first evidence that this resistance is associated with an increased enzymatic activity and transcript accumulation of the *PR-1* gene. In many plants, the chemical BABA has been shown to enhance disease resistance and to increase salt, drought, and thermo tolerance (Jakab et al., 2005; Slaughter et al., 2008; Ton et al., 2005; Ton and Mauch-Mani, 2004; Zimmerli et al., 2000, 2001, 2008). Typically, following infection by *Pseudomonas syringae* pv. compared to tomato DC3000, the salicylic acid (SA)-dependent defense marker pathogen related gene 1 (*PR*1) is induced earlier and stronger in BABA-treated Arabidopsis (Ton et al., 2005; Zimmerli et al., 2000).

BABA does not activate a defense response directly but rather sensitizes plants to respond more quickly and strongly to biotic and abiotic stresses. This process is referred to as priming (Conrath et al., 2002; Conrath et al., 2006). Given that accumulation of these proteins after pathogen infection correlates with induced resistance.

PAL activity in plant tissue might be rapidly changed under the influence of various factors, e.g. pathogen attack and treatment with elicitors. PAL and POD activities were enhanced several fold in tomato roots by a biotic elicitor *Fusarium mycelium* extract derived from Fol (Mandal and Mitra, 2007). Enhancement of PAL and POD activities in SA-treated asparagus plants upon *F. oxysporum* f. sp. *asparagi* infection resulted in reinforcement of the cell wall and restricted subsequent fungal penetration and infection (He and Wolyn, 2005). Addition of 20 mM salicylic acid to Saussurea medusa cell cultures resulted in a 7.5-fold increase in PAL activity (Yu et al., 2006). SA spraying on Ya Li pear plants increased PAL and POD activities greatly and contributed to protection of pear fruits against postharvest diseases (Cao et al., 2006). In the present investigation the activities of PAL and POD were also increased to a great extent in the BABA treated plants compared to non-BABA-treated plants, probably contributing in enhanced resistance of cucurbit to *Podosphaera xanthii*. In this study, PAL activity was increased after inoculation. Mauch- Mani and Slusarenko (1996) reported that PAL is involved in the synthesis of SA and precursors of lignification in Arabidopsis.

SAR, induced biologically and chemically in plants, is associated with an ability of plants to resist pathogen attack by enhanced activation of cellular defense mechanisms (Mé Traux et al., 2002). The results indicate that the induced resistance observed in cucurbit against *P. xanthii* may be a case of SA-dependent SAR. In conclusion, it seems that BABA feeding does reduce susceptibility of squash plants to *P. xanthii*, likely due to induction of SAR accompanied by increased activities of the defense enzymes PAL and POD.

The results of this investigation show that BABA may be used as a potential inducer of SAR against *P. xanthii*. Fungicide resistance and public concern over environment contamination has resulted in identification of biocompatible products for managing cucurbit powdery mildew with low animal toxicity and low potential risk to the environment. BABA would be a valuable contribution to disease management.

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