European Journal of Plant Pathology (2005) 112:361–369
DOI 10.1007/s10658-005-6234-1

Induction of oxidants in tomato leaves treated with DL-β-Amino butyric acid (BABA) and infected with *Clavibacter michiganensis* ssp. *michiganensis*

Ömür Baysal[1], Y. Ziya Gürsoy[2], Hakan Örnek[2], and Ahmet Duru[2]
[1]*West Mediterranean Agricultural Research Institute (WMARI), P.B. 35 07100, Antalya, Turkey; (E-mail: obaysal@ttnet.net.tr);* [2]*Bornova Plant Protection Research Institute (BPRI), 35040, Bornova, Izmir, Turkey*

Accepted 22 April 2005

Key words: clavibacter, DL-β-amino butyric acid (BABA), induced resistance, oxidative burst

Abstract

Bacterial canker is an economically important disease of tomato. Resistance induced by DL-β-Amino butyric acid against bacterial canker caused by *Clavibacter michiganensis* ssp. *michiganensis* in tomato plants was investigated. Different doses of DL-β-Amino butyric acid (250–1000 μg ml^{-1} doses) were tested on 3-week old plants inoculated with a 10^8 CFU ml^{-1} bacterial suspension, and disease development was evaluated after inoculation and treatment. Although *in vitro* growth of the bacteria was not affected by DL-β-Amino butyric acid treatment, foliage sprays of 500 μg ml^{-1} DL-β-Amino butyric acid significantly suppressed disease development up to 54% by day 14 after inoculation at the four different doses tested. Bacterial populations were reduced by 84% in BABA-treated plants compared to water-treated plants by day 4 after inoculation. Inoculated BABA-treated plants showed significantly higher phenylalanine ammonia-lyase activity, peroxidase activity, and H_2O_2 concentration than inoculated water-treated plants during day 1 after treatment. These findings suggest that the DL-β-Amino butyric acid treatment resulted in an increase of these enzymes and in H_2O_2 concentration *in planta*, and was associated with induction of resistance to bacterial canker.

Abbreviations: AOS – active oxygen species; BABA – DL-β-amino butyric acid; PAL – phenylalanine ammonia-lyase; POX – peroxidase; SAR – systemic acquired resistance

Introduction

Clavibacter michiganensis ssp. *michiganensis* (*Cmm*) is a gram-positive bacterium that causes bacterial canker characterized by vascular infections, wilting, chlorosis and death of the plant. *Cmm* is a recurrent and serious disease problem in tomato (*Lycopersion esculentum)* (Ark, 1994), of both field and greenhouse-grown crops in several countries (Gleason et al., 1993). Disease control is difficult due to a lack of commercial resistant tomato cultivars. Chemical control of the disease relies on the use of antibiotics (such as streptomycin) and copper compounds, which prevent bacterial multiplication and further infection. However, the use of antibiotics is banned or severely limited in many countries for control of bacterial plant diseases. Therefore, alternate measures for bacterial canker of tomato is of great importance, particularly to control the disease in greenhouse crops in southern Turkey.

A plant defense response can be activated upon infection with pathogens as well as following treatment with elicitors. Various substances have been demonstrated to be elicitors of disease resistance in plants. Systemic acquired resistance (SAR) is characterized by a reduction in the number and severity of lesions following challenge

inoculation with a normally virulent pathogen. Salicylic acid (SA) is recognized as an inducer of SAR when sprayed on plants and, it fulfills all of the criteria of an elicitor. However, reports of SA-mediated resistance have shown restricted effects in treated tissue, indicating that SA does not translocate efficiently throughout the plants when applied exogenously (Enyedi and Raskın, 1993). Therefore, synthetic chemicals such as 2,6-dichloroisonicotinic acid and acibenzolar-s-methyl have been developed that induce a reaction similar to that to induced by SA (Oostendorp et al., 2001). The synthetic, non-protein, aminoacid, DL-β-aminobutyric acid (BABA), has been thoroughly studied by several research groups. It induces resistance against diseases caused by downy mildew as well as by necrothrophic fungi and bacteria in many crops (Zimmerli et al., 2000). Its mechanism of action is uncertain because the reactions induced depend not only on the pathosystem but also on the application method (Jakab et al., 2001). The translocation of BABA and its accumulation in young leaves of a plant has been demonstrated with its protective effect against fungal and bacterial pathogens (Cohen, 2002).

