Bai *et al. Applied Biological Chemistry* (2021) 64:83
https://doi.org/10.1186/s13765-021-00654-x

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE Open Access

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

Yujia Bai[1,2,3], Zuoshan Feng[1,2,3*], Maerhaba Paerhati[4*] and Jin Wang[2,3]

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

Introduction

Xinjiang is the largest production area for high-quality muskmelon (*Cucumis melo* L.) in the world [1]. Jiashi County in the Kashi area of Xinjiang is known as "Melon Township". Throughout their growth, melons may be infected by *Alternaria alternata* (*A. alternata*) [2]. According to reports, black spot disease is caused by *A. alternata*, which is potentially harmful to melon storage [3–5]. When fruits and vegetables are infected by pathogens, many physiological changes occur [6–9], which leads to a series of pathological changes. The defense ability of fruits and vegetables reflects their disease resistance. When a pathogen infection starts, it produces signals that irritate fruits and vegetables, and toxins start to accumulate slowly in the host [10–12]. Plants respond to bacterial invasion by activating defense responses associated with accumulation of several enzymes and inhibitors, which prevent pathogen infection. The interaction between the pathogen and the host plant induces some changes in cell metabolism, which are mainly changes in enzyme activity [13, 14]. This study focused on the role of phenylpropanoid pathway especially the PAL [EC 4.3.1.24], C4H [EC 1.14.13.11] and 4CL [EC 6.2.1.12] in imparting resistance to *A. alternata*. PAL is the primary enzyme in the phenylpropanoid metabolism pathway, which leads to the conversion of L-phenylalanine to *trans*-cinnamic acid with the elimination of ammonia. It is the key enzyme in the synthesis of several defense-related secondary compounds such as phenols and lignin [15]. C4H has high activity in various tissues of plants. In the second step of the phenylpropanoid metabolism

*Correspondence: fengzuoshan@126.com; marhaba524@126.com
[2] College of Food Science and Pharmacy, Xinjiang Agricultural University, Urumqi 830052, Xinjiang, China
[4] Institute of Quality Standards and Testing Technology for Agro-Products, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, Xinjiang, China
Full list of author information is available at the end of the article

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

Riped 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℃, the annual average rainfall is 64.6 mm, the annual average evaporation is 2051.5 mm, the annual extreme maximum temperature is 41.1℃, and the annual extreme minimum temperature is − 22.1℃. 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 rinsen 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 ℃ in the dark with sterile distilled water containing 0.01% Tween-20. The spore suspension was adjusted to 1×10^4 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 ℃ 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 ℃. 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 ℃ in 5 mL extract A [50 $mmolL^{-1}$, pH 8.9 Tris–HCl buffer, 15 $mmolL^{-1}$β-mercaptoethanol, 4 $mmolL^{-1}$ $MgCl_2$, 5 $mmolL^{-1}$ ascorbic acid, 10 $\mu molL^{-1}$Leupeptin, 1 $mmolL^{-1}$ PMSF, 0.15% (w/v) PVP, 10% (w/v) glycerol]. The homogenates were centrifuged at 12,000×*g* at 4℃ 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 $\mu molL^{-1}$ trans-Cinnamic acid, 50 $mmolL^{-1}$ pH 8.9 Tris–HCl buffer, 2 $\mu molL^{-1}$ $NADPNa_2$, 5 $\mu molL^{-1}$ 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 $0.01\triangle OD_{340}{\cdot}h^{-1}{\cdot}g^{-1}$ 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℃ in 3 mL 0.1 $molL^{-1}$, pH 8.8 boric acid buffer [10% (w/v) PVPP, 1 $mmolL^{-1}$ EDTA, 50 $mmolL^{-1}$ β-mercaptoethanol]. The homogenates were centrifuged at 15,000×*g* for 30 min at 4 ℃. 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 $molL^{-1}$ L-type phenylalanine solution was prepared with boric acid buffer). When reaction started 30 min at 30 ℃ immediately added 200 µL, 6 $molL^{-1}$ 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 $0.01\triangle OD_{290}{\cdot}h^{-1}{\cdot}g^{-1}$ 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℃ in 3 mL 0.2 mol L^{-1} pH 8.0 Tris–HCl buffer [25% glycerol, 0.1mol L^{-1} DTT]. The homogenates were centrifuged at 15,000×g for 20 min at 4℃. The supernatant was collected and immediately used for 4CL enzyme activity determination. Reaction system: 0.45 mL 15 $\mu molL^{-1}$ Mg^{2+} (Mg_2SO_4 or MgCl), 0.15 mL 5 µmol mL^{-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℃, then measured by the absorbance change at 333 nm. The reference was the extract without p-coumaric acid. 4CL activity was expressed in units $0.01\triangle OD_{333}{\cdot}h^{-1}{\cdot}g^{-1}$ 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 TransZol 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 Trlzol and added 200 µL chloroform mixed well. Above liquid were centrifuged at 12,000×*g* for 15 min at 4℃. The supernatant was collected and added isopropanol, centrifuged at 12,000×*g* for 15 min at 4℃. 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 delectrophoresed 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 systemin the microtube, 25℃ for 10 min, 42℃ for 30 min, 85℃ 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 40cycles 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

