The Transcription Factor LaMYC4 From Lavender Regulates Terpenoid Biosynthesis

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Research Article

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Abstract

Background

The basic helix-loop-helix (bHLH) transcription factors (TFs), as one of the largest families of TFs, are important regulators of plant terpenoid biosynthesis and response to stresses. Lavender has more than 75 volatile terpenoids, yet few TFs have been identifified to be involved in the terpenoid biosynthesis.

Results

Based on RNA-Seq, reverse transcription-quantitative polymerase chain reaction, and transgenic technology, this study characterized the stress-responsive transcription factor LaMYC4 regulates terpenoid biosynthesis. Treatment with methyl jasmonate (MeJA) increased volatile terpenoid emission, and the differentially expressed gene *LaMYC4* was isolated. *LaMYC4* expression level was higher in leaves than in other tissues. The expression of *LaMYC4* decreased during flower development. The promoter of *LaMYC4* contained hormone and stress-responsive regulatory elements and was responsive to various treatments, including UV, MeJA treatment, drought, low temperature, *Pseudomonas syringae* infection, and NaCl treatment. *LaMYC4* overexpression increased the levels of sesquiterpenes, including caryophyllenes, in *Arabidopsis* and tobacco plants. Furthermore, the expression of key node genes involved in terpenoid biosynthesis and glandular trichome number and size increased in transgenic tobacco.

Conclusions

we have shown that the stress-responsive MYC TF LaMYC4 from ‘Jingxun 2’ lavender regulates terpenoid synthesis. This study is the first to describe the cloning of *LaMYC4*, and the results help understand the role of LaMYC4 in terpenoid biosynthesis.

Background

Volatile terpenoids are the most abundant class of volatile metabolites in plants and are involved in defense responses. Plants are exposed to environmental stresses, including abiotic (such as salt and drought) and biotic (such as pathogens and herbivores) stresses [1, 2], and adopt multiple defense mechanisms against stresses for growth and survival [3]. Volatile terpenoids protect plants against herbivores [4, 5] and thermal and oxidative stress [8] and mediate chemical communication [6, 7]. Moreover, plants synthesize monoterpenoids and sesquiterpenoids [9–13]. Among them, (-)-thujopsene and β-caryophyllene promote lateral root formation and induce plant resistance to microbes [9, 14, 15]. The sesquiterpenoid β-caryophyllene binds to the transcriptional co-repressor TOPLESS complex and modulates jasmonic acid (JA)-mediated signaling [16]. And caryophyllene induces defense responses via JA signaling [17].

Terpenoid biosynthesis begins with the formation of isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl diphosphate (DMAPP), through the mevalonate pathway in the cytosol and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway in plastids [18]. The enzymes 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGR), 1-deoxyxylulose- 5-phosphate synthase (DXS), and deoxyxylulose 5-phosphate reductoisomerase (DXR) control terpenoid synthesis [19, 20]. All monoterpenes are derived from geranyl diphosphate (GPP; C10), which is synthesized in a head-to-tail condensation reaction of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) by GPP synthases (GPPS). Then, farnesyl pyrophosphonate synthase (FPPS) adds two IPP molecules to DMAPP to form the C15 diphosphate precursor of sesquiterpenes and triterpenes [21].

Terpenoid biosynthesis is regulated by structural genes and transcription factors (TFs). TFs modulate gene expression by changing transcription rates [22, 23]. Basic helix-loop-helix (bHLH) TFs play a pivotal role in plant growth and development, stress response, and the biosynthesis of secondary metabolites [24]. MYC family members are bHLH TFs [25]. Some MYC TFs control terpenoid biosynthesis in plants [26]. For instance, CpMYC2 and AtMYC2 regulate caryophyllene synthesis and the transcription of terpenoid biosynthetic genes in *Arabidopsis thaliana* [27, 28], and SlMYC1 controls terpenoid emission in tomato (*Solanum lycopersicum*) [29]. MYC TFs have been characterized in *A. thaliana* [27, 30], *S. lycopersicum* [29], *Artemisia annua* [31], and other plants [28, 32, 33] but not in lavender.

