BMC Plant Biology

RESEARCH

Open Access

# Functional characterization of PcMYB25, a candidate regulator of Patchouli alcohol biosynthesis in *Pogostemon cablin*, via exogenous hormone-elicited transcriptome analysis

Liqiong Feng[1], Xuanxuan Zhou[1], Peihan Chen[1], Xiuzhen Chen[1,2], Kun Guo[1], Ziqian Chen[1], Xiaojin Ge[1], Ruoting Zhan[1] and Likai Chen[1*]

## Abstract

**Background** Plant hormones often cause changes in secondary metabolites. Patchouli alcohol (PA) is a bioactive compound of the medicinal plant *Pogostemon cablin*. Similar to other secondary metabolites, its synthesis is also regulated by transcription factors. While the PA biosynthetic pathway has been characterized, its regulatory mechanisms remain incompletely understood.

**Results** Transcriptome analysis revealed that exogenous hormone application significantly altered the transcriptional landscape of *Pogostemon cablin*, particularly affecting genes involved in terpenoid metabolism. Through weighted gene co-expression network analysis (WGCNA), we identified the R2R3-MYB transcription factor gene PcMYB25 as a key candidate regulator. Transient overexpression analysis showed that overexpression of *PcMYB25* significantly upregulated *PcPTS* expression and select upstream MVA pathway genes, and increased PA content by 85% compared to empty vector controls. In addition, yeast one-hybrid assays confirmed that PcMYB25 binds to the *PcPTS* promoter, and transcriptional activation assays demonstrated its strong transactivation activity.

**Conclusions** We conclude that exogenous hormone treatment triggers an extensive transcriptional response in patchouli, and integrated co-expression analysis identifies PcMYB25 as a candidate regulatory factor involved in plant hormone signaling and the accumulation of PcPTS-derived PA and sesquiterpenoid compounds. These findings not only provide new insights into the transcriptional regulation of PA biosynthesis, but also lay the foundation for further genetic engineering strategies for PA production.

**Keywords** *Pogostemon cablin*, WGCNA, Patchouli alcohol, MYB, Coexpression analysis

*Correspondence:
Likai Chen
chenlk@gzucm.edu.cn
[1]Research Center of Chinese Herbal Resource Science and Engineering, Guangzhou University of Chinese Medicine, Key Laboratory of Chinese Medicinal Resource from Lingnan (Guangzhou University of Chinese Medicine), Ministry of Education, Joint Laboratory of National Engineering Research Center for the Pharmaceutics of Traditional Chinese Medicines, Guangzhou 510006, P. R. China
[2]Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China

*Pogostemon cablin* (Blanco) Benth., a herbaceous plant of the Lamiaceae family, is distributed in Southern and Southeast Asia [1]. This plant exhibits a wide range of pharmacological activities, including anti-inflammatory, analgesic, antioxidant, antibacterial, and antiviral properties, which have led to its extensive use in traditional and modern medicine [2, 3]. In addition to its medicinal uses, *Pogostemon cablin* is also valued in daily life and industrial applications due to its essential oil. Patchouli oil has a distinctive fragrance, primarily attributed to its major component, patchouli alcohol (PA). Studies have shown that the content of PA in patchouli oil can reach up to 55.7% [4, 5]. Because of its unique and long-lasting aroma, PA is widely used in personal care products and cosmetics, such as perfumes, facial toners, and moisturizing creams. Globally, the annual consumption of pure PA for cosmetic and household purposes reaches several thousand kilograms [6]. Due to the significant application and commercial value of PA, traditional methods of cultivating patchouli plants to extract the compound are no longer sufficient to meet growing demand. Therefore, genetic engineering strategies aimed at enhancing PA production have emerged as a promising alternative.

PA is synthesized throughout the entire plant, primarily via the mevalonate (MVA) pathway [7, 8]. Its biosynthesis involves three main steps. First, the precursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are synthesized through the MVA pathway. In this step, six enzymes are involved: acetoacetyl-CoA thiolase (AACT), hydroxymethylglutaryl-CoA synthase (HMGS), hydroxymethylglutaryl-CoA reductase (HMGR), mevalonate kinase (MVK), phosphomevalonate kinase (PMK), and mevalonate diphosphate decarboxylase (MVD). The generated IPP can be reversibly converted to DMAPP under the action of isopentenyl diphosphate isomerase (IPPI). In the second step, farnesyl pyrophosphate synthase (FPPS) catalyzes the condensation of IPP and DMAPP to form farnesyl diphosphate (FPP), which serves as the direct precursor for PA biosynthesis. Finally, in the last catalytic step, patchoulol synthase (PTS) converts FPP into PA [9, 10]. The biosynthesis of terpenoids is a complicated process and is affected by many rate-limiting enzymes. Notably, transcription factors can simultaneously modulate multiple genes in terpenoid metabolism to enhance metabolite accumulation. For instance, in *Curcuma wenyujin*, jasmonate (JA)-induced overexpression of the transcription factor *CwbHLH27* increases the accumulation of the sesquiterpenoids β-elemene and β-caryophyllene by approximately 2.8-fold [11]. The AP2/ERF family transcription factor AaORA in *Artemisia annua* is a positive regulator of genes such as *AaADS*, *AaCYP71AV1*, *AaDBR2*, and *AaERF1*. It can positively regulate the expression levels of these genes to increase the content of artemisinin. After *AaORA* overexpression, the content of artemisinin and dihydroartemisinic acid increased 40–53% and 22–35%, respectively [12, 13]. Wang et al. demonstrated that the transcription factor PcWRKY44 can bind to *Pogostemon cablin* farnesyl pyrophosphate synthase gene (*PcFPPS*). Its overexpression upregulates the expression levels of *PcFPPS* and *PcPTS*, thus promoting the biosynthesis of PA [14]. Wu et al. discovered that the PcENO3 protein can interact with *PcPTS* and enhances its enzymatic activity, thereby regulating PA biosynthesis [15].

In natural environments, the growth and development of plants are affected by many factors, such as temperature, humidity, and salinity. When sensing environmental changes, plants respond through hormone changes [16, 17]. These hormonal fluctuations modulate the activity of specific transcription factors, thereby regulating the synthesis and accumulation of secondary metabolites [18, 19]. As a sesquiterpenoid secondary metabolite, PA biosynthesis is similarly influenced by hormonal and other signaling pathways. Chen et al. showed through dual-luciferase experiments that PatSWC4 transcription factor can bind to the promoter of *PatPTS* to increase its transcription activity. Yeast two-hybrid (Y2H) results indicated that PatSWC4 and PatJAZ4 proteins can interact, and suggested that PatSWC4 plays a part in the JA response network [20]. The SA-responsive transcription factor PatWRKY71 can regulate the synthesis of PA [21].

Substantial research has been applied to reveal the regulatory network of PA biosynthesis for increasing its production. The rapid development of transcriptome sequencing technology and the continuous reduction in costs have led to a large amount of data support for seeking the functional genes. Weighted gene co-expression correlation network analysis (WGCNA) is a systematic biological analysis method that can correlate phenotypic trait data with specific genes. Currently, WGCNA plays a significant role in the analysis of plant secondary metabolism. Huang et al. used WGCNA analysis to indicate that transcription factors such as AP2/ERF, WRKY, MYB and bHLH play an important role in MJ-induced monoterpenoid biosynthesis of *Mentha arvensis* L [22].

In this study, we integrated transcriptome data from *Pogostemon cablin* leaves treated with various plant hormones and used PA content as a phenotypic trait to construct a gene co-expression network using WGCNA. This allowed us to link PA accumulation to specific gene modules. From these modules, we identified the R2R3-MYB transcription factor PcMYB25. Yeast one-hybrid assays revealed that PcMYB25 can bind to the promoter of *PcPTS*. Transient overexpression experiments confirmed that overexpression of *PcMYB25* significantly increased both PA content and the expression level of *PcPTS*, consistent with PcMYB25 functioning as a candidate positive regulator of PA accumulation. These findings provide

new insights into the transcriptional regulation of PA biosynthesis and lay the foundation for further metabolic engineering strategies.

## Materials and methods

### Plant materials and treatment

*Pogostemon cablin* (Blanco) Benth. was obtained from a cultivated plantation in Yangjiang, Guangdong, China (21.87°N, 111.98°E). The plant material was authenticated by Professor Ruoting Zhan (Guangzhou University of Chinese Medicine) based on morphological characteristics. A voucher specimen (No. 819727) has been deposited at the Herbarium of the College of Traditional Chinese Medicine, Guangzhou University of Chinese Medicine. The plant material was subsequently cultivated in controlled-environment chambers at the Research Center of Chinese Herbal Resource Science and Engineering, Guangzhou University of Chinese Medicine (23.03°N, 113.23°E). The temperature and humidity of the growth chamber were maintained at 26 °C/75% during the day and at 21 °C/65% at night. The light/dark photoperiod was maintained 16 h light/8 h dark.

*Pogostemon cablin* plants at the six-leaf stage were used for hormone treatment. Patchouli plants with similar growth state were divided randomly into control (0.5% ethanol) and five phytohormone-treatment groups: methyl jasmonate (MeJA), salicylic acid (SA), ethylene (ETH), abscisic acid (ABA), and MeJA + ETH co-treatment. Each treatment included 3 biological replicates. Control ethanol and various hormones (dissolved in 0.5% ethanol) were sprayed on patchouli leaves until the solution covered the surface of the leaves and just dribbled. Transparent containers enclosed treated plants to prevent hormone volatilization. Eight hours after spraying, leaves collected from each treatment group were flash-frozen in liquid nitrogen and stored at −80 °C for RNA-Seq and extraction of PA. Concentrations used for various hormones were 300 μM (MeJA, SA, and ABA) or 500μM (ETH).