The development of SAR is often associated with various cellular defense responses, such as synthesis of pathogenesis-related (PR) proteins, phytoalexins, accumulation of active oxygen species (AOS), and rapid alterations in the cell wall, which enhance the activity of various defense-related enzymes (Ryals et al., 1996). In recent studies, AOS have been explored during expression of SAR. There is ample evidence indicating that AOS, H_2O_2 in particular, perform several important functions in early defense responses of the plant against pathogens, including direct antimicrobial action, lignin formation, phytoalexin production, and triggering of SAR (Mehdy et al., 1996; Lamb and Dixon, 1997). In another study, systemic activation of SAR in non-infected tissue has been associated with enhanced levels of reactive oxygen intermediates with increases in antioxidant levels (Alvarez et al., 1998). AOS, produced via an oxidative burst, are under control of enzymes such as NADPH oxidase and peroxidases (POXs) (Wojtaszek, 1997). POXs have been implicated in the hypersensitive response (Bestwick et al., 1998) and the formation of papilla and polymerization of lignin from monomeric lignols (Nicholson and Hammerschmidt, 1992). Furthermore, peroxidases have been implicated in the cross-linking reactions of cell wall associated proteins, involving hydroxyproline-rich or glycine-rich glycoproteins. As a result of oxidative cross-linking reactions, cell walls may be strengthened and function as physical barriers against invading pathogens (Brisson et al., 1994).

Phenylalanine ammonia-lyase (PAL) is one of the biochemical markers of induced resistance. It is considered to be the principal enzyme of the phenylpropanoid pathway, which is the prime intermediary in the biosynthesis of phenolics and flavonoids (Hahlbrock and Scheel, 1989; Dixon and Lamb, 1990). It catalyses the conversion of L-phenylalanine to *trans*-cinammic acid in the first step of the phenylpropanoid pathway and regulates the production of precursors for lignin biosynthesis along with other phenolic protectants in plant cells (Nicholson and Hammerschmidt, 1992). This pathway has been associated with the initiation of resistance in plants (Sticher et al., 1997).

The main objective of the present investigation was to evaluate evidence for the involvement of AOS in resistance to *Cmm* infection in BABA-treated tomato plants. Therefore, disease development and changes in two enzymes, POX, PAL, and concentration of H_2O_2 were followed in plants, in order to relate levels and times of occurrence of these activities with application of BABA at non-phytotoxic levels, and its role investigated in the initiation of induced resistance.

Materials and methods

Plant material

Greenhouse-grown 3 week-old tomato seedlings cv. Rio Grande with four fully expanded leaves were used for all experiments. Plants were grown in pots in a soil mix containing sand, perlite, and peat compost in the greenhouse at 25 $\pm$ 5 °C with 68–80% RH. The soil mix also contained a slow-release fertilizer (14-12-14, N-P-K). Natural light was supplemented by a single 1000-W sodium vapour lamp during a 16 h photoperiod.

Bacterial strain and inoculation

The strain of *Cmm*, ICPM7200, was preserved on modified Nutrient Yeast Dextrose Agar (NYA) at

4 °C (Gross and Vidaver, 1979). Inoculum suspension was prepared from early log-phase cells which were obtained by growing the bacterium in nutrient yeast extract broth supplemented with appropriate antibiotics (rifampycine) in 25 ml sterile tubes and incubated at 27 °C on an orbital shaker at 200 rpm for 24 h. Bacteria were subsequently pelleted by centrifugation (twice, each at 3500 *g* for 5 min) and washed in sterile distilled water (SDW). Their concentration was adjusted to 10^8 cfu ml^{-1} by dilution to give OD_{640} of 0.12. The two youngest leaves of the seedlings were cut at the tip and inoculated by dipping into a suspension of 10^8 cfu ml^{-1} as described by Baysal et al. (2003).