Lavender is a model for studying the regulation of terpenoid synthesis [34]. More than 75 volatile terpenoids were identified in *Lavandula angustifolia* [35, 36]. One hundred terpene synthases (TPSs) have been identified in lavender, of which 11 were characterized, and some are induced by methyl jasmonate (MeJA) [13, 37]. Recently, a reference genome for the ‘Jingxun 2’ lavender cultivar was created [37].

This study isolated the MYC TF LaMYC4, which regulates terpenoid biosynthesis. The expression of LaMYC4 was upregulated by UV, low temperature, drought, MeJA, and *Pseudomonas syringae* infection. Moreover, LaMYC4 overexpression increased the levels of terpenoids (especially caryophyllene) and the number and size of glandular trichomes (GTs) in transgenic plants. These results demonstrate that LaMYC4 can be a candidate gene for *L. angustifolia* molecular breeding.

Results

MeJA affects terpenoid biosynthesis

Lavender plants were treated with or without 8 mM of MeJA, and volatile terpenoids were analyzed by solid-phase microextraction gas chromatography/mass spectrometry (SPME-GC-MS). The results revealed that MeJA induced volatile terpenoid emission, and production was significantly higher in leaves (Fig. 1). Furthermore, MeJA promoted the emission of β-myrcene, β-cis-ocimene, and caryophyllene in lavender sepals and leaves (JAS and JAL) (Additional file 2: Fig. S2).

Isolation and bioinformatics analysis of LaMYC4

Twenty-six MYCs were previously identified (unpublished) in *L. angustifolia* based on genome data (PRJNA642976), and the MYC gene *LaMYC4* was differentially expressed by MeJA treatment (Fig. 2a). The level of *LaMYC4* expression was significantly higher in leaves than in other tissues and decreased during flower development (Fig. 2b, c). The 1422-bp open reading frame of *LaMYC4* encoded 473 amino acids (Additional file 3: Fig. S3). Bioinformatics analysis indicated that the LaMYC4 protein contained a bHLH-MYC sequence between amino acids 38 and 211, corresponding to the N-terminal region of MYB and MYC TFs, and DNA-binding domains between amino acids 299 and 373 (Fig. 2e). Physicochemical characterization using ExPASy showed that LaMYC4 had a molecular mass of 52.24 kDa and an isoelectric point of 5.75. A phylogenetic tree was constructed with LaMYC4 and 22 MYCs from different plants (Additional file 9: Table S2) and showed that LaMYC4 was most closely related to NaMYC4 and BpMYC4 (Fig. 2d).

Analysis of the *LaMYC4* promoter sequence and response to stresses

The 2000-bp promoter upstream of the 5'-untranslated region (5' UTR) was analyzed using PlantCARE software (Additional file 10: Table S3). Four abscisic acid response elements were found at +1432, -1469, -1467, and +1687 bp, three light or abscisic acid response elements (G-box) were located at −1431, +1469, and −1686 bp, four low-temperature response elements were situated at −104, −1478, +614, and −1809 bp, one TC-rich repeating element involved in defense and stress response was located at −1617 bp, and one gibberellin response element (TATC-box) was located at −1953 bp (Fig. 3a and Additional file 10: Table S3).

Gene expression studies have shown that MYC transcription increased in response to biotic and abiotic stresses. *LaMYC4* expression levels were quantified by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The results showed that *LaMYC4* expression was significantly upregulated in lavender leaves by UV (~4-fold), cold (~3-fold), drought (~6-fold), MeJA (~5-fold), and *Pst* DC3000 (~6-fold) and downregulated 3-fold by NaCl (Fig. 3b).

Subcellular localization and transactivation activity of LaMYC4

The subcellular localization of the LaMYC4 protein was assessed using a transient expression assay in tobacco (*Nicotiana benthamiana*) leaves. The results showed that 35S::GFP was found in the cytoplasm and nucleus of plant cells, whereas LaMYC4 fusion proteins were only present in the nucleus (Fig. 4a), suggesting that LaMYC4 localizes to the nucleus.

The yeast strain AH109 and the pGBKT7 vector containing the DNA-binding domain of GAL4 were used to measure the transactivation activity of LaMYC4. Yeast cells transformed with any vector were cultivated in SD/-Trp medium. Yeast cells transformed with the fusion plasmid (pGBKT7-LaMYC4) or positive control plasmid (pGBKT7-p53) and cultivated in SD/-Trp/X-α-Gal medium appeared blue, whereas yeast cells transformed with the negative control plasmid pGBKT7 did not turn blue (Fig. 4b), indicating that LaMYC4 has transactivation activity in yeast.

