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Analysis of metabolic and transcription levels provides insights into the interactions of plant hormones and crosstalk with MAPKs in the early signaling response of cherry tomato fruit induced by the yeast cell wall

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ABSTRACT

Yeast cell walls (YCW) are promising bio-based elicitors for controlling post-harvest fruit decay. In this study, 1% YCW induction increased the resistance of cherry tomato fruits, reducing disease incidence by 66%. This study aimed to explore the interaction of hormones and crosstalk with MAPKs (mitogen-activated protein kinases) in the early response of resistance regulation in cherry tomato fruits treated with YCW and U0126. We analyzed the temporal changes in hormone content, the expression of critical genes involved in phytohormone biosynthesis, and signal transduction in cherry tomato fruits response to the induction. Results revealed that jasmonic acid (JA) and brassinosteroids (BR) significantly regulated early resistance response in fruit induced by 1% YCW. The salicylic acid (SA) pathway is inhibited by the activation of the JA pathway. JA and SA signaling pathway crosstalk with the MAPK3 pathway. BR plays an essential role in the regulation of fruit resistance. The BR pathway may function independently when JA/SA and MAPK3 pathways are inhibited.

1. Introduction

Fruit, an essential part of the human diet, is prone to decay during storage and transportation, resulting in significant economic losses. Fungal diseases are the leading cause of post-harvest fruit and vegetable loss. Chemical synthetic fungicides are the most crucial control method. However, their long-term use can cause pathogen resistance, high residues, and human health issues (Petriacq et al., 2018). Therefore, there is an increased need to develop safe, environmentally friendly, and inexpensive post-harvest fruit disease and decay control strategies. Inducing fruit disease resistance with bio-based components could be an effective alternative to chemical fungicides and has gained widespread attention (Romanazzi et al., 2016). The response of microbial or pathogen-associated molecular patterns (MAMPs or PAMPs) to activate PAMP-triggered immunity (PTI) is the primary responsibility of the plant defense system. It plays an indispensable role in pathogen-induced immune responses (Pusztahelyi, 2018). Yeast cell walls, composed of polysaccharides (chitin, glucose polymers, and mannoproteins), act as MAMP and induce a defense response in plants (Narusaka et al., 2015). In recent years, yeast cell walls and their components have been widely studied as biological activators to induce post-harvest resistance of pear and tomato to *Penicillium expansum* (Sun et al., 2018) and *Botrytis cinerea* (Guo et al., 2021).

Biological elicitors (antagonistic yeast, cell wall extract components, and metabolites) can participate in plant resistance responses by activating plant-hormone-mediated signal transduction cascades (Angulo et al., 2015). Generally, ISR (induced systemic resistance) is induced by non-pathogen organisms and relies on JA signaling, while pathogen-induced SAR (systemic acquired resistance) is dependent on SA signaling (Xie et al., 2020). JA promotes the production of primary defense-related secondary metabolites and the expression of some pathogenesis-related (PR) proteins (Campos et al., 2014). SA is an essential endogenous signaling molecule in plant immunity (Shigenaga & Argueso, 2016a). BRs also play a crucial role in protecting plants from

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various stresses (such as pathogen invasion). Exogenous brassinolide (BL) enhances tobacco defense against the viral pathogen tobacco mosaic virus (TMV) and fungal pathogen *Oidium sp*. (Nakashita et al., 2003).

Previous studies on hormones have focused on the physiological regulation of plant growth and seed germination under abiotic (cold, drought, and high temperature) and biological stresses (insect pests) (Wani et al., 2016). Recently, the regulation of post-harvest fruit resistance and fruit shelf-life extension by plant hormones has been highlighted. Plant hormones can extend fruit shelf-life by activating the expression of fruit defense genes, repressing the sensitivity of senescence-related phytotropins, stimulating the activity of antioxidant enzymes, and maintaining cell membrane integrity (Xiang et al., 2020). However, there is a lack of comprehensive analysis of plant hormone profiles and resistance regulation mechanisms in post-harvest fruit in response to bio-based compound elicitor induction.

In the early stages of the plant immune response, MAMP/PAMP can trigger the mitogen-activated protein (MAP) kinase signaling cascade, reactive oxygen species (ROS) burst, synthesis, and/or signaling of hormones. Subsequently, the expression of downstream defense-related genes is activated (Lazar et al., 2014). MAPK3 is related to biotic and abiotic stress responses and various developmental processes and is considered a crucial regulator of plant defense signal transduction (Dóczi & Bögre, 2018). Activation of the MAPK cascade alters the phosphorylation state of many substrate proteins, such as the downstream resistance-related transcription factors ERF and WRKY (Zhou et al., 2022), which could directly improve the expression of resistance genes.

The MAPK cascade has been reported to interact with hormones, such as JA, SA, ethylene (ET), and BRs, among which MAPK3 and MKK3-MPK6 (Wang et al., 2013) are involved in JA synthesis and signal transduction. However, the interactions and the crosstalk between hormones and MAPKs could vary with plant species and induction treatment. Our previous study revealed that 1 % YCW could activate MAPK3 to regulate the expression of transcription factors and downstream PR genes, thus promoting fruit disease resistance (Guo et al., 2021). To clarify the regulatory mechanisms that operate under induced resistance conditions, it is essential to characterize phytohormone metabolism, signaling, and crosstalk with MAPKs.

To understand the roles of plant hormones, the interactions of hormones, and crosstalk with MAPK pathway in the early signaling response of cherry tomato fruits induced by 1 % YCW, the following aspects were studied: (1) analyze the roles and interactions of plant hormones in induced resistance response by analyzing the temporal profile of the hormones through targeted metabolomics, along with the temporal profile of the transcriptional expression levels of genes related to hormone signaling pathway; (2) explore the crosstalk and regulatory networks between MAPK3 and hormones in induced resistance response by measuring the levels of major plant hormones and transcription expression levels of hormone synthesis and signal transduction related genes in cherry tomato fruits treated with U0126 (1,4-diamino-2,3-dicyano-1,4-bis (o-amino-phenylmercapto) butadiene).