Twenty-four hours before inoculation the plants were uniformly sprayed with BABA as an aqueous solution (250, 500, 750 and 1000 μg ml^{-1}) and water (control) on foliage of seedlings and covered with plastic bags after treatment. The plants were inoculated with *Cmm* bacterial suspension according to van den Bulk et al. (1991). Symptom development in seedlings was evaluated at 14 days after inoculation (dai) by using a 0–5 rating scale. Ratings were as follows: 0 = no leaves showing wilting; 1 = slight marginal wilting, 1–10% of leaves with wilt; 2 = 11–25% of leaves with wilt; 3 = sectored wilting, 26–49% of leaves showing wilting associated with chlorosis; 4 = pronounced leaf collapse, 50–74% of leaves showing wilting; 5 = whole leaf wilted. A mean disease index (DI) was calculated from each treatment by combining the score of the 60 plants (3 replicates of 20 plants for each treatment), and expressing the value as a percentage using the formula described by Anfoka et al. (2000).

Effect of BABA on bacterial growth in planta

Bacteria were recovered from inoculated tissues, treated with either BABA or water 1 day before inoculation, by removing 5 mm diameter leaf discs aseptically from the region of inoculation. Excised discs were homogenized in 1 ml of sterile 0.06% NaCl solution, and bacterial concentrations determined as colony forming units (cfu) by dilution plating. Bacterial numbers *in planta* were calculated for each of the dilution plates, and a mean value obtained from pooling of all values for all replicates. Each dilution from each leaf disc was duplicated. Results presented are means for two separate experiments in which three leaf discs were homogenized from each treatment. Young leaves were sampled from seedlings for physiological assays. The plants sprayed with the optimum dose of BABA (500 μg ml^{-1}) were used to determine the enzymatic activities.

Preparation of samples for physiological assays

The first group consisted of uninoculated plants and seedlings sprayed with BABA or water as previously described. In a second group, the plants were treated with BABA or water and inoculated with the pathogen 1 day after treatment. Tissues were taken from inoculated leaves at the actual site of inoculation with *Cmm*. Tissues were taken from control plants at sites similar to inoculated leaves. Samples for enzyme extraction were taken separately 1, 3, 5 and 7 days after treatment. To avoid possible side effects of leaf cutting, both cut edges (about 2 mm) of the leaf segments were removed and the adjacent tissue was immersed in liquid N_2. The frozen leaf segments were homogenized (1:5 w/v) in an ice-cold mortar using 50 mM potassium phosphate buffer (pH 7.0) containing 1 M NaCl, 1% polyvinylpyrrolidone, 1 mM EDTA and 10 mM β-mercaptoethanol. Thereafter, the homogenates were centrifuged at 17,000 *g* for 20 min at 4 °C and finally, the supernatant (crude enzyme extract) was collected and divided into 1.5 ml portions. When not immediately used for enzyme assays, enzyme extracts were stored at −20 °C. Protein concentrations were determined by the method of Bradford (1976) using BSA as a standard. Extracts were obtained from two different lots of leaf samples (1 g fresh weight each) for each treatment. All assays were performed at 25 °C in a UV/visible light spectrophotometer (JAS Co. UV VIS750).

Phenylalanine ammonia-lyase (PAL) activity

Stored leaf samples were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrolidone. The extract was filtered through cheesecloth and the filtrate was centrifuged at 16,000 *g* for 15 min. The supernatant was used as the enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to *trans*-cinnamic acid at 290 nm

(Dickerson et al., 1984). Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The amount of *trans*-cinnamic acid synthesized was calculated (Bradford, 1976). Enzyme activity was expressed as nmol *trans*-cinnamic acid min mg protein^{-1}.

Peroxidase activity

Peroxidase (EC 1.11.1.7) activity was determined from the crude extract according to a procedure described by Maehly and Chance (1954) using guaiacol as a common substrate for peroxidases. The reaction mixture consisted of 0.5 ml of enzyme extract, 0.5 ml of 50 mM sodium acetate buffer (pH 5.6), 0.5 ml of 20 mM guaiacol and 0.5 ml of 60 mM H_2O_2. The linear increase in absorbance at 480 nm due to the formation of tetraguaiacol was monitored for 4 min at 30 °C. The enzyme activity was calculated from the change in absorbance and was expressed as mmol tetraguaiacol produced per min and per mg of proteins using a molar extinction coefficient of 26.6 $mM^{-1}cm^{-1}$. Sodium acetate buffer was used as the blank.

