BIOLOGIA PLANTARUM 49 (4): 599-604, 2005

Acibenzolar-S-methyl induced resistance to *Phytophthora capsici* in pepper leaves

Ö. BAYSAL*[1], C. TURGUT** and G. MAO***

*Turkish Ministry of Agriculture and Rural Affairs, WMRAI, BATEM, P.B. 35, 07100 Antalya, Turkey**
*Faculty of Agriculture, University of Aydin Menderes, 09100 Aydin, Turkey***,
*The University of Tennesse Knoxville, 2407 River Drive, Knoxville, TN 37996, USA****

Abstract

The leaves of pepper (*Capsicum anuum* L.) were inoculated with *Phytophthora capsici* Leonian 3 d after treatment with acibenzolar-S-methylbenzo [1,2,3]thiadiazole-7-carbothioic acid-S-methyl ester (ASM) and resistance to *Phytophthora* blight disease was investigated. Results showed that *P. capsici* was significantly inhibited by ASM treatment by up to 45 % *in planta*. The pepper plants responded to ASM treatments by rapid and transient induction of L-phenylalanine ammonia-lyase (PAL), increase in total phenol content and activities of chitinase and β-1,3-glucanase. No significant increases in enzyme activities were observed in water-treated control plants compared with the ASM-treated plants. Therefore it may be suggested that ASM induces defense-related enzymes, PAL activity, PR proteins and phenol accumulation in ASM-treated plants and contribute to enhance resistance against *P. capsici*.

Additional keywords: blight disease, chitinase, β-1,3-glucanase, PAL activity, phenols, PR proteins.

Introduction

Phytophthora capsici Leonian is a soilborne pathogenic protist (phylum *Oomycota*) that infects many solanaceous plants. The disease can occur on the plant at any stage causing damping-off, seedling blight, foliar blight, and plant death preceded by wilting. Infection on mature plants is seen as a dark, rapidly expanding, water-soaked lesions. Control of *P. capsici* infection remains an ongoing agricultural problem and is most commonly accomplished by the application of biocides, such as methyl bromide or metalaxyl, to the soil. The ability of oospores to persist in soil for long periods obviates the use of crop rotation as an antipest strategy. Alternatively, more environmentally benign methods of control will likely have to target host-specific stages of the infectious cycle, since the pathogen is so persistent. Therefore, induction of plant resistance against pathogen is considered as a potential method in reducing and control of the disease severity.

The defense responses occurring in plants exhibiting induced resistance (IR) reflect genetically fixed mechanisms typical for each plant species. Defense responses in systemic acquired resistance (SAR) include lignication and formation of cell wall appositions (papillae) at the sites of attempted penetration of fungal pathogens, hypersensitive reactions and the accumulation of pathogenesis-related (PR) proteins (Hammerschmidt and Kuć 1995, Van Loon 1997). Recently, the best studied resistance activator is acibenzolar-S-methylbenzo [1,2,3]thiadiazole-7-carbothioic acid-S-methyl ester (ASM). It has been developed as a potent SAR activator which does not have antimicrobial properties themselves, but instead increase crop resistance to disease by activating the SAR signal transduction pathway besides the natural products such as salicylic acid (SA) and DL-β-amino-n-butyric acid (Reuveni *et al*. 2001). Induction of SAR by ASM was reported against fungal, viral and bacterial diseases in many plants (Siegrist *et al*. 1997, Cole 1999, Anfoka 2000, Oostendorp *et al.* 2001, Lopez and Lucas 2002). It has been commercially released in some countries as a plant health promoter of annual crops under the name of *Bion*® or *Actigard*™.

