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Cloning and Expression Analysis of 3-Hydroxy-3-Methylglutaryl-CoA Reductase Gene from *Matricaria chamomilla*

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Abstract

Chamomile (*Matricaria chamomilla* L.) is renowned for its production of essential oils, which major components are sesquiterpenoids. As the important enzyme in the sesquiterpenoid biosynthesis pathway, the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) catelyzes the first committed step of isoprenoids biosynthesis in MVA pathway. In this study, a full-length cDNA encoding HMGR (designated as *McHMGR*, GenBank Accession No. KX925561) was successfully isolated from leaves of *M. chamomilla* by PCR. The full-length cDNA of *McHMGR* was 2276 bp long and contained a 1743 bp open reading frame (ORF) encoding a 580-amino-acid protein. The sequence of the McHMGR protein was highly homologous to those of HMGR proteins from other plant species. Phylogenetic tree analysis indicated that McHMGR shared the same ancestor in evolution with HMGRs and had a further relationship with other Dicotyledoneae species. qRT-PCR analysis revealed that the expression level of *McHMGR* is highest in the flowers and lowest in the stems. The isolation and characterization of *McHMGR* gene will be helpful to further study the role of *McHMGR* gene in the biosynthesis of sesquiterpenoids on *M. chamomilla*.

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Matricaria chamomilla
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Introduction

Chamomile (*Matricaria chamomilla* L.) is one of the most predominant medicinal plants in Europe (Raal et al., 2012; Sayadi et al., 2014). The essential oil of chamomile flowers has significant antiphlogistic, spasmolytic and antimicrobial activity and is therefore used for several pharmaceutical, nutritional and cosmetic applications (Pirzad et al., 2006). *M. chamomilla* flowers contain 0.24-2.0 percent volatile oil that is blue in color. The two key constituents, a-bisabolol and chamazulene, account for 50-65 percent of total volatile oil content. a-bisabolol, a sesquiterpene, has pharmacological actions and calming, carminative, and spasmolytic properties (Murti et al., 2012; Son et al., 2014). In addition, it is a popular ingredient in topical health and beauty products for its soothing and anti-inflammatory effects on skin (Chadwick et al., 2013; Bonifacio et al., 2012; Su et al., 2015).

In nature, isoprenoids are synthesized from just two universal C5 precursors: isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) (Attia et al., 2012; Pitera et al., 2007). Although IPP and DMAPP are the universal precursors for isoprenoid biosynthesis, there are two distinct pathways to biosynthesize isoprenoid in plant kingdom: the cytosolic mevalonate (MVA) pathway and the plastidic 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway

(Vranová et al., 2013; Liu et al., 2006). The MVA pathway starting from 3 acetyl-CoA to finally yield IPP, is responsible for synthesizing sesquiterpenoids and sterols (Miziorko, 2011; Buhaescu and Izzedine, 2007). The MEP pathway producing IPP and DMAPP from pyruvate and D-glyceraldehyde 3-phosphate (GAP) is mainly responsible for forming monoterpenoids, diterpenoids constituents (Zhao et al., 2012).

The 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGR), which catalyzes the conversion of HMG-CoA to MVA, is a rate-limiting enzyme in isoprenoid biosynthesis via MVA pathway (Rui et al., 2012; Dai et al., 2011). In view of its significance in isoprenoid metabolism, genes encoding HMGRs have been isolated and extensively characterized from many plants including *Withania somnifera* (Akhtar et al., 2013), *Coffea arabica* (Pleschberger et al., 2011), *Paris fargesii* (Liang et al., 2014), *Ginkgo biloba* (Liao et al., 2015) and *Camptotheca acuminata* (Burnett et al., 1993). Here, we report for the first time the cloning and characterization of a new functional *HMGR* gene from *M. chamomilla*. The expression profile of *McHMGR* in different organs is also described.

Materials and methods

Plant materials

Matricaria chamomilla was grown at 25/18°C in a controlled growth chamber (16 h light/8 h dark). *M. chamomilla* leaves were collected from botanical garden at Yangtze University, and immediately placed in a -80°C until use. Primer synthesis and DNA sequencing were performed by Shanghai Sangon Biotechnology Company, in China. Agarose Gel DNA extraction Kit Ver 4.0, pMD18-T vector kit, AMV Reverse Transcriptase, dNTPs, RNasin, and Taq DNA polymerase were purchased from Takara Company (Dalian, China).

