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Department of Biochemistry and Molecular Biology, Federal University of Ceará, Ceará, Brazil

Benzo-thiadiazole-7-carbothioic Acid *S*-methyl Ester does not protect Melon Fruits against *Fusarium pallidoroseum* Infection but Induces Defence Responses in Melon Seedlings

Darcy M. F. Gondim[1], Daniel Terao[2], Aparecida S. Martins-Miranda[1], Iika M. Vasconcelos[1] and José T. A. Oliveira[1]

Authors' addresses: [1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, PO Box 6033, 60451-970, Fortaleza, Ceará, Brazil; [2]Embrapa Semi-Árido, PO Box 23, CEP 56302-970, Petrolina, PE, Brazil (correspondence to J. T. A. Oliveira. E-mail: jtaolive@ufc.br)

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Abstract

The present study investigated the potential of benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) to protect postharvest melons var. 'Orange Flesh' from the fruit rot caused by *Fusarium pallidoroseum.* It was noticed that melon fruits immersed in BTH and postinoculated with the fungus presented the same pattern of disease incidence/severity and activity of the defence-related enzymes superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, phenylalanine ammonia-lyase, and β-1,3-glucanase of controls, indicating that BTH was ineffective in protecting melons from the fruit rot disease. However, the preflowering application of BTH in melon seedlings induced stunted growth, probably related to enhanced lignification which is related to the plant cell wall reinforcement and increase of resistance against invading pathogens, and alterations of the activity of the studied defence-related enzymes in comparison with controls, suggesting that this strategy could probably be effective for the control of the postharvest rot of melon fruits through activation of systemic resistance.

Introduction

Commercialization of melon (*Cucumis melo* L.) fruit destined for internal consumption and export constitutes one of the main segments of the Brazilian economy. Melon production is exported particularly to European Union countries which require high quality, undamaged, and phytopathogen-free fruits. *Fusarium pallidoroseum* is an important fungus which provokes rot in melon fruits (Gadelha, 2002). This disease represents a serious obstacle in its commercialization, particularly as a foreign commodity. In addition, the disease control is difficult due to the great aggressiveness of the pathogen.

Many reports have shown that induced disease resistance in plants by biotic and abiotic elicitors is an effective method for restricting the spread of fungal infection (Smith-Becker et al., 2003; Buzi et al., 2004; Yao and Tian, 2005). Benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) or acibenzolar-*S*-methyl has been developed as an inducer of systemic acquired resistance (SAR) which promotes plant resistance towards several plant pathogens. For example, BTH was shown to suppress the postharvest infection of cut *Freesia hybrida* L. flowers by *Botrytis cinerea* (Darras et al., 2006) and to reduce the severity and development of grey mould disease on strawberry fruit caused by *B. cinerea* (Terry and Joyce, 2000, 2004a). Further, a preflowering and unique foliar spray of BTH without postharvest fruit treatment decrease the incidence and extent of postharvest disease caused by *Alternaria* sp. and *Fusarium* sp. in rock melons cv. Eldorado (Huang et al., 2000). Moreover, induced resistance in melon to *Didymella bryoniae*, *Sclerotinia sclerotiorum* (Buzi et al., 2004), *Colletotrichum lagenarium* and cucumber mosaic virus (Smith-Becker et al., 2003) were also reported.

The development of inducible resistance is associated with various defence responses in plant cells. These include synthesis of pathogenesis-related proteins (Iriti and Faoro, 2003; Baysal et al., 2005; Malolepsza, 2006), alterations in cell walls such as lignification (Vanacker et al., 2000; Zhao et al., 2005), generation of reactive oxygen species (ROS) (Iriti and Faoro, 2003; Malolepsza, 2006), changes in the expression of oxidative stress-related enzymes (Wendehenne et al., 1998; Liu et al., 2005) and synthesis of the key enzyme of the phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL) (Chen et al., 2005; Wen et al., 2005).

As the use of fungicide has been severely restricted and melon cultivars resistant to *F. pallidoroseum* are not available, the aim of the present study was to address the question whether BTH could protect post-harvest melons from the fruit rot caused by *F. pallidoroseum* by functioning as an elicitor of biochemical defence responses on both infected melon fruits and healthy melon plants (*Cucumis melo* L.) var. 'Orange Flesh'.

Materials and Methods

Biological material

Melon fruits var. 'Orange Flesh' were harvested from a commercial plantation at the commercial ripening stage and selected for uniformity of size and ripeness and absence of injuries. Fruits were brought to the laboratory within 24 h after harvesting and sprayed with 70% ethanol, air-dried and stored at room temperature (25 ± 2°C). *Fusarium pallidoroseum* was isolated from infected melon fruits and maintained on potato dextrose agar medium. The conidial suspension for inoculation was obtained from seven-day-old cultures incubated at 28°C. The spore concentration was adjusted to 10^5 spores/ml with sterile Milli-Q grade water.

BTH treatment and fungal infection of melon fruits

Melon fruits were soaked in BTH (50% wettable granule formulation, Bion®, Syngenta, Brazil) solutions, prepared in 0.05% Tween 80 and containing 0.5, 1.0 and 2.0 mM active compound or in 0.05% Tween 80 (controls), for 5 min, and then air-dried at room temperature (25 ± 2°C). Sixty hours after treatments, four cavities (2 mm diameter, 3 mm deep) were produced on the surface of each melon fruit using a device containing a set of five sterile needles joint together and 20 μl conidial suspension (10^5 spores/ml) were deposited per cavity. Then they were kept into paper boxes (48 × 33 × 15 cm), four fruits per box (one experimental unit), at room temperature, and covered with plastic bags for 24 h to increase humidity and allow spores to germinate. Disease incidence and lesion diameter on each fruit were recorded daily from 1 to 10 days after inoculation. Determinations were carried out with four replicates for each treatment.