Phenylalanine ammonia-lyase (PAL) is considered to

Received 20 January 2004, *accepted* 17 February 2005.
Abbreviations: AMS - acibenzolar-S-methylbenzo[1,2,3]thiadiazole-7-carbothioic acid-S-methyl ester, dai - days after inoculation; IR - induced resistance; PAL - L-phenylalanine ammonialyase; PR - pathogenesis-related; SA - salicylic acid; SAR - systemic aquired resistance.
[1] Corresponding author; fax: (+90) 242 3341861 e-mail: obaysal@ttnet.net.tr

be the principal enzyme of the phenylpropanoid pathway catalysing the transformation, by deamination, of L-phenyalanine into trans-cinnamic acid, which is the prime intermediary in the biosynthesis of phenolics (Dixon and Lamb 1990). An important first line plant defense response against infection is often suggested to be the very rapid synthesis of phenolic compounds and their polymerization in the cell wall (Matern and Kneusel 1988). In recent studies with ASM treatment the production of phenolic compounds, such as flavonoids and phenylpropanoids against fungal pathogens has been demonstrated (Stadnik and Buchenauer 2000). Despite the numerous reports regarding the effect of ASM application and its ability to induce resistance, to date, there is no information available regarding the physiological changes during the induced resistance in ASM treated pepper seedlings against the root and crown rot disease caused by *P. capsici.* Here, we present study evidence that, the application of the ASM led to induced resistance effect against *P. capsici*. Hence, PAL and total phenol content and PR protein activites (chitinase and β-1,3-glucanase) were compared in uninfected and *P. capsici* infected pepper plants and with ASM treated plants infected with pathogen.

Materials and methods

Plants: Greenhouse-grown pepper (*Capsicum anuum* L.) seedlings (cvs. Arikanda and Charliston) with four fully-expanded leaves were used for all experiments. Plants were grown in 10 cm pots in soil mix containing sand, *Perlite* and peat compost in the greenhouse at 25 ± 5 °C with 68 - 80 % RH. Natural light was supplemented by a single 1000 W sodium vapour lamp during a 16-h photo-period (irradiance of 350 μmol m^{-2} s^{-1}).

Application of ASM and fungal pathogen inoculation: ASM (*Bion®*, *Syngenta*, Frankfurt, Germany) was dissolved in distilled water to obtain a concentration of 200 mg dm^{-3} and then sprayed on whole seedlings (*ca*. 0.2 cm^3 per seedling). Isolate of *Phytopthora capsici* was maintained on cornmeal agar plates *Difco CMA* (Detroit, MI, USA) at 15 °C. To induce sporulation and zoospore release, the isolate was grown on clarified 10 % V8 vegetable juice agar and incubated for 3 to 6 d at 25 °C. Sporangial formation in *P. capsici* was induced by removing uncolonized agar from around the mycelium and incubating the culture for an additional 1 to 2 d at 25 °C under fluorescent lights (12-h photoperiod). Zoospores were released from the sporangia by flooding the plates with sterile deionized water and incubating them at room temperature for 20 to 30 min. The zoospores were filtered through four layers of cheesecloth to remove sporangial cases and mycelial fragments. After the treatment, the seedlings were maintained in a greenhouse as described above. The pepper plants were wounded by making 1-cm longitudinal slits on the stems 1 cm from the soil surface. Sterile cotton was dipped in zoospore suspension (1×10^5 zoospores cm^{-3}) and placed on the wounded sites on the stem. The inoculated sites were covered with plastic tape to maintain a moist condition. Plants in the first group were treated with ASM and inoculated with pathogen 3 d after treatment. Plants in the second group were treated with water and inoculated as previous group. Controls were seedlings sprayed with ASM or water but not inoculated.

The level of the resistance induced in seedlings against the *P. capsici* was evaluated at 2, 3, 4, 5, and 7 d after inoculation (dai) by using a 0 - 5 arbitrary scale (Sunwoo *et al.* 1996). Disease severity on the pepper plants was rated daily after inoculation, based on a scale: 0 - no visible disease symptoms, 1 - leaves slightly wilted with brownish lesions beginning to appear on the stems, 2 - 30 to 50 % of the entire plant diseased, 3 - 50 to 70 % of the entire plant diseased, 4 - 70 to 90 % of the entire plant diseased, 5 - plant dead. Disease indices (DI) were calculated by summing the score of the 30 plants (three replicates of 10 plants per treatment). Time course changes in disease severity and differences in disease severity of control-inoculated plants and of ASM treated-inoculated plants were examined.

