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Cloning and characterization of a farnesyl pyrophosphate synthase from *Matricaria recutita* L. and its upregulation by methyl jasmonate

S.S. Su, H.M. Zhang, X.Y. Liu, G.F. Pan, S.P. Ling, X.S. Zhang, X.M. Yang, Y.L. Tai and Y. Yuan

College of Life Sciences, Anhui Agricultural University, Hefei, China

Correspondence author: Y. Yuan
E-mail: zhiwuxue239@163.com

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ABSTRACT. *Matricaria recutita* (L.), commonly known as chamomile, is one of the most valuable medicinal plants because it synthesizes a large number of pharmacologically active secondary metabolites known as α-bisabolol and chamazulene. Although the plant has been well characterized in terms of chemical constituents of essential oil as well as pharmacological properties, little is known about the genes responsible for biosynthesis of these compounds. In this study, we report a new full-length cDNA encoding farnesyl diphosphate synthase (FPS), a key enzyme in the pathway of biosynthesis of isoprenoids, from *M. recutita*. The cDNA of MrFPS comprises 1032 bp and encodes 343 amino acid residues with a calculated molecular mass of 39.4 kDa. The amino acid sequence homology and phylogenetic analysis indicated that MrFPS belongs to the plant FPS super-family and is closely related to FPS from the Asteraceae family. Expression of the MrFPS gene in *Escherichia coli* yielded FPS activity. Using real-time quantitative PCR, the expression pattern of the MrFPS gene was analyzed in different

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tissues of *M. recutita* as well as in response to methyl jasmonate. The expression analysis demonstrated that MrFPS expression varies in different tissues (with maximal expression in flowers and stems) and was significantly elevated in response to methyl jasmonate. This study will certainly enhance our understanding of the role of MrFPS in the biosynthesis and regulation of valuable secondary metabolites in *M. recutita* at a molecular level.

Key words: Farnesyl pyrophosphate synthase; Secondary metabolite; Methyl jasmonate; *Matricaria recutita* L.

INTRODUCTION

Chamomile [*Matricaria recutita* (L.) Rauschert, Asteraceae] is one of the oldest and most agronomically important medicinal plant species in Europe. The essential oil of chamomile flowers has significant antiinflammatory (Jellinek, 1984; Tubaro et al., 1984; Lal et al., 1993), antiseptic (Musselman, 1996), antiphlogistic (Isaac, 1979; Jakovlev et al., 1979), and spasmolytic (Maschi et al., 2008) properties and is therefore used in pharmaceutical, perfume, cosmetics, aromatherapy, and food industries (Lal et al., 1993). To evaluate the pharmaceutical value, analysis of essential oil and extracts from the flowers of *M. recutita* revealed the high presence of sesquiterpene derivatives (75-90%) including (E)-β-farnesene (4.9-8.1%), terpene alcohol (farnesol), chamazulene (2.3-10.9%), α-bisabolol (4.8-11.3%), and α-bisabolol oxides A (25.5-28.7%) as well as α-bisabolol oxides B (12.2-30.9%) (Pothke and Bulin, 1969; Isaac, 1979; Gasic et al., 1983; Schilcher et al., 2005) and these components are known for their antiinflammatory, antiseptic, antiphlogistic, and spasmolytic properties. Although extensive studies have been carried out regarding the pharmacological importance (Antonelli, 1928; Gould et al., 1973; Salamon, 1992; Pasechnik, 1996) of the essential oil constituents, little is known about the genes responsible for biosynthesis of these terpenoid molecules.

Farnesyl diphosphate synthase (FPS) is a key enzyme in isoprenoid biosynthesis (Chappell, 1995). FPS catalyzes the sequential 1'-4 condensation of dimethylallyl diphosphate (DMAPP) with two molecules of isopentenyl diphosphate (IPP) to form FPP, which is the precursor of all sesquiterpenes (Ogura and Koyama, 1998). Not only is FPP a common precursor for sesquiterpene compounds, but the branching point of pathways also results in the biosynthesis of diterpenes, tetraterpenes, and polyterpenes (Wang et al., 2004). Therefore, cloning and characterization of the FPS gene from *M. recutita* serves as an initial step to further study the sesquiterpenoids and their derivatives in *M. recutita*.

Because of the crucial role of FPP, a large group of FPS genes have been cloned and characterized from a variety of plant species, including *Arabidopsis* (Closa et al., 2010), *Artemisia annua* (Matsushita et al., 1996; Hemmerlin et al., 2003), maize (Cervantes et al., 2006), *Chimonanthus praecox* (Xiang et al., 2010), *Withania somnifera* (Gupta et al., 2011), and *Hedychium coronarium* (Lan et al., 2013). Nevertheless, as far as we know, there was only one coding sequence of the *fps* gene found in *M. recutita* and insufficient discussion. To better understand the role of FPS in sesquiterpenoid biosynthesis in *M. recutita*, we needed to first identify the *fps* gene and its expression profile.

