

# Comparative transcriptome analysis identifies jasmonic pathway genes related to anther dehiscence in *Solanum melongena* L.

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## Research article

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# Abstract

**Background:** Anther indehiscence is an important form of functional male sterility that can facilitate the production of hybrid seeds. However, the molecular mechanisms of anther indehiscence-based male sterility in eggplant (*Solanum melongena* L.) have not been thoroughly explored. Therefore, we performed RNA-seq analysis to investigate the molecular mechanisms of anther dehiscence in eggplant.

**Results:** We used transcriptome sequencing and qRT-PCR assays to compare the anthers of normally developing (F142) and anther indehiscent (S12) eggplant. We identified 2670 differentially expressed genes between lines. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses identified 31 differentially expressed genes related to hormone biosynthesis. We therefore measured the contents of the phytohormones jasmonic acid (JA), IAA, GA3 and ABA in S12 and F142. There were no significant differences in IAA, GA3 or ABA levels between S12 and F142. However, JA levels were significantly lower in S12 than in F142. Five key genes in the JA signaling pathway were differentially expressed in S12 vs. F142. Of these, SmJAZ and SmJAR-like were significantly up-regulated and SmDAD1, SmLOX and SmCOI1 were down-regulated in S12 vs. F142. In DNA-protein interaction studies, SmLOX, SmAOC, SmOPR3, SmCOI1 and SmJAZ1 failed to be enriched on the chromatin of SmDAD1. Protein–protein interaction studies identified a direct interaction between SmDAD1 and SmLOX, but SmDAD1 failed to interact with SmAOC, SmOPR3, SmCOI1 and SmJAZ1.

**Conclusion:** JA is an essential factor affecting anther dehiscence in eggplant. SmDAD1 interacts with SmLOX to alter JA levels, thereby regulating anther dehiscence. Our data represent a valuable resource for further exploring the regulatory mechanisms underlying anther dehiscence in eggplant.

## Introduction

Eggplant (*Solanum melongena* L.), a popular vegetable crop that is thought to have originated in Africa, is widely cultivated in Africa, Asia, Europe, and the Near East [1]. The major characteristics of eggplant exhibit obvious heterosis, and the early use of hybrid vigor in the breeding of eggplant cultivars has been described. The use of reliable male-sterile systems could simplify the process and reduce the labor, cost, and time involved in producing hybrid eggplant seeds [2]. Functional genic male sterility (GMS) was first reported in eggplant in 1954 [3–6]. These GMS lines show anther indehiscence in which the anthers do not open to release pollen, thereby disabling pollination [7]. Anther dehiscence is a vital process in which mature pollen grains are released from the locules of the anther, thus enabling pollination [8, 9]. Although the morphological changes in anthers during dehiscence have been thoroughly described [8, 10], the molecular mechanisms controlling anther dehiscence remain relatively unknown.

Jasmonic acid (JA) is a lipid-derived hormone that functions as an important regulator of plant responses to various stresses as well as development [11]. Analysis of plants showed that JA affects wheat development, including germination, growth, flowering time, senescence, and alters tolerance to environmental stresses [12]. Wheat plants with high JA levels are characterized by delayed germination, slower growth, late flowering and senescence, and improved tolerance to short-term freezing [13]. The application of exogenous jasmonate significantly stimulated root hair elongation [1]. JA plays an important role in regulating anther dehiscence

[14, 15]. JA signal transduction pathways have been investigated in *Arabidopsis thaliana* [16, 17]. JA biosynthesis originated from fatty acids in chloroplasts, and then its metabolic compounds are produced from 12 different pathways in peroxisomes and cytosol, respectively [16].

Gene mutations involved in JA biosynthesis cause failure or delay of anther dehiscence and may lead to male sterility. Several of these genes have been identified, such as anther dehiscence defect 1 (DAD1) [14], AOS [18]; LOX [19]; COI1 [20]; DEHISCENCE 1 (DDE1)/OPR3 [21, 22]; and the triple mutation (fad3 fad7 and fad8) [23]. In addition, JAR1 (a JA-amino acid synthetase) has the biological function in regulating the flower opening and closure and anther dehiscence in rice [15]. Some studies have found possible mechanisms that JA is a significant regulator of anther dehiscence. Through activating DAD1 in *Arabidopsis*, a RING-type E3 ligase controls anther dehiscence [24]. However, the exact mechanisms of JA activity regulating anther dehiscence remain to be elucidated. It is also unclear how genes in the JA pathway are mutually regulated.

In the current study, we performed transcriptome analysis to identify differentially expressed genes (DEGs) in eggplant S12 (indehiscent anthers) and F142 (normal anther development) in order to uncover differences in the anther indehiscence network. Enrichment analysis of the DEGs and endogenous hormone measurements highlighted the effect of JA signal transduction pathways in anther dehiscence. Finally, we analyzed the relationships between JA pathway genes by yeast two-hybrid and yeast one-hybrid analyses. Our results lay the foundation for further uncovering the molecular mechanisms and biological function of anther dehiscence in eggplant.

## Results

### Morphological Comparison Of F142 And S12

Anther indehiscence is an important form of functional male sterility. Morphological analysis indicated that eggplant accession S12 produced indehiscent anthers, whereas F142 produced normal anthers (Figs. 1A, B, C and D). We used the 2, 3, 5-triphenyl tetrazolium chloride staining method to further investigate pollen viability. The activity of S12 pollen was similar to that of F142 pollen (Fig. 1E, F). These results indicate that S12 produces anthers with an abnormal appearance but has normal pollen viability.

### Transcriptome Assembly

To detect potential molecular differences between eggplant accessions S12 and F142, we performed transcriptome sequencing on anther tissue. The genome-directed stratagem Trinity was used to assemble transcriptome sequences and align single RNA-seq library data with the *S. melongena* genome (SME\_r2.5.1). As a result, the mean of clean reads from three biological repeats per sample was more than 20 million, the mean of mapped reads and uniquely mapped reads were far greater than 10 million, and the highest localization ration of each library was roughly 50% in the *S. melongena* genome (Table 1 and Table S2). After being assembled the average length of the contig was 1204 bp, while the contig of N50 was 1538 bp. We found that the average GC content in the libraries reached 40–50% (Table S2 and S3). When redundant and

short reads were removed, we obtained about 257,800 transcript assembly contigs (TAC) > 100 bp (Table S2). Overall the abundant transcriptome data are enough for further analysis.

Table 1  
Statistical analysis of RNA-seq reads mapped to the ancestor genome

Samples	Total Reads	Mapped Reads	Uniq Mapped Reads	Multiple Map Reads	Clean Reads	GC Content	%≥Q30
F142	42,791,674.67	39,368,088	38,019,098	1,348,989	21,395,837	42.68%	94.41%
S12	43,133,323.33	39,694,750	38,266,857	1,427,894	21,566,662	42.93%	94.70%

## Analysis Of Biological Processes In S12

We identified 2,670 DEGs in S12 vs. F142 (Table S4 and Figure S2), including 1,928 up-regulated and 742 down-regulated DEGs (Figure S2). We constructed a heatmap representing the differential expression of the 2670 DEGs (Figure S5) in S12 vs. F142. We detected dynamic changes in the transcriptomes during anther development in S12 (anther indehiscence). In the heatmap, the original gene expression data were transformed into log<sub>2</sub> fold change values (Table S4). Further cluster analysis revealed significant differences in gene expression between F142 and S12.

To characterize the DEGs in detail, we performed Gene Ontology (GO) analysis to uncover their putative functions. We constructed histograms based on the categories of DEGs in anther indehiscent eggplant (S16), including biological processes, molecular functions, and cellular compartments (Figure S3). Metabolic process was the major group in the biological processes category, including GO terms cellular, single-organism, response to stimulus, and biological regulation. The molecular functions category contained at least 882 DEGs involved in nucleic acid binding transcription factor activity and more than 888 DEGs involved in signal transducer activity, transcription factor activity, and protein binding. Most of the DEGs were present in the top three groups, including the cell, organelle, and membrane (Figure S4). We performed KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis to categorize all annotated genes. Most of the 2,670 DEGs were categorized into six pathways (Figure S4). The most highly enriched biological processes in anther indehiscent eggplant were plant hormone signal transduction, protein processing in endoplasmic reticulum, amino sugar and nucleotide sugar metabolism, biosynthesis of amino acids, carbon metabolism and plant–pathogen interactions (Fig. 2 and S4).

## Enrichment Analysis Of Degs In Hormone Signal Transduction Pathways

We performed KEGG pathway analysis to investigate the major regulatory pathways of the DEGs. The DEGs in S12 vs. F142 were mainly enriched in the pathways inositol phosphate metabolism (16 DEGs), plant hormone signal transduction (31 DEGs), flavonoid biosynthesis (12 DEGs), amino sugar and nucleotide

sugar metabolism (39 DEGs), and fatty acid biosynthesis (25 DEGs) (Figs. 2 and 3). To investigate the hormonal control of anther indehiscence in more detail, we analyzed the expression levels of key DEGs in the JA, auxin (IAA), gibberellin (GA), abscisic acid (ABA), cytokinin (CTK), ethylene (ETH) and brassinosteroid (BR) signaling pathways (Fig. 4 and Table 2). Five key genes were identified in the JA signaling pathway, including DAD1, LOX, COI1, JAZ1 and JAR-like, including two that were significantly upregulated and three that were downregulated in S12 vs. F142. One key gene in the CTK signaling pathway and two genes in the ABA signaling pathway were differentially expressed in S12 vs. F142. Two key genes in the GA signaling pathway were significantly upregulated and two were significantly down-regulated in S12 vs. F142. Four key genes in the IAA signaling pathway were differentially expressed in S12 vs. F142. Four key genes in the ETH signaling pathway were also differentially expressed in S12 vs. F142; all four were significantly up-regulated in S12 vs. F142. Four genes in the BR pathway were significantly upregulated in S12 vs. F142 as well. Finally, nine genes in other hormone signaling pathways were differentially expressed in S12 vs. F142, including two that were significantly down-regulated in the indehiscent accession (Fig. 4).