Biochemical analyses: From 2 inoculated leaves (4, 5, 6 and 7 dai) tissues were taken at the actual site of inoculation. From control plants, tissues were taken from sites similar to inoculated leaves. Fresh leaves were homogenized with liquid nitrogen in a pre-chilled mortar and pestle. The homogenized samples were stored at -70 °C.

For estimation of total phenol content stored leaf samples were homogenised in 80 % methanol and agitated for 15 min at 70 °C (Zieslin and Ben-Zaken 1993). The methanolic extract (1 cm^3) was added to 5 cm^3 of distilled water and 0.25 cm^3 of Folin-Ciocalteau reagent (1 M) and the solution was kept at 25 °C. The absorbance of developed blue colour was measured using UV/visible spectrophotometer (*JAS.CO 7850 PC*, Tokyo, Japan) at 725 nm. Catechol was used as the standard. Protein concentration was determined as described by the Bradford (1976), using bovine serum albumin (BSA) as a standard.

For estimation of phenylalanine ammonia-lyase (PAL) activity stored leaves samples were homogenized in 3 cm^{-3} of 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrolidone (ice cold). The extract was filtered through cheesecloth and the filtrate was

centrifuged at 16 000 *g* for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L-phenyalanine to transcinnamic acid at 290 nm. (Dickerson *et al.* 1984). Samples containing 0.4 cm^{-3} of enzyme extract were incubated with 0.5 cm^{-3} of 0.1 M borate buffer, pH 8.8 and 0.5 cm^{-3} of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The amount of transcinnamic acid synthesized was calculated (Dickerson *et al.* 1984).

For estimation of chitinase activity and 1,3-β-glucanase activities the frozen leaf segments were homogenized in an ice-cold mortar using 50 mM potassium phosphate buffer (1:5 m/v; 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 cm^{-3} portions. Protein concentrations were determined by the method of Bradford using BSA as a standard. The extract, obtained from two different lots of leaf samples (1 g fresh mass each) for each treatment, was used to determine the activity of chitinase and glucanase.

Chitinase activity was determined by the method of Wirth and Wolf (1990). High polymeric carboxymethyl-substituted chitin, labelled covalently with Remazol Brilliant Violet 5R (*CM-Chitin-RBV*, *Biochemica*, Loewe, Germany) was used as substrate for chitinase activity. Potassium acetate buffer (0.2 cm^{-3} 0.1 M, pH 5.0) and 0.1 cm^{-3} of suitably diluted crude extract were added to a micro-centrifuge tube and equilibrated to 37 °C for 10 min. The reaction was initiated after adding aqueous CM-Chitin-RBV solution (2 mg cm^{-3}), the mixture was incubated at 37 °C for 2 h. The reaction was terminated by addition of 0.1 cm^{-3} 2 M HCl, which precipitated the undergraded substrate. Tubes were cooled on ice for 10 min then centrifuged for 5 min at 9 000 *g*. Absorbance of the supernatant was recorded at 550 nm. Blanks were prepared similarly with Na-acetate buffer instead of the homogenate.

β-1,3-glucanase activity was determined by the method of Wirth and Wolf (1992). Remazol Brilliant Violet 5R (*CM-Curdlan-RBV*) was used as substrate for glucanase activity. Potassium acetate buffer (0.2 cm^{-3} 0.1 M, pH 5.0) and suitably diluted crude extract were added to a micro-centrifuge tube and equilibrated to 37 °C for 10 min. The reaction was initiated after adding 0.1 cm^{-3} aqueous *CM-Curdlan-RBV* solution (2 mg cm^{-3}), the mixture was incubated at 37 °C for 1 h. The reaction was terminated by adding 0.1 m 2 M HCl. Tubes were cooled on ice for 10 min then centrifuged for 5 min at 9 000 *g*. Absorbance of the supernatant was recorded at 600 nm. Blanks were prepared similarly with Na-acetate buffer instead of the homogenate. Each sample of extract was measured twice in each replicate, and at least two replications were performed per analysis.