Methyl jasmonate (MeJA) is a plant-specific signaling molecule that mediates a diverse set of physiological processes in plants (Liechti and Farmer, 2002). In previous studies, MeJA have been shown to induce the accumulation of useful secondary metabolites in several plant species, including triterpene saponins from cultured *Panax ginseng* cells (Lu et al., 2001), alkaloid pilocarpine in the leaves of *Pilocarpus jaborandi* (Avancini et al., 2003), galantamine in *Lycoris radiata* (Jiang et al., 2011), and sesquiterpene lactone artemisinin content in *A. annua* (Wang et al., 2010). However, there has not been a similar report on the effect of MeJA on the regulation of *fps* gene expression in *M. recutita* to produce useful secondary metabolites.

In this study, we described the cloning and functional analysis of the FPS gene from *M. recutita,* its expression pattern in different tissues and in flowers of various stages, and the leaves treated by 100 μM MeJA. Our results provide an important foundation for further understanding the expression and function of FPS in the control of sesquiterpenoid biosynthesis in *M. recutita*.

MATERIAL AND METHODS

Plant materials

The *M. recutita* seeds were provided by YuePing Limited and grown in Farmers Extraction Garden of Anhui Agricultural University. Flower development was divided into three stages. At stage 1, ligulate flowers unrolled. At stage 2, ligulate flowers were half-open. At stage 3, ligulate flowers fully opened. Floral tissue samples (roots, stems, leaves, tubular flowers, and ligulate flowers) were taken from *M. recutita* at stage 3. All plant material was harvested and frozen in liquid nitrogen and stored at -80°C for RNA extraction.

MeJA treatment

To evaluate the transcriptional activity of MrFPS in leaves of MeJA-treated plants, 8-week-old plants were sprayed with a water solution of 100 μM MeJA (Sigma, USA) in 0.8% ethanol. Control plants were only treated with an equivalent amount of ethanol and were analyzed in parallel with the experimental samples. The plants treated were left in the open air for 2 h to completely evaporate the remaining MeJA solution, followed by transfer into artificial greenhouses under the same conditions. Leaves were quickly harvested at 0, 3, 6, 12, 24, 36, 48, 60, and 72 h after treatment of both control and experimental populations. The harvested leaf samples were flash frozen in liquid nitrogen and stored at -80°C for RNA extraction.

RNA extraction and cDNA synthesis

Flowers of *M. recutita* were used for RNA extraction. RNAiso Plus (TaKaRa, Japan) was used for extraction of total RNA according to manufacturer instructions. The quality and concentration of the extracted RNA were determined using a NanoDrop 2000 (NanoDrop Technologies, USA) and agarose gel electrophoresis. The reverse transcription reaction was carried out with the PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, Japan) using 500 ng total RNA and 50 μM oligo dT following the manufacturer protocol.

Cloning of the *fps* gene

The primary synthesized ss-cDNA was used as template for PCR amplification by using *fps* gene-specific primers. FPS-F: 5'-CGGGATCCATGAGTATCGTGGATCTGAAATCA AAGTTTT-3' and FPS-R: 5'-CGAGCTCCTACTTTTGCCTCTTGTAGATTTTACCCAAG-3' were designed based on the *fps* gene of *M. recutita* (GenBank accession No. EF675758), the underlined sequences are the *Bam*HI and *Sac*I restriction sites, respectively. PCR was performed according to the following protocol: an initial delay at 94°C for 3 min, 39 cycles of denaturation at 94°C for 30 s, 63°C for 30 s, 72°C for 1 min, and a final elongation step of 72°C for 10 min. PCR products were resolved on 1% agarose gel by electrophoresis and then purified. The purified fragment was cloned into the pEASY-T1 cloning vector (TransGen, China) after DNA purification and then transferred into the *Escherichia coli* Trans1-T1 (TransGen, China). The inserted fragment was sequenced using standard M13 sequencing primers at Sangon Biotech (China).

Expression of FPS in *E. coli* and purification of recombinant protein

The recombinant plasmid pEASY-T1-FPS was digested with *Bam*HI and *Sac*I. The inserted fragment was isolated and ligated into the expression vector pET30a (+) and then transferred into *E. coli* BL21 (DE3) cells (TransGen, China) for ultimate expression. The recombinant plasmid was denominated as pET-FPS and then the inserted sequence was confirmed by sequencing and restriction enzyme digesting. The confirmed clone was grown in kanamycin-supplemented LB media to an OD_{600} of 0.5 at 37°C and then induced with 1 mM isopropyl thiogalactoside (IPTG) for 15 h at 25°C.