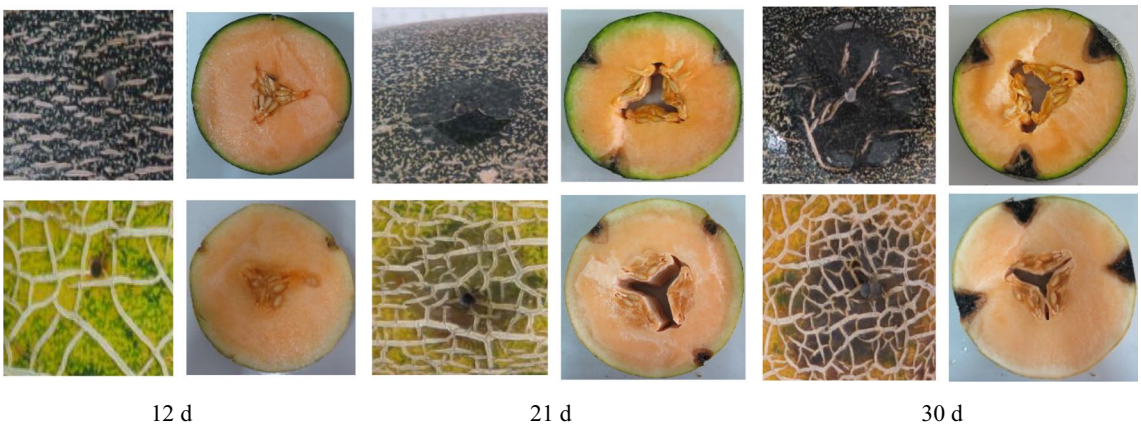

Fig. 1 Diameter changes of lesion diameter in melon fruit inoculated with *A. alternata* (The upper row was Jiashi melon, the lower row was 86-1 melon). Jiashi and 86-1 melons were cleaned after harvested and dried in air. Afterwards, Jiashi and 86-1 melons were inoculated with *A. alternata* (1×10^4 conidia per milliliter) or sterile distilled water (control) and storage at 7 ℃, 85–90% RH

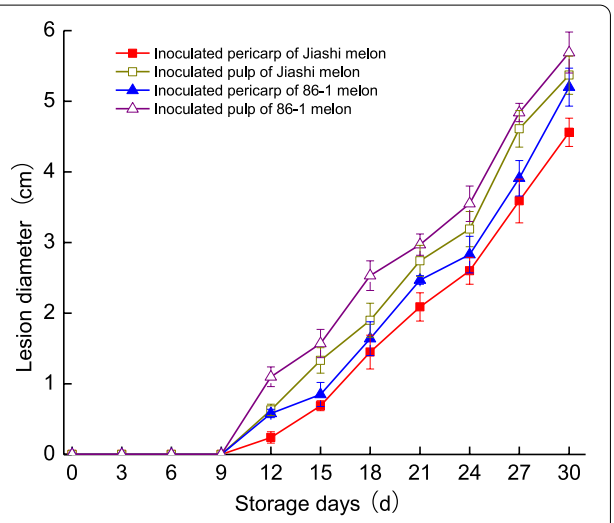

Fig. 2 Effects of *A. alternata* infected on the lesions diameter of two melons. Jiashi and 86-1 melons were washed after harvested and dried in air. Afterwards, Jiashi and 86-1 melons were inoculated with *A. alternata* (1×10^4 conidia per milliliter) or sterile distilled water (control) and storage at 7 ℃, 85–90% RH. Each point represents the mean of three replicates

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 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.

