



Jasmonate increases terpene synthase expression, leading to strawberry resistance to *Botrytis cinerea* infection

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Abstract

Key message Jasmonate induced *FaTPS1* to produce terpene, and overexpression *FaTPS1* led to fruit resistant against *B. cinerea* infection, *FaMYC2* induced *FaTPS1* by binding to its promoter that downstream of jasmonate.

Abstract Jasmonic acid (JA) and its derivatives are associated with plant defence responses against pathogenic organisms. In the present study, a total of 10,631 differentially expressed genes, 239 differentially expressed proteins, and 229 differential metabolites were screened and found to be mainly involved in pathogen perception, hormone biosynthesis and signal transduction, photosynthesis, and secondary metabolism. In strawberry fruits, methyl jasmonate (MeJA) induced *FaTPS1* expression and quickly increased the terpene content. Furthermore, *FaTPS1* overexpression increased the emission of sesquiterpenes, especially germacrene D, and improved strawberry resistance against *Botrytis cinerea* infection, although the knockdown of *FaTPS1* increased its susceptibility to the same pathogen. Using a yeast one-hybrid assay and transient expression analysis, we demonstrated that *FaMYC2* can bind to the G-box element in the promoter region of *FaTPS1*, thus inducing *FaTPS1* expression. MeJA also stimulated *FaMYC2* expression and regulated downstream signalling cascades. Moreover, we presented a possible model of the new signalling pathway of MeJA-mediated strawberry resistance to *B. cinerea*.

Keywords Jasmonate · *Botrytis cinerea* · Terpenes · Omics analysis · *FaMYC2* · *FaTPS1*

Introduction

Strawberry (*Fragaria × ananassa*) is cultivated worldwide and has a broad market prospect and great economic benefits. Its fruits are delicious, juicy, and nutritious, and they are very popular among consumers. However, strawberry is highly susceptible to many pathogens and is one of the crops most dependent on fungicides (Petrasch et al. 2019).

Strawberry grey mould, caused by the ubiquitous necrotrophic fungal pathogen *B. cinerea*, has the greatest impact on the economic feasibility of strawberries (Petrasch et al. 2019). Its negative effect includes weight loss, colour fading, accelerated softening, and reduction of shelf life, all of which result in severe economic losses (García-Pastor ME et al.). The main strategy for the prevention and control of fungal diseases in plants is the application of various chemical fungicides. Pesticide residues cause environmental pollution, but also negatively affect human health (Jankowska et al. 2016). Therefore, it is imperative to explore safe and effective methods for the prevention and control of strawberry grey mould to reduce its yield losses.

Many studies have shown that jasmonic acid (JA) plays a key role in plant defence against disease infection, and its role in plant innate immunity has been fully demonstrated, especially in the model plant *Arabidopsis thaliana*; however, it has not been demonstrated in horticultural plants (Grant and Jones 2009; Robert-Seilaniantz et al. 2011; Howe et al. 2018; Przydacz et al. 2020). JA was first discovered in the essential oil of *Jasminum grandiflorum*, and structurally, it is a cyclopentanone derivative synthesised from

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α -linolenic acid (Wasternack and Kombrink 2010; Demole et al. 1962). JA has been shown to be an indispensable signal for resistance/susceptibility to multiple diseases caused by fungal, bacterial, and viral pathogens (Wasternack and Strnad 2016; Zhang et al. 2017). JA signalling pathways have been reported to be involved in grey mould inhibition (Yu et al. 2019). *Arabidopsis* JA-knockout mutants showed increased susceptibility to *Botrytis* species (Thomma BP et al. 1998; Zander M et al. 2009). Consistently, overexpression mutants with increased levels of endogenous JA showed increased resistance to *B. cinerea* (Merz PR et al. 2015; AbuQamar S et al. 2017). A recent study found that when plants are infected by *B. cinerea*, JA cannot only directly inhibit the growth of pathogens, but it can also act as a signalling substance, activating the disease-resistance pathway and inducing the expression of related defence genes, for example, promoting the upregulation of genes encoding chitinase β -1,3-glucanase and polygalacturonase inhibitors (Valenzuela-Riffo et al. 2020). It was reported that the resistant ‘Syrah’ grapes displayed higher basal levels of jasmonoyl-isoleucine (JA-Ile) than those of the susceptible ‘Trincadeira’ grapes, along with higher expression of MYC2 (Myelocytomatosis protein 2) and JAZ8 (Jasmonate ZIM-domain 8), although compared to that in uninfected berries, the synthesis of jasmonic acid increased in infected berries of both cultivars (Coelho et al. 2019). Furthermore, the JA signalling pathway was activated against necrotrophs through key regulators, such as transcription factor MYC2 (Liu et al. 2018). Under internal or external stimuli, elevated JA-Ile levels promote the degradation of SCF COI1-dependent (jasmonate-ZIM domain) JAZ inhibitors, thereby activating MYC2 of JA response genes to direct transcription. MYC2-coordinated transcriptional reprogramming is the central topic of JA signalling (Kazan and Manners 2013; Wasternack and Hause, 2013; Zander et al. 2020). However, the specific mechanism of MYC2 involvement in strawberry resistance to *B. cinerea* remains unclear.

In addition, exogenous MeJA treatment induced the release of volatile terpenes, which suggested that JA may improve plant resistance to fungi by inducing the synthesis of terpene compounds (Martin et al. 2003; Hampel et al. 2005; Moenne et al. 2021). Terpene synthase (TPS) is a large family which includes more than 100 genes known today, and it generates a diversity of terpenoids by catalysing the conversion of acyclic prenyl diphosphate substrates into hydrocarbons (Tholl 2006; Karunanithi and Zerbe 2019). Many studies have reported that besides having antibacterial effects, some terpene compounds are also considered to be plant toxins produced by fungi (Mastelic et al. 2005; Pinedo et al. 2008; Ergüden, 2021).

Plant omics is an emerging field of system science and it offers prospects for evidence-based evaluation of the effect of plant hormones against various diseases. Therefore, in

the present study, we used multi-omics, including RNA-seq, TMT quantitative proteomics, and LC–MS/MS metabolomics, to analyse the genetic, protein, and metabolic changes in strawberries infected with *B. cinerea* after MeJA pretreatment. Furthermore, we used GC–MS and LC–MS/MS to analyse the changes in metabolite contents and composition in response to *Botrytis* infection after transient expression of *FaTPS1* in strawberry fruits. Using yeast one-hybrid, we found that FaMYC2 can bind to cis-regulatory elements, including E-box and G-box elements in the promoter regions of *FaTPS1*, and consequently induce *FaTPS1* expression under MeJA. Based on the above data, we presented a new mechanism of plant resistance induced by JA, in which the effects of *B. cinerea* infection in strawberries are decreased, and fruits are preserved from fungal infection.

Materials and methods

MeJA treatment and plant material collection

Healthy and complete strawberry leaves (*Fragaria × ananassa* ‘Sweet Charlie’) were collected from the experimental field of Nanjing Agricultural University. Then, the leaves were disinfected with 2% (v/v) sodium hypochlorite solution, washed with distilled water three times, and dried at room temperature. The leaves were divided into two groups, basing on the pre-experiment, one group was sprayed with 10 $\mu\text{mol/L}$ of MeJA solution, and the other group was sprayed with water as a control. 0.1% Tween 80 was added to the solution to improve adhesion. 24 h later, the leaves were then placed in a constant temperature incubator at 22 °C and 95% relative humidity, and sprayed with suspension spores of *B. cinerea* (10^5 mL^{-1}). 3 days later, leaves were collected for RNA-Seq (transcriptome) and proteome analysis, and 12 days later, leaves were collected for metabolomic analysis. All treatments were repeated three times, and each replicate contained five leaves. The physiological indicator determination are presented in the supplementary file.

Library preparation and RNA-Seq

Library preparation and RNA-Seq were performed by the Beijing Novogene Technology Corporation (Beijing, China). After cluster generation, the library preparations were sequenced on an Illumina Novaseq platform. Clean reads were obtained by removing raw reads containing adapters through in-house Perl scripts, and all downstream analyses were based on high-quality clean reads. Meanwhile, Q20, Q30, and GC content of the clean reads was calculated. The clean reads were then mapped to the grape reference genome using Hisat2 (https://www.rosaceae.org/species/fragaria_x_ananassa/genome_v1.0.a1). The mapped reads of each

sample were assembled by StringTie (v1.3.3b) (Pertea et al. 2015) using a reference-based approach. All treatments were composed of three biological replicates. Fragments per kilobase of transcript per million mapped reads (FPKM) value of each gene was calculated based on the length of the gene and read count mapped to each gene. Differential expression analysis of the two groups was performed using the DESeq2 R package. The obtained p values were adjusted to q values using Benjamini and Hochberg's approach for controlling the false discovery rate (FDR) (Benjamini and Hochberg 1995). Genes with q values < 0.001 and absolute fold changes > 2 were assigned as differentially expressed genes (DEGs). Gene Ontology (GO) and KEGG enrichment analysis of DEGs were performed using the clusterProfiler R package, in which gene length bias was corrected. GO terms and KEGG pathways with corrected q values < 0.05 were considered significantly enriched by DEGs. The raw data were deposited in the NCBI database (PRJNA727866).

