Phenylpropanoid metabolism enzyme activities and gene expression in postharvest melons inoculated with *Alternaria alternata*

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**Abstract**

This study explored the mechanism of melon resistance to *Alternaria alternata* (*A. alternata*) infection in Jiashi and 86-1 melons. Melons were inoculated with *A. alternata* and the change in lesion diameter was measured. The changes in cinnamic acid-4-hydroxylase (C4H), phenylalanine ammonia lyase (PAL), and 4-coumaric acid coenzyme A ligase (4CL) activity and gene expression were studied in the pericarp tissues of Jiashi and 86-1 melons. The lesion diameter was smaller in Jiashi melon than in 86-1 melon, and the pericarp lesions were smaller than pulp lesions, indicating that Jiashi melon can resist *A. alternata* infection better than 86-1 melon. After inoculation with *A. alternata*, the C4H, PAL, and 4CL activities of Jiashi and 86-1 melons peaked in the middle and late storage period, and the peak was higher in Jiashi melons. The gene expression changes were consistent with the enzyme activity. The C4H, PAL, and 4CL gene expression was significantly higher in Jiashi melon pericarp than in 86-1 melon, and the C4H, PAL, and 4CL activities in Jiashi melon were positively correlated with their gene expression, confirming the role of phenylpropanoid metabolism enzymes in resistance to *A. alternata*.

**Keywords:** Melon, *Alternaria alternata*, Disease resistance, Phenylpropanoid metabolism
pathway, through the catalysis of C4H, trans-cinnamic acid can further generate p-coumaric acid [16]. 4CL is the last step in the phenylpropanoid metabolism pathway, which catalyzing various hydroxycinnamic acids to generate corresponding thioesters. These thioesters are at the branch point of the phenylpropanoid metabolism pathway and various end product specific synthesis pathways [17].

Xinjiang Jiashi melon is more resistant to infection in storage. Researchers in the early stage have summarized the pathogenic mechanism of A. alternata through a lot of work, that is, the formation process of toxins, the mechanism of action of degrading enzymes in the cell wall, and changing the pH value in the storage environment [18–21]. When fruits resist infection by pathogens, such as black spot caused by A. alternata, pink rot caused by Rhodotorula rosea, as well as Fusarium oxysporum, the wound will form special components to make the structure heal. The resistance on fruits involves primary metabolism, secondary metabolism and signal transduction [22, 23]. Among them, the researchers concluded that the phenylpropane pathway including C4H, PAL and 4CL are the main disease resistance-related enzymes. Previous studies showed that increases in activities of C4H, PAL and 4CL, played key roles in inducing disease resistance, which contributed to the enhancement of disease resistance against pathogen invasion, and subsequently suppressed disease development and prolonged the shelf-life of harvested fruit [13, 24–26].

In this study, Jiashi and 86-1 melons were inoculated with A. alternata to study their resistance to infection. The change in lesion diameter was measured and the changes in C4H, PAL, and 4CL activity in the pericarp tissues were quantified. The changes in the expression of these enzymes shed light on the physiological changes that occur in infected melons and provide a foundation for studying melon defense mechanisms during A. alternata infection.

Methods

Chemicals and reagents

All reagents used in this work were of analytical reagent grade or better. Sodium borate decahydrate, boric acid, PVPP-K30, Anhydrous ethanol, Isopropyl alcohol and chloroform were obtained from Tianjin Fuyu Fine Chemical. L-phenylalanine, β-mercaptoethanol was obtained from Solarbio. Ethylenediamine tetraacetic acid, DEPC and Agarose were obtained from Sangon Biological Engineering. Column type total RNA extraction and Purification Kit were obtained from TransGen Biotech. Real-time fluorescence quantitative PCR kit were obtained from Qiagen.

Equipments

UV–visible spectrophotometer: TU-1810PC (Beijing), Nucleic acid protein quantifier: K5500 (Beijing), Electrophoresis tank: DYCZ-21 (Beijing), Gel imaging system: 2500 (Shanghai), PCR machine: MyCycler Thermal Cycler (USA), Real Time PCR machine: ABI QuantStudio™ 6 Flex Real-Time (USA).

