Journal of Food Safety

Journal of Food Safety ISSN 1745-4565

DEFENSE RESPONSES OF SALICYLIC ACID IN MANGO FRUIT AGAINST POSTHARVEST ANTHRACNOSE, CAUSED BY *COLLETOTRICHUM GLOEOSPORIOIDES* AND ITS POSSIBLE MECHANISM

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Received for Publication January 11, 2016
Accepted for Publication May 2, 2016

doi: 10.1111/jfs.12294

ABSTRACT

The effects and mechanisms of salicylic acid (SA) on defense response to *Colletotrichum gloeosporioides* from mango fruits were investigated by *in vitro* and *in vivo* test. *In vitro* experiment results showed that SA significantly reduced mycelial growth of *C. gloeosporioides* in a concentration-dependent manner. SA effectively controlled anthracnose decay on inoculated mango fruit, as well as natural infection. Disease incidence and lesion diameter in SA-treated fruit were significantly lower than those of the control fruit. SA treatment increased the activities of chitinase, β-1,3-glucanase, phenylalanine ammonia-lyase and polyphenoloxidase, and the content of total phenolic compounds and lignin in mango fruit. Moreover, SA treatment effectively maintained fruit firmness by suppressing conversion of insoluble protopectin into water soluble pectin. And correlation analysis showed there is a higher negative correlation between fruit firmness and disease incidence. These findings suggest that the effect of SA on postharvest diseases was attributed to its direct antimicrobial activity and the elicitation of resistant responses, as well maintaining the firmness in mango fruit. Therefore, SA treatment is a promising measure for controlling postharvest anthracnose rot in mango.

PRACTICAL APPLICATIONS

Induction of fruit resistance against pathogenic infection with biological or chemical elicitors has been thought to be a promising approach for controlling of postharvest diseases and reducing the use of synthetic fungicides. Anthracnose, caused by *Colletotrichum gloeosporioides* is the predominant postharvest disease in mango production that causes severe postharvest losses and fruit quality deterioration. SA is a natural plant substance involved in plant defense responses to biotic stresses. The present results from *in vitro* and *in vivo* experiment suggested that SA treatment could effectively inhibit mycelial growth of *C. gloeosporioides*, enhance resistance of mango fruit against the pathogen and reduce anthracnose rot, as well maintain fruit firmness. Hence, SA can be applied in mango storage and preservation.

INTRODUCTION

Anthracnose, caused by *Colletotrichum gloeosporioides* is the major postharvest disease in mango, leading to serious quality deterioration and economic losses (Dodd *et al.* 1997; Zhang *et al.* 2013). Although postharvest anthracnose rot could be controlled by synthetic fungicides, application of fungicides is increasingly limited because of the development

of fungicide resistance by pathogens, and public concern about the potential harmful effects of fungicide residues on the environment and human health (Dubey *et al.* 2007; Dessalegn *et al.* 2013). There is an urgent need for effective and safe non-fungicide means of controlling postharvest diseases with limited side effects.

Induction of fruit resistance against pathogenic infection with biological or chemical elicitors has been thought to be a promising approach for control of postharvest diseases (Cao *et al.* 2013). Salicylic acid (SA) is an endogenous plant substance regulating many processes of plant growth and development. It is also involved in plant defense responses to postharvest fungal pathogens (Giménez *et al.* 2014). Many researchers have reported that exogenous application of SA at nontoxic concentration could enhance disease resistance and decrease spoilage in various fruits such as mangoes (Zeng *et al.* 2006), sweet cherries (Xu and Tian 2008), Chinese winter jujube (Cao *et al.* 2013), peach (Xu *et al.* 2008; Panahirad *et al.* 2012) and citrus fruits (Zhou *et al.* 2014). The mechanisms of disease resistance by SA in postharvest fruit included oxidative burst, production of antimicrobial phytoalexins and synthesis of pathogenesis-related (PR) proteins (Xu and Tian 2008; Asghari and Aghdam 2010; Cao *et al.* 2013; Xue *et al.* 2014). In addition, some *in vitro* tests showed that SA can act directly as an antimicrobial compound by inhibiting conidial germination and mycelial growth of fungi. For example, SA could significantly inhibit the development of *Rhizopus stolonifer* colonies and reduce the mycelial growth and the mycelial growth was completely inhibited by 5 mM SA (Panahirad *et al.* 2012). SA at higher concentration markedly inhibited the spore germinations of *Botrytis cinerea* (Zhang *et al.* 2010a). However, it has not been reported whether SA has a direct inhibitory effect on the pathogen of anthracnose.

Cell wall is the first barrier of pathogens infection, and they may play an important role in maintaining the disease resistance of fruit and preventing decay development (Yuan *et al.* 2013). Fruit softening has been correlated with solubilization and depolimerization of cell wall constituents (Brummell and Harpster 2001). Excessive softening would influence shelf life and infections by postharvest pathogens (Vicente *et al.* 2005). It is reported that SA could retard the fruits firmness loss and decay incidence, such as sugar apple (Mo *et al.* 2008) and peach (Tareen *et al.* 2012). However, the effects of SA on the degradation of cell wall are not well understood yet. And the relationship between these changes during storage period with pathogen resistance in still not clear.

The objectives of this study are to evaluate: (1) the effects of SA on growth of *C. gloeosporioides in vitro* and *in vivo*; (2) the effects of SA treatment on the PR enzymes and antifungal compounds involved in host defense functions; (3) the effects of SA treatment on the natural infections, postharvest fruit firmness and cell wall component.

MATERIALS AND METHODS

Fruit Materials

Mango (*Mangifera indica* L. *cv.* Tainong) fruits at the green-mature stage were harvested from an orchard in Hainan Province and immediately transported to the research laboratory at Guizhou University, China. Fruits of uniform size and free from visual blemishes and diseases were used for the experiments.

Pathogen

Colletotichum gloeosporioides was isolated from naturally infected "Tainong" mango fruit showing typical anthracnose lesions through culture and re-culture of single macroconidia on potato dextrose agar (PDA). After identifying morphologically, physiologically and pathogenically, a single-spore culture was again isolated and maintained on PDA medium at 4°C. Fresh cultures were cultured on PDA at 25 ± 1°C for 2 weeks before use. Fungal spores were obtained by flooding the surface of the culture with sterile distilled water containing 0.05% (v/v) Tween-80. The suspension was filtered through four layers of sterile cheesecloth and adjusted to a concentration of 1×10^6 spores/mL using a hemocytometer.