2. Materials and methods

2.1. Materials

Cherry tomatoes are grown in greenhouses at 24 °C with light for 16 h per day and a light intensity of 2000 Lx. Cherry tomato fruits are harvested at the red maturity stage. Red-ripening cherry tomato fruits (*Lycopersicon esculentum Mill*, Minny Tomato) of uniform size without mechanical damage were selected. The fruits were soaked in 0.1 % (v/v) sodium hypochlorite for 2 min, rinsed thrice and air dried at 25 °C.

Cherry tomatoes were randomly divided into four groups. Each group had three replicates, with each replicate containing 20 fruits. Each fruit was punctured through the peel with a sterile borer to create a wound (5 mm diameter and ~3 mm deep). Each wound of the four fruit groups was treated with 15 μL of (1) sterile distilled water as control, (2) 0.5 % (w/v) YCW, (3) 1 % (w/v) YCW, (4) 2 % (w/v) YCW, respectively. After 3 h, 15 μL of *Botrytis cinerea* suspension (1×10^4 spores mL^{-1}) was added to each wound. After drying at room temperature, fruits were individually packed in plastic boxes and stored at 25 °C and 90 % relative humidity (RH). Disease incidence was recorded at 24, 48, and 72 h after inoculation.

Cherry tomatoes were randomly divided into three groups. The fruit treatment was the same as described above. Each wound of the three fruit groups was treated with 15 μL of (1) sterile distilled water as control, (2) 0.5 % (w/v) YCW, (3) 10 μM U0126 (Sigma-Aldrich, St. Louis, USA), respectively. After treatment, five biological replicates of fruits were sampled from each group at each time point. The (1) and (2) treated fruits were sampled at 0.5, 4, and 24 h, and the (3) treated fruits were sampled at 0.5 and 4 h for targeted metabolomics analysis.

Cherry tomato fruits were randomly divided into four groups and treated for Quantitative Real-time PCR (RT-qPCR) analysis. Three of the four treatments are consistent with the above-mentioned target metabolomics analysis experiments. An additional set of treatments inoculated with 15 μL U0126 combined with 1 % YCW were added. Each group contained 30 fruits sampled at 0, 4, 12, 24, 48, and 72 h. The biological replicates at each time point were replicated five times.

2.2. Targeted metabolite profiling

2.2.1. Standard curve

Standards were obtained from Olchemim (Olomouc, Czech Republic). The standard substance was serially diluted with a methanol aqueous solution. The labeling curve was established using the isotope internal standard method.

2.2.2. Metabolite extraction

Samples were ground in liquid nitrogen. An 80 ± 5 mg sample was placed in a 2 mL centrifuge tube. Subsequently, 50 μL of the internal standard solution and 1 mL of acetonitrile solution were added. The mixture was vortexed for 3 min. The supernatant was extracted in darkness for 12 h (4 °C) and centrifuged at 14,000*g* for 10 min. The supernatant (800 μL) was dried with nitrogen, redissolved in 200 μL acetonitrile–water (1:1, v/v), and centrifuged at 14,000*g* for 10 min. It was further analyzed.

2.3. Chromatography-mass spectrometry analysis

2.3.1. Ultra high performance liquid chromatography conditions

Waters I-Class LC Ultra Performance Liquid Chromatography (Waters, Taunton, Massachusetts (USA)) was used to separate samples. Mobile phase: Liquid A consisted of 0.05 % FA aqueous. Liquid B consisted of 0.05 % FA acetonitrile. Samples were placed in an automatic sampler (4 °C). The column temperature was 45 °C, the flow rate was 400 μL/min, and the sample size was 4 μL. Gradient conditions: 0 min, 98 % A combined with 2 % B; 10 min, 2 % A combined with 98 % B; 11.1 min, 98 % A combined with 2 % B; 13 min, 98 % A combined with 2 % B. A quality control (QC) sample was set in the sample queue for several experimental samples at every interval to verify the stability and repeatability of the system.

2.3.2. Mass spectrometry analysis

The positive and negative ion modes were analyzed using a 5500 QTRAP mass spectrometer (AB SCIEX, Framingham, MA (USA)). 5500 QTRAP ESI source conditions: source temperature 500 °C, ion source Gas1 (Gas1): 45, Ion source Gas2 (Gas2): 45, Curtain gas (CUR): 30, Ion Sapary Voltage Floating (ISVF) −4500 V; multiple reaction monitoring (MRM) model was used to detect ion pairs. Information on the ion pairs of all plant hormones is provided in Supplement 1.

2.4. Mass spectrometry data analysis and processing

The plant hormone content was calculated following the standard curve after processing with MultiQuant software to obtain the peak area and retention time.

2.5. RNA extension and transcriptome analysis

Total RNA was extracted using RNAiso Plus (9108, TaKaRa, Dalian, China) following the manufacturer's instructions with minor modifications. The Colibri Microvolume Spectrometer (Nano Photometer spectrophotometer) measured the concentration and quality. The PrimeScript RT reagent Kit with gDNA Eraser reagent (RR047A, TaKaRa, Dalian, China) was used to synthesize cDNA, according to the manufacturer's instructions.

The Real-time PCR (RT-qPCR) detections were performed using a BIO-RAD CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Singapore) with TB Green Premix Ex Taq (Tli RNase H Plus) using the following reaction conditions: 95 °C for 30 s, 95 °C for 5 s, and 60 °C for 34 s (40 cycles). The melting curve was then obtained. Gene transcription abundances in different induction groups are expressed as fold-changes in multiples relative to 0 h for each time point. Fold changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Supplement 2.

2.6. Statistical analysis

Each measurement was repeated thrice. All experiments were repeated twice. All data presented in this article are the results of a single experiment but represent two independent experiments with similar results. The data were analyzed using SPSS/PC version II.x (SPSS Inc. Chicago, Illinois, USA) using Duncan's multiple range test. Statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. Induction of disease resistance against B. cinerea in cherry tomato fruits by 1 % YCW

As shown in Fig. 1, the disease incidence in the treatment with 1 % and 2 % YCW was significantly lower than that of control ($P < 0.05$) after 24 h. The disease incidence in the treatments with 1 % and 2 % YCW was significantly lower than that in 0.5 % YCW. The incidence was 100 % in the control group, 34 % in the 1 % YCW treatment group, 48 % in the 0.5 % YCW treatment group, and 36 % in the 2 % YCW treatment group at 72 h. Therefore, 1 % YCW treatment was used for subsequent studies.