Assay of hydrogen peroxide concentration

The concentration of H_2O_2 in the leaves was determined according to a modified method of Capaldi and Taylor (1983). Leaves were ground in 5% TCA (2.5 ml per 0.5 g leaf tissue) with 50 mg active charcoal at 0 °C and centrifuged for 10 min at 15,000 *g*. Supernatant was collected, neutralised with 4 N KOH to pH 3.6 and used for the H_2O_2 assay. The reaction mixture contained 200 µl of leaf extract, 100 µl of 3.4 mM 3-methylbenzothiazoline hydrazone. The reaction was initiated by adding 500 µl of horseradish peroxidase solution (90 U 100 ml^{-1}) in 0.2 M sodium acetate (pH 3.6). Two minutes later 1400 µl of 1 N HCl was added. Absorbance of A_{630} was read after 15 min.

Experimental design and statistical analyses

All greenhouse experiments were arranged in a completely randomized split-plot design with three replicates of 20 plants for each treatment and repeated at least twice. The Pearson's correlation coefficient was used to analyse the relationship between DI and days after treatment. A simple multiple linear regression (MLR) was performed to explain variations in the response variable of bacterial growth as a function of the explanatory variables of the treatments and the days after inoculation. Each sample of extract was measured twice in each replicate, and at least four replications were performed per analysis. Significant differences between mean values were determined. Analysis of variance (ANOVA) was carried out, and significant differences among treatments were determined according to Duncan's Multiple Range Test ($P < 0.05$) with SPSS 11.0 Software, edition 2002.

Results

The effect of BABA on symptom development

BABA treatment resulted in a significant reduction of canker symptoms caused by *Cmm* on plants at four tested doses (250–1000 µg ml^{-1}) (Table 1). Although 1000 µg ml^{-1} BABA gave the best protection on tomato plants, 500 and 750 µg ml^{-1} also provided good protection, and 250 µg ml^{-1} showed a lower DI than water-treated plants, but was not significantly different. While the DI of inoculated water-treated plants reached 4.42, the index for BABA-treated plants was between 2.15 and 1.93 (Table 1). With time, most inoculated water-treated plants showed severe wilting and the DI reached 4.42 by day 14 after inoculation. However, the DI of BABA-treated plants was dose-dependent and significantly lower than water-treated plants; the 500 µg ml^{-1} dosage reduced disease by up to 54% on tomato seedlings (Table 1). Therefore, although all tested doses of BABA showed no phytotoxic effects the 500 µg ml^{-1}dose was selected for all physiological studies with BABA.

Bacterial multiplication in planta

A significant reduction of bacterial multiplication was found in inoculated BABA-treated plants compared to the inoculated water-treated plants (Figure 1). Bacterial populations were significantly lower in BABA-treated plants than at 2, 4 and 7 dai. Bacterial multiplication was reduced by the BABA treatment up to 86, 84 and 71% compared

Table 1. The effect of BABA treatment on canker development in tomato

Treatment	Doses (μg ml^{-1})	DI	% Efficacy
Control	0	4.42[a]	–
BABA treatment (24 h before inoculation)	250	3.87[a]	17
	500	2.15[b]	54
	750	2.12[b]	54
	1000	1.93[c]	56

Tomato seedlings were inoculated with *Cmm* 24 h after treatment with BABA or water (control). Disease symptoms were evaluated 14 days after inoculation (dai). Disease index (DI) was calculated for each treatment by combining the score of 60 plants (3 replicates of 20 plants for each treatment) based on a 0–5 scale described in the materials and methods section. Efficacy was calculated as the percent disease reduction compared to control plants. The values followed by different letter are significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

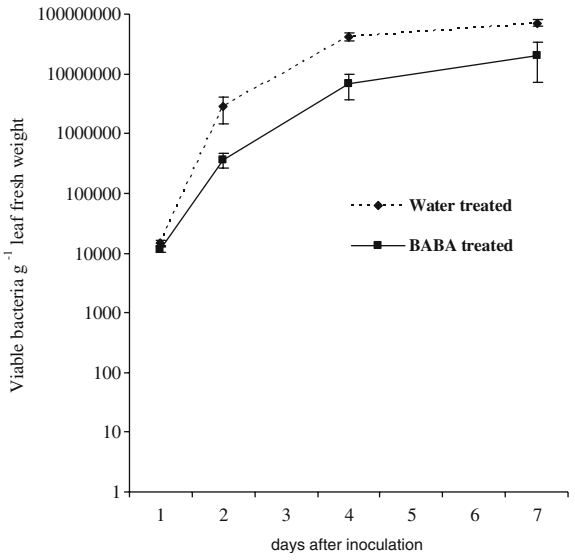

Figure 1. The effect of BABA on growth of *Cmm*. (ICP7200) in tomato leaves. The seedlings were inoculated with the *Cmm* isolate ICP7200, 24 h after treatment with BABA, or water. Data are the mean of two independent experiments, and the values represent standard deviations. Effect of BABA treatment on bacterial growth was significant according to Student's two-sample *t*-test ($P < 0.05$).