Effects of SA on Mycelial Growth of *C. gloeosporioides In Vitro*

The effect of SA on the *in vitro* growth of *C. gloeosporioides* was tested following the method described by Zhang *et al.* (2013). Briefly, SA solution or sterile water (control) was mixed with PDA to give a total volume of 15 mL per Petri plate (90 mm diameter). SA concentrations in the PDA were 0 (as control), 0.5, 1, 2 and 5 mM. After the PDA had solidified, a 5-mm plug of 7-d culture of *C. gloeosporioides* was placed in the center of each Petri plate containing PDA and SA. Mycelial growth as expressed by diameter (mm) was recorded after 4 days of incubation at 25 ± 1°C. Each treatment contained 10 replicates, and the experiment was repeated twice.

Efficacy of SA in Inhibiting the Anthracnose Rot of Mango Fruits

Test fruits were disinfected with sodium hypochlorite solution (0.2% of active chlorine) for 3 min, rinsed with distilled water and then air-dried. SA solutions at different concentrations of 0 (as control), 0.5, 1, 2 or 5 mM were prepared with distilled water containing 0.05% (v/v) Tween-80 as surfactant. Twenty-four hours after treatment with SA, a uniform

wound (3 mm deep × 5 mm wide) was made at the equator of each fruit using a sterile nail. Then, 20 μL of spore suspension of *C. gloeosporioides* (1×10^6 spores/mL) was inoculated into the wounded site using a pipette. Inoculated fruits were stored at 25 ± 1°C. Disease incidence was recorded at 4 days after inoculation. When the visible rot zone outside the wounded area on fruit was more than 1 mm wide, it was counted as decayed fruit. Disease incidence was defined as number of decayed fruits/total amount of fruits. Lesion diameter was recorded at 4, 6 and 8 days after inoculation. Three replicates were performed for each treatment, and each replicate contained 20 fruits.

Effects of SA on Antifungal Compounds and Enzyme Activities of Mango Fruits

Fruits Treatment and Sampling. Based on the results of disease incidence and antifungal activity *in vivo* and *in vitro*, the fruit treated with SA at the optimum concentration of 2 mM was used for analysis of enzymes and antifungal compounds. Mango fruits were treated with 0 or 2 mM SA and inoculated with 20 μL of spore suspension of *C. gloeosporioides* (1×10^6 spores/mL) according to the method described above, and then stored at 25 ± 1°C and 90–95% RH. The fruits were sampled at 1, 3, 5 and 7 days after inoculation, and immediately frozen at −80°C.

Total Phenolics Content. Total phenolics content was determined spectrophotometrically using Folin-Ciocalteu reagent following the method of Li *et al.* (2008). The total phenolics content of sample was expressed as μg gallic acid equivalent per g of fresh weight (FW).

Lignin Content. Lignin content was determined according to the method of Zhang *et al.* (2014) with some modifications. Sample tissue (2 g) was homogenized with 2 mL of 95% ethanol. The homogenate was centrifuged at 12,000 × g for 10 min. The resulting cell wall pellet was washed thrice with 95% ethanol and thrice with ethanol: hexane (1:2, v: v). The precipitate was then dried, suspended in 2 mL of 25% bromized acetyl-acetic acid, and incubated at 70°C in water for 30 min. The reaction was terminated with 1 mL of 2 M NaOH, metered with glacial acetic acid to 10 mL and centrifuged at 4,000 × g for 10 min at 4°C. The supernatant was collected, and the absorbance was measured at 280 nm, with lignin content expressed as OD_{280} per g of FW.

Phenylalanine Ammonialyase Activity. Sample tissue (2 g) was homogenized on ice with 3 mL of 100 mM sodium borate buffer, pH8.8, containing 5 mM β-mercaptoethanol, 2 mM ethylene diaminetetraacetic acid (EDTA) and 4% (w/v) polyvinylpyrrolidine. The homogenate was centrifuged at 12,000 × g at 4°C for 30 min. The supernatant was collected for the enzyme assay according to Cao *et al.* (2007). The phenylalanine ammonialyse (PAL) activity was spectrophotometrically determined and expressed as 0.01 increases in absorbance at 290 nm per hour per g of FW.

Polyphenol Oxidase Activity. Polyphenol oxidase (PPO) was extracted using the method of Zhu *et al.* (2010) with some modifications. Sampled tissue (2 g) was homogenized with 2 mL of 0.1 M sodium phosphate buffer (pH5.5), containing 1 mM polyethylene glycol and 1% Triton X-100, then centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was used for enzyme assay. Each 3 mL reaction mix contained 1 mL of 50 mM catechol, 2 mL of 50 mM sodium phosphate buffer (pH5.5) and 100 μL enzyme extract. The increase in absorbance at 420 nm was measured immediately. One unit (U) of PPO activity was defined as 1.0 increase in absorbance at 420 nm per min per g of FW.

Chitinase Activity. Chitinase (CHI) was extracted using the method of Cao *et al.* (2007). Sampled tissue (2 g) was homogenized with precooled 2 mL of 100 mM sodium acetate buffer (pH5.2), containing 1 mM EDTA and 5 mM β-mercaptoethanol. The homogenate was centrifuged at 12,000 × g at 4°C for 30 min and the supernatant was collected as crude extract. CHI activity was expressed as U/g FW, where one unit was defined as 10^{-9} mol N-acetyl-D-glucosamine produced per second per g FW.

β-1,3-Glucanase Activity. β-1,3-glucanase (GLU) was assayed by measuring the amount of reducing sugar released from the substrate by the dinitrosalicylate method of Cao *et al.* (2007). Sampled tissue (2 g) was homogenized with pre-cooled 2 mL of 100 mM sodium acetate buffer (pH5.2), containing 1 mM EDTA, 5 mM β-mercaptoethanol and 0.1% (w/v) L-ascorbic acid. The homogenate was centrifuged at 12,000 × g at 4°C for 30 min and the supernatant was collected as crude extract. The amount of reducing sugars was measured spectrophotometrically at 540 nm. The GLU activity was expressed as U/g FW, where one unit was defined as the reducing sugar equivalent to 10^{-9} mol of glucose produced per second per g FW.

