

INDUCTION OF DISEASE RESISTANCE AND ANTIOXIDANT ENZYMES BY ACIBENZOLAR-S-METHYL AGAINST BACTERIAL CANKER (*CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS*) IN TOMATO

E.M. Soylu¹, S. Soylu¹ and Ö. Baysal²

¹University of Mustafa Kemal, Faculty of Agriculture, Department of Plant Protection, 31034 Antakya, Hatay, Turkey

²Turkish Ministry of Agriculture and Rural Affairs, Bornova Plant Protection Research Institute, 35040 Bornova, Izmir, Turkey

SUMMARY

The plant defence activator acibenzolar-S-methyl (Bion) was assayed on tomato seedlings for its ability to induce multicomponent defence response against *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). Pre-treatment of plants with ASM reduced the severity of the disease as well as the growth of the bacteria *in planta*. In ASM-treated plants, reduction in disease severity (up to 75%) was correlated with the suppression of bacterial growth (up to 68.2%) during the time course of infection. In plants treated with ASM, activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione S-transferase (GST) were estimated as markers of resistance. The results indicated that ASM treatment led to enhanced activities of SOD and GST in tomato leaves. A slightly increase in SOD and GST activities was also found in *Cmm*-infected leaves. However, the increase in enzyme activities occurred much more rapidly and was more strongly enhanced in *Cmm*-infected leaves that were previously treated with ASM. The involvement of both enzymes in ASM mediated resistance in tomato is discussed.

Key words: antioxidative protection, benzothiadiazole, induced resistance, *Clavibacter michiganensis* subsp. *michiganensis*, tomato.

INTRODUCTION

Plants possess a range of active defence responses that contribute to resistance against a variety of pathogens. Pre-treatment of susceptible plants with avirulent pathogens (biotic inducers) or chemical compounds (abiotic inducers) can enhance resistance to subsequent attack not only at the site of treatment, but also in tissues distant from the initial infection sites. Typically, this inducible resistance system known as systemic acquired resistance (SAR) is effective against diverse pathogens including viruses, bacteria and fungi (Ryals *et al.*, 1996). SAR is characterized by a reduction

in the disease severity following challenge inoculation with a virulent pathogen. Certain synthetic or natural compounds with no direct anti-microbial effect such as 2,6-dichloroisonicotinic acid (INA), potassium salts, and amino butyric acid (BABA) were reported to induce SAR in plants (Oostendorp *et al.*, 2001).

Recently, the benzothiadiazole derivative benzo (1,2,3) thiadiazole-7-carbothioic acid-S-methyl ester (acibenzolar-S-methyl, ASM or BTH) has been developed as a potent SAR activator which does not have antimicrobial properties, but instead increases crop resistance to diseases by activating the SAR signal transduction pathway in several plant species such as bean, cauliflower, cucumber, tobacco, apple and pear (Siegrist *et al.*, 1997; Cole, 1999; Godard *et al.*, 1999; Brisset *et al.*, 2002). It has been commercially released in some countries as a plant health promoter of plant under the name of Bion[®] or Actigard[™].

The development of SAR is often associated with the various cellular defence responses, such as synthesis of pathogenesis-related (PR) proteins, phytoalexins, and accumulation of active oxygen species (AOS), rapid alterations in cell wall and enhanced activity of various defence-related enzymes (Ryals *et al.*, 1996). In recent studies, AOS were explored during expression of SAR. There is ample evidence indicating that AOS, H₂O₂ in particular, perform several important functions in early defence responses of the plant against pathogens, including direct antimicrobial action, lignin formation, phytoalexin production, and triggering of SAR (Mehdy *et al.*, 1996). AOS have also harmful effects on cells. AOS species such as O₂, OH[·] and H₂O₂ are commonly produced under stress conditions (Scandalios, 1993) and are strong oxidizing species that can rapidly attack all types of bio-molecules and damage. For the protection from oxidative damage, plant cells contain both oxygen radical detoxifying enzymes such as catalase, peroxidase and superoxide dismutase (SOD), and non-enzymatic antioxidants such as ascorbate peroxidase and glutathione-S-transferase (GST) (Alscher *et al.*, 1997). These enzymes play a crucial role in the protection of the plant cell from oxidative damage at the sites of enhanced AOS generation (Kuzniak and Sklodowska, 2001).

Bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), is a recurrent and serious

Corresponding author: S. Soylu
Fax: +90.326.2455832
E-mail: soylu@mku.edu.tr

disease of field and greenhouse-grown tomatoes (*Lycopersicon esculentum*) in several countries (Gleason *et al.*, 1993). Disease control is difficult because of lack of commercially acceptable resistant tomato cultivars. Chemical control of the disease relies upon the use of antibiotics (such as streptomycin) and copper compounds, which prevent bacterial multiplication and further infection. Unfortunately, the antibiotics have led to the selection of resistant bacterial populations. The induction of resistance might be a method for reducing the severity of such disease.

The aims of this study were to test ASM for its ability to induce resistance in tomato plants against *Cmm* and to characterize the mechanisms involved in ASM mediated resistance. The analysis of the two antioxidant enzymes SOD and GST was undertaken in tomato tissue, to assess a possible relationship between the activation of these enzymes and the observed protection in seedlings exhibiting induced resistance following treatment with ASM.

MATERIALS AND METHODS

Plant material. Tomato seedlings (*L. esculentum* cv. F144) with four fully expanded leaves were used for all experiments. Plants were grown in 10 cm pots in a soil mix containing sand, perlite, and peat compost under a 16 h photoperiod at $400 \mu\text{E m}^{-2} \text{s}^{-1}$ light intensity at $25 \pm 3^\circ\text{C}$ with 70-80% RH.

Bacterial strain and inoculation. The strain of *Cmm*, ICP7200, was preserved on modified Nutrient Yeast Dextrose Agar (NYA) at 4°C . Inoculum suspension was prepared from early log-phase bacterial cells grown in nutrient yeast extract broth 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, suspended in sterile distilled water, and their concentration was adjusted to 10^8 cfu ml^{-1} ($\text{OD}_{640}=0.1$). The two youngest leaves of the seedlings were cut at the tip and inoculated by dipping into the bacterial suspension as described by Gleason *et al.* (1993).

Application of ASM. ASM (Bion[®], Syngenta, Germany, as 50% active ingredients in WP formulation) was dissolved in distilled water (0.2 mg ml^{-1}) and then sprayed on tomato seedlings (ca. 200 μl per seedling) in the greenhouse. To determine the most efficient induction interval time conferred by ASM, seedlings were first treated with ASM or water (control) and experimentally inoculated 1, 2, 3 or 4 days after treatment. The resistance level induced in seedlings against *Cmm* was evaluated at 4, 7, 11 and 14 days after inoculation (dai) by using a 0-5 arbitrary scale. On such a scale, rating 0 was assigned to leaves showing no sign of wilting. The scale classes 1 = small marginal wilting, 1-10% of leaves showing wilting; 2 = 11-25% of leaves showing

wilting; 3 = sectored wilting, 26-49% of leaves showing wilting associated with chlorosis; 4 = pronounced collapse as leaf extended, 50-74% of leaves showing wilting; 5 = whole leaf wilted. A mean disease severity index (DSI%) was calculated from each treatment by summing the score of the 60 plants (three replicates of 20 plants per treatment), and expressing the value as a percentage using the formula described by Anfoka (2000).

Effect of ASM on bacterial growth *in planta*. Bacterial colony forming units (cfu) were recovered from inoculated tissues, treated with either ASM or water 3 days before inoculation, by removing leaf tissues aseptically from the region of inoculation. Excised tissues were homogenized in 1 ml of sterile 0.06% NaCl solution, diluted serially in 10-fold dilutions. Aliquots of alternate dilutions were plated on to NYA agar plates. Plates were incubated at 26°C for 48 h, and emerging colonies counted on all dilution plates showing bacterial growth.