Melons and treatments

Ripened Jiashi and 86-1 melons were harvested from a melon patch in Jiashi County, Kashgar Prefecture. Jiashi County is located in the southwest of Xinjiang Uygur Autonomous Region (China). Located between 39°16′–40°00′ north latitude and 76°20′–78°00′ east longitude. The annual average temperature is 11.7°C, the annual average rainfall is 64.6 mm, the annual average evaporation is 2051.5 mm, the annual extreme maximum temperature is 41.1°C, and the annual extreme minimum temperature is −22.1°C. All melons were picked in mid-July 2017 and transported to Urumqi (Capital city of Xinjiang Uygur Autonomous Region) by train, and selected for uniformity of size and absence of defects. Jiashi melon weighted about (4.0 ± 0.5) kg, diameter of the largest cross section were (19 ± 1.2) cm, and the soluble solids of melons were about (11.3 ± 1.22)%%. 86-1 melon weighted about (3.5 ± 0.5) kg, diameter of the largest cross section were (18 ± 0.8) cm, and the soluble solids of melons were about (11.0 ± 1.21)%.

The melons need to be washed before inoculated with A. alternata suspension. All melons were washed with running water to clean the dirt and dust from the melons. Then use 2% hydrogen peroxide solution to wash and soak for 30 s for disinfection. Finally rinse melons with sterile water 3 times, and let them dry naturally. A. alternata was isolated from decayed melon fruit and maintained on potato dextrose agar medium (PDA). Conidial suspension of the pathogen was prepared by flooding the 7-day-old culture dishes incubated at 27°C in the dark with sterile distilled water containing 0.01% Tween-20. The spore suspension was adjusted to 1 × 10⁴ CFU mL⁻¹ (conidia per milliliter) with sterile distilled water using a hemo cytometer. Three wounds with a depth of 5 mm (diameter of 3.5 mm) were equally stabbed on the equatorial line of the melons, and 20 μL of the spore suspension was sucked into the wound. The control group melons were inserted into the same amount of sterile water. All melons’ wound was covered with plastic film. After treatment, both inoculated and control melons (80 melons per treatment, three replicates) were kept in boxes and stored at 7°C and 85–90% RH. For the enzyme activities and gene expression, melons samples were
obtained from pericarp (about 6 mm thick) from 5 to 15 mm around the wound were taken at every 3 days and frozen in liquid nitrogen at −80 °C. Lesion diameter on each melon was recorded every 3 days.

Enzyme extraction and assay
C4H enzyme activity was assayed according to Lamb et al. [27]. 1 g of frozen pericarp tissue were fully ground at 4 °C in 5 mL extract A [50 mmol L−1, pH 8.9 Tris–HCl buffer, 15 mmol L−1 β-mercaptoethanol, 4 mmol L−1 MgCl2, 5 mmol L−1 ascorbic acid, 10 μmol L−1 Leupeptin, 1 mmol L−1 PMSF, 0.15% (w/v) PVPP, 10% (w/v) glycerol]. The homogenates were centrifuged at 12,000 × g at 4 °C for 20 min and then got the supernatant as the crude C4H enzyme extract. Reaction system: 0.8 mL C4H enzyme extract, 2.2 mL buffer A [2 μmol L−1 trans-Cinnamic acid, 50 mmol L−1 pH 8.9 Tris–HCl buffer, 2 μmol L−1 NADPNa2, 5 μmol L−1 G-6-pNa]. The reference was the extract without enzyme (adding 0.8 mL distilled water). Measured by the absorbance change at 340 nm, and the result were expressed in units 0.01 ΔOD340 h−1 g−1 Fresh Weight (FW).

For PAL assay, the method of Liu et al. was followed [28]. 1 g of frozen pericarp tissue were fully ground at 4 °C in 3 mL 0.1 mol L−1, pH 8.8 boric acid buffer [10% (w/v) PVPP, 1 mmol L−1 EDTA, 50 mmol L−1 β-mercaptoethanol]. The homogenates were centrifuged at 15,000 × g for 30 min at 4 °C. The supernatant was collected and immediately used for PAL enzyme activity determination. Reaction system: 0.2 mL PAL enzyme extract, 2 mL mixed liquids (0.02 mol L−1 L-type phenylalanine solution was prepared with boric acid buffer). When reaction started 30 min at 30 °C immediately added 200 μL, 6 mol L−1 HCl to stop the reaction. The reference was the extract without enzyme (adding 0.2 mL distilled water). The optical density was measured at 290 nm. PAL activity was expressed in units 0.01 ΔOD290 h−1 g−1 FW.