Cloning the full-length cDNA of *McHMGR*

Total RNA of *M. chamomilla* leaves was isolated using the TaKaRa MiniBEST Plant RNA Extraction kit (Dalian, China) according to the manufacturer's instructions. The extracted RNA was purified using oligo-dT-attached magnetic beads. The purified mRNA was cleaved into small pieces by adding fragmentation buffer. Cleaved mRNAs were used as templates to construct RNA-seq library according to the manufacturer's protocol. The transcriptome sequencing libraries were generated using a NEB Next® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA). Sequencing run was performed at Biomark Biomarker Co., Beijing, China using Illumina Hiseq 2500 platform. The assembled unigenes were searched against the Nr and Nt databases, and the Swiss-Prot protein and COG/KOG databases using BLAST with an cutoff E-value of $1e^{-5}$. To assign functional annotations, the unigenes were searched against Pfam using HMMER 3.0 (Finn et al., 2011) with E-value of $1e^{-2}$. Based on the annotation information of the unigenes of *M. chamomilla*, a pair of specific primers (McHMGRu: 5'-ATGGATGTCCGACGAGGATC-3' and McHMGRd: 5'-TCCACCAGAGATCTTGCTTCC-3') were designed and synthesized according to the sequence of HMGR unigene. Single strand cDNA was synthesized using PrimeScript cDNA Synthesis Kit (TaKaRa, Japan). McHMGR cDNA was amplified with the one-step RT-PCR kit (Dalian TaKaRa, Dalian, China) under the following conditions: 94°C for 3 min, followed by 32 cycles of amplification at 94°C for 1 min, 54°C for 30 s, and 72°C for 1 min; and extension for 10 min at 72°C. The PCR product was purified and cloned into the pMD18-T vector, and transformed into *Escherichia coli* strain DH5a followed by sequencing.

Bioinformatics and molecular evolution analysis

Sequence assembly was performed with programs of DNAstar (http://www.dnastar.com). The nucleotide sequence, deduced amino acid sequence and open reading frame (ORF) were analyzed, and the sequence comparison was conducted through database search (http://www.ncbi.nlm.nih.gov). The calculated isoelectric point (pI) and molecular weight of the McHMGR protein were computed with the software of Compute pI/Mw Tool at http://web.expasy.org/compute_pi/. Multiple sequence alignment was performed with the software Vector NTI 11.5 program. Phylogenetic analysis of McHMGR and other HMGR from other plants were aligned with CLUSTALX 2, and subsequently, a phylogenetic tree was constructed by the neighbor-joining (NJ) method with MEGA 6 software (Kumar et al., 2001).

Differential expression analysis of *McHMGR* by qRT-PCR

The expression level of McHMGR was determined by real-time PCR (qRT-PCR). Aliquots of 1 μg total RNA was used as the template for qRT-PCR. qRT-PCR was performed using a Bio-Rad Mini Opticon™ Real-time PCR Mini Cycler (BioRad, Hercules, CA, USA) with SYBR Premix Ex Taq™ II Kit (Dalian TaKaRa) according to the

method of Xu et al. (2014). The primers for McHMGR (McHMGRu: 5'-GCTTCACAATCTGCTTGCCTAA-3', McHMGRd: 5'-TTGACGAGTTGTCCTGCTGAAA-3') and referential gene 18S rRNA gene (18SU: 5'-ACCGAGCGTCGAGTGGATTAA-3' and 18SD: 5'-CTAGTTCGTGCGTCCGTCAAA-3') were designed using the Sequence Detection System software. Raw data were analyzed with MiniOpticon™ Real-time PCR Detection system, and expression level was normalized into 18S gene to minimize the variation in the cDNA template levels. qRT-PCR data were technically replicated with error bars, representing mean ± SD (n = 3). The relative expression fold of each sample was calculated by its Ct value normalized to the Ct-value of reference gene using the $2^{-\Delta\Delta Ct}$ method described by Livak and Schmittgen (2001).

