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Reduction of Latent Infection and Enhancement of Disease Resistance in Muskmelon by Preharvest Application of Harpin

Junjie Wang,[†,‡] Yang Bi,[*,†] Zhengke Zhang,[†] Huaiyu Zhang,[†] and Yonghong Ge[†]

[†]College of Food Science and Engineering, Gansu Agricultural University, Lanzhou, Gansu 730070, People's Republic of China

[‡]College of Life Science and Engineering, Northwest University for Nationalities, Lanzhou, Gansu 730124, People's Republic of China

ABSTRACT: Latent infection was analyzed when harpin at 50 mg/L was sprayed on muskmelon 4 times at four different stages: florescence, young fruit, fruit enlarging, and netting periods. Results showed that the latent infection was significantly lower in sprayed muskmelons than in the control fruit. Meanwhile, the activities of peroxidase, phenylalanine ammonia-lyase, β-1,3-glucanase, and chitinase increased significantly in the fruit treated with harpin. It also resulted in an increase of contents of total phenolic compounds, flavanoids, and lignin. In addition, the treatment of harpin increased the activity of superoxide dismutase, enhanced the content of hydrogen peroxide, and reduced catalase activity. Furthermore, harpin treatments contributed to the reinforcement of cell walls of pericarp in fruit, the reduction of postharvest decay, and the improvement of postharvest qualities. These results suggested that harpin effectively participated in inducing resistance and could be a new strategy for preventing latent infection in muskmelon.

KEYWORDS: Fruit, elicitor, latent infection, induce resistance

INTRODUCTION

Muskmelon (*Cucumis melo* L. cv. Yindi) is one of the most important cultivated crops in the northwest of China. The fruits are susceptible to postharvest decay caused by pathogenic fungi. Alternaria and Fusarium rots, caused by *Alternaria alternata* and *Fusarium* spp., respectively, account for most of the postharvest losses of muskmelons.[1] The two pathogens often infect the flowers and young fruit in the field and remain quiescent until storage when lesions progressively appeared, which were called latent infection.[2] Therefore, reduction of latent infection would be beneficial to controlling postharvest rots. Several fungicides by spraying before harvest, including iprodione and azoxystrobin, can effectively control the latent infection in melon.[1] However, because of increasing concerns about chemical usage in food and the environment, there is a worldwide trend to explore new alternatives to reduce application of synthetic fungicides.[3]

Induction of natural disease resistance in harvested horticultural crops is being considered as a preferred strategy for disease management.[1] An emerging strategy is the chemical induction resistance in postharvest fruit and vegetables.[4] Harpin, a bacterial hypersensitive response (HR) elicitor, is a heat-stable, glycine-rich protein that was first described in *Erwinia amylovora*, which causes fire blight of apple, pear, and other members of Rosaceae.[5] It is commercially released in some countries as a plant health promoter under the name of Messenger. Application of harpin could induce systemic-acquired resistance response in cucumber.[6] The elicitor has also been applied effectively as a postharvest treatment to suppress decay in apples,[7] pears,[8] melons,[1] and oranges.[9] The inhibition of rots was correlated with the induction of some defense mechanisms involved in resistance-related enzymes or metabolites.[1,8,9] However, no information is available on its effect as a control of latent infection on fruit.

The purpose of this study was to investigate the effect of preharvest treatment by harpin on latent infection, to determine whether harpin sprays have an effect on resistance-related enzyme activities and metabolite contents, to observe the change of the cell-wall structure of pericarp, and to evaluate the efficiency of harpin treatment on postharvest qualities in muskmelon fruit.

MATERIALS AND METHODS

Preharvest Treatment of Harpin. The melon seeds (cv. Yindi) were cultivated in the open field of Quanshan, Minqin County, Wuwei, Gansu, China, during 2005−2006. The experimental design for both years was a randomized complete block with three replications of eight treatments; plots were single rows, and each plot was 8 m long, with a spacing of 30 cm between rows and 100 cm between plants within rows. The treatments consisted of harpin (Messenger) (Eden Bioscience Co., Bothell, WA) at 50 mg/L (on the basis of the previous study; data not shown) and deionized water as a control sprayed 4 times on plants in the field. The spray time was at flowering, young fruit period [14 days after flowering (daf)], fruit enlarging period (28 daf), and netting period (42 daf). A total of 1 L of solution treated 45−50 plants, 30 plants per treatment, repeated 3 times at least.

Fruit Sampling. Fruit were sampled 2 weeks after treatment, at young fruit period, enlarging period, netting period, and mature period (56 daf) for investigation. The sampled fruits were sorted for uniform size and absence of obvious injuries, put in the sampling boxes, and transported to the laboratory within 24 h.