Induction of biochemical defence response in melon fruits

To carry out this experiment new fruits were collected, treated, inoculated and kept as previously described. Based on the responses of the melon fruits treated with the BTH concentrations stated above and postinoculated with *F. pallidoroseum*, melon fruits were treated only with the highest BTH concentration used (2.0 mM). Then 3, 7 and 10 days after fungal inoculation, plant tissue samples (2 cm diameter × 1 cm deep) close to the inoculation sites were excised using a sterile cork borer, weighed, frozen in liquid N_2 and kept at −82°C until use for crude extract preparation and enzyme analyses. This experiment was repeated thrice and each experimental unit comprised six melons. Enzyme assays were carried out in crude extracts prepared from the frozen cuts homogenized (1:1 m/v) for 10 min in an ice-cold mortar with 0.05 M sodium acetate buffer, pH 5.2, containing 0.15 M NaCl. After centrifugation at 20 000 × g at 4°C for 10 min, the supernatant (crude enzyme extract) was collected, dialysed (for 48 h, five changes) against the extracting buffer, and stored at −20°C for subsequent analyses.

Induction of biochemical defence response in melon seedlings

Melon (var. 'Orange Flesh') seeds were germinated (eight seeds per pot) and grown in plastic pots (1 l capacity) containing river sand as substrate exhaustively washed in tap water and autoclaved (120°C, 1.5 kg/cm^2, 30 min). Seedlings were maintained under greenhouse conditions (27–35°C, 40–60% relative humidity) exposed to natural light. Seedlings were daily irrigated with distilled water up to 3 days after emergence and subsequently, at 3-day intervals, with 10, 5, and 2 times diluted Hoagland and Arnon (1950) nutritive solution, modified as described by Silveira et al. (2001). Thereafter, undiluted nutritive solution was used until the end of the experimental period. Eight days after planting, the seedling foliage was sprayed (300 μl per seedlings with aqueous solutions of BTH, containing 0.3, 0.5 and 1.0 mM active compound. Control plants were sprayed with sterile water. Melon plants were harvested 2, 4, 6, 8, 10 12, and 14 days after BTH or water treatment and then weighed. Secondary leaves were collected, frozen in liquid N_2 and kept at −82°C until use for biochemical analyses. Determinations were carried out with three replicates for each treatment.

To analyse BTH-induced defence enzymes, frozen secondary leaves were homogenized (1:3, m/v) in an ice-cold mortar using 0.05 M sodium acetate buffer, pH 5.2, containing 0.15 M NaCl. The suspension was centrifuged at 20 000 × g for 10 min at 4°C, and the supernatant collected, dialysed (for 24 h, five changes) against the extracting buffer and the resulting leaf enzyme extracts stored at −20°C for subsequent analyses.

Protein concentration and enzyme analyses

Protein concentration was determined according to Bradford (1976) using bovine serum albumin as standard.

Superoxide dismutase (SOD) (EC 1.15.1.1) isoenzymes were examined in native polyacrylamide gel electrophoresis following the procedures of Beauchamp and Fridovich (1971) and Martinez et al. (2000). Equal protein quantities (4.5 μg) of leaf crude extracts were loaded in each lane of a single gel. Protein bands were visualized with 0.25 mg/ml nitro blue tetrazolium plus 0.1 mg/ml riboflavin in the presence of a 32 W fluorescent light.

For ascorbate peroxidase (APX) (EC 1.11.1.11) assay (Peixoto et al., 1999), 300 μl leaf crude extract was incubated with 600 μl 0.05 M potassium phosphate buffer, pH 6.0, 0.5 mM ascorbate and 100 μl 2 mM H_2O_2, for 3 min, at 30°C. The variation of one

absorbance unit, measured at 290 nm, within the reaction time, was defined as APX activity unit and expressed per gram of leaf fresh weight per minute (AU g/FW/min).

For guaiacol peroxidase (POX) (EC. 1.11.1.7) assay (Urbanek et al., 1991), 20 μl leaf extract was incubated with 980 μl extracting buffer without NaCl, 500 μl 0.02 M guaiacol and 500 μl 60 mM H_2O_2, at 30°C, for 3 min. Absorbance was measured at 480 nm and the variation of one unit of absorbance was defined as POX activity unit. Enzyme activity was expressed as activity unit per gram of leaf fresh mass per minute (AU g/FM/min).

To analyse phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5) activity (Mori et al., 2001; El-Shora, 2002), 200 μl leaf extract was incubated with 200 μl 0.04 M/L-phenylalanine, 20 μl 0.05 M β-mercaptoethanol and 480 μl 0.1 M Tris–HCl buffer, pH 8.8, at 30°C, for 60 min. Reaction was stopped by addition of 100 μl 6 M HCl. After centrifugation (20 000 × ***g***, 5 min) the absorbance of the collected supernatant was measured spectrophotometrically at 290 nm. Activity was expressed as the amount of *trans*-cinnamic acid produced per gram leaf fresh mass per second (pmol cinnamic acid g/FM/s).