Results

Cloning and characterization of the full-length cDNA of *McHMGR* gene

A total of 82,946 unigenes have been identified in *M. chamomilla* RNA-Seq database through annotation against public protein databases (data not published). Among these unigenes, one HMGR member was identified based on the annotation information. Using a pair of specific primers based on the HMGR unigene (Gene ID: McBMK_36655) of transcriptome data, we performed PCR using cDNA as the template, and a 2276-bp fragment was amplified. Using an RT-PCR method, a cDNA fragment encoding *HMGR*, designated as *McHMGR* (GenBank Accession No. KX925561), was isolated and characterized. The nucleotide sequence of *McHMGR* had high similarity with *HMGR* genes of other plants (Table 1). The results indicate that the gene we cloned is a member of the *HMGR* gene family. As shown in Fig. 1, *McHMGR* cDNA contained a 1743 bp open reading frame (ORF) encoding a 580 amino acid protein.

Table 1. Nucleotide sequence of *McHMGR* similarity to the *HMGR* genes of other plant species.

Species	Accession no.	Homology (%)
Taraxacum kok-saghyz	HQ857601.1	81
Ligularia fischeri	DQ916106.1	84
Camellia sinensis	KF649853.1	78
Prunus mume	XM_008237609.2	77
Gossypium hirsutum	XM_016860153.1	79
Glycine max	XM_003534178.3	77