### Identification of differentially expressed genes (DEGs) and the co-expression network

Total RNA was extracted from the patchouli leaves stored at −80 °C as mentioned above. The concentration and purity of the extracted RNA were assessed using NanoDrop2000, and the RNA quality number (RQN) value was determined by Agilent5300. Total RNA samples were required to meet the following criteria: 1 μg of total RNA, a concentration of ≥ 30 ng/μL, an RQN value > 6.5, and an OD260/280 ratio between 1.8 and 2.2. The high-quality RNA samples were used to enrich mRNA and fragment mRNA through Oligo dT, reverse transcribe to synthesize cDNA, ligate the adapter sequence, purify the products, amplify the fragments by PCR, construct the sequencing library, and sequence using the NovaSeq X Plus platform.

Using the Trinity software assembly sequencing data, and through TransRate (http://hibberdlab.com/transrate/) and CD-HIT (http://weizhongli-lab.org/cd-hit/) optimized filter assembly results, assembly evaluation was conducted by BUSCO (http://busco.ezlab.org). Get reads and compare the reference sequence used to express the quantitative software RSEM at the quantitative level of gene and transcriptome, and quantitative analysis, according to the transcripts per million reads (TPM) method to calculate the expression level of each transcript. DESeq2 was used for differential expression analysis, and genes with $p < 0.05$ and FC ≥ 2 or FC ≤ 0.5 (genes up-regulated twice or down-regulated more than two times) were considered DEGs. DEGs were annotated with GO and KEGG databases, and enrichment analyses were performed.

The WGCNA was used to construct the gene co-expression network. The DEGs whose expression level is less than one and whose coefficient of variation is less than 0.1 are filtered. Then, the soft-thresholding power of the signed co-expression was adjusted to 12, and the modules were identified. The core co-expression modules were visualized using Cytoscape software.

### Sequence alignment and phylogenetic tree construction of MYB25-related sequences

The MYB25-related sequences from other species were identified from the NCBI database using PcMYB25 as bait. Sequence alignment was conducted using DNAMAN with default parameters and a phylogenetic tree was constructed using MEGA 7.0.

### Transient overexpression in Patchouli leaves

Patchouli plants with six leaves were used for transient overexpression by agrobacteria-mediated transformation. The 969-bp open reading frame (ORF) of *PcMYB25* was cloned into the NotI/PacI sites of the pJLTRBO expression vector [23]. Both the recombinant pJLTRBO-*PcMYB25* and empty vector control (pJLTRBO) were transformed into *Agrobacterium tumefaciens* GV3101 (pSoup p19) (Weidi Biotechnology, Shanghai). Then the transformed agrobacteria were plated on Luria-Bertani plates containing 50 μg/mL kanamycin and 25 μg/mL rifampicin for 48 h for plasmid selection.

Agrobacterium-mediated transient expression was performed as previously described [24] The agrobacteria were cultured in Luria-Bertani medium supplemented with 50 μg/mL kanamycin and 25 μg/mL rifampicin for 24 h until the OD600 reached 0.6–0.8, then the agrobacteria were harvested by centrifugation and resuspended in the induction medium (10 mM MES, pH 5.7, 10 mM MgCl2, 200 μM acetosyringone) at an OD600 of 1.0.

Cells were incubated at room temperature for 2–4 h. Two to three leaves per plant were infiltrated on the abaxial sides using 1 mL needleless syringe. After infection, the plants were incubated at 25 °C for 96 h. Then the leaves were collected and stored at −80 °C for subsequent RNA extraction and PA content detection.

### RNA extraction and quantitative reverse-transcription PCR (RT-qPCR)

Total RNA was extracted from patchouli leaves using the Favor Prep™ Plant Total RNA Mini Kit (Favor Biotech, China). Then HiScript III RT Super Mix for qPCR (+gDNA wiper) (Vazyme Biotech, China) was used for reverse transcription. For each sample, 200 ng RNA was used for reverse transcription. AceQ Universal SYBR qPCR Master Mix (Vazyme Biotech, China) was used for qPCR. The internal reference gene 18 S rRNA was used to normalize the relative expression for tested genes. The values shown are averages of three biological replicates. The primers used are listed in Table S1.

### PA extraction and quantification

The method of PA extraction and quantification used has been described previously [24]. Patchouli leaves were ground in liquid nitrogen, and 200 mg of the powder was ultrasonically extracted with 1.5 mL hexane at 60 Hz for 30 min. Then, the mixture was placed in a 56 °C water bath for 1 h. After a short centrifugation, the supernatant was filtered with a 0.22 μm organic microporous membrane, then the filtrate was taken as the test solution for GC-MS detection.

Agilent 7890B/5977A gas chromatography-mass spectrometry (GC-MS) and HP-5ms capillary column (30 m × 0.25 mm × 0.25 μm) was used for the separation and detection of PA. The procedure was set with an initial temperature of 50 °C and kept for 2 min, then the temperature was increased to 130 °C at a rate of 20 °C/min, and the temperature was increased to 150 °C at 2 °C/min, and maintained for 5 min at 150 °C. After that, the temperature increased to 230 °C at a rate of 20 °C/min. In addition, commercial PA (Feiyu, Nantong, China) was used as a standard.

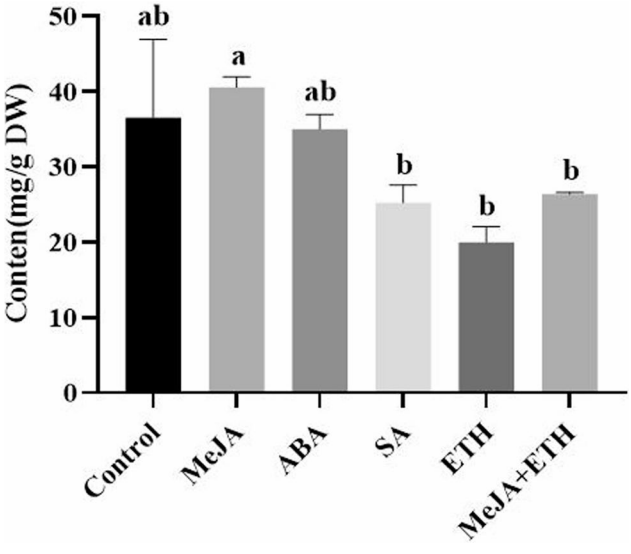


**Fig. 1** PA content in leaves treated with various hormones

### Transcriptional activation activity of PcMYB25 and yeast one-hybrid (Y1H) assay

For the detection of PcMYB25 transcriptional activation, the open reading frame (ORF) of *PcMYB25* was cloned into the expression vector pGBKT7 using a one-step cloning method. The recombinant pGBKT7-PcMYB25 was then transformed into yeast strain Y2HGold as the experimental group, while an empty pGBKT7 vector served as the negative control. These were cultured on SD/-Trp medium, SD/-Trp medium supplemented with 50 μg X-α-Gal, and SD/-Trp medium with 50 μg X-α-Gal plus 200 ng/mL aureobasidin A (AbA), observing the growth of colonies.

For the Y1H assay, the promoter region of *PcPTS* was defined as the 1,865-bp sequence upstream of the ATG start codon and was cloned into the pHIS2 vector to generate the reporter construct pHIS2-PcPTSpro. This construct was introduced into the Y187 yeast strain using the PEG/LiAc transformation method to evaluate potential self-activation activity. The full-length coding sequence of *PcMYB25* was amplified and cloned into pGADT7, resulting in pGADT7-PcMYB25. This construct was co-transformed with pHIS2-PcPTSpro into the Y187 yeast strain as the experimental group. An empty vector and pHIS2-PcPTSpro were co-transformed into the Y187 yeast strain as the negative control group. These transformants were initially grown on SD agar plates lacking tryptophan and leucine (-Trp/-Leu), and positive clones were transferred onto SD/-Trp/-His/-Leu plates containing various concentrations of 3-amino-1,2,4-triazole (3-AT) for further growth observation.

## Results

### PA content altered in Patchouli leaves treated with various hormones

The biosynthesis of PA mainly occurs in the leaves of patchouli and the content of secondary metabolites is often affected by exogenous plant hormones [25]. The six-leaf-stage patchouli leaves were treated with 0.5% ethanol (control) or various hormones (MeJA, SA, ETH, ABA, and MeJA + ETH). Among different hormone treatments, the mean PA content was highest under MeJA and lowest under ETH. The PA content observed with the MeJA exhibited significant differences from that observed with the SA, ETH, and MeJA + ETH (Fig. 1).