After harvesting, the cells obtained after centrifugation were resuspended in 1/10 of the original culture volume in lysis buffer containing 50 mM NaH_2PO_4, 300 mM NaCl, and 10 mM imidazole and lysed by sonication. The $(His)_6$-tagged recombinant protein was purified by passing the supernatant through Ni-NTA resin (Qiagen, Germany) following manufacturer instructions. Elution was carried out with elution buffer containing 250 mM imidazole, 50 mM NaH_2PO_4, and 300 mM NaCl. Both crude lysate and the purified protein were checked for the presence of recombinant protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue staining of the gel. FPS protein concentration was determined by the Bradford method (Bradford, 1976).

Western blotting analysis

For Western blot analysis, recombinant protein samples were subjected to SDS-PAGE based on the method described by Laemmli (Laemmli, 1970) and then transferred onto a polyvinylidene difluoride membrane (Sigma, USA) by an electrophoretic transfer system (Bio-Rad, USA). The membrane was blocked with phosphate-buffered saline containing 0.1% Tween-20 and subsequently incubated with anti-His tag antibodies for 2 h at room temperature, washed with phosphate buffered saline Tween-20, and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (Sigma, USA) for 1 h at room temperature (Zhu and Wu, 2008). The final detection was performed with an HRP-diaminobenzidine Chromogenic Substrate Kit (Tiangen, China).

Enzyme assays

For identification of reaction products, large-scale assays (1 mL) were performed with 250 µL purified FPS protein using 1 mg/mL IPP and 1 mg/mL GPP (Sigma, Germany) in assay buffer (50 mM Tris-HCl, pH 7.6, 2 mM DTT, 5 mM $MgCl_2$). The reactions were incubated for 30 min at 30°C; subsequently, 50 µL 3 M HCl was added to stop the reaction.

High performance liquid chromatography (HPLC) analysis was performed on a Waters 600E HPLC system with an ultraviolet detector (Waters Corporation, USA). The analysis was monitored at 275 nm and performed using a XTerra MS C_{18} column (4.6 x 50 mm) with a binary gradient mobile phase profile (acetonitrile, water) by applying the following gradient system: 0 min, 20% acetonitrile and 80% water; 15 min, 40% acetonitrile and 60% water; 35 min, 60% acetonitrile and 40% water; and 55 min, 90% acetonitrile and 10% water. The column was maintained at 30°C. The flow rate was set at 0.8 mL/min, and the injection volume was 20 µL. Authentic standards of FPP (Sigma, USA) were prepared in 85% methanol at a final concentration of 20 µg/mL and used for the preparation of the calibration graphs. Quantification was repeated three times for each sample.

Real-time quantitative PCR analysis of *MrFPS* gene expression

Expression profiles of MrFPS in three different developmental stages of flowers and various plant tissues (roots, stems, leaves, tubular flowers, and ligulate flowers) as well as under MeJA treatments were studied using real-time quantitative PCR. Total RNA from each tissue and treated sample was separately isolated as described above. Then, 500 ng total RNA was reverse-transcribed in a total volume of 10 µL reaction with PrimeScript™ RT Master Mix (Perfect Real Time) (Takara, Japan) according to the manufacturer protocol.

Real-time quantitative PCR was performed using the iQ5 real-time PCR detection system (Bio-Rad, USA). Gene-specific primers for real-time PCR were designed with Primer Premier 5.0 (forward 5'-TAAGTGCTCCTGGTTAGTTGTCA-3' and reverse: 5'-GACTGCTTTGCTTGGGTGA-3'). The 18S rRNA gene (GenBank accession No. KC816562) of *M. recutita* was amplified along with the target gene as an endogenous control to normalize expression between different samples. Control primers included 18S-F: 5'-TTCTTGTTTGCCCTCTTATTCT-3' and 18S-R: 5'-AACCAGGTAGCATTCCTCTTC-3'. Reactions were performed by an initial incubation at 95°C for 3 min and then cycled at 95°C for 10 s and 56°C for 30 s for 39 cycles. Real-time RT-PCRs were run in triplicate per sample per run. The relative quantification of the MrFPS transcript levels was calculated by the ΔΔCt method.

Results are generally reported as means ± the standard error from the values of three independent tests. Groups of data were analyzed by the Student *t*-test. A difference between means was considered to be significant at a value of $P < 0.05$.