Preparation of samples for determining enzyme activities. Seedlings were sprayed with ASM or water and inoculated 3 days afterwards with *Cmm* as previously described. Control treatments consisted of seedlings sprayed with water and inoculated (*i*), seedlings sprayed with ASM (*ii*) or water (*iii*) but not inoculated. From inoculated leaves, tissues were taken at the actual site of inoculation with *Cmm*. From control plants, tissues were taken from sites as similar to inoculated leaves. Samples for enzyme extractions from all treatments (inoculated or not) were separately taken 1, 2, 4, 7 and 14 days after inoculation. 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 using BSA as a standard (Bradford, 1976). The extract, obtained from two different lots of leaf samples (1 g fresh weight each) for each treatment, was used to determine the activity of SOD and GST.

Enzyme assays. Spectrophotometric analyses were conducted on UV/visible light spectrophotometer (Shimadzu UV1601 PC). The activity of SOD (EC 1.15.1.1) was assayed by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA and 20 μl enzyme extract. Ri-

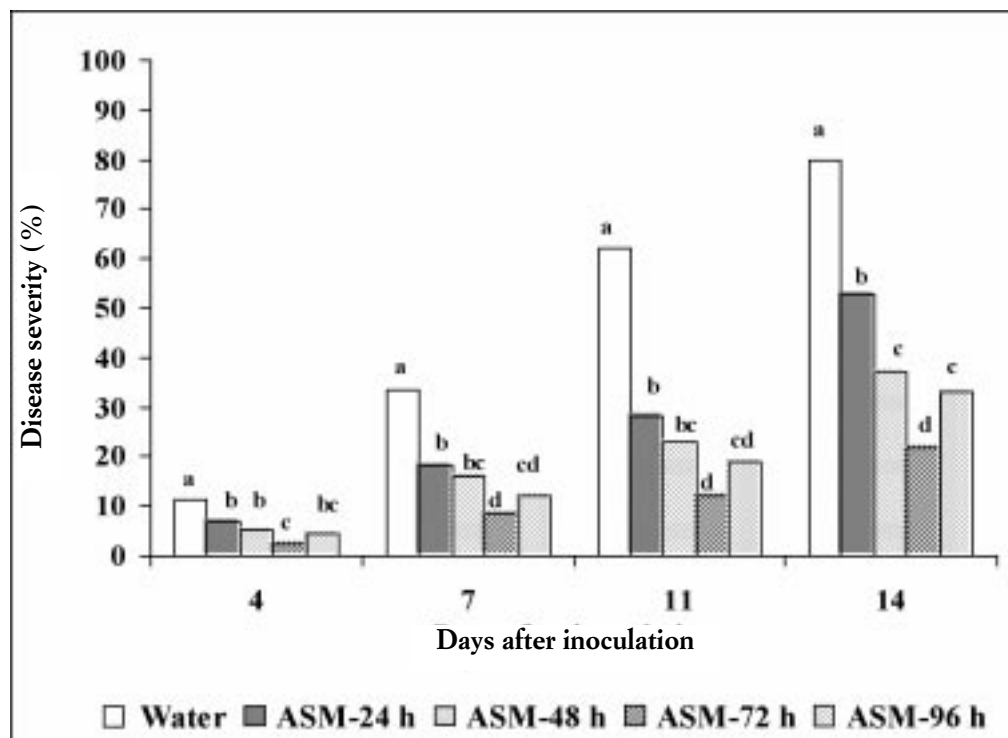


Fig. 1. Effect of ASM treatment on the severity of the disease symptoms caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). Seedlings were inoculated with *Cmm* 24-96 h after treatment with ASM or water (control). Inoculated leaves were scored at 4, 7, 11 and 14 days after inoculation (dai) using the 0-5 scale as described in Materials and Methods section. A mean disease severity (%) was calculated from each treatment by summing the score of the 60 plants (three replicates of 20 plants per treatment), and expressing the value as a percentage using the formula described by Anfoka (2000). Data are presented as the mean of the two independent experiments. Bars with the same letters represent values that are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