β-1,3-glucanase (βGLU) (E.C. 3.2.1.39) activity was assayed using 2.0 mg/ml laminarin (Sigma-Aldrich, Inc., St Louis, MO, USA) as substrate (Boller, 1993). The reaction mixture consisted of 100 μl leaf extract and 900 μl laminarin solution (2.0 mg/ml). After incubation at 50°C for 30 min, the amount of glucose liberated was determined from absorbance readings taken at 520 nm. Activity was expressed as nanokatal (nkat) per gram leaf fresh mass (nkat/g FM) and defined as the enzyme catalysing the formation of 1.0 nM glucose equivalent per second.

Statistical analysis

Data were subjected to analysis of variance followed by Duncan's test ($P \leq 0.05$) using the SAS package (Statistical Analysis Systems Inc., Cary, NC, USA).

Results

Effect of BTH treatment on melon fruits

The effects of BTH treatment on the protection of postharvest melon fruits against *F. pallidoroseum* is shown in Fig. 1. It is depicted that BTH at 0.5, 1.0 or 2.0 mM concentration had no effect on the incidence (a) and severity (b) of the rot caused by *F. pallidoroseum* compared with control fruits during the experimental period examined. Thus this suggests that BTH was ineffective in protecting melon fruits from the fruit rot disease.

Zymogram analysis revealed at least four SOD isoforms being present in melon fruits (Fig. 2). Although their activities increased during the course of the experimental period such increases seemed to be due to fruit maturation and not related to chemical elicitation, except at 10 days after BTH-treatment (DAT) when a slight increase of b, c and d isoforms, compared with the respective control, was noticed. Regarding the enzyme activities of APX, POX, PAL and βGLU in melon fruits treated with 2.0 mM BTH, no significant differences in their activities were observed by comparison with the corresponding controls at 3, 7 and 10 DAT (Fig. 3). Overall, in this case, there was a fruit maturation-dependent increase in POX, PAL and βGLU, and decrease in APX activity. Nevertheless,

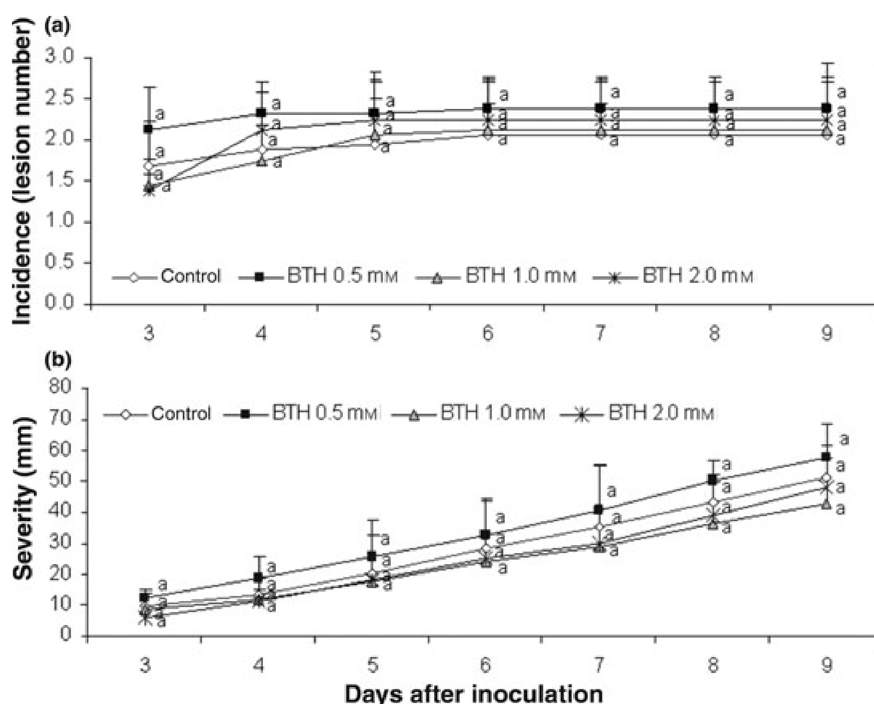

Fig. 1 Effect of benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) on the incidence (a) and severity (b) of melon fruit rot caused by *Fusarium pallidoroseum*. Equal letters at each time-point indicate that values are not statistically different (Duncan's test, $P > 0.05$). Bars indicate SE

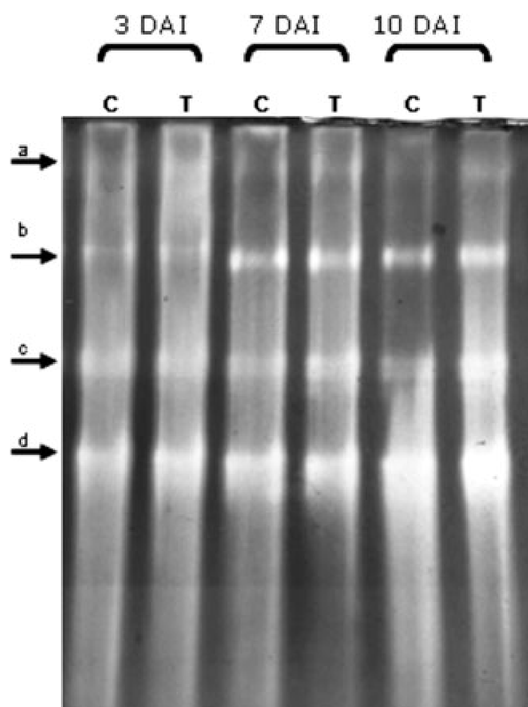

Fig. 2 Native-polyacrylamide gel electrophoresis showing the zymogram of superoxide dismutase (SOD) from melon fruits treated with benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) and post-inoculated with *Fusarium pallidoroseum*. Arrows indicate SOD isoforms. C = control; T = 2.0 mM BTH treatment; DAI = days after inoculation