4CL enzyme activity was measured according to the method of Zhu et al. with some modifications [29]. 1 g of frozen pericarp tissue were fully ground at 4 °C in 3 mL 0.2 mol L−1 pH 8.0 Tris–HCl buffer [25% glycerol, 0.1 mol L−1 DTT]. The homogenates were centrifuged at 15,000 × g for 20 min at 4 °C. The supernatant was collected and immediately used for 4CL enzyme activity determination. Reaction system: 0.45 mL 15 μmol L−1 Mg2+ (MgSO4 or MgCl), 0.15 mL 5 μmol L−1 p-coumaric acid, 0.15 mL 50 μmol mL−1 ATP, 0.15 mL 1 μmol mL−1 CoA, 0.5 mL 4CL enzyme extract. The reaction continued 10 min at 40 °C, then measured by the absorbance change at 333 nm. The reference was the extract without p-coumaric acid. 4CL activity was expressed in units 0.01 ΔOD333 h−1 g−1 FW.

RNA extraction and first strand cDNA synthesis reaction
Total RNA was isolated from pericarp tissue on Jiashi and 86-1 melons collected and frozen in liquid nitrogen immediately every 3 days, using the protocol from the Tranzol Up (TransGen Biotech, ET111, Beijing, China). Specially, total RNA was isolated from liquid nitrogen-frozen pericarp tissue (0.5 g) by the addition of 1 mL of Trizol and added 200 μL chloroform mixed well. Above liquid were centrifuged at 12,000 × g for 15 min at 4 °C. The supernatant was collected and added isopropanol, centrifuged at 12,000 × g for 15 min at 4 °C. The supernatant was drained, followed by the addition 75% ethanol. The RNA was precipitated for 1 h using RNase-free water. All samples of total RNA were denatured an electrophoresed in 1.8% (w/v) agarose gels. Used the protocol from the TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, AT311, Beijing, China). Prepared according to the reaction system in the microtube, 25 °C for 10 min, 42 °C for 30 min, 85 °C for 5 s.

Real-time fluorescence quantitative PCR test
PCR amplification and quantitative analysis were performed in a Quanti Nava SYBR Green Kit (QIAGEN, 208054, Beijing, China). Reaction system includes 5 μL 2 × SYBR Green Select Mix, 0.7 μL Forward Primer, 0.7 μL Reverse Primer, 0.05 μL ROX, 1 μL cDNA, and RNase-free Water L. Followed by 40 cycles of repeated denaturation (5 s at 95 °C) and annealing/extension (60 s at 65 °C).

Statistical analysis and experiment replicates
All data were collected and analyzed by one-way analysis of variance (ANOVA) using the statistical software of SPSS 23 for Windows (SPSS Inc., Chicago, IL, USA) using a general linear model. Mean separations were performed using the least significant difference method (LSD test), and a difference at the 1% level was considered as significant. Each experiment had three replicates and all experiments were run twice with similar results.

Results
Lesion diameter
As the duration of storage increased, the disease of Jiashi and 86-1 melons gradually worsened (Fig. 1). In the late stage, the pericarp and pulp lesions blackened. As the lesion diameter increased, the pericarp tissue appeared sunken and soft, and brown water-stained edges gradually appeared around the pulp lesions, indicating that the lesion spread from the pericarp to the pulp.

On day 12, both kinds of melon began to show black lesions (Fig. 2). The diameters of the lesions in the
pericarp and pulp gradually increased, although the pericarp lesions were always larger than the pulp lesions. Compared with 86-1 melon, the Jiashi melon lesion diameter increased relatively slowly. On day 30, the diameter of the lesions in the inoculated pericarp and pulp of 86-1 melon was 1.14 and 1.06 times that of the respective Jiashi melon lesions. Although the pericarp and pulp of inoculated Jiashi and 86-1 melons began to show disease spots on the same day, the lesion diameters remained smaller in Jiashi melons. After inoculation with *A. alternata*, the order of lesion diameter of the two melons was as follows: infected Jiashi melon pericarp < infected 86-1 melon pericarp < infected Jiashi melon pulp < infected 86-1 melon pulp. Therefore, Jiashi melon could better resist *A. alternata* infection than 86-1 melon. The pericarps of the two melons significantly (P < 0.05) inhibited the increase in *A. alternata* lesion diameter compared with the pulp.