boflavin was added at the end and the tubes were shaken and placed 30 cm below a light source consisting of two 15 W fluorescent lamps. The reaction was initiated by turning the lights on and reduction of NBT was followed by reading A_{560} for 10 min. Blanks were run the same way but without illumination. The volume of enzyme extract corresponding to 50% inhibition of the reaction was considered as one enzyme unit. Each sample of extract was measured twice in each replicate, and at least two replications were performed per analysis.

Glutathione S-transferase (EC 2.5.1.18) activity was determined according to the method described by Mozzer *et al.* (1983). The model substrates 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) were used to measure GST activity. The reaction mixture contains final concentrations of 10 mM reduced GSH and 1 mM CDNB which are dissolved in 2.5% ethanol, and potassium-phosphate buffer (0.1 M, pH 6.5). GST activity was determined by absorbance readings at A_{340} nm. Change in absorbance at A_{340} was measured as 1-chloro-2,4-dinitrobenzene conjugated with reduced glutathione using an extinction coefficient of $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$.

Experimental design and statistical analyses. The ex-

periment was arranged in a completely randomized split-plot design with three replicates of 20 plants per treatment. Data obtained at various numbers of days after inoculation, and which usually included typical disease symptom, are presented. Experiments were repeated at least twice. Standard analysis of variance (ANOVA) was carried out by using the SPSS Statistical computer software program (Version 10.0). Significance was determined according to Duncan's Multiple Range Test ($P < 0.05$).

RESULTS

The effect of the ASM treatment on disease resistance. Resistance induced in tomato seedlings by ASM is shown in Fig. 1. Initial symptoms appeared on control plants as small marginal wilting 4 days after inoculation (dai). The mean DSI in these plants was 11%. The progression of the disease in control plants increased with time and by 14 dai, most of the leaves developed severe wilting; the mean DSI in these plants reached 80%. After application of ASM, a remarkable reduction in the DSI was observed (Fig. 1). The time between initial treatment with ASM and subsequent inoculation with

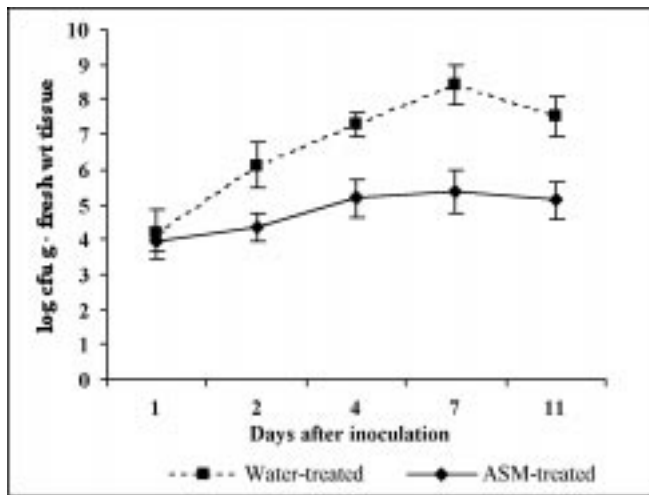


Fig. 2. Effect of ASM treatment on the bacterial growth of *Cmm* in tomato leaves. After treatment with ASM or water (control), seedlings were inoculated with *Cmm* 3 days after the induction. Data are the mean of two independent experiments, and bars represent standard deviation of the means.