Table 2  
Differentially expressed genes involved in hormone signaling pathways

Gene_ Id	Function Description	Up_Down
Jasmonate Acid		
Sme2.5_03464.1_g00003	jasmonate ZIM-domain protein 1	up
Sme2.5_01200.1_g00003	defective in anther dehiscence1	down
Sme2.5_03723.1_g00002	coronatine-insensitive 1	down
Sme2.5_00363.1_g00004	lipoxygenase	down
Sme2.5_06810.1_g00003	jasmonic acid-amido synthetase JAR1-like	up
Auxin		
Sme2.5_01815.1_g00004	auxin influx carrier (LAX family), LAX1	up
Sme2.5_00736.1_g00017	auxin responsive GH3 gene family, indole-3-acetic acid-amido synthetase GH3.1	up
Sme2.5_00946.1_g00005	auxin-responsive protein IAA26-like	down
Sme2.5_04620.1_g00003	auxin responsive GH3 gene family, indole-3-acetic acid-amido synthetase GH3.6	up
Brassinosteroid		
Sme2.5_00033.1_g00010	brassinosteroid resistant 1-like	up
Sme2.5_02777.1_g00002	brassinosteroid insensitive 2, shaggy-related protein kinase eta-like	up
Sme2.5_00067.1_g00001	brassinosteroid insensitive 1, BRI1	up
Sme2.5_16526.1_g00002	brassinosteroid resistant 1, BES1/BZR1 homolog protein 2-like	up
Ethylene		
Sme2.5_02344.1_g00010	ethylene receptor 2-like	up
Sme2.5_04397.1_g00003	ethylene-insensitive protein 3, EIN3	up
Sme2.5_04397.1_g00004	ethylene-insensitive protein 3, EIN3-like	up
Sme2.5_07322.1_g00001	ethylene receptor 1, ETR1	up
Cytokinin		
Sme2.5_05437.1_g00002	histidine kinase 2-like isoform X1 (cytokinin receptor)	up
Abscisic Acid		

Gene_Id	Function Description	Up_Down
Sme2.5_01218.1_g00005	abscisic acid-insensitive responsive element binding factor, ABA 5-like protein 7	up
Sme2.5_04709.1_g00002	ABA responsive element binding factor, G-box-binding factor 4	up
Gibberellin		
Sme2.5_00083.1_g00005	gibberellin receptor GID1B-like	down
Sme2.5_00038.1_g00002	F-box protein GID2	up
Other Hormone		
Sme_newGene_256	SAUR family protein, uncharacterized LOC107006819	down
Sme_newGene_493	Myb-like DNA-binding domain, two-component response regulator ARR14	up
Sme2.5_00575.1_g00014	protein phosphatase 2C 6-like	up
Sme2.5_03790.1_g00001	Myb-like DNA-binding domain, Two-component response regulator ARR18-like	up
Sme2.5_01620.1_g00003	serine/threonine-protein kinase SRK2	up
Sme2.5_00225.1_g00038	somatic embryogenesis receptor kinase 3B precursor, SERK3B	up
Sme2.5_11093.1_g00002	SAUR family protein, uncharacterized protein LOC104880086	down
Sme2.5_02487.1_g00002	serine/threonine-protein kinase, SAPK3	up
Sme2.5_13238.1_g00001	two-component response regulator ARR17-like	up

## Endogenous hormone measurements and validation of the expression patterns of several key genes

We collected independent anther samples from the plants and performed qRT-PCR analysis to validate the expression levels of several key JA-related genes. In total, we measured the expression levels of seven JA-related unigenes via qRT-PCR. JAZ1 and JAR1 were significantly up-regulated and DAD1, LOX, OPR3, AOC and COI1 were significantly down-regulated in S12. The expression patterns of these genes corresponded well with the FPKM values obtained by RNA-seq (Fig. 6), suggesting that the expression patterns of most unigenes were consistent between the two methods. Finally, we measured JA, IAA, GA<sub>3</sub> and ABA levels in S12 and F142 (Fig. 5). There were no significant difference in IAA, GA<sub>3</sub> or ABA levels in S12 vs. F142. By contrast, JA levels were significantly lower in S12 than in F142. These findings suggest that JA is an important factor for anther dehiscence in eggplant.

# The SmDAD1 promoter does not directly interact with SmLOX, SmAOC, SmOPR3, SmCOI1 or SmJAZ1

The JA biosynthesis and signal transduction pathways involve the induction and inhibition of a series of genes [11, 12, 25, 26]. However, it is unclear whether these genes interact to mediate anther dehiscence. We performed yeast one-hybrid assays to measure the enrichment of SmLOX, SmAOC, SmOPR3, SmCOI1, SmJAZ1 on the chromatin of SmDAD1 (Fig. 7A). None of the proteins bound to the promoter of SmDAD1. Consistent with the yeast one-hybrid results, a Dual-Glo Luciferase assay revealed that SmLOX, SmAOC, SmOPR3, SmCOI1, SmJAZ1 protein failed to bind to the SmDAD1 promoter in vivo (Fig. 7B), as the relative ratio of LUC to REN in all experiments using pGreen II 62SK-SmLOX, 62SK-SmAOC, 62SK-SmOPR3, 62SK-SmCOI1, and 62SK-SmJAZ1 combined with pGreen II 0800LUC-DAD1 were not significantly different from that of the negative control.

## Interaction Of Smdad1, Smlox, Smaoc, Smopr3, Smcoi1 And Smjaz1

Finally, since SmLOX, SmAOC, SmOPR3, SmCOI1 and SmJAZ1 failed to interact with the SmDAD1 promoter, we performed yeast two-hybrid assays to detect the interactions of SmDAD1 protein with SmLOX, SmAOC, SmOPR3, SmCOI1, and SmJAZ1 (Fig. 8A). SmDAD1 directly interacted with SmLOX but not with SmAOC, SmOPR3, SmCOI1 or SmJAZ1 (Fig. 8A). We also performed pull-down assays to determine whether SmDAD1 interacts with SmAOC, SmOPR3, SmCOI1 and SmJAZ1 (Fig. 8B). SmLOX-GST was pulled down by SmDAD1-HIS, but SmAOC-GST, SmOPR3-GST, SmCOI1-GST and SmJAZ1-GST were not. Therefore, our yeast two-hybrid results were replicated in the pull-down assays (Fig. 8).

## Discussion

Transcriptome analysis facilitates the comprehensive investigation of altered gene expression patterns in genetic variants and provides insights into the molecular basis of specific biological processes [27]. Transcriptome sequencing provides a systemic approach for studying gene expression patterns and network interactions underlying various processes in plants. In the current study, to explore the molecular mechanism underlying anther dehiscence in eggplant, we generated a high-quality transcriptome dataset from anthers of normally developing (F142) and anther indehiscent (S16) eggplant. The most highly enriched biological processes among the DEGs in indehiscent anthers were plant hormone signal transduction, protein processing in endoplasmic reticulum, amino sugar and nucleotide sugar metabolism, biosynthesis of amino acids, carbon metabolism and plant–pathogen interaction.

We identified 31 DEGs (Table 2) involved in hormone signal transduction pathways, including JA, IAA, GA, ABA, CTK, ETH and BR. Plant hormones play essential roles in regulating plant growth and development as well as plant fertility. The accumulation or deficiency of auxin in plants is related to the occurrence of male sterility. The IAA content in a cytoplasmic male sterile rapeseed line was consistent with that of normal plants under low temperature conditions. However, the IAA content of the sterile line increased with

increasing temperature, whereas no change in IAA levels was detected in fertile plants [28]. This phenomenon was also observed in tomato mutants [29]. Horner suggested that auxin accumulation causes male sterility in crops, as high IAA levels in pepper induced the production of ETH, which induced male sterility [30]. In addition, Li Yingxian et al. studied K and T types of sterile wheat and determined that during pollen abortion, IAA levels were lower in sterile anthers than in fertile anthers [31]. This phenomenon was also observed in rice [32], citrus [33], and mustard [34].

Moreover, CTK levels were lower in cytoplasmic male sterile barley lines than in their maintainer lines [35]. The excess ABA in the leaves and anthers of cytoplasmic male sterile cabbage lines might be related to the occurrence of microspore abortion [36]. Liu found that in male sterile wheat lines (induced by GENESIS), after induction, ETH levels were significantly higher in sterile lines than in fertile lines during the mononuclear, dinuclear and trinuclear stages of anther development [37]. The rate of infertility increased with increasing induction, and the rate of infertility and the ETH release rate also increased. Finally, wild-type Arabidopsis plants treated with GA and double mutants in the GA signaling repressors RGA and GAI exhibited a loss of fertility [38].