Sequence analysis of the *fps* gene

Sequence homology and deduced amino acid sequence comparisons were carried out using National Center for Biotechnology Information basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast). Theoretical isoelectric point (pI) and molecular weight (Mw) of proteins were carried out using Compute pI/Mw Tool (http://www.expasy.org/tools/pi_tool.html). Multiple alignments of amino acid sequences were carried out with

the sequence analysis software Clustal X. Phylogenetic analysis of FPS sequences was performed using the neighbor-joining method in the MEGA 5 software. Bootstrapping was performed by resampling from the data 1000 times.

RESULTS

Cloning of the *fps* gene from *M. recutita*

The cDNA encoding the *fps* gene was amplified by RT-PCR according to the known *M. recutita fps* nucleotide sequence (GenBank: EF675758). The nucleotide sequence we obtained contained an open reading frame of 1032 bp (data not shown), encoding a protein of 343 amino acids with a predicted molecular mass of 39.4 kDa (pI 5.59). The DNA sequence of the *fps* gene was then deposited in GenBank (accession No. KJ130321). The obtained *fps* gene encoded a protein that was almost identical to the other *M. recutita fps* reported (99% overall identity) except for two amino acid differences. There were several other differences in the DNA sequences of these clones but these did not affect the predicted protein sequences.

Sequence and phylogenetic analysis of MrFPS

An amino acid BLAST search showed that the MrFPS protein was 90-96% identical to the FPS proteins from *A. annua, Helianthus annuus, Artemisia spiciformis, Parthenium argentatum, Tanacetum coccineum*, and *Panax notoginseng*. The alignment of the deduced polypeptide with other plant FPS proteins suggested the presence of highly conserved peptide domains (I-VII) that are important for catalytic/functional activity (Szkopinska and Pochocka, 2005) in MrFPS (Figure 1A). The motifs FARM (First Asp-Rich Motif; domain II) with DDXX(XX)D and SARM (Second Asp-Rich Motif, domain VI) with the sequence DDXXD are also present in MrFPS, which are thought to be binding sites for the pyrophosphate moieties of IPP and allylic substrates (Ohnuma et al., 1996).

A phylogenetic tree was constructed based on the deduced amino acid sequences of MrFPS and other FPS proteins from different organisms, including fungi, plants, chordates, and bacteria, to investigate evolutionary relationships (Figure 1B). As expected, the results showed that MrFPS belonged to the plant clade kingdom and was closely related to other FPS of the Asteraceae family. The FPS from Asteraceae including *M. recutita* and *T. coccineum* grouped into one cluster, which means they had the closest evolutionary relationships in the plant kingdom.

Functional characterization of the MrFPS protein

To characterize the properties of FPS, the entire reading frame of MrFPS was cloned into vector pET30a and expressed in *E. coli* Rosetta (DE3) cells to obtain the MrFPS protein. Upon induction by IPTG, a recombinant protein with a molecular weight of about 43 kDa was detected by SDS-PAGE. Meanwhile, the recombinant fusion proteins were purified by affinity chromatography, and a protein band corresponding to the predicted molecular weight of 43 kDa was detected (Figure 2A). Western blotting analysis showed that a 43-kDa consensus protein band was found after induction by IPTG but not in the empty vector control group, and its expression was not influenced by different IPTG concentrations (Figure 2B). These results indicate the successful expression of the recombinant protein in *E. coli* cells.