Investigation of Latent Infection. The sampled fruit were washed and air-dried. Discs of peel and flesh (5 mm in diameter and 2 mm thick) were prepared using a cork borer. Disc sampling was performed in three parts at the top, stem, and middle around the fruit. A total of 10 discs were sampled from each part. The discs were

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disinfected with 70% ethanol for 30 s, then were dipped in a solution of 0.1% mercuric chloride for 2 min, washed 3 times in sterile water, and dried on sterile filter paper. The discs was placed, peel side down, in a Petri dish containing potato dextrose agar and 40 μg/mL streptomycin as a culture medium and incubated at 25 °C for 5−7 days, and the category and number of fungi that appeared were recorded. The latent infection was assayed according to Prusky et al.[10] A total of 50 fruits were used from each treatment, repeated 3 times.

$$I = \text{percentage of latent infections of fruit} = \frac{\sum \text{percentage of infected discs in different parts}}{\text{number of parts}}$$

$$S = IW^{2/3}$$

S expressed the relative latent infected surface, and W expressed the weight of a single fruit.

Enzyme Assays. Approximately 3 g of tissue was removed 5.0−10.0 mm below the skin with a stainless-steel cork borer around the equator for the fruit. Each sample was packed, frozen in liquid nitrogen, and kept at −80 °C until enzyme extraction.

All procedures of enzyme extracts were conducted at 4 °C. Each 3 g of fruit flesh was homogenized with 3 mL of extracting buffer as follows. Peroxidase (EC 1.11.1.7) was extracted using 50 mM phosphate buffer (pH 7.5) containing 8% (w/v) polyvinylpolypyrrolidone, 1 mM polyethyleneglycol, 0.01% (v/v) Triton X-100, and 1 mM phenylmethanesulfonyl fluoride. Phenylalanine ammonia-lyase (EC 4.3.1.5) was extracted with 0.2 M boric acid buffer (pH 8.8) containing 10% (w/v) polyvinylpolypyrrolidone, 1 mM ethylene diamine tetraacetic acid, and 50 mM β-mercaptoethanol. β-1,3-Glucanase (EC 3.2.1.6) and chitinase (EC 3.2.1.14) were extracted with 50 mM sodium acetate buffer (pH 5.0) containing 2% polyvinylpolypyrrolidone. Superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6) were extracted using 100 mM sodium phosphate buffer (pH 7.5) containing 5 mM dithiothreitol and 2% polyvinylpolypyrrolidone. The extracts were then centrifuged at 17000*g* for 30 min at 4 °C. The supernatants were used for the enzyme assays.

Peroxidase activity was assayed according to Chance and Maehly,[11] with some modifications. Supernatants were diluted in 0.05 M sodium phosphate buffer at pH 7.5 (1:3, v/v). This diluted extract (60 μL) was added to a 3.6 mL reaction mixture that contained 0.05 M guaiacol and 200 μL of 2% hydrogen peroxide (H_2O_2). Oxidation of guaiacol to tetraguaiacol was monitored by a spectrophotometer at 470 nm for 3 min at 25 ± 2 °C. Peroxidase activity was expressed as units per milligram of protein (unit/mg of protein), where 1 unit is equal to 1 nM tetraguaiacol produced per minute using 6165.41 as a molar extinction coefficient.

Phenylalanine ammonia-lyase activity was determined using a modified method of Koukol and Conn.[12] A total of 300 μL of the extract was incubated with 1 mL of 0.02 M L-phenylalanine and 2 mL of the phenylalanine ammonia-lyase extracting buffer at 24 °C for 2 min, and absorbance at 290 nm was measured in an ultraviolet spectrophotometer. The activity was expressed as unit/mg of protein, where unit/mg of protein = $0.01\Delta A_{290}$ (mg of protein)$^{-1}$ min^{-1}.

β-1,3-Glucanase and chitinase activities were measured according to the method of Abeles et al.,[13] with some modifications. β-1,3-Glucanase activity was assessed by measuring the amount of reducing sugar released from the substrate by incubating 250 μL of enzyme preparation for 60 min at 37 °C with 250 μL of 0.5% (w/v) laminarin. The reaction was terminated by adding 250 μL of 3,5-dinitrosalicylate and boiling for 5 min in a water bath. The solution was diluted with 4 mL of distilled water, and the amount of reducing sugars was measured by a spectrophotometer at 540 nm. A total of 1 unit was defined as the amount of enzyme catalyzing the formation of 1 μmol of glucose equivalents s^{-1} L^{-1} (mg of protein)$^{-1}$. Chitinase activity was assayed by mixing 2 mL of crude enzyme solution with 1 mL of 2% dye-labeled carboxymethyl chitin (w/v) in 50 mM sodium acetate buffer (pH 5.0). After 1 h of incubation at 37 °C, the reaction was stopped by adding 1 mL of 1.0 M HCl. The mixture was cooled and centrifuged, and then the absorbance of the supernatant was measured at 550 nm with a spectrophotometer. A total of 1 unit of chitinase activity was defined as the amount of enzyme required to catalyze the formation of 1 nmol/min of product.