TMT quantitative proteomics

Four volumes of lysis buffer (containing 8 M urea, 1% Triton-100, 10 mM dithiothreitol, and 1% protease inhibitor cocktail) were added to the sample powder. After sonication, an equal volume of Tris was added and the mixture was centrifuged at $5500 \times g$ for 10 min at 4 °C. Then, the supernatant was collected, and five volumes of 0.1 M ammonium acetate/methanol were added for precipitation overnight. The protein precipitate was washed with methanol and acetone and finally reconstituted with 8 M urea. Dithiothreitol was added to the protein solution to obtain a final concentration of 5 mM, and the mixture was reduced at 56 °C for 30 min. Then, iodoacetamide was added to a final concentration of 11 mM, which was incubated at room temperature for 15 min in the dark. Finally, trypsin was added at a 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h digestion. After trypsin digestion, the peptides were desalted with a Strata X C18 SPE column (Phenomenex, USA) and vacuum-dried. The peptides were dissolved in 0.5 M tetraethylammonium bromide (TEAB) and processed according to the manufacturer's protocol for the TMT kit. The peptides were fractionated by high pH reverse-phase HPLC using an Agilent 300Extend C18 column (5 μ m particle diameter, 4.6 mm inner diameter, 250 mm length). The tryptic peptides were dissolved in 0.1% formic acid (solvent A) and directly loaded onto a home-made reversed-phase analytical column (15 cm length, 75 μ m i.d.). The gradient comprised solvent B whose concentration was increased from 6 to 23% (0.1% formic acid in 98% acetonitrile) over 26 min, 23 to 35% in 8 min, and up to 80% in 3 min; subsequently, it was held at 80% for the last 3 min, all at a constant flow rate of 400 nL/min on an EASY-nLC 1000 UPLC system.

The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q Exactive™ Plus (Thermo, USA) coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The m/z scan range was 350–1800 for full scan, and intact peptides were detected in Orbitrap (Thermo, USA) at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 28, and the fragments were detected in Orbitrap at a resolution of 17,500. A data-dependent procedure alternated between one MS scan followed by 20 MS/MS scans with a 15.0 s dynamic exclusion. Automatic gain control (AGC) was set at 5E4. Fixed first mass was set at 100 m/z .

Liquid chromatography-mass spectrometry (LC-MS/MS) analysis

The plant tissue powder ground with liquid nitrogen was added to an EP tube and resuspended in an aqueous solution containing 80% methanol and 0.1% formic acid. The samples were placed in an ice bath for 5 min, and then centrifuged at $15,000 \times g$ at 4 °C for 10 min. The supernatant was collected, diluted with mass spectrometry-grade water to a methanol content of 53%, and then centrifuged at $15,000 \times g$ at 4 °C for 10 min. Finally, the supernatant was injected into the LC-MS/MS system for analysis. In addition, QC was prepared by mixing an equal volume of samples from each sample. UHPLC-MS/MS analyses were performed at Novogene Co, Ltd. (Beijing, China) using a Vanquish UHPLC system (Thermo Fisher, Germany) coupled with an Orbitrap Q Exactive™ HF mass spectrometer (Thermo Fisher, Germany). Compound Finder 3.0 (CD 3.0, Thermo Fisher) was used to process the raw data files to perform peak alignment, peak selection, and quantification for each metabolite. A univariate analysis (t test) was used to calculate the statistical significance (p value). Metabolites with $VIP > 1$, p value < 0.05 , and fold change ≥ 2 or $FC \leq 0.5$ were considered to be differential metabolites.

Volatile analysis using GC-MS

Samples (1.5 g) were ground and transferred to a headspace bottle (15 mL). Three millilitres of saturated NaCl solution were added to the samples, and 32.84 ng 3-nonanone was added as an internal standard. GC-MS analysis was performed automatically using a TSQ™ 9000 Triple Quadrupole GC-MS/MS System (Thermo Scientific, Nanjing, China) with a 50/30 μ m DVB/CAR/PDMS Fibre (Supelco, USA) that was maintained at 40 °C for 30 min. The MS conditions were as follows: EI mode: voltage, 70 eV; ion source temperature, 230 °C; scanning rate, 2.88 scan·s⁻¹; mass spectrometry detection range, 29–540 $m \cdot z^{-1}$; carrier gas, helium; flow rate, 1.0 mL min⁻¹. The column temperature was programmed

as follows: the initial temperature was set at 50 °C for 6 min and then increased to 250 °C at 6 °C min⁻¹, which was held for 3 min. The chromatographic and spectral data were evaluated using the TraceFinder software (Thermo Scientific, USA).

Transient expression of *FaTPS1* by agrobacterium infiltration

FaTPS1 (maker-Fvb4-4-augustus-gene-45.47) was cloned using primers *FaTPS1*-F and *FaTPS1*-R, and ligated to pRI101-AN vector digested with Sall and KpnI restriction enzymes. Antisense interference vector was constructed referred to our previous method (Jia et al. 2016), and the empty PRI101 AN was used as control. These constructs were transferred into EHA105 and cultured on LB medium (100 mg ml⁻¹ kanamycin and 50 mg ml⁻¹ rifampicin). The positive cells were collected by centrifugation and suspended in 50 ml of infiltration buffer (10 mM MgCl₂, 10 mM MES, 200 μm acetosyringone, pH 5.6). After standing at room temperature for 3 h, the suspension was slowly injected into the strawberry fruit through a 1 ml syringe until the whole fruit was infiltrated. Ten strawberry fruits were used for the injection experiment and repeated three times.

Yeast one-hybrid (Y1H) and dual-luciferase assay

The *FaTPS1* promoter was cloned using primers *FaTPS1*-Pr-F and *FaTPS1*-Pr-R and ligated to the PAbAi vector digested with *Hind* III and *Kpn* I restriction enzymes. *FaMYC2* was cloned using primers *FaMYC2*-F and *FaMYC2*-R and ligated to pGADT7 AD vector digested with *EcoR* I and *BamH* I restriction enzymes. Y1HGold-GAL4-AbA system was used to detect the interaction between DNA and protein (Mou et al. 2018). PAbAi-TPS was linearized using BstBI and transformed into Y1HGold Competent Cell (YC1001, WeidiBiotechnology, Shanghai). Matchmaker™ Insert Check PCR Mix (Cat. 630,496, Takara, Beijing) was used to confirm the integration site of the pAbAi vector containing DNA sequence.

The full-length sequence of *FaMYC2* was inserted into the pSAK277 vector. The *FaTPS1* promoter sequence was inserted into the pGreenII dual-luciferase target expression vector. The constructed vector was introduced into *Agrobacterium tumefaciens*, suspended, infiltrated and the subsequent process of *Arabidopsis* protoplast. A dual-luciferase reporter gene assay system (Promega, Madison, WI) was used for luminescence measurement. Cytation 3 cell imaging multi-mode reader (BioTek, Santa Barbara, California) was used to measure the activity levels of firefly luciferase

(Luc) and Renilla luciferase (Ren), and each measurement was repeated at least 10 times.

Statistical analysis

Statistical analysis was performed using SPSS Statistics software (IBM®SPSS®Statistics, New York, NY, USA) to calculate the significant differences of the recorded data, and was repeated in triplicate, significant differences were less than 0.05 at p values. In the figure legends they were expressed as mean ± standard error. Duncan's multiple range tests was used for the comparison of means.

Results

MeJA enhanced strawberry resistance to *B. cinerea* through the inhibition of spore germination and growth

We documented the process of *B. cinerea* infection in strawberry leaves and calculated the disease incidence and disease index (Fig. 1a). On the 6th day of infection, necrotic spots were observed on the control leaves, and the infection area significantly expanded on the 12th day. In contrast, MeJA-treated strawberry leaves remained healthy without obvious browning. Compared with the control, MeJA treatment significantly reduced the disease incidence and disease index of strawberry leaves, which indicated that MeJA treatment inhibited the damage of strawberry leaves by *B. cinerea* (Fig. 1b, c).

To determine the effect of MeJA on the growth of *B. cinerea*, we calculated the rate of spore germination and mycelial growth. The results showed that MeJA inhibited the growth of *B. cinerea* spores in vitro up to 80% (Fig. 1d). Compared to the control, MeJA treatment significantly decreased the spore length in the fruit and leaf extracts of strawberries (Fig. 1e, f). Furthermore, MeJA treatment significantly decreased the number of spores and the area of infection compared to those in the control (Fig. 1g, h). These results showed that MeJA inhibited *B. cinerea* spore germination and growth under in vivo and in vitro conditions.

Furthermore, we measured the antioxidant enzyme activity and stress physiological indices in strawberry leaves on the 12th day of infection. The results showed that MeJA treatment significantly improved the activities of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), in strawberry leaves and fruits (Table 1). MeJA treatment significantly increased the content of proline and reduced malondialdehyde (MDA) content and the relative conductance rate (Table 1). Collectively, these results showed that

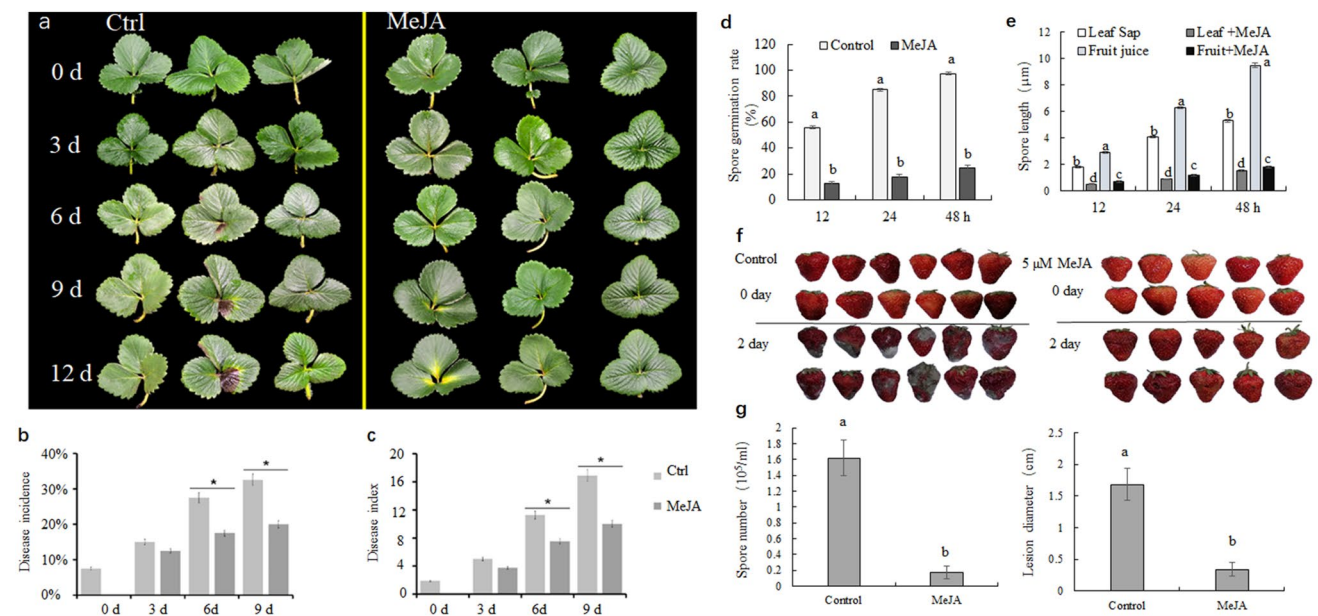


Fig. 1 Changes in the extent of *Botrytis* infection and effect of MeJA on the spore germination of *B. cinerea* under in vitro and in vivo condition in strawberry leaves. **a** Pretreatment of strawberries leaves with and without MeJA treatment before infection, **b** disease incidence, **c** disease index. **d** Spore germination under MeJA in vitro. **e** The extract of leaf and fruit inhibited spore length under in vitro condition. **f** Fruits were inoculated by *B. cinerea* in the pretreated samples