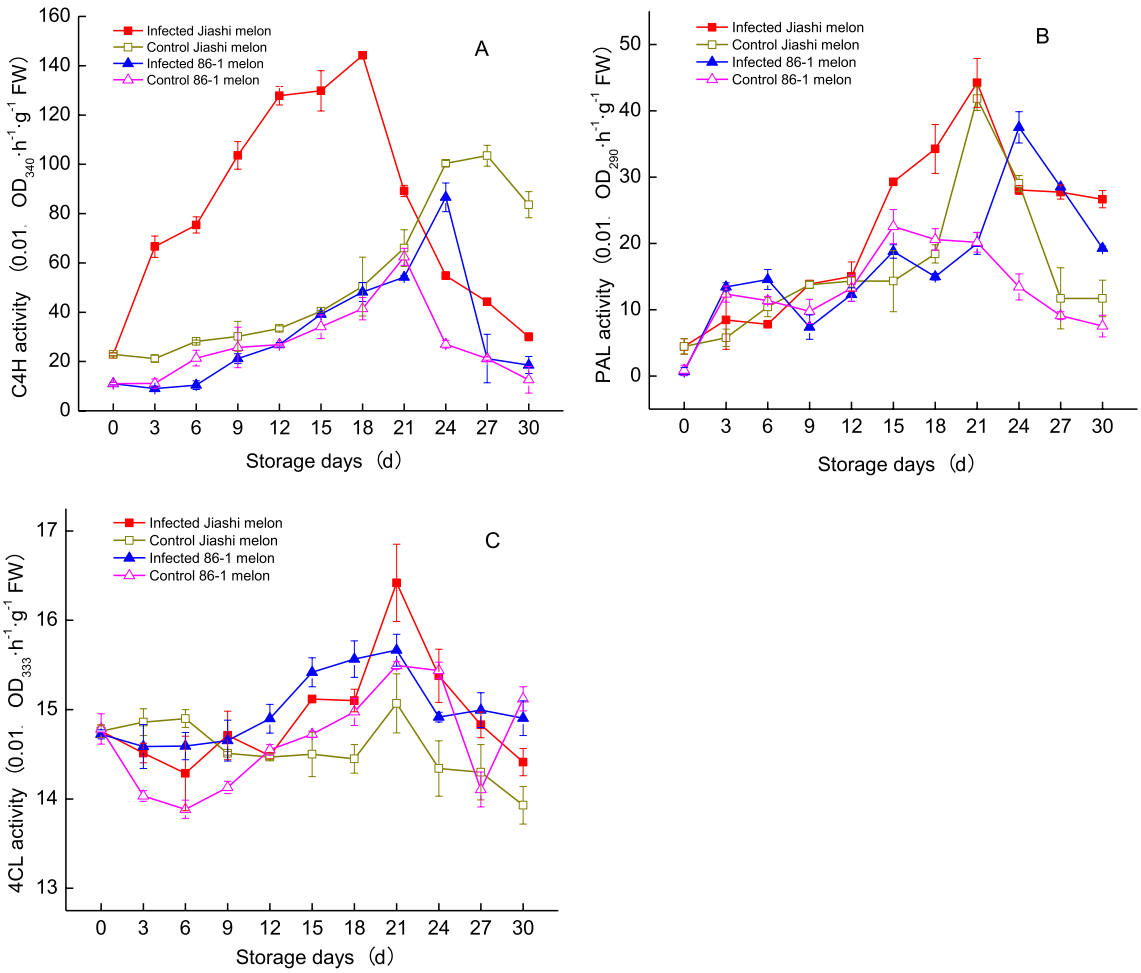

Fig. 3 Effects on C4H (**A**), PAL (**B**) and 4CL (**C**) activities in Jiashi and 86-1 melons infected with *A. alternata*. Jiashi and 86-1 melons were washed after harvested and dried in air. Afterwards, Jiashi and 86-1 melons were inoculated with *A. alternata* (1×10^4 conidia per milliliter) or sterile distilled water (control) and storage at 7 ℃, 85–90% RH. Each point represents the mean of three replicates