Superoxide dismutase activity was assayed according to Prockazkova et al.[14] The reaction mixture (3 mL) contained 100 mM sodium phosphate buffer (pH 7.5), 13 mM methionine, 75 μM nitro blue tetrazolium (NBT), 10 μM ethylenediaminetetraacetic acid, 10 μM riboflavin, and 0.1 mL of enzyme extract. The mixtures were illuminated with a fluorescent lamp (15 W) for 10 min, and then the absorbance was determined at 560 nm. Identical solutions held in the dark served as blanks. The specific activity was expressed as units per milligram of protein, where 1 unit was defined as 50% inhibition of the NBT reduction under light by the enzyme.

Catalase activity was assayed according to Milosevic and Slusarenko.[15] The activity was determined by adding 0.2 mL of enzyme preparation to 2.8 mL of 40 mM hydrogen peroxide (dissolved with 100 mM sodium phosphate buffer at pH 7.5) as a substrate and was expressed as unit/mg of protein, where unit = ΔOD_{240}/min.

The protein content in the crude enzyme extracts was determined according to the method of Bradford,[16] using bovine serum albumin (BSA) as the standard protein.

Determination of the Metabolite Content. Contents of total phenolic compounds and flavonoids were measured according to Pirie and Mullins.[17] Fruit samples (1.0 g) were homogenized with 5 mL of cold 1% HCl/ethanol (v/v) on ice, and the homogenates were centrifuged at 12000*g* for 10 min at 4 °C. The absorbance of the supernatant was measured at 280 nm for total phenolic compounds and 325 nm for flavonoids. The contents of total phenolic compounds and flavonoids were expressed as micrograms of gallic acid per gram of flesh weight (FW) and OD_{325} per gram of FW, respectively.

The H_2O_2 content was assayed according to the method of Patterson et al.,[18] with slight modification. A total of 3 g of fresh tissue was homogenized with 3 mL of cold acetone and then centrifuged at 12000*g* for 15 min at 4 °C. The supernatant was measured by monitoring the absorbance of the titanium peroxide complex at 410 nm. Absorbance values were calibrated against a standard curve (generated using known concentrations of H_2O_2) and expressed as micromoles per milligram of FW.

The lignin content was determined according to the method of Morrison.[19] A total of 3 g of fresh tissue was homogenized with 3 mL of cold 95% alcohol and then centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was collected immediately for lignin analysis, and the lignin content was expressed as OD_{280} per gram of FW.

All biochemical experiments mentioned above were performed with a minimum of three tissue samples replicated per treatment and per time point. Each experiment was performed at least 3 times.

Transmission Electron Microscopy (TEM). The ultrastructure of the cell wall was investigated by TEM according to the method of He et al.,[20] with slight modifications. At a mature period, approximately 2−3 mm^2 pericarp samples were excised from the center of the fruit treated by harpin or water spraying 4 times. The samples were immediately fixed in 3% (v/v) glutaraldehyde and 4% formaldehyde, subsequently rinsed twice in 0.1 M sodium phosphate buffer at pH 7.2, and then post-fixed for 2 h in 1% OsO_4 buffered in 0.1 M sodium phosphate buffer. After aqueous washing (twice), samples were dehydrated through a graded series of ethanol and embedded in Epon 812/Araldite resin. Following polymerization, thin sections (60 nm) were cut on an ultramicrotome, post-stained with uranyl acetate (ethanolic) and lead citrate, and viewed

under an electron microscope (JEOL Co., Tokyo, Japan) operating at 80 kV. Micrographs were taken by a charge-coupled device (CCD) camera (Gatan Co., Pleasanton, CA).