Effects of SA on Disease Index, Fruit Firmness and Pectin Content of Mango Fruits

Fruits Treatment and Sampling. Mango fruits were disinfected with sodium hypochlorite solution (0.2% of active chlorine) for 3 min, rinsed with distilled water and then air-dried. Then, the fruits were treated with 0 or 2 mM SA as described above, but without inoculation pathogens, and then stored at 25 ± 1°C. During storage period, the fruits were sampled at 0, 7, 14 and 21 days, and immediately frozen at −80°C.

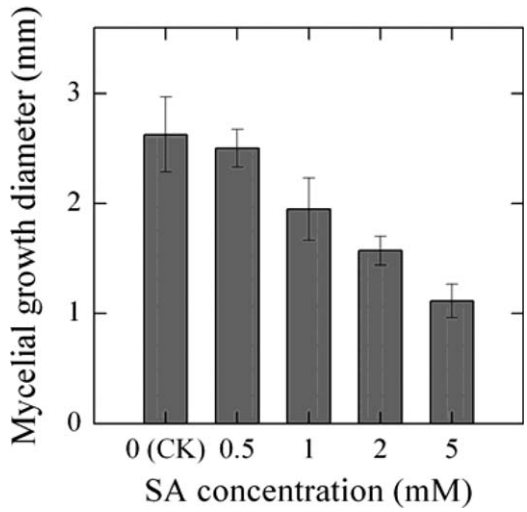

FIG. 1. EFFECT OF DIFFERENT SA CONCENTRATIONS ON MYCELIAL GROWTH OF *C. GLOEOSPORIOIDES IN VITRO* AFTER 4 DAYS OF INCUBATION AT 25 ± 1°C

Disease Index. Natural disease index was evaluated in according to the method of Chen *et al.* (2007) at 0, 7, 14 and 21 days after treatment. Three independent replicates (each replicate contained 30 fruits) were conducted in each treatment. The disease index was expressed with the following equation:

$$\text{Disease index} = \frac{\sum (\text{rank} \times \text{quantity})}{\text{number of total fruits} \times \text{highest disease scale}} \times 100$$

Fruit firmnesss. Fruit firmness was determined at four equatorial regions on the flesh of mango using a hand penetrometer that was fitted with a 10 mm long probe (GY-3, Top Instrument Inc., Hangzhou, China). Three independent replicates (each replicate contained six fruits) were conducted in each treatment. The result was expressed as kg/cm^2.

Water Soluble Pectins and Protopectin. Pectin was extracted and determined as described by Cao *et al.* (2007). Sampled tissue (1 g) was homogenized in 50 mL of 95% ethanol. The homogenate was boiled for 30–40 min and then centrifuged at 8,000 × g for 15 min. The insoluble material was washed two more times with 95% ethanol. The residue dissolved in 40 mL double distilled water and warmer at 50°C for 30 min, and then centrifuged at 8,000 × g for 15 min to obtain the Water Soluble Pectins. The pellet dissolved in 100 mL 0.5 M H_2SO_4 and boiled for 1 h, and then centrifuged at 8,000 × g for 15 min to obtain the protopectin. The uronic acid content was determined according to Cao *et al.* (2007) using galacturonic acid as standard substance. The pectin content was expressed as galacturonic acid equivalents. Results are expressed as grams of galacturonic acid equivalents per 100 g FW.

Statistical analysis

Data were expressed as mean ± SD. One-way ANOVA analysis of variance (statistical analyses) was performed with SPSS 17.0 (SPSS Inc., Chicago, IL). Differences at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of SA on Mycelial Growth of *C. gloeosporioides In Vitro*

In the *in vitro* test, all applied SA concentrations more than 0.5 mM significantly inhibited mycelial growth of *C. gloeosporioides* in a concentration-dependent manner ($P < 0.05$, Fig. 1). Mycelial growth of *C. gloeosporioides* was inhibited by 25.9, 40.3 and 57.6% after 4 days of treatment by 1, 2 and 5 mM SA, respectively. However, 0.5 mM SA had no significant antifungicidal effect on mycelial growth of *C. gloeosporioides* ($P > 0.05$). It has been previously reported that SA has direct inhibitory effect on many plant pathogens at high concentration (Mandal *et al.* 2009; Dessalegn *et al.* 2013). The results confirmed previous findings on antifungal

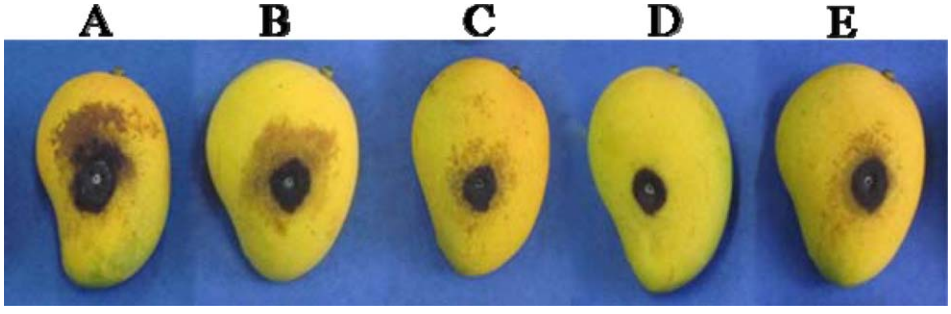

FIG. 2. ANTHRACNOSE SYMPTOMS IN MANGO FRUIT TREATED WITH DIFFERENT CONCENTRATION OF SA, INOCULATED WITH *C. GLOEOSPORIOIDES* AND INCUBATED AT 25 ± 1°C FOR 8 DAYS. A, CK; B, 0.5 mM SA; C, 1 mM SA; D, 2 mM SA; E, 5 mM SA

TABLE 1. EFFECT OF DIFFERENT SA CONCENTRATIONS ON DISEASE INCIDENCE AT 4 DAYS AFTER INOCULATION WITH *C. GLOEOSPORIOIDES* AND INCUBATED AT 25 ± 1°C

	SA concentration (mM)				
	CK	0.5	1	2	5
Disease incidence (%)	55 ± 5.21a	52.6 ± 6.15a	46 ± 5.07ab	41.9 ± 3.09c	45.1 ± 4.41bc

Note: Data are expressed as means ± SD of disease incidence (%) for different concentrations of SA and control. The values with different letters in the same row are significantly different ($P < 0.05$; Least Significant Difference (LSD)).

activity of SA against *Penicillium expansum* (Yu and Zheng 2006), *Alternaria alternate* (Qin *et al.* 2003), *Monilinia fructicola* (Xu *et al.* 2008), *B. cinerea* (Yu *et al.* 2007), *Aspergillus flavus* (Panahirad *et al.* 2014), and *Rhizopus stolonifer* (Panahirad *et al.* 2012).

