Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
Impact Factor (GIF): 0.615
Impact Factor (SJIF): 2.092
June-August 2015; 3(2): 272-278
Available online at http://www.jpcbs.info

Original Research Article

Cloning and Sequence Analysis of a Terpene Synthase Gene (*McTPS3*) from *Matricaria chamomilia*

Xiaomeng Liu, Li Zhu, Tingting Tao, Qiling Song, Weiwei Zhang, Qiangwen Chen, Feng Xu*

College of Horticulture and Gardening, Yangtze University, Jingzhou, 434025, China

***Corresponding Author:** Feng Xu, College of Horticulture and Gardening, Yangtze University, Jingzhou, 434025, China

Received: 25 July 2015 Revised: 02 August 2015 Accepted: 06 August 2015

ABSTRACT

Terpene synthases are the primary enzymes in the biosynthesis of terpene metabolites. Here a terpene synthase gene, designated as *McTPS3*, was cloned from *Matricaria chamomilia* based on EST sequence by RT-PCR. The *McTPS3* cDNA contains a 1689 bp length ORF encoding 563 amino acid protein. The sequence of McTPS3 protein was highly homologous to those of TPS proteins from other plant species. The predicted isoelectric point and molecular weight of McTPS3 protein are 5.01 and 63.9 kDa respectively. Phylogenetic analysis indicated that McTPS3 has closer relationship with TPSs from other compositae plants than other plants. The cloning and sequence analysis of *MrTPS3* provided foundation for further stud the role of this gene in the biosynthesis of terpenoids in *M. chamomilia*.

Keyword: *Matricaria chamomilia*; *McTPS3*; cloning; sequence analysis

INTRODUCTION

Terpene products have important biological functions in plants. Terpene metabolites are not only essential for plant growth and development (e.g. gibberellin phytohormones) but also represent important tools in the various interactions of plants with the environment [1]. Volatile and non-volatile terpenes are implicated in the attraction of both pollinators and predators of herbivores, in protection against photooxidative stress, in mediating thermo tolerance, and in direct defense against microbes and insects [2]. Nowadays human has used terpenes that are extracted from plants for many different purposes as fragrances and masque, as pharmaceutical agents and as insecticides [3]. Thus, it is important to improve their commercial value.

Chamomile *(Matricaria chamomilla* L.) is one of the most important herbal medicine plants [4]. Analysis of extracts from chamomile revealed that high presence of sequiterpene derivatives including chamazulene, terpene alcohol, (E)-β-farnesene, α-bisabolol and α-bisabolol oxides A, which are used for pharmaceutical, nutritional and cosmetic applications [5-9]. Therefore, increase in the terpenoid content of chamomile can significantly improve the quality and economic value of chamomile. Previous studies show that the use of genetic engineering technology to enhance the expression of key genes of secondary metabolism is an effective way to increase the desired products in plant [10-12]. Therefore, it is an effective strategy to enhance the content of terpenoid in chamomile using genetic engineering to up-regulate the expression of key genes involved in biosynthesis pathway of sequiterpene. Son et al has screened six terpene synthase (TPS) genes from the transcriptome database of chamomile, and clarified the functions of these six genes in formation of sesquiterpenoid a-bisabolol [13]. However, the diversity of plant terpenes are mainly caused by the TPS species diversity, there may exist a variety of *TPS* genes in chamomile. As a key enzyme in the biosynthesis of terpenoids, TPS has become one of the most in-depth study enzymes in terpene biosynthetic pathway. In this study, we cloned a *TPS* gene (*McTPS3*) from chamomile. The sequences analysis including the alignment of amino acid predicted from *McTPS3* cDNA and the evolutional relationship are also presented ,which will be useful for unveiling biosynthesis pathway of the terpenoids and providing the gene resource for improving the content of terpenoids in chamomile.

MATERIAL AND METHODS

Plant materials and reagents

M. chamomilia was grown in the botanical garden at Yangtze University, the flowers were collected, immediately frozen in liquid nitrogen, and kept at freezer (at -80°C) prior to RNA extraction. Both the primers synthesis and DNA sequencing were performed by Shanghai Sangon Biotechnology Company, in China. Agarose Gel DNA purifieation Kit Ver.4.0, pMD18-T vector kit, AMV Reverse Transcriptase, dNTPs, RNasin and *Taq* DNA polymerase were purchased from Takara, Dalian, in China.