Evaluation of Postharvest Quality. Fruit quality was evaluated after storage. General appearance was evaluated visually as described by Aharoni et al.[21] for freshness of the fruit, decay, and skin blemishes on a scale of 1−5, with 1 = poor, 3 = good, and 5 = excellent quality. Fruit with a rating of less than 2.5 were considered unfit for marketing. Total soluble solids were determined by squeezing juice from the central section of flesh directly on an Abbe refractometer (10481 S/N, Division of Warner-Lambert Co., Ann Arbor, MI). Three readings were taken from each melon. Firmness was measured using a hand-held penetrometer (Hangzhou Top Instruments Co., Ltd., Zhejiang, China) at the equator of fruit where a section of rind (4 × 4 cm and 1 cm deep) had been removed. Three readings were taken for each melon. Fruit were considered to be decayed if there was fungal growth on the surface of the stem end and the rind. The percentage of fruit with decay was determined. Each treatment was applied to 3 replicates of 10 melons, and the experiment was performed twice.

Statistical Analysis. The statistical analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL). To test for the effect of the treatment, the data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered as significant. The data were also expressed as the means [±standard error (SE)].

RESULTS

Effect of Harpin Treatment on Latent Infection. According to our previous studies, the isolates including *A. alternata* and *Fusarium* spp. had the ability of causing postharvest diseases in muskmelon fruit, which proved that the two pathogens were the major latent infectious fungi of muskmelon fruit.[2,22] Therefore, the present study only conducted the investigation of the effect of the harpin treatment on the latent infection.

Both the total rate of latent infection and the area of relative latent infection increased along with the fruit development. At the two later periods of fruits, the total rate of latent infection and the area of relative latent infection were both significantly ($p < 0.05$) reduced by one harpin treatment and decreased further with more harpin treatments (panels A and B of Figure 1). In mature fruit treated with harpin 4 times, for example, both the total rate of latent infection and the area of relative latent infection were 80, 73, 69, and 42% lower than those in control fruit and fruits treated with harpin 1, 2, and 3 times, respectively.

Influence of Harpin Treatment on Activities of Resistance-Related Enzymes. Preharvest treatment with harpin strongly affected the activities of peroxidase, phenylalanine ammonia-lyase, β-1,3-glucanase, chitinase, superoxide dismutase, and catalase in developing fruit compared to the control, and the effect of harpin increased with times of applications (Figure 2). Peroxidase activity were enhanced in treated fruit during each period, and the effect increase gradually with the times of harpin treatments (Figure 2A). In the control fruit, phenylalanine ammonia-lyase activity exhibited an initial increase from young fruit to the enlarging and netting period and then declined in the mature period. Harpin treatment noticeably affected phenylalanine ammonia-lyase activity in developing melons. Consistent with changes in the control fruit, phenylalanine ammonia-lyase activity in fruit treated with harpin declined in the mature period compared to those in the netting period but declined less after more treatments with harpin (Figure 2B).

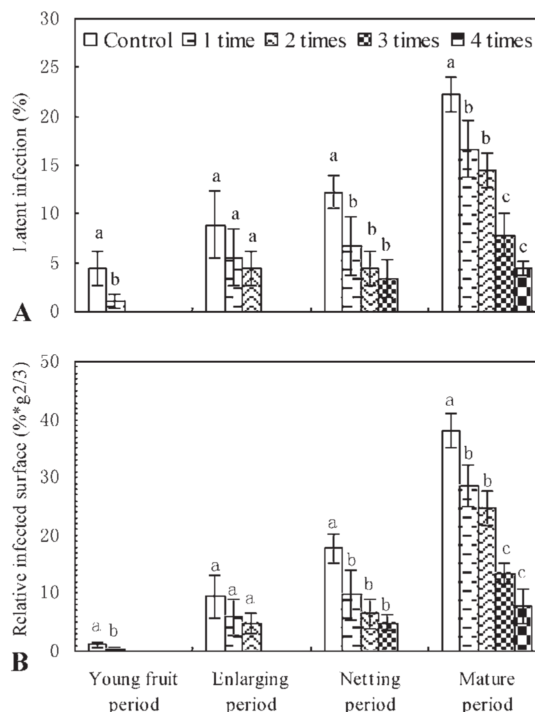

Figure 1. Effect of harpin treatment on the (A) latent infection rate and (B) relative infected surface in melons (cv. Yindi). Bars represent SE. Values followed by the same letter are not significantly different by Duncan's test at $p < 0.05$ in the same period.

The activity of β-1,3-glucanase was obviously raised in developing fruit treated with harpin. The melons had more activity of β-1,3-glucanase with the increase of spray times, except 3 times of spray in the netting period (Figure 2C). Figure 2D indicated that the chitinase activity increased gradually during young fruit and enlarging fruit and subsequently decreased sharply in netting and mature fruit. Harpin treatment had no effect ($p > 0.05$) on the chitinase activity in the young fruit and netting period. However, the chitinase activity was increased only by harpin preharvest treated 2 times in the enlarging period and 4 times in the mature period.