Experimental design and statistical analyses: All experiments were arranged in a completely randomized split-plot design with three replicates of 10 plants per treatment. All experiments were repeated at least twice. Data obtained during different days after inoculation, which usually included a typical disease development were analysed using $P < 0.05$ by *Minitab Software*, *version 13.32* (*Minitab Inc.*, State College, USA). The Pearson's correlation coefficient was used to account for the direction of the relationship between disease index, treatments and days after inoculation. For disease severity studies, significance of differences among the treatments was determined by a non-parametric Mann-Whitney Rank Sum Test. For enzyme activities, analysis of variance (*ANOVA*) was carried out, and the significance of differences among the treatments was determined according to Duncan's Multiple Range Test.

Results

The effect of the ASM treatment on symptom development: The progress of the disease in control plants increased with time and by 2 dai most of the plant leaves developed severe wilting. Mean disease index (DI) in these plants reached to 4.67 at 7 dai (Table 1). The resistance induced by the ASM treatment was already evident 2 dai and lasted for the entire experimental period. At 7 dai DI was nearly by 45 % lower in ASM-trested plants than in water-treated plants. Since the induced resistance was observed at a time interval of 3 d between treatment and inoculation, this interval was also taken into consideration in order to assay of enzyme experiments.

Table 1. The effect of ASM treatment on the disease index caused by *Phytophthora capsici*. 3 d after treatment with ASM or water seedlings were inoculated *P. capsici*. Disease symptom was evaluated at 2, 3, 4, 5 and 7 dai. Disease index was calculated from each treatment by summing the score of the 30 plants (three replicates of 10 plants per treatment) by using the 0 - 5 scale as described in Materials and methods. Medians followed by different letter are significantly different according to Mann-Whitney test ($P < 0.05$).

Treatment	2 dai	3 dai	4 dai	5 dai	7 dai
Water treated	0.53^{a}	1.67^{a}	3.10^{a}	4.07^{a}	4.67^{a}
ASM treated	0.17^{b}	0.43^{b}	1.73^{b}	2.07^{b}	2.57^{b}