Effect of SA on the Disease Incidence and Anthracnose Lesion Diameter *In Vivo*

As showed in Tables 1 and 2, all SA concentration decreased the disease incidence and lesion diameter of *C. gloeosporioides*. Among the test concentrations, 2 mM SA showed the strongest inhibitory efficacy on the disease incidence and lesion development of *C. gloeosporioides* ($P < 0.05$), with disease incidence was 23.8% lower than that of control fruits after 4 days of inoculation (Table 1), and the average lesion diameters on days 4, 6 and 8 after inoculation were 10.2, 13.3 and 17.6% lower than that of control, respectively (Table 2 and Fig. 2). Compared with control fruit, the fruit treated with 0.5 mM SA showed no significant difference in disease incidence and lesion diameter during storage period after inoculation ($P > 0.05$). The results of the current study illustrated that SA at optimum concentration has the effective performance for the control of *C. gloeosporioides* growth in mango fruits. Similar result was obtained by Zeng *et al.* (2006) in "Matisu" mango and Panahirad *et al.* (2012) in peach fruits.

Effects of SA Treatment on CHI and GLU Activities in Mango Fruit

Synthesis of PR proteins are considered to be the common defense responses of plants to pathogenic attack (van Loon *et al.* 2006). In the PR proteins, CHI and GLU are the most fully characterized enzymes that are capable of hydrolyzing chitin and β-1,3-glucan in the cell walls of fungi (Xu *et al.* 2008). As shown in Fig. 3a,b, the activities of CHI and GLU increased gradually in both control and SA treated fruit during storage period after inoculation with *C. gloeosporioides*. In SA-treated fruit, the level of both enzyme activities was significantly higher than those in control fruit at the same time except GLU after 3 days of inoculation ($P < 0.05$). After 7 days of inoculation, fruits treated with SA and inoculated with *C. gloeosporioides* showed 2.2 times higher activity of CHI and 1.2 times higher activity of GLU than those in control fruit. Similarly, SA treatment increased the activities of GLU and CHI in citrus fruits against blue and green mold decay caused by *P. italicum* and *P. digitatum* (Zhou *et al.* 2014), jujube fruit against black mold rot caused by *A. alternate* (Cao *et al.* 2013), peach fruit against brown rot caused by *M. fructicola* (Xu *et al.* 2008), sweet cherry against decay caused by *P. expansum* (Xu and Tian 2008).

Effects of SA Treatment on PAL and PPO Activities in Mango Fruit

PAL is the first enzyme of phenylpropanoid pathway, which is directly involved in the synthesis of phenols, phytoalexins and lignin that are associated with the localized resistance processes (Chen *et al.* 2014). PPO is involved in lignification of host plant cells and considered as key enzyme related to defense reaction against pathogen infections (Chen *et al.* 2014). As shown in Fig. 4a,b, activities of PAL and PPO increased in both control and SA-treated fruit during storage period after inoculation, while both enzyme activities in SA-treated fruit were significantly higher than those of control fruit ($P < 0.05$). After 7 days of inoculation, the activities of

TABLE 2. EFFECTS OF DIFFERENT SA CONCENTRATIONS ON LESION DIAMETER OF MANGO FRUIT INOCULATED WITH *C. GLOEOSPORIOIDES* AND INCUBATED AT 25 ± 1°C FOR 8 DAYS

		SA concentration (mM)				
	Days after inoculation	CK	0.5	1	2	5
Lesion diameter (mm)	4	6.97 ± 0.46a	6.76 ± 0.42a	6.56 ± 0.89a	6.25 ± 0.51a	6.49 ± 0.37a
	6	11.32 ± 0.93a	10.69 ± 0.57a	10.11 ± 0.78ab	9.82 ± 0.54b	10.28 ± 0.81ab
	8	16.45 ± 0.90a	15.30 ± 0.84ab	14.24 ± 0.83bc	13.55 ± 0.72c	14.56 ± 0.82bc

Note: Data are expressed as means ± SD of lesion diameter (mm) for different concentrations of SA and control. The values with different letters in the same row are significantly different ($P < 0.05$; LSD).

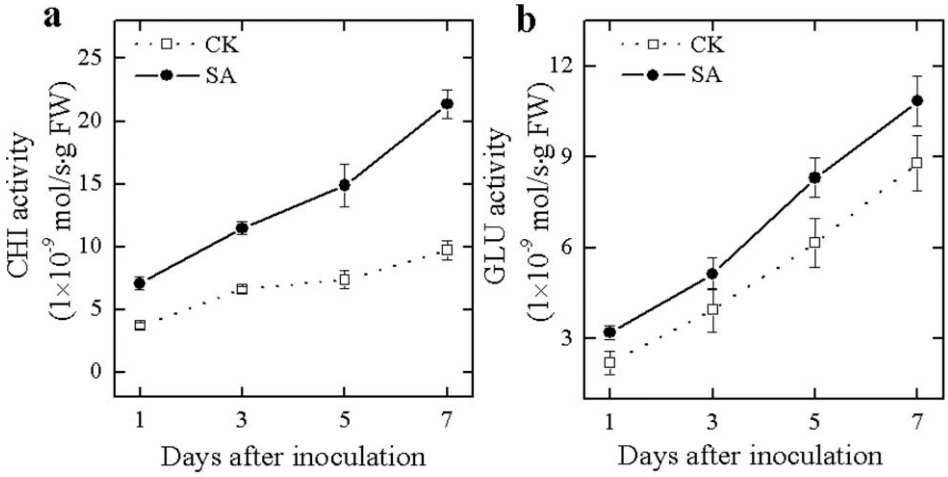

FIG. 3. EFFECTS OF SA TREATMENT ON CHI (A) AND GLU (B) ACTIVITIES IN MANGO FRUITS INOCULATED WITH *C. GLOEOSPORIOIDES* AND INCUBATED AT 25 ± 1°C FOR 7 DAYS

PAL and PPO in the SA-treated fruits were 75.7 and 81.1% higher than those of control fruit, respectively. Similarly, previous studies also confirmed that SA increased activities of PAL and PPO, which were correlated with the onset of resistance induction (Qin *et al.* 2003; Zhang *et al.* 2010b; Cao *et al.* 2013; Ojaghian *et al.* 2013).