with and without MeJA. **(g, h)** The fungi spore and lesion area were quantified in the pretreated samples with and without MeJA. Vertical bars represented means \pm standard deviations (SD). *represents significant differences compared to control at $p=0.05$. Different letters indicated a statistical difference at $p<0.05$ as determined by Duncan's multiple range test ($n=3$)

MeJA treatment significantly improved the antioxidant capacity of strawberry leaves and reduced the damage caused by *B. cinerea*.

MeJA increased the content of phenolic compounds after *B. cinerea* infection

To evaluate how MeJA treatment inhibited *B. cinerea* infection, we determined the changes in phenolic compounds. The results showed that compared to the control, MeJA treatment significantly increased the total phenolic and flavonoid contents in strawberries (Table 2). It also increased the content of gallic acid in strawberries compared to that in the control; however, this was not observed for other components (Table 2). Moreover, the contents of cell wall metabolism-associated components of pectin and cellulose were increased and decreased under MeJA, respectively, compared to those under control, and β -1,3-glucanase content was decreased, suggesting that the cell wall was strengthened by MeJA. The biotic stress-associated enzymes phenylalanine ammonia-lyase (PAL), POD, chitinase, and polyphenol oxidase (PPO) were increased under MeJA treatment compared to those under control (Table 2), indicating that MeJA can activate

the stress signal to induce fruit resistance to *B. cinerea* infection.

MeJA upregulated JA biosynthesis-associated gene expression and increased JA content

To study the effect of exogenous MeJA on the endogenous JA pathway, we measured the expression of JA biosynthesis genes and JA content. The results showed that *FaLOX* (Lipoxygenase, maker-Fvb4-3-augustus-gene-100.49), *FaAOC* (Allene oxide cyclase, maker-Fvb6-1-augustus-gene-51.40), *FaAOS* (Allene oxide synthase, augustus_masked-Fvb2-4-processed-gene-262.8), and *FaOPDAI* (12-oxophytodienoic acid, maker-Fvb5-3-snap-gene-62.56) were increased under MeJA treatment compared to those under the control, and JA signalling pathway genes such as *FaCOI1* (COR-insensitive 1), *FaMYC2* (augustus_masked-Fvb5-3-processed-gene-147.1) were increased and *FaJAZ1* (maker-Fvb5-4-augustus-gene-79.38) was decreased, indicating that MeJA affected JA biosynthesis and signalling pathways, consequently affecting endogenous JA in response to *B. cinerea* infection. We also showed that under MeJA treatment, JA accumulated and was consistent with the expression of JA biosynthesis-associated genes (Figure S1).

Table 1 Antioxidant enzyme activity and MDA content of strawberry leaves and fruits

	SOD (U/g)	POD (U/g)	CAT (U/g)	APX (U/g)	MDA (nmol/g)	Proline content (mg/Kg)	Relative conductance rate (%)
Leaf	66.3 ± 4.62	8.32 ± 1.24	17.65 ± 2.31	16.74 ± 1.83	2.38 ± 0.42	10.25 ± 1.33	21.3 ± 2.36
Leaf + MeJA	109.65 ± 7.32*	15.73 ± 1.31*	37.95 ± 3.52*	29.33 ± 1.61*	1.67 ± 0.23*	16.78 ± 1.87*	17.8 ± 1.85*
Fruit	121.52 ± 5.42	13.22 ± 0.91	18.943 ± 1.01	22.31 ± 1.07	3.25 ± 0.31	6.89 ± 1.01	45.8 ± 2.35
Fruit + MeJA	197.21 ± 8.51*	17.61 ± 1.83*	29.75 ± 1.89*	30.11 ± 1.57*	1.88 ± 0.36*	13.77 ± 1.35*	37.6 ± 2.01*

* Significant differences compared with the control sample at < 0.01, using Student's test

Table 2 Effect of MeJA on phenolic compounds and cell wall composition in strawberry fruit

Physiological changes	Control	MeJA (5 µM)
PAL (U mg ⁻¹ FW)	83.55 ± 5.66	112.8 ± 8.77*
POD (U g ⁻¹ min ⁻¹ FW)	205.6 ± 11.56	334.7 ± 20.56*
Chitinase (U g ⁻¹ min ⁻¹ FW)	8.5 ± 0.8	13.5 ± 1.6*
β-1,3-Glucanase (U g ⁻¹ FW)	3.8 ± 0.6	5.2 ± 0.4*
Cellulase content (%)	1.56 ± 0.33	1.05 ± 0.27*
Flavonoid (µg g ⁻¹ FW)	1.88 ± 0.35	2.56 ± 0.21*
Pectin content (%)	0.28 ± 0.08	0.39 ± 0.06*
Total soluble solid (%)	7.9 ± 0.21	8.9 ± 0.16*
Gallic acid (µg g ⁻¹ FW)	17.3 ± 0.89	20.6 ± 0.68*
Caffeic acid (µg g ⁻¹ FW)	8.33 ± 0.88	8.56 ± 1.01
Protocatechuic acid (µg g ⁻¹ FW)	10.21 ± 0.87	9.95 ± 0.96
Syringic acid (µg g ⁻¹ FW)	1.88 ± 0.11	1.92 ± 0.21
p-Coumaric acid (µg g ⁻¹ FW)	5.88 ± 0.88	5.65 ± 0.69
Total phenol (µg g ⁻¹ FW)	145.6 ± 6.65	188.9 ± 9.56*
PPO (U g ⁻¹ FW)	2.33 ± 0.23	3.58 ± 0.43*

* Significant differences compared with the control sample at < 0.01, using Student's test

Transcriptome data on strawberry treated with MeJA

To study the processes at the molecular level, we performed RNA-seq analysis. Six samples divided into two treatments were sequenced, with three biological replicates per treatment. This analysis produced 51.15 G data, with an average of 8.53 G data per sample (Supplementary Table S1). An average of 60,705,769 raw reads were generated, among which 56,839,143 were clean reads with a filtration rate of 97.8%. We used HISAT to compare clean reads to the reference genome (https://www.rosaceae.org/species/fragaria_x_ananassa/genome_v1.0.a1). The total mapping rate was 91.33%, and the unique mapping rate was 76.9%, which indicated that most of the clean reads were matched to the reference genome. In addition, we conducted correlation analysis and PCA of the sequencing results to compare the repeatability between samples (Figure S2a, b). The results

showed that the repeatability of samples with the same treatments met the requirements, and that the difference in sample repeatability was significant between the control and MeJA-treated samples.

To analyse the expression distribution of DEGs, we performed cluster analysis and constructed volcano maps for the expression of DEGs. Cluster analysis was used to study differences among the samples, and we found that the biological replicates of the treatment had similar expression patterns, and there was a significant difference between the treatments (Figure S2c). Furthermore, a total of 71,643 genes were quantified in the present study, among which 1653 were identified as novel genes. A total of 10,631 DEGs were identified, of which 5534 were upregulated and 5097 were downregulated (Figure S2d, Supplementary Table S2). The distribution of the DEGs was illustrated using a volcano graph. Compared with the number of downregulated genes, the number of upregulated genes was higher and the difference was more significant.

To explore the function of DEGs, we annotated the GO and KEGG pathways for the differential genes. A total of 1290 GO items were enriched, of which 147 were significantly enriched. The GO annotation enrichment map contained the 30 most enriched items, divided into three groups: molecular functions, cellular processes, and cellular composition (Figure S2e). GO enrichment showed that the DEGs were mainly involved in photosynthesis, response to biotic stimulus, and substance metabolism. The KEGG pathway was used to map the genes expressed in different pathways. Twenty KEGG pathways with the highest enrichment degree were selected to create a bubble chart, and we found that the KEGG enrichment results were similar to the GO enrichment results (Figure S2f). The DEGs were mainly involved in photosynthesis, plant disease perception, JA biosynthesis, and secondary biomass metabolism, including 'alpha-linolenic acid metabolism', 'MAPK signalling pathway-plant', and 'phenylpropanoid biosynthesis'.

To verify the reliability of the RNA-seq results, we randomly selected 10 DEGs for qRT-PCR. The results showed that the expression pattern of qRT-PCR was similar to the

sequencing results, which indicated that the RNA-seq results were reliable (Figure S3).

Proteome data on strawberry treated with MeJA

A total of 527,503 secondary spectra were obtained by mass spectrometry. After searching the protein library, the number of available effective spectra was 46,240, and the spectrum utilisation rate was 8.8%. A total of 21,221 peptides were identified by spectral analysis, of which 20,354 were specific peptides. Furthermore, a total of 4553 proteins were identified, of which 3406 could be quantified (Figure S4a, Supplementary Table S3). Samples were also analysed by repeatability tests (Figure S4b), and the results showed that repeatability met the requirements.