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

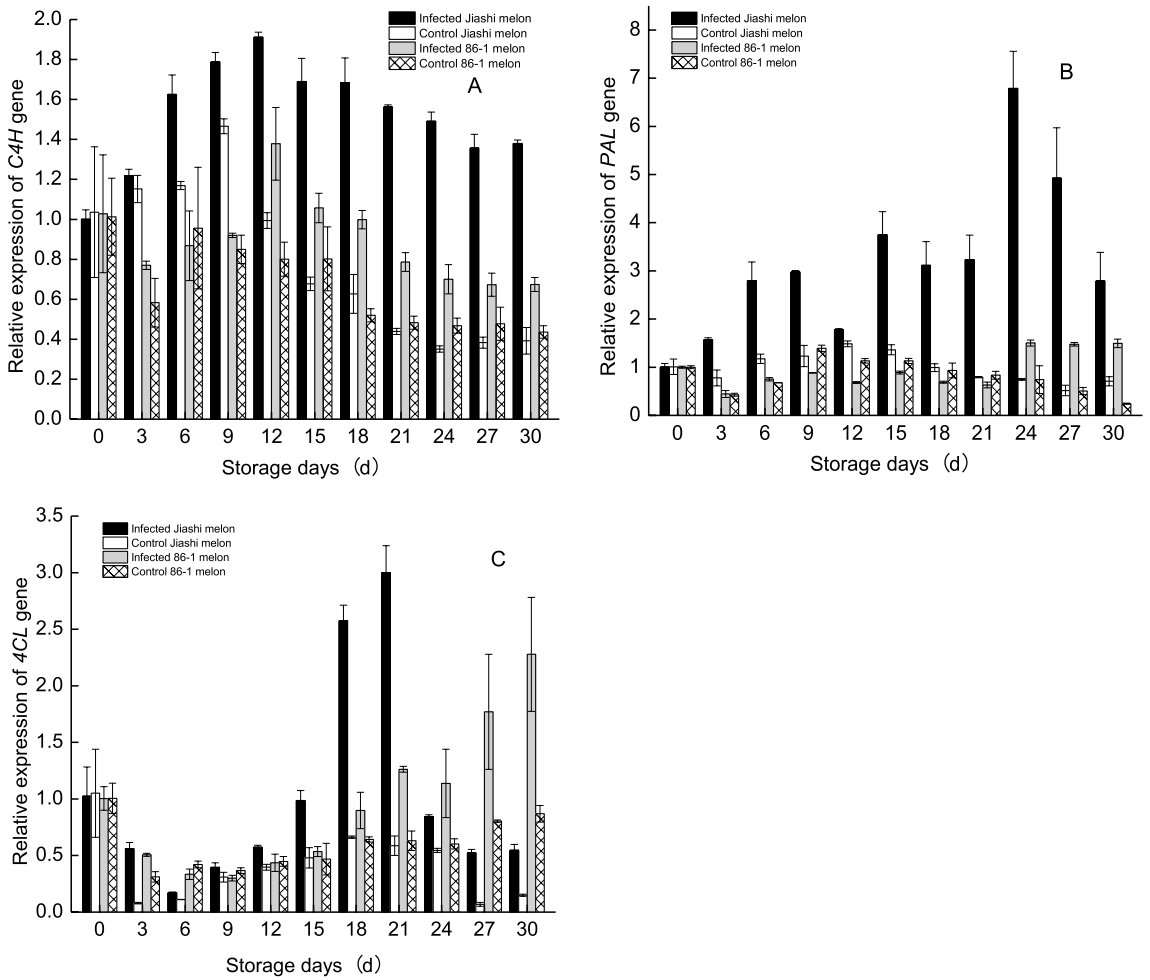

Fig. 4 Changes of *C4H* (**A**), *PAL* (**B**) and *4CL* (**C**) gene relative expression in Jiashi and 86-1 melons after infected with *A. alternata*. Jiashi and 86-1 melons were washed cleaned after harvested and dried in air. Afterwards, Jiashi and 86-1 melons were inoculated with *A. alternata* (1×10^4 conidia per milliliter) or sterile distilled water (control) and storage at 7℃, 85–90% RH. Each column represents the mean of three replicates. PCR amplification and quantitative analysis were performed in aQuantiNava SYBR Green Kit (QIAGEN, 208054, Beijing, China)

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

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	0.826**	− 0.138	–	–	–	–
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

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, frostbite, 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 (*Citrusreticulata*) 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]. C4H and

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 *Fusariumoxysporum*; 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*.

Acknowledgements

Thank you for your help and support from the Crop Science Xinjiang Agricultural University Postdoctoral Programme in terms of experimental conditions.

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 YJB. Statistical analysis was carried out by JW. All authors read and approved the final manuscript.