**C4H, PAL, and 4CL activities**

The C4H activity was greater in infected Jiashi melons than in control Jiashi melons, with the peak C4H activity occurring 9 days earlier in the former. During days 24–30, the C4H activity of infected Jiashi melon was lower than in the control. The peak C4H activity in infected 86-1 melon occurred on day 24, and its activity exceeded that of infected Jiashi melon only on this day (Fig. 3A).

There was no significant difference in PAL activity between infected and control Jiashi melons in the early stage of storage, but the difference became significant when the activity peaked on day 21 (P < 0.01). The PAL activity of infected 86-1 melon fluctuated significantly throughout the storage period, increasing initially and then decreasing. The PAL activity of infected 86-1 melon also peaked on day 24, although the PAL activity peak of infected Jiashi melon was 4.52 times that of infected 86-1 melon (P < 0.01) (Fig. 3B).

As Fig. 3C shows, the 4CL activity of both Jiashi and 86-1 melons increased initially and then decreased during storage. The 4CL activity of infected Jiashi melon peaked on day 21; the 4CL activity of infected 86-1 melon did not change much in the early storage period, while its activity also peaked on day 21, and the peak value was higher than the control.
PCR products

PCR products matching the target C4H, PAL, and 4CL genes and internal reference GAPDH gene were identified. The fluorescence signals of the four genes first increased and then decreased with increasing temperature. When the temperature rose to the DNA dissolution temperature, the fluorescence signal strengthened and peaked. There was no primer–dimer formation, the fluorescence quantitative PCR amplification product was specific, and the primer specificity was strong.

C4H, PAL, and 4CL gene expression

As shown in Fig. 4A, the relative expression of the C4H gene in Jiashi melon during storage increased, peaking on day 12, and then decreased. Compared with the control, the relative C4H expression in inoculated Jiashi melon decreased slowly in the late storage period and it was relatively high. In 86-1 melon, the C4H gene expression fluctuated during storage, first decreasing, then increasing, and finally decreasing; it also peaked on day 12.

During storage, the relative expression of the PAL gene in Jiashi melon increased initially and then decreased (Fig. 4B). The PAL expression in inoculated Jiashi melon peaked on day 24 compared with day 12 in control melons, and the peak in the inoculated Jiashi melon was significantly (P<0.01) higher. In inoculated 86-1 melon, the PAL expression fluctuated throughout the storage period, peaking on day 24, and continued to be high until the end of storage. The PAL expression peaked earlier in the control 86-1 melon and the expression was lower than in the inoculated melon.

During storage, the relative 4CL expression in Jiashi melon decreased initially, then increased, and finally decreased (Fig. 4C). The increase was faster in inoculated Jiashi melon than the control and peaked on day 21. In 86-1 melon, the 4CL expression decreased initially and...
then increased during storage; the expression was greater in inoculated 86-1 melon than in the controls, especially late in storage.

**Correlation between phenylpropanoid metabolic activity and melon gene expression**

Used SPSS software ([version 23]; SPSS Inc., Chicago, IL, USA) to analyse statistical. The correlation between phenylpropanoid metabolic activity and the relative gene expression in infected Jiashi and 86-1 melons was compared. The phenylpropanoid metabolism enzyme activities of inoculated Jiashi melons were positively correlated throughout the storage period, and the activities of C4H (P < 0.01, sig = 0.826) and 4CL (P < 0.01, sig = 0.817) were significantly positively correlated with the relative gene expression. The PAL and 4CL activities of inoculated 86-1 melons were positively correlated with their relative gene expression levels throughout storage, while the C4H activity (sig = −0.138) was negatively and non-significantly correlated (Table 1).