Effects of SA Treatment on Content of Antifungal Compounds of Mango Fruit

Presence of lignin and phenolics in plant are recognized as a critical factor that serves as biochemical barrier and antimicrobial substances against pathogen invasion (Wang *et al.* 2014; García *et al.* 2015). In this study, total phenolic content in control fruit after inoculation with *C. gloeosporioides* gradually increased to maximum after 5 days of inoculation, and then slightly decreased (Fig. 5a). SA treatment significantly induced an accumulation of the total phenolics, and the content in SA-treated fruit was averagely 13.2% higher than in control fruit during storage after inoculation.

Lignin content showed a trend of decrease after inoculation in both control and SA treatment (Fig. 5b). Compared with control fruit, SA treatment significantly promoted the increase in lignin content during storage period after inoculation. The lignin content was 22.9% higher in SA-treated fruits than in control fruit after 7 days of inoculation. Previous studies had demonstrated that SA treatment increases the resistance in citrus fruit (Zhou *et al.* 2014) and mango fruit (Zeng *et al.* 2006) against pathogenic attack by inducing the accumulation of large amounts of phenolic compounds. Similarly, enhanced resistance against anthracnose rot caused by *C. gloeosporioides* in relation to promoted lignin was also

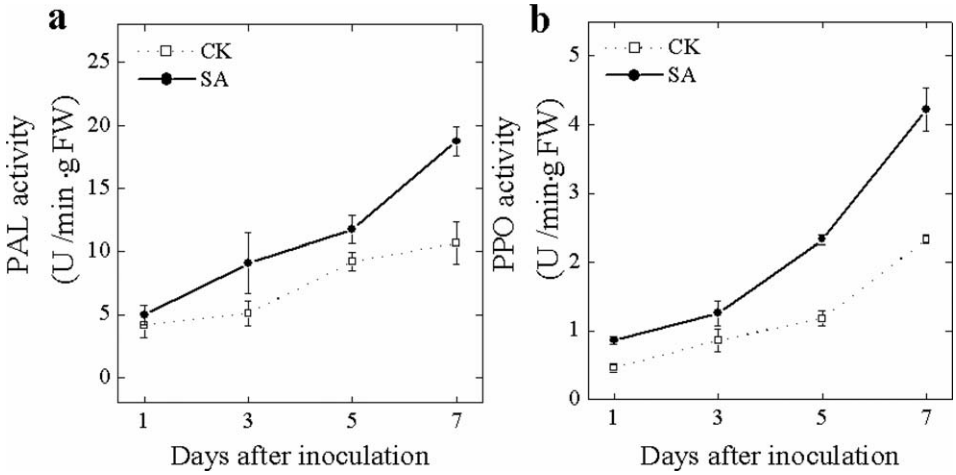

FIG. 4. EFFECTS OF SA TREATMENT ON PAL (A) AND PPO (B) ACTIVITIES IN MANGO FRUITS INOCULATED WITH *C. GLOEOSPORIOIDES* AND INCUBATED AT 25 ± 1°C FOR 7 DAYS

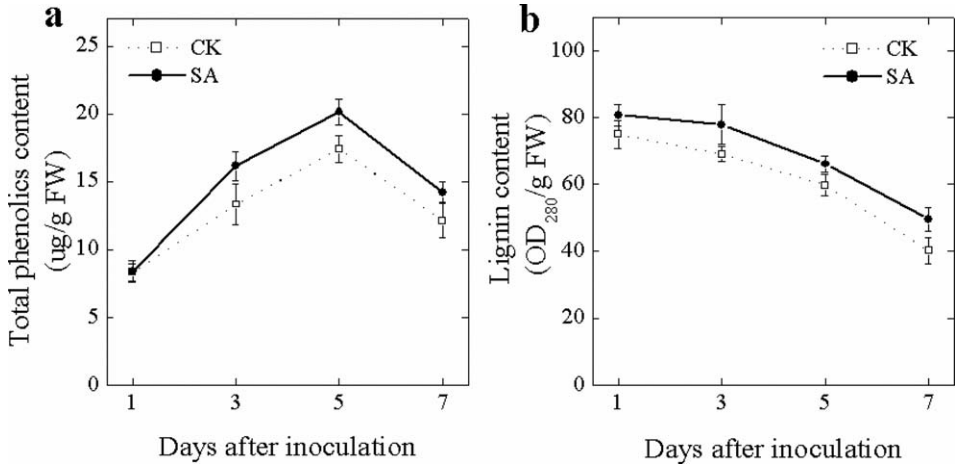

FIG. 5. EFFECTS OF SA TREATMENT ON TOTAL PHENOLICS CONTENT (A) AND LIGNIN CONTENT (B) IN MANGO FRUITS INOCULATED WITH *C. GLOEOSPORIOIDES* AND INCUBATED AT 25 ± 1°C FOR 7 DAYS

observed in NO-treated "Guifei" mango fruit (Hu *et al.* 2014). Based on this finding, it was suggested that enhancement of total phenolic and lignin biosynthesis in SA-treated mango fruits on *C. gloeosporioides* infection may result in the reinforcement of the cell wall and formation of an efficient physical barrier to restrict subsequent fungal penetration and infection.