Funding

Funds for this study were provided by the 61st Batch of General Programs of the China Postdoctoral Science Foundation "Postdoctoral Talent Funding Program in Western China" in 2017 (Grant number: 2017M613303XB).

Availability of 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.

Author details
[1]Crop Science Xinjiang Agricultural University Postdoctoral Programme, Xinjiang Agricultural University, Urumqi 830052, Xinjiang, China. [2]College of Food Science and Pharmacy, Xinjiang Agricultural University, Urumqi 830052, Xinjiang, China. [3]Xinjiang Key Laboratory for Postharvest Science and Technology of Fruits, Xinjiang Agricultural University, Urumqi 830052, Xinjiang, China. [4]Institute of Quality Standards and Testing Technology for Agro-Products, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, Xinjiang, China.

Received: 1 June 2021 Accepted: 22 November 2021
Published online: 07 December 2021

References

1. Maerhaba P, Bai YJ, Li M, Li J, Zhang MM, Wang J, Feng ZS (2019) Effects of pre-storage illumination treatment on the storage quality and some defense enzyme activities of Jiashi melon. Sci Technol Food Ind 40:271–275
2. Wang ZY, Yao T, Fan YW, Zhang Y, Wang YS (2018) Morphological characterization and rDNA ITS sequence analysis of filamentous fungi isolated from wallace melon. Food Sci 39:112–118
3. Ge YH, Bi Y, Li X, Li M (2018) Induces resistance against Fusarium and Pink Rots by Acibenzolar-S-Methyl in harvested muskmelon (*cv*. Yindi). Agric Sci China 7:58–64
4. Zhao J, Bao S, Ma G, Wu X (2016) Characterization of *Alternaria* species associated with muskmelon foliar diseases in Beijing municipality of China. J Gen Plant Pathol 82:29–32
5. Zhao LH (2020) Study on the mechanism and control method of Hami melon rot caused by *Alternaria alternata* and *Trichothercium roseum*. Dissertation, Shenyang Agricultural University
6. Maerhaba P, Bai YJ, Wang J, Zhu WT, Adilai T, Feng ZS (2018) Infection of *Alternaria alternata* in postharvest Jiashi melon and 86-1 melon. Food Mach 34:135–140
7. Li M, Feng ZS, Maerhaba P, Zhang MM, Wang HR, Xia X et al (2018) The relationship between the difference of the resistance capacity and the reactive oxygenspecies related metabolism in muskmelon fruit inoculated with *Alternaria alternata*. Sci Technol Food Ind 39:272-277+285
8. Yan XL (2020) Effects of *Fusariumoxysporum* infection on physiological metabolism of postharvest asparagus and substance metabolism of resistance to disease in asparagus. Dissertation, Jiangsu University
9. Wang J, Bai YJ, Feng ZS, Zhu WT, Zeng YRK, Jiang LY (2021) Study on the gene expression and enzyme activity of the Pathogenesis-related proteins in postharvest muskmelon infected by *Alternaria alternata*. Food Sci 42(14):94–102
10. Li M, Feng ZS, Zhang MM, Maerhaba P, Wang YH, He Y et al (2018) Resistance difference of muskmelon fruiton infection of *Alternaria alternata*. Sci Technol Food Ind 39:281–286
11. Jiang XJ, Lin HT, Lin MS, Chen Y, Wang H, Lin Y et al (2018) A novel chitosan formulation treatment induces disease resistance of harvested litchi fruit to *Peronophythora litchii* in association with ROS metabolism. Food Chem 266:299–308
12. Tao X, Mao L, Li J, Chen J, Lu W, Huang S (2016) Abscisic acid mediates wound-healing in harvested tomato fruit. Postharvest Biol Technol 118:128–133
13. Wang B, Jiang H, Bi Y, He XF, Prusky D (2019) Preharvest multiple sprays with sodium nitroprusside promote wound healing of harvested muskmelons by activation of phenylpropanoid metabolism. Postharvest Biol Technol 158:110988
14. Zheng XY, Wang TL, Zhang JR, Jiang H, Wang B, Bi Y (2019) Using chlorine dioxide treatment to promote wound healing of postharvest muskmelon fruit. Sci Agric Sin 52(3):133–141