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1       CTAACTGTATACAAGATTCAAATCAAGCGTGTAGTTATCCATATACCTATAAATAAAGCC
61      ACCATATTTACAACCATTTTGCAAATAAGCTACTTTACAAATCCCATTATCTGTATTCGT
121     TCATCCTCAACGAACTCGTCTTCGGCTCCATCCCATCTACCCAGTAGTACTACCGTAGCA
181     AAAGGGATGGATGTCCGACGAGGATCTTCGTTGAAACCTTCTTTTAAGAATCATGTTTCA
61            M  D  V  R  R  G  S  S  L  K  P  S  F  K  N  H  V  S
241     GATGATAATAAGATGTTTGAAGCCAAGGACAAAAAAGTGGTGGACGGTTCTGTCCCACTT
81       D  D  N  K  M  F  E  A  K  D  K  K  V  V  D  G  S  V  P  L
301     TCTTTAGGGATTTCAAATGGTGTTTTCTTTACTGTTTTCTTTTCGGTTACGTATTTTCTT
101      S  L  G  I  S  N  G  V  F  F  T  V  F  F  S  V  T  Y  F  L
361     CTTACTAGATGGCGTGAAAAGATCCGTAACTCGACTCCACTCCATGTTGTCACCATGTCG
121      L  T  R  W  R  E  K  I  R  N  S  T  P  L  H  V  V  T  M  S
421     GAGATCGCTGCAATCTTCTTGTTTGTGGCGTCGTTTATCTACCTTATTGGCTTCTTTGGG
141      E  I  A  A  I  F  L  F  V  A  S  F  I  Y  L  I  G  F  F  G
481     ATGAGTTTTGTTAACCCTACGCCTTATTCAGACGATGAAGAAGAAGAGGAGATAGAAGGT
161      M  S  F  V  N  P  T  P  Y  S  D  D  E  E  E  E  E  I  E  G
541     GACGTGAACGAAATTGTGCGTAAGGAAGACACTCGTGTGACCTCTTGTGGTGCAGCCCTA
181      D  V  N  E  I  V  R  K  E  D  T  R  V  T  S  C  G  A  A  L
601     GACTGTGAGTCAGATGTGGTCGTAAAACACGTTATAAAGAAAGACTTGGAGCATTTACCA
201      D  C  E  S  D  V  V  V  K  H  V  I  K  K  D  L  E  H  L  P
661     ACTGAAACAGTTCTTACGGAAGAGGATGAGGAGGTAATAAAAGCGGTTGTTTCGGGTAAA
221      T  E  T  V  L  T  E  E  D  E  E  V  I  K  A  V  V  S  G  K
721     ACACCATCTTACTCGTTGGAATCCAAGCTTGGGGATTGTAAACGTGCCGCTTTCATAAGG
241      T  P  S  Y  S  L  E  S  K  L  G  D  C  K  R  A  A  F  I  R
781     CGAGTAGCCCTGGAGAGGATTACTGGGAAATCTCTTGATGGTTTGCCTCTAGAAGGGTTT
261      R  V  A  L  E  R  I  T  G  K  S  L  D  G  L  P  L  E  G  F
841     GATTACGAATCGATATTGGGACAGTGTTGTGAGATGCCAGTTGGTTATGTTCAAATACCC
281      D  Y  E  S  I  L  G  Q  C  C  E  M  P  V  G  Y  V  Q  I  P
901     GTTGGTATTGCTGGTCCGATGTTGTTGGATGGACAGGAGTTTACCGTGCCCATGGCGACT
301      V  G  I  A  G  P  M  L  L  D  G  Q  E  F  T  V  P  M  A  T
961     ACTGAAGGGTGTCTTGTGGCTAGTACCAATAGGGGTTGTAAGGCCATTTATGTATCTGGT
321      T  E  G  C  L  V  A  S  T  N  R  G  C  K  A  I  Y  V  S  G
1021    GGTGCAACTAGTGTCCTACTTAAAGATGGCATGACTCGAGCTCCGGTTGTTAGGTTTGGA
341      G  A  T  S  V  L  L  K  D  G  M  T  R  A  P  V  V  R  F  G
1081    ACCGCGAAGAGGGCTGCTGATTTGAAGTTCTTCTTGGAGGAACCACTCAACTTTGATACA
361      T  A  K  R  A  A  D  L  K  F  F  L  E  E  P  L  N  F  D  T