Effects of SA Treatment on Cell Wall Degradation and Disease Index of Mango Fruit

The cell wall is a dynamic structure that plays a critical role in growth and development as well as in preventing wounding and pathogen attack (Bellincampi *et al.* 2014). The solubilization and depolymerization of the cell wall constituents would facilitate the infections by postharvest pathogens, to increase postharvest decay and to decrease the quality of fruit. The pectin is the principal components of fruit cell wall, which makes important contributions to the texture of fruits (Liu *et al.* 2014). Figure 6a,b showed that content of protopectin decreases while the content of water soluble pectin increases during mango storage. Compared with control fruit, the rate of decrease in protopectin and increase in water soluble pectin in SA treated fruits were slower than those of control. After 21 days of the storage, protopectin content in SA treated fruit was 2.8 times higher than that in the control fruit, and water soluble pectin content in SA treated fruit was about 82.0% of the control. With the solubilization of pectin, fruit firmness gradually decreased during storage (Fig. 7a). However, compared with control fruit, SA treatment maintained higher firmness. At the end of storage, the fruit firmness in SA-treated fruit was 1.5 times greater than that of the control. These data corroborated previous studies in other fruits (Mo *et al.* 2008; Kazemi *et al.* 2011; Tareen *et al.* 2012).

SA treatment significantly reduced disease index of the mango fruit during storage (Fig. 7b). Disease index in SA treated fruit was 50.0 and 58.8% ($P < 0.05$) lower than those in the control fruit after 14 and 21 days of the storage, respectively (Fig. 7b). The results indicated that pathogen resistance of "Tainong" mango was remarkably enhanced by SA treatment. These results were in agreement with the

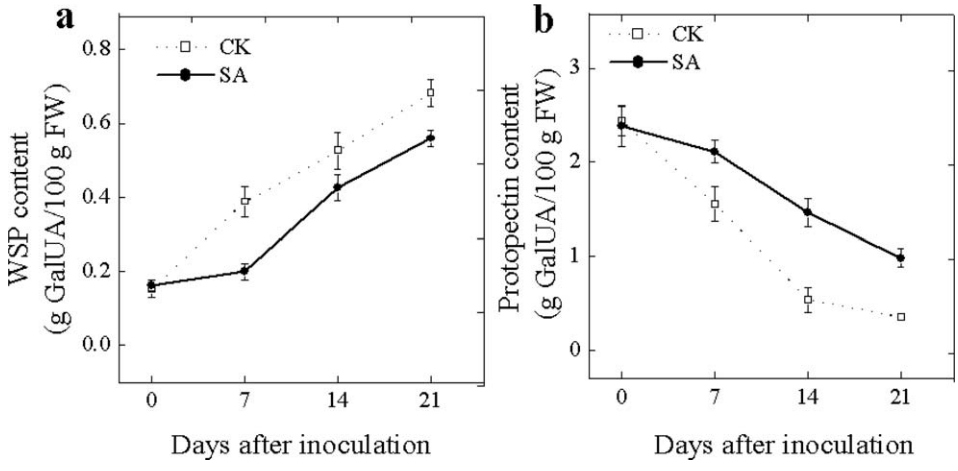

FIG. 6. EFFECTS OF SA ON CONTENTS OF WATER SOLUBLE PECTIN (A) AND PROTOPECTIN (B) IN MANGO FRUITS DURING STORAGE FOR 21 DAYS

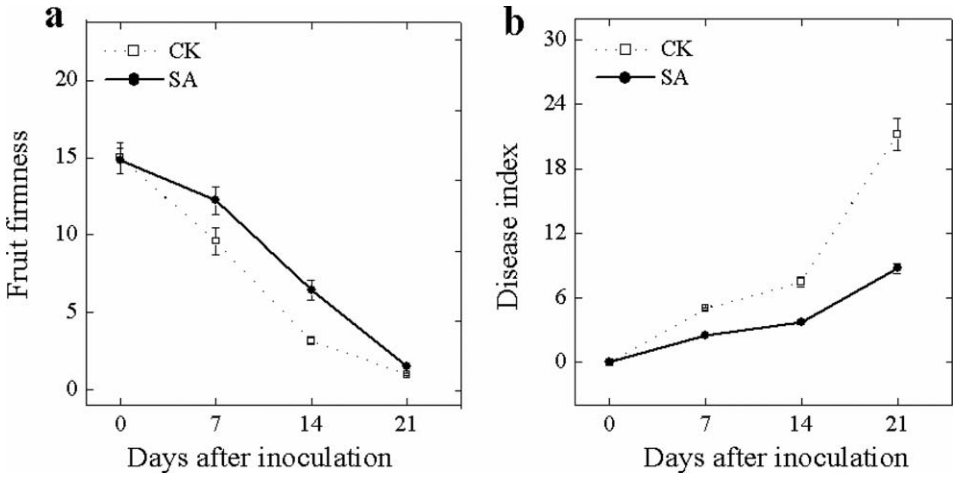

FIG. 7. EFFECTS OF SA ON FRUIT FIRMNESS (A) AND DISEASE INDEX (B) OF MANGO FRUIT DURING STORAGE FOR 21 DAYS

outcome of Tareen *et al.* (2012) in peaches and Cao *et al.* (2013) in jujube fruit that SA treatment significantly lowered the fruit decay.

Recently, Yuan *et al.* (2013) and Dessalegn *et al.* (2013) found that there is a high correlation between firmness and decay incidence, and that nature disease incidence is very high whenever firmness is decreased. In control fruit, a high and a negative linear correlation coefficient (−0.81755) was found between firmness and disease index in mango fruits (Fig. 8), indicating that maintaining the fruit firmness is important to prevent the disease development and prolong the storage time. In the present study, SA slowed down fruit softening process (Fig. 7a). Therefore, a lower disease index in treated fruit was observed (Fig. 7b), and firmer mango fruit treated with SA might be due to decrease in pectin solubilization (Fig. 6a,b).