Plant development and responses to environmental signals are coordinated by complex multicomponent signaling networks. JA, a phytohormone derived from fatty acids, is an important component of this regulatory system. JA participates in all stages of plant growth and development. JA also regulates anther dehiscence [15, 39]. In the current study, the transcript levels of JA biosynthesis genes were always lower in anther indehiscent plants than in plants with normal anther development. The JA content was also significantly lower in these plants than in normal eggplant. This observation, which is consistent with the results of transcriptome sequencing, confirms the notion that JA is an essential factor affecting anther dehiscence. This finding validates the results of previous studies. For example, mutations in genes involved in JA biosynthesis typically cause delayed or failed anther dehiscence, including DAD1 [14, 40] and OPR3 [21, 41].

Based on the current transcriptome data for genes in the JA biosynthesis pathway, we propose that feedback regulation of JA signaling in anther-indehiscent eggplant alters the expression patterns of genes at the mRNA level during anther development [42–44]. In this study, we identified 5 JA pathway genes (DAD1, LOX, COI1, JAZ1, and JAR-like) that were differentially expressed in S12 vs. F142 eggplant. DAD1, LOX and COI1 were clearly downregulated in S12, whereas JAZ1 and JAR-like were upregulated in S12 vs. F142. DAD1 is crucial for JA biosynthesis. Arabidopsis DAD1 (At2g44810) encodes the first chloroplastic lipase identified. This enzyme is involved in supplying  $\alpha$ -linolenic acid for the JA-biosynthetic pathway [14]. Mutations in DAD1 reduce JA levels in flower buds, causing a delay in their development, failed anther dehiscence during flower opening, and the lack of pollen grain maturation [14]. In the Arabidopsis coi1, opr3, and dad1 mutants, the anthers fail to crack and the filaments are short: these phenotypes were significantly altered by the external application of JA [21, 45].

The JA pathway involves a series of genes-encoded hormone-related factors involved in anther dehiscence [15, 25, 46]. Previous gene expression analysis demonstrated that overexpressing AtOPR3 selectively affected the expression of various genes of the endogenous jasmonate system, while the expression of other genes remained unaltered. Transgenic wheat plants with high AtOPR3 expression levels exhibited notably

altered plant growth and development, including delayed germination, slower growth, and anther indehiscence [47]. These findings indicate that these plant phenotypes are regulated by direct or indirect interactions of these genes.

In the current study, we measured the enrichment of SmLOX, SmAOC, SmOPR3, SmCOI1, and SmJAZ1 on the chromatin of SmDAD1. None of these proteins bound to the promoter of SmDAD1. Maeli (2008) demonstrated that the physical interaction between COI1 and JAZ proteins could be effectively promoted by treatment with biologically active jasmonates (JA-Ile) [28, 48]. In addition, AtMYC2 interacts with JAZs [25, 49]. Here, we demonstrated that SmDAD1 interacts with SmLOX1 in vitro and in Y2H assays. However, how these proteins regulate anther dehiscence remains unclear and should be addressed in future studies.

## Conclusion

The transcriptomes compared results of the normally developing anthers (F142) and indehiscent (S12) anther of eggplant showed that the number of genes differentially expressed in S12 was 2670 and mainly enriched in inositol phosphate metabolism, plant hormone signal transduction, flavonoid biosynthesis, amino sugar and nucleotide sugar metabolism. Gene Ontology (GO) database and Kyoto Encyclopedia of Genes and Genomes (KEGG) database analyses identified 31 genes related to hormone biosynthesis. The contents of JA, IAA, GA<sub>3</sub> and ABA were measured in F142 and S12. It was found that there was no significant difference in IAA, GA<sub>3</sub> and ABA between F142 and S12. On the contrary, the content of JA in S12 was significantly lower than that of F142. Five key genes were identified in the jasmonic acid (JA) signaling pathway, of which SmJAZ and SmJAR-like were significantly upregulated and SmDAD1, SmLOX and SmCOI1 were downregulated. In addition, DNA-protein interaction studies identified failed to enrich of SmLOX, SmAOC, SmOPR3, SmCOI1 and SmJAZ1 on the chromatin of SmDAD1. Protein-protein interaction studies identified a direct interaction between SmDAD1 and SmLOX, but SmDAD1 failed to interact with SmAOC, SmOPR3, SmCOI1 and SmJAZ1. This test provides valuable information for subsequent related research. Furthermore, the specific regulatory mechanisms of these genes in anther dehiscence need further study.

## Materials And Methods

### Plant Materials and Growth Conditions

The S12 (anther indehiscent) and F142 (normal plant) *S. melongena* L. lines were provided and grown at the Institute of Vegetables and Flowers, Chongqing Academy of Agricultural Sciences (Chongqing, China) from 2017 to 2019. The eggplant seeds were sterilized and sown in trays. Then, the seedlings were transferred and grown under normal conditions. Selected anthers were immediately frozen in liquid nitrogen and stored at -80°C until they were used for further analysis.

### RNA extraction, library construction, and RNA-Seq

Total RNA of each sample was extracted from the anther of eggplant according to the instruction manual of the TRIzol Reagent (Life technologies, California, USA). RNA integrity and concentration were examined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). The mRNA was isolated by NEBNext Poly (A) mRNA Magnetic Isolation Module (NEB, E7490). The cDNA library was constructed following the manufacturer's instructions of NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, E7530) and NEBNext Multiplex Oligos for Illumina (NEB, E7500). In brief, the enriched mRNA was fragmented into approximately 200nt RNA inserts, which were used to synthesize the first-strand cDNA and the second cDNA. The double-stranded cDNA were performed end-repair/dA-tail and adaptor ligation. The suitable fragments were isolated by Agencourt AMPure XP beads (Beckman Coulter, Inc.), and enriched by PCR amplification. Finally, the constructed cDNA libraries of the eggplant were sequenced on a flow cell using an Illumina HiSeq™ sequencing platform.

## Transcriptome analysis using reference genome-based reads mapping

Low quality reads, such as only adaptor, unknown nucleotides >5%, or Q20 <20% (percentage of sequences with sequencing error rates <1%), were removed by perl script. The clean reads that were filtered from the raw reads were mapped to eggplant genome (SME\_r2.5.1) using Tophat2 [50] software. The aligned records from the aligners in BAM/SAM format were further examined to remove potential duplicate molecules. Gene expression levels were estimated using FPKM values (fragments per kilobase of exon per million fragments mapped) by the Cufflinks software [51].

## Identification of differential gene expression

DESeq and Q-value were employed and used to evaluate differential gene expression between F142 and S12. After that, gene abundance differences between those samples were calculated based on the ratio of the 2 FPKM values. The false discovery rate (FDR) control method was used to identify the threshold of the P-value in multiple tests in order to compute the significance of the differences. Here, only gene with an absolute value of log<sub>2</sub> ratio ≥2 and FDR significance score <0.01 were used for subsequent analysis.

## Sequence Annotation

Genes were compared against various protein database by BLASTX, including the National Center for Biotechnology Information (NCBI) non-redundant protein (Nr) database, Swiss-Prot database with a cut-off E-value of 10<sup>-5</sup>. Furthermore, genes were searched against the NCBI non-redundant nucleotide sequence (Nt) database using BLASTn by a cut-off E-value of 10<sup>-5</sup>. Genes were retrieved based on the best BLAST hit (highest score) along with their protein functional annotation.

To annotate the gene with gene ontology (GO) terms, the Nr BLAST results were imported into the Blast2 GO program. GO annotations for the genes were obtained by Blast2 GO. This analysis mapped all of the

annotated genes to GO terms in the database and counted the number of genes associated with each term. Perl script was then used to plot GO functional classification for the unigenes with a GO term hit to view the distribution of gene functions. The obtained annotation was enriched and refined using TopGo (R package). The gene sequences were also aligned to the Clusters of Orthologous Group (COG) database to predict and classify functions [52]. KEGG (kyoto encyclopedia of genes and genomes) pathways were assigned to the assembled sequences by perl script.

## Hormone extraction and determination

Endogenous hormone extraction and purification are based on the method of Yu mei et al. The high performance liquid chromatograph is Shimadzu LC-60A. Chromatographic conditions: mobile phase was methanol 0.8% glacial acetic acid solution = 55/45, column temperature was 30°C, flow rate was 0.8 mL/min, detection wavelength was 254 nm, injection volume was 10 µL. Each sample was tested 3 times and averaged. The data was statistically analyzed using Graphpad Prism 8 and SPSS 22.0.

## Determination of pollen vigor

The pollen viability was determined by TTC method [53]. A small amount of pollen was placed on a clean glass slide, a drop of 0.5% TTC solution was added, stirred, covered with a cover glass, and stained in a 35°C incubator for 20 min. Observe with a microscope and calculate pollen vigor.