After the significance test, a total of 229 proteins were identified as differentially expressed proteins, of which 75 were upregulated and 154 were downregulated (Figure S4c). The volcano map showed that the expression of most proteins did not change significantly depending on the treatment, and the fold changes in differentially expressed proteins were less than 2 (Figure S4d).

To explore the function of differentially expressed proteins, we annotated the proteins to the KEGG and COG databases and predicted their locations in the subcellular structure. KEGG enrichment results showed that these proteins were enriched in disease signal transduction and secondary biomass synthesis, including ‘plant-pathogen interaction’, ‘flavonoid biosynthesis’, and ‘phenylpropanoid biosynthesis’ (Figure S5a, b). This indicated that JA may regulate disease signal transduction and secondary metabolism. The COG database uses homology to predict protein function, and the classification results showed that most proteins were associated with ‘posttranslational modification’, ‘protein turnover’, and ‘chaperones’ (Figure S5c). In addition, many proteins were enriched in ‘energy production and conversion’, ‘carbohydrate transport and metabolism’, and ‘secondary metabolite biosynthesis, transport, and catabolism’. The prediction of subcellular localisation showed that chloroplasts contained the most proteins, followed by cytoplasm and nucleus (Figure S5d). In conclusion, functional annotation of these proteins indicated that the differentially expressed proteins were mainly involved in plant photosynthesis and disease-resistance signal transduction.

Metabolome data on strawberry treated with MeJA

Using LC–MS, we analysed the changes in metabolite content after MeJA treatment. Principal component analysis (PCA) was used to evaluate the metabolic differences and variability between the samples within the group (Figure S6a, b). The results showed that treatment repeatability met the requirements for further analysis. A total of 680

substances were detected in the positive ion mode, and 553 substances were detected in the negative ion mode. We found 239 substances with significant differences in content, of which 169 were upregulated and 70 were downregulated. The volcano map shows the number and distribution of different metabolites in negative and positive ion modes (Figure S6c, d, Supplementary Table S4). The number of upregulated metabolites was higher than the number of downregulated metabolites. All substances with different contents could be divided into 14 categories, of which the most were carbohydrates, followed by acids, amides, and lipids. In addition, we discovered 19, 15, and three compounds belonging to terpenes, phenols, and alkaloids, respectively (Supplementary Table S4).

Correlation analysis between transcriptome and metabolome

Metabolomics is a comprehensive analysis of all metabolites, and it can directly and accurately reflect the physiological state of an organism. To study the relationship between gene expression and metabolites, we integrated the KEGG annotation results of the transcriptome and metabolome (Figure S7a, b). The results showed that the pathways involved in amino acid synthesis and metabolism, such as ‘tryptophan metabolism’, ‘histidine metabolism’, and ‘lysine biosynthesis’, were co-enriched. In addition, the pathways involved in plant hormone synthesis and signalling, such as ‘alpha-linolenic acid metabolism’, ‘phenylpropanoid biosynthesis’, and ‘plant hormone signal transduction’, were also co-enriched.

Plant hormone biosynthesis and signal transduction under MeJA

Plant hormones play important roles in regulating plant developmental processes and signalling networks. MeJA and ethylene are considered to play a key role in plant defence responses to necrotic pathogens. In the present study, in MeJA biosynthesis, a total of 15 DEGs were annotated into the ‘alpha-linolenic acid metabolism’ pathway, of which 12 (including AOS, OPR3, and ACX) were upregulated. The expression of jasmonate O-methyltransferase (JMT) was also upregulated after MeJA treatment compared to that under control at the transcriptome and proteome levels. Metabolomic data showed that MeJA content significantly increased after MeJA treatment compared to that before this treatment. In ethylene biosynthesis, the expression of key enzymes, including 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO), was upregulated after MeJA treatment compared to control. According to proteomic data, MeJA treatment downregulated the expression of ACO. In addition, DEGs

involved in gibberellin biosynthesis, including ent-kaurenoic acid oxidase (KAO), gibberellin 20 oxidase (GA20OX), and gibberellin 2-beta-dioxygenase (GA2OX), were significantly upregulated after MeJA treatment. We found that the expression of beta-ring hydroxylase and zeaxanthin epoxidase (ZEP) was significantly downregulated, and these genes were involved in the biosynthesis of abscisic acid (ABA) (Xiong and Zhu 2003).

A total of 46 DEGs were enriched in the ‘plant hormone signal transduction’ pathway, of which 32 were upregulated. The expression of JAZ, MYC2, ETR, EBF1, and ERF1 was upregulated after MeJA treatment, and they were considered to cascade the signalling related to JA and ethylene. In SA signalling, the expression of NPR1 was downregulated, and the expression of PGA was upregulated after MeJA treatment. In particular, the expression of auxin influx carrier and auxin response factor (ARF) was upregulated after MeJA treatment, indicating that JA activates the auxin signalling pathway. In addition, MeJA treatment upregulated the expression of gibberellin-insensitive Dwarf1 (GID1), which is considered to be a receptor for GA. JA also regulated ABA signalling because MeJA treatment upregulates the abscisic acid receptor PYL and serine/threonine-protein kinase SAPK2 (Supplementary Table S2).

Pathogen perception and signalling transduction under MeJA

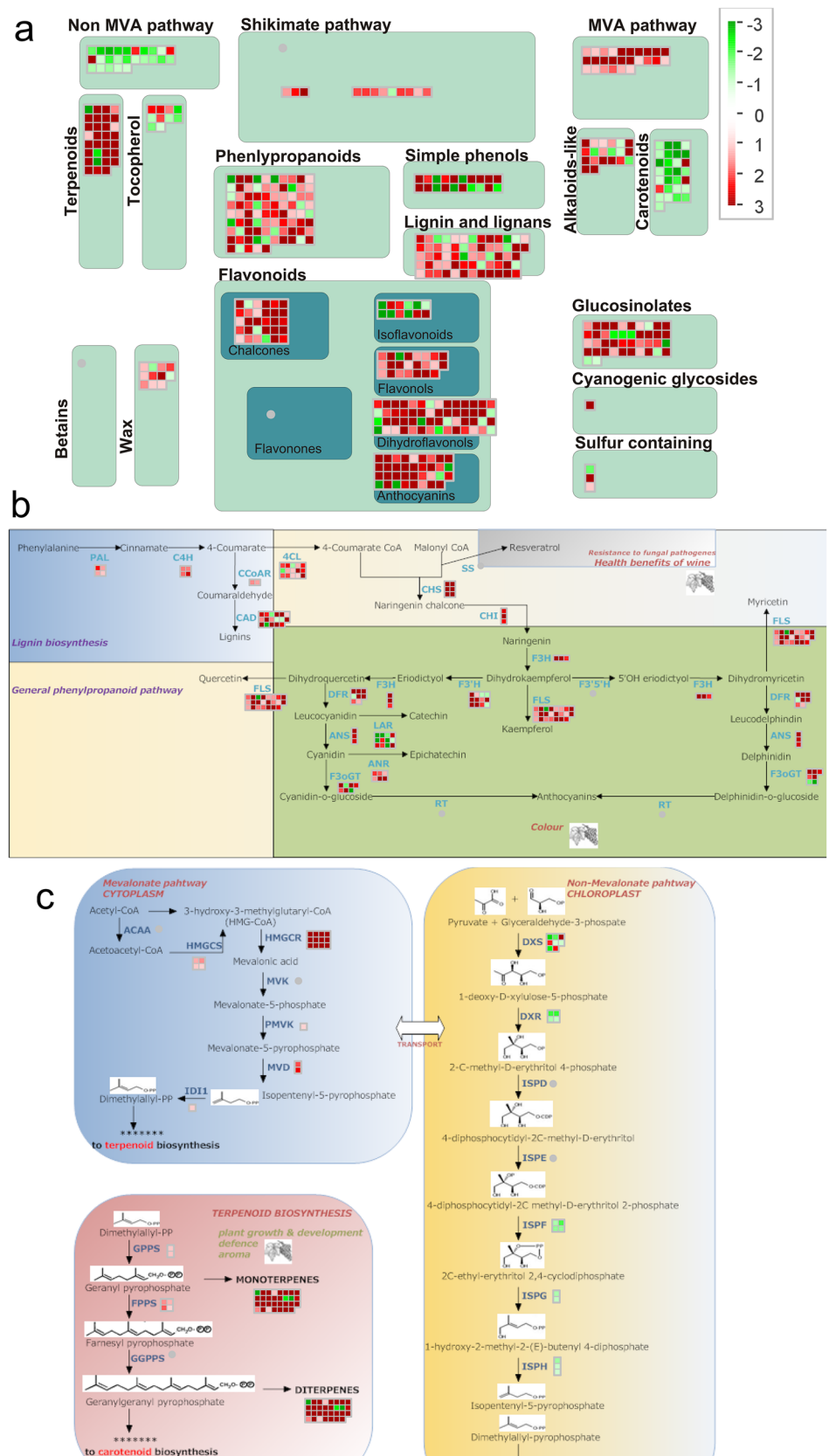
Plants perceive the flux of chemical signature components related to pathogens through pattern recognition receptors (PRRs), which are pathogen-associated molecular patterns (PAMPs) (Sun et al. 2013). According to our gene annotation results, MeJA treatment significantly upregulated the expression of flagellin-sensitive 2 (FLS2), a leucine-rich repeat receptor kinase (LRR-RK). In the MAPK signalling cascade, the expression of MEKK1 and MKK4/5 was upregulated after MeJA treatment. The expression of the transcription factor WRKY33, a downstream signal of MAPK, was also upregulated after MeJA treatment. In addition, 38 DEGs were annotated as a pathogenesis-related (PR) protein family, of which 34 were upregulated. In Ca^{2+} signalling, the expression of calcium-dependent protein kinase (CDPK) was significantly downregulated. Meanwhile, two DEGs were annotated as respiratory burst oxidase homologs (RBOH), one of which was upregulated and the other was downregulated. Furthermore, four DEGs were annotated as CAM, two of which were upregulated and the others were downregulated (Figure S8a, b). In conclusion, MeJA treatment significantly regulated the perception of pathogen attack in strawberry leaves.