LaMYC4 overexpression increases sesquiterpenoid biosynthesis in *A. thaliana*

Under the control of the CaMV 35S promoter, *LaMYC4* was overexpressed in transgenic *A. thaliana* by *Agrobacterium tumefaciens*-mediated transformation. Terpenoid levels were measured in transgenic plants from the T3 generation. The results indicated that total terpenoid and monoterpenoid contents did not change significantly in transgenic lines (Fig. 5a, b). In contrast, sesquiterpenoid levels increased 0.5-1.0-fold in transgenic lines overexpressing *LaMYC4* (#2, #7) compared with the empty vector group (Fig. 5c). In addition, caryophyllene was the most abundant sesquiterpenoid in *A. thaliana*, and its emission was more than 2-fold higher in transgenic *A. thaliana* than the control groups (wild-type and empty vector plants) (Fig. 5d and Additional file 4: Fig. S4).

Overexpression of *LaMYC4* increases terpenoid biosynthesis in tobacco

Under the CaMV 35S promoter, *LaMYC4* was overexpressed in tobacco by *Agrobacterium tumefaciens*-mediated transformation. Terpenoid concentrations were quantified in transgenic plants from the T2 generation using SPME-GC-MS. The results indicated that total terpenoid and sesquiterpenoid contents increased 1-2-fold and 2-3-fold in transgenic tobacco, respectively, compared with the control (Fig. 6a, c), whereas monoterpenoid contents increased significantly only in transgenic line #5 (Fig. 6b). Caryophyllene concentrations were higher in lines #3 and #5 than in control plants (Fig. 6 d). Caryophyllene levels were ~5-fold higher in transgenic lines overexpressing *LaMYC4* (#3 and #5) than in empty vector plants (Additional file 5: Fig. S5). Furthermore, transgenic tobacco plants (35S:: LaMYC4) showed reduced flower color and increased plant height (Additional file 7: Fig. S7) compared with control plants.

LaMYC4 overexpression upregulates genes related to terpenoid synthesis in tobacco

To assess the effect of LaMYC4 on the expression of genes related to terpene synthesis, we investigated HMGR, FPPS, DXS, DXR, and GPPS (the sequences are shown in Additional file 11: Table S4), which are key enzymes in the MVA and MEP pathways. The expression of genes *HMGR*, *FPPS*, *DXS*, *DXR*, and *GPPS* increased 1.3- to 3.8-fold (Fig. 7b) in *LaMYC4*-overexpressing transgenic tobacco flowers. In addition, *DXR* expression was strongly associated with the expression of *LaMYC4*. These results indicate that LaMYC4 regulates terpenoid synthesis and *DXR* expression and indirectly controls the expression of *HMGR*, *FPPS*, *DXS*, and *GPPS*. However, this result needs to be validated.

LaMYC4 overexpression increases the number and size of GTs

GTs are a physical defense to insect herbivores in response to mechanical stimulation. Moreover, evidence indicates that glandular secretory trichomes (GSTs) synthesize and store terpenoids. Since LaMYC4 regulates terpenoid biosynthesis in transgenic lines, we examined GT morphology by scanning electron microscopy. GTs on the stems of the fourth fully grown internode of 35S::LaMYC4 tobacco plants had longer stalks and larger glandular heads than control plants (Fig. 8). Moreover, the number of GTs was 0.4-fold higher in 35S::LaMYC4 tobacco plants than in control plants (Fig. 8d).

Discussion

Plants utilize various physiological and biochemical processes to survive and respond to stresses [38, 39]. Plant bHLH proteins play a pivotal role in stress responses. For instance, OsbHLH148 and OsbHLH006 (RERJ1) respond to drought stress through the JA signaling pathway [40, 41]. *Vitis vinifera* bHLH1 responds to drought and salinity via the accumulation of flavonoids and is the regulation of abscisic acid (ABA) synthesis [42]. RsICE1 interacts with CBF/DREB1 in rice plants to improve cold tolerance [43]. We identified the promoter region of LaMYC4 by genomic analysis [37]. This region contains stress-related cis-elements that allow LaMYC4-encoded TFs to adapt to the environment. In additional, the results of UV, MeJA treatment, drought, low temperature, *Pseudomonas syringae* infection, and NaCl treatment indicated that LaMYC4 responed to multiple stresses.