3.2. Targeted metabolomics analysis of plant hormone profiles in cherry tomato fruit induced by 1 % YCW

Recently, improving the disease resistance of post-harvest fruits to fungal pathogens by inducing resistance has attracted considerable attention. Previous studies have focused on the expression of resistance genes and the activity of antioxidase in the later stages of resistance effect (Zhang et al., 2020). Studies on the resistance response and regulatory network of early signals are lacking. Plant hormones, essential signaling molecules in the early resistance response, participate in regulating plant resistance response and form complex crosstalk in the regulation process to cope with different external stresses (Bari & Jones, 2009). Similar to MAMPs, yeast cell wall extract induced upregulated expression of PR genes, which could be regulated by SA and JA/ET pathways in plants and enhanced plant disease resistance (Narusaka et al., 2015). However, the regulatory network mechanism of hormones in yeast cell wall-induced fruit resistance remains unclear.

To understand the plant hormone metabolism in cherry tomato fruits induced by 1 % YCW, we measured the temporal profile of plant hormones. Fig. 2 displays that 1 % YCW induced the content changes of jasmonates (*cis*-OPDA (12-oxophytodienoic acid), JA), brassinosteroid (BL, CS (castasterone)), ethylene precursor ACC (1-aminocyclopropyl-1-carboxylic acid), and SA in cherry tomato fruits.

The JA and *cis*-OPDA levels were substantially higher than that of the control during the entire period. The *cis*-OPDA content increased and

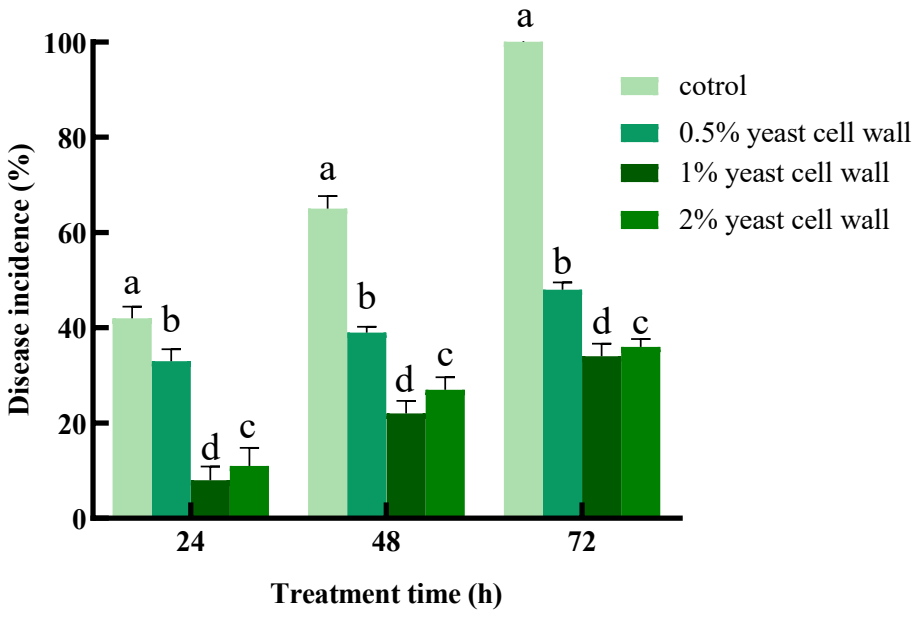

Fig. 1. Effect of 5 %, 1 % and 2 % yeast cell wall on the gray mold incidence in cherry tomato fruits. The bars represent standard errors. The columns with different letters are statistically different according to the Duncan's multiple range test at $P = 0.05$ at each time point.