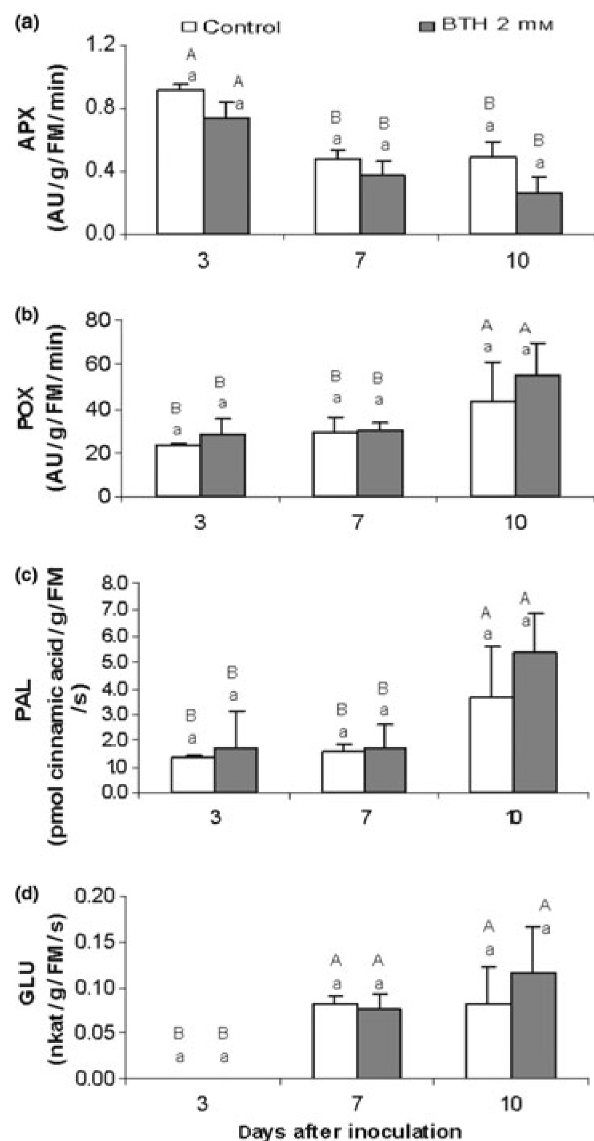

Fig. 3 Ascorbate peroxidase (APX) (a), guaiacol peroxidase (POX) (b), phenylalanine ammonia-lyase (PAL) (c), and β-1,3-glucanase (βGLU) (d) activities of melon fruits treated with benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) and postinoculated with *Fusarium pallidoroseum*. Data are compared within each time-point (small letters) and during the course of the experimental period (capital letters). Different letters indicate significant differences (Duncan's test, P ≤ 0.05). Bars indicate SE

BTH treatment of melon fruits followed by *F. pallidoroseum* inoculation did not act as an elicitor of host biochemical defence responses under the conditions of the present study.

Effect of BTH treatment on melon seedlings

This experiment evaluated whether BTH treatments affect the plant growth and the kinetics of some defence-related enzymes in healthy melon seedlings that had not been inoculated with *F. pallidoroseum*. All BTH concentrations used (0.3, 0.5 and 1.0 mM) reduced the seedling growth particularly from day 6 onwards (Fig. 4). This effect was more pronounced in both the shoot height and the size of the secondary leaves which were smaller than those of controls (figures not shown). As a consequence, the seedling total fresh masses were decreased by around 21% already at 6 DAT. Maximal differences were achieved at 12 DAT when fresh masses decreased, on average, by 35% of controls at all BTH concentrations used (Fig. 4).

BTH-treatment also altered the expression of some defence enzymes. Zymogram analysis of SOD revealed the presence of at least four isoforms in the secondary leaves of melon seedlings which were induced upon BTH-treatment (Fig. 5). However, the most prominent increases in SOD activity occurred with band c, particularly from 6 to 12 DAT. At the end of the experimental period (14 DAT), no differences in intensities were visualized amongst the bands in control and treated seedlings, apparently because the inducing effect of BTH has ceased.

A significant effect of BTH-treatment on APX was noted only at 2 and 12 DAT (Fig. 6a). At 2 DAT, the decrease in APX activity occurred only with 1.0 mM treatment, whereas at 12 DAT, the decreases were more pronounced with 0.5 and 1.0 mM BTH concentrations applied, representing around 61% and 65% of those of control seedlings, respectively.

Figure. 6b shows that POX activity steadily increased from day 4 to day 8, when it displayed the highest activity, comprising 3066%, 5200% and 3690% over those of controls, at 0.3, 0.5 and 1.0 mM BTH treatments, respectively. Thereafter POX