Harpin treatment significantly enhanced ($p < 0.05$) superoxide dismutase activity in developing fruit. The melons had more activity of superoxide dismutase with the increase of spray times, except 2 times of spray in the mature period (Figure 2E). The catalase activity was decreased in developing fruit sprayed with harpin. The melons had less activity of catalase with the increase of spray times, except 1 time of spray in the enlargement, netting, and mature periods (Figure 2F).

Effect of Harpin Treatment on Contents of Resistance-Related Metabolites. The contents of total phenolic compounds and flavonoids in the control fruit gradually increased during the growth of fruit. The levels of these metabolites increased significantly ($p < 0.05$) after harpin treatment and increased with a greater number of treatments (panels A and B of Figure 3). Treatment with harpin markedly affected the contents of H_2O_2 and lignin. A dramatic accumulation of H_2O_2 contents was found in fruit sprayed 1 time in young fruit period, 2 times in the enlargement period, 3 times in the netting period, and 3 and 4 times in the mature period (Figure 3C). Except for the enhancement along with the time of harpin spray in the enlarging period, the spray times of harpin had no effect ($p > 0.05$) on the content of lignin in other periods (Figure 3D).

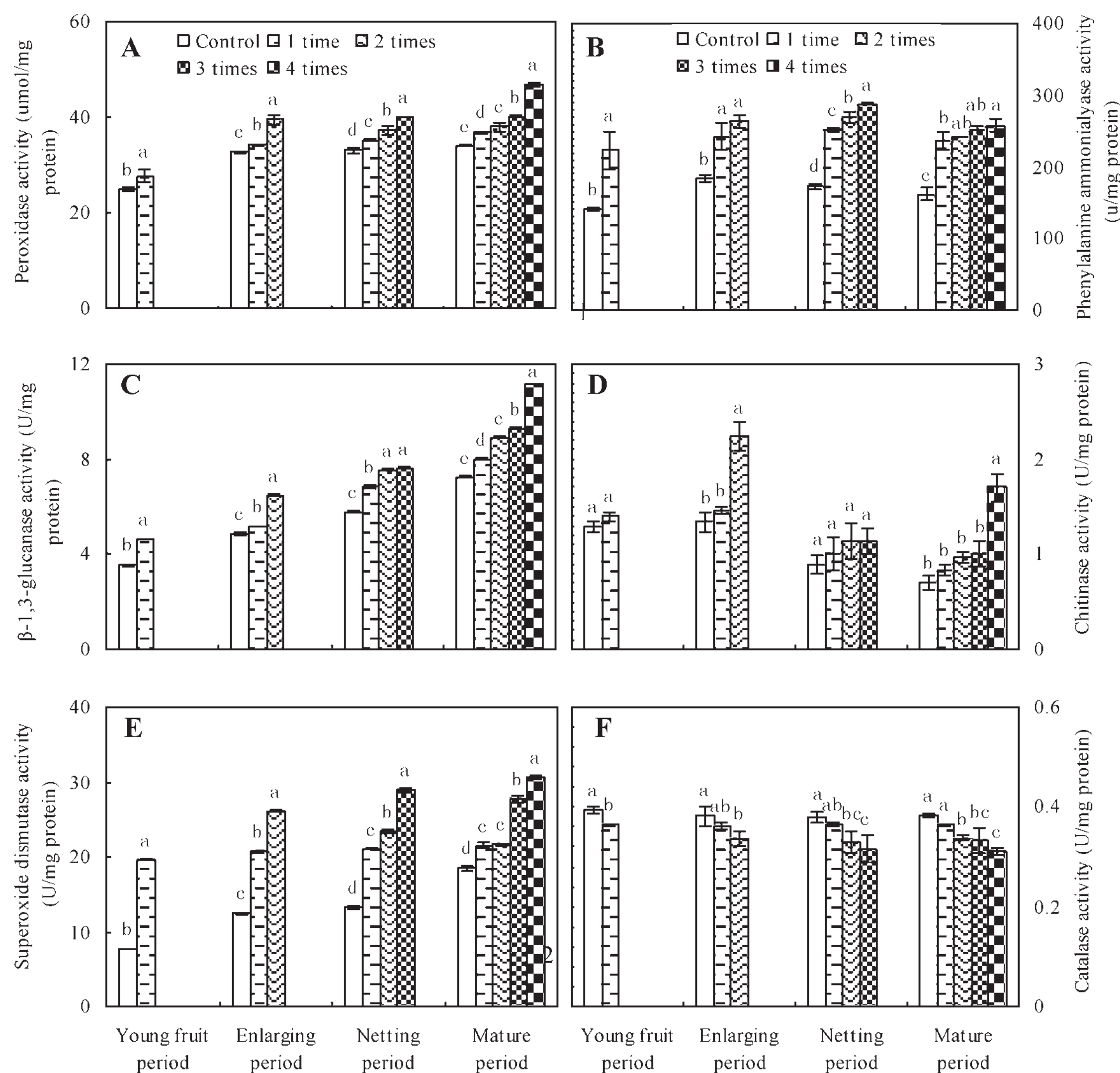

Figure 2. Effect of preharvest harpin treatment on the activity of peroxidase, phenylalanine ammonia-lyase, β-1,3-glucanase, chitinase, superoxide dismutase, and catalase in melons (cv. Yindi). Bars represent SE. Values followed by the same letter are not significantly different by Duncan's test at $p < 0.05$ in the same period.