Cmm significantly affected the efficacy of the induced resistance. Although all interval times significantly reduced the DSI, the highest level of protection was given by ASM treatment 72 h before inoculation (Fig. 1). The resistance induced by the ASM treatment was already evident 4 dai and lasted for the entire experimental period (until 14 dai). The disease index was reduced by 75% in ASM-treated seedlings 7 dai, and this was maintained at the same level until 14 dai. DSI of control seedlings were 80% whereas those of ASM-treated seedlings were only 21-53%, at 14 dai. Since the highest induced resistance was observed at a time interval of 3 days between treatment and inoculation, this interval was taken into consideration in order to determine the bacterial growth and enzyme assay experiments.

Bacterial multiplication in planta. The growth of *Cmm* was markedly reduced in ASM-treated seedlings compared to the water-treated control (Fig. 2). This inhibitory effect was first observed 4 dai and monitored until 7 dai. The bacterial population was reduced by 62.8, 68.2 and 60.5% by ASM, compared to control plants at 4, 7 and 11 dai, respectively (Fig. 2).

Changes in SOD and GST activities. In tomato plants treated with ASM, the expression of induced resistance was associated with enhanced enzyme activities (Fig. 3). The seedlings treated with ASM and challenged 3 days afterward with *Cmm* had considerably higher levels of SOD activity than other three treatments. SOD activity increased with time and reached the highest-level 4 dai. The activity was significantly different from other treatments (Fig. 3A). Increased activity was followed by a decrease 7 dai. In seedlings treated with ASM but not challenged with *Cmm*, SOD activity was not as high as found in ASM-treated and inoculated

seedlings. The levels of SOD activity in the water-treated seedlings (inoculated or not) increased gradually, but never reached to the level of activity in ASM-treated plants during the time course (Fig. 3A).

Treatment of seedlings with ASM has also resulted in a marked increase of GST activity (Fig. 3B). The activity of GST in ASM-treated and inoculated plants was significantly higher than that observed in other three treatments at all intervals. In seedlings treated with ASM but not challenged with *Cmm*, GST activity increased with time. The GST activity observed at day 7 was significantly different from water-treated control plants (inoculated or not). The levels of GST activities remained low in both inoculated and uninoculated water-treated seedlings during the time course (Fig. 3B).

DISCUSSION

The current study assessed the effect of the plant activator ASM on the bacterial canker caused by *Cmm*. Results clearly showed that ASM may induce a resistance response in tomato seedlings. Similar enhanced disease resistance induced by ASM was observed in tomato plants against potential fungal, bacterial, viral disease agents and insects (Benhamou and Belanger, 1998; Anfoka, 2000; Scarponi *et al.*, 2001). Beside tomato, induction of resistance by ASM was also demonstrated in a number of plant species against a wide spectrum of fungal, bacterial and viral pathogens (Siegrist *et al.*, 1997; Cole, 1999; Godard *et al.*, 1999; Brisset *et al.*, 2002).

For the development of resistance, plants need an interval period before being challenged with a pathogen. In most cases, this interval was reported to lie between 1 and 7 days. In our study, the best protection against *Cmm* was obtained when ASM was applied 3 days before inoculation: a delayed challenge with the pathogen did not show higher level of protection. This result is comparable to the findings of Godard *et al.* (1999) in the cauliflower/*Peronospora* pathosystem. In cocoa, however, a longer interval period was needed for the development of resistance against *Verticillium* wilt and witches' broom diseases: the best protection was obtained by treatment 15 and 30 days prior to inoculation (Resende *et al.*, 2002).

The effect of ASM treatment on bacterial growth was evaluated. The results revealed that reduction in the DSI was related to considerable lower bacterial growth in treated plant leaves. In ASM treated seedlings, a significantly lower bacterial population was observed up to 7 dai compared to water-treated plants. These data indicated that ASM did not only limit symptom progression but also inhibited the *Cmm* multiplication in planta. Experiments with a diverse set of fungal species showed that neither ASM nor its major metabolites exhibit *in vitro* antimicrobial activity even at the concentrations exceeding the levels shown to be efficacious in plants (Cole, 1999; Godard *et al.*, 1999; Anfoka, 2000).