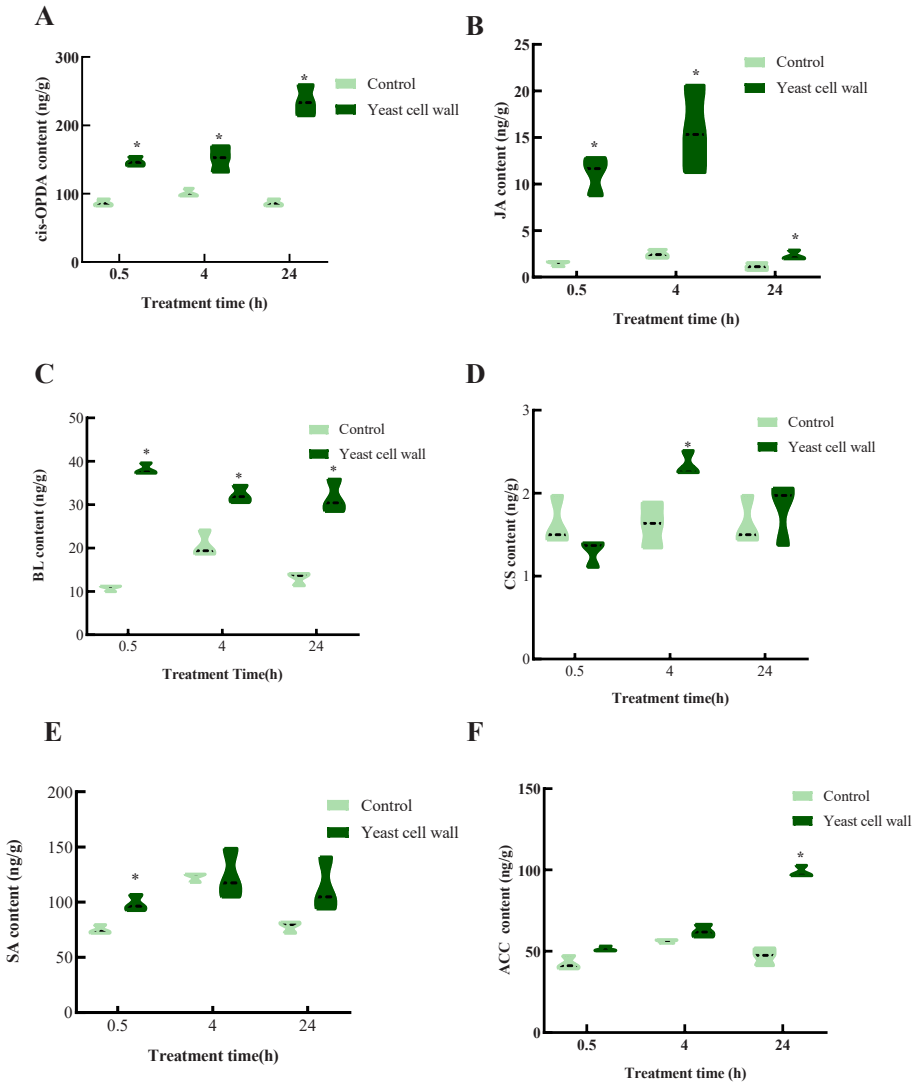

Fig. 2. Plant hormone content in cherry tomatoes after induction with 1 % yeast cell wall at 0.5, 4, and 24 h. (A) *cis*-OPDA, (B) JA, (C) BL, (D) CS, (E) SA, (F) ACC. The asterisk (*) indicates relative transcript levels that were significantly ($P < 0.05$) higher than those of the control.

reached the highest value at 24 h (235.71 ng/g, 2.72 times control), while JA reached the highest at 4 h (15.72 ng/g, 6.35 times control). JA contributes to plant host defense against various pathogenic fungi, such as *Alternaria brassicicola* and *B. cinerea*. Verhage reported that JA could be quickly induced to produce an effective defense response (Verhage et al., 2011). JA synthesis begins with α-linolenic acid. Subsequently, α-linolenic acid is oxidized by 13-lipid oxidase (13-LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC) to generate 12-oxygen-phytodienoic acid (OPDA). Jasmonic acid was obtained by reducing OPDA reductase 3 (OPR3) and triple β-oxidation (Dar et al., 2015). In this study, the rapid accumulation of JA and *cis*-OPDA may play a key role in regulating the resistance response. In brassinosteroids, the BL content peaked at 0.5 h (38.15 ng/g, 3.54 times control) and displayed a downward trend from 0.5 h. However, this was higher than the control during the entire period. The CS content was substantially higher than that of the control at 4 h. BRs enhance the resistance response of plants against pathogen infection. Exogenous application of BRs can reduce the susceptibility of rice to bacterial blight diseases and activate the resistance of *Nicotiana tabacum* to *Tobacco mosaic virus* (TMV) and other pathogens (Nakashita et al., 2003). Castosterone (CS) is the immediate precursor of BL, and its synthesis relies on the canical (CN) pathway. Compared with the control group, the CS content increased at 4 h, while the BL content increased significantly during the entire experimental period. Another CN-independent pathway for BL synthesis is catalyzed by DWF4, DET2, and other enzymes (Ohnishi et al., 2012). The lack of continuous accumulation of CS may be related to the activation of the CN-independent pathway.

There is a synergy between JA and ET in the plant-induced resistance (Lorenzo et al., 2003). As the precursor of ET, ACC is transformed into ET by ACC oxidase (ACO) (Mou et al., 2020). Fig. 2 shows that the ACC content increased significantly after 24 h in the 1 % YCW induction group, which indicated that ET would regulate the resistance response in the late stage, and JA functions in the entire regulation process. This study also demonstrates that the SA content was increased at 0.5 h after the induction of 1 % YCW. No significant changes were observed in the later period (Fig. 2). According to a report, SA and JA have antagonistic effects on induced plant resistance (Shigenaga & Argueso, 2016a). We speculated that the rapid and continuous increase in JA induced by 1 % YCW might inhibit the further synthesis of SA and subsequent signal transduction and resistance regulation.

Therefore, the JA and BR contents increased significantly after 1 % YCW treatment during the entire testing period. These results indicate that JA and BL may play prominent regulatory roles in the early stages of induced resistance.

3.3. Effect of 1 % YCW on the relative expression level of JA, SA, and BRs synthesis and signal transduction genes

The transcriptional expression changes of key genes in the synthesis and signal transduction of hormones were conducted by RT-qPCR to further analyze the regulatory mechanism of hormones as early signals in induced resistance by 1 % YCW.

3.3.1. The relative expression level of JA synthesis and signal transduction genes

When plants are attacked by pathogens, JA and its derivatives are rapidly synthesized through the lipoxygenase biosynthesis pathway to enhance plant resistance. Lipoxygenase (LOX) is a crucial component of this mechanism (Gfeller et al., 2010). Fig. 3A shows that the relative expression level of *SlLOX* upregulated significantly at 1 h in the treatment group of 1 % YCW and reached a maximum at 24 h (up-regulated by 3.47 times), suggesting that the 1 % YCW could enhance the fruit resistance response by promoting JA biosynthesis. As the JA receptor, the COI protein recognizes the biologically active jasmonic acid molecule and turns on the jasmonic acid signal transduction (Sheard et al., 2010). Fig. 3A demonstrated that the relative expression of *SlCOI1* was considerably upregulated when it reached its maximum value at 72 h (2.96-fold). COI1 can further interact with the negative regulator JAZ to produce a complex, which is subsequently ubiquitinated by the 26S proteasome, along with the cleavage of JAZ proteins to activate downstream transcription factors (Lorenzo et al., 2003). Fig. 3A shows that *SlJAZ* was considerably downregulated and *SlMYC2* was significantly upregulated at 72 h (3.07-fold). The MYC2 transcription factor is activated by JA signaling, and the MYC2-dependent JA signaling pathway is critical in the plant resistance response against the pathogen *Botrytis cinerea* (Du et al., 2017). In addition to MYC2, other transcription factors such as NAC, ERF, and WRKY can also be activated by JAZ degradation. JA can protect *Arabidopsis* against *Botrytis cinerea* by activating the expression of *ERF1* and *ORA59* (Lorenzo et al., 2003). Our previous results indicated that 1 % YCW could increase the expression of *SlERF1* in cherry tomatoes (Guo et al., 2021). These results indicated that 1 % YCW could enhance the fruit resistance response by promoting the biosynthesis and signal transduction pathways of JA. Simultaneously, the gene expression variation trend was consistent with the targeted metabolomics results. The JA signal transduction pathway has also been activated (Fig. 3A).