Plant bHLH TFs play vital roles in terpenoid biosynthesis. For instance, AtMYC2 binds to the promoter of the caryophyllene biosynthetic pathway genes *TPS21* and *TPS11* and stimulates gene expression [27], and CrBIS2 plays an essential role in the generation of monoterpenoid indole alkaloids [44]. LaMYC4 overexpression enhanced terpenoid synthesis, especially sesquiterpenoid caryophyllene (Fig.s 5 and 6). In addition, the transcript levels of the structural genes *HMGR*, *FPPS*, *DXR*, *DXS*, *GPPS* from the terpenoid biosynthesis pathway were significantly increased in *LaMYC4*-overexpressing lines (Fig. 7). *LaMYC4* overexpression enhanced the flux of terpenoid biosynthetic pathways, and the decrease in anthocyanin accumulation in transgenic plants produced light-colored flowers (Additional file 7: Fig. S7). Anthocyanin production is metabolically expensive, and the overexpression of *VvmybA1* resulted in the accumulation of anthocyanins in leaves, whereas the concentration of most volatile compounds decreased in the leaves of transgenic plants [45]. The overexpression of *CpbHLH13* increased the concentration of volatile terpenoids and decreased anthocyanin accumulation [28]. These results indicated that LaMYC4 modulated volatile terpenoid biosynthesis, especially sesquiterpenoid caryophyllene, and influenced carbon flow in the terpenoid pathway.

MYC3 and MYC4 activate JA-regulated responses and act synergistically with MYC2 to control different subsets of JA-dependent transcriptional activity [46]. Different volatile compounds are involved in JA-associated stress response [47–50]. Our MeJA treatment confirmed the result. And this study found that *LaMYC4* overexpression in transgenic tobacco increased ABA and GA3 contents and decreased JA and IAA levels (Additional file 6: Fig. S6). Abe et al. [30] have shown that AtMYC2 acts as a transcriptional activator in ABA signaling in *Arabidopsis*. Moreover, GA is involved in cell elongation [51]. Plant height increased in *LaMYC4*-overexpressing tobacco (Additional file 7: Fig. S7a, b). The morphology of stem epidermal cells was examined by scanning electron microscopy. The length of these cells was 0.3-fold higher in transgenic plants than in control plants (Additional file 7: Fig. S7). These results indicate that LaMYC4 promotes the elongation of epidermal cells by upregulating GA, increasing plant height.

Trichomes serve as physical barriers to insect herbivores [29]. Evidence indicates that GSTs produce and accumulate terpenoids [52]. In tomato, SlMYC1 regulates GT formation and terpenoid biosynthesis [29]. *LaMYC4*-overexpressing in tobacco confirmed the results that MYC play a pivotal role in plant GT

formation and terpenoid biosynthesis. In addition, the increase in terpenoid levels was significantly higher in *LaMYC4*-overexpressing tobacco than in *LaMYC4*-overexpressing *A. thaliana*, which may be because there is a lack of GTs in *A. thaliana*. In conclusion, we have shown that the stress-responsive MYC TF LaMYC4 from 'Jingxun 2' lavender regulates terpenoid synthesis. *LaMYC4*-overexpressing plants accumulated more terpenoids, especially sesquiterpenoid caryophyllene. In addition, LaMYC4 may be involved in regulating GT formation, increasing terpenoid biosynthesis and accumulation.

Conclusions

This study provides, to our knowledge, the first to describe the cloning of *LaMYC4*. We successfully profiled the tissue-specific expression patterns based on RNA-Seq. Different stress treatments and analysis of the *LaMYC4* promoter sequence shown that *LaMYC4* response to multiple stress to adapt to the environment. Furthermore, *LaMYC4*-overexpression increased the levels of terpenoids (especially caryophyllene) and the number and size of GTs in transgenic plants. These results demonstrate that LaMYC4 can be a candidate gene for *L. angustifolia* molecular breeding. And our study serve as a basis for future studies on the regulation of terpenoid synthesis and stress responses by MYCs.