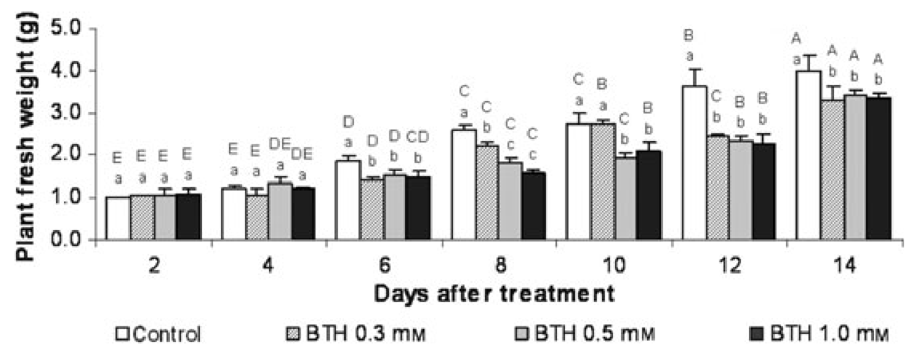

Fig. 4 Effect of benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) on the fresh weight of melon seedlings. Data are compared within each time-point (small letters) and during the course of the experimental period (capital letters). Different letters indicate significant differences (Duncan's test, P ≤ 0.05). Bars indicate SE

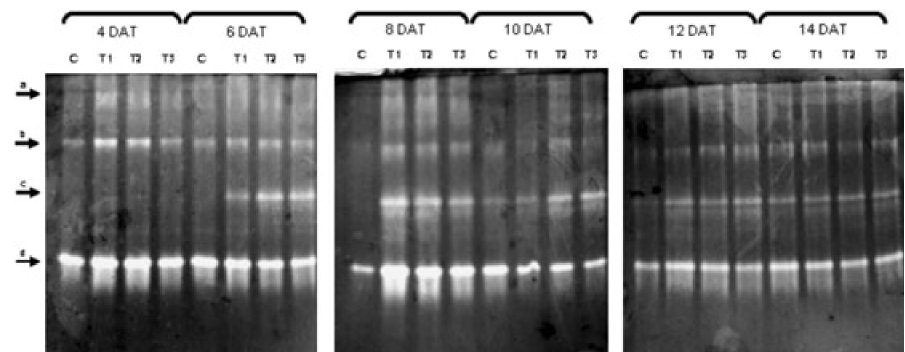

Fig. 5 Native-polyacrylamide gel electrophoresis showing the zymogram of superoxide dismutase (SOD) from melon seedlings treated with benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH). Arrows indicate different isoforms. C = control; T1, T2, and T3 = 0.3 mM, 0.5 mM and 1.0 mM BTH concentrations, respectively; DAT = days after treatment

decreased slightly until the end of the experimental period, but still kept higher levels of activity compared with control seedlings.

Phenylalanine ammonia-lyase activity (Fig. 6c) showed to be unresponsive to BTH as it showed a trend of decreasing activity as the seedlings aged.

On the other hand, BTH was able to significantly induce *β*GLU activity from 8 to 14 DAT (Fig. 6d), particularly at 0.5 and 1.0 mM concentrations. At 8 DAT, *β*GLU activity increased by at least 666% over those of controls.

Discussion

Relatively little work has addressed pathogen resistance of postharvest fruits, especially related to melons. However, the melon rot caused by *F. pallidoroseum* represents a constraint to its commercialization and searches to find out new strategies to control this disease is imperative. BTH is considered an environmentally friendly crop protection agent and resistance activator for a great number of plant species against a large number of pathogens (Iriti and Faoro, 2003; Baysal et al., 2005; Malolepsza, 2006). Based on this premise BTH has been tentatively employed to reduce the use of agrotoxic substances while maintaining crop yields. However, the results of the present work indicate that the protective effects of BTH against pathogens cannot be taken for granted and that this effect on a particular crop should be independently evaluated. Indeed, application of BTH in melon fruits, var. 'Orange Flesh', did not result in any effective reduction of the incidence and severity of the melon rot caused by *F. pallidoroseum*, under the experimental conditions of the present work (Fig. 1). Actually, the use of this chemical inducer, even at 2.0 mM active ingredient concentration, did not modify the activities of the defence-related enzymes SOD (Fig. 2), APX, POX, PAL and *β*GLU in melon fruits (Fig. 3) which is in agreement with the ineffectiveness of BTH in protecting the melon fruits against the postharvest rot. Few reports have also shown the unsuccessful use of BTH in inducing resistance. Indeed, BTH was ineffective in reducing the number and diameters of lesions provoked by *Botrytis cinerea* on *Freesia hybrida* cv. 'Cote d'Azur' flower (Darras et al., 2007). Similarly, field application of BTH had no significant effect on *B. cinerea* disease severity on two Geraldton Waxflower (*Chamelaucium uncinatum*) cultivars (Dinh et al., 2007). Thus, altogether, the present as well as some previous results provide evidence that in some plant-pathogen systems BTH is not able to induce resistance.

To control postharvest diseases of several important fruits a number of approaches have been tried towards the development of alternative methods of protection against postharvest decays other than the use of fungicides. These generally include manipulation of temperature and humidity of fruit storage to suppress pathogen development, hot-air or hot-water treatments or induction of natural resistance by environmentally friendly elicitors, and UV-C irradiation (Janisiewicz and Korsten, 2002; Terry and Joyce, 2004b). Unfortunately, none of these methods alone have provided satisfactory control of decays. Therefore, more efforts are necessary to discover new alternatives to overcome the drawbacks that postharvest decays bring to the fruit industries.