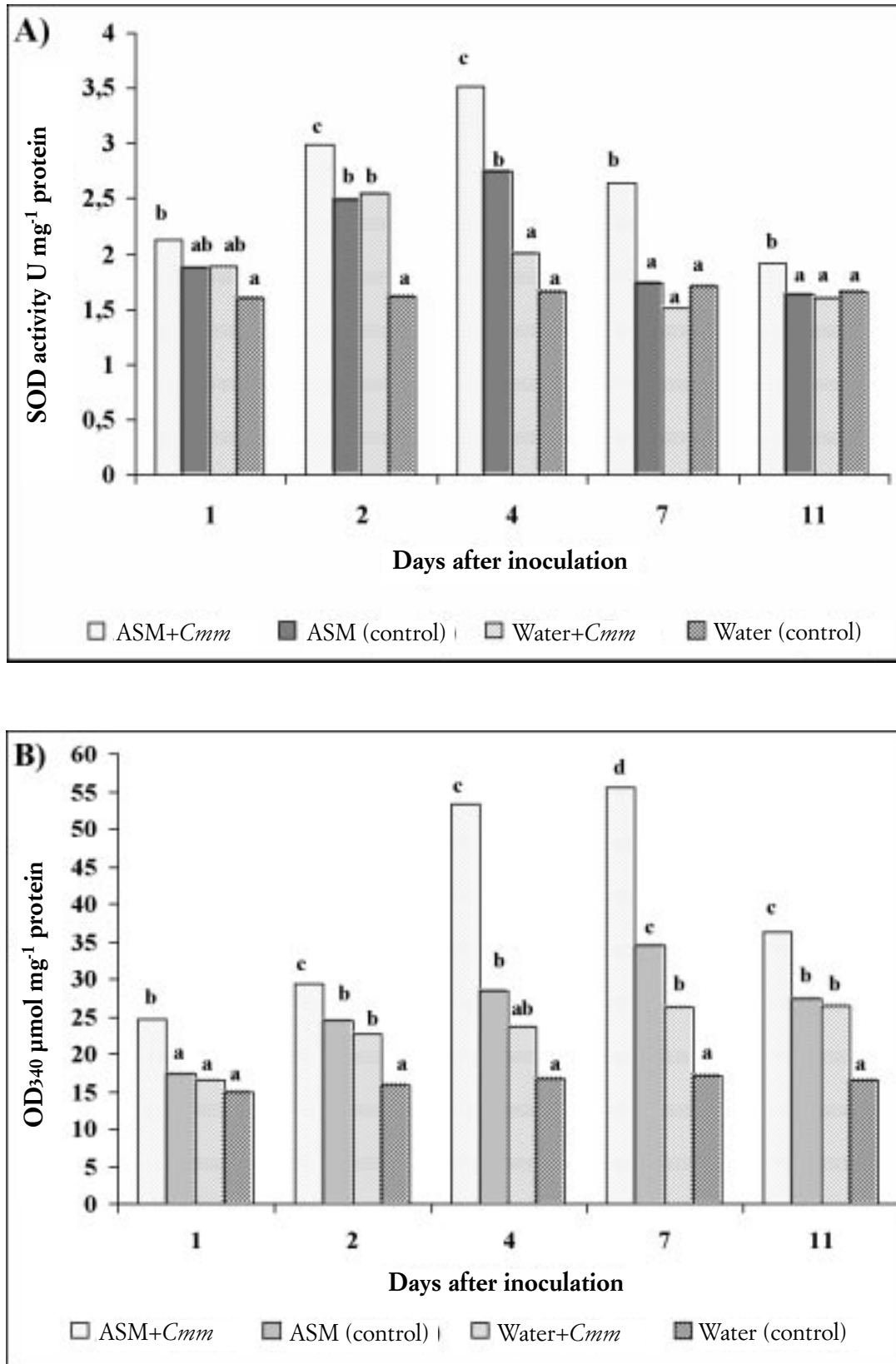


Fig. 3. Effect of the ASM treatment on the SOD (A) and GST (B) activities in tomato leaves. Leaves, treated with ASM or water, were inoculated with the *Cmm* 3 days after the induction. As a control, leaves were sprayed with water or ASM but not inoculated. Both inoculated and uninoculated leaves were removed at the indicated periods and processed as described in Materials and Methods. The results are expressed as the mean of two separate experiments (in each experiment two different extractions were pooled at every time point). Bars with the same letters represent values that are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

Increased production of AOS is a common feature of defence responses to challenge by avirulent pathogens and elicitors. When plants are subjected to environmental stress such as drought, salt, extreme temperatures, nutrient deficiency, air pollution, herbicides and pathogen infection, the balance between the production of AOS and the quenching activity of antioxidant may be upset and oxidative damage may result (Scandalios, 1993; Alscher *et al.*, 1997). Failure to quench or inactivate the AOS may lead to degradation of membrane lipids, proteins and DNA. The coordinated induction of antioxidant enzymes such as SOD, catalase, glutathione S-transferase (GST) and glutathione peroxidase, in response to environmental stress has been reported in tomato (Kuzniak and Sklodowska, 2001; Mittova *et al.*, 2002). The study of the role of the antioxidant system in the mechanisms induced by chemical activators such as ASM is relatively recent. Recently ASM, as a functional analogue of salicylic acid, has been reported to inhibit catalase and ascorbate peroxidase, the two key H_2O_2 scavenger enzymes, increasing H_2O_2 level in treated tobacco leaves (Wendehenne *et al.