Phenol and terpenoid biosynthesis under MeJA

Secondary metabolites in plants are considered to play an important role in the defence against herbivores and pathogens. To explore the regulation of JA on plant secondary metabolism, MapMan was used to annotate DEGs to the secondary metabolism pathway, and most DEGs (393/533) were upregulated (Fig. 2). According to MapMan annotation, after MeJA treatment, the DEGs annotated to the MVA pathway and terpenoids were significantly upregulated (46/49), whereas the DEGs annotated to the non-MVA pathway and carotenoid metabolism were significantly downregulated (48/54) (Fig. 2a). The annotated results of the ‘flavonoid biosynthesis’ pathway showed that the key enzymes of phenolic compounds, including PAL, C4H, 4CL, CHS, and CHI, were upregulated after MeJA treatment (Fig. 2b). Meanwhile, proteome data demonstrated that the expression of PAL, 4CL, and trans-cinnamate 4-monooxygenase was also upregulated after MeJA treatment. A total of 13 phenols were significantly upregulated after MeJA treatment, including eight phenylpropanes and five flavonoids, while only 4-coumaric acid was downregulated (Table S3).

We found that the expression of GPPS and FPPS was significantly upregulated, and they were directly involved in the synthesis of precursors such as GPP and FPP (Fig. 2c). Based on the multi-omics data, we screened a candidate gene family, terpene synthase (TPS), which plays a key role in fruit response to *B. cinerea* infection. TPS is the main enzyme involved in the terpene biosynthesis pathway. Twenty-nine DEGs were annotated as TPS, of which 26 were upregulated (Fig. 3b). Therefore, we believe that TPS responded positively to MeJA. The phylogenetic tree showed that 23 TPS genes were closely related, of which 16 genes were considered to be germacrene D synthase enzymes, five genes were alpha-pinene synthase enzymes, and two genes were serine/threonine-protein kinases TIO (Fig. 3a). In addition, STRING was used to assess the protein–protein interaction network, and the results showed that most TPS genes interacted with squalene synthase (Fig. 3c). These results showed that TPS may be an important gene for plant resistance to *B. cinerea* infection under JA. To better elucidate the effect of exogenous MeJA treatment on terpenoid biosynthesis, we annotated the DEGs and differential metabolites into the terpenoid biosynthesis pathway (Fig. 3d). A total of 19 types of terpenes were detected in the metabolome, including two monoterpenes, two sesquiterpenes, two diterpenes, two terpene glycosides, and 11 triterpenes.

Fig. 2 MapMan visualization of DEGs involved in secondary metabolism (a), phenolic metabolism (b) and terpenoid metabolism (c). Heat maps show genes with statistically significant differential expression identified by comparing MeJA and control leaves treated with *Botrytis* infection. Different squares represent DEGs, where red indicates upregulation and green indicates down-regulation



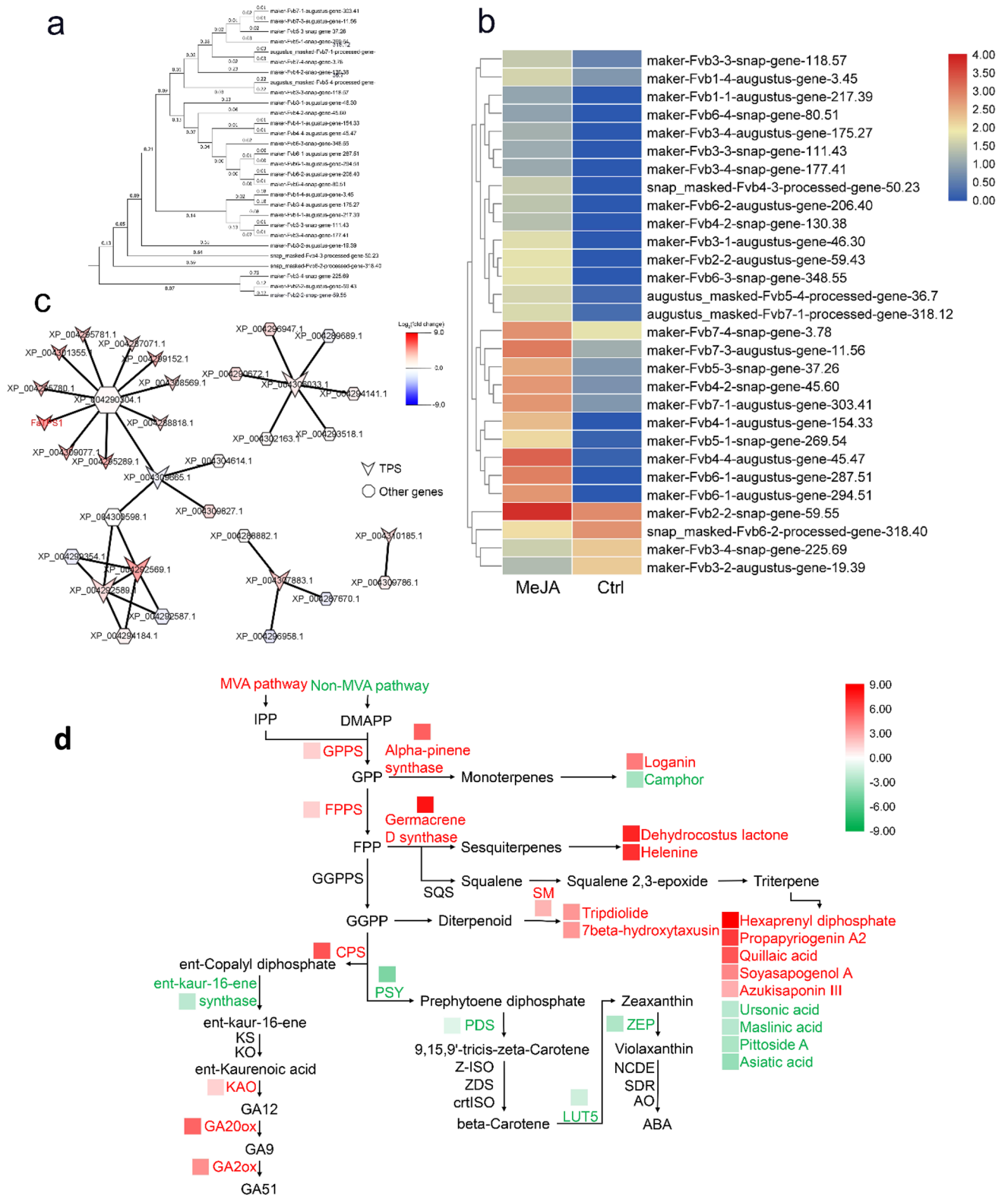


Figure 3 TPS expression and interaction protein analysis after MeJA treatment. **a** Evolutionary tree of DEGs annotated as terpene synthase family, **b** Cluster heat map of TPS family gene expression, **c** Interaction analysis between DEGs and TPS based on RNA-seq data. **d** DEGs and differential content metabolites were annotated to the pathway of terpenoid biosynthesis. GPPS: geranyl diphosphate synthase. FPPS farnesyl diphosphate synthase, GPS geranylgeranyl pyrophosphate synthase, SQS Squalene synthase, SM Squalene monooxygenase, CPS ent-copalyl diphosphate synthase, KS ent-kaurene synthase, KO ent-kaurene oxidase, KAO ent-kaurenoic acid oxidase, GA20OX gibberellin 20 oxidase, GA2OX gibberellin 2-beta-dioxygenase, LUT5 LUTEIN DEFICIENT 5, PDS phytoene desaturase, PSY phytoene synthase, CRTISO Prolycopene isomerase, Z-ISO:15-cis-zeta-carotene isomerase; AO aldehyde oxidase, NCED 9-cis-epoxycarotenoid dioxygenase, SDR short-chain dehydrogenase/reductase, ZEP zeaxanthin epoxidase

Terpene synthase *FaTPS1* underlies the resistance of strawberry fruits against *B. cinerea*

To explore the role of *FaTPS1* in the resistance of strawberry fruits to pathogen attack, we regulated *FaTPS1* expression in strawberry fruits and infected them with *B. cinerea* to evaluate the disease indices in fruits (Fig. 4a). Our results showed that *FaTPS1* was significantly upregulated in TPSO (overexpression), but significantly downregulated in TPSI (RNA interference) compared to that in PRI (control) (Fig. 4d). We found that TPSO significantly reduced the disease incidence and disease indices of strawberry fruits, whereas TPSI showed the opposite effect (Fig. 4b, c). Compared to the control, we observed an increase and decrease in the activities of antioxidant enzymes (including SOD, POD, CAT, and APX) in strawberry fruits with TPS overexpression (TPSO) and interference (TPSI), respectively (Supplementary Table S5). In addition, the MDA content, relative conductance rate, and proline content of strawberry fruits in TPSO were significantly lower and higher than those in the control and TPSI, respectively (Supplementary Table S5). In conclusion, *FaTPS1* overexpression improved the resistance of strawberry fruits to *B. cinerea* as well as their antioxidant capacity.

On the 5th day post-infection, *B. cinerea* hyphae were observed on the surface of some strawberry fruits, especially in TPSI strawberry fruits (Fig. 4a). After longitudinal sectioning, browned flesh was observed in fruits infected with *B. cinerea*. Meanwhile, in TPSI and PRI, we clearly observed the expansion of infection from the inoculation part, whereas in TPSO, infection was observed only in the wound. We used a transmission electron microscope to scan strawberry fruits infected by *B. cinerea*, and we observed the invasion of *B. cinerea* into the pulp cells in TPSI and PRI (Fig. 4e). Meanwhile, the pulp cells of TPSO were intact, and no obvious damage was observed (Fig. 4f). We believe that the overexpression of TPS delayed *B. cinerea* infection and improved strawberry resistance to *B. cinerea*.