**Discussions**

Disease resistance in plants is associated with activation of a wide array of defense responses that slow down or halt infection at certain stages of the host–pathogen interaction. The defense mechanisms include preexisting physical and chemical barriers that interfere with pathogen establishment [30]. Our results clearly showed that in terms of variety, Jiashi melons have better ability to resist *A. alternata* infection than 86-1 melons. From the
structural point of view, the pericarp of melons can resist A. alternata infection better than the pulp. The pericarp of the melon is covered with a cuticle, which is the first natural barrier against germs [31]. Most pathogens can invade plants through natural pores (such as stomata, lenticels, water holes, nectaries, etc.) and epidermal wounds (such as insect wounds, bruises, cuts, saws, frost-bite, etc.) in the plant epidermis [32]. Seen from the surface, the color of Jiashi melons pericarp were dark green with no or little netting. However, the pericarp of 86-1 melons were mixed with yellow and green, and the whole fruits were covered with netting. We speculate that the excessive netting of the 86-1 melons pericarp makes A. alternata more easily invaded. Studies have described the disease process in detail following A. alternata inoculation: the pericarp becomes dull and brown; spongiform lesions appear in the pulp; and the surrounding tissues blacken and are clearly demarcated from uninfected pulp tissue [33]. Our observations of Jiashi and 86-1 melons inoculated with A. alternata were consistent with this description. When Wenzhou tangerines (Citrus reticulata) were infected with A. alternata, the lesion diameter increased gradually over time and A. alternata toxin accumulated in the fruit; this toxin could also be detected in non-diseased parts [34]. Once the fruit is infected, the pathogen slowly spreads, facilitated by the fruit’s nutrient environment. Even if the surface does not appear to be infected, the pathogen toxin spreads. We found that with increased storage time, the melon pulp gradually developed brown water-stained edges around the lesions. This was likely due to the disease spreading and A. alternata toxin accumulating in this area, affecting the subsequent processing and use of the melons.

The synthetic pathway from phenylalanine to hydroxycinnamic acid and its derivatives is the phenylpropanoid metabolic pathway, which is a very important pathway in plant metabolism. All substances containing the phenylpropanoid framework are directly or indirectly generated through this pathway [35]. Studies have found that there are a variety of enzymes involved in the phenylpropanoid metabolic pathway, including PAL, C4H and 4CL [36, 37]. We found that after the melons were inoculated with A. alternata, the activity of phenylpropanoid metabolism enzymes increased in the pericarp tissue, which plays an important role in protecting melons from pathogenic bacteria. C4H, PAL and 4CL activities increased initially and then decreased during storage. The peak activity of these three enzymes roughly appears in 18–24 days. The enzyme activity peak of infected Jiashi melon is always higher than infected 86-1 melon. This indicates that Jiashi melons have a more active performance in defending against A. alternata infection than 86-1 melons. When melons resist A. alternata infection, the phenylpropanoid metabolic pathway is stimulated, and the related enzyme activity increases to resist the infection, which is an instinctive reaction to resist infection. There are a large number of researchers have reported the importance of phenylpropanoid metabolism enzyme activities in plant growth and development, disease (insect) resistance, stress response and other biological processes [38]. As a key enzyme and rate-limiting enzyme in the metabolic pathway of phenylpropanoid, PAL activity is positively correlated with plant disease resistance. After pathogens infect different resistant varieties, the PAL activity of disease-resistant varieties is much stronger than that of susceptible varieties, and the greater the PAL activity, the stronger the disease resistance of the variety [39]. Relevant studies have found that when wheat is infected with powdery mildew [40] and stalk rust [41], the PAL activity does not change significantly at the initial stage of infection. With the extension of the infection time, the PAL activity continues to rise to the maximum value, and then decreases rapidly. This is consistent with the changes in PAL activity after potato infection with late blight [42], and is also similar to the results of this paper. Phenylpropanoid metabolic pathway can divert large amounts of carbon into the biosynthesis of phenylpropanoid-derived compounds, especially lignin [43].

### Table 1: Correlation analysis between the activity of phenylpropanoid metabolism related enzymes and the relative expression of genes inoculated Jiashi and 86-1 melons

|          | C4H activity | PAL activity | 4CL activity |
|----------|--------------|--------------|--------------|
|          | Jiashi melon | 86-1 melon   | Jiashi melon | 86-1 melon   | Jiashi melon | 86-1 melon   |
| C4H gene | 0.626**      | −0.138       | −0.56        | 0.547        | 0.575        | −0.817**     | 0.226        |
| PAL gene | −            | −            | 0.547        | 0.575        | −            | −            | −            |
| 4CL gene | −            | −            | −            | −            | 0.817**      | −            | 0.226        |

**SPSS software (version 23; SPSS Inc., Chicago, IL, USA) was were performed using the least significant difference method (LSD test), and a difference at the 1% level was considered as significant.**