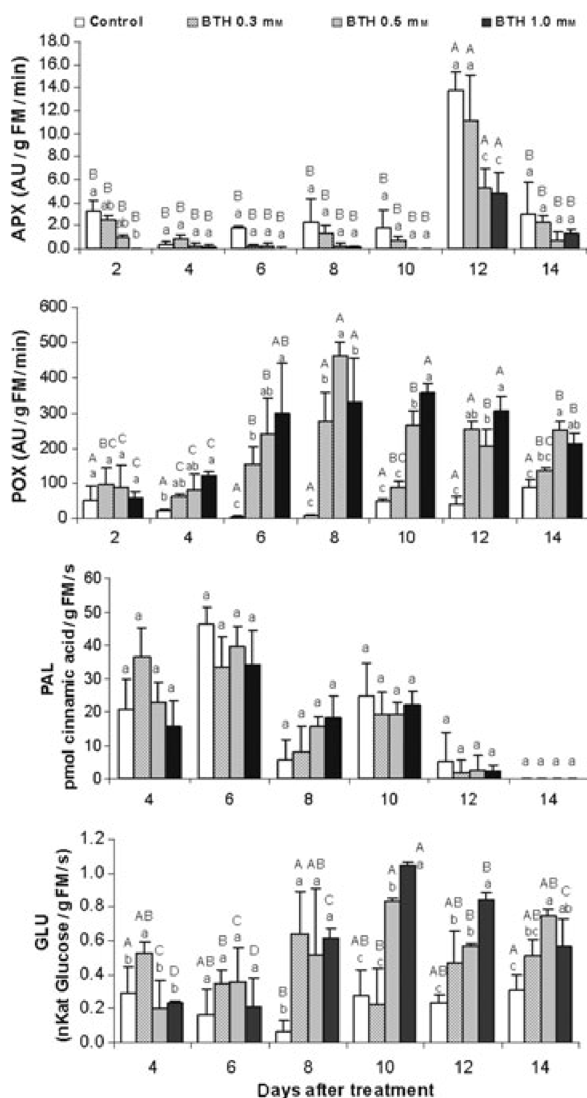

Fig. 6 Ascorbate peroxidase (APX) (a), guaiacol peroxidase (POX) (b), phenylalanine ammonia-lyase (PAL) (c), and β-1,3-glucanase (βGLU) (d) activities of melon seedlings treated with benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH). Data are compared within each time-point (small letters) and during the course of the experimental period (capital letters). Different letters indicate significant differences (Duncan's test, P ≤ 0.05). Bars indicate SE

The present study showed that soaking melon fruits in BTH was not efficient in protecting them against the rot caused by *F. pallidoroseum*. Therefore, an experiment in which melon seedlings were sprayed with BTH aiming at triggering defence-related enzymes was carried out. The rationale behind this approach was to verify if BTH could induce effective defence responses SAR in melon plants.

The melon seedlings treated with BTH showed stunted growth from 6 DAT in comparison with controls (Fig. 4). This reduction could be the result of increased lignification of the cell wall of melon seedlings which might have restricted the meristematic cell division and elongation. Lignification and cell elongation are amongst the broad range of processes that POX is involved in plants (Fournand et al., 2003; Passardi et al., 2006). In this present study, BTH was able to induce overexpression of POX in melon seedlings (Fig. 6b). Similar BTH-induction effect on POX activity had previously been documented (Iriti and Faoro, 2003; Soylu et al., 2003; Liu et al., 2005; Malolepsza, 2006) and it has been suggested that the increase in POX activity is related to the plant cell wall reinforcement and increase of resistance against invading pathogens (Soylu et al., 2003).

Phenylalanine ammonia-lyase activity was showed to be unresponsive to BTH when compared with control plants (Fig. 6c). As PAL acts upstream of salicylic acid (SA) in the phenylpropanoid pathway, a-SA analogue would not be expected to induce PAL. However it has been reported increases in PAL activity few hours after plants had been challenged with pathogens, a response associated with the onset of the hypersensitive response (HR) and later to the synthesis of phenolic compounds and cell lignification (Silva et al., 2002; Barreto et al., 2007). Thus, pathogen-induced SAR results in increased PAL activity, but not SA or BTH treatment.

Various biological processes in living organisms produce ROS. However, in stress situations as under pathogen attack, plants may rapidly generate ROS as one of the earliest events linked with plant resistance (Hammond-Kosack and Jones, 1996; Kotchoni and Gachomo, 2006). To prevent cellular damages caused by excessive ROS, plants rely on antioxidant enzymes such as SOD and APX to regulate their levels (Shigeoka et al., 2002). Increased expression of isoforms of SOD, as revealed by gel electrophoresis, was noticed in the present work (Fig. 5). SOD converts superoxide radicals into less harmful H_2O_2 protecting the plant cells from the oxidative damage induced by biotic stresses. Conversely the APX activity was consistently lower compared with control plants (Fig. 6a) indicating that BTH-treatment down-regulated this enzyme, particularly at 0.5 and 1.0 mM concentrations. Inhibitory effect of BTH on both antioxidant enzymes APX and catalase (CAT) is a well documented effect (Wendehenne et al., 1998). These results suggest that BTH might indirectly contribute to H_2O_2 accumulation in plant as it inhibits the metabolic degradation of H_2O_2 by APX and CAT. In this present study, the BTH-induction of SOD as well as its inhibitory effect upon APX might have contributed to H_2O_2 accumulation. H_2O_2 participates in the plant defence strategies as its accumulation could directly kill the invading pathogen (Hunt et al., 1996), commence HR and programmed cell death (Hancock et al., 2002; De Pinto et al., 2006) and trigger the increase of gene defence expression (Borden and Higgins, 2002; Mlickova et al., 2004).