FaTPS1 expression alters the profiling of fruit volatile compounds

We evaluated and compared the profiling of volatile compounds in strawberry fruits by GC–MS when *FaTPS1* was overexpressed (TPSO) and silenced (TPSI). To determine the type of substance, the screening conditions were set to SI and RSI values greater than 700. The mass spectrum data obtained by GC–MS were matched to those in the database (Fig. 5b). A total of 204 compounds were detected, of which TPSO, PRI, and TPSI included 116, 131, and 131 compounds, respectively (Fig. 5a, Supplementary Table S6). The component with the highest content was ‘3(2H)-furanone and 4-methoxy-2,5-dimethyl-’, which is considered to be a mildew, sweet, and caramel compound, accounting for an average of 15.14%. In addition, esters were the main components responsible for the strawberry aroma, with an average share of 85%, followed by hydrocarbons, aldehydes, and alcohols. Moreover, we found that more esters were detected in PRI and TPSI, accounting for 88 and 86%, respectively, than in TPSO (only 81%). A total of six terpenes were detected by GC–MS, including four monoterpenes and two sesquiterpenes. Among them, five terpenes were found in TPSO and PRI, and only three were found in TPSI, and their content was the highest in TPSO (Fig. 5d). In particular, the content of germacrene D was significantly upregulated in TPSO compared with that in PRI and TPSI (Fig. 5c).

FaTPS enhanced the stability of cell wall and expression of defense related genes against *B. cinerea*.

Cell wall is the first line of defense against foreign fungal infection and is responsible for biotic stressors affecting the physiological parameters in fruits. We found the *FaTPSO* fruit had a firmer in cell wall compared to control, and the cell wall components, such as pectin, water soluble pectin (WSP), GalA-EDTA soluble pectin (ESP), GalA-HCl soluble pectin (HSP), and neutral sugar content increased in the *FaTPSO* fruit, indicating that *FaTPS* could strengthen the cell wall in response to environmental stressors (Supplementary table S7). On the contrary, the cell wall metabolism enzymes, such as PG, PME, β -galactosidase, α -arabinofuranosidase, and degree of pectin esterification decreased in *FaTPSO* compared to control (Supplementary table S7), while *FaTPSI* had an opposite effect. These indicated that *FaTPS* delayed fruit softening, inhibited cell wall metabolism, and positively induced resistance against *B. cinerea* infection.

To detect whether the *B. cinerea* hyphal was affected after *FaTPS* modulation fruits, we observed *B. cinerea* growth in the strawberry fruit surface. In the *FaTPSI* fruit, the *B. cinerea* mycelium grew normally with control, which it was slender, straight, uniform in thickness and smooth in line

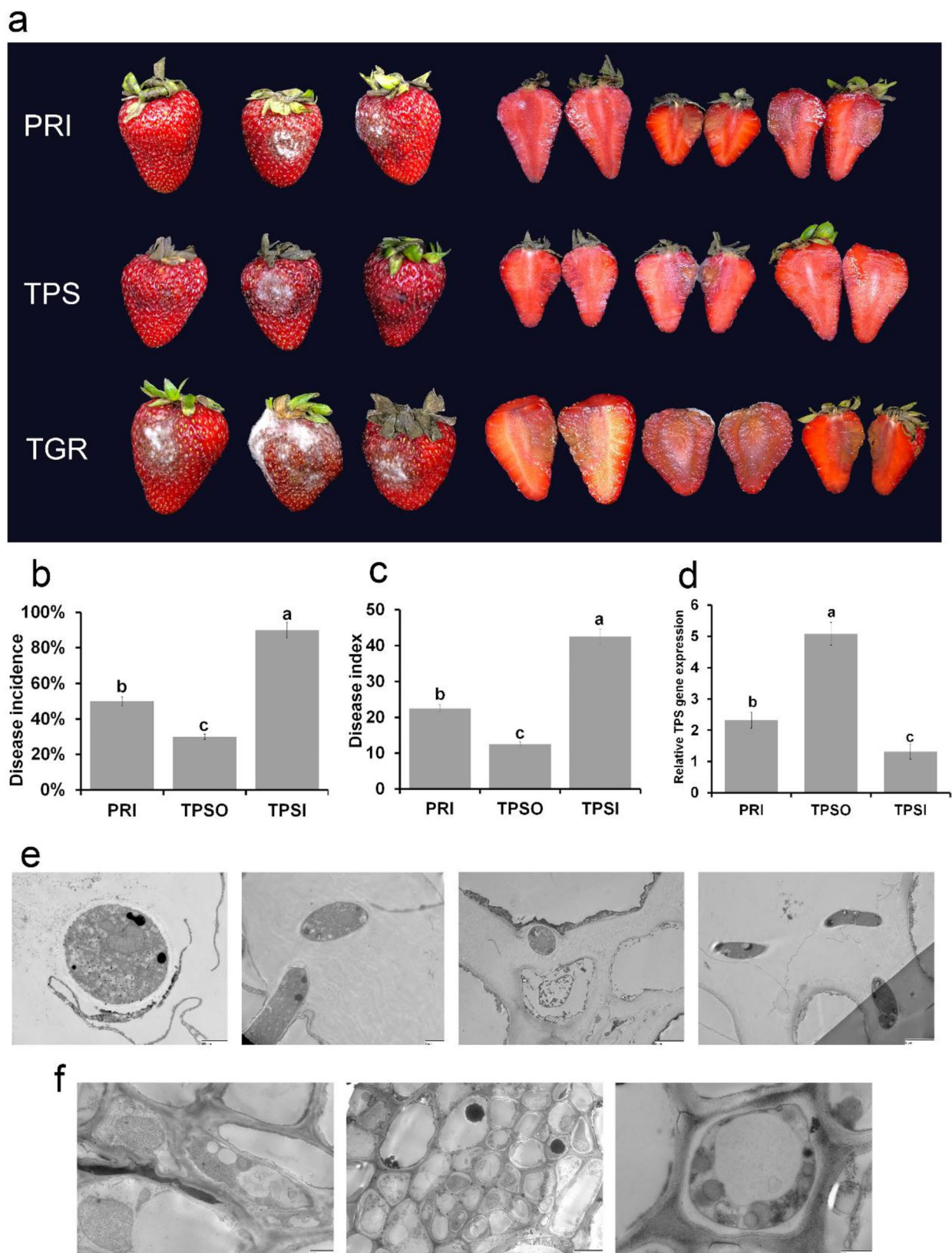


Fig. 4 a Changes in the extent of *Botrytis* infection in strawberry fruits after overexpression or interference with *FaTPS1* expression. **b** Disease index, **c** disease incidence, **d** Relative gene expression of

FaTPS1. **e** The results of transmission electron microscope scanning the fruits of TPSI (TGR) and PRI. **f** The results of transmission electron microscope scanning the fruits of TPSO (TPS)

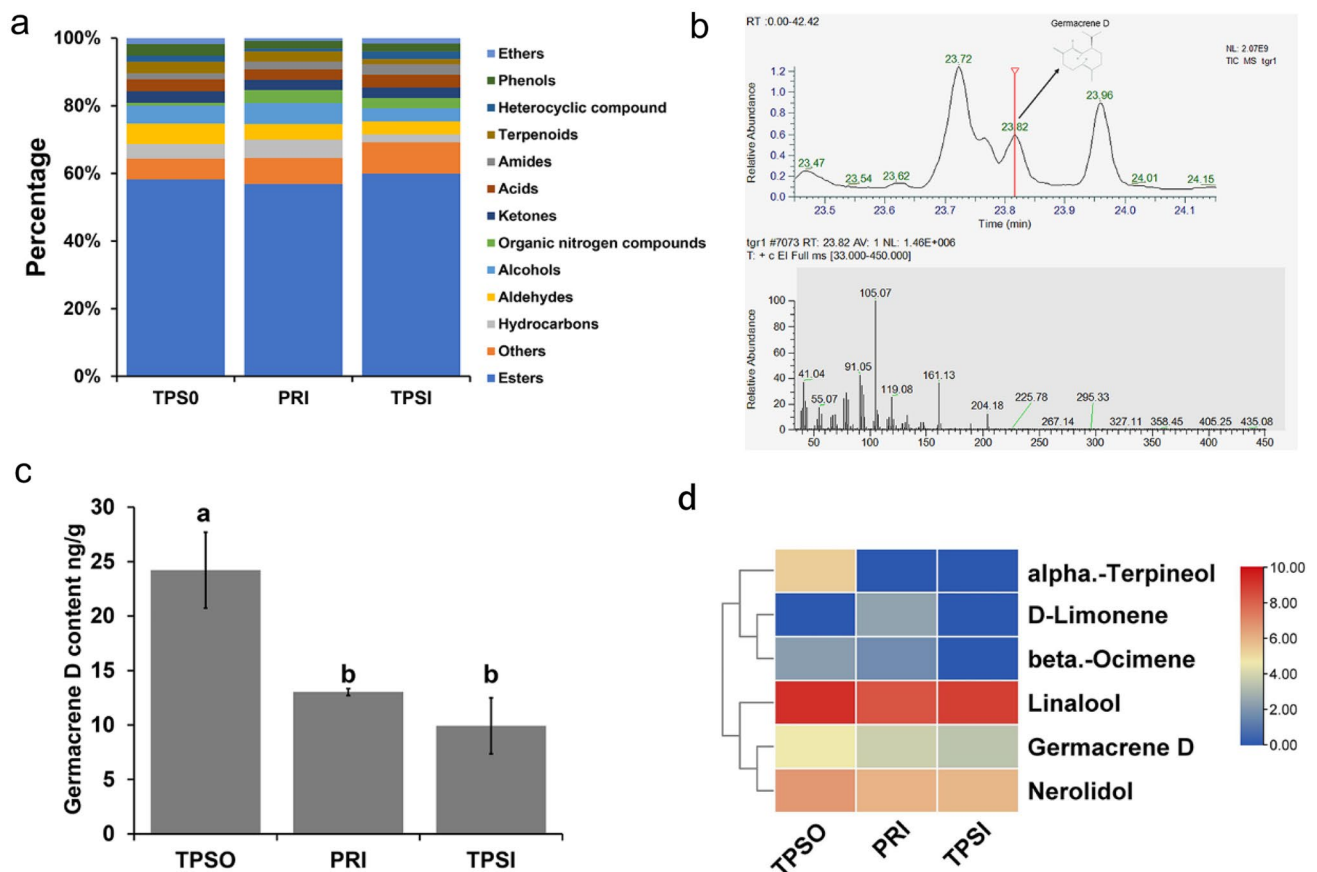


Fig. 5 GC–MS detection of strawberry fruits after overexpression or interference with FaTPS1. **a** Composition of strawberry aroma, **b** Mass spectrum of Germacrene D detected by GC–MS. **c** The content of Germacrene D. **d** Cluster heat map of terpenoid content detected by GC–MS

(Figure S9a). However, in FaTPSO fruit, the *B. cinerea* mycelial growth was damaged. The mycelium was disordered, the growth rate was slow, most of them collapsed, much branches were appeared at the growth point and branching spacing became shorter, irregular with swelling or candida-shaped, and exudation of inclusions (Figure S9a).