## Quantitative real-time PCR

The qRT-PCR was performed as previously described [54]. The primers used to test the transcript levels of all genes are shown in Table S4, using GAPDH as the internal reference. The qRT-PCR mixtures contained 2 µL primers, 2 µL cDNA, 10 µL SsoFast™EvaGreen®Supermix (Bio-Rad) and distilled water to a final of 20 µL. The reaction conditions were as follows: 95 °C for 3 min (one cycle); 95 °C for 10 s, 59 °C for 30 s and 72 °C for 30 s (39 cycles).

## Yeast one-hybrid assay

The SmLOX, SmAOC, SmOPR3, SmCOI1, SmJAZ1 genes were ligated into the pGADT7 vector to construct the recombinant plasmid of pGADT7-SmLOX, pGADT7-SmAOC, pGADT7-SmOPR3, pGADT7-SmCOI1, pGADT7-SmJAZ1. The promoter of SmDAD1 in eggplant was inserted individually into the pAbAi vector and formed plasmids of pAbAi-SmDAD1 which was transformed into Y1HGold for screening the optimal resistance concentrations of AbA. Then the pGADT7-SmLOX, pGADT7-SmAOC, pGADT7-SmOPR3, pGADT7-SmCOI1, pGADT7-SmJAZ1 were respectively transformed into Y1H (pAbAi-DAD1) for examining the interactions between pGADT7-SmLOX, pGADT7-SmAOC, pGADT7-SmOPR3, pGADT7-SmCOI1, pGADT7-SmJAZ1 protein and promoter of *SmDAD1* on media lacking Leu (SD/-Leu) supplemented with the optimal AbA of 400 ng/mL to identify the motif or sequence region of DNA-protein interactions

## Dual-Glo® Luciferase assay

The interactions of SmLOX, SmAOC, SmOPR3, SmCOI1, SmJAZ1 protein with promoter of SmDAD1 were verified using the Dual-Glo® Luciferase Assay system (Promega, Madison, USA). The SmLOX, SmAOC, SmOPR3, SmCOI1, SmJAZ1 were subcloned and then were recombined into pGreenII 62-SK. Meanwhile, the promoters of SmDAD1 was ligated into pGreenII 0800-LUC. All the above recombinants were transformed into agrobacterium (GV3101) then co-infiltrated the leaves of Nicotianabenthamian for measuring the activities of fluorescein enzyme via GLOMAX® multifunctional instrument (Promega, USA).

## Yeast two-hybrid assay

SmDAD1, SmLOX, SmAOC, SmOPR3, SmCOI1, SmJAZ1 were separately sub-cloned into the activation domain of pGADT7 and pGBKT7 using the BamHI and XhoI sites and then ligated into pGADT7 or pGBKT7 to construct the recombinant plasmids. Using Matchmaker™ Gold Yeast Two-Hybrid System, the recombinants were respectively transformed into Y187 and Y2H (Clontech) for detecting the protein-protein interactions. In addition, toxicity and self-activation detection of the recombinant plasmids had been performed previously.

## Pull-down assay

SmDAD1 was sub-cloned into the pET32a(+) vector, while SmLOX, SmAOC, SmOPR3, SmCOI1, SmJAZ1 were cloned into the pGEX-4T-1 vector. Then, the plasmids were transformed into E. coli Rosetta (DE3) competent cells and 1.0 mM isopropyl β-D-thiogalactoside was added before incubation at 37°C for 3.5 h. The SmDAD1-HIS protein was purified using BeaverBeads IDA-Nickel Kit-10 (Beaver). The SmLOX-GST, SmAOC-GST, SmOPR3-GST, SmCOI1-GST and SmJAZ1-GST proteins were purified by BeaverBeads GSH (Beaver). The protein-protein interactions were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

## Abbreviations

GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; COG: Clusters of Orthologous Group; JA: Jasmonic acid; IAA: auxin; GA: gibberellin; ABA: abscisic acid; CTK: cytokinin; ETH: ethylene; BR: brassinosteroid; qPCR: Real time quantitative PCR; RNAseq: RNA-sequencing; FPKM: fragments per kilobase of exon per million fragments mapped; 13-HPOT: 13(S)-hydroperoxy-9, 11, 15 octadecatrienoic acid; 12-OPDA: 12-oxo-phytodienoic acid; GMS: Functional genic male sterility; DAD1: DEFECTIVE IN ANther DEHISCENCE 1; AOS: allene oxide synthase; OPR3/ (DDE1): DEHISCENCE 1; COI1: coronatine insensitive 1.

## Declarations

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## **Author's Contributions**

ZM W and Y N designed research, SW Z and C Y performed the experiments, QL T, DY W, Y N and SB T analyzed the data, ZM W wrote and modified the paper. All authors read and approved the manuscript.

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## **Availability of data and materials**

The data charts supporting the results and conclusions are included in the article and additional files.

## **Ethics approval and consent to participate**

Not applicable.

## **Consent for publication**

Not applicable.

## **Competing interests**

The authors declare that they have no competing interests.

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## References

1. Weese TL, Bohs L. Eggplant origins: Out of Africa, into the Orient. *Taxon*. 2010;59(1):49-56.
2. Mennella G, Rotino GL, Fibiani M, D'Alessandro A, Francese G, Toppino L, Cavallanti F, Acciarri N, Lo Scalzo R. Characterization of health-related compounds in eggplant (*Solanum melongena* L.) lines derived from introgression of allied species. *J Agric Food Chem*. 2010; 58(13):7597-7603.
3. Jasmin JJ. Male sterility in *Solanum-Melongena* L - preliminary report on a functional type of male sterility in eggplants. *P Am Soc Hortic Sci*. 1954;63(6):443-443.
4. Nuttall VW. Inheritance and possible usefulness of functional male sterility in *Solanum Melongena* L. *Can J Genet Cytol*. 1963;5(2):197-199.
5. Burke JJ, Kalttorres W, Swafford JR, Burton JW, Wilson RF. Studies on genetic male-sterile soybeans. *Plant physiology*. 1984;75(4):1058-1063.
6. Filippone E, Lurquin PF. Stable transformation of eggplant (*Solanum-Melongena*-L) by cocultivation of tissues with agrobacterium-tumefaciens carrying a binary plasmid vector. *Plant Cell Reports*. 1989; 8(6):370-373.
7. Cheng Q, Li T, Ai Y-X, Lu Q-H, Wang Y-H, Sun L, Shen H-L. Complementary transcriptomic and proteomic analysis reveals a complex Network regulating pollen abortion in GMS (msc-1) pepper (*Capsicum annuum* L.). *Int J Mol Sci*. 2019;20(7):1789.
8. Sanders PM, Bui AQ, Le BH, Goldberg RB. Differentiation and degeneration of cells that play a major role in tobacco anther dehiscence. *Sex Plant Reprod*. 2005;17(5):219-241.
9. Sanders PM, Bui AQ, Weterings K, McIntire KN, Hsu YC, Lee PY, Truong MT, Beals TP, Goldberg RB. Anther developmental defects in *Arabidopsis thaliana* male-sterile mutants. *Sex Plant Reprod*. 1999;11(6):297-322.
10. Beals TP, Goldberg RB. A novel cell ablation strategy blocks tobacco anther dehiscence. *The Plant Cell*. 1997; 9(9):1527-1545.
11. Scott RJ, Spielman M, Dickinson HG. Stamen structure and function. *The Plant Cell*. 2004; 16:S46-S60.
12. Wasternack C. Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of Botany*. 2007;100(4):681-697.
13. Lannoo N, Vandenborre G, Miersch O, Smagge G, Wasternack C, Peumans WJ, Van Damme EJM. The jasmonate-induced expression of the *Nicotiana tabacum* leaf lectin. *Plant and Cell Physiology*. 2007;48(8):1207-1218.
14. Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K. The *DEFECTIVE IN ANther DEHISCENCE1* gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which

- synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. *The Plant Cell*. 2001;13(10):2191-2209.
15. Xiao Y-G, Chen Y, Charnikhova T, Mulder PPJ, Heijmans J, Hoogenboom A, Agalou A, Michel C, Morel JB, Dreni L et al. *OsJAR1* is required for JA-regulated floret opening and anther dehiscence in rice. *Plant Molecular Biology*. 2014;86(6):19-33.
  16. Wasternack C, Strnad M. Jasmonates—News on occurrence, biosynthesis, metabolism and action of an ancient group of signaling compounds. *Int J Mol Sci*. 2018;19(9):2539.
  17. Schaller A, Stintzi A. Enzymes in jasmonate biosynthesis - Structure, function, regulation. *Phytochemistry*. 2009;70(13-14):1532-1538.
  18. Bae HK, Kang H-G, Kim GJ, Eu HJ, Oh SA, Song J-T, Chung I-K, Eun MY, Park SK. Transgenic rice plants carrying RNA interference constructs of *AOS* (allene oxide synthase) genes show severe male sterility. *Plant Breeding*. 2010;129(6):647-651.
  19. Caldelari D, Wang G, Farmer EE, Dong X. *Arabidopsis* *lox3 lox4* double mutants are male sterile and defective in global proliferative arrest. *Plant Mol Biol*. 2011;75(11): 25-33
  20. Xie D-X, Feys BF, James S, Nieto-Rostro M, Turner JG. *COI1*: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science*. 1998;280(5366):1091-1094.
  21. Stintzi A, Browse J. The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97(19):10625-10630.
  22. Schaller F, Biesgen C, Mussig C, Altmann T, Weiler EW. 12-oxophytodienoate reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis. *Planta*. 2000;210(6):979-984.
  23. McConn M, Browse J. The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant. *The Plant Cell*. 1996; 8(3):403-416.
  24. Peng YJ, Shih CF, Yang JY, Tan CM, Hsu WH, Huang YP, Liao PC, Yang CH. A RING-type E3 ligase controls anther dehiscence by activating the jasmonate biosynthetic pathway gene *DEFECTIVE IN ANTHER DEHISCENCE1* in *Arabidopsis*. *Plant Journal*. 2013;74(2):310-327.
  25. Chini A, Boter M, Solano R. Plant oxylipins: COI1/JAZs/MYC2 as the core jasmonic acid-signalling module. *Febs Journal*. 2009;276(17):4682-4692.
  26. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu GH, Nomura K, He SY, Howe GA, Browse J. JAZ repressor proteins are targets of the SCFCO11 complex during jasmonate signalling. *Nature*. 2007;448(7154):661-671.
  27. Liu H, Gu J-Z, Lu Q, Li H-F, Hong Y-B, Chen X-P, Ren L, Deng L, Liang X-Q. Transcriptomic analysis reveals the high-oleic acid feedback regulating the homologous gene expression of Stearoyl-ACP Desaturase 2 (SAD2) in peanuts. *Int J Mol Sci*. 2019;20(12):3091.
  28. Singh S, Sawhney VK, Pearce DW. Temperature effects on endogenous Indole-3-Acetic-Acid levels in leaves and stamens of the normal and male sterile stamenless-2 mutant of tomato (*Lycopersicon-Esculentum Mill*). *Plant Cell and Environment*. 1992;15(3):373-377.
  29. Shukla A, Sawhney VK. Metabolism of dihydrozeatin in floral buds of wild-type and a genic male-sterile line of rapeseed (*Brassica-Napus L*). *Journal of Experimental Botany*. 1993; 44(266):1497-1505.

30. Horner HT. A comparative light-and electron-microscopic study of microsporogenesis in male-fertile and cytoplasmic male-fertile sunflower (*Helianthus annuus*). *Am J Bot.* 1977;64(6):745-759.
31. Li Y-X, Zhang A-M, Huang T-C. Relationship between wheat cytoplasmic male sterility and the content of endogenous hormones in the anther. *Journal of Agricultural Biotechnology.* 1996;(4):307-313.
32. Xu M-L, Liu W-F, Xiao Y-H. The content change of IAA of HPGMR during its panicle development. *Journal of Huazhong Agricultural University.* 1990;25(4):381-386.
33. Tian C-G, Duan J, Liang C-G, Huang Y-W, Liu H-G. Change in phytohormones in cytoplasmic male sterile rice and its maintainer line during panicle development. *J Trop Subtrop Bot.* 1998;2(6):137-143.
34. Kojima K. Changes of ABA, IAA and GAs levels in reproductive organs of citrus. *Jarq-Jpn Agr Res Q.* 1997;31(4):271-280.
35. Chen Z-J, Zhang M-F, Wang B-L, Dong W-M, Huang S-Q. A study on fertility and agronomic characters of CMS lines for tuber mustard. *Acta Horticulturae Sinica.* 1995;22(1):40-46.
36. Shi G-G, Hou X-L, Hu W. Changes of endogenous hormones and polyamines in cytoplasmic male sterile non-heading Chinese cabbage during anther development. *Acta Bot. Boreal.-Occident. Sin.* 2004;24(11):2109-2112.
37. Liu H-W, Zhang G-H, Wang J-W, Wang X-L, Fang Z-W. Relation between ethylene of young spike and male sterility induced by GENESIS in wheat. *Jour. of Northwest Sci-Tech Univ. of Agri. and For. (Nat. Sci. Ed.).* 2003;31(3):39-42.
38. Dill A, Sun TP. Synergistic derepression of gibberellin signaling by removing RGA and GAI function in *Arabidopsis thaliana*. *Genetics.* 2001;159(2):777-785.
39. Shih CF, Hsu WH, Peng Y-J, Yang C-H: The NAC-like gene ANOTHER INDEHISCENCE FACTOR acts as a repressor that controls anther dehiscence by regulating genes in the jasmonate biosynthesis pathway in *Arabidopsis*. *Journal of Experimental Botany.* 2014;65(2):621-639.
40. Qin Y-G, Lei J-J, Cao B-H, Chen G-J, Zhai Y-L, Yang C-Q. Cloning and sequence analysis of the *defective in anther dehiscence1 (DAD1)* gene fragment of Chinese kale. *Afr J Biotechnol.* 2011;10(56):11829-11831.
41. Chini A, Monte I, Zamarreno AM, Hamberg M, Lassueur S, Reymond P, Weiss S, Stintzi A, Schaller A, Porzel A et al. An OPR3-independent pathway uses 4,5-didehydrojasmonate for jasmonate synthesis. *Nature Chemical Biology.* 2018;14(2):171-178.
42. Zhao D-Z, Ma H. Male fertility: A case of enzyme identity. *Current Biology.* 2000;10(24):R904-R907.
43. Ma H. Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. *Annual Review of Plant Biology.* 2005;56:393-434.
44. Sanders PM, Lee PY, Biesgen C, Boone JD, Beals TP, Weiler EW, Goldberg RB. The *Arabidopsis DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. *The Plant Cell.* 2000;12(7):1041-1061.
45. An LK, Ahmad RM, Ren H, Qin J, Yan Y-X. Jasmonate Signal receptor gene family ZmCOIs restore male fertility and defense response of *Arabidopsis* mutant *coi1-1*. *J Plant Growth Regul.* 2019;38(2):479-493.
46. Grunewald W, Vanholme B, Pauwels L, Plovie E, Inze D, Gheysen G, Goossens A. Expression of the *Arabidopsis* jasmonate signalling repressor JAZ1/TIFY10A is stimulated by auxin. *Embo Rep.*

2009;10(8):923-928.

47. Pigolev AV, Miroshnichenko DN, Pushin AS, Terentyev VV, Boutanayev AM, Dolgov SV, Savchenko TV. Overexpression of *Arabidopsis* in hexaploid wheat (*Triticum aestivum* L.) alters plant development and freezing tolerance. *Int J Mol Sci.* 2018;19(12): 3989.
48. Melotto M, Mecey C, Niu Y, Chung H-S, Katsir L, Yao J, Zeng W-Q, Thines B, Staswick P, Browse J et al. A critical role of two positively charged amino acids in the Jas motif of *Arabidopsis* JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. *Plant Journal.* 2008;55(6):979-988.
49. Wasternack C. The Trojan horse coronatine: the COI1-JAZ2-MYC2,3,4-ANAC019,055,072 module in stomata dynamics upon bacterial infection. *New Phytologist.* 2017; 213(3):972-975.
50. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology.* 2013;14(4):R36.
51. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks (vol 7, pg 562, 2012). *Nat Protoc.* 2014;9(10):2513-2513.
52. Tatusov RL, Galperin MY, Natale DA, Koonin EV. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic acids research.* 2000;28(1):33-36.
53. Roath WW, Hockett EA. Genetic male sterility in barley. III. Pollen and Anther Characteristics. 1971;11(2):200-203.
54. Matsumura H, Nirasawa S, Kiba A, Urasaki N, Saitoh H, Ito M, Kawai-Yamada M, Uchimiya H, Terauchi R. Overexpression of Bax inhibitor suppresses the fungal elicitor-induced cell death in rice (*Oryza sativa* L.) cells. *Plant Journal.* 2003;33(3):425-434.