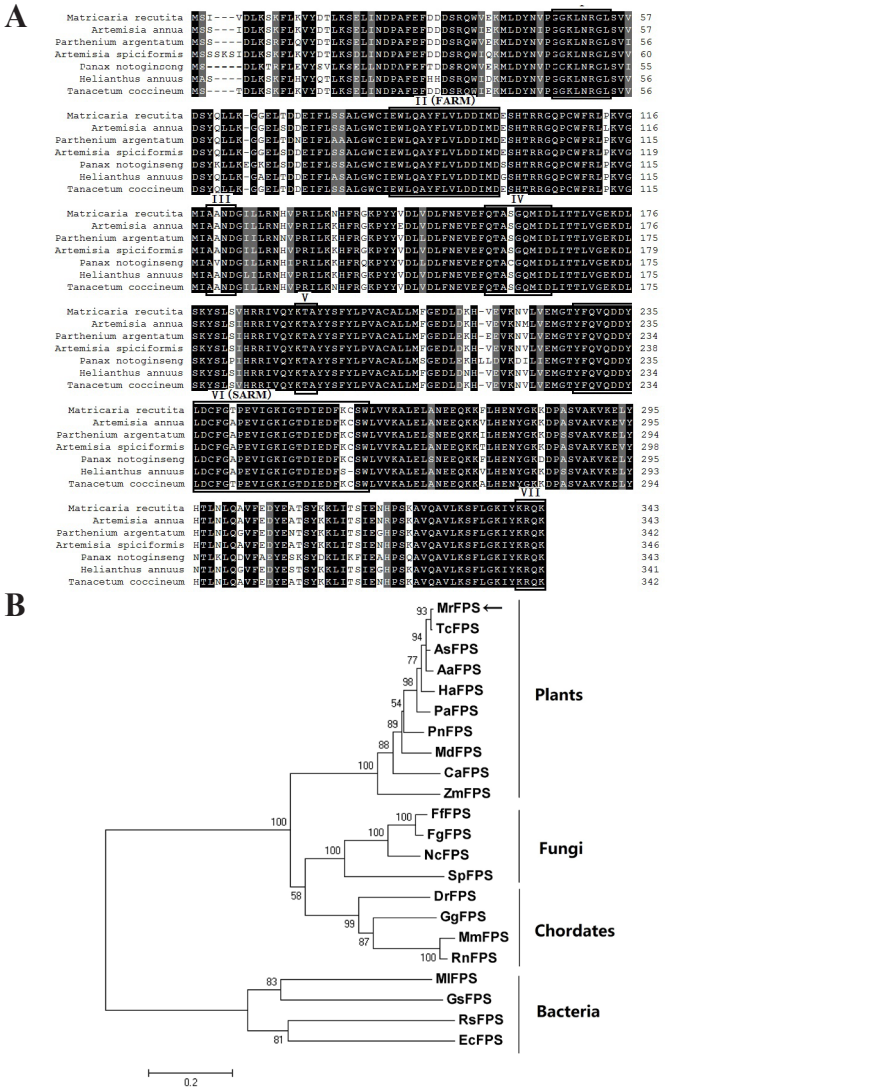

Figure 1. A. Alignment of the deduced full-length amino acid sequence of known FPS with MrFPS. The MrFPS sequence was aligned with FPS sequences from *Artemisia annua* (ADJ67472), *Parthenium argentatum* (CAA57892), *Artemisia spiciformis* (AAP74720), *Panax notoginseng* (AAY53905), *Helianthus annuus* (AAC78557), and *Tanacetum coccineum* (AFW98435) using ClustalW. The seven conserved motifs (I-VII) that are essential for FPS activity are shown in boxes and numbered. The highly conserved aspartate-rich motifs (FARM and SARM) are present in domains II and VI, respectively. **B.** Phylogenetic tree of the amino acid sequences of FPS of different organisms constructed by the neighbor-joining method using MEGA 5. The accession numbers of the sequences are as follows: *T. coccineum* (TcFPS, AFW98435); *A. annua* (AaFPS, ADJ67472); *A. spiciformis* (AsFPS, AAP74720); *Helianthus annuus* (HaFPS, AAC78557); *P. argentatum* (PaFPS, CAA57892); *P. notoginseng* (PnFPS, AAY53905); *Malus domestica* (MdFPS, AAM08927); *Capsicum annuum* (CaFPS, CAA59170); *Zea mays* (ZmFPS, P49353); *Fusarium fujikuroi* (FfFPS, Q92235); *Fusarium graminearum* (FgFPS, XP386960); *Neurospora crassa* (NcFPS, EAA32305); *Schizosaccharomyces pombe* (SpFPS, NP593299); *Danio rerio* (DrFPS, AAH97112); *Gallus gallus* (GgFPS, P08836); *Mus musculus* (MmFPS, AAL09445); *Rattus norvegicus* (RnFPS, AAH59125); *Micrococcus luteus* (MlFPS, BAA25265); *Geobacillus stearothermophilus* (GsFPS, Q08291); *Rhodovulum sulfidophilum* (RsFPS, BAA96459); and *Escherichia coli* (EcFPS, BAA00599). The numbers at nodes represent 1000 bootstrap replicates.

When the purified protein was incubated with GPP and IPP in the presence of the cosubstrate Mg^{2+} and analyzed by HPLC, a comparable retention time (~47 min) was obtained between the reaction product and FPP standard product (Figure 3). This indicated that only FPP formed in the reaction. Generally, FPP synthase has the functions of both geranyltransferase which forms FPP from GPP and IPP, and dimethylallyl transferase which forms GPP from DMAPP and IPP. Therefore, MrFPS encodes FPP synthase.