Meantime, the expression levels of *FaPAL*, *FaPPO*, *FaSOD* (Superoxide dismutase), *FaPOD*, *FaChitinase*, *FaPLA*, *FaPLB*, *FaPLC*, *FaB-Gall1*, *FaB-Gal4*, and *FaAral* were upregulated in *FaTPSO* fruit that led to fruit resistant against *B. cinerea* infection compared to control. However, in *FaTPSI*, these genes were downregulated (Figure S9b). These suggested that *FaTPS* could induce disease-related genes expression to control specialized metabolites in fruit, finally improve the ability of fruit against *B. cinerea* infection during fruit development.

FaTPS1 responds to MeJA through binding FaMYC2 to its promoter

MeJA could induce resistance to *B. cinerea* through the expression of TPS1 in strawberry fruits, but the molecular

mechanism of MeJA was unclear. To know which gene regulated the TPS expression, we have a screen on the TPS promoter and found three responsive elements to JA including G-box and E-box elements, that 389 bp of *FaTPS1* promoter was connected to pAbAi for Y1H blast (Fig. 6a). Results showed that *FaMYC2* (XM_004306579.2) could bind to the *FaTPS1* promoter (Fig. 6b). In addition, the transformed yeast strain with 0.9% sodium chloride suspension was diluted and spotted to SD/- Leu/ + AbA (200 ng/ml) (Fig. 6d), and we found it grew normally, indicating that the bait PAbAi-*FaTPS1* + PGADT7-*FaMYC2* could interact with together and activate the expression of the host bacteria reporter gene AbAr/AUR-C.

To further confirm whether *FaMYC2* could specifically bind to the *FaTPS1* promoter, transient expression assay was conducted using *Arabidopsis* protoplast transient expression system for transforming LUC reporter gene. Results showed that the LUC/REN ratios were significantly higher in the transformed protoplasts with the effector and G/E containing reporter than those in the control (Fig. 6e) and this promoter activity was enhanced by MeJA. However, when G-box element or E-box in the promoter of *FaTPS1* was mutated, the

LUC/REN ratios were resumed to the control (Fig. 6e). To confirm the results of the Y1H assay, strawberry fruit transient expression analysis was conducted using *FaMYC2* as an effector. The three repeated G-box or E-box sequences were fused upstream of the minimal 35S GUS (m35S-GUS) to form reporter (G-box/E-box-m35S-GUS) using m35S-GUS as a control reporter (Fig. 6f). The fruits infiltrated with only the reporters (either G-box/E-box-m35S-GUS or m35S-GUS) or the effector did not display blue color using GUS staining, while co-infiltrated with the effector and G-box/E-box-m35S-GUS were stained blue in fruits (Fig. 6g). The GUS activity was also showed that *FaMYC2* could bind to the G-box or E-box to activate downstream gene expression. These suggest that *FaMYC2* could bind to the promoter of FaTPSs to regulate FaTPSs response to *B. cinerea* infection.

Discussion

Plants rely on transmembrane pattern recognition receptors to respond to PAMPs, such as FLS2, which is the basis of induced basic resistance (Lu et al. 2011). The MAPK cascade is a universal signal transduction module which connects extracellular stimulus signals and cellular responses (Group et al. 2002). In the present study, MeJA improved the signalling pathway from perception to transduction signals by upregulating the expression of FLS2, MAPK cascade, and WRKY33. In addition, the expression of a large number of PR proteins, which are considered to be directly involved in plant defence responses, was also significantly upregulated (Ali et al. 2018). The increase in Ca^{2+} concentration in free cells is considered an early event in plant defence signalling and it activates the expression of downstream defence genes, such as ROS genes (Lecourieux et al. 2006). MeJA treatment may inhibit the transmission of Ca signals to inhibit the production of ROS, which may reduce the damage caused by *B. cinerea*. We found that MeJA treatment improved the antioxidant enzyme activities of strawberry leaves, including the activities of SOD, POD, CAT, and APX. Previous studies have suggested that necrotic pathogens benefit from programmed cell death caused by ROS accumulation (Glazebrook 2005). We concluded that MeJA treatment may regulate plant perception and signal transmission to pathogenic bacteria and improve plant defence responses.

It is generally believed that SA mediates plant resistance to vegetative pathogens, while the combination of JA and ethylene mediates plant resistance to necrotic pathogens (Glazebrook 2005). In the present study, MeJA treatment promoted the biosynthesis of endogenous JA and ethylene by upregulating the expression of key genes, such as ACS, AOS, and OPR3. In addition, we found that MeJA treatment upregulated the expression of JMT, which is considered to

be a key enzyme regulating plant responses, to promote the conversion of endogenous JA to MeJA (Seo et al. 2001). Furthermore, MeJA treatment upregulated the expression of ERF1 and MYC2, which coordinately regulated the cascade of JA and ethylene signalling and activated the expression of downstream defence genes, such as PDF1.2 (Wasternack and Hause 2013). In addition, MeJA treatment may suppress SA signalling by downregulating the expression of NPR1, which is considered to be the core of the SA signalling pathway and negatively regulates JA signalling (Spoel et al. 2003). We found that MeJA treatment affected the expression of key genes involved in the biosynthesis of GAs and ABA. Interestingly, GA and ABA play opposing roles in leaf senescence. ABA is considered to be a senescence-promoting hormone, whereas GA delays senescence (Zhang and Zhou 2013). Exogenous MeJA may delay leaf senescence to improve leaf resistance to *B. cinerea*, because senescence is one of the most important factors affecting the susceptibility of tissues to *B. cinerea* (Elad and Evensen 1995).

Plant secondary metabolites do not directly participate in the main processes of basic growth and development, but they regulate plant acclimatisation to adverse environmental conditions (Kroymann 2011; Yang et al. 2012). In the present study, we found that MeJA treatment significantly improved the secondary metabolism of strawberry leaves, especially the biosynthesis of phenols and terpenoids. Many studies have reported that the positive effects of phenolic compounds in improving plant disease resistance include limiting the growth of *B. cinerea* and eliminating active oxygen (Zhang et al. 2015; Xu et al. 2018). In the present study, the key genes involved in phenol metabolism, including PAL, C4H, 4CL, CHS, and CHI, were significantly upregulated after MeJA treatment, and the accumulation of phenolic compounds was also detected in the metabolome. In addition, MeJA treatment increased antioxidant enzyme activity in leaves, which may also be related to the upregulation of secondary metabolism.

Terpenoids play an important role in the interaction between plants and the environment, especially in pollination and defence against herbivores (Pichersky and Gershenzon 2002). We found that MeJA treatment significantly regulated terpenoid biosynthesis, while the overexpression of the TPS gene increased the resistance of strawberry fruits to *B. cinerea*. Plants synthesise IPP through two pathways: MEP and MVA. (Talapatra and Talapatra 2015). Interestingly, MeJA treatment activates the MVA pathway and inhibits the MEP pathway. The MVA pathway is located in the cytoplasm and is responsible for the synthesis of sesquiterpenes and sterols, whereas the MEP pathway is located in the plastids and is responsible for the synthesis of diterpenoids and carotenoids (Vranová et al. 2013). In addition, we found that MeJA treatment also upregulated the expression of FPPS and GPPS to promote the synthesis of FPP and GPP,

