Effect of benzo-thiadiazole-7-carbothioic acid S-methyl ester (BTH) treatment on the resistant substance in postharvest mango fruits of different varieties

Yong-Gui Pan*1,2 and Xin-Hua Liu1

1College of Food, Hainan University, Haikou 570228, P. R. China.
2South Subtropical Crops Research Institute, Chinese Academy of Tropical Agricultural Sciences, Zhanjiang, Guangdong 524091, P. R. China.

Accepted 19 October, 2011

Nang klangwan and Tainong mango fruits were treated with benzo-thiadiazole-7-carbothioic acid S-methyl ester (BTH). The effect of BTH treatment on the resistant substance in postharvest fruits was evaluated to explore the enhanced disease-resistance mechanisms of mango fruits by BTH from different varieties. Results indicate that the main resistant substance such as total phenolic, flavonoid, lignin and hydroxyproline-rich glycoprotein (HRGP) content were obviously increased by BTH treatment in different varieties. However, the increase in resistant substances of Tainong mango fruit was higher than that in Nang klangwan fruit. At the same time, Tainong fruit without BTH treatment had relatively higher level of these resistant substances than Nang klangwan mango fruit, especially in the contents of phenolic compounds and lignin.

Key words: Mango, total phenolic, flavonoid, lignin, hydroxyproline-rich glycoprotein (HRGP).

INTRODUCTION

Postharvest decay in fruits and vegetables is a serious problem in the world. Chemical fungicides are the major weapon against postharvest diseases. However, fungicides are becoming less effective because of the development of pathogen resistance, along with consumer concerns about possible risks associated with the use of chemicals (Wilson et al., 1994). A number of new strategies are being investigated to control postharvest decay without the pollution of the environment and risk to public health. As such, induced resistance in harvested crops is promising (Wilson et al., 1994; Terry and Joyce, 2004). This phenomenon is also known as systemic acquired resistance (SAR). SAR can be activated by exogenous treatments with chemical inducers such as benzo-thiadiazole-7-carbothioic acid S-methyl ester (BTH) (Gorlach et al., 1996).

Most studies have shown the effectiveness of BTH in protecting different plant species against diseases caused by viral, bacterial and fungal pathogens (Gorlach et al., 1996; Benhanou and Belanger, 1998; Cole, 1999). Most studies have used vegetative tissues as materials to elucidate how BTH may affect disease resistance, and little work has been done in horticultural products such as fruit and vegetables (Liu et al., 2005). But some researches have shown that BTH is also effective to induce resistance in some fruits and vegetables such as beet (Burketová et al., 2003), tomato (Anfoka et al., 2000), cucumber (Li et al., 2005), peach (Liu et al., 2005), banana (Wang, 2005; Zhu et al., 2007), strawberry (Terry et al., 2000) loquat (Zhang et al., 2009) and so on. In the previous study, we found that BTH treatment can also reduce the incidence of postharvest mango fruit anthracnose (Liu et al., 2009) but the effective concentration of BTH is different according to the mango varieties. The most effective concentration of BTH treatment...
was 200 and 50 mg/L for the Nang klangwan fruit and Tainong fruit, respectively (Liu et al., 2009).

Although BTH is highly effective in inducing enhanced disease resistance, its mechanism of action and cellular targets are less known (Liu et al., 2005), especially in fruits and vegetables. In addition, many studies have focused on the effect of BTH treatment on the defense-related enzymes peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), β-1,3-glucanase, and chitinase. However, researches about the effect of BTH treatment on the resistant substance are generally lacking. In addition, are there some correlations between different concentration of BTH, different mango varieties and resistance substance? Therefore, Nang klangwan and Tainong mango fruits were used as the experimental fruits to study the effects of BTH on the resistance substance in different mango varieties.

MATERIALS AND METHODS

Plant material

Nang klangwan and Tainong mango fruits were harvested from a commercial orchard in the Guangba farm, Dongfeng city, Hainan Province, China, at the earliest stage of commercial ripening (green mature). Fruit were transported to the laboratory, selected for uniformity of size, ripeness and absence of defects.

BTH treatment

BTH (50% wettable granule formulation, Bion®, Novartis Ltd., Basel, Switzerland) solution was prepared with sterile deionized water plus 0.05% Tween 80.