### RNA-sequencing, de Novo assembly and functional annotation

To identify the critical transcription factors regulating the biosynthetic pathway of PA, patchouli leaves in triplicate were treated with 0.5% ethanol (control), MeJA, ETH, ABA, SA, and MeJA + ETH respectively. A total of 154,779 unigenes were assembled, with a size of 223,580,816 bp and an N50 of 1058. These data indicated that the quality of RNA sequencing is sufficient for downstream analysis. To assess the expression pattern of the patchouli transcriptomes for various hormone treatments, the assembled transcriptome sequences were annotated and compared with six databases: NR, Swiss-Prot, Pfam, COG, GO and KEGG. Of the total 154,779 unigenes annotated: 60,039, 55,123, and 43,196 unigenes were annotated from NR, Swiss-Prot, and Pfam, respectively. We used GO, COG, and KEGG databases to classify unigenes. In order to understand the function and classification of unigenes in a global view, GO functional annotations were performed. Of 43,477 unigenes annotated to this database, three main categories were grouped: biological process (BP), cellular component (CC), and molecular function (MF) (Fig. S1). For biological processes, organic substance metabolic process (GO:0071704), primary metabolic process (GO:0044238), cellular metabolic process (GO:0044237) were the most enriched items. For cellular components, unigenes related to membrane part (GO:0044425), cell part (GO:0044464), intrinsic component of membrane (GO:0031224) were the most abundant items. For molecular function category, organic cyclic compound binding (GO:0097159), heterocyclic compound binding (GO:1901363), and ion binding (GO:0043167) were the most highly represented GO terms. Of 8,162 unigenes annotated with COG, functional groups were classified into 24 categories (Fig. S2): 758 unigenes were assigned to Translation, ribosomal structure and biogenesis (the J category). Moreover, 35,368 unigenes were mapped into the KEGG pathways. The most representative pathways were "translation", "Carbohydrate metabolism" and "Folding, sorting and degradation" (Fig. S3).

### Identification of differentially expressed genes (DEGs)

To identify potential transcription factors regulating the biosynthetic pathway of PA, we analyzed DEGs among the five hormone treatment groups by comparing MeJA + ETH, MeJA, SA, ABA, and ETH with the control and by comparing MeJA + ETH with ETH or MeJA. Comparative analysis revealed 14,419 DEGs total, with MeJA + ETH treatment inducing the most significant transcriptional changes—9,563 unigenes comprising 5,086 upregulated and 4,477 downregulated genes. In contrast, SA treatment showed minimal differential expression with only 77 upregulated and 54 downregulated unigenes relative to control, as detailed in Figure S4. GO and KEGG enrichment analyses were performed on DEGs to predict their biological functions. The GO enrichment analysis divided 14,419 differentially expressed unigenes into three categories: cellular component, molecular function, and biological process (Fig. 2A). Cellular components were dominated by membrane-associated terms, representing 19.6% and 18.98% of annotations for membrane and membrane parts respectively. Molecular functions primarily involved catalytic activity and binding, accounting for 30% and 26.87% of annotations. Metabolic processes constituted the most enriched biological process at 17.22%, followed closely by cellular processes at 16.57%. The results reflected that more DEGs are involved in metabolic processes or cellular processes and have catalytic and binding functions. KEGG pathway analysis further identified 151 unigenes associated with plant hormone signal transduction, 46 with terpenoid backbone biosynthesis, and 34 specifically involved in sesquiterpene and triterpenoid biosynthesis pathways (Fig. 2B).

### Construction of the WGCNA and the identification of hub genes

The DEGs and identification module were evaluated using WGCNA. Here, the processed genes were divided into 10 modules (Fig. 3A). Association of the PA content (trait) with 10 modules was analyzed to identify key modules and potentially involved transcription factors (Fig. 3B). Among these 10 modules, four modules, MEmagenta ($r$=−0.643, $p$-value = 0.004), MEgreen ($r$=−0.591, $p$-value = 0.0098), MEpink ($r$ = 0.544, $p$-value = 0.0196), and MEblack ($r$=−0.525, $p$-value = 0.0253), were identified as the key modules associated with the PA content ($P < 0.05$) (Fig. 3B). While MEmagenta showed the strongest correlation with PA content, it contained no transcription factors. We therefore focused on analyzing MEgreen due to its significant correlation with PA content ($r$=−0.591, $p$-value = 0.0098). A total of 358 genes are included in this module, including 7 structural genes related to PA synthesis (Table S4) and 14 transcription factors (Table S3). Notably, we found a key transcription factor, TRINITY_DN73869_c4_g1 (kME = 0.892, where kME indicates the correlation between the expression profile of a gene and the module eigengene), named *PcMYB25*. As a crucial gene, *PcMYB25* ranked top 30 in connectivity in MEgreen module (degree = 44.11) (Table S2). Co-expression network analysis revealed PcMYB25's close association with terpenoid metabolism-related genes (Fig. 3C). Given the established role of MYB transcription factors in plant secondary metabolism [26], these findings suggest PcMYB25 may be a candidate regulator of PA biosynthesis.

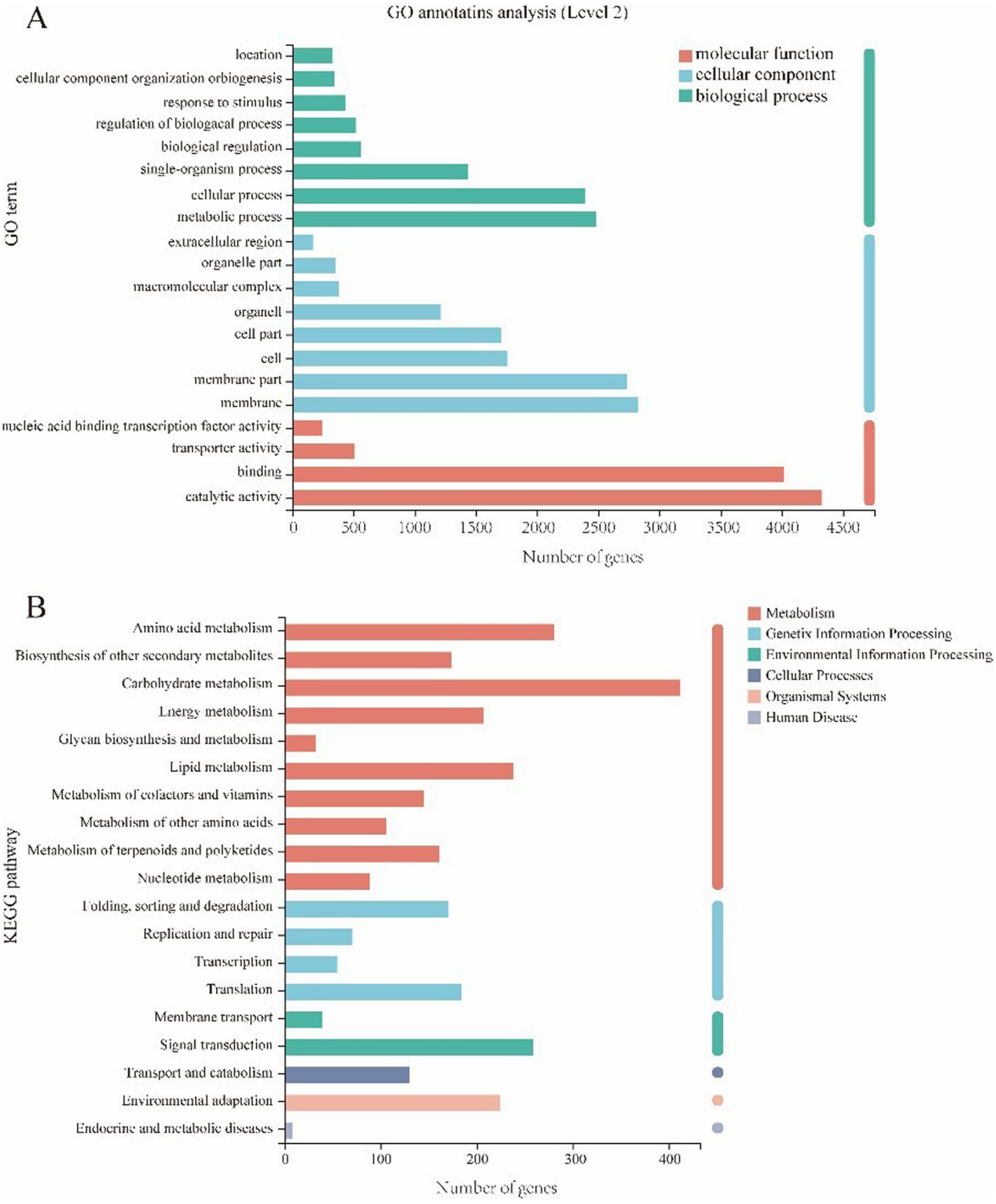


**Fig. 2** GO and KEGG enrichment analysis of DEGs. **A** GO function classification of DEGs. The ordinate on the left of the figure represents the secondary classification of GO, the abscissa represents the number of unigenes included in the secondary classification, and the three colors on the right represent the three major branches of GO (BP, CC, MF). **B** KEGG classification of DEGs. The ordinate shows the names of the KEGG metabolic pathways and the abscissa indicates the number of unigenes