3.3.2. The relative expression level of SA synthesis and signal transduction genes

Phenylalanine ammonia lyase (PAL) is a common pathway for SA synthesis (Shigenaga and Argueso, 2016b). The relative expression of PAL was up-regulated from 0.5 to 48 h, which was inconsistent with the trend that SA content significantly accumulated only at 0.5 h (Fig. 3B). This may be because in addition to participating in SA synthesis PAL can also act as a key enzyme in secondary metabolite synthesis and

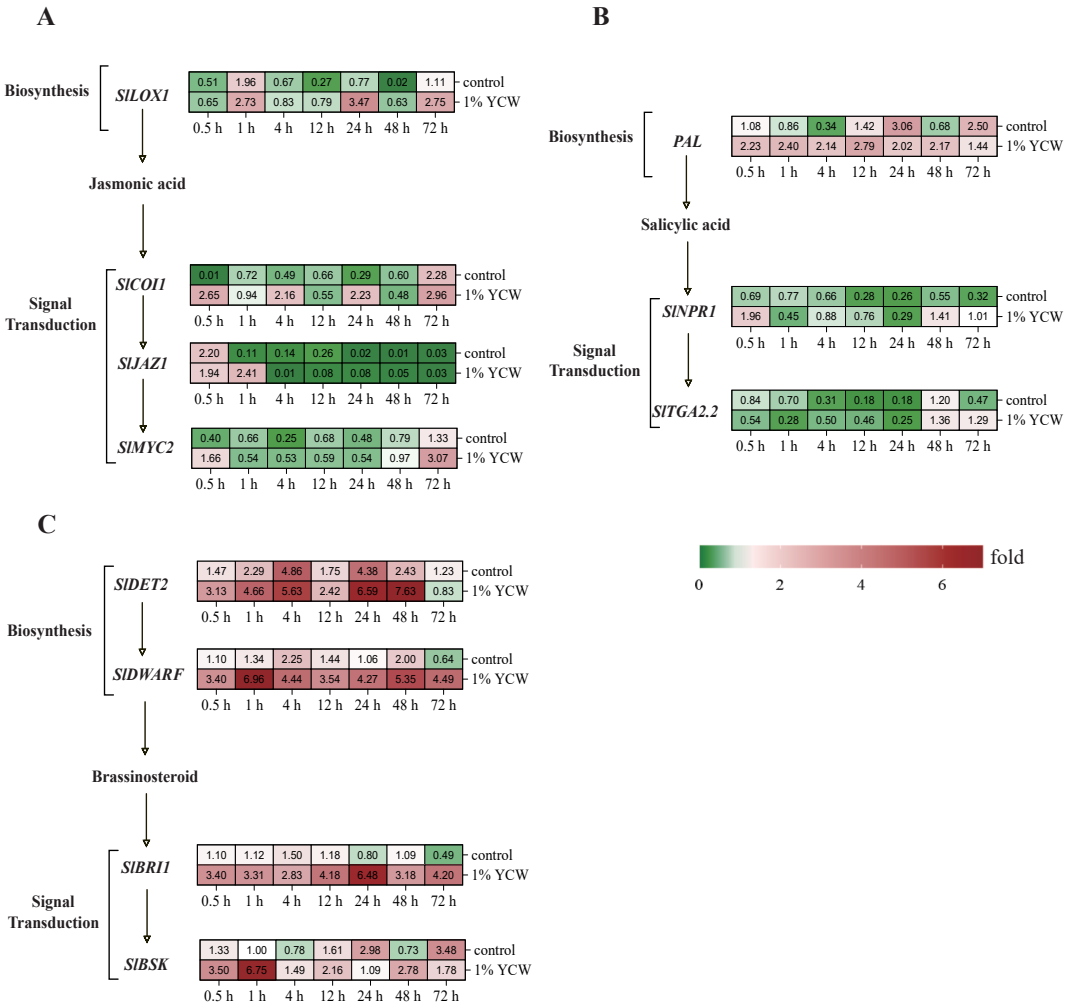

Fig. 3. Effect of 1 % yeast cell wall on the relative expression level of genes associated with JA, including *SlLOX*, *SlCOI1*, *SlJAZ1*, and *SlMYC2* (A); genes associated with SA, including *PAL*, *SlNPR1*, and SlTGA2.2 (B); genes associated with BRs, including *SlDET2*, *SlDWARF*, *SlBAK1*, and *SlBSK* (C) in cherry tomato fruits. Values were normalized to those of the control at 0 h.

participate in plant responses to biotic and abiotic stresses (de Jong et al., 2015). *SlNPR1* is a transcriptional coactivator. *SlNPR1*, as a positive regulator of the SA signaling pathway, can activate downstream transcription factors, such as WRKYs and TGAs, which promote the expression of related defense genes and initiate the resistance of plants to pathogens (Wang et al., 2020). Fig. 3B demonstrates that the relative expression of *SlNPR1* is considerably decreased from 1 to 24 h after treatment with 1 % YCW. Whereas, the relative expression of *SlTGA2.2* decreases from 1 to 24 h after treatment with 1 % YCW. It was deduced that SA synthesis was promoted and its signal transduction was inhibited after 1 % YCW treatment. This may be due to the antagonistic effects of SA and JA on the regulation of plant resistance. Simultaneously, the expression levels of JA synthesis and signal transduction-related genes were significantly up-regulated in this study. Elicitors (oral secretions of *Spodoptera exigua* and *Manduca sexta*) produced by herbivores can enhance JA signal transduction-related gene expression in tobacco to inhibit the SA signal transduction pathway (Poppenberger et al., 2011). In tobacco, exogenous JA effectively inhibited the expression of genes associated with the SA synthesis (Imanishi et al., 2000). These results were similar to the results of this study.