Methods

Plant materials and treatments

The *L. angustifolia* cultivar used in this study was 'Jingxun 2' from the Institute of Botany, Chinese Academy of Sciences. The voucher specimen of 'Jingxun 2' was kept at the Chinese national herbarium, Institute of Botany, Chinese academy of sciences (voucher specimen: 02308796). All wild-type *Arabidopsis* and tobacco seeds used come from key laboratory of plant resources. And all plant material was used in accordance with relevant guidelines and regulations. Transcriptome data were obtained from a previous study [13, 37]. For *Pseudomonas syringae* pv. tomato (*Pst*) DC3000, UV, MeJA, salinity (NaCl), cold, and drought treatments, 12 one-year-old potted plants of the same cultivar (for each treatment) were grown in a greenhouse. Leaves were removed from potted plants for the all treatments. *Pst* DC3000 inoculation was performed for 6 h as described previously [53]. UV treatment lasted 10 minutes a day for three days. MeJA treatment was with 8 mM for 12 h. NaCl treatment with 300 mM was once every three days, twice in total, sampling on the seventh day, and watering thoroughly each time. cold (16°C) and or drought treatments were for 7 days. Sepals were harvested and treated with 8 mM MeJA for 12 h. *L. angustifolia*, *A. thaliana* (Col-0), and tobacco (*Nicotiana benthamiana* and *N. tabacum*) were grown under a 16 h photoperiod at 22 ± 2°C. Abbreviations corresponding to samples are as follows: sepal (S), leaf (L), root (R), stem (S), opening flower (F), glandular trichomes (GTs), flower bud (FB). FB0, FB1, FB2, F3, F4, and F5 correspond to different stages of flower development, as described previously [13].

RNA extraction and qPCR analysis

Total RNA was extracted from frozen samples using the HiPure Plant RNA Mini Kit (Magen, China) according to the manufacturer's instructions. RNA quality and concentration were analyzed by gel

electrophoresis and spectrophotometry. RNA was stored at − 80°C until use. cDNA was synthesized according to the manufacturer's instructions (Vazyme, China). Gene expression was measured by reverse transcription-quantitative PCR (RT-qPCR) on an Mx3000P system (Agilent Stratagene). Primers were designed using primer-BLAST (https://www.ncbi.nlm.nih.gov/ tools/primer-blast) (Additional file 8: Table S1). PCR and data analyses were performed as described previously [54].

LaMYC4 cloning and sequence analysis

Primers were designed based on the *LaMYC4* sequence obtained from the lavender genome (PRJNA642976) [37] (Additional file 8: Table S1), and the gene was amplified by PCR. The PCR product was cloned into the pBM16K vector and sequenced by TsingKe (Tianjin, China). Amino acid sequences homologous to LaMYC4 were retrieved from the NCBI database. Phylogenetic analysis was performed in MEGA software version 7.0 using the neighbor-joining method. The reliability of the neighbor-joining tree was estimated by bootstrap analysis using 1,000 bootstrap replications. The properties of the deduced amino acid sequence were predicted using ExPASy (http://web.expasy.org/compute_pi/).

Subcellular localization and the transactivating activity of LaMYC4

The full-length cDNA of LaMYC4 was cloned using primers (Additional file 8: Table S1) containing KpnI restriction sites and was ligated into the expression vector pCAMBIA2300 to produce a fusion protein (35S::LaMYC4-GFP). The empty vector (pCAMBIA2300) and the recombinant vector (35S::LaMYC4-GFP) were transformed into *Agrobacterium tumefaciens* GV3101 by heat shock. Four-week-old *N. benthamiana* plants were transformed with the 35S::LaMYC4-GFP vector or 35S::GFP vector, as described previously [55]. After 3 days of transformation, leaves were removed and analyzed on a confocal laser scanning microscope equipped with a standard filter set (Leica TCS SP5).

For the transactivation assay, the full-length cDNA of *LaMYC4* was cloned into the pGBKT7 vector containing EcoRI and BamHI restriction sites. The negative control (pGBKT7), positive control (pGBKT7-p53), and recombinant vector were expressed in the yeast strain AH109 following the manufacturer's instructions.