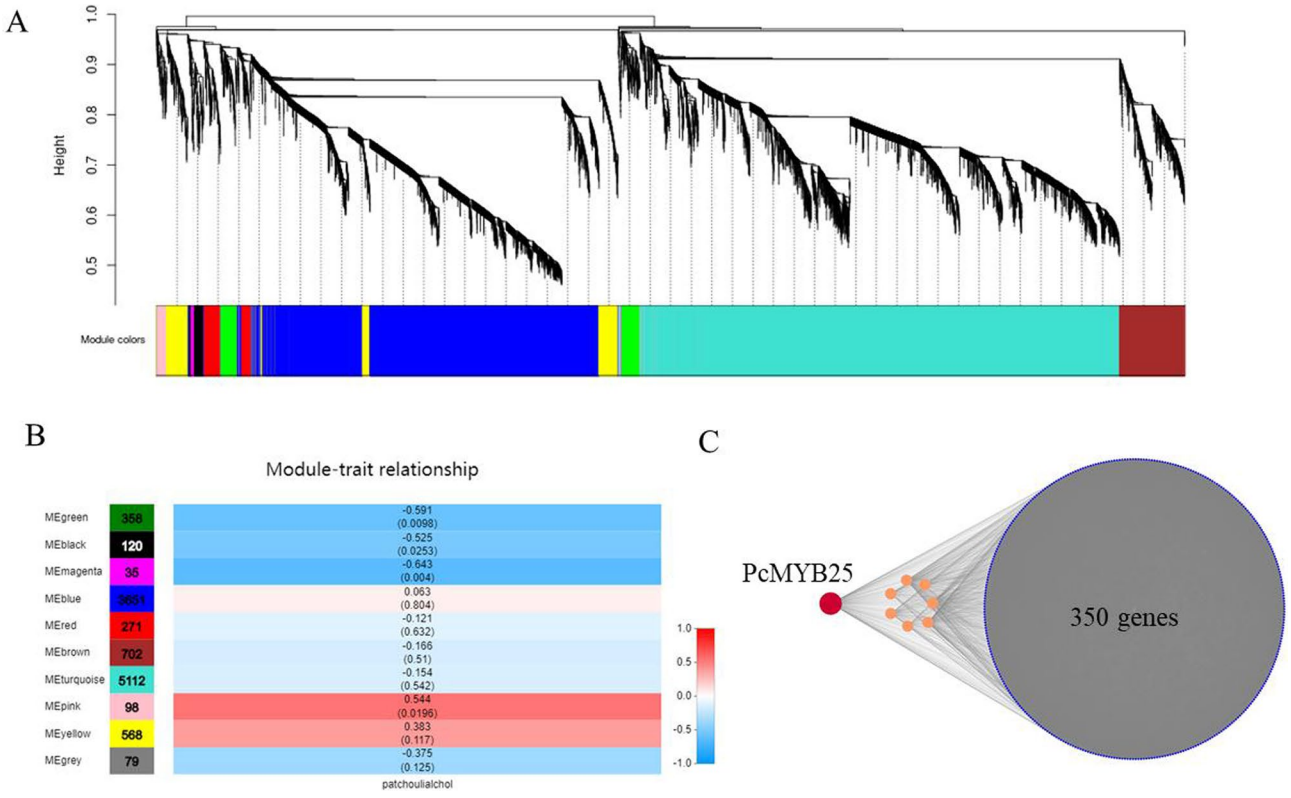


**Fig. 3** Network analysis dendrogram shows the modules identified by Weighted Gene Co-expression Network Analysis (WGCNA). **A** Tree diagram with color annotations. A kind of color represents a module. **B** Module and phenotype correlation heat map. Module-PA weight correlation and corresponding *P-value*. The left panel shows 10 modules. The color scale on the right shows the correlation of module characteristics from – 1 (blue) to 1 (red). **C** Network analysis of *PcMYB25*, genes related with terpenoid and other genes in MEgreen module. Red spot represents *PcMYB25*, orange spot represents terpenoid related genes, blue spot represents other 358 genes in MEgreen

### PcMYB25 codes a R2R3-MYB protein

We cloned the full-length coding sequence of *PcMYB25* from patchouli cDNAs. After sequence analysis, the ORF including the stop codon of PcMYB25 was determined to be 969 bp in length and encoded 322-amino acid. The molecular weight of this protein was predicted to be 38.4 kDa, and the isoelectric point was 7.6. Sequence alignment confirmed that *PcMYB25* shares high similarity with R2R3-MYB proteins from *Sesamum indicum*, *Salvia splendens*, and *Salvia miltiorrhiza*. Structural analysis identified characteristic R2 and R3 DNA-binding domains at the N-terminus, confirming its classification as a canonical R2R3-MYB transcription factor (Fig. 4A). To predict potential functions of *PcMYB25*, we constructed a phylogenetic tree using MEGA 7 (Fig. 4B). The results showed that PcMYB25 and SmMYB25 clustered together with high bootstrap support. *PcMYB25* also showed high similarity (95%) to the R2-R3 conserved domains of *AtMYB25* from *Arabidopsis thaliana* (Fig. 4B). A neighbor-joining (NJ) phylogenetic tree was constructed using *PcMYB25* and 125 R2R3-MYB transcription factors from *Arabidopsis thaliana*. Phylogenetic analysis classified *PcMYB25* into the S23 subgroup of *Arabidopsis thaliana* R2R3-MYB proteins, where it formed a monophyletic clade with *AtMYB25*, *AtMYB1*, and *AtMYB109* with high bootstrap support (Fig. S5).

### Overexpression of *PcMYB25* is associated with upregulated expression of terpenoid pathway genes and increased PA accumulation

To investigate the function of *PcMYB25* in patchouli, we transiently overexpressed *PcMYB25* in patchouli leaves using the pJLTRBO vector through Agrobacterium-mediated transformation (Fig. 5A). The efficiency of this overexpression was evaluated by RT-qPCR four days after the injection. Compared with the empty-vector control pJLTRBO group, the expression level of *PcMYB25* in the leaves in the experimental groups increased by 2.55-fold (Fig. 5B). Analysis of PA biosynthetic pathway genes revealed significant upregulation of *AACT* and *PMK* transcripts, while *HMGR* expression showed marked reduction. Most notably, the expression level of *PcPTS* was significantly 4.09-fold higher than that of the control group (Fig. 6). Consistent with these changes, GC-MS analysis revealed an 85% increase in PA content in PcMYB25-overexpressing leaves compared to controls (Fig. 5C and D).

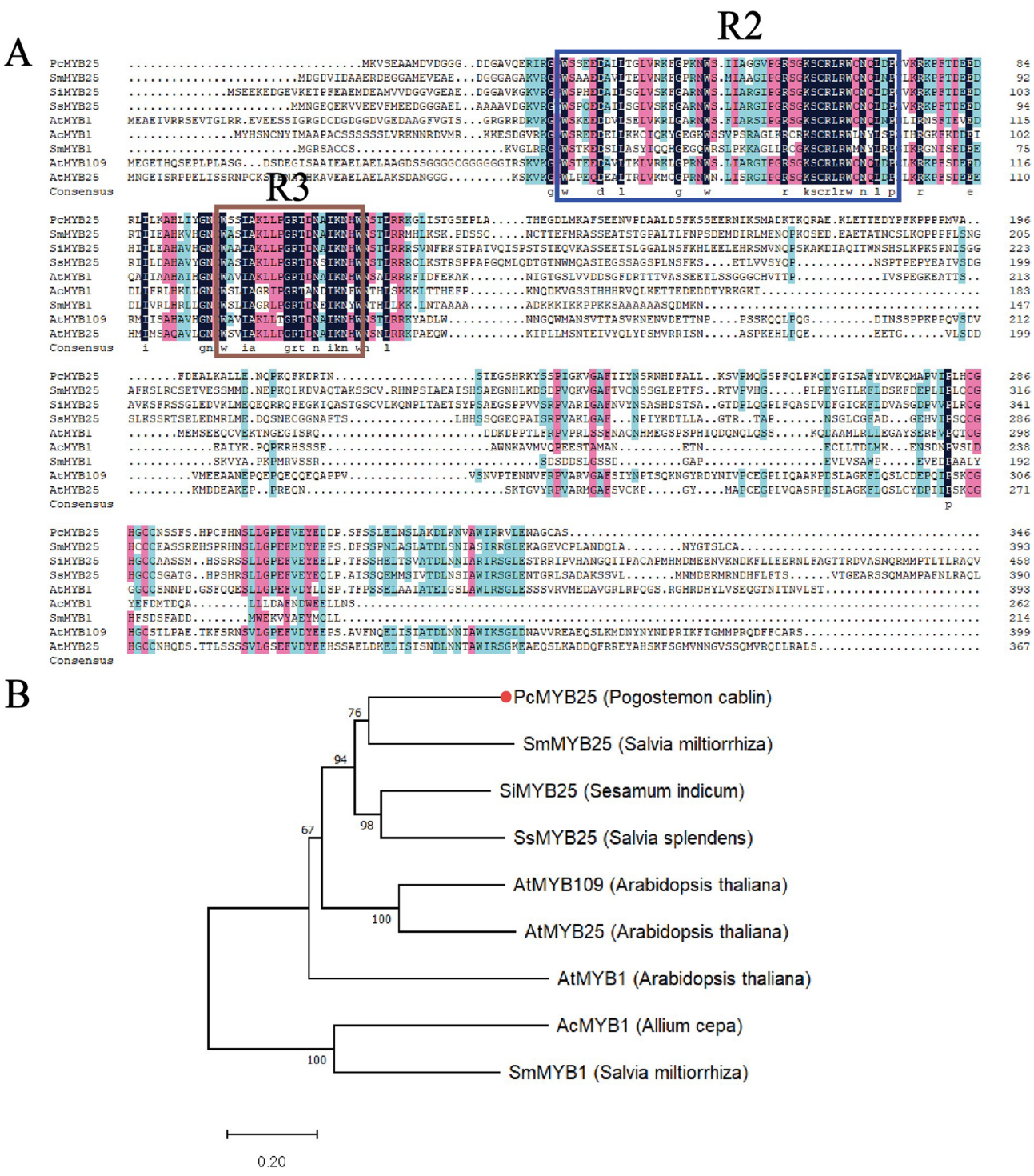


**Fig. 4** Phylogenetic analysis of PcMYB25. **A** Sequence analysis of PcMYB25 and other MYB transcription factors. In order to compare the MYB sequence between different plant species, *PcMYB25* was used as a bait gene, and MYB transcription factors with similar sequences in *Sesamum indicum*, *Salvia miltiorrhiza*, *Salvia splendens*, and *Arabidopsis thaliana* were identified from the NCBI database. **B** Phylogenetic tree of MYB transcription factors. Some MYB proteins share similar sequences for phylogenetic analysis. A phylogenetic tree was constructed by MEGA 7