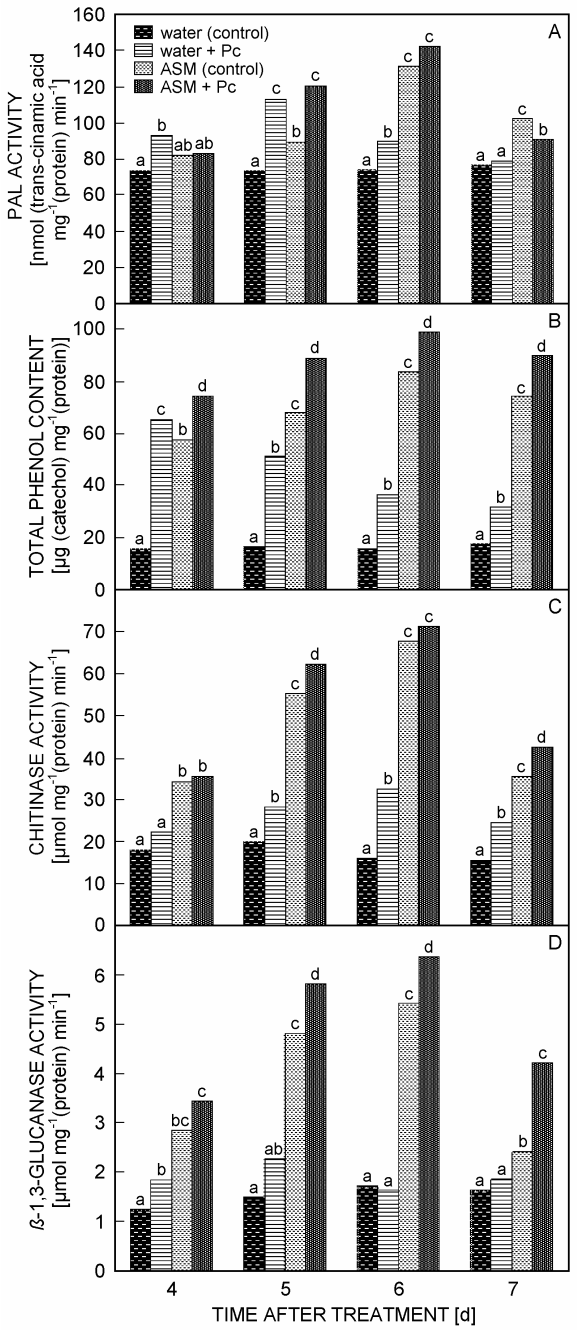

Fig. 1. The effect of the ASM treatment on the PAL activity (*A*), total phenol content (*B*), chitinase activity (*C*), and β-1,3-glucanase activity (*D*) in pepper leaves. Leaves, treated with ASM or water, were inoculated with the *P. capsici* (Pc), 3 d after the induction. Means of two separate experiments (in each experiment two different extractions were pooled at every time point). The values with the same letters represent values that are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

Changes in PAL activity and total phenol content in ASM treated plants: Increased PAL activity was observed in ASM-treated and water-treated pepper plants after inoculation with *P. capsici* (Fig. 1*A*). These increases in PAL activity were found significant at 5 dai and they were higher in water-treated than ASM-treated plants. There was no marked change in PAL activity in uninoculated water-treated plants during the time course of experimental period but it remained in lower compared to ASM-treated uninoculated plants.

ASM treatment resulted in increase of phenol content in uninoculated and inoculated plants (Fig. 1*B*). At 4 dai the phenol content reached maximum in ASM-treated inoculated plants (nearly 5 times higher than water-treated uninoculated plants) and the activity was high in ASM-treated inoculated plants at 5 dai. Afterwards the phenol content in water-treated inoculated plants decreased. The phenol content in ASM-treated uninoculated and inoculated plants was significantly higher compared to water-treated ones at 6 dai (Fig. 1*B*).

Changes in chitinase and 1,3-β-glucanase activities: Chitinase activity was also significantly higher in ASM-treated uninoculated and inoculated plants compared to water-treated inoculated and uninoculated plants (Fig. 1*C*). In the ASM treated and uninoculated plants the activity was nearly 1.9 and 4.1 times higher than that of water-treated uninoculated plants at dai 4 and 6, respectively. The activities were also significantly higher in ASM-treated than water treated inoculated plants at 7 dai.

β-1,3 glucanase activities also showed significant differences between in ASM-treated and water-treated plants in the experimental period (Fig. 1*D*). The activity was significantly higher (nearly 3 times) in ASM-treated inoculated plants than water-treated uninoculated plants at 4 dai and in ASM treated inoculated plants nearly 4 times higher than water-treated inoculated plants at 5 dai.

Discussion

In the present study PAL activity, total phenol content and activities of chitinase and β-1,3 glucanase were examined in attempt to determine their roles in resistance reactions of pepper plants to infection with *P. capsici* induced by ASM. For the development of resistance, plants need a period before being challenged with a pathogen. Therefore in our study ASM treatment 3 d before inoculation was selected. According to Siegrist *et al.* (1997), a minimum interval period of 96 h was necessary for ASM to induce resistance on bean leaves against fungal and bacterial pathogens. In most cases this interval was reported to lie between 1 and 7 d (Doubrawa

et al. 1998).

The present study clearly indicated an increase in activity of PAL, the accumulation of phenolic compounds and increase in activities of chitinase and β-1,3 glucanase in plant after ASM treatment and induction of resistance by ASM treatment. Certain experiments revealed the ASM to be a potent SAR inducers providing protection against a wide spectrum of plant pathogens (Lawton *et al.* 1996). A common response of plant cells to stress such as infection, elicitation or wounding, is the induced incorporation of phenolic material into the cell wall (Matern and Grimming 1994). The present findings showed that ASM treatment of pepper seedlings maybe resulted in the accumulation of phenolics in the walls of exposed pepper cells. Further by application of abiotic inducers such as salicylic acid (SA), dichloroisonicotinic acid (analog of SA) an increase in phenols in the cell wall of plant has been reported (Siegrist *et al.* 1997). It may be assumed that these phenolic compounds cause some inhibition of the pathogen (Heath 1980). Generally, many resistance reactions of the plant against pathogens are characterized by very rapid synthesis of phenolics and their polymerisation at the cell wall and phenolic compounds have often been found to be involved in defense mechanism of plants (Matern and Kneusel 1988). In our study the accumulation of phenolic compounds in pepper plant tissue might protect it against *P. capsici* invasion.