3.3.3. The relative expression level of BL synthesis and signal transduction genes

In addition to SA and JA, BRs have been shown to participate in plant immune processes (Nakashita et al., 2003). The expression of genes involved in BRs biosynthesis after 1 % YCW treatment was analyzed in a follow-up experiment. *DET2* and *DWARF* are essential genes in the BRs pathway. Fig. 3C shows that compared with 0 h, 1 % YCW significantly increased the relative expression level of *SlDET2*, while the relative expression level of *SlDWARF* increased significantly during the whole experiment. *DET2*-encoded reductase participates in reducing 22 of 23-di OH-4-EN-3-One to 6-deoxO3DT (3-dihydro-6-deoxoteasterone) to accelerate the BR biosynthesis (Poppenberger et al., 2011). *DWARF*, another BRs biosynthetic enzyme, encodes CYP80 to mediate c-6 oxidation of 6-deoxybrassinosterol (CR) to testosterone (CN) in tomatoes and participates in the synthesis of BRs (Bishop et al., 1999). BRI1 can activate the signaling of BRs. BSK is a positive regulator of signal transduction. It has been reported that the combination of BRs and the receptor kinase BRI1 promotes the autophosphorylation of BRI1. Activated BRI1 interacts with BAK1 and phosphorylates each other to activate BRI1 completely. Activated BRI1 phosphorylates the positive regulator BSK to activate BRs signal transduction, BSK1 associates with the PRR FLAGELLIN SENSING2 (FLS2) to activate downstream signal transduction in plant immunity (Wang et al., 2008). In our study, the expression levels of *SlBRI1* and *SlBSK* were markedly upregulated in the 1 % YCW treatment group, indicating that BRs signals maintained a continuous transduction state. Overall, the expression levels of genes related to BL synthesis and signal transduction were upregulated, consistent with the BRs accumulation trend. Thus, it could be considered that BL is involved in the induced resistance response of 1 % YCW.

The above results indicate that the hormone, as an early signal of resistance, can rapidly respond to 1 % YCW induction to boost the resistance of cherry tomato fruits. Multiple hormones regulate the resistance response of cherry tomatoes. There is an interaction network, as shown in Supplement 2, which quickly responds in the early stage and plays a major part in the entire resistance response. After induction, both JA and BL signaling pathways were activated, which further activated downstream transcription factors and resistant proteins and stimulated the defense response of fruits. The ACC content increased at 24 h, indicating that ET was involved in regulating resistance in the late induction period. JA in the JA/ET pathway played a major role in the induction of early signal response. There is antagonism between the synthesis and signal transduction of SA and JA. The synthesis and signal transduction activation of JA suppresses the synthesis and signal transduction of SA.

3.4. Targeted metabolomics analysis of plant hormone profiles in cherry tomato fruit treated with U0126

Activation of the MAPK cascade is an early critical event in defense signaling. Previous studies by our group showed that *SlMAPK3* regulates the fruit resistance induced by 1 % YCW (Guo et al., 2021). A previous report suggested that there is an interaction between hormones and the MAPK pathway (Smékalová et al., 2014). However, the specific mechanism that regulates the resistance response is still unclear. Therefore, in subsequent experiments, tomatoes were treated with U0126 to analyze the crosstalk between plant hormones and the MAPKK1/2-MAPK3 pathway.

The SA content significantly increased after U0126 treatment throughout the induction period (Fig. 4). A previous report showed that the knockdown of *SlMAPK3* increased SA synthesis and inhibited JA synthesis (Zhang et al., 2018), consistent with our results. In jasmonates, the *cis*-OPDA content was lower than that in the control group except at 0.5 h. The JA content was higher than that in the control group except at 0.5 h. In brassinosteroids, BL content was markedly higher than that of the control at 0.5 h, whereas CS content was not significantly different from that of the control. These results suggest that SlMAPK3 inhibition may promote BL synthesis (Fig. 4). It has been reported that the inhibition of MAPK3 can promote the decline of ACS2/6 by the 26S proteasome and inhibit the synthesis of ET (Han et al., 2010). Targeted metabolomics showed that ACC content decreased significantly at 4 h, indicating that U0126 inhibited the production of ACC, inhibiting ET synthesis.

3.5. Effects of U0126 and U0126 combined with 1 % YCW on the relative expression of genes associated with JA, SA, and BL synthesis and signal transduction

To further analyze the interaction network between hormones and the MAPKK1/2-MAPK3 pathway in combination with the metabolome results, the expression of genes involved in the synthesis and signal transduction of JA, SA, and BRs at the transcriptional level was detected under U0126 and U0126 combined with 1 % YCW treatment.

3.5.1. The relative expression level of JA synthesis and signal transduction genes

As indicated in Fig. 5A, the expression of *SlLOX1* began to be substantially downregulated after 12 and 4 h, respectively, in U0126 and U0126 combined with 1 % YCW treatment groups, indicating that inhibition of *SlMAPK3* can reduce JA synthesis in cherry tomatoes. As a key gene involved in JA synthesis, the relative expression of *SlLoxC* decreased in tomato mutants with the *SlMAPK3* gene knocked out (Zhang et al., 2018), which was consistent with our results. Following U0126 treatment, the relative expression of *SlJAZ* significantly increased, except at 4 and 48 h. Expression of *SlCOI1* and *SlMYC2* decreased before 24 and 12 h, respectively, with little change at other periods in the U0126 treatment. This demonstrated that the U0126 treatment inhibited the JA signal transduction pathway. A similar result showed that the knockout of *SlMAPK3* could also inhibit JA signal transduction. *JAZ1* showed higher expression. *JAZ1* functions as a negative regulator of JA signal transduction in the mutants (Zhang et al., 2018). Similar results were observed in the U0126 combined with the 1 % YCW treatment group, the relative expression of *SlLOX* decreased significantly from 4 h. The relative expression of *SlJAZ* did not change significantly (except for a significant increase at 72 h). The relative expression of *SlCOI1* and *SlMYC2* remained considerably downregulated before 48 h. Therefore, 1 % YCW induction after inhibitor treatment did not stimulate the synthesis and signal transduction of JA.