Figure. 6d shows that BTH was able to significantly induce βGLU activity from day 8 after spraying, particularly at 0.5 and 1.0 mM concentrations, compared with controls. Other works have demonstrated this same capacity of BTH in inducing βGLU expression (Bokshi et al., 2003; Buzi et al., 2004). Participation of

this enzyme in the plant defence mechanism is associated with its ability to degrade pathogenic fungus cell wall. Moreover, the products of the βGLU hydrolytic activity can function as elicitors of defence response (Rivera et al., 2002). Generally, the content of this enzyme is low in healthy plants. However, when plants are infected by pathogens or treated with elicitors of defence mechanisms, the βGLU activity increases quickly. In melon plants as βGLU activity increases this implies that SAR has been induced, as PR-protein expression is taken as a marker of active defence (Bargabus et al., 2004).

In conclusion the present study showed that BTH did not act as a protection agent of melon fruits against the fruit rot caused by *F. pallidoroseum*. However, as the preflowering and single application of BTH modified the activities of the defence-related enzymes SOD, APX, POX and β-GLU in melon seedlings, this strategy could probably be effective for the control of the postharvest rot of melon fruits through activation of systemic resistance. For instance, similar treatment significantly reduced the incidence of the postharvest disease caused by *Alternaria* sp. and *Fusarium* sp. in postharvest melons (Huang et al., 2000).

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References

Bargabus RL, Zidack NK, Sherwood JE, Jacobsen BJ. (2004) Screening for the identification of potential biological control agents that induce systemic acquired resistance in sugar beet. *Biol Control* **30**:342–350.

Barreto ALH, Vasconcelos IM, Grangeiro TB et al. (2007) Infection process and host defense responses in compatible and incompatible interactions between cowpea (*Vigna unguiculata*) and *Colletotrichum gloeosporioides*. *Int J Plant Sci* **168**:193–203.

Baysal O, Turgut C, Mao G. (2005) Acibenzolar-S-methyl induced resistance to *Phytophthora capsici* in pepper leaves. *Biol Plant* **49**:599–604.

Beauchamp C, Fridovich I. (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* **44**:276–287.

Bokshi AI, Morris S, Deverall B. (2003) Effects of benzothiadiazole and acetylsalicylic acid on β-1,3-glucanase activity and disease resistance in potato. *Plant Pathol* **52**:22–27.

Boller T. Biochemical analysis of chitinases and β-1,3-glucanases. In: Gurr SJ, McPherson MJ, Bowles DJ (eds), *Molecular Plant Pathology*, New York, NY, USA, IRL Press, 1993, pp. 23–29.

Borden S, Higgins VJ. (2002) Hydrogen peroxide plays a critical role in the defence response of tomato to *Cladosporium fulvum*. *Physiol Mol Plant Pathol* **61**:227–236.

Bradford MM. (1976) A rapid and sensitive method for quantification of micrograms quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248–254.

Buzi A, Chilosi G, De Sillo D, Magro P. (2004) Induction of resistance in melon to *Didymella bryoniae* and *Sclerotinia sclerotiorum* by seed treatments with acibenzolar-S-methyl and methyl jasmonate but not with salicylic acid. *J Phytopathol* **152**:34–42.

Chen JY, Wen PF, Kong WF et al. (2005) Effect of salicylic acid on phenylpropanoids and phenylalanine ammonia-lyase in harvested grape berries. *Postharvest Biol Technol* **40**:64–72.

Darras AI, Joyce DC, Terry LA. (2006) Acibenzolar-S-methyl and methyl jasmonate treatments of glasshouse-grown freesias suppress post-harvest petal specking caused by *Botrytis cinerea*. *J Hortic Sci Biotechnol* **81**:1043–1051.

Darras AI, Joyce DC, Terry LA, Pompodakis NE, Dimitriadis CI. (2007) Efficacy of postharvest treatments with acibenzolar-S-methyl and methyl jasmonate against *Botrytis cinerea* infecting cut *Freesia hybrida* L. flowers. *Australas Plant Pathol* **36**:332–340.

De Pinto MC, Paradiso A, Leonetti P, De Gara L. (2006) Hydrogen peroxide, nitric oxide and cytosolic ascorbate peroxidase at the crossroad between defence and cell death. *Plant J* **48**:784–795.

Dinh SQ, Joyce DC, Irving DE, Wearing AH. (2007) Field application of three different classes of known host plant defence elicitors did not suppress infection of Geraldton waflower by *Botrytis cinerea*. *Australas Plant Pathol* **36**:142–148.

El-Shora HM. (2002) Properties of phenylalanine ammonia-lyase from Marrow cotyledons. *Plant Sci* **162**:1–7.

Fournand D, Cathala B, Lapierre C. (2003) Initial steps of the peroxidase-catalyzed polymerization of coniferyl alcohol and/or sinapyl aldehyde: capillary zone electrophoresis study of pH effects. *Phytochemistry* **62**:139–146.

Gadelha JC. (2002) *Controle preventivo e curativo da podridão pós-colheita de frutos de melão com produto alternativo*. Master Thesis. Ceará, Brazil, Federal University of Ceará, pp. 37.

Hammond-Kosack KE, Jones JDG. (1996) Resistance gene-dependent plant defense responses. *Plant Cell* **8**:1773–1791.

Hancock JT, Desikan R, Clarke A, Hurst RD, Neill SJ. (2002) Cell signaling following plant/pathogen interactions involves the generation of reactive oxygen and reactive nitrogen species. *Plant Physiol Biochem* **40**:611–617.

Hoagland DR, Arnon DI. *The Water Culture Method for Growing Plants Without Soil*, Berkeley, CA, USA, California Agriculture Experimental Station, 1950.