### PcMYB25 exhibits transcriptional activation activity and binds to the *PcPTS* promoter

The *PcMYB25* gene was cloned into pGBKT7 as the experimental group. Compared to the negative control group, both the experimental and positive control groups were able to grow normally and exhibited blue coloration on SD/-Trp medium supplemented with 50 μg/mL X-α-Gal and 200 ng/mL AbA, while the negative control

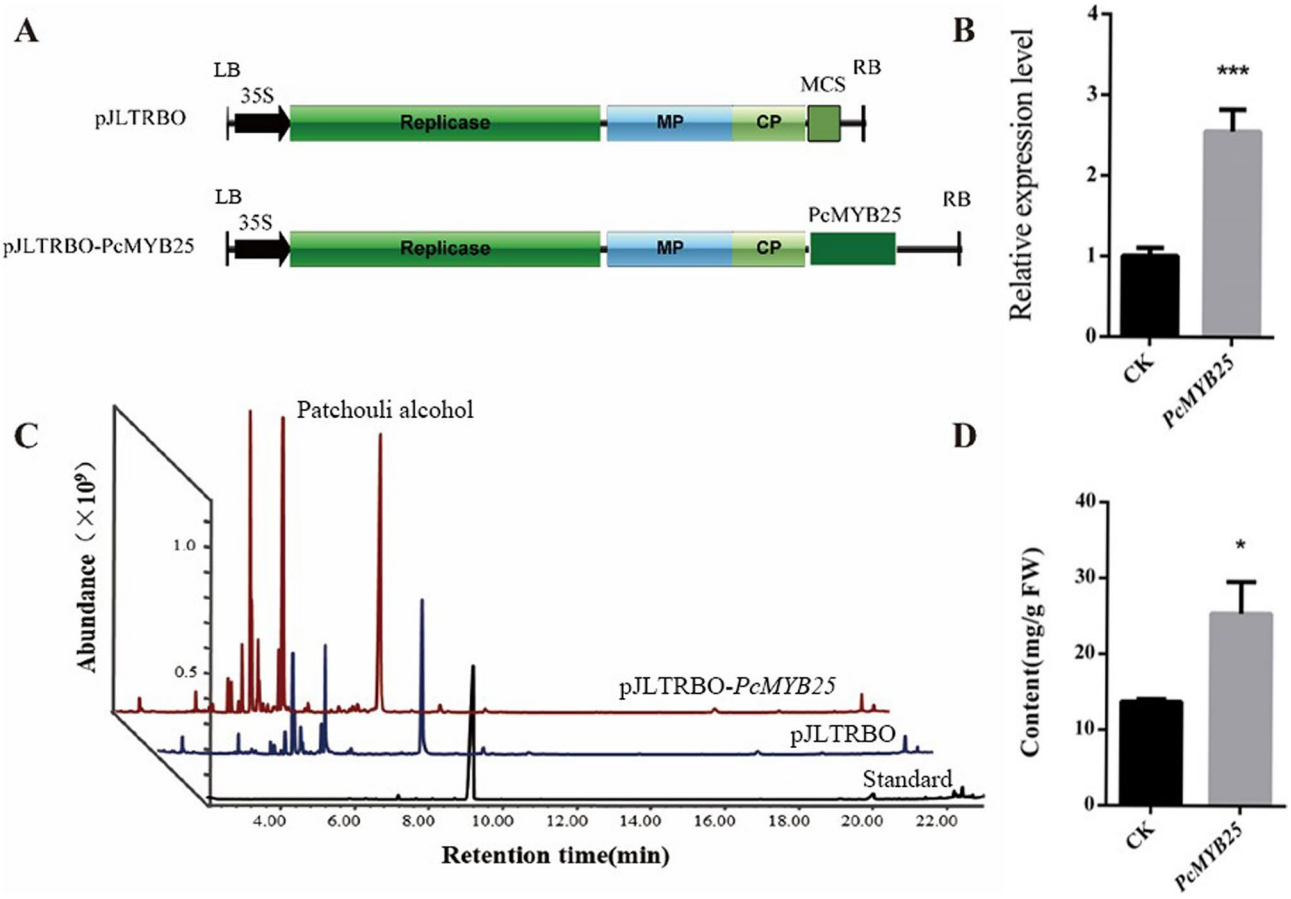


**Fig. 5** Transient overexpression analysis of *PcMYB25* in patchouli leaves. **A** *PcMYB25* was cloned into overexpression vector pJLTRBO to form pJLTRBO-*PcMYB25* construct with the restriction enzyme sites *PacI* and *NotI*. **B** Analyzed by RT-qPCR, the expression level of *PcMYB25* in the leaves was significantly increased than the empty-vector control pJLTRBO (CK). **C** GC-MS chromatogram of the standard of patchouli alcohol, extracts of the leaves treated with the empty-vector control pJLTRBO, the overexpression vector pJLTRBO-*PcMYB25*. **D** After overexpression of *PcMYB25*, the content of PA in the overexpressed group increased significantly compared with the CK group (*** $p < 0.001$ ** $p < 0.01$, * $p < 0.05$). FW, fresh weight; CK, the empty-vector control pJLTRBO

(empty vector) could not grow. These results confirm that PcMYB25 has strong transcriptional activation activity (Fig. S6A).

The yeast strain Y187[pHIS2-PcPTSpro] was able to grow on SD/-Trp/-His medium but could not grow on SD/-Trp/-His + 10 mM 3-AT or SD/-Trp/-His + 20 mM 3-AT. This shows that 10 mM 3-AT is sufficient to inhibit its background expression, and therefore, media containing 10 mM or higher concentrations of 3-AT can be used for subsequent Y1H screening (Fig. S6B). Y1H assays showed that, on plates with different concentrations of 3-AT, the experimental group strains grew better than the negative control group, suggesting a potential interaction between PcMYB25 and the *PcPTS* promoter (Fig. S6C).

## Discussion

### Changes of Patchouli under different hormone treatments

Plants are often exposed to various adversity stresses during their growth. These adverse stresses usually lead to changes in plant hormone levels, thereby affecting the synthesis and accumulation of secondary metabolites [27]. Like most secondary metabolites, the synthesis of PA has previously been found to be associated with phytohormone. Tang et al. used MeJA, ABA, SA to treat the leaves of patchouli. It was found that the expression of *HMGS*, *PMV* and *MVK* on the MVA pathway have increased [28]. In comparison with control, content of secondary metabolites including PA were found to be significantly different in patchouli leaves treated with MeJA, ETH, or MeJA + ETH. After treatments, 254, 229, and 400, respectively, differentially expressed proteins were identified. These differentially expressed proteins are mainly involved in photosynthesis, secondary metabolites, carbohydrate and energy metabolism [29]. These previous studies have shown expression levels or content of genes, proteins and secondary metabolites changed when patchouli leaves were treated with exogenous hormones. To determine the effect of various hormones on PA content, PA content of patchouli leaves treated with