***Indicate that the difference was extremely significant (P < 0.01) among the treatments (n = 3); *"Indicate didn't compare.
4CL are not only important enzymes for the metabolism of phenylpropanoid, but also key enzymes in the biosynthetic pathway of lignin. The role of lignin is to modify the cell wall and increase the ability of plant cell walls to resist fungal penetration [44]. We speculate that the increase of C4H and 4CL enzymes of the infected melons in this study promoted the synthesis of lignin. After the melons pericarp is lignified, a dense and impenetrable physical barrier is formed. The higher the lignin anabolism, the stronger the melon's disease resistance will be. Jiang et al. found that the phenylpropanoid metabolic activity of potato tubers was better maintained by shallow wounds than by deep wounds; during healing, the shallowly wounded tubers accumulated more lignin and suberin than the deeply wounded tubers [45]. Similarly, pathogen infection promoted the accumulation of flavonoids, total phenols, and lignin in the melon pericarp [46], which would play a same role in the defense against A. alternata infection.

Real-time fluorescent quantitative PCR can be used to explore plant gene functions and regulatory mechanisms [47]. The phenylpropanoid metabolism genes are involved in plant disease resistance. Pathogenic bacteria increased gene expression in plants resisting pathogen infection [48]. In this study, when A. alternata infected melon, C4H, PAL, and 4CL gene expression peaked in the middle and late stages of storage, when the defenses were strongest. The interaction between plant pathogens and pathogens will affect the gene expression of plant-related enzymes. The expression of C4H gene is regulated by a variety of factors, such as light, injury, elicitor, pathogen infection, etc., and it is closely related to the lignification process of plants. By regulating the expression of C4H and 4CL genes, the synthesis of lignin can be affected [49]. The function of C4H was studied and found that during the growth and development of plants and stimulated by various external factors (such as elicitors, fungal infections, mechanical damage, chemical inducers, etc.), the change trend of the mRNA accumulation level of its coding genes was similar to that of PAL, 4CL tends to be the same. The expression of 4CL genes in plants is regulated by development and induction. In addition, 4CL genes are also activated in different plants by external stimuli such as trauma, pathogen infection, ultraviolet radiation, etc. [50]. Julio found that Citrus sinensis inoculated with Xanthomonas citri subsp. citri expressed many disease-resistance genes, but the expression such genes were lowest with severe disease [51]. In our study, the peak enzyme activity occurred 3–6 days earlier in infected Jiashi melon than in infected 86-1 melon, indicating that Jiashi melon has a faster response after being inoculated with A. alternata, while the response of 86-1 melon was slower. This difference may be related to the disease resistance of the melons themselves.

In infected Jiashi melon, there was a positive correlation between phenylpropanoid metabolism enzyme activities and relative gene expression throughout the storage period, while the PAL and 4CL activities were positively correlated with gene expression in infected 86-1 melon. The expression level of the 4CL gene in infected Jiashi melon pericarp and that of the PAL gene in infected 86-1 melon pericarp, were consistent with the peaks of enzyme activity in the middle and late storage periods. This indicated that the changes in molecular expression were closely related to physiological changes, which jointly contributed to the defense against A. alternata infection. Comparing the two melons, the peak of gene expression was higher in infected Jiashi melon than in infected 86-1 melon. This was confirmed after melons were inoculated with Fusarium oxysporum; the PAL and 4CL enzyme activities were greatly increased in melons inoculated with F. oxysporum [52]. A significant difference in 4CL expression between lodging-resistant and -susceptible rape was also found, which showed that plant gene expression was related to the resistance difference [53]. Our correlation analysis showed that the C4H and 4CL activities were significantly positively correlated with the relative gene expression in infected jiashi melon throughout the storage period. While the PAL and 4CL activities in infected 86-1 melons were positively correlated with the relative gene expression, none of the correlations were significant. Therefore, Jiashi melon inoculated with A. alternata can resist pathogen stress more actively than 86-1 melon, confirming that phenylpropanoid metabolism enzymes play a role in resisting A. alternata infection. This may partially explain why Jiashi melon is more resistant to A. alternata than 86-1 melon.

**Abbreviations**

C4H: Cinnamic acid hydroxylase; PAL: Phenylalanine ammonia-lyase; 4CL: 4-Coumaric acid-coenzyme A ligase; A. alternata: Alternaria alternata.

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**Authors’ contributions**

The research was conceptualized and designed by YJB, ZSF, and PM, who also participated in acquiring data and drafting the manuscript. The data analysis and interpretation were done by PM. The study was designed and statistical analysis was carried out by JW. All authors read and approved the final manuscript.

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Available data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

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