Nang klangwan and Tainong mango fruits were soaked in 200 and 50 mg/L BTH solution for 10 min, respectively. The control fruit including Nang klangwan and Tainong mango fruits were soaked in sterile deionized water plus 0.05% Tween 80 for 10 min. After the treatment, both BTH-treated and control fruit were kept in trays covered with plastic film and incubated at 15°C, and 75 to 85% RH. Each treatment had 60 fruit with three replicates.

Inoculation and infection

Colletotrichum gloeosporioides were obtained from the Environment and Plant Protection Institute Chinese Academy of Tropical Agricultural Sciences, Hainan Province, China, and then cocultured on potato dextrose agar (PDA) for eight days; the cultural temperature was 28°C.

The inoculations were carried out according to Liu et al. (2005). 72 h after the BTH treatment, both the BTH-treated and control fruit were sterilized with 70% ethanol, and then wounded with a syringe at three points (1 mm deep × 3 mm wide) on the equator of each fruit. Thereafter, agar disk containing C. gloeosporioides (φ1.5 mm) was stuck to the wounded site following 24 h moisturization with cotton, and the fruit were incubated at 15°C, and 75 to 85% RH.

Disease incidence (the percentage of fruit with visible disease development) and lesion diameter on each fruit were recorded daily. When the visible rot zone beyond the wounded area on each fruit was more than 1 mm wide, it was counted as an affected fruit. Tissue samples outside the wounded and infected pulp were collected on the days as indicated in the results and stored at -20°C.

Measurements of phenolic compounds, flavonoid, lignine and hydroxyproline-rich glycoprotein (HRGP)

Phenolic compounds and flavonoid were measured according to the methods of Pirie and Mullins (1976). 1 g of frozen tissue was homogenized with 5 ml ice-cold 1% HCl–methanol solution and then centrifuged at room temperature for 2 h, and then filtered with filter paper. The filtrate was diluted 10 times with 1% HCl–methanol solution, and absorbance was measured at 280 nm and 325 nm using 1% HCl–methanol solution as a reference. The content of phenolic compounds was calculated with the standard curve obtained on the basis of content of gallic acid expressed as μg/g FW. Flavonoid content was expressed as OD₅₂₅ nm/g FW.

Lignin content was determined according to Morrison (1972). 1 g of frozen tissue was homogenized with 10 ml 95% alcohol solution and then centrifuged at 4°C for 10 min at 12,000× g. Extraction was repeated twice, but the second was extracted with alcohol; n-hexane = 1:2 (V/V), precipitation was collected, then heated at 60°C for 2 h, and then dissolved in 1 ml 25% brominated acetyl glutamic acid solution, and put in a 70°C water bath for 30 min. Furthermore, 1 ml of 2 mol/L NaOH was added to stop the reaction. 2 ml glacial acetic acid and 0.4 ml of 2 mol/L oxammonium hydrochloride were added and then centrifuged at 4°C for 10 min at 12,000× g. 5 ml of glacial acetic acid was put in 0.1 ml supernatant, and the optical density at 280 nm was measured. The contents of lignin content were calculated according to calibration curves of sinapyl alcohol, and expressed as μg/g FW.

HRGP content is positively related to hydroxyproline content; thus, the Hyp content of the cell wall is to represent the relative content of the HRGP.

1 g of frozen tissue was homogenized with 5 ml of 0.1 mol/L phosphate buffer (pH 7.2) solution and then centrifuged at 4°C for 10 min at 12,000× g. Precipitation was centrifuged, cleaned three times in the same buffer, one time with TritonX-100 (0.5%), two times with water, one time with 1 mol/L NaCl, following three times with water, and finally washed one time with acetone. The residue was dried and the cell wall product was obtained (Hu et al., 1999).