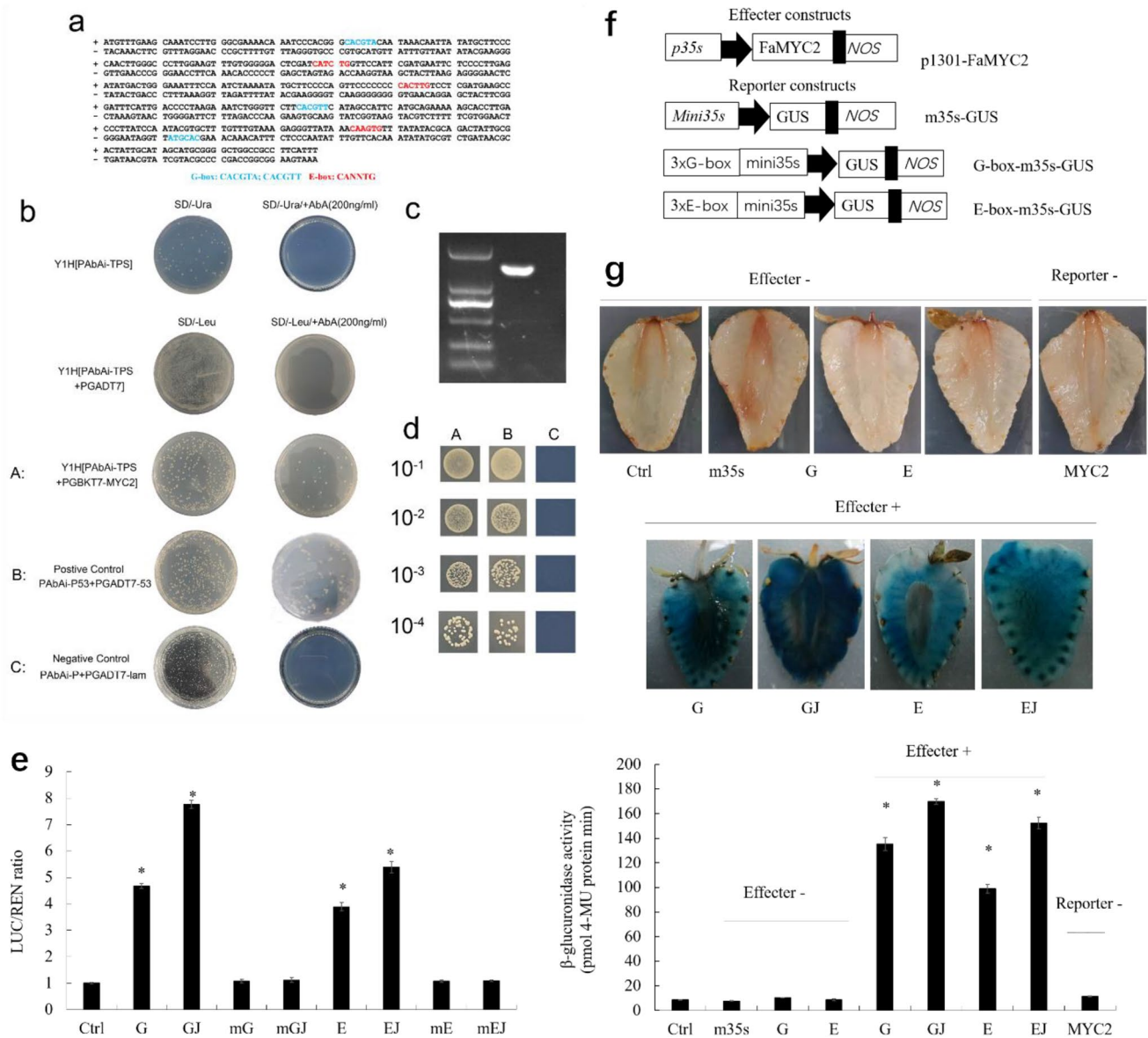


Fig. 6 FaMYC2 binds to and activates the promoters of FaTPS1. **a** Analysis of cis-acting elements of FaTPS1 promoter. **b** Yeast single hybrid. **c** The amplification of FaTPS1 promoter. **d** different concentration of yeast to verify the interaction of FaMYC2 with FaTPS1 promoter. **e** Transient expression assay of the promoter activity using Arabidopsis protoplasts co-transformed with the effector and each of the reporters containing the normal G-box or E-box element or containing mG-box or mE-box element. GJ or EJ was G-box or E-box

adding MeJA (10 μM). **f** Schematic structures of the effector (p1301-FaMYC2) and reporter vector (G/E-box-m35S-GUS or m35S-GUS) used for transient expression analysis. **g** GUS staining of strawberry fruits infiltrated with only reporters (top panel) or those co-infiltrated with the effector and the reporters (G/E-m35S-GUS; Middle panel). GUS activities was also measured with the infiltration fruits (bottom panel). – and + was the absence or present of effector or reporters, respectively

which are used as substrates by the TPS gene to synthesise monoterpenes and sesquiterpenes (Keeling and Bohlmann 2006). We also found that TPS accelerated the production of sesquiterpenes, especially germacrene D, which improves plant resistance against *B. cinerea* infection. Previous studies have reported that JA induces sesquiterpene synthesis genes and some sesquiterpenes with antifungal activity (Song et al. 2019; Taniguchi et al. 2014). In addition, some studies have

reported that MYC2 binds to G-box and E-box elements and is also considered to be the binding site of bHLH transcription factors (Hou et al. 2010; Hong et al. 2012). In the present study, we found that *FaMYC2* regulates the expression of *FaTPS1* by binding to G-box elements. *FaMYC2* and *FaTPS1* increased under MeJA treatment, which was downstream of the JA signalling pathway. Therefore, we

believe that MeJA improves plant resistance to *B. cinerea* by activating sesquiterpene biosynthesis.

Disease incidence, disease index, and transmission electron microscopy data showed that compared with PRI, TPSO delayed the infection of *B. cinerea*, whereas TPSI accelerated it. Compared with those in TPSO, more abundant and higher peaks along with more esters were observed in TPSI and PRI, which may be related to the further ripening of strawberries and release of more esters (Vandendriessche et al. 2012). Previous research has reported that *B. cinerea* may hijack maturity by inducing the biosynthesis of hormones, including ABA and ethylene (Petrasch et al. 2019). Strawberry fruits overexpressing *FaTPS1* hiked more terpenoid biosynthesis, especially germacrene D, according to GC–MS. In grapes, *FaTPS1* is considered to be a sesquiterpene synthase which promotes the biosynthesis of germacrene D (Lücker et al. 2004).

Based on our results, we established a possible model describing how MeJA treatment regulates leaf resistance to *B. cinerea* in strawberry (Fig. 7). This model provides a schematic presentation of different pathways, including pathogen perception, hormone biosynthesis and signal transduction, photosynthesis, and secondary metabolism, in response to *Botrytis* infection when the plants were pre-treated with MeJA. We believe that MeJA enhanced the

perception and signal transduction of *Botrytis* infection, which promoted plant hormone biosynthesis. MeJA treatment significantly inhibited leaf photosynthesis, including photoreaction and carbon assimilation. *FaMYC2* regulated the expression of *FaTPS1* by combining with the G-box element to promote the synthesis of terpenoids. In addition, MeJA significantly promoted the synthesis of phenolics and enhanced the activity of plant antioxidant enzymes to improve strawberry resistance to *B. cinerea*.

Conclusion

According to integrating transcriptome, proteome and metabolome data, MeJA treatment significantly activates pathogen signal transduction, increases endogenous JA biosynthesis, and promotes the biosynthesis of phenols and terpenoids. MeJA treatment significantly upregulated genes related to terpene precursors and terpene biosynthesis, especially the MVA pathway and sesquiterpenes. In particular, the expression of *FaTPS1* was significantly upregulated, and overexpression of *FaTPS1* significantly improved the resistance of strawberry fruits against *B. cinerea*. Results of yeast monohybrid and luciferase

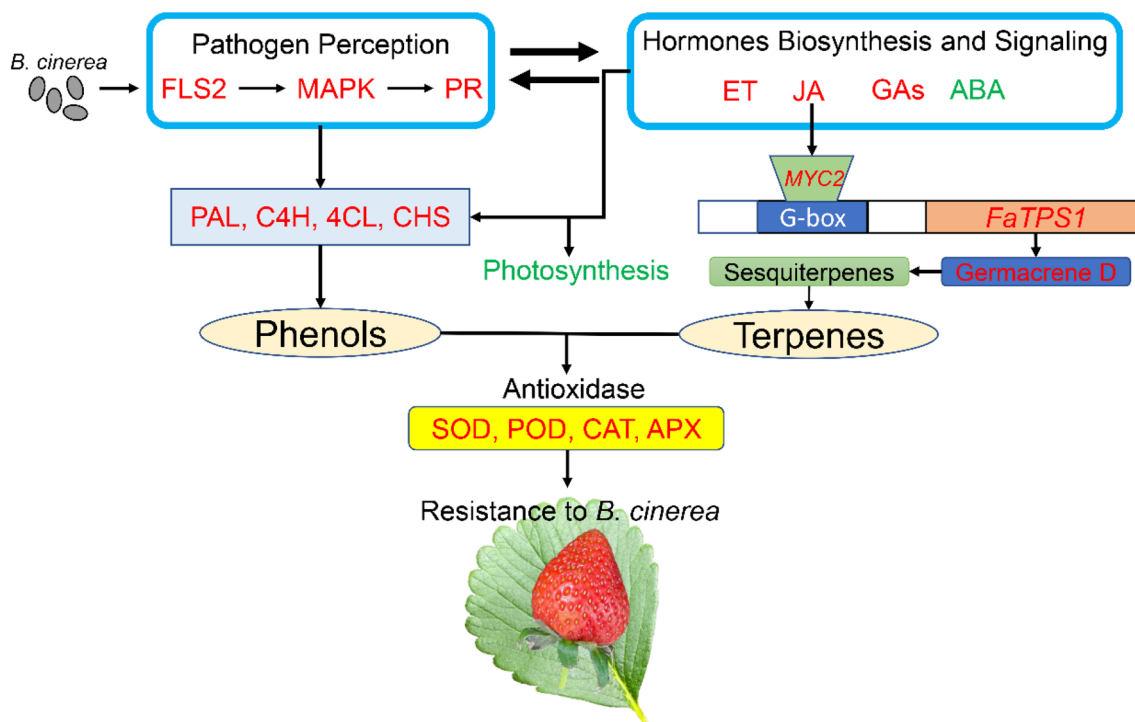


Fig. 7 Schematic model of the effect of exogenous MeJA on intracellular mechanism in the molecular and metabolic levels when *Botrytis* fungus attacks strawberry leaves. Red and green color of genes is related to up- and down-regulation, respectively. *FLS2*, flagellin-sen-

sitive 2; *MAPKs*, mitogen-activated protein kinases; *PR*, pathogenesis-related protein; *PAL*, phenylalanine ammonia-lyase; *C4H*, Cinnamate 4-hydroxylase; *4CL*, 4-coumarate-CoA ligase; *CHS*, Chalcone synthase

showed that *FaMYC2* combined with G-box cis-acting elements to regulate the expression of *FaTPS1*. GC–MS analysis showed that the overexpression of *FaTPS1* increased the content of sesquiterpenes, especially Germacrene D.

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Author contribution statement HFJ and JGF: Conceptualization and Funding acquisition; ZBZ and WBY: Validation and Writing—original draft; SE and SWL Writing—review a editing; YPZ, and HFJ: Formal analysis and Investigation.

Declarations

Conflict of interest The authors declare no competing financial interest.

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