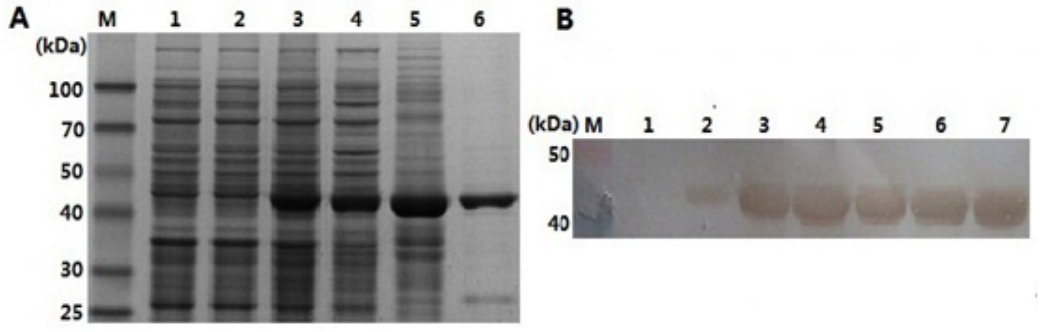

Figure 2. A. SDS-PAGE analysis of recombinant MrFPS protein expressed in *E. coli. Lane M* = low weight marker; *lane 1* = empty pET30a vector; *lane 2* = non-induced; *lane 3* = induced with 0.5 mM IPTG; *lane 4* = the supernatant of the MrFPS protein after induction; *lane 5* = the sediment of the MrFPS protein after induction; and *lane 6* = purified recombinant proteins. **B.** Western blot analysis of recombinant proteins in the *E. coli* BL21 (DE3) cells with anti His-Tag antibody. A protein band with a molecular mass of about 43 kDa was detected by Western blotting using anti His-Tag antibody. No immunoreactive band was found in the control group. *Lane 1* = empty pET30a vector; *lanes 2-7* = induced with 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mM IPTG, respectively.

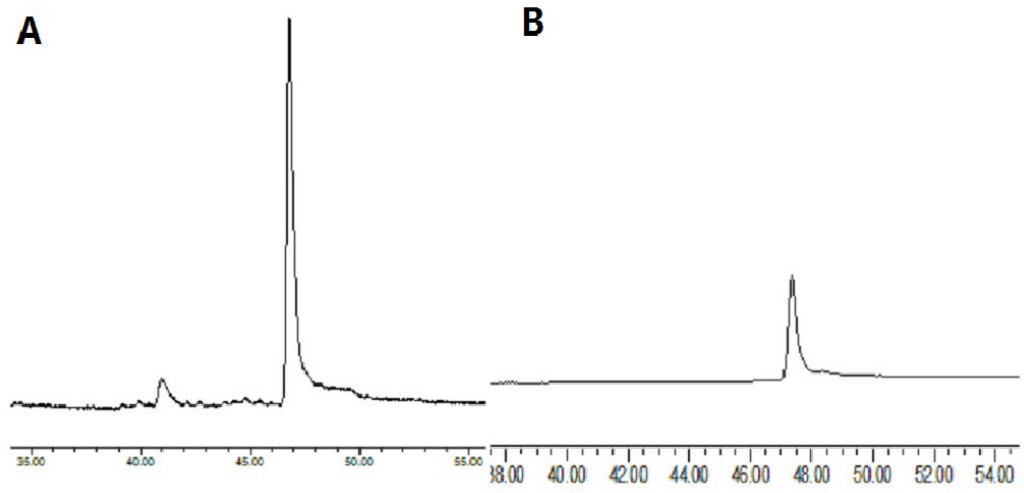

Figure 3. HPLC analysis of the reaction products catalyzed by purified recombinant MrFPS incubated with GPP and IPP. **A.** Standard (FPP) chromatogram. **B.** Sample (purified recombinant MrFPS incubated with GPP and IPP) chromatogram.

Expression analyses of different tissues and leaf development stages

Earlier investigations of the oil of *M. recutita* showed that, in addition to the flowers, the shoot (leaves and stems) and root of the plant also contain essential oil (Reichling et al., 1979; Schilcher et al., 2005), and the components of essential oil vary among different tissues. In particular, some tissue-specific components, such as chamazulene and α-bisabolol, were only present in flowers and not found in other tissues. This prompted us to investigate the expression pattern of FPS in different tissues of *M. recutita* in more detail to explore its tissue expression pattern by real-time PCR analyses. When MrFPS transcript values in the samples were adjusted to the expression levels of Mr-18S for each respective stage, the lowest level of MrFPS mRNA expression was found in the roots. Its value was set as 1, and the MrFPS expression in other tissues was evaluated relative to the root level (Figure 4). The results showed that the MrFPS was ubiquitously expressed in all of the tissues examined, but at different levels. The highest transcript levels of MrFPS were observed in tubular flowers, about 7.4-fold higher than those in roots. MrFPS showed lower expression in ligulate flowers and leaves, about 3 and 1.7-fold compared with that in roots, respectively. Interestingly, stems and tubular flowers had little discernible expression. Consistent with the findings that MrFPS is a key enzyme of floral scent volatile biosynthesis as well as biosynthesis of other terpenoids, such as membrane constituents, photosynthetic pigments, growth substances, electron transport carriers, and plant hormones (Okada et al., 2001).