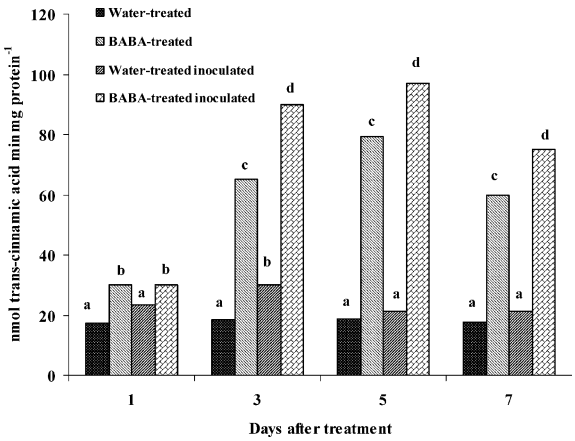

Figure 2. Changes in PAL activity of BABA and water-treated tomato seedlings. Leaves were inoculated with the *Cmm* isolate ICPM7200 24 h after treatment with BABA or water. Bars with the same letters represent values that are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

to water treated plants at 2, 4 and 7 dai respectively (Figure 1).

Phenylalanine ammonia-lyase (PAL) activity

In a time-course experiment, the occurrence of PAL activity was studied from early to late stages of infection on BABA-treated plants. BABA treatment resulted in a significant increase of PAL activity compared to uninoculated water-treated plants. Activity of PAL in tomato leaves is shown (Figure 2) at different times after BABA treatment. PAL activity increased in BABA-treated plants 1 day after treatment and was found to be significantly different from water-treated plants. At day 3 after BABA treatment, the activity was found to be nearly five times higher than in uninfected water-treated plants. *Cmm* inoculated BABA-treated plants showed maximum activity at day 5 after treatment. Uninoculated BABA-treated plants resulted in significantly higher PAL activity compared to uninoculated water-treated plants. PAL activity of inoculated BABA-treated plants was higher than uninoculated BABA-treated plants. PAL activity did not significantly change in water-treated plants during the experimental time-course (Figure 2). Untreated plants infected with the pathogen showed slightly increased PAL activity

by day 3 after treatment and then subsequently decreased.

Peroxidase (POX) activity

BABA treatment caused induction of a progressive and significant increase of POX activity compared to water-treated plants at the day 1 after treatment. POX activity was significantly higher and reached the maximum (about 3.3 and 3.4 times) in BABA-treated plants at day 3 after treatment (Figure 3). POX activity reached a higher level in infected plants compared to uninoculated water-treated plants throughout the experimental period. There was no marked change in plants treated with uninoculated water-treated plants during the experimental period.

In inoculated water-treated plants, the activity started to increase at day 3 after treatment. In inoculated BABA-treated plants POX activity was maximum (two-fold increase in activity) at day 3 after treatment and continued at a high level to day 7 after treatment (Figure 3). There was no marked change in inoculated water-treated plants between day 3 and 7 after treatment.

Hydrogen peroxide concentration

When the leaves of seedlings were analyzed for H_2O_2 concentration, uninoculated water-treated and inoculated seedlings did not show higher activity after day 1 of treatment. However, the activity in BABA-treated plants was 1.5 times higher than control plants (water-treated). The concentration of H_2O_2 increased by three-fold in BABA treated plants by day 3 after treatment. The level in inoculated plants exceeded that in uninoculated plants but was only significant for BABA treated plants at day 3 after treatment (Figure 4). By day 7 after treatment, H_2O_2, levels had almost returned to the level at day 1, after treatment.