Huang Y, Deverall BJ, Tang WH, Wang W, Wu FW. (2000) Foliar application of acibenzolar-S-methyl and protection of postharvest rock melons and Hami melons from disease. *Eur J Plant Pathol* **106**:651–656.

Hunt MD, Neuenschwander UH, Delaney TP et al. (1996) Recent advances in systemic acquired resistance research – a review. *Gene* **179**:89–95.

Iriti M, Faoro F. (2003) Benzothiadiazole (BTH) induces cell-death independent resistance in *Phaseolus vulgaris* against *Uromyces appendiculatus*. *J Phytopathol* **151**:171–180.

Janisiewicz MJ, Korsten L. (2002) Biological control of postharvest diseases of fruits. *Annu Rev Phytopathol* **40**:411–441.

Kotchoni SO, Gachomo EW. (2006) The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. *J Biosci* **31**:389–404.

Liu H, Jiang W, Bi Y, Luo Y. (2005) Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by Penicillium expansum and enhances activity of fruit defense mechanisms. *Postharvest Biol Technol* **35**:263–269.

Malolepsza U. (2006) Induction of disease resistance by acibenzolar-S-methyl and o-hydroxyethylorutin against *Botrytis cinerea* in tomato plants. *Crop Prot* **25**:956–962.

Martinez C, Baccou JC, Bresson E et al. (2000) Salicylic acid mediated by the oxidative burst is a key molecule in local and systemic responses of cotton challenged by an avirulent race of *Xanthomonas campestris* pv *malvacerum*. *Plant Physiol* **122**:757–766.

Mlickova K, Luhova L, Lebeda A, Mieslerova B, Pec P. (2004) Reactive oxygen species generation and peroxidase activity during O*idium neolycopersici* infection on *Lycopersicon* species. *Plant Physiol Biochem* **42**:753–761.

Mori T, Sakurai M, Sakuta M. (2001) Effects of conditioned medium on activities of PAL, CHS, DAHP syntase (DS-Co and Ds-Mn) and anthocyanin production in suspension cultures of *Fragaria ananassa*. *Plant Sci* **160**:355–360.

Passardi F, Tognolli M, De Meyer M, Penel C, Dunand C. (2006) Two cell wall associated peroxidases from *Arabidopsis influence* root elongation. *Planta* **223**:965–974.

Peixoto PHP, Cambraia J, Sant'anna R, Mosquim PR, Moreira MA. (1999) Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. *Rev Bras Fisiol Veg* **11**:137–143.

Rivera ME, Conida JC, Olea F, Vicente A, Pérez-Garcia A. (2002) Differential expression of β-1,3-glucanase in susceptible and resistant melon cultivars in response to infection by *Shaerotheca fusca*. *Physiol Mol Plant Pathol* **61**:257–265.

Shigeoka S, Ishikawa T, Tamoi M et al. (2002) Regulation and function of ascorbate peroxidase isoenzymes. *J Exp Bot* **53**:1305–1319.

Silva MC, Nicole M, Guerra-Guimarães L, Rodrigues CJ. (2002) Hypersensitive cell death and post-haustorial defence responses arrest the orange rust (*Hemileia vastatrix*) growth in resistant coffee leaves. *Physiol Mol Plant Pathol* **60**:169–183.

Silveira JAG, Costa RCL, Oliveira JTA. (2001) Drought-induced effects and recovery of nitrate assimilation and nodule activity in cowpea plants inoculated with *Bradyrhizobium* spp. under moderate nitrate level. *Braz J Microbiol* **32**:187–194.

Smith-Becker J, Keena NT, Becker JO. (2003) Acibenzolar-S-methyl induces resistance to *Colletotrichum lagenarium* and cucumber mosaic virus in cantaloupe. *Crop Prot* **22**:769–774.

Soylu S, Baysal O, Soylu EM. (2003) Induction of disease resistance by the plant activator, acibenzolar-S-methyl (ASM), against bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) in tomato seedlings. *Plant Sci* **165**:1069–1075.

Terry LA, Joyce DC. (2000) Suppression of grey mould on strawberry fruit with the chemical plant activator acibenzolar. *Pest Manag Sci* **56**:989–992.

Terry LA, Joyce DC. (2004a) Influence of growing conditions on efficacy of acibenzolar and botryticides in suppression of *Botrytis cinerea* on strawberry fruit. *Adv Strawb Res* **23**:11–19.

Terry LA, Joyce DC. (2004b) Elicitors of induced disease resistance in postharvest horticultural crops: a brief review. *Postharvest Biol Technol* **33**:1–13.

Urbanek H, Kuzniak-Gebarowska E, Herka K. (1991) Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturonase. *Acta Physiol Plant* **13**:43–50.

Vanacker H, Carver TLW, Foyer CH. (2000) Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hypersensitive response in the barley-powdery mildew interaction. *Plant Physiol* **123**:1289–1300.

Wen PF, Chen JY, Kong WF, Pan QH, Wan SB, Huang WD. (2005) Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. *Plant Sci* **169**:928–934.

Wendehenne D, Durner J, Chen Z, Klessig DF. (1998) Benzothiadiazole, an inducer of plant defenses, inhibits catalase and ascorbate peroxidase. *Phytochemistry* **47**:651–657.

Yao H, Tian S. (2005) Effects of pre- and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. *Postharvest Biol Technol* **35**:253–262.

Zhao H, Wang BC, Zhao HC, Wang JB. (2005) Stress stimulus induced resistance to *Cladosporium cucumerinum* in cucumber seeding. *Colloids Surf B: Biointerfaces* **44**:36–40.