*, 1998). Increased levels of H_2O_2 , which might result from inhibition of these enzymes would serve as a second messenger for the induction of defence response. If higher H_2O_2 levels or changes in the cellular redox status play a role in ASM mediated activation of defence responses, then antioxidants should counteract the effect of these compounds. H_2O_2 has been implicated not only in triggering hypersensitive cell death, but also in limiting the spread of cell death by inducing the expression of cell protecting genes in surrounding cells (Levine *et al.*, 1994). Close relationships were found between ASM-induced resistance and the high levels of both antioxidant enzyme activities. Synchronous action of both SOD and GST as a part of the antioxidative system has been reported to protect plants against AOS by reducing H_2O_2 and free fatty acid hydroxyl peroxide (Kuzniak and Sklodowska, 2001). Increased activities of these enzymes may correlate with increased protection from damage associated with oxidative stress as suggested (Levine *et al.*, 1994; Kuzniak and Sklodowska, 2001). At present, it is unclear whether ASM inhibition of catalase is involved in the signal transduction pathways leading to activation of oxidative burst in our system. In bean, however, histochemical studies clearly showed that ASM induce H_2O_2 accumulation in bean plants (Iriti and Faoro, 2003). Similar induction might have been occurred in tomato following treatment with ASM. Further studies will be necessary to determine whether ASM induce AOS in tomato. In addition to antioxidative enzymes, low nutritional status and/or accumulation of antimicrobial compounds, such as phytoalexins and antibacterial peptides, in ASM-treated tomato tissues may also account for this resistance.

ASM, therefore, proved to be an efficient activator of several plant defence mechanisms, and seems to be a useful tool to induce resistance in tomato, as observed in other plant species. Along with conventional fungi-

cides, biocontrol organisms and improved seed varieties, ASM may be new option for disease control.