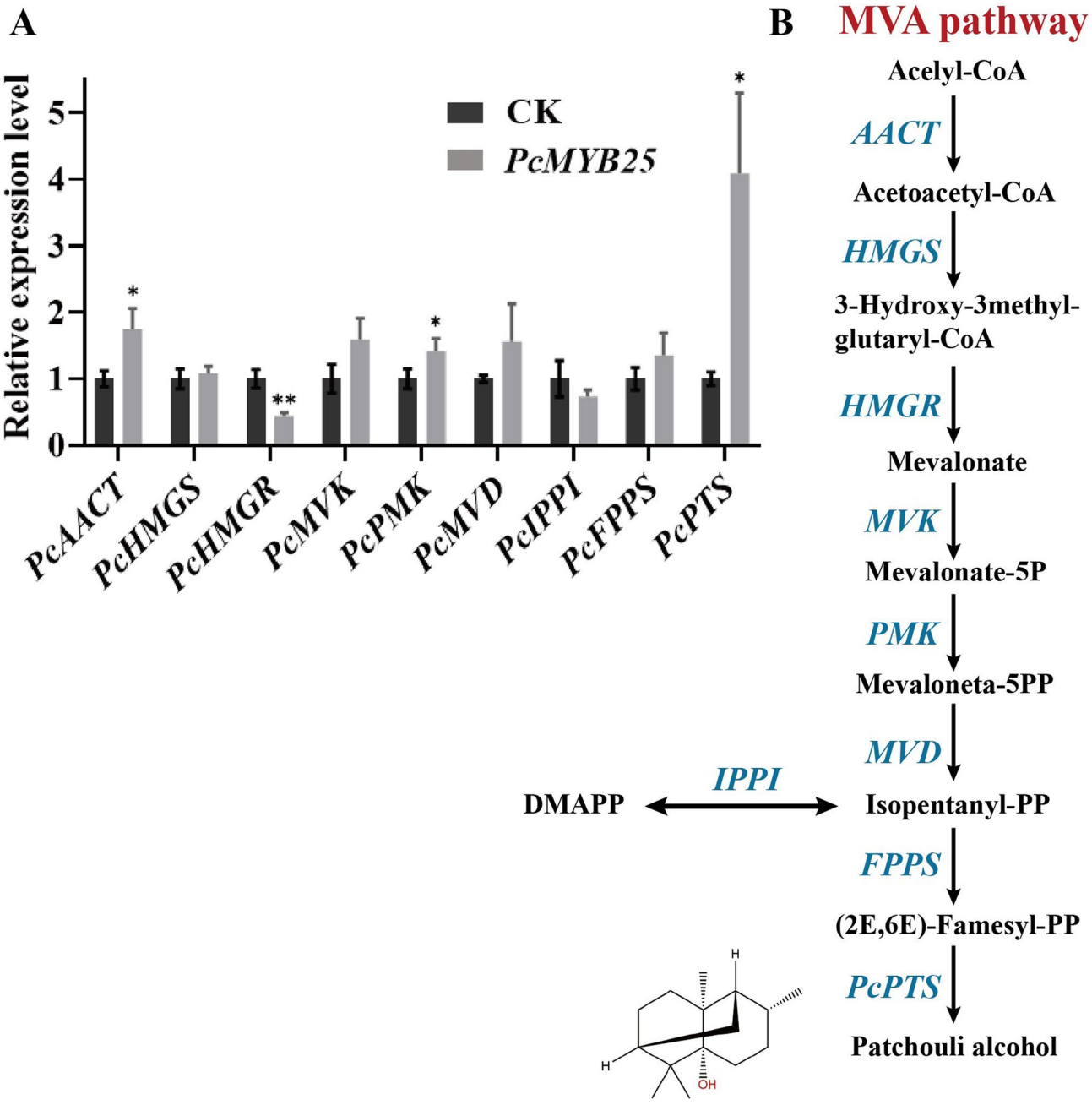


**Fig. 6** The expression level of genes on the PA synthesis pathway after transient overexpression of *PcMYB25*. **A** The expression level of genes on the MVA pathway was determined by RT-qPCR, and CK represents the empty-vector control pJLTRBO. Using *Pc18S* as the internal reference gene, the relative expression level of the gene was calculated according to the $2^{-\triangle\triangle Ct}$ method. Asterisks represent significant differences (*$P < 0.05$, **$P < 0.01$).**B** Schematic representation of the MVA pathway leading to the synthesis of patchouli alcohol

various hormones was determined. The highest content of PA was found in MeJA-treated leaves, and the lowest content was associated with ETH-treated. PA content in MeJA-treated leaves differed significantly from that of the SA-, ETH-, and MeJA + ETH -treated.

RNA-Seq is a powerful tool to identify the transcriptional regulation mechanism of secondary metabolism. This study provides a transcriptome survey to understand the functions of DEGs. DEGs identified are mainly enriched in metabolic and cellular processes, and are mainly involved in catalysis and binding. Enriched pathways included plant hormone signal transduction, MAPK signaling, and α-linolenic acid metabolism. Notably, a considerable number of DEGs are involved in terpenoid backbone biosynthesis, indicating that exogenous hormone application exerts a broad influence on

terpenoid metabolic pathways. This extensive transcriptional reprogramming is consistent with previous observations in patchouli following glutathione treatment, where hormonal elicitors also induced genome-wide transcriptional changes, leading to altered production of secondary metabolites [25].

### Co-expression facilitates the identification of PA-related transcription factors

The MYB proteins represent a large and functionally diverse family of transcription factors in eukaryotes, and participate in cell proliferation [30], differentiation [31], apoptosis [32], abiotic and biotic stress [33] and secondary metabolism [34]. The N-termini of the MYB transcription factors contain 1–3 conserved DNA binding domains: R1, R2, R3, and/or R4. Based on the presence of specific DNA binding domains, MYB transcription factors can be divided into four categories: R1-MYBs, R2R3-MYBs, R1R2R3-MYBs, and R4-MYBs. Notably, R2R3-MYBs are transcription regulators for synthesizing secondary metabolites and are involved in plant hormone response [35]. For example, PpMYB140 works as a transcriptional repressor to directly inhibit the expression of anthocyanin-related genes and prevent anthocyanin over-accumulation in bananas [36]. *CaMYB48* encodes an R2R3 transcription factor to activate the promoters of *AT3* and *KasIa*, leading to increases in Cap and DhCap [37]. However, although the genes involved in the PA biosynthetic pathway have been identified, many of the transcription factors regulating this process remain to be discovered and characterized. Studies on the regulatory role of MYB in *Pogostemon cablin* and its secondary metabolites remain limited.

In this study, *Pogostemon cablin* was treated with different plant hormones, and through WGCNA, we identified two modules—MEmagenta and MEgreen—that showed high correlations with PA content. The MEmagenta module exhibited the strongest association with PA accumulation. However, no transcription factors were directly identified within this module. Further analysis revealed several genes that still warrant attention. For instance, TRINITY_DN60695_c1_g1, TRINITY_DN63434_c1_g2, TRINITY_DN76152_c3_g5, TRINITY_DN80278_c5_g2, and TRINITY_DN80278_c5_g4 are annotated as encoding LOX21, which catalyzes the hydroperoxidation of linoleate and linolenate to generate JA precursors [38]. These JA precursors subsequently influence PA synthesis through the JA signaling pathway. Additionally, CLT2, identified in this module, regulates glutathione (GSH) levels, thereby affecting PA content [25, 39]. Some genes, such as L-ascorbate oxidase (AAO), have also been found to respond to environmental stresses and modulate the plant's redox state, potentially influencing PA indirectly [40]. Subsequently, we analyzed the MEgreen module, which contains 14 transcription factors distributed among the MYB, bHLH, WRKY, NAC, CCAAT, and AP2/ERF gene families. Among them, we identified a MYB transcription factor, which showed a strong correlation with PA content. It has a high degree value and kMe value, with a Pearson correlation coefficient of 0.926. Homology analysis revealed that this transcription factor shares the highest similarity with SmMYB25 from *Salvia miltiorrhiza*, and it was therefore designated as PcMYB25. An *Arabidopsis* T-DNA-inserted MYB25-null mutant exhibited deformed pollen cell wall, two-celled pollen, and misarranged male germ unit, indicating a role of AtMYB25 in *Arabidopsis* pollen development [41]. GhMYB25 can promote the differentiation of cotton ovule epidermal cells into fibrocytes, thereby regulating the length of cotton fibers. After silencing *GhMYB25*, cotton showed shorter seed fiber, less trichomes, and less seed in other parts [42]. To date, functional studies on MYB25 remain relatively limited. Phylogenetic analysis indicated that PcMYB25 belongs to the S23 subgroup of the R2R3-MYB family, which also includes members such as AtMYB1, AtMYB25, and AtMYB109. These transcription factors have been reported to be involved in plant responses to both biotic and abiotic stress pathways [43]. Therefore, we speculate that PcMYB25 may have a similar function in responding to stress and regulating plant growth and development in *Pogostemon cablin*. However, further detailed studies are still needed to confirm this hypothesis. Notably, although PcMYB25 is phylogenetically distant from certain secondary metabolic regulators such as AcMYB1 from *Allium cepa*, which regulates anthocyanin biosynthesis, and AaMYB1 from *Artemisia annua*, which positively regulates artemisinin synthesis [44, 45] (Fig. S7). However, they share identical protein domains, suggesting that PcMYB25 may have similar transcriptional regulatory potential.

Unlike previous studies focusing on developmental roles, our findings suggest a novel role for PcMYB25 in secondary metabolism, specifically PA biosynthesis. Further analysis revealed a correlation coefficient of 0.897 between the expression levels of *PcMYB25* and *PcPTS*. The strong correlation suggests a potential influence of PcMYB25 on *PcPTS* expression. After the overexpression of *PcMYB25*, the PA content in plant leaves was increased by 85%. Gene expression analysis of the PA biosynthetic pathway showed that *PcMYB25* overexpression led to a significant and specific upregulation of *PcPTS*, increasing its expression level by 4.09-fold. Furthermore, *PcMYB25* overexpression also significantly enhanced the expression of the upstream MVA pathway genes *AACT* and *PMK*. This suggests that PcMYB25 may exert broader regulatory effects on terpenoid metabolism and could modulate other genes in the pathway—directly or indirectly—to promote PA biosynthesis. During the

transient overexpression experiment, GC-MS analysis detected not only PA but also three other secondary metabolites: β-caryophyllene, α-guaiene, and pogostolene. According to Deguerry et al., these compounds are all catalyzed by *PcPTS* [9]. GC-MS analysis revealed that the levels of these three metabolites were increased in plants overexpressing *PcMYB25* compared to the control group. These findings collectively indicate that *PcMYB25* overexpression enhances the accumulation of multiple PcPTS-derived sesquiterpenoids, further supporting its role in modulating terpenoid biosynthesis. However, the module and trait correlation heat map show a negative correlation ($r$=−0.591, *p-value* = 0.0098) for the green module with PA (Fig. 3B). A similar situation exists in Pan's research [46]. Pan et al. performed a WGCNA of all genes of Y12-4 (cold-tolerance genotypes). Modules-trait relation showed that *LTG5* in the module greenyellow ($r$ = 0.35, p-value = 0.2) is a key gene in regulating cold tolerance at the germination stage. Overexpression of *LTG5* can increase the germination rate of rice at low temperature. However, there are *COLD1* [47], *CTB4a* [48], *LTG1* [49], *Ctb1* [50], *ICE1* [51] in the blue module ($r$=−0.98, *p-value* = $2e^{-10}$), *qLTG3–1* [52] in the brown module ($r$=−0.92, *p-value* = $2e^{-06}$). These genes can improve the tolerance of rice at low temperature conditions as well.