Effect of Harpin Treatment on the Cell-Wall Structure of Fruit. In the mature period, the cell wall from pericarp of the fruit treated with harpin or water 4 times was examined by electron microscopy. The cell walls of fruit, regardless of the non-netting side (panels A and B of Figure 4) and netting side (panels C and D of Figure 4), were strikingly thickened by harpin treatment. Furthermore, quantitation of the cell-wall thickness showed that the width or thickness of the cell wall of treated fruit by harpin on the non-netting and netting sides were more than 2- and 4-fold higher than that of the control, respectively (data not shown).

Effect of Harpin Treatment on Postharvest Decay Incidence and Qualities. Decay incidence and qualities involved in general appearance, firmness, and total soluble solids of fruits harvested at the mature period followed by 10 days of storage are shown in Table 1. Except for 1 time treatment, decay incidence was significantly ($p < 0.05$) reduced by harpin treatment, and it was decreased further with more harpin treatments. For general appearance and firmness, the values were significantly ($p < 0.05$) enhanced by harpin treatment, but no significant differences ($p > 0.05$) were observed between harpin treatment 2, 3, and 4 times. However, harpin treatment had no effect ($p > 0.05$) on the content of total soluble solids of fruit (Table 1).

DISCUSSION

Preharvest spray with harpin proved effective for reducing latent infection in melons, and the effect in the reduction of latent infection was in proportion to the amount of harpin treatments (Figure 1). A similar result was found by Ge et al.,[22] who indicated Fusarium and pink rots of melon could be decreased by treatment with harpin spray 1 week before harvest.

In the present study, the control of latent infection clearly correlated with the activation of defense mechanisms. The data provided evidence that harpin strengthened the defense system in muskmelon fruit by increasing the contents of pathogenesis-related proteins, enforcing the structure of cell walls, activating the phenylpropanoid pathway, and accumulating the content of H_2O_2 quickly.

Oxidative burst, which generated rapidly reactive oxygen species, including H_2O_2, has been considered as one of the most