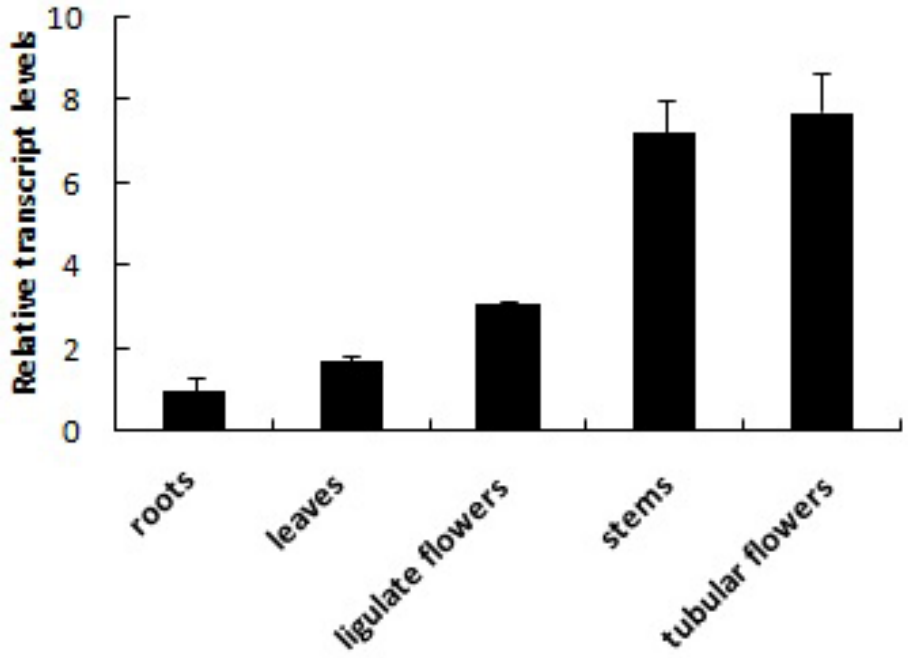

Figure 4. MrFPS expression in different tissues of *Matricaria recutita*. Total RNA from roots, stems, leaves, tubular flowers, and ligulate flowers was utilized to determine MrFPS expression by RT-PCR. MrFPS expression values were normalized to the levels of Mr-18S RNA expression in respective tissues. MrFPS expression in roots was set as 1. Data are reported as means ± SE from three experimental replicates.

Real-time PCR analysis with flowers in different developmental stages was also carried out to examine the developmental expression of the MrFPS gene in tubular flowers. The lowest level of MrFPS mRNA expression was found in stage 2. Its value was set as 1 and the MrFPS expression in other two stages was evaluated relative to the stage 2 level (Figure 5). As expected, the highest transcript levels of MrFPS were observed in blooming flowers (stage 3). Expression of MrFPS in stages 1 and 3 was 1.3- and 1.5-fold higher than that in stage 2, respectively.

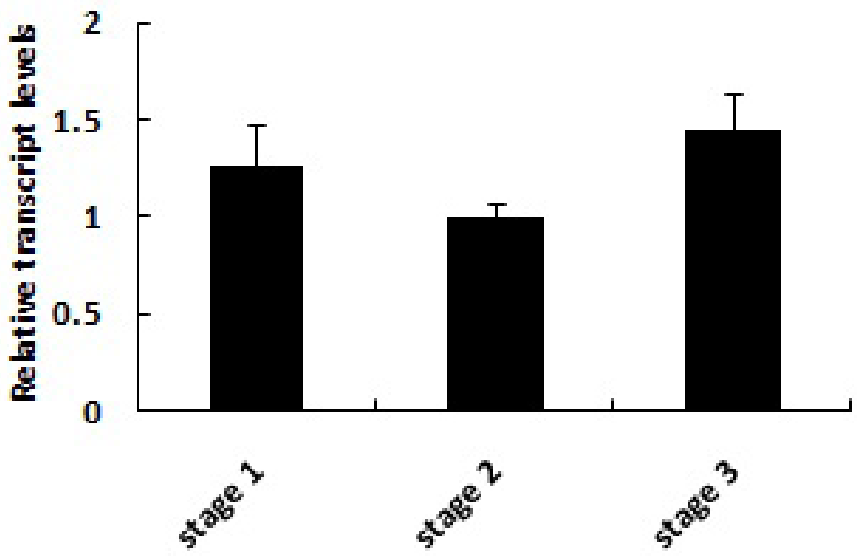

Figure 5. mRNA expression level of MrFPS in *Matricaria recutita* tubular flowers during flower development. MrFPS expression values were normalized to the levels of 18S RNA expression in the respective stages. MrFPS expression in stage 2 was set as 1. All data are reported as means ± SE from three experimental replicates.