The hydroxyproline content was then assayed according to Kivirikko et al. (1967). 20 mg cell wall product and 5 ml of 6 mol/L HCl were placed in a screw-capped tube and the tube was heated at 110°C for 18 h. After the hydrolysis, 2 ml of the hydrolyzed supernatant solution was placed in another screw-capped tube and the sample was adjusted to pH 7.0. The sample was then oxidized by the addition of exactly 1 ml of 0.8 mol/L boric acid and 1.0 ml of the 0.2 mol/L chloramine T solution, and the sample was well mixed immediately. The tube was allowed to stand at room temperature for 25 min with occasional mixing, and 2.0 ml of 3 mol/L sodium thiosulfate was added. 2.5 g KCl was added in order to allow complete saturation. 30 min. Furthermore, 3 ml of toluene was added to the tube, the tube was then shaken for 5 min and allow to stand in order to separate the toluene layer. The toluene layer was removed with suction. The tube was tightly capped and placed in a briskly boiling water bath for 30 min, then cooled with running tap water, and exactly 3.5 ml of toluene was added. After enough immingle and extraction, exactly 2 ml of the toluene extract was placed in a clean test tube and 1.0 ml of the Ehrlich’s reagent was added, and rapidly mixed with the sample. After 20 min of standing at room temperature, the absorbance at 560 nm was determined.

Statistical analysis

All statistical analyses were performed with SPSS 10.0. Data were
analyzed by one-way analysis of variance (ANOVA). Mean separations were performed using the least significant difference method (LSD test). Each experiment had three replicates and all experiments were run three times with similar results. Measurements from all the replicates were combined and treatment effects were analyzed.

RESULTS

Effect of BTH treatment on phenolic content

Phenolic compounds are inherent constituent of many plants themselves and are also the plant secondary metabolites. They play an important role in plants resistant mechanism. Phenolic content of *Nang klangwan* and *Tainong* mango fruits decreased at first and then increased after *C. gloeosporioides* was inoculated (Figure 1). The phenols content that was decreased could be due to the improvement of polyphenol oxidase activity in fruits which catalyzed polyphenols oxidization to quinonoids, etc (antifungal substances) to resist further expansion of the pathogen. Along with the enhancement of the secondary metabolism, the phenols content continuously increased. Changes in phenol of fruits treated with BTH

Figure 1. Effects on phenolic compounds contents in (a) “Nang klangwan” mango and (b) “Tainong” mango by BTH treatment during damnifying inoculation.
Figure 2. Effects on flavanoid contents in (a) "Nang klangwan" and (b) "Tainong" by BTH treatment during damaging inoculation.

had similar trend as that of the control. However, phenol content of BTH treated fruits was higher and phenol content of Tainong mango fruits increased more significantly especially in the early time of storage. The results show that BTH treatment promoted the accumulation of total phenol content in fruits. Meanwhile, it could be seen that phenol content of Tainong mango fruits was higher than Nang klangwan fruits without BTH treatment. It may be one of the reasons why Tainong mango fruits had lower incidence than Nang klangwan.

Effect of BTH treatment on flavonoids content

Many studies indicate that host cell has plenty of flavonoids accumulation after the invasion of pathogens, so the flavonoids could be used as a biochemical marker of resistance to pathogen (Yu et al., 2001). As shown in Figure 2, the flavonoids content except ascending and decrease later during storage for Nang klangwan and
Tainong mango fruits. BTH treatment enhanced the levels of flavonoids in the fruits. Flavonoids accumulation speed in Tainong mango fruits was obviously higher than those in Nang klangwan mango fruits. Also, the peak values appearance of flavonoids in Nang klangwan mango fruits was earlier than that in Tainong mango fruits. This was probably because the fruits were earlier attacked by pathogen and the induced flavonoids were quickly synthesized to prevent the expansion of pathogen. Flavonoids decrease was depleted to prevent the expansion of pathogen during storage.

**Effect of BTH treatment on lignin content**

Lignin is the cross-linking molecules of many phenylpropane which together always combine with other saccharides on cellular wall. Lignin is easily deposited in the cell walls to form cork which can stop pathogen infection and spread (Yang et al., 2003). The lignin content in both control and BTH-treated fruit showed a decrease trend. However, the contents of lignin in Tainong fruit were obviously higher (about 2 times) than that in Nang klangwan mango fruit (Figure 3), which was helpful to strengthen the disease resistance of Tainong fruit. The lignin content of fruits treated with BTH maintained higher level throughout the storage especially in Tainong fruit. It proved that BTH treatment had more evident effect to induce lignin synthesis in Tainong fruit.