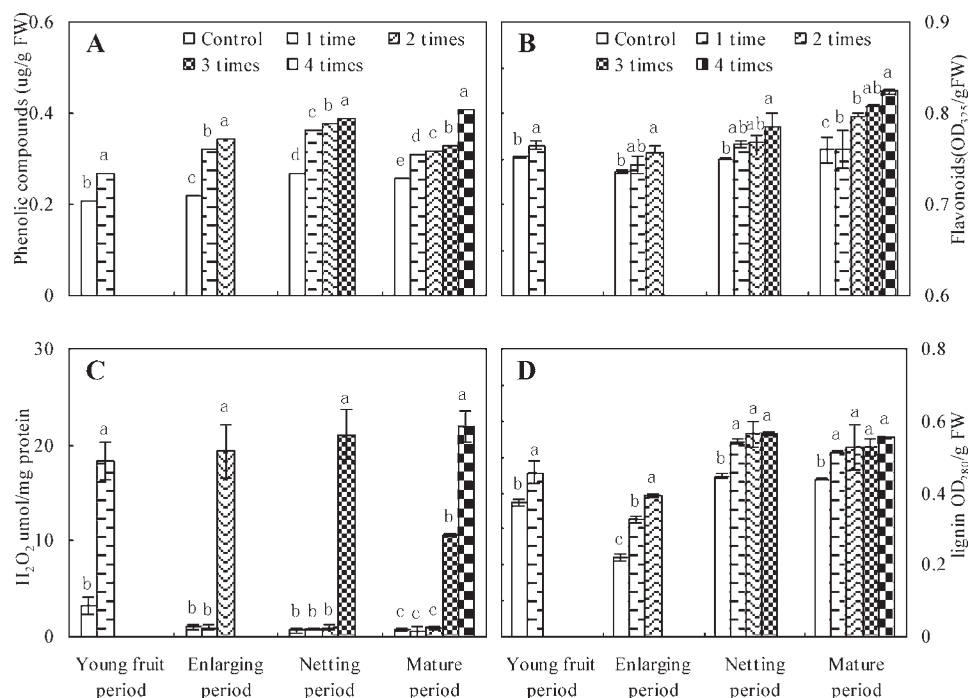

Figure 3. Effect of preharvest harpin treatment on the content of total phenolic compounds, flavonoids, H_2O_2, and lignin in melon (cv. Yindi). Bars represent SE. Values followed by the same letter are not significantly different by Duncan's test at $p < 0.05$ in the same period.

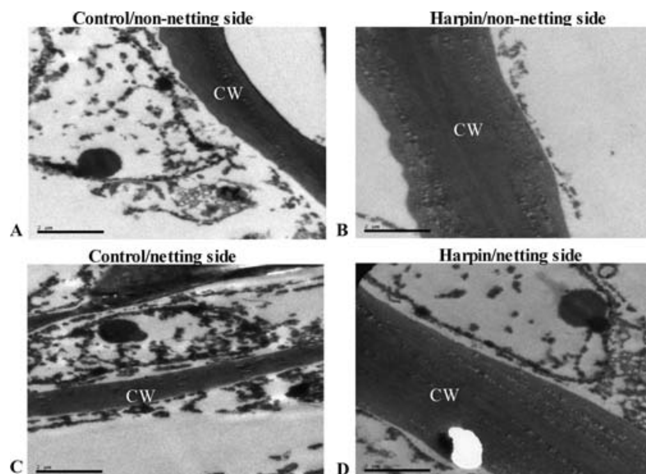

Figure 4. Effect of harpin on the ultrastructure in pericarp of muckmelon fruit. The plants were treated with harpin (50 mg of AI/L) or water (as a control) at flowering, young fruit, enlarging, and netting periods during growth and collected at the mature stage. Electron micrographs show the increased thickness of the cell wall (CW) from fruit pericarp cells on the (A and B) non-netting side and (C and D) netting side (scale bars = 2 μm).

prominent and earliest defense responses in the plant−pathogen interaction during infection.[23] In the present study, in comparison to the control, the level of H_2O_2 content in melon fruit was significantly enhanced by harpin (Figure 3C). Meanwhile, a marked increase of superoxide dismutase activity (Figure 2E) and a marked decrease of catalase activity (Figure 2F) were observed in fruit treated with harpin, The superoxide dismutase and catalase, which work together with other enzymes of the ascorbate−glutathione cycle, can promote the balance of reactive oxygen species.[24] Superoxide dismutase catalyzes the dismutation of superoxide anion (O_2^-) into H_2O_2 and O_2, while catalase protects cells from H_2O_2 by catalyzing its decomposition into O_2 and H_2O.[23] This rapid accumulation of H_2O_2 possibly hinders microorganism penetration in plant tissues by facilitating peroxidase reactions catalyzing intra- and intermolecular cross-links between structural components of cell walls and lignin polymerization. In addition, H_2O_2 acts as a secondary messenger to induce expression of pathogenesis-related proteins and initiates the development of systemic-acquired resistance.[24]

Phenylalanine ammonia-lyase is a key enzyme in the first step of the phenylpropanoid pathway, which is directly involved in the synthesis of phenols, phytoalexins, and lignin that are correlated with localized resistance processes.[15] In the present study, we found that phenylalanine ammonia-lyase could be activated by treatment with harpin (Figure 2B), consequently accumulating phenolic compounds (Figure 3A) and flavonoids (Figure 3B) in muskmelon. Flavonoids, as a kind of phytoalexins, are associated with the restriction of pathogen development and directly involved in defense that has a measurable benefit for the plant.[25] As several kinds of important resistance metabolites, accumulation of phenolic compounds at the infection site limits the development of the pathogen and occurs as a result of rapid (hypersensitive) cell death.[25] Defense systems in plants are finally carried out by biosynthesizing metabolites, such as phytoalexins and phenols, to form lignin or toxic quinones directly against pathogen infection.[15] Therefore, in our present study, harpin treatment could increase lignin contents in muskmelon (Figure 3D). Lignin is formed by dehydrogenative polymerization of precursors produced in the phenylpropanoid pathway.[26] Furthermore, lignin is extremely resistant to microbial degradation and, thus, constitutes one of the most effective barriers