15. Wang Y, Yang Q, Jiang H, Wang B, Bi Y, Li YC et al (2020) Reactive oxygen species-mediated the accumulation of suberin polyphenolics and lignin at wound sites on muskmelons elicited by benzo (1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester. Postharvest Biol Technol 170:111325
16. Obianom C, Romanazzi G, Sivakumar D (2019) Effect of chitosan treatment on avocado postharvest diseases and expression of phenylalanine ammonia-lyase, chitinase and lipoxygenase genes. Postharvest Biol Technol 147:214–221
17. Legay S, Guerriero G, André C, Guignard C, Cocco E, Charton S (2016) MdMyb93 is a regulator of suberin deposition in russeted apple fruit skins. New Phytol 212:977–991
18. Zheng FL, Zheng WW, Li LM, Li L, Pan S, Liu M et al (2017) Chitosan controls postharvest decay and elicits defense response in kiwifruit. Food Bioprocess Technol 10:1937–1945
19. Lee HB, Patriarca A, Magan N (2015) *Alternaria* in food: ecophysiology, mycotoxin production and toxicology. Mycobiology 43:93–106
20. Hajnal E, Jasna M, Bagi F, Dejan O, Budakov D, Kos J et al (2019) Effect of wheat milling process on the distribution of Alternaria toxins. Toxins 11(3):139
21. Heryani H, Putra MD (2017) Kinetic study and modeling of biosurfactant production using *Bacillus sp*. Electron J Biotechnol 27(C):49–54
22. Zhang JR, Wang B, Jiang H, Wang Y, Li X, Si M et al (2018) Postharvest benzothiadiazole treatment promotes wound healing in pear Fruit. Food Sci 39:190–195
23. Jiang H, Wang B, Ma L, Zheng XY, Gong D, Xue HL et al (2019) Benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid s-methyl ester (BTH) promotes tuber wound healing of potato by elevation of phenylpropanoid metabolism. Postharvest Biol Technol 153:125–132
24. Li Z, Xue S, Xu X, Bin W, Zheng XY, Li BJ (2021) Preharvest multiple sprays with chitosan accelerate the deposition of suberin poly phenolic at wound sites of harvested muskmelons. Postharvest Biol Technol 179(12):111565
25. Anita H, Simona DF, Michele S, Laura L, Alessandra B (2017) *Fusarium oxysporum f. Sp.* melonis-melon interaction: effect of grafting combination on pathogen gene expression. Eur J Plant Pathol 194(4):787–796
26. Wei X, Guan W, Yang Y, Shao YL, Mao LC (2021) Methyl jasmonate promotes wound healing by activation of phenylpropanoid metabolism in harvested kiwifruit. Postharvest Biol Technol 175:111472
27. Lamb CJ, Rubery PH (1975) A spectrophotometric assay for *trans*-cinnamic acid 4-hydroxylase activity. Anal Biochem 68:554–561
28. Liu HX, Jiang WB, Bi Y, Luo YB (2005) Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by *Penicillium expansum* and enhances activity of fruit defense mechanisms. Postharvest Biol Technol 35:263–269
29. Zhu MH, Ou Yang GC, Xue YL (1990) Changes of G6PD, PAL, 4CL, PO activity and lignin content in cucumber during immune induction. J Shanghai Agric 2:23–27
30. Bacete L, Mélida H, Mie DSE, Molina A (2017) Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. Plant J 93:614–636
31. Mishra S, Singh A, Sharma R, Gogna N, Dorai K (2020) NMR-based metabolomic profiling of the differential concentration of phytomedicinal compounds in pericarp, skin and seeds of Momordica charantia (bitter melon). Nat Product Res. 2020. https://doi.org/10.1080/14786419.2020.1762190
32. Yabes JE, Vij S, Arulmozhi P, Rajalakshmi S (2018) Screening the antimicrobial potential of twelve medicinal plants against venereal diseases causing pathogens. Acta Ecol Sin 39:1–6
33. Chen CK, Wang WS, Gao YH, Feng JQ, Dong CH, Wang DL (2008) Separation and identification of pathogenic fungi from postharvest thick-skinned melon. Storage Process 8:54–56
34. Jiang LY, Gong L, Ge ZX, Liu YY, Ma L, Zhao QY et al (2017) Production and distribution of *Alternaria* mycotoxins in Satsuma mandarin (*Citrus unshiu* Marc.) artificially inoculated with *Alternariaalternate*. Food Sci 38:251–257