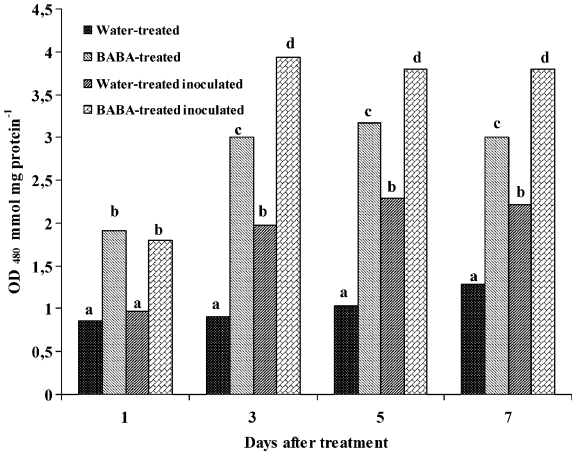

Figure 3. The effect of BABA treatment on peroxidase activity in tomato leaves. Leaves were inoculated with the *Cmm* isolate ICP7200 24 h after treatment with BABA or water. Bars with the same letters represent values that are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

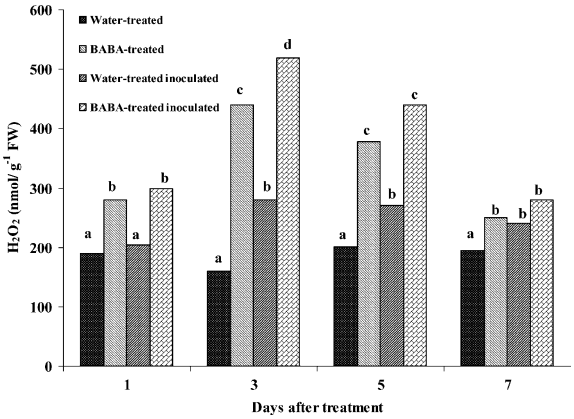

Figure 4. Changes in H_2O_2 concentration on BABA-treated tomato seedlings. Leaves were inoculated with the *Cmm* 24 h after treatment with BABA or water. The values with the same letters represent values that are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

Discussion

The study reported here describes the protective effect of BABA on tomato plants against bacterial canker caused by *Cmm*. BABA induced substantial levels of disease resistance and decreased symptom development in infected tomato seedlings. In previous studies BABA was described as a SAR inducer in tomato against fungal pathogens (Zimmerli et al., 2000; Cohen, 2002). In the present study, the protection of BABA was shown against *Cmm* when it was applied to tomato plants 1 day before inoculation. The *Cmm* population was considerably lower in the leaves of inoculated BABA-treated plants than in inoculated water-treated plants 7 days after inoculation. Moreover, this study showed that BABA (500 μg ml^{-1}) induced resistance and related enzymatic activities

reached the maximum level at day 3 after treatment. SAR requires an induction time of 2–3 days, during which the plant is conditioned for a rapid response to pathogen attack. Therefore in our study the BABA treatment 1 day before inoculation was selected as an interval period before being challenged with a pathogen. According to Siegrist et al. (1997), a minimum interval period of 96 h is necessary for acibenzolar-s-methyl or 2,6-dichloroisonicotinic acid to induce resistance on bean leaves against fungal and bacterial pathogens. In recent studies acibenzolar-s-methyl has been shown to be an efficient SAR inducer; it reduced foliar disease severity and the incidence of bacterial spot and anthracnose on fruit, while increasing yield of marketable fruit (Abbasi et al., 2002). In another study, acibenzolar-s-methyl was tested on many different crops to control a number of diseases including bacterial spot (*Xanthomonas axonopodis* pv. *vesicatoria*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*) of tomato in field experiments (Louws et al., 2001). In our previous study (Baysal et al., 2003) 72 h was found to be an efficient interval between application of acibenzolar-s-methyl and inoculation with *Cmm* for disease control. In this present study, BABA treatment suppressed bacterial growth *in planta* and significantly reduced the DI on inoculated tomato plants. Although BABA treatment (500–1000 μg ml^{-1}) did not result in substantial disease control on tomato plants, the greenhouse experiments showed that BABA treatment significantly reduced disease development compared to water-treated plants.