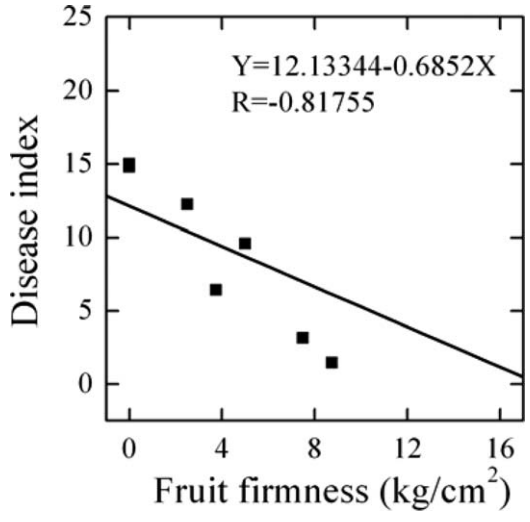

FIG. 8. CORRELATION ANALYSIS BETWEEN FIRMNESS AND DISEASE INDEX IN MANGO FRUIT

CONCLUSION

In conclusion, the present study demonstrated that SA effectively enhanced resistance of mango against postharvest anthracnose rot caused by *C. gloeosporioides*. The enhanced disease resistance may be attributed to a direct antifungal capacity and the induction of fruit defense-related responses. Furthermore, it was found that the SA treatment effectively reduced disease incidence which might be associated with maintained fruit firmness by suppressing degradation of pectin. These results suggested that postharvest SA treatment could be a potential alternative to conventional control methods to prevent postharvest anthracnose in mango fruit.

ACKNOWLEDGMENTS

This work was funded by the National Natural Science Foundation of China (31360413, 30901011) and Technology Foundation for Selected Overseas Chinese Scholar of Guizhou Province (201307).

REFERENCES

ASGHARI, M. and AGHDAM, M.S. 2010. Impact of salicylic acid on post-harvest physiology of horticultural crops. Trends Food Sci. Tech. *21*, 502–509.

BABALAR, M., ASGHARI, M., TALAEI, A. and KHOSROSHAHI, A. 2007. Effect of pre- and postharvest salicylic acid treatment on ethylene production, fungal decay and overall quality of Selva strawberry fruit. Food Chem. *105*, 449–453.

BELLINCAMPI, D., CERVONE, F. and LIONETTI, V. 2014. Plant cell wall dynamics and wall-related susceptibility in plant–

pathogen interactions. Front. Plant Sci. *5*, 228. doi: 10.3389/fpls.2014.00228

BRUMMELL, D.A. and HARPSTER, M.H. 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol. Biol. *47*, 311–340.

CAO, J.K., JIANG, W.B. and ZHAO Y.M. 2007. *Experiment Guideline of Postharvest Physiology and Biochemistry of Fruits and Vegetables*, pp. 84–152, China Light Industry Press, Beijing, China.

CAO, J.K., YAN, J.Q., ZHAO. Y.M. and JIANG, W.B. 2013. Effects of postharvest salicylic acid dipping on *Alternaria* rot and disease resistance of jujube fruit during storage. J. Sci. Food Agric. *93*, 3252–3258.

CHAN, Z.L., WANG, Q., XU, X.B., MENG, X.H., QIN, G.Z., LI, B.Q. and TIAN, S.P. 2008. Functions of defense-related proteins and dehydrogenases in resistance response induced by salicylic acid in sweet cherry fruits at different maturity stages. Proteomics *8*, 4791–4807.

CHEN, S.H., WANG, W.M. and XIA, X.Z. 2007. Study on effect of chitosan film coating on mango storage. Food Sci. Technol. *3*, 236–238 (in Chinese).

CHEN, J.P., ZOU, X., LIU, Q., WANG, F., FENG, W. and WAN, N. 2014. Combination effect of chitosan and methyl jasmonate on controlling *Alternaria alternata* and enhancing activity of cherry tomato fruit defense mechanisms. Crop Prot. *56*, 31–36.

DESSALEGN, Y., AYALEW, A. and WOLDETSADIK, K. 2013. Integrating plant defense inducing chemical, inorganic salt and hot water treatments for the management of postharvest mango anthracnose. Postharvest Biol. Technol. *85*, 83–88.

DODD, J.C., PRUSKY, D. and JEFFRIES, P. 1997. Fruit diseases. In *The Mango: Botany, Production and Uses* (R.E. Litz, ed.) pp. 257–280, CAB International press, Wallingford, UK.

DUBEY, R.K., KUMAR, R., JAYA and DUBEY N.K. 2007. Evaluation of *Eupatorium cannabinum Linn.* oil in enhancement of shelf life of mango fruits from fungal rotting. World J. Microb. Biot. *23*, 467–473.

GARCÍA, T., GUTIÉRREZ, J., VELOSO, J., GAGO-FUENTES, R. and DÍAZ, J. 2015. Wounding induces local resistance but systemic susceptibility to *Botrytis cinerea* in pepper plants. J. Plant Physiol. *176*, 202–209.

GIMÉNEZ, M.J., VALVERDE, J.M., VALERO, D., GUILLÉN, F., MARTÍNEZ-ROMERO, D., SERRANO, M. and CASTILLO, S. 2014. Quality and antioxidant properties on sweet cherries as affected by preharvest salicylic and acetylsalicylic acids treatments. Food Chem. *160*, 226–232.

HU, M.J., YANG, D.P., HUBER, D.J., JIANG, Y.M., LI, M., GAO, Z.Y. and ZHANG, Z.K. 2014. Reduction of postharvest anthracnose and enhancement of disease resistance in ripening mango fruit by nitric oxide treatment. Postharvest Biol. Technol. *97*, 115–122.

KAZEMI, M., ARAN, M. and ZAMANI, S. 2011. Effect of $CaCl_2$ and salicylic acid treatments on quality characteristics of Kiwi fruit (*Actinidia deliciosa* cv. Hayward) during storage. Am. J. Plant Physiol. *6*, 183–189.

LI, J., N, J.Y., LI, H.F., XU, G.F, WANG, X.D., WU, Y.L. and WANG, Z.X. 2008. On determination conditions for total polyphenols in fruits and its derived products by Folin-phenol methods. J. Fruit Sci. *25*, 126–131.

LIU, K., WANG, X.L. and YOUNG, M. 2014. Effect of bentonite/potassium sorbate coatings on the quality of mangos in storage at ambient temperature. J. Food Eng. *137*, 16–22.

MANDAL, S., MALLICK, N. and MITRA, A. 2009. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. Lycopersici in tomato. Plant Physiol. Bioch. *47*, 642–649.