35. Patil SV, Kumudini BS (2019) Seed priming induced blast disease resistance in finger millet plants through phenylpropanoid metabolic pathway. Physiol Mol Plant Pathol 108:101428
36. Gouvinhas I, Martins-Lopes P, Carvalho T, Barros A, Gomes S (2019) Impact of *Colletotrichum acutatum* pathogen on olive phenylpropanoid metabolism. Agriculture 9(8):173
37. Jiang H, Wang Y, Wang B, Zhang JR, Zheng XY, Bi Y (2018) A comparison study of healing ability of shallow and deep wounds in potato tubers 'Qingshu 168.' Acta Horticult Sin 45:908–918
38. Barros J, Dixon RA (2019) Plant phenylalanine/tyrosine ammonia-lyases. Trends Plant Sci 25(1):66–79
39. Mirzaee H, Peralta N, Carvalhais LC, Dennis PG, Schenk PM (2021) Plant-produced bacteriocins inhibit plant pathogens and confer disease resistance in tomato. New Biotechnol 63:54–61
40. Yuan HJ, Zeng XQ, Yang QF, Xu QJ, Wang YL, Jabu DZ (2018) Gene coexpression network analysis combined with metabonomics reveals the resistance responses to powdery mildew in Tibetan hulless barley. Sci Rep 8:1–13
41. Hovhannisyan NA, Dulloo ME, Yesayan AH, Knüpffer H, Amri A (2011) Tracking of powdery mildew and leaf rust resistance genes in *Triticum boeoticum* and *T. urartu*, wild relatives of common wheat. Czech J Genet Plant Breed 47(2):45–57
42. Sunny A, Sangha MK, Astha JM (2016) Effect of salicyclic acid on defence related enzymes and late blight in potato. Plant Dis 31:52–58
43. Pascual MB, Jorge EA, De L (2016) Biosynthesis and metabolic fate of phenylalanine in conifer. Front Plant Sci 7:1030
44. Cheng XC, Cui XY, Qin Z, Liu HM, Wang XD, Liu YL (2021) Effect of drying pretreatment methods on structural features and antioxidant activities of Brauns native lignin extracted from Chinese quince fruit. Process Biochem 106:70–77
45. Jiang H, Bi Y, Li CJ, Wang Y, Li SE, Liu YN et al (2017) Comparison of healing ability on potato tuber cultivars "Qingshu No. 168" and "Longshu No. 3." Chin Agric Sci 50:774–782
46. Mascia T, Santovito E, Gallitelli D, Cillo F (2010) Evaluation of reference genes for quantitative reverse-transcription polymerase chain reaction normalization in infected tomato plants. Mol Plant Pathol 11:805–816
47. Nikalje GC, Srivastava AK, Sablok G, Pandey GK, Suprasanna P (2017) Identification and validation of reference genes for quantitative real-time PCR under salt stress in a halophyte, *Sesuvium portulacastrum*. Plant Gene 13:18–24
48. Jousset A, Rochat L, Lanoue A, Bonkowski M, Keel C, Scheu S (2011) Plants respond to pathogen infection by enhancing the antifungal gene expression of root-associated bacteria. Mol Plant Microbe Interact 24(3):352–358
49. Tang W, Bai Y, Hu YQ, Wang C, Cui MJ, Zhang WY (2019) Characterization of grape lignin-related genes *PAL*,*4CL*,*CAD* and the role of GA in the berry development. Journal of Nanjing Agricultural University
50. Soltani BM, Ehlting J, Hamberger B, Douglas CJ (2006) Erratum: Multiple cis-regulatory elements regulate distinct and complex patterns of developmental and wound-induced expression of Arabidopsis thaliana *4CL* gene family members. Planta 224(5):1239–1240
51. Julio M, Silva SR, Ferro JA, Varani AM (2020) A genomic and transcriptomic overview of MATE, ABC, and MFS transporters in *Citrus sinensis* interaction with *Xanthomonas citri* subsp. *citri*. Plants 9(6):1–21
52. Liu HJ, Li YP, Liu W, Shao Q, Qi HY (2016) Effects of *Fusariumoxysporum* f. sp. melonis on lignin, activities of lingin-related enzymes and genes expressions of *CmCADs* in Oriental Melon (*Cucumismelo* var. makuwa Makino). Sci Agric Sin 49:2153–2163
53. Liu XT, Wang J, Hou XF, Li Q, Jia DH, Gu YG et al (2019) Progress of functions of lignin and relevant genes in plant lodging resistance. Mol Plant Breed 17:655–662

Publisher's Note