In addition, constructing a co-expression network, the correlation between gene expression level and trait is considered, but the influence of gene interaction on trait is not considered. Modules clustering heat map showed that, genes in the green module were highly expressed in MeJA + ETH treatment group, lowly expressed in ETH treatment group, and expressed irregularly in other treatment groups. However, the PA content was the lowest under the MeJA + ETH treatment, so the WGCNA analysis showed that the module was negatively correlated with the gene. This indicates that the expression patterns of the green module genes under different hormone treatments are complex and diverse. It may reflect that this might be due to the influence of gene interactions on the regulation of the synthesis of PA. In addition, the module is negatively correlated with genes, but there may be differences of genes in the green module, which could be the reason for the positive correlation between *PcMYB25* and PA. The Y1H assay suggested a potential interaction between PcMYB25 and the *PcPTS* promoter. MYB transcription factors are known to form protein complexes with other partners to regulate gene expression. For example, a study by Cao et al. demonstrated that MrMYB5 and MrMYB5L interact with MrbHLH2 to form MYB5-bHLH complexes, which synergistically activate the expression of *MrF3'5'H* and *MrFLS1*, promoting myricetin accumulation in *Morella rubra* [53]. In addition, Luan et al. study revealed that the PsMYBPA2-PsbHLH1-3 and PsMYB308-PsbHLH1-3 complexes cooperatively regulate spot formation in tree peony [54]. Therefore, combined with the overexpression results, PcMYB25 may function in concert with other transcription factors to modulate terpenoid biosynthesis. Its effect on *PcPTS* expression could be either through direct cooperative regulation as part of a transcriptional complex, or indirectly via regulation of upstream signaling or metabolic pathway components.

In conclusion, our research results indicate that PcMYB25 as an important R2R3-MYB transcription factor, responds to hormonal signals and is involved in the biosynthesis of PA in *Pogostemon cablin*. Through co-expression analysis and functional validation, we provided preliminary evidence for its regulatory role, suggesting that PcMYB25 may directly or indirectly promote the biosynthesis of PA and related sesquiterpenes by influencing terpene metabolic pathway genes. This study will further comprehensively elucidate the function of *PcMYB25* and its regulatory network. In subsequent studies, it is still necessary to employ techniques such as Virus-Induced Gene Silencing (VIGS) or dual-luciferase reporter (LUC) assays for in-depth functional validation, and to identify interacting proteins that may cooperate with it in regulating PA biosynthesis.

## Conclusions

In this study, transcriptome sequencing of *Pogostemon cablin* treated with various phytohormones revealed extensive changes of genes involved in signal transduction and secondary metabolism. Combined with WGCNA analysis, led to the identification of a key R2R3-MYB transcription factor, PcMYB25, which is highly correlated with PA biosynthesis. Homology analysis revealed that PcMYB25 is most similar to SmMYB25 from *Salvia miltiorrhiza*. Experimental evidence demonstrated that PcMYB25 exhibits transcriptional activation activity. In addition, yeast one-hybrid assays showed that PcMYB25 can bind to the promoter of *PcPTS*. Transient overexpression assays show that PcMYB25 significantly enhances the expression of *PcPTS* and select upstream MVA pathway genes, and promotes the accumulation of PA and other sesquiterpenoids. These findings suggest that PcMYB25 may act as a candidate regulatory factor that plays a role in plant hormone signaling and contributes to the accumulation of PcPTS-derived PA and sesquiterpenoids. This discovery lays the foundation for further research into the transcriptional network controlling PA biosynthesis and highlights the potential for metabolic engineering to enhance PA yields.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-07585-5.

Supplementary material 1.

**Acknowledgements**
Not applicable.

**Authors' contributions**
RZ and LC designed the study; LF, XZ and XC performed experiments; LF, XZ,PC and XC analyzed the data; KG, ZC, and XG helped the field works; LF,PC and XZ wrote the manuscript. LC edited the manuscript and provided guidance during this experimentation. All authors read and approved the final manuscript.

**Funding**
Financial support for this research was provided in part by the Natural Science Foundation of Guangdong Province (No. 2023A1515030194), National Natural Science Foundation of China (No. 82373976), and Special Project for Rural Revitalization Strategy in Guangdong Province (No. 2024CXTD24, 2024-NPY-00-041 and 2023-NBH-00-022).

**Data availability**
The datasets generated and analysed during the current study are available in the NCBI (https://www.ncbi.nlm.nih.gov/) repository, SRX5546201-3, SRX5546617-9, SRX5560046-8, SRX5565335-7, SRX5565333-5, SRX5558061-3.

## Declarations

**Ethics approval and consent to participate**
No animals or humans were involved in this study, and there are no ethical issues involved in this paper.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

Received: 22 April 2025 / Accepted: 15 October 2025
Published online: 11 November 2025