The biosynthesis and accumulation of secondary metabolites in plants is closely associated with the transcription level of the relevant genes in organs and tissues as well as in different developmental stages. Specific accumulation of sesquiterpenoids that was consistent with FPS expression levels has been recorded in some plant species, such as *W. somnifera* (Gupta et al., 2011), *C. praecox* (Xiang et al., 2010), and *H. coronarium* (Lan et al., 2013). Therefore, we can speculate that, during flower development, expression levels of the MrFPS gene is correlated with sesquiterpenoid production. Unfortunately, since we did not determine the FPS protein levels in different flower development stages of *M. recutita*, no direct evidence indicates that MrFPS regulates the volatile sesquiterpenes in *M. recutita* flowers. However, as described above, this strong expression of the MrFPS gene might cause specific accumulation of floral volatile sesquiterpenes in the chamomile flowers at the blooming stage. Therefore, MrFPS might indirectly regulate the formation of sesquiterpenoids in chamomile flowers.

Transcriptional expression of MrFPS in response to MeJA treatment

The transcriptional level of the MrFPS gene was studied under 100 μM MeJA induction. Transcription accumulation was obvious in MrFPS gene expression under all MeJA treat-

ments. MrFPS expression was slightly induced (1.6- to 1.9-fold) after treatment with MeJA in a short time (3 to 6 h). However, its expression was sharply induced up to 9.8-fold at 12 h and then declined thereafter (about 3- to 4-fold) compared with the control sample (Figure 6).

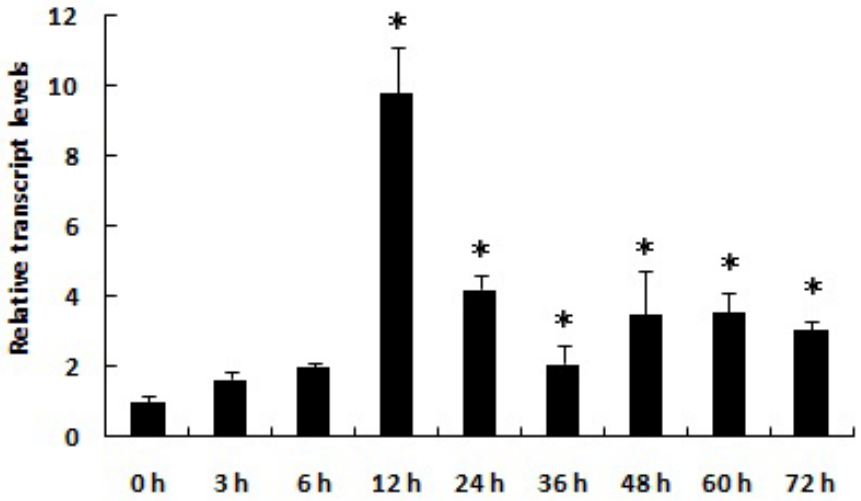

Figure 6. Real-time PCR analysis of MrFPS in MeJA-treated samples. MrFPS expression in the leaves of plants before (0 h) and 3, 6, 12, 24, 36, 48, 60, and 72 h after MeJA treatment was measured through real-time quantitative PCR. Values are reported as means ± SE relative ratio of expression levels compared with the 0-h sample. Groups of data were compared by the Student *t*-test, $P < 0.05$, $N = 3$.

CONCLUSIONS

Plant FPS is a branch-point enzyme that is involved in isoprenoid biosynthesis that synthesizes sesquiterpene precursors for several classes of essential metabolites, including sterols, dolichols, ubiquinones, and carotenoids required in the essential biological processes such as growth, development, reproduction, and adaptation to environmental challenges. This study described the cloning and characterization of a cDNA-encoding FPS from flowers of *M. recutita* for the first time. An expression analysis of different tissues was performed by using real-time quantitative PCR, and the results showed that the MrFPS gene was ubiquitously expressed in all of the examined tissues and highest in the flowers. This finding may explain the molecular basis for the high-sesquiterpene derivatives in the flowers. When the *M. recutita* leaves were treated with 100 μM MeJA, the mRNA expression profile of MrFPS was significantly elevated and reached maximum expression after 12 h. The characterization and expression analysis of MrFPS would not only enhance our understanding of the biosynthesis and regulation of plant secondary metabolites in *M. recutita* but also provide molecular information for biotechnological improvement of this medicinal plant.

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