Plant transformation and identification of transgenic lines

Bacterial colonies containing the 35S::LaMYC4-GFP vector were selected and transformed into the *Arabidopsis* Col-0 cultivar using a floral dip method [56] or tobacco plants using the leaf disk method [57]. Plants containing the empty vector served as a control. Explants were incubated in a growth chamber at 23°C under a 16 h light/8 h dark photoperiod. Primary transformants were selected on half-strength Murashige and Skoog medium containing 50 μg ml^{-1} kanamycin, and the presence of the transgene was confirmed by PCR.

Measurement of volatile terpenoid concentrations

The volatile compounds released from lavender, tobacco, and *Arabidopsis* plants were collected by solid-phase microextraction (SPME) [28, 37]. Fresh sepals (10 mg), fresh leaves (100mg) from lavender, and fresh flowers (2 g) from tobacco were placed into headspace vials and kept at 40°C (lavender sepals and leaves) or 60°C (tobacco flowers) for 40 min and exposure to a DVB/CAR/PDMS fiber for 20 min, followed by analyte desorption at 250°C for 3 min. A total of 0.25 μg of 3-octanol was added to these samples as an internal standard. To measure the release of volatiles by *Arabidopsis* plants, the plants were placed in a 25 cm x 38 cm plastic bag (EasyOven) and incubated at 23 ± 2°C via DVB/CAR/PDMS fiber for 3 h, followed by analyte desorption at 250°C for 3 min. The relative concentration of the target compounds was determined using standard curves, which were generated by three repeats: y = 10-7x + 0.0024 and R2 = 0.92 (Additional file 1: Fig. S1).

GC-MS analysis was performed via splitless injection using an Agilent 7890B GC system and an Agilent Technologies 7000C Inert XL Mass Selective Detector equipped with an HP-5MS UI column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies), as described previously [37].

Products were identified based on retention times and electron ionization mass spectra obtained from the National Institute of Standards Technology (NIST) Mass Spectral Library (NIST-14.0) and literature data [35, 58, 59].

Trichome morphology and number

Samples were examined on a field-emission scanning electron microscope (Hitachi S-4800), and the number and size of stem trichomes from the fourth fully grown internode of each plant were determined.

Measurement of the level of anthocyanins and endogenous hormones

Twelve plants from each line were selected for measuring plant growth and total anthocyanin concentration. Total anthocyanins in tobacco flowers (500 mg) were measured as described previously [28]. GA, ABA, IAA, ZR, and JA in tobacco leaves were measured by enzyme-linked immunosorbent assay (ELISA). Hormones were extracted and purified according to He [60] and quantified by ELISA based on Yang et al. [61].

Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by independent-samples *t*-test or Fisher's least-significant difference test using SPSS software version 17.0. Data were expressed as the mean ± standard deviation of at least three independent experiments. *P*-values smaller than 0.05 were considered statistically significant.

Declarations

Ethics approval and consent to participate

Not applicable. All of the material is owned by the authors and/or no permissions are required.

Consent to publish

Not applicable.

Availability of data and materials

The raw genome and transcriptome sequencing data reported in this paper have been deposited in the National Center for Biotechnology Information (NCBI) database under project number PRJNA642976. And the data and materials in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest

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Authors contribution

Conceptualization, HT.B., H.L. and L.S, methodology, YM.D. and D. W, software, YM.D. and D. W, validation, YM.D., WY.Z. and JR.L, resources, HT.B, data curation, H.L, writing—original draft preparation, YM.D, writing—review and editing, H.L. and L.S, visualization, YM.D, supervision, H.L. and L.S. All authors have read and agreed to the published version of the manuscript.

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Figures

Figure 1

Emission of volatile terpenoids (μg g-1 fresh weight) in lavender with 8 mM MeJA. (**a**) GC-MS/MS chromatograms from the lavender sepal. (**b**) GC-MS/MS chromatograms from the lavender leaf. (**c, d, e**) Terpenoid contents from the lavender sepal (control, CKS, treatment, JAS). (**f, g, h**) Terpenoid contents from lavender leaves (control, CKL, treatment, JAL). Values shown are mean ± SD at least three replicates, and standard errors are indicated as vertical lines on the top of each bar, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *t*-test.