1141    CTTGCATCTGTTTTCAACAAATCAAGCCGATTTGGGAGGCTTCAGAGAATTCAATGTGCG
381      L  A  S  V  F  N  K  S  S  R  F  G  R  L  Q  R  I  Q  C  A
1201    ATAGCCGGGAAGAATCTGTACGTAAGGTTTACATGCAGCACTGGTGATGCAATGGGGATG
401      I  A  G  K  N  L  Y  V  R  F  T  C  S  T  G  D  A  M  G  M
1261    AACATGGTTTCCAAAGGTGTTCAAAATGTTCTAGACTATCTCCAAGCTGATTTCCCCGAC
421      N  M  V  S  K  G  V  Q  N  V  L  D  Y  L  Q  A  D  F  P  D
1321    ATGGACGTCATTGGCATATCTGGAAACTATTGTTCGGATAAGAAACCGGCAGCGGTGAAT
441      M  D  V  I  G  I  S  G  N  Y  C  S  D  K  K  P  A  A  V  N
1381    TGGATAGAAGGGAGAGGTAAATCAGTGGTGTGTGAGGCAATCATAAAGGAAGAAATAGTG
461      W  I  E  G  R  G  K  S  V  V  C  E  A  I  I  K  E  E  I  V
1441    AAAAAGGTATTGAAAACAACTGTAGCTTCCTTGGTCGAACTGAACATGCTCAAGAACCTC
481      K  K  V  L  K  T  T  V  A  S  L  V  E  L  N  M  L  K  N  L
1501    ACGGGATCCGCTATGGCTGGTGCTCTTGGTGGCTTCAACGCGCATGCAAGTAACATCGTG
501      T  G  S  A  M  A  G  A  L  G  G  F  N  A  H  A  S  N  I  V
1561    TCAGCTGTGTTCCTTGCTACTGGACAAGATCCAGCTCAGAACGTCGAAAGCTCTCATTGT
521      S  A  V  F  L  A  T  G  Q  D  P  A  Q  N  V  E  S  S  H  C
1621    ATCACCATGATGGAAGCTGTGAACGATGGCAAAGACCTTCACGTGTCTGTGACCATGCCA
541      I  T  M  M  E  A  V  N  D  G  K  D  L  H  V  S  V  T  M  P
1681    TCAATTGAGGTTGGGACTGTGGGTGGTGGAACTCAGTTGGCTTCACAATCTGCTTGCCTA
561      S  I  E  V  G  T  V  G  G  G  T  Q  L  A  S  Q  S  A  C  L
1741    AACTTGTTGGGAGTGAAGGGTGCAAACAAAGAACTTGCTGGATCAAATGCCCGACAATTG
581      N  L  L  G  V  K  G  A  N  K  E  L  A  G  S  N  A  R  Q  L
1801    GCTAAGGTTGTCGCAGCCGCAGTTCTAGCTGGAGAGTTATCTCTCATGTCGGCAATTTCA
601      A  K  V  V  A  A  A  V  L  A  G  E  L  S  L  M  S  A  I  S
1861    GCAGGACAACTCGTCAAAAGTCACATGAAATACAACCGCTCCACCAGAGATCTTGCTTCC
621      A  G  Q  L  V  K  S  H  M  K  Y  N  R  S  T  R  D  L  A  S
1921    AAGGCTTAAGCTTCTCTCGATTTGTTTGTTGTCATATTGGAGGGCAGCTATCAGTTATTC
641      K  A  *
1981    TTGTTTCTACTGGTCTTTTTGAGCTCTTGTTCTTCCACTGATCTTCGTTCAGCTCTTGGT
2041    TGTGAGCAATTCGAGTTTAAGGTTCAAATATATACTATACCTTAAGTATTTGAAGTGCAT
2101    GTTAAGCATTTGGTATATGAATAATAAAGTGTTTAATAATTAGCAATACGTGTGATTGCG
2161    ATTGTAAATGACTATCAAAATCAAGTATTGTCGCACATTAAGCTCTAATTTGGTCCCATT
2221    TTTGTAGTCTAATCTACAAATAGGCGTAAAAGATATACAAAATTGATGGTTTCAAG
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Fig. 1: The nucleotide acid sequence and deduced amino acid sequence of *McHMGR*. The underline for specific primers McHMGRu and McHMGRd.