In plants, development of induced resistance is associated with the co-ordinate expression of a complex set of so-called 'SAR genes' which include genes for some of the PR proteins (Conrath *et al.* 2001). The β-1,3-glucanase (PR-2) and chitinase (PR-3) posses direct antimicrobial activity by degrading microbial cell wall components (Stintzi *et al.* 1993). Furthermore, over-expression of several PR-genes in transgenic plants has been shown to enhance their resistance to certain fungal pathogens (Zhu *et al.* 1994). Therefore, the expression of PR genes and the associated accumulation of the encoded PR proteins have often been considered as the molecular basis of induced resistance. In our studies a correlation was also found between the pepper resistance and activities of chitinase and β-1,3-glucanase in leaves. In previous studies the selected bacterial strains were also tested against *P. capsici* and *Alternaria alternata* in sweet pepper plants and in these studies the inhibitory effect of the selected microorganisms was detected (Sid *et al.* 2003). Further, in other immunohistological study on the induced proteins the systemic distribution of induced PR-proteins (chitinase and β-1,3 glucanase) was also followed in BTH and SA treated leaves of sugar beet (Burketová *et al.* 2003). Induction of these proteins in ASM-treated pepper leaves corresponds with the finding of Friedrich *et al*. (1996) and Suo and Leung (2001) who also found increased accumulation of chitinase and glucanase gene activations in *Arabidopsis*, tobacco and rose. Accumulation of PR protein by abiotic stress and inducers was also reported in other plant pathogen interactions (Cohen *et al.* 1994, Dann *et al.* 1996, Görlach *et al.* 1996, Burketová *et al*. 1999).

In conclusion, ASM can stimulate resistance in the pepper plant to *P. capsici.* Therefore along with conventional fungicides, biocontrol organisms and improved cultivars, ASM may provide the farmer a new option for disease control.

References

Anfoka, G.H.: Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum* Mill. cv.Volledung) to cucumber mosaic virus. - Crop Protect. **19**: 401-405, 2000.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Burketová, L., Šindelářová, M., Šindelář, L.: Benzothiadiazole as an inducer of β-1,3-glucanase and chitinase isozymes in sugar beet. - Biol. Plant. **42**: 279-287, 1999.

Burketová, L., Štillerová, K., Feltlová, M., Šindelářová, M.: Immunolohistological analysis of chemically induced proteins in sugar beet. - Biol. Plant. **47**: 243-251, 2003.

Cohen, Y., Niederman, T., Mösinger, E., Fluhr, R.: 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 Physiol. **104**: 59-66, 1994.

Cole, D.L.: The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance against bacterial and fungal diseases of tobacco. - Crop Protect. **18**: 267-273, 1999.

Conrath, U., Thulke, O., Katz, V., Schwindling, S., Kohler, A.: Priming as a mechanism in induced systemic resistance of plants. - Eur. J. Plant Pathol. **107**: 113-119, 2001.

Dickerson, D.P., Pascholati, S.F., Hagerman, A.E., Butler, L.G., Nicholson, R.L.: Phenylalaninelyase and hydroxy-cinnamate; CoA ligase in maize mesocotyls inoculated with *Helminthosporium carbonum.* - Physiol. Plant Pathol. **25**: 111-123, 1984.

Dixon, A.R., Lamb, C.J.: Molecular communication in interaction between plants and microbial pathogens. - Annu. Rev. Plant Physiol. Plant mol. Biol. **41**: 339-367, 1990.

Doubrava, N.S., Dean, R.A., Kuć, J.: Induction of systemic resistance to antracnosis caused by *Collectotrichum lagenarium* in cucumber by oxalate and extracts of spinach and rhubarb leaves. - Physiol. mol. Plant Pathol. **33**: 69-79, 1988.

Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Rella, M.G., Meier, B., Dincher, S., Staub, T., Uknes, S., Metraux, J.-P., Kessmann, H., Ryals, J.: A benzothiadiazole derivative induces systemic acquired resistance in tobacco. - Plant J. **10**: 61-70, 1996.

Görlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., Ryals, J.: Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. - Plant Cell **8**: 629-643, 1996.

Hammerschmidt, R., Kuć, J.: Induced Resistance to Disease in Plants. - Kluwer Academic Publishers, Dordrecht 1995.

Heath, M.C.: Reactions of nonsuscepts to fungal pathogens. - Annu. Rev. Phytopathol. **18**: 211-236, 1980.

Lawton, K.A., Friedrich, L., Hunt, M., Weymann, K., Delaney, T., Kessmann, H., Staub, T., Ryals, J.: Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway.- Plant J. **10**: 71-82, 1996.

Lopez, A.M.Q., Lucas, J.A.: Effects of plant defence activators on anthracnose disease of cashew. - Eur. J. Plant Pathol. **108**: 409-420, 2002.

Matern, U., Grimming, B.: Natural phenols as stress metabolites. - Acta Hort. **381**: 448-462, 1994.

Matern, U., Kneusel, R.: Phenolic compounds in plant disease resistance. - Phytoparasitica **16**: 153-170, 1988.

Oostendorp, M., Kunz, W., Dietrich, B., Staub, T.: Induced disease resistance in plants by chemicals. - Eur. J. Plant Pathol. **107**: 19-28, 2001.

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

Sid, A., Ezziyyani, M., Egea-Gilabert, C., Candela, M.E.: Selecting bacterial strains for use in the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternata* in sweet pepper plants. - Biol. Plant. **47**: 569-574, 2003.

Siegrist, J., Glenewinkel, D., Kolle, C., Schmidtke, M.: Chemical induced resistance in green bean against bacterial and fungal pathogens. - J. Plant Dis. Protect. **104**: 599-610, 1997.

Stadnik, M.J., Buchenauer, H.: Inhibition of phenylalanine ammonia-lyase suppresses the resistance induced by benzothiadiazole in wheat to *Blumeria graminis* f. sp. *tritici*. - Physiol. mol. Plant Pathol. **57**: 25-34, 2000.

Stintzi, A., Heitz, T., Prasad, V., Wiedemann-Merdinoghi, S., Kauffmann, S., Geoffroy, P., Legrand, M., Fritig, B.: Plant 'pathogenesis-related' proteins and their role in defence against pathogens. - Biochemistry **75**: 687-706, 1993.

Sunwoo, J.Y., Lee, Y.K., Hwang, B.K.: Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-β-amino-n-butyric acid. - Eur. J. Plant Pathol. **102**: 663-670, 1996.

Suo, Y., Leung, D.W.M.: Elevation of extracellular β-1,3-glucanase and chitinase activities in rose in response to treatment with acibenzolar-S-methyl and infection by *D. rosae*. - J. Plant Physiol. **158**: 971-976, 2001.

Van Loon, L.C.: Induced resistance in plants and the role of pathogenesis-related proteins. - Eur. J. Plant Pathol. **103**: 753-765, 1997. 1997

Wirth, S.J., Wolf, G.A.: Dye-labelled substrates for the assay and detection of chitinase and lysozyme activity. - J. Micro-methods **12**: 197-205, 1990.

Wirth, S.J., Wolf, G.A.: Micro-plate colorimetric assay for endo-acting cellulase, xylanase, chitinase β-1,3-glucanase and amylase extracted from forest soil horizons. - Soil Biol. Biochem. **24**: 511-519, 1992.

Zhu, Q., Maker, E.A., Masond, S., Dixon, R.E., Lamb, C.: Enhanced protection against fungal attack by constitutive co-expression of chitinase and glucanase in transgenic tobacco. - Bio-Technology **12**: 807-812, 1994.

Zieslin, N., Ben-Zaken, R.: Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. - Plant Physiol. Biochem. **31**: 333-339, 1993.