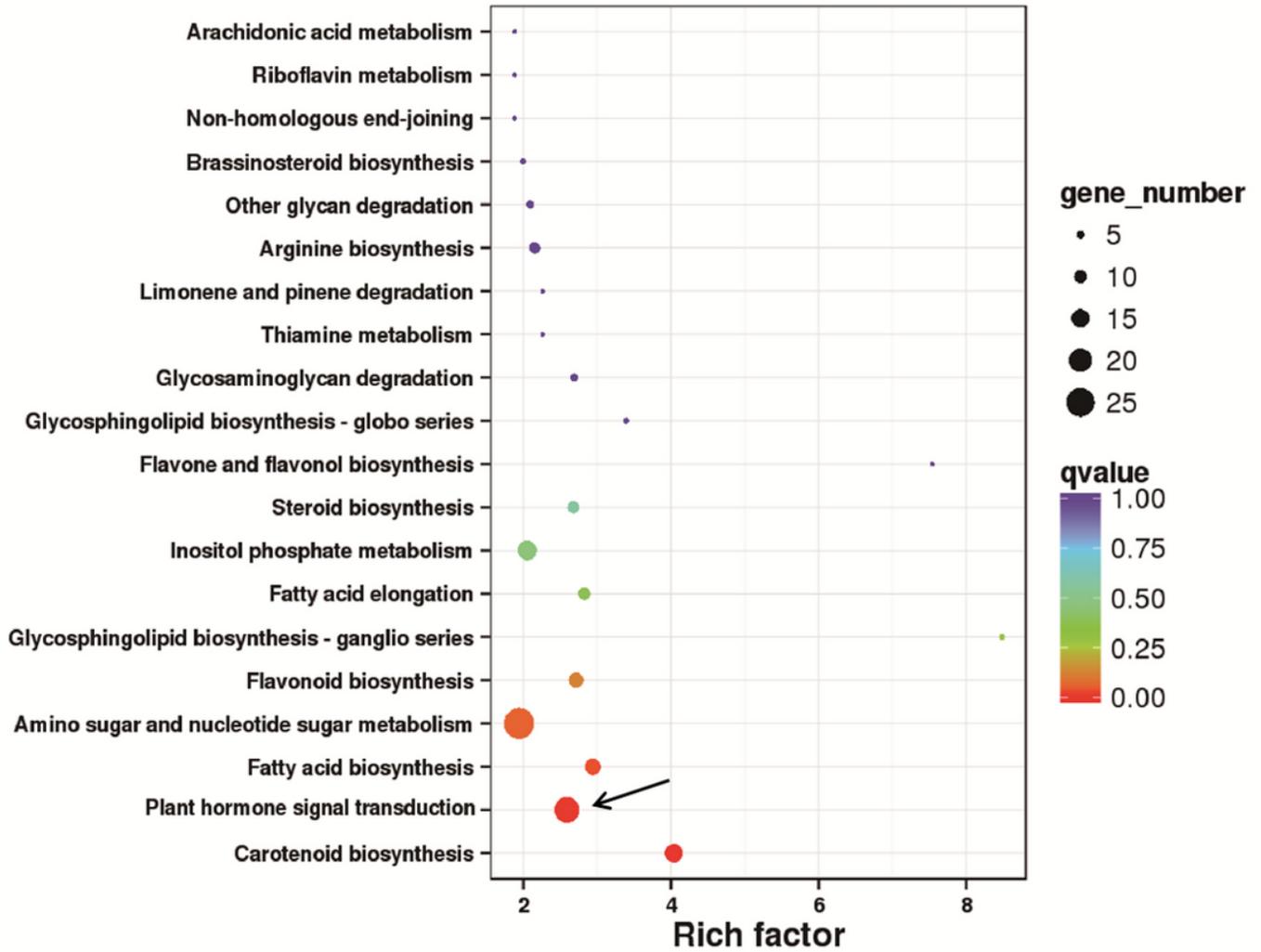
## Figures



### Figure 1

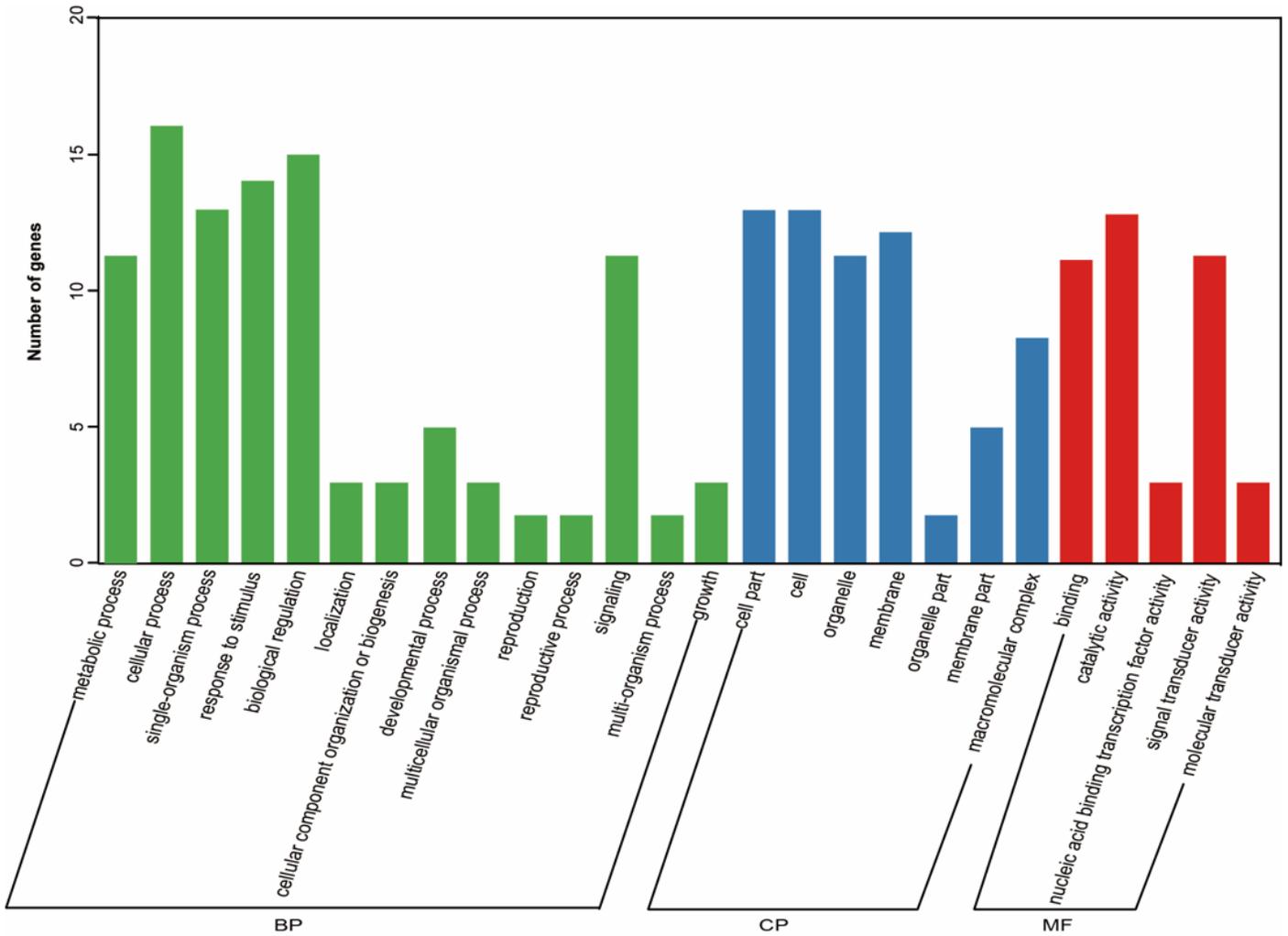
Morphological analysis of *Solanum melongena* L. F142 (A, C) and S16 (B, D) flowers and anthers. Pollen vitality identification in F142 (E, 200x) and S12 (F, 200x).

## Statistics of Pathway Enrichment



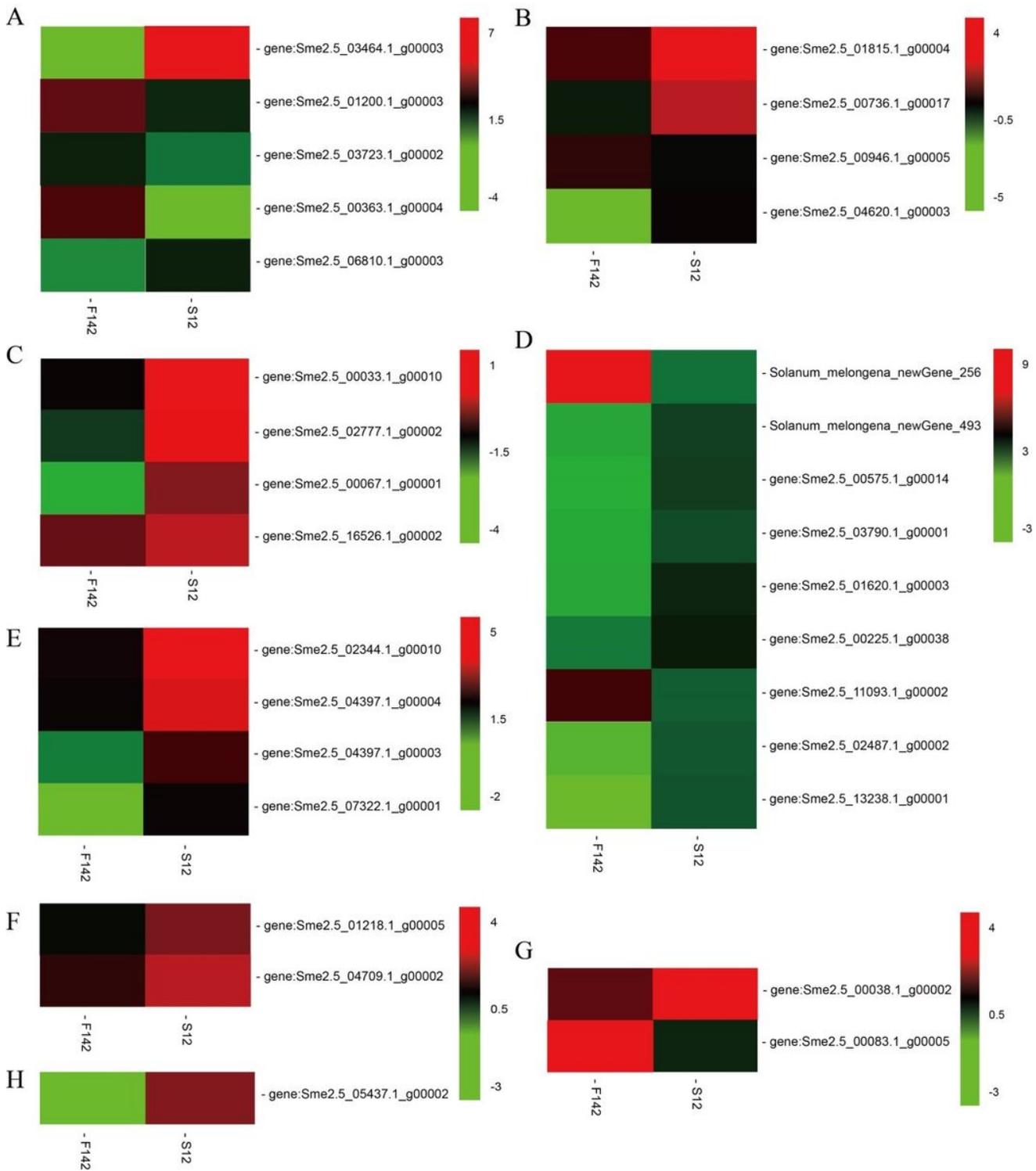
**Figure 2**

KEGG pathway enrichment analysis of 2670 DEGs. The pathway of plant hormone signal transduction was mainly enriched (black arrows).