REFERENCES

- Alscher R.G., Donahue J.L., Cramer C.L., 1997. Reactive oxygen species and antioxidants: relationships in green cells. *Physiologia Plantarum* **100**: 224-233.
- Anfoka G.H., 2000. 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 Protection* **19**: 401-405.
- Benhamou N., Belanger R., 1998. Benzothiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato. *Plant Physiology* **118**: 1203-1212.
- Beauchamp C., Fridovich I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44**: 276-287.
- Bradford M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* **72**: 248-257.
- Brisset M.N., Faize M., Heintz C., Cesbron S., Chartier R., Tharaud M., Paulin J.P., 2002. Induced resistance to *Erwinia amylovora* in apple and pear. *Acta Horticulturae* **590**: 335-338.
- Cole D.L., 1999. The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance against bacterial and fungal diseases of tobacco. *Crop Protection* **18**: 267-273.
- Gleason M.L., Gitaitia R.D., Ricker M.K., 1993. Recent progress in understanding and controlling bacterial canker of tomato in eastern North America. *Plant Disease* **77**: 1069-1076.
- Godard J.P., Ziadi S., Monot C., Le Corre D., Silue D., 1999. Benzothiadiazole (BTH) induces resistance in cauliflower (*Brassica oleracea* var *botrytis*) to downy mildew of crucifers caused by *Peronospora parasitica*. *Crop Protection* **18**: 397-405.
- Iriti M., Faoro F., 2003. Benzothiadiazole (BTH) induces cell-death independent resistance in *Phaseolus vulgaris* against *Uromyces appendiculatus*. *Journal of Phytopathology* **151**: 171-180.
- Kuzniak E., Sklodowska M., 2001. Ascorbate, glutathione and related enzymes in chloroplasts of tomato leaves infected by *Botrytis cinerea*. *Plant Sciences* **160**: 723-731.
- Levine A., Tenhaken R., Dixon R., Lamb C., 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* **79**: 583-593.
- Mehdy M.C., Sharma Y.K., Sathasivan K., Bays N.W., 1996. The role of activated oxygen species in plant disease resistance. *Physiologia Plantarum* **98**: 365-374.
- Mittova V., Tal M., Volokita M., Guy M., 2002. Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. *Physiologia Plantarum* **115**: 393-400.

- Mozer T.J., Tiemeier D.C., Jaworski E.G., 1983. Purification and characterization of corn glutathion S-transferase. *Biochemistry* **22**:1068-1072.
- Oostendorp M., Kunz W., Dietrich B., Staub T., 2001. Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology* **107**: 19-28.
- Resende M.L.V., Nojosa G.B.A., Cavalcanti L.S., Aguilar M.A.G., Silva L.H.C.P., Perez J.O., Andrade G.C.G., Carvalho G.A., Castro R.M., 2002. Induction of resistance in cocoa against *Crinipellis pernicioso* and *Verticillium dahlia* by acibenzolar-S-methyl (ASM). *Plant Pathology* **51**: 621-628.
- Ryals J.A., Neuenschwander U.H., Willits M.G., Molina A., Steiner H.Y., Hunt M.D., 1996. Systemic acquired resistance. *Plant Cell* **8**: 1809-1819.
- Scandalios J.G., 1993. Oxygen stress and superoxide dismutase. *Plant Physiology* **101**: 7-12.
- Scarponi L., Buonaurio R., Martinetti L., 2001. Persistence and translocation of a benzothiadiazole derivative in tomato plants in relation to systemic acquired resistance against *Pseudomonas syringae* pv *tomato*. *Pest Management Science* **57**: 262-268.
- Siegrist J., Glenewinkel D., Kollé C., Schmidtke M., 1997. Chemical induced resistance in green bean against bacterial and fungal pathogens. *Journal of Plant Disease and Protection* **104**: 599-610.
- Wendehenne D., Durner J., Chen Z., Klessig D.F., 1998. Benzothiadiazole, an inducer of plant defenses, inhibits catalase and ascorbate peroxidase. *Phytochemistry* **47**: 651-657.

Received 11 April 2003

Accepted 8 August 2003