Table 1. Decay Incidence and Quality of Muskmelons (cv. Yindi) 10 Days after Harvest at Room Temperature

treatment	decay incidence (%)	general appearance[a]	firmness (kg/cm^2)	total soluble solids (%)
before storage	0	4.5 ± 0.41	7.0 ± 0.35	11.7 ± 0.27
control	43.5 ± 4.16 a[b]	2.0 ± 0.18 c	3.7 ± 0.61 b	9.7 ± 0.72 a
1 time	40.7 ± 2.87 a	2.5 ± 0.26 b	3.8 ± 0.54 b	10.1 ± 0.40 a
2 times	34.8 ± 2.34 b	3.0 ± 0.21 a	4.8 ± 0.32 a	9.4 ± 1.16 a
3 times	27.3 ± 4.12 c	3.5 ± 0.35 a	4.7 ± 0.29 a	10.3 ± 0.67 a
4 times	25.6 ± 3.65 c	3.5 ± 0.43 a	4.9 ± 0.47 a	10.5 ± 0.51 a

[a] General appearance: 1 = poor, 3 = good, and 5 = excellent. [b] The data were based on 3 replicates of 10 fruit each. Values followed by the same letter are not significantly different according to the least significant difference test ($p < 0.05$).

against pathogenic invasion.[15,26] An increase of these metabolites has a similar trend with the enhancement of phenylalanine ammonia-lyase activity in muskmelon fruit, as reported in pear[8] and Hami melon[1] fruit treated with harpin. This indicated that the enhancement of resistance metabolites through enzymatic catalysis could be a component of the mechanism of harpin resistance induced in melon fruit. In addition, similar to the trend of several enzyme activities above, the results have also shown that defense-related metabolites increase along with the number of harpin treatments.

An increase of peroxidase activity is typical of the process of pathogenesis and oxidative enzymes. Peroxidase participates in cell-wall reinforcement and is involved in the final steps of lignin biosynthesis and the cross-linking of the cell-wall protein.[27] Our results showed a significant accumulation of peroxidase in melons by harpin treatment (Figure 2A), which could increase the resistance against pathogens by accelerating the reinforcement of the cell wall. The fungus establishes and spreads in the intercellular spaces of parenchyma cells, and cell-wall fortification could limit its progression. Furthermore, it was also found that cell walls could be thickened by harpin treatment in our study (Figure 4), which was associated with the activation of peroxidase and the accumulation of phenol, H_2O_2, and lignin contents. In the presence of peroxidase, H_2O_2 can be involved in the oxidation of phenolic compounds and the enhancement of the physical barrier in the cell wall to arrest the development of pathogens in the infected site.[27] Meanwhile, lignin is a rigid and hydrophobic polymer usually present in the cell wall. Plant cells were surrounded by complex composite walls that are important preformed barriers to pathogen infection.[28] Wagstaff et al.[29] found that modification of cell-wall properties in lettuce can inhibit infection by bacteria. However, there are several other possible mechanisms that may contribute to cell-wall thickening. For example, inhibition of the disassembly of the cell wall in fruits by alternation of some cell-wall-degrading enzymes could also reinforce the cell-wall structure.[28]

β-1,3-Glucanase and chitinase, as two kinds of pathogenesis-related proteins, play a very important role in the reaction between host and pathogen. The two enzymes, which hydrolyze polymers of fungal cell walls, are thought to be involved in plant defense mechanisms against fungal infection.[30] Therefore, it is possible that the significant accumulation of β-1,3-glucanase (Figure 2C) and chitinase (Figure 2D) was an indication of an induction of overall resistance against the pathogen. Enhancements in activities of defense enzymes, which are related to resistance by harpin treatment, were also reported comprehensively in pears[8] and melons.[1]

Because the latent infection was controlled by application of harpin before harvest, postharvest decay incidence of muskmelon can be significantly inhibited (Table 1). Meanwhile, in our present studies, it is also found that harpin had no negative effect on the qualities of fruit (Table 1). On the one hand, some qualities including general appearance and firmness can be improved, which may be attributed to the enhancement of the cell wall. However, on the other hand, the total soluble solids content of fruit has not been increased or decreased by harpin.

In conclusion, the present result has demonstrated that harpin has an ability of controlling latent infection in melon fruit. The chemical has also an ability of inducing disease resistance, and the increase of this effect can be obtained with more harpin treatments before harvest. Because harpin has no direct effect on the pathogen but activates systemic-acquired resistance mechanisms,[1] harpin spray could be evaluated as a new strategy for preventing latent infection in fruit.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-931-7632-360. Fax: +86-931-7631-201. E-mail: beyang62@163.com.

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