Figure 2

Characterization of LaMYC4. (**a**) Transcriptional changes in response to MeJA (**b**) The expression levels of *LaMYC4* in different tissues of *L. angustifolia* (LAR, root; LAS, stem; LAL, leaf; LAF, flower; LAGT, glandular trichome). (**c**) The expression levels of *LaMYC4* during flower development. (**d**) Phylogenic tree analysis of LaMYC4 and MYC TFs from *Arabidopsis thaliana*, *Artemisia annua*, *Oryza sativa*, etc. The phylogenic tree was constructed on MEGA7.0 by using the neighbor-joining method, and the bootstrap values were obtained for 1000 replications. (**e**) Multiple alignments of LaMYC4 with related MYC proteins from other plant species. Values shown are mean ± SD of three replicates, and standard errors are indicated as vertical lines on the top of each bar.

Figure 3

Analysis of *LaMYC4* promoter sequence and transcriptional abundance of *LaMYC4* under different stress conditions in lavender leaves. (**a**) Putative cis-acting regulatory elements were identified in the promoter sequence of *LaMYC4* using the PlantCARE database. (**b**) Treatments included UV, cold, NaCl, drought, MeJA and *Pst* DC3000. The relative expression of *LaMYC4* was quantified by qRT-PCR. Values shown are the means ± SD at least three replicates, and standard errors are indicated as vertical lines on the top of each bar. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *t*-test.

Figure 4

Subcellular localization of LaMYC4 in *N. benthamiana* and transcriptional activity of LaMYC4 in a yeast assay system. (**a**) Tobacco leaves were transformed with constructs including control (GFP) or fusion plasmids (LaMYC4::GFP). (**b**) Yeast cells (strain AH109) transformed with the positive control vector (top panel), the fusion vector-containing LaMYC4 (middle panels) and the negative control vector (bottom panels).

Figure 5

SPME-GC-MS analysis of VOCs from the *Arabidopsis* plants. wild type (WT), transformed by the empty vector pCAMBIA2300S (2300) and overexpressed LaMYC4 gene (35S::LaMYC4) plants (#2, #7). (**a**) Total contents. (**b**) monoterpenoids. (**c**) sesquiterpenoids. (**d**) GC trace of caryophyllene. The products were identified by comparison with compounds in the library NIST14 and reference standards. The values shown are mean ± SD at least three replicates. Standard errors are indicated as vertical lines on the top of each bar, and bars annotated with different letters were significantly different according to Fisher's LSD test ($P < 0.05$) after ANOVA.

Figure 6

SPME-GC-MS analysis of VOCs from the tobacco floral. wild type (WT), transformed by the empty vector pCAMBIA2300S (2300) and overexpressed *LaMYC4* gene (35S::LaMYC4) plants (#3, #5). (**a**) Total contents. (**b**) monoterpenoids. (**c**) sesquiterpenoids. (**d**) GC trace of caryophyllene. The products were identified by comparison with compounds in the library NIST14 and reference standards. The values

shown are mean ± SD at least three replicates. Standard errors are indicated as vertical lines on the top of each bar, and bars annotated with different letters were significantly different according to Fisher's LSD test ($P < 0.05$) after ANOVA.

Figure 7

Transcript levels of *LaMYC4* and genes related to terpenoid synthesis in the tobacco floral (**a**) Schematic representation of terpenoid synthesis. (**b**) Relative expression levels of these genes related to terpenoid synthesis were determined. The values shown are mean ± SD at least three replicates. Standard errors are indicated as vertical lines on the top of each bar, and bars annotated with different letters were significantly different according to Fisher's LSD test ($P < 0.05$) after ANOVA.

Figure 8

Morphology and number of glandular trichomes on tobacco stems (**a-c**) Glandular trichomes of wild-type (WT), empty vector pCAMBIA2300 (2300) and overexpression of *LaMYC4* transgenic plants (35S::LaMYC4) on stem surfaces. (**d**) Number of glandular trichomes on the stem surfaces of wild-type (WT), empty vector pCAMBIA2300 (2300) and overexpression of *LaMYC4* transgenic plants (35S::LaMYC4). Red arrows indicate glandular trichomes. The bars represent the mean values (± SD), calculated from three to four scanning-electron micrographs of stems from different plants. Standard errors are indicated as vertical lines on the top of each bar, and bars annotated with different letters were significantly different according to Fisher's LSD test ($P < 0.05$) after ANOVA.

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