To determine the reasons for disease reduction and inhibition of bacterial multiplication, physiological changes in leaves of BABA-treated and control plants were evaluated. The physiological studies were limited to 7 days because it was in this time frame that the bacterial population in BABA-treated plants (500 μg ml^{-1}) was lower than in water-treated plants. Significant changes were found in the enzymatic activities of plants after treatment with BABA. The changes in PAL activity were followed and found to be considerably higher in BABA-treated plants compared to water-treated plants. Therefore, PAL activity may be associated with production of SA in BABA-treated plants and SA, along with H_2O_2, may promote its synthesis. The first oxidative burst generates AOS that induce SA synthesis, which in turn potentiates the formation of H_2O_2, which then activates synthesis of more SA (Van Camp et al., 1998). Increase of PAL activity however, was also found in inoculated water-treated plants (Figure. 2), and this finding may be correlated with those of another study conducted on susceptible rice plants inoculated with *Xanthomonas oryzae* pv. *oryzae*. In this study the inoculation alone resulted in increased PAL activity within a short period after inoculation and then showed a drastic decrease (Li et al., 1999).

POX plays several important roles in the disease resistance expressed against a number of pathogens and is responsible for the generation of reactive oxygen species (Wojtaszek, 1997). Increases in POX activity are often associated with the progressive incorporation of phenolic compounds within the cell wall during incompatible plant-microbe/elicitor interactions. In tomato, POX is one of the enzymes believed to catalyse the last step in lignification (Brisson et al., 1994). On the other hand, peroxidase activity may contribute to the production of H_2O_2 and other activated oxygen species and may generate antimicrobial phenolics (Bestwick et al., 1998). A first series of measurable effects of induced resistance include a transient accumulation of H_2O_2, callose deposition, the activation of PR-genes and the synthesis of anionic peroxidase. Increase in POX activity of BABA-treated plants may cause oxidative cross-linking of proteins to increase the resistance against bacterial pathogens. However, the role of the peroxidases in plant-bacterial interactions is not clear. There is some evidence that the peroxidases accumulate at the site of bacterial attachment to plant cells, and locally accumulated H_2O_2 may directly damage bacteria (Brown et al., 1998). The burst of H_2O_2 production at the plant cell surface drives rapid peroxidase-mediated oxidative cross-linking of structural proteins in the cell wall, thereby reinforcing this physical barrier against pathogen ingress (Scheel, 1998). Our findings showed that BABA treatment increased the activities of these enzymes. The increase in H_2O_2 concentration was found to be significantly higher in the leaves of BABA-treated plants at the early phase (day 1–3 of treatment), and it showed a close relationship between induced resistance and the activity of POX. Induction of POX has been implicated in the production of toxic radicals, such as O^{-2}, and H_2O_2. In plants, the increased pro-

duction of both the superoxide radical and H_2O_2 is a common feature of defense responses to challenge by avirulent pathogens and elicitors (Lamb and Dixon, 1997). There is ample evidence indicating that H_2O_2 performs several important functions in disease resistance (Mehdy et al., 1996). H_2O_2 has been implicated not only in triggering hypersensitive cell death, but also in limiting the spread of cell death by inducing cell protectant genes in surrounding cells (Levine et al., 1994). We have found a correlation between increases of PAL, POX and H_2O_2 concentrations in plants and the reduction of disease in BABA-treated plants.

In conclusion, we suggest that treatment of tomato plants with BABA may provide a protective effect against bacterial canker, and along with conventional biocontrol agents and improved seed varieties, may be a useful tool to decrease the disease on tomato.

References

Abbasi P, Al-Dahmani J, Sahin F, Hoitink HAJ and Miller SA (2002) Effect of compost amendments on disease severity and yield of tomato in conventional and organic production systems. Plant Disease 86: 156–161

Alvarez ME, Pennell RI, Meijer PJ, Ishikawa A, Dixon RA and Lamb C (1998) Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92: 773–784

Anfoka GH (2000) Benzo-(12,3)-thiadiazole−7-carbothioic acid s-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum* Mill. cv. *volledung*) to cucumber mosaic virus. Crop Protection, 19: 401–405

Ark PA (1994) Studies on bacterial canker of tomato. Phytopathology 34: 394–400

Baysal Ö, Soylu ME and Soylu S (2003) Induction of defense related enzymes and resistance by the plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* spp. *michiganensis*. Plant Pathology 52: 747–753

Bestwick CS, Brown IR and Mansfield JW (1998) Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a non-host hypersensitive reaction in lettuce. Plant Physiology 118: 1067–1078

Bradford M (1976) A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein dye binding. Analytic Biochemistry 72: 248–250

Brisson LF, Tenhaken R and Lamb CJ (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. Plant Cell 6: 1703–1712