**Effect of BTH treatment on HRGP content**

HRGP is the structural proteins in plants which may play certain roles on resistant diseases. The resistance mechanism of HRGP is due to the effect of clusterin. HRGP could combine with pathogenic bacteria, and have effect of barrier on cell wall. So it can prevent pathogenic bacteria from invading and spreading between cells. As shown in Figure 4, HRGP content increased both in fruits inoculated with P. expansum of the control fruit and of BTH-treated fruit during storage whereas, the HRGP content increased rapidly during the latter storage period (Figure 4a). Treatment with BTH significantly increased the HRGP content either in Nang klangwan mango fruit and Tainong fruit. It indicates that BTH treatment is helpful in increasing the accumulation of HRGP and decreasing the disease.

**DISCUSSION**

Phenol compounds are not only toxic to pathogen but also can be oxidized to more harmful quinonoids in vivo. Thus, they play an important role in plants defense response. In addition, phenols compounds are the precursor of the disease-resistant material such as lignin and phytoalexin. In general, lots of phenolics were rapidly synthesized and lignified (Yuan et al., 1995). Flavonoids are the most important class of phenolic substances which have very strong antibacterial action in plant and pathogen interactions. Flavonoids can be used as a kind of self-defense substances that can provide protein to reduce or remove excess free radicals produced from stress condition (Wang, 2007).

One defense mechanism against the pathogen infection in plants is through the synthesis of lignin, leading to tissue lignification. Lignin accumulation in host plants is a biological active response to pathogens in a number of plant–pathogen interactions. It is a common phenomenon that lignin content increases in plants after infection by pathogens. Hydroxy-proline-rich glycoprotein is the main structure protein in plants cell wall. Some researches showed that HRGP in plants can be induced and accumulated because of pathogen infection, and plays some part in plant resistance. It is considered that HRGP is related with plant resistance mechanism (Fan et al., 2005; Cheng et al., 2006). It was also found that HRGP and lignin content increased significantly in muskmelons by preharvest BTH spraying, and enhanced the disease resistance of fruits (Zhang et al., 2006). This was also confirmed in muskmelon cultivar Yindi (Li et al., 2005).

Our results indicate that BTH significantly increased the total phenolic, flavonoid, lignin and HRGP content of mango fruits. Therefore, it enhanced the fruit resistance and reduced the incidence of a disease and the expansion of disease spot in fruits. The same results were obtained for peach (Liu, 2004), Pear (Cao, 2005) and banana (Ma et al., 2006) fruits treated with BTH. These results indicate that the antibacterial substance played a very important role in the induction resistance of fruits. Our results also show that accumulation of total phenolic, flavonoid and lignin in Nang klangwan mango fruit were much slower than that in Tainong fruit. The contents of total phenolic, flavonoid and lignin in Tainong fruit were significantly higher than those in Nang klangwan mango fruit both in the control and treated fruits. Thus, Tainong fruit had much more antibacterial substance than Nang klangwan fruit. Tainong fruit had stronger resistance during storage. At the same time, this might be the reason why lower BTH concentrations can induce the efficient disease-resistant of Tainong fruits.

**Conclusion**

BTH treatment increased the content of the main resistant substances such as total phenolic, flavonoid, lignin and HRGP both in Nang klangwan and Tainong mango fruit. However, the increase in resistant substance contents of Tainong mango fruit was higher than that of Nang klangwan fruit. At the same time, these resistant substances especially phenolic compounds and lignin of
Figure 3. Effects on lignin contents in (a) "Nang klangwan" mango and (b) "Tainong" mango by BTH treatment during damaging inoculation.

*Tainong* fruit without BTH treatment were relatively higher than that of *Nang klangwan* mango fruit. It indicates that, *Tainong* fruits had stronger resistance to disease than *Nang klangwan* fruits.
Figure 4. Effects on hydroxyl-proline-rich glycoprotein (HRGP) content in (a) "Nang klangwari" mango and (b) "Tainong" mango by BTH treatment during damnifying inoculation.

Acknowledgment

This work is supported by the Natural Science Foundation of Hainan Province (Project No. 310032) and Higher School Science Research Aid Project of Hainan Province (Project No. Hjkj2010-07).