3.5.2. The relative expression level of SA synthesis and signal transduction genes

The relative expression of *PAL* in U0126 and U0126 combined with

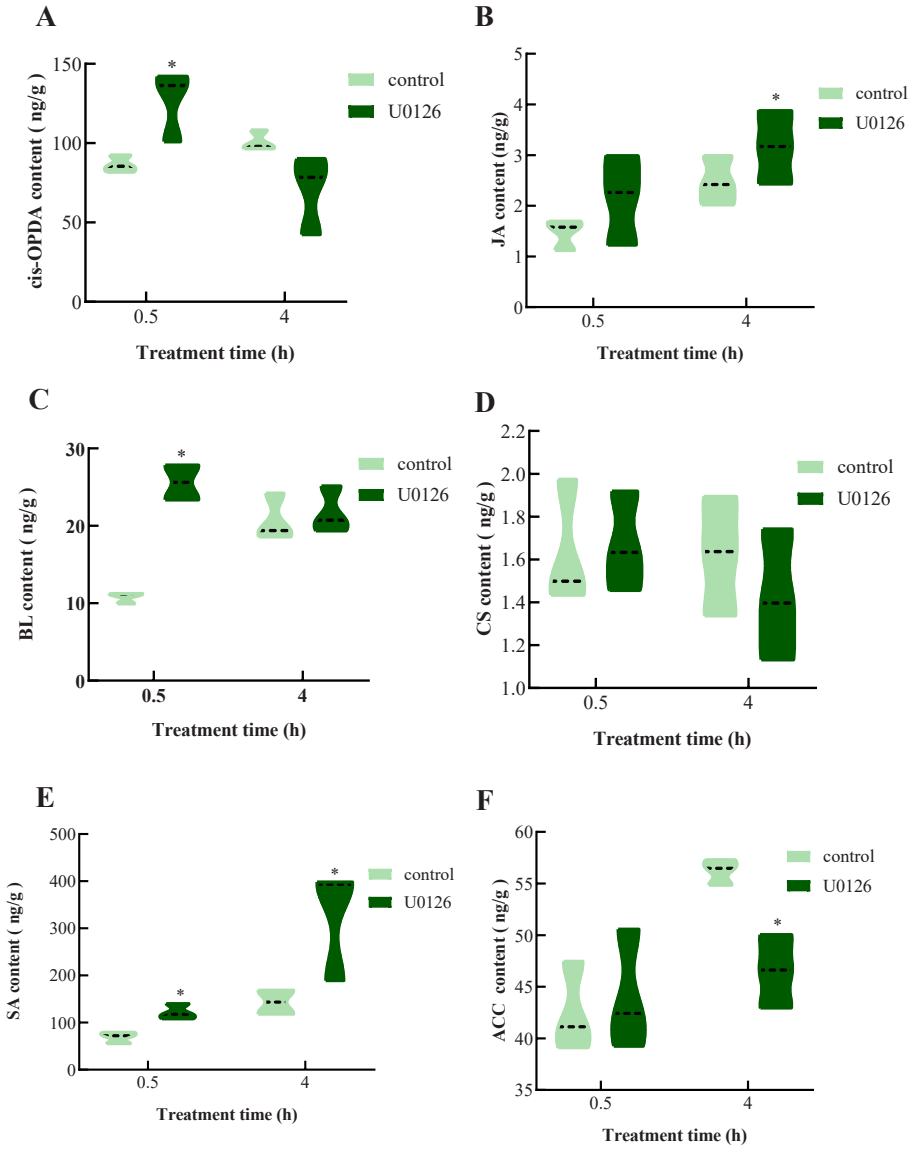

Fig. 4. Plant hormone content in cherry tomatoes after induction with U0126 at 0.5 and 4 h. (A) *cis*-OPDA, (B) JA, (C) BL, (D) CS, (E) SA, (F) ACC. The asterisk (*) indicates relative transcript levels that were significantly ($P < 0.05$) higher than those of the control.

1 % YCW treatment groups was up-regulated at 0.5, 24, 48 h and 0.5, 4, 24, and 48 h, respectively (Fig. 5B). These results indicate that the U0126 treatment increased SA synthesis by inhibiting *SlMAPK3* expression. In *Arabidopsis*, *AtMAPK3* functions as a negative regulator of the flg22-induced salicylic acid accumulation (Nicolas et al., 2014). Zhang et al. demonstrated that the knockout of *SlMAPK3* upregulated the expression of SA synthesis-related genes and increased SA content, supporting our results. *SlNPR1* and *SlTGA2.2* are the primary genes involved in SA signal transduction. The relative expression levels of *LeNPR1* (4 and 12 h) and *SlTGA2.2* (from 1 to 24 h) were significantly downregulated in the U0126 treatment group. There was no significant change at other time points. U0126 combined with 1 % YCW treatment considerably downregulated the expression of *LeNPR1* (except for 0.5 and 72 h) and *SlTGA2.2* (except for 0.5, 48, and 72 h) at other time points. Therefore, U0126 and U0126 combined with 1 % YCW could up-regulate the expression of SA synthesis-related genes at the transcriptional level but inhibited the relative expression level of genes related to SA signal transduction. SA signal transduction was inhibited after *MAPK3* was inhibited. These results are similar to Li et al. (Li et al., 2017), indicating that *SlMAPK3* silencing weakened the expression of defense-related genes in SA-mediated pathways.