Characterization of the deduced McHMGR protein

By using the software of Compute pI/Mw Tool, the calculated isoelectric point (pI) and molecular weight of the McHMGR were predicted to be 5.90 and 62.4 kDa,

respectively. A database search with BlastP and multi-alignment by Vector NTI 11.5, results showed that McHMGR exhibited high similarity to HMGR proteins from other plants (Fig. 2). The deduced McHMGR protein sequence showed 83, 86, 75, 75, 75 and 77 % identities to the counterparts of *Cynara cardunculus* var. *scolymus*, *Taraxacum kok-saqhyz*, *Sesamum indicum*, *Gossypium raimondii*, *Prunus meme*, *Ricinus communis*, thereby indicating that McHMGR belongs to plant HMGR superfamily.

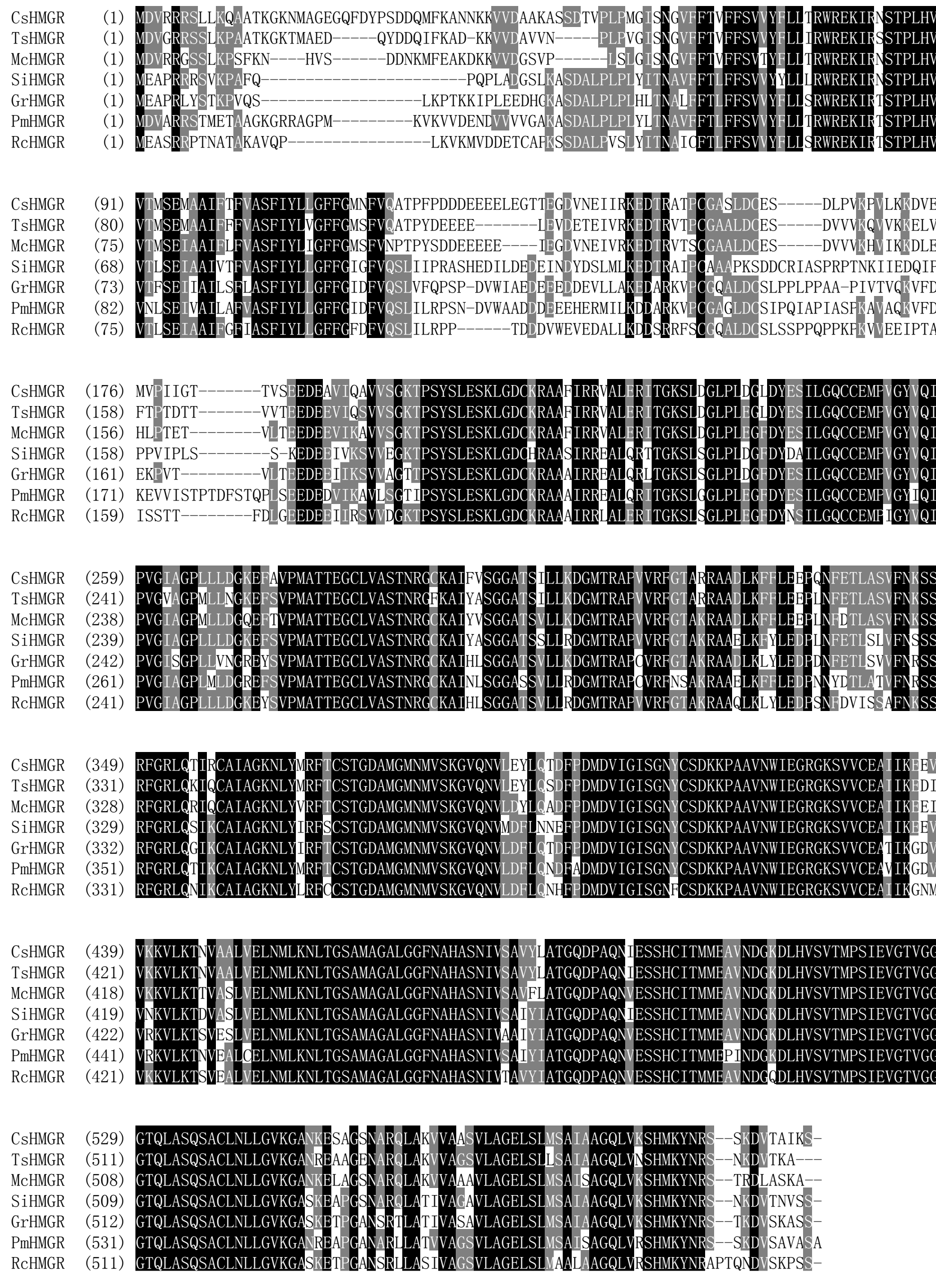

Fig. 2: Multiple alignment of amino acid sequences of McHMGR along with the selected HMG-CoA reductases. The completely identical amino acids are indicated with white foreground and black background. The conserved amino acids are indicated with black foreground and grey background. Non-similar amino acids are indicated with black foreground and white background. The species, protein names and GenBank accession numbers are following: *Cynara cardunculus* var. *scolymus*: CsHMGR (KVI04181.1); *Taraxacum kok-saqhyz*: TsHMGR (AEA92686.1); *Sesamum indicum*: SiHMGR (XP_011092919.1); *Gossypium raimondii*: GrHMGR (XP_012479905.1); *Prunus meme*: pmHMGR (XP_008235831.1); *Ricinus communis*: RcHMGR (XP_002514400.1).

Molecular evolution analysis

To investigate the evolutionary relationships among McHMGR and HMGR proteins from other plants, the phylogenetic tree was constructed using neighbor-jointing method. As shown in Fig. 3, McHMGR belonged to Dicotyledoneae in the branch of Dicotyledoneae, Monocotyledoneae and Gymnospermae. The McHMGR protein has the closest relationship to LfHMGR and TsHMGR of Asteraceae. The results suggest that McHMGR shares a common evolutionary with other plant HMGR proteins based on conserved structure and sequence characteristics, such as amino acid homologies and conserved motifs.