REFERENCES


Prunus Persica u W, Wen J (1999). The relation between the accumulation of
Cao JK (2005). Effects of SA, ASM, INA and citric acid on postharvest
disease resistance and quality of Yali Pear fruit. Beijing: China Agric. 
Univ. Ph.D.Dissertation.
between BTH-induced resistance to downy mildew in cucumber leaves 
and benzothiadiazole treated sugar beet roots. Physiol. Mol. Plant 
Pathol. 63: 47-54.
Cao JK (2005). Effects of SA, ASM, INA and citric acid on postharvest 
disease resistance and quality of Yali Pear fruit. Beijing: China Agric. 
Univ. Ph.D.Dissertation.
localization of chitinase and β-1,3-glucanase in rhizomania diseased 
and benzothiadiazole treated sugar beet roots. Physiol. Mol. Plant 
Pathol. 63: 47-54.
Cole DL (1999). The efficacy of acibenzolar-S-methyl, an inducer of 
systemic acquired resistance, against bacterial and fungal diseases of 
researches on induced resistance of plant activator. J. Plant Prot. 
32(1): 87-92.
(1996). Benzoïothiadiazole, a novel class of inducers of systemic 
acquired resistance, activates gene expression and disease 
Hu J, Zhu W, Wen J (1999). The relation between the accumulation of 
hrpg and lignin in cell wall of poplars and the resistance to poplar 
Krivriķi Ko, Laitinen O, Pracckop DJ (1967). Modifications of a specific 
Krivriķi Ko, Laitinen O, Pracckop DJ (1967). Modifications of a specific 
induces resistance of peach (Prunus persica L cv. Jiubao) fruit to 
infection by Penicillium expansum and enhances activity of fruit 
Liu HY (2004). Effects of 1-MC, BTH and PHC on postharvest 
benzothiadiazole and methyl jasmonate induced resistance of 
treatment on latent infection of muskmelon cultivar Yind. J. Gansu 
Liu XH, Pan YG, Zu H, Yang DH, Zhu F (2009). The optimum 
concentration of BTH treatment for different varieties of postharvest 
Mango fruit against anthracnose. J. Southwest Univ (Natural Sci 
Li YH, Cheng ZH, Chen XG (2005). Effect of several chemicals on 
induction resistance to downy mildew in cucumber seedlings. 
benzothiadiazole and methyl jasmonate in relation to activities of 
Morrison IM (1972). A semi-micro method for the determination of lignin 
and its use in predicting the digestibility of forage crops. J. Sci. Food 
Agric. 23(4):455-463.
Piun A, Mullins MG (1976). Changes in anthocyanin and phenolic 
content of grapevine leaf and fruit tissue treated with sucrose,nitrate 
Terry LA, Joyce DC (2000). Suppression of grey mould on strawberry 
with the chemical plant activator acibenzolar. Pest Management Sci. 
Terry LA, Joyce DC (2004). Elicitors of induced disease resistance in 
Wang JH (2005). Effects of BTH on the control of anthracnose of 
harvested banana fruit and the mechanisms of systemic acquired 
resistance (SAR). Guangzhou: South China Agric. Univ.
Wang WY (2007). Studies on the salicyli-acid-induced effect of disease-resistance to ralstonia solanacearum and physiological and 
resistance to control postharvest diseases of fruits and vegetables. 
Plant Dis. 78: 837-842.
Yang YF, Liang YC, Lou YS, Yao QJ (2003). Influences of silicon on 
peroxidase, superoxide dismutase activity and lignin content in 
leaves of wheat (Triticum aestivum L.) and its relation to resistance to 
Yu Q, Liu Y (2001). Study on efficacy and mechanism of tobacco anti-
virus agent applied in the field. J. Yurman Agric. Univ. 16(1):9-12.
Yuan ZH, Qu JM (1995). A preleminary study on the biochemical 
game of glandless cotton resistance to fusarium wilt. Acta 
Gossypii. Sin.7(2):100-104.
enzymes and disease resistance substances induction in 
muskmelons by preharvest benzothiazole(BTH) spraying. J. Gansu 
Zhang ZW, Zhu SJ (2009). Effects of benzothiadiazole on diseases and 
Zhu S, Ma B (2007). Benzothiadiazole-or methyl jasmonate induced 
resistance to Colletotrichum musae in harvested banana fruit is 
related to elevated defense enzyme activities. J. Hort. Sci. 
Biotecnol. 82: 500-506.