3.5.3. The relative expression level of BRs synthesis and signal transduction genes

The expression of *SlDET2* was considerably increased (except at 24 and 48 h) whether U0126 and U0126 were combined with 1 % YCW treatment groups. Whereas expression of *SlDWARF* only significantly increased at the early stage (0.5 and 1 h). Considering the genes related to BRs signal transduction, the expression of *SlBRI1* was upregulated at 0.5 and 1 h in the U0126 treatment group and 1 and 4 h in the U0126, combined with a 1 % YCW treatment group. The relative expression of *SlBSK* in the U0126 treatment group was significantly up-regulated at 0.5 and 12 h. In comparison, upregulated expression of *SlBSK* was observed in the U0126 combined with a 1 % YCW treatment group at 12 h (no significant changes were observed in other periods). Fig. 5C demonstrates that U0126 had no immediate impact on BRs synthesis and signal transduction. MAPK3 did not participate in regulating the BRs pathway (Song et al., 2018).

Our results suggest that MAPK3 is involved in regulating SA and JA synthesis and signal transduction. As shown in Supplement 3, U0126 inhibited JA and SA signal transduction by inhibiting the MAPKK1/2-MAPK3 pathway. We speculated that BRs could regulate the fruit defense response independent of the SA and JA/ET signaling pathways.

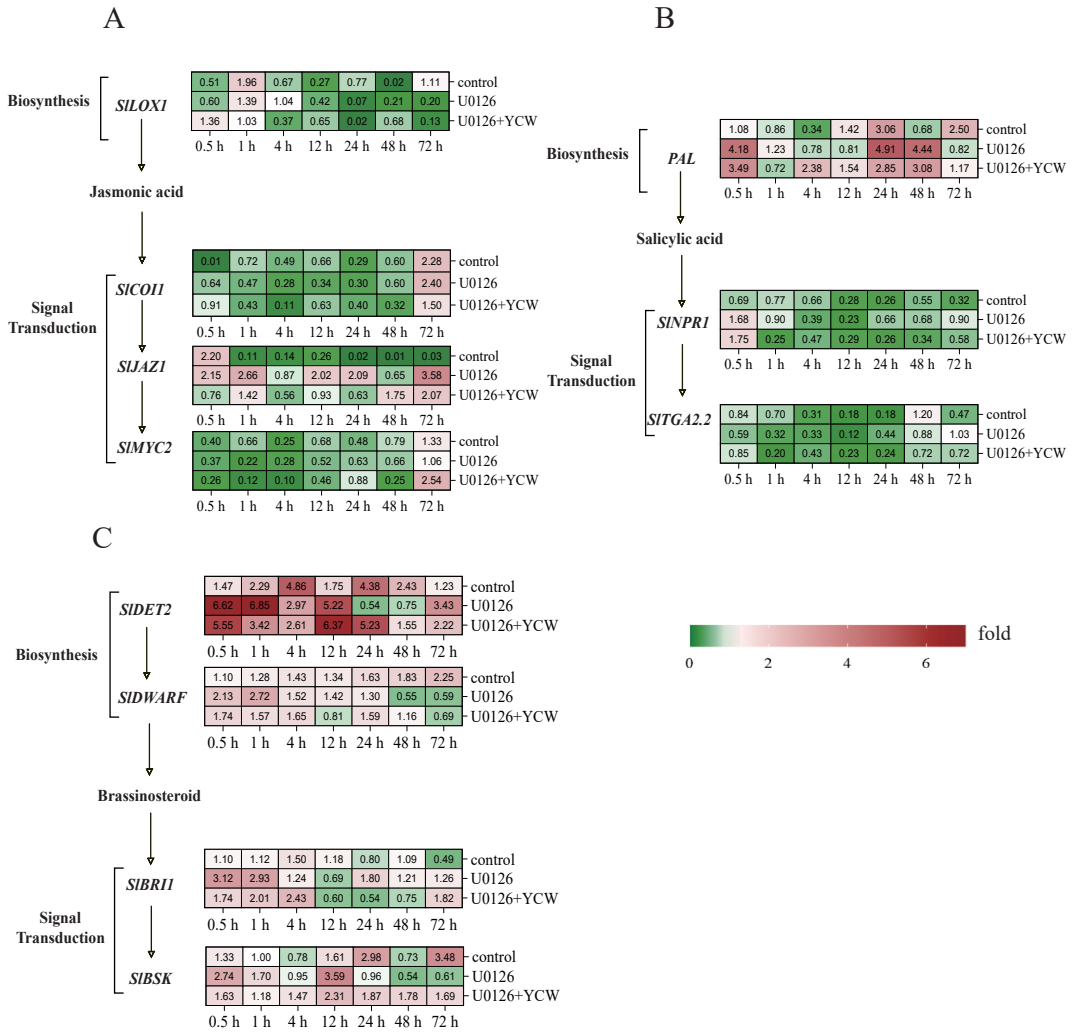

Fig. 5. Effect of U0126 and U0126 combined with 1 % yeast cell wall on the relative expression level of genes associated with JA, including *SlLOX*, *SlCOI1*, *SlJAZ1*, and *SlMYC2* (A); genes associated with SA, including *PAL*, *SlNPR1*, and SlTGA2.2 (B); genes associated with BRs, including *SlDET2*, *SlDWARF*, *SlBAK1*, and *SlBSK* (C) in cherry tomato fruits. Values were normalized to those of the control at 0 h.

4. Conclusion

Jasmonates and brassinosteroids, the primary early response signals, were rapidly induced by 1 % YCW and switched on the expression of signal transduction and resistance genes to improve cherry tomato fruits resistance against *B. cinerea*. The synthesis and signal transduction of SA were inhibited by the activation of the JA pathway induced by 1 % YCW. JA and SA signaling pathway crosstalk with the MAPK3 pathway. There is no clear regulatory relationship between *SlMAPK3* and its BRs. The BR pathway may function independently in the regulation of fruit resistance when JA/SA and MAPK3 pathways are inhibited. Therefore, the early signals involved in the response to induced resistance include the interaction between resistance hormones and crosstalk with MAPKs.

CRediT authorship contribution statement

Keyu Sun: Investigation, Writing – original draft. **Xue Zhang:** Data curation. **Ze Wei:** Validation. **Ziwuzhen Wang:** Methodology. **Jifeng Liu:** Project administration. **Jian Liu:** Project administration. **Jianhua Gao:** Project administration. **Jun Guo:** Conceptualization, Writing – review & editing. **Xin Zhao:** Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2022.100160.

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