**References**

1. Fatima S, Farzeen I, Ashraf A, et al. A comprehensive review on pharmacological activities of pachypodol: a bioactive compound of an aromatic medicinal plant *Pogostemon cablin* Benth. Molecules. 2023;28(8): 3469. https://doi.org/10.3390/molecules28083469
2. Mrisho II, Musazade E, Chen HB et al. Unlocking the therapeutic potential of Patchouli leaves: A comprehensive review of phytochemical and Pharmacological insights [J]. Plants-Basel, 2025;14(7):1034.
3. Chen JR, Xie XF, Li MT et al. Pharmacological activities and mechanisms of action of Pogostemon Cablin benth: a review [J]. Chin Med, 2021;16(1):5.
4. Yan WP, Yang YZ, Wu YG, et al. Isopentenyl diphosphate isomerase (IPI) gene silencing negatively affects patchouli alcohol biosynthesis in *Pogostemon cablin* [J]. Plant Mol Biol Rep. 2021;39(3):557–65.
5. Lee HS, Lee J, Smolensky D, et al. Potential benefits of Patchouli alcohol in prevention of human diseases: a mechanistic review. Int Immunopharmacol. 2020;89: 107056. https://doi.org/10.1016/j.intimp.2020.107056
6. Bhatia SP, Letizia CS, Api AM. Fragrance material review on Patchouli alcohol [J]. Food Chem Toxicol. 2008;46(11):S255–6.
7. Bouvier F, Rahier A, Camara B. Biogenesis, molecular regulation and function of plant isoprenoids [J]. Prog Lipid Res. 2005;44(6):357–429.
8. Liao P, Hemmerlin A, Bach TJ, et al. The potential of the mevalonate pathway for enhanced isoprenoid production [J]. Biotechnol Adv. 2016;34(5):697–713.
9. Deguerry F, Pastore L, Wu SQ, et al. The diverse sesquiterpene profile of patchouli, *Pogostemon cablin*, is correlated with a limited number of sesquiterpene synthases. Arch Biochem Biophys. 2006;454(2):123–36.
10. Luo GJ, Lin Y, Chen ST, et al. Overproduction of patchoulol in metabolically engineered *Komagataella phaffii*. J Agric Food Chem. 2023;71(4): 2049-2058. https://doi.org/10.1021/acs.jafc.2c08228
11. Wu SY, Lan K, Wang Q, et al. Comprehensive characterization of the bHLH transcription factor family in curcuma Wenyujin and functional Elucidation of CwbHLH27 in jasmonate-regulated sesquiterpenoid biosynthesis [J]. Plant Physiology and Biochemistry; 2025 ;220:109527.
12. Shen Q, Yan TX, Fu XQ, et al. Transcriptional regulation of artemisinin biosynthesis in *Artemisia annua* L. Sci Bull. 2016;61(1):18–25.
13. Lu X, Zhang L, Zhang FY, et al. AaORA, a trichome-specific AP2/ERF transcription factor of *Artemisia annua*, is a positive regulator in the Artemisinin biosynthetic pathway and in disease resistance to *Botrytis cinerea* [J]. New Phytol. 2013;198(4):1191–202.
14. Wang XB, Tang Y, Huang HL, et al. Functional analysis of *Pogostemon cablin* farnesyl pyrophosphate synthase gene and its binding transcription factor PcWRKY44 in regulating biosynthesis of Patchouli alcohol [J]. Front Plant Sci. 2022;13: 946629. https://doi.org/10.3389/fpls.2022.946629
15. Wu DD, Chen L, Zhong BY, et al. PcENO3 interacts with Patchoulol synthase to positively affect the enzymatic activity and Patchoulol biosynthesis in *Pogostemon cablin*. Physiol Plant. 2023;175(6): e14055. https://doi.org/10.1111/ppl.14055
16. Li GJ, Hu SQ, Zhao XY et al. Mechanisms of the morphological plasticity induced by phytohormones and the environment in plants [J]. Int J Mol Sci, 2021; 22(2):765.
17. Mir ZA, Ali S, Manzoor S, et al. Plant defense hormones: thermoregulation and their role in plant adaptive immunity. J Plant Growth Regul. 2025;1-18. https://doi.org/10.1007/s00344-024-11620-4
18. Kumari S, Nazir F, Maheshwari C, et al. Plant hormones and secondary metabolites under environmental stresses: enlightening defense molecules [J]. Plant Physiology and Biochemistry; 2024 206:108238.
19. Mubeen B, Murtaza S, Yaqoob S, et al. Harnessing plant defense: elicitors, hormones and immunity-driven production of medicinally valuable secondary metabolites [J]. S Afr J Bot. 2025;179:280–92.
20. Chen XZ, Li JR, Liu YT et al. PatSWC4, a Methyl jasmonate-responsive MYB (v-myb avian myeloblastosis viral oncogene homolog)-related transcription factor, positively regulates Patchoulol biosynthesis in Pogostemon Cablin [J]. Ind Crops Prod, 2020 ;154:112672.
21. Li J, Huang HC, Zuo YQ et al. PatWRKY71 transcription factor regulates Patchoulol biosynthesis and plant defense response [J]. BMC Plant Biol, 2024, 24(1):8.
22. Huang TT, Men W, Myanganbayar A, et al. Transcriptome analysis reveals regulatory mechanism of Methyl jasmonate-induced monoterpenoid biosynthesis in *mentha arvensis* L. Front Plant Sci. 2025;15: 1517851. https://doi.org/10.3389/fpls.2024.1517851
23. Lindbo JA. TRBO: A high-efficiency tobacco mosaic virus RNA-Based overexpression vector [J]. Plant Physiol. 2007;145(4):1232–40.
24. Wang XB, Chen XZ, Zhong LT et al. PatJAZ6 acts as a repressor regulating JA-Induced biosynthesis of Patchouli alcohol in Pogostemon Cablin [J]. Int J Mol Sci, 2019 ;20(23):6038.
25. Mo MZ, Lin WY, Ul Haq MZ, et al. Exogenous glutathione (GSH/GSSG) promoted the synthesis of Patchoulol and pogostone, main active components of Pogostemon Cablin (Patchouli) [J]. Industrial Crops and Products; 2024;222:119788.
26. Bhatt PA, Gurav TP, Kondhare KR, et al. MYB proteins: versatile regulators of plant development, stress responses, and secondary metabolite biosynthetic pathways. Int J Biol Macromol. 2025;138588. https://doi.org/10.1016/j.ijbiomac.2024.138588
27. Mahajan M, Kuiry R, Pal PK. Understanding the consequence of environmental stress for accumulation of secondary metabolites in medicinal and aromatic plants [J]. J Appl Res Med Aromat Plants. 2020;18: 100255. https://doi.org/10.1016/j.jarmap.2020.100255
28. Tang Y, Zhong LT, Wang XB, et al. Molecular identification and expression of sesquiterpene pathway genes responsible for patchoulol biosynthesis and regulation in *Pogostemon cablin*. Bot Stud. 2019;60(1): 11. https://doi.org/10.1186/s40529-019-0259-9
29. Li JR, Chen XZ, Zhong LT et al. Comparative iTRAQ-based proteomic analysis provides insight into a complex regulatory network of Pogostemon Cablin in response to exogenous MeJA and ethrel [J]. Ind Crops Prod, 2019 ;140:111661.
30. Yusenko MV, Trentmann A, Andersson MK, et al. Monensin, a novel potent MYB inhibitor, suppresses proliferation of acute myeloid leukemia and adenoid cystic carcinoma cells. Cancer Lett. 2020;479:61–70.
31. Piao CL, Gao ZR, Yuan SM, et al. The R2R3-MYB gene CgMYB4 is involved in the regulation of cell differentiation and fiber development in the stamens of chelone glabra L [J]. Protoplasma. 2022;259(6):1397–407.

32. Chen JY, Ji ZL, Wu D, et al. MYBL2 promotes cell proliferation and inhibits cell apoptosis via PI3K/AKT and BCL2/BAX/cleaved-caspase-3 signaling pathway in gastric cancer cells [J]. Sci Rep. 2025;15(1): 9148. https://doi.org/10.1038/s41598-025-93022-4
33. Wang XP, Niu YL, Zheng Y. Multiple functions of MYB transcription factors in abiotic stress responses [J]. Int J Mol Sci, 2021; 22(11):6125.
34. Luo YR, Xu XY, Yang LF, et al. A R2R3-MYB transcription factor, FeR2R3-MYB, positively regulates anthocyanin biosynthesis and drought tolerance in common buckwheat (Fagopyrum esculentum) [J]. Plant Physiology and Biochemistry; 2024 217:109254.
35. Zhang DW, Zhou HP, Zhang Y et al. Diverse roles of MYB transcription factors in plants [J]. J Integr Plant Biol, 2025 ;67(3):539-562.
36. Ni JB, Premathilake AT, Gao YH, et al. Ethylene-activated PpERF105 induces the expression of the repressor-type R2R3-MYB gene PpMYB140 to inhibit anthocyanin biosynthesis in red Pear fruit [J]. Plant J. 2021;105(1):167–81.
37. Sun B, Zhou X, Chen C, et al. Coexpression network analysis reveals an MYB transcriptional activator involved in capsaicinoid biosynthesis in hot peppers [J]. Hortic Res. 2020;7(1):162.
38. Czernicka M, Chlosta I, Keska K, et al. Protuberances are organized distinct regions of long-term callus: histological and transcriptomic analyses in Kiwifruit [J]. Plant Cell Rep. 2021;40(4):637–65.
39. Lim B, Meyer AJ, Cobbett CS. Development of glutathione-deficient embryos in Arabidopsis is influenced by the maternal level of glutathione [J]. Plant Biol. 2011;13(4):693–7.
40. Skorupa M, Szczepanek J, Yolcu S, et al. Characteristic of the ascorbate oxidase gene family in beta vulgaris and analysis of the role of AAO in response to salinity and drought in beet. Int J Mol Sci. 2022;23(21): 12773. https://doi.org/10.3390/ijms232112773
41. Renák D, Dupl'áková N, Honys D. Wide-scale screening of T-DNA lines for transcription factor genes affecting male gametophyte development in Arabidopsis. Sex Plant Reprod. 2012;25(1):39–60.
42. Walford SA, Wu YR, Llewellyn DJ, et al. GhMYB25-like: a key factor in early cotton fibre development [J]. Plant J. 2011;65(5):785–97.
43. Beathard C, Mooney S, Al-Saharin R, et al. Characterization of *Arabidopsis thaliana* R2R3 S23 MYB transcription factors as novel targets of the ubiquitin proteasome-pathway and regulators of salt stress and abscisic acid response. Front Plant Sci. 2021;12: 629208 https://doi.org/10.3389/fpls.2021.629208
44. Schwinn KE, Ngo H, Kenel F, et al. The Onion (Allium cepa L.) R2R3-MYB Gene MYB1 Regulates Anthocyanin Biosynthesis [J]. Front Plant Sci. 2016;7:1865.
45. Matías-Hernández L, Jiang WM, Yang K, et al. AaMYB1 and its orthologue AtMYB61 affect terpene metabolism and trichome development in Artemisia annua and Arabidopsis Thaliana [J]. Plant J. 2017;90(3):520–34.
46. Pan YH, Liang HF, Gao LJ, et al. Transcriptomic profiling of germinating seeds under cold stress and characterization of the cold-tolerant gene LTG5 in rice [J]. BMC Plant Biol. 2020;20(1): 371 https://doi.org/10.1186/s12870-020-02569-z
47. Ma Y, Dai XY, Xu YY, et al. COLD1 confers chilling tolerance in rice [J]. Cell. 2015;160(6):1209–21.
48. Zhang ZY, Li JJ, Pan YH, et al. Natural variation in CTB4a enhances rice adaptation to cold habitats [J]. Nat Commun. 2017;8:14788.
49. Lu GW, Wu FQ, Wu WX, et al. Rice LTG1 is involved in adaptive growth and fitness under low ambient temperature [J]. Plant J. 2014;78(3):468–80.
50. Saito K, Hayano-Saito Y, Kuroki M, et al. Map-based cloning of the rice cold tolerance gene Ctb1 [J]. Plant Sci. 2010;179(1–2):97–102.
51. Chinnusamy V, Zhu J, Zhu JK. Cold stress regulation of gene expression in plants. Trends Plant Sci. 2007;12(10):444–51.
52. Fujino K, Iwata N. Selection for low-temperature germinability on the short arm of chromosome 3 in rice cultivars adapted to Hokkaido, Japan [j]. Theor Appl Genet. 2011;123(7):1089–97.
53. Cao YL, Zhang RN, Xing MY, et al. Synergistic actions of three MYB transcription factors underpins the high accumulation of myricetin in Morella rubra. Plant J. 2023;115(2): 577-594 https://doi.org/10.1111/tpj.16247
54. Luan YT, Tao J, Zhao DQ. Synergistic actions of 3 MYB transcription factors underpin blotch formation in tree peony [J]. Plant Physiol. 2024;196(3):1869–86.


## Publisher's Note