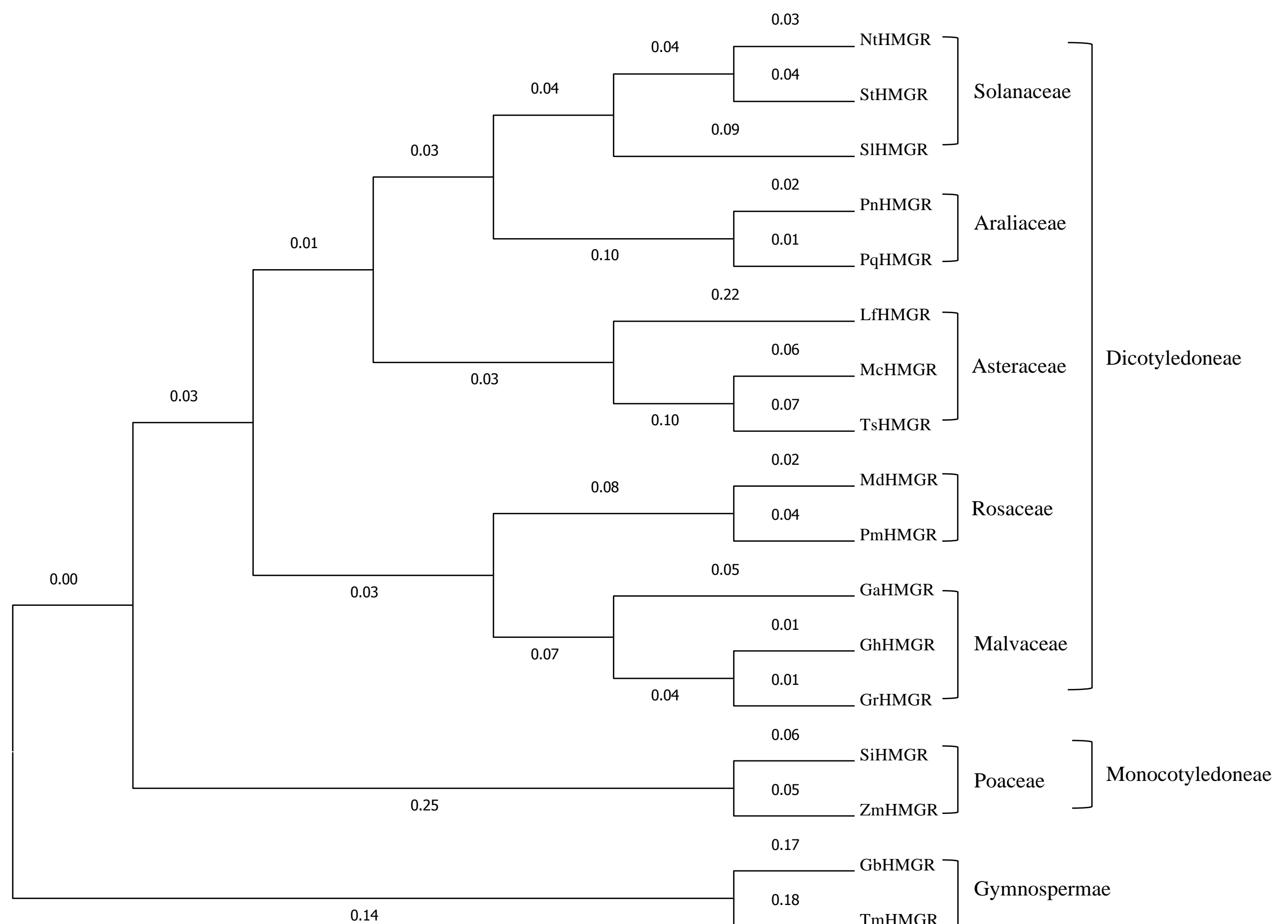

Fig. 3: Phylogenetic tree of HMGR from different species using the Neighbor-joining method. The numbers of nodes represent the percentage of boot strap value obtained from 1000 sampling. *Bar* 0.01 shows the substitutions per nucleotide position. The species, protein names and GenBank accession number are as following: *Solanum lycopersicum*: SlHMGR (NP_001234846); *Nicotiana tabacum*: NtHMGR (XP_016447095.1); *Solanum tuberosum*: StHMGR (NP_001275084.1); *Panax notoginseng*: PnHMGR (AKP55621.1); *Panax quinquefolius*: PqHMGR (NP_001234846); *Ligularia fischeri*: LfHMGR (ABJ16394.1); *Taraxacum kok-saghyz*: TsHMGR (AEA92686.1); *Malus domestica*: MdHMGR (XP_008348952.1); *Prunus mume*: PmHMGR (XP_008235831.1); *Gossypium arboreum*: GaHMGR (XP_017636398.1); *Gossypium hirsutum*: GhHMGR (XP_016728010.1); *Gossypium raimondii*: GrHMGR (XP_012479904.1); *Setaria italica*: SiHMGR (XP_004957395.1); *Zea mays*: ZmHMGR (CAA70440.1); *Ginkgo biloba*: GbHMGR (AAU89123.1); *Taxus* x *media*: TmHMGR (AAQ82685.1).