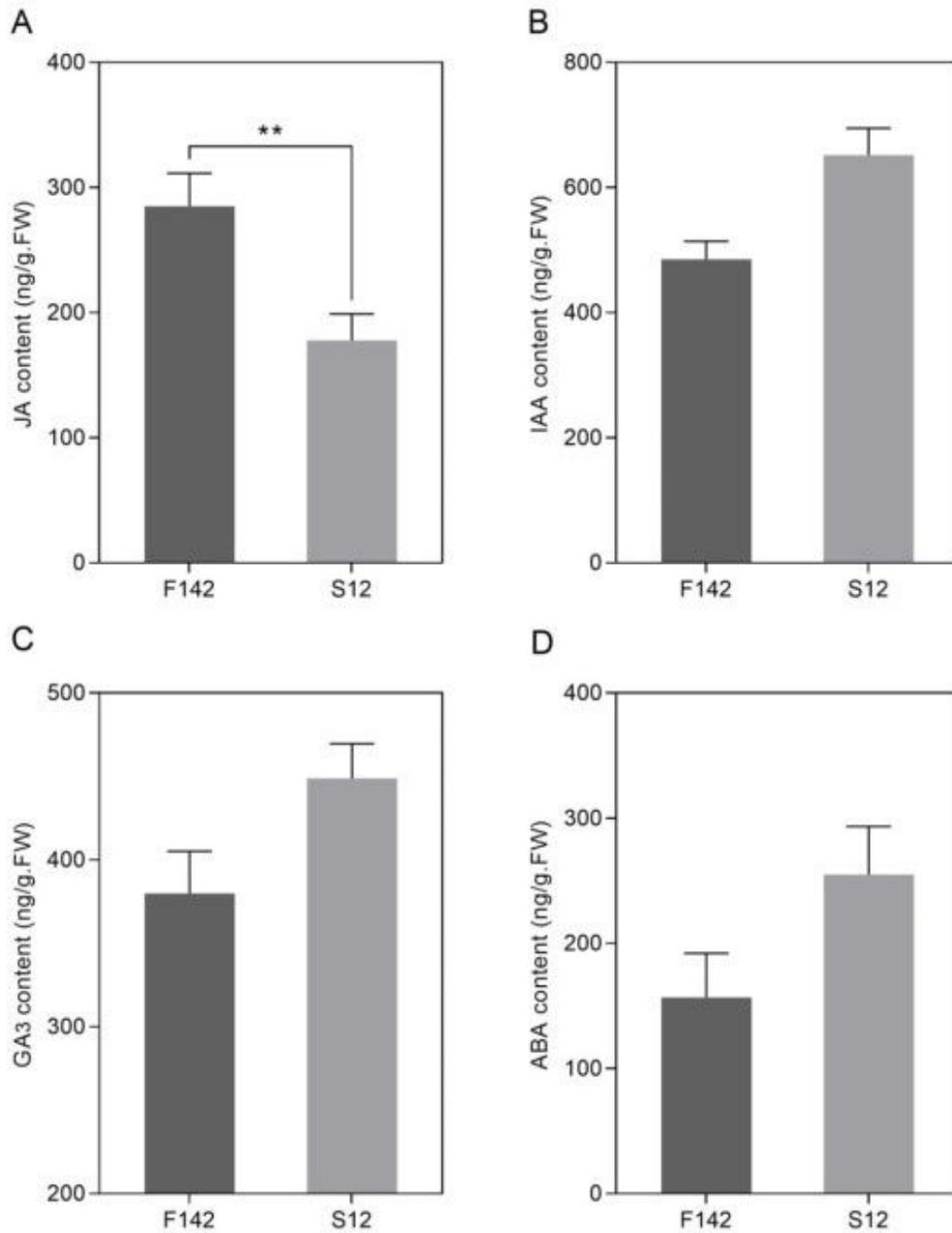
**Figure 3**

GO (gene ontology) enrichment analysis of 31 hormone DEGs. BP: biological processes, CP: cellular compartments, MF: molecular functions.



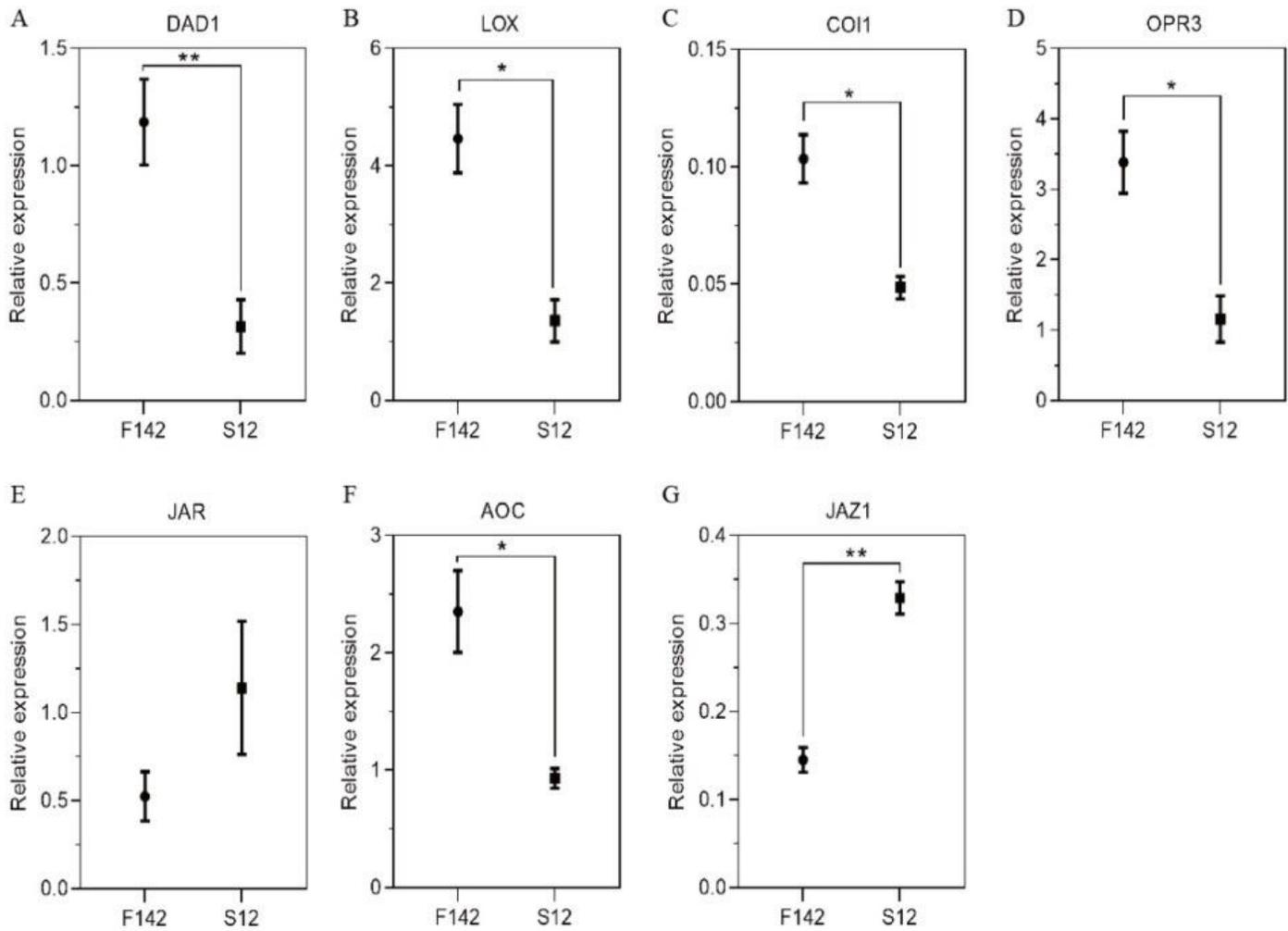
**Figure 4**

Expression changes of the genes involved in the jasmonic acid (A), ethylene (B), auxin (C), other hormones (D), Brassinolide (E), cytokinin (F), gibberellin (G) and abscisic acid (H) signaling pathways in F142 and S12.