Brown IR, Trethowan J, Kerry M, Mansfield J and Bolwell GP (1998) Localization of components of the oxidative cross linking of glycoproteins and of callose in papillae formed during the interactions between non-pathogenic strains of *Xanthomonas campestris* and French bean mesophyll cells. Plant Journal 15: 333–343

Camp W, Van Montagu M and Inzé D (1998) H_2O_2 and NO: Redox signals in disease resistance. Trends in Plant Science 3: 330–334

Capaldi DJ and Taylor KE (1983) A new peroxidase color reaction: Oxidative coupling of 3-methyl−2-benzothiazolinone hydrazone (MBTH) with its formaldehyde azine. Application to glucose and choline oxidases. Analytic Biochemistry 129: 329–336

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

Dickerson DP, Pascholati SF, Hagerman AE, Butler LG and Nicholson RL (1984) Phenylalanine ammonia-lyase and hydroxycinnamate: Coa ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiological Plant Pathology 25: 111–123

Dixon AR and Lamb CJ (1990) Molecular communication in interaction between plants and microbial pathogens. Annual Review of Plant Physiology Plant Molecular Biology 41: 339–367

Enyedi AJ and Raskin I (1993) Induction of UDP glucose salicylic acid glucosyl-tranferase activity in tobacco mosaic virus-inoculated tobacco (*Nicotiana tabacum*) leaves. Plant Physiology, 101: 1375–1380

Gleason ML, Gitaitia RD and Ricker MK (1993) Recent progress in understanding and controlling bacterial canker of tomato in eastern. North America Plant Disease 77: 1069–1076

Gross DC and Vidaver AK (1979) A semiselective medium for isolation of *Corynebacterium nebraskense* from soil and plant parts. Phytopathology 69: 82–87

Hahlbrock K and Scheel D (1989) Physiology and molecular biology of phenylpropanoid metabolism. Annual Review of Plant Physiological Plant Molecular Biology 40: 347–364

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

Lamb CJ and Dixon RA (1997) The oxidative burst in plant disease resistance. Annual Review of Plant Physiology Plant Molecular Biology 48: 251–275

Levine A., Tenhaken R., Dixon R and Lamb C (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79: 583–593

Li A, Luo K and Liu YJ (1999) Studies on physiological indicators of near-isogenic rice lines in interaction with *Xanthomonas oryzae* pv. *oryzae*. Acta Phytopatologica Sinica 26: 35–39

Louws FJ, Wilson M, Campbell HL, Cuppels DA, Jones JB, Shoemaker PB, Sahin F and Miller SA (2001) Control of bacterial spot and bacterial speck of tomato using a plant activator. Plant Disease 85: 481–488

Maehly AC and Chance B (1954) The assay of catalase and peroxidases. In: Glick D (ed.) Methods of Biochemical Analysis, Vol. 1 (pp 357–424). Interscience Publishers Inc., New York

Mehdy MC, Sharma YK, Sathasivan K and Bays NW (1996) The role of activated oxygen species in plant disease resistance. Plant Physiology : 365–374

Nicholson RL and Hammerschmidt R (1992) Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30: 369–389

Oostendorp M, Kunz W, Dietrich B and Staub T (2001) Induced disease resistance in plants by chemicals. European Journal of Plant Pathology 107: 19–28

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY and Hunt MD (1996) Systemic acquired resistance. Plant Cell 8: 1809–1819

Scheel D (1998) Resistance response physiology and signal transduction. Current Opinion in Plant Biology 1: 305–310

Siegrist J, Glenewinkel D, Kolle C and Schmidtke M (1997) Chemical induced resistance in green bean against bacterial and fungal pathogens. Journal of Plant Disease Protection 104: 599–610

Sticher L, Mauch-Mani B and Metraux JP (1997) Systemic acquired resistance. Annual Review of Phytopathology 35: 235–270

Van den Bulk RW, Jansen J, Lindhaut WH and Lsffler HJM (1991) Screening of tomato somaclones for resistance to bacterial canker (*Clavibacter michiganensis* subsp. Michiganensis). Plant Breeding 107: 190–196

Wojtaszek P (1997) The oxidative burst: A plant's early response against infection. Biochemistry Journal 322: 681–692

Zimmerli L, Jacob G, Metraux JP and Mani BM (2000) Potentiation of pathogen-specific defence mechanism in *Arabidopsis* by β-aminobutyric acid. Proceedings Natural Academic Science: 12920–12925