Expression analysis of *HMGR* in different tissue of *Matricaria chamomilla*

The expression analysis revealed that *McHMGR* is ubiquitously expressed in all four different tissues tested but with a strong expression in flowers. Stems recorded the lowest expression level among all the tissue tested (Fig. 4). This result suggested that flowers may a vital tissue for sesquiterpenoid biosynthesis in *M. chamomilla*.

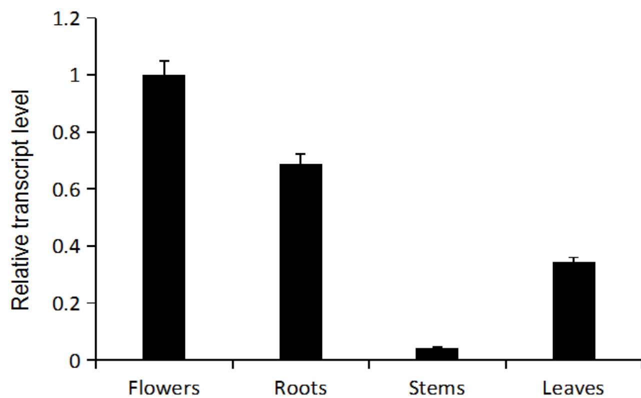

Fig. 4: Real-time PCR for *McHMGR* gene expression analysis among different tissues of *Matricaria chamomilla.*

Discussion

In recent years, there has been a remarkable progress in the understanding of the molecular regulation of isoprenoid biosynthesis in plant (Shen et al., 2006; Wang et al., 2007). 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) plays an important role in catalyzing the first committed step of isoprenoids biosynthesis in MVA pathway (Chen et al., 2012). Thus, we attempted to examine the molecular biology of sesquiterpenoid biosynthesis pathways in *M. chamomilla* by cloning and characterization of the full-length cDNA of *McHMGR*.

In this study, we reported the molecular characterization of *HMGR* gene from *M. chamomilla* for the first time. McHMGR is highly similar to HMGR sequences from other species. Multiple sequence alignment analysis of McHMGR also confirmed structural and functional conservation when compared with other plant HMGRs. The phylogenetic tree was constructed considering the HMGRs from plant species other than Asteraceae family to elucidate the evolutionary relationships among the HMGRs which revealed that McHMGR is more distinct and ancient in terms of evolutionary relationship than HMGRs of other plant species.

M. chamomilla is a widely used medicinal plant with great exploitative value for its α-bisabolol (Sayadi et al., 2014). *M. chamomilla* flower is the most commonly used plant part for medicinal purposes (Irmisch et al., 2012). Besides flowers, roots, stems and leaves are also rich in essential oil containing chamazulene, α-bisabolol oxide B and α-bisabolol oxide A (Hajjaj et al., 2013; Formisano et al., 2015; Roby et al., 2013). Therefore, it is interesting to investigate whether or not *McHMGR* transcript is possibly correlated with the accumulation of the active compounds in different tissues of *M. chamomilla*. Several studies showed that the expression pattern of *HMGR* in plant tissues greatly varies across different plants. For example, *HMGR* is mainly expressed in the node, leaf and stem but is seldom expressed in the root of *Centella asiatica* (Kalita et al., 2015). However, *HMGR* exhibited the highest expression level in the leaf and lowest expression level in the stem of *Panax quinquefolius* (Wu et al., 2012). The expression pattern of *McHMGR* in *M. chamomilla* revealed that the gene is expressed in all tissues, but is expressed at higher levels in the flowers. This trend is consistent with the chemical constituent analysis from earlier reports (Farhoudi, 2013), which showed that significant higher contents of the major sesquiterpenoids were observed in the flowers than other parts of *M. chamomilla*.

Conclusion

In conclusion, the cloning of *McHMGR* provides a foundation for further studies of the biosynthesis of sesquiterpenoids in *M. chamomilla*. In this present investigation, we have successfully cloned and characterized the gene encoding *HMGR* involved in the biosynthesis of α-bisabolol, known as sesquiterpenoids in *M. chamomilla*. Meanwhile, McHMGR is also a committed enzyme in the biosynthetic pathway engineering of active sesquiterpenoids in *M. chamomilla*. Further research will be necessary to clarify the process of catalysis in plants to fully understand the biosynthesis of metabolites. And other relevant genes involved in sesquiterpenoids synthesis in *M. chamomilla* need to be detected.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

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