MO, Y.W., GONG, D.Q., LIANG, G.B., HAN, R.H., XIE, J.H. and LI, W.C. 2008. Enhanced preservation effects of sugar apple fruits by salicylic acid treatment during post-harvest storage. J. Sci. Food Agric. *88*, 2693–2699.

OJAGHIAN, M.R., ALMONEAFY, A.A., CUI, Z.Q., XIE, G.L., ZHANG, J.Z., SHANG, C.L. and LI, B. 2013. Application of acetyl salicylic acid and chemically different chitosans against storage carrot rot. Postharvest Biol. Technol. *84*, 51–60.

PANAHIRAD, S., ZAARE-NAHANDI, F., SAFARALIZADEH, R. and ALIZADEH-SALTEH, S. 2012. Postharvest control of *Rhizopus stolonifer* in peach (*Prunus persica* L. Batsch) fruits using salicylic acid. J. Food Saf. *32*, 502–507.

PANAHIRAD, S., ZAARE-NAHANDI, F., MOHAMMADI, N., ALIZADEH-SALTEH, S. and SAFAIE, N. 2014. Effects of salicylic acid on *Aspergillus flavus* infection and *aflatoxin* B1 accumulation in pistachio (*Pistacia vera* L.) fruit. J. Sci. Food Agric. *94*, 1758–1763.

QIN, G.Z., TIAN, S.P., XU, Y. and WAN, Y.K. 2003. Enhancement of biocontrol efficacy of antagonistic yeasts by salicylic acid in sweet cherry fruit. Physiol. Mol. Plant P. *62*, 147–154.

TAREEN, M.J., ABBASI, N.A. and HAFIZ, I.A. 2012. Postharvest application of salicylic acid enhanced antioxidant enzyme activity and maintained quality of peach *cv.* 'Flordaking' fruit during storage. Sci. Hortic. *142*, 221–228.

VAN LOON, L.C., REP, M. and PIETERSE, C.M. 2006. Significance of inducible defence-related proteins in infected plants. Annu. Rev. Phytopathol. *44*, 135–162.

VICENTE, A.R., COSTA, M.L., MARTÍNEZ, G.A., CHAVES, A.R. and CIVELLO, P.M. 2005. Effect of heat treatments on cell wall degradation and softening in strawberry fruit. Postharvest Biol. Technol. *38*, 213–222.

WANG, K.T., JIN, P., HAN, L., SHANG, H.T., TANG, S.S., RUI, H.J., DUAN, Y.F., KONG, F.Y., KAI, X. and ZHENG, Y.H. 2014. Methyl jasmonate induces resistance against *Penicillium citrinumin* Chinese bayberry by priming of defense responses. Postharvest Biol. Technol. *98*, 90–97.

XU, X.B. and TIAN, S.P. 2008. Salicylic acid alleviated pathogen-induced oxidative stress in harvested sweet cherry fruit. Postharvest Biol. Technol. *49*, 379–385.

XU, X.B., CHAN, Z.L., XU, Y. and TIAN, S.P. 2008. Effect of *Pichia membranaefaciens* combined with salicylic acid on controlling brown rot in peach fruit and the mechanisms involved. J. Sci. Food Agric. *88*, 1786–1793.

XUE, R.F., WU, J., WANG, L.F., BLAIR, M.W., WANG, X.M., GE, W.D., ZHU, Z.D. and WANG, S.M. 2014. Salicylic acid enhances resistance to *Fusarium oxysporum* f. sp. *phaseoli* in common beans (*Phaseolus vulgaris* L.). J. Plant Growth Regul. *33*, 470–476.

YU, T. and ZHENG, X.D. 2006. Salicylic acid enhances biocontrol efficacy of the antagonist *Cryptococcus laurentii* in apple fruit. J. Plant Growth Regul. *25*, 166–174.

YU, T., CHEN, J.H., CHEN, R., HUANG, B., LIU, D.H. and ZHENG, X.D. 2007. Biocontrol of blue and gray mold diseases of pear fruit by integration of antagonistic yeast with salicylic acid. Int. J. Food Microbiol. *116*, 339–345.

YUAN, L., BI, Y., GE, Y.H., WANG, Y., LIU, Y.Y. and LI, G.L. 2013. Postharvest hot water dipping reduces decay by inducing disease resistance and maintaining firmness in muskmelon (*Cucumis melo* L.) fruit. Sci. Hortic. *161*, 101–110.

ZENG, K.F., CAO, J.K. and JIANG, W.B. 2006. Enhancing disease resistance in harvested mango (*Mangifera indica* L. cv. 'Matisu') fruit by salicylic acid. J. Sci. Food Agric. *86*, 694–698.

ZHANG, H.Y., MA, L.C., JIANG, S., LIN, H.T., ZHANG, X.Y., GE, L.L. and XU, Z.L. 2010a. Enhancement of biocontrol efficacy of *Rhodotorula glutinis* by salicylic acid against gray mold spoilage of strawberries. Int. J. Food Microbiol. *141*, 122–125.

ZHANG, H.Y., MA, L.C., TURNER, M., XU, H.X., ZHENG, X.D., DONG, Y. and JIANG, S. 2010b. Salicylic acid enhances biocontrol efficacy of *Rhodotorula glutinis* against postharvest *Rhizopus* rot of strawberries and the possible mechanisms involved. Food Chem. *122*, 577–583.

ZHANG, Z.K., YANG, D.P., YANG, B., GAO, Z.Y., LI, M., JIANG, Y.M. and HU, M.J. 2013. β-Aminobutyric acid induces resistance of mango fruit to postharvest anthracnose caused by *Colletotrichum gloeosporioides* and enhances activity of fruit defense mechanisms. Sci. Hortic. *160*, 78–84.

ZHANG, M.A., WU, G.B. and CHEN, F.H. 2014. Effect of nitric oxide fumigation on lignification of loquat fruits during cold storage. Food Sci. *35*, 232–237.

ZHOU, Y.H., MING, J., DENG, L.L. and ZENG, KF. 2014. Effect of *Pichia membranaefaciens* in combination with salicylic acid on postharvest blue and green mold decay in citrus fruits. Biol. Control *74*, 21–29.

ZHU, L.Y., WU, W.W., SUN, J. and DU, T.T. 2010. Enzymatic properties of polyphenol oxidase from kuerle pear. Food Sci. *31*, 275–278.