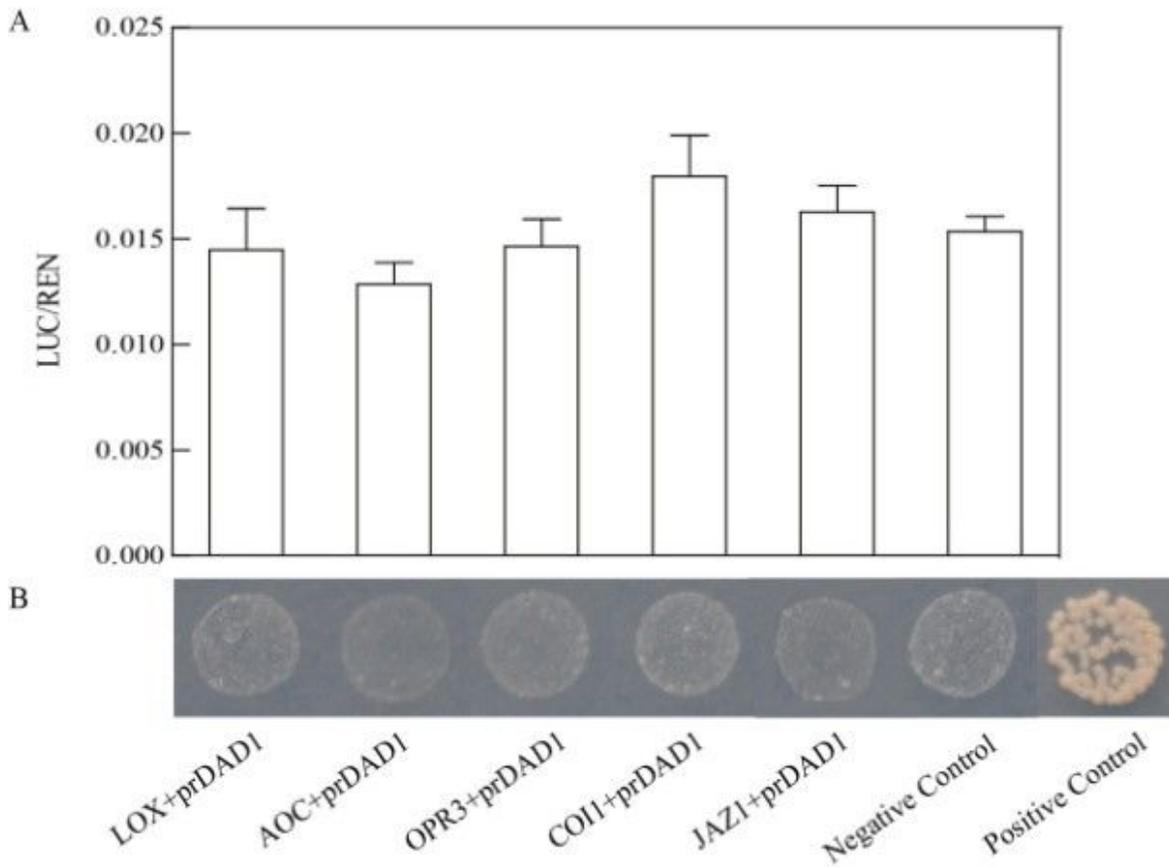
**Figure 5**

Changes in JA, IAA, GA3 and ABA concentrations in F142 and S12. (A) JA concentration. (B) IAA concentration. (C) GA3 concentration. (D) ABA concentration. Three independent biological replicates were used. Three biological replicates were assessed. Paired t-tests, \*  $p < 0.05$ , \*\*  $p < 0.01$ .



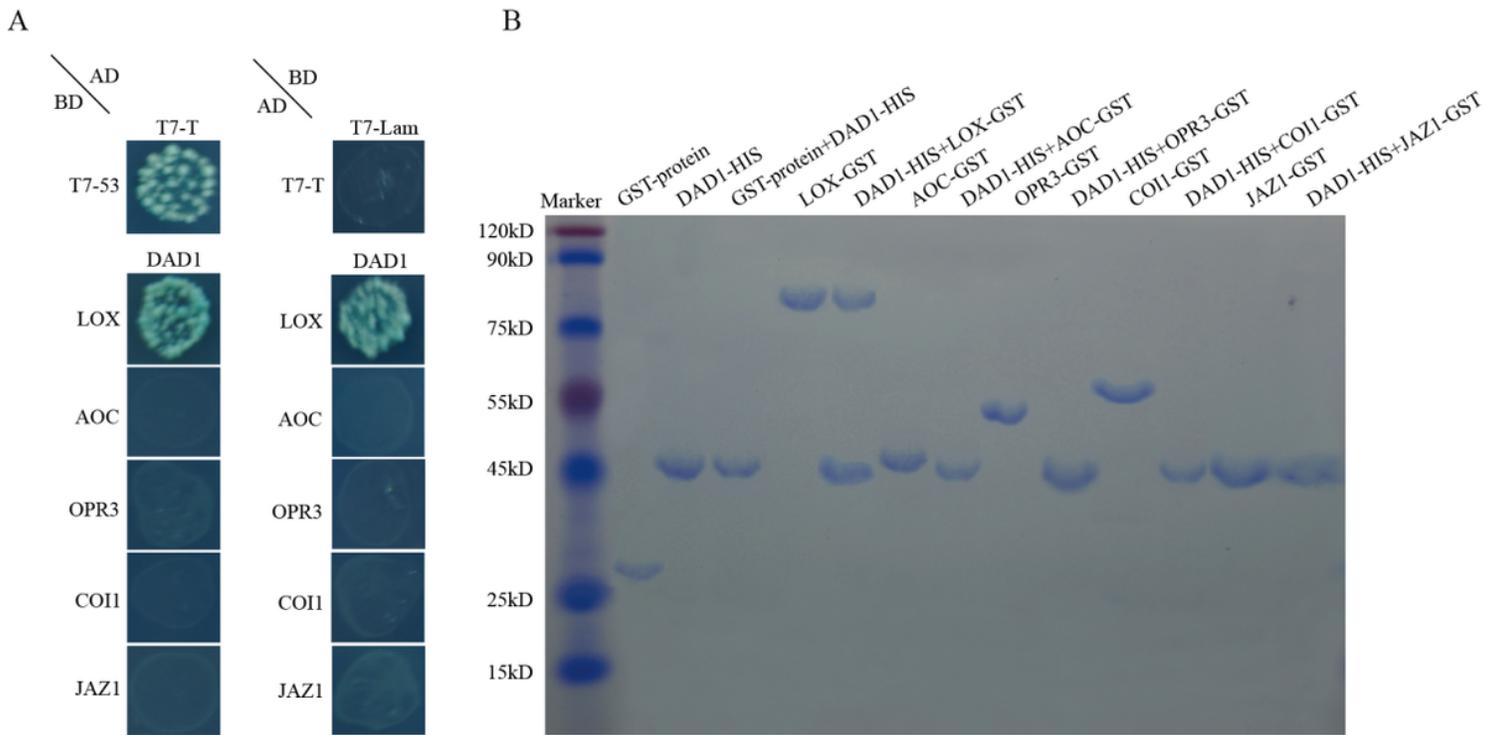
**Figure 6**

The transcript accumulation of DAD1 (A), LOX (B), COI1 (C), OPR3 (D), JAR (E), AOC (F) and JAZ1 (G). Total RNA was extracted in anthers and used for RT-qPCR analyses. Three biological replicates (each including three technical repeats) were assessed. Paired t-tests, \*  $p < 0.05$ , \*\*  $p < 0.01$ .



**Figure 7**

Interactions of SmLOX, SmAOC, SmOPR3, SmCOI1 and SmJAZ1 with the promoters of DAD1. (A) In vivo Dual-Glo® Luciferase assay. The ratio of firefly luciferase (LUC) and renilla luciferase (REN) of the empty vector (62-SK) mixed with 0800LUC- DAD1 was taken as negative controls, respectively. Error bars indicated S.E. from at least six replicates. (B) Yeast one-hybrid assays. Transformed yeast cells were grown on SD/- Leu with 400ng/mL. Y1H (p53-AbAi+ pGADT7-53) was used as positive control. Y1H (DAD1+ pGADT7) was used as negative control.



**Figure 8**

Protein interactions of SmDAD1 with SmLOX, SmAOC, SmOPR3, SmCOI1 and SmJAZ1. (A) Detecting interactions of SmCOI1 with SmLOX, SmAOC, SmOPR3, SmCOI1 and SmJAZ1 by yeast two-hybrid assay. Transformed yeast cells were plated on SD/-Ade/-His/-Leu/-Trp/X-a-Gal medium to grow at 30°C for 3-5 days. The pGBKT7-T53 (T7-T53) and pGBKT7-lam (T7-lam), combined with pGADT7-T (T7-T), were used as positive and negative controls, respectively. (B) Examining the interactions of SmDAD1 with the other proteins by Pull-down. The HIS-tagged SmDAD1 protein was generated by cloning into the pET32a (+) vector (19 kDa). The GST-tagged proteins of SmLOX, SmAOC, SmOPR3, SmCOI1 and SmJAZ1 were generated by cloning into the pGEX-4T-1 vector (26 kDa). Bound proteins were eluted and stained with Coomassie Brilliant Blue 250, then separated by 12.5% SDS-PAGE.

## Supplementary Files

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