RNA extraction and isolation of *McTPS3*

Total RNA was isolated from frozen plant tissues using the TaKaRa MiniBEST Plant RNA Extraction kit (Dalian). The specific primer McTPS3-F (5'-ATGGCAGCCATTCAAGCTAATGTG-3') and reverse primer McTPS3-R (5'-TCACATGGGTAGAGAACCCACAAA-3') were designed according to the EST sequence of chamomile *TPS* gene. One-step reverse transcription PCR (RT-PCR) was performed using the one-step RT-PCR kit (Dalian TaKaRa, China) under the following conditions: 50 °C for 30 min and 94 °C for 3 min, followed by 32 cycles of amplification at 94 °C for 1 min, 48 °C for 30 s, and 72 °C for 1 min; followed by an extension for 10 min at 72 °C. The PCR product was purified, cloned into the pMD18-T vector, and then sequenced by Shanghai Sangon Biotechnology Company.

Bioinformatic analysis

Sequence assembly was performed with programs of DNAstar (http://www.dnastar.com). Protein and DNA homology searches were performed by using TBLASTN, TBLASTX, BLASTP and BLASTN programs (http://www.ncbi.nlm.nih.gov/BLAST/).

Multiple sequence alignment was performed with the software Vector NTI 11.5 program. Phylogenetic analysis of *McTPS3* from *M. chamomilia* and TPSs from other plants was performed by using software CLUSTAL X 2 and MEGA 6 with the neighbor-joining (NJ) method [14].

RESULTS

Cloning of the cDNA fragement of *McTPS3*

Using One-step reverse transcription PCR method, a cDNA fragment of McTPS3 gene was finally obtained from M. chamomilia. The length of McTPS3 cDNA is 1692 bp, contained G/C content of 40%, and encoded a 563 amino acids protein (Fig. 1). The cDNA sequence of McTPS3 had high similarity with other TPS genes. As shown in Table 1, the nucleotide sequence of McTPS3 was 94%, 93%, 93%, 91%, 91%, 86%, 85%, 85% identical to TPS gene from *Tanacetum cinerariifolium*, *Artemisia annua*, *Tanacetum parthenium, Artemisia absinthium, Artemisia annua, Cichorium intybus, Lactuca sativa, and Helianthus annuas*, Indicating that McTPS3 was one member of TPS gene family in *M. chamomilia*. Furthermore, the homologous sequence of TPS genes among different plant species showed that TPS genes might keep a strong conservation during the molecular evolution [15].

Table 1: Nucleotide sequence of *McTPS3* similarity to the TPS genes of other plant

Species	GenBank No.	Homology	E-value
Tanacetum cinerariifolium	AGO03788.1	94	0.0
Artemisia annua	BAN81913.1	93	0.0
Tanacetum parthenium	AEH41844.1	93	0.0
Artemisia absinthium	BAN81912.1	91	0.0
Artemisia annua	AFK93531.1	91	0.0
Cichorium intybus	Q8LSC2.1	86	0.0
Lactuca sativa	AAM11626.1	85	0.0
Helianthus annuas	ACZ50512.1	85	0.0

Characterization of the deduced McTPS3 protein

The deduced amino acid sequence for McTPS3 polypeptide was shown in Fig. 1. Using BLastx, and online website (http://web.expasy.org/protparam/) for protein sequence analysis showed the isoelectric point and molecular weight of McTPS3 protein are 5.01, and 63.9 kDa, respecitively. A database search with BlastP (NCBI) and multialignment by Vector NTI 10.0 showed that the McTPS3 protein had high similarity with TPSs form other plant species (Fig. 2). The amino acid sequence of McTPS3 was 93%, 92%, 92%, 91%, 86%, 85%, 85% similarity to TPSs from *Tanacetum cinerariifolium, Artemisia annua, Tanacetum parthenium, Artemisia absinthium, Cichorium intybus, Lactuca sativa, Helianthus annuas,* respectively. Multiple sequence comparison analysis by DNAMAN showed McTPS3 protein had high similarity with other TPSs from other plant species (Fig.2). All the results mentioned above suggested that McTPS3 was a member of TPS family

Molecular evolution analysis of the McTPS3

In order to analyze the evolutionary relationships among McTPS3 and other TPS proteins, a phylogenetic tree was constructed based on the predicted McTPS3 amino acid sequences and other plant species TPS proteins selected from the GenBank. The phylogenetic tree was constructed by using Neighbor-Joining (NJ) method with Clustal X 2.0 and MEGA 6.0 software. As showed in Fig. 3, the evolutionary tree was divided into two distinct categories.

The results highlighted all plants derived from a common ancester in the evolution using TPS as outgroup, no matter whether they belonged to the xylophyta or herb plants.

```
1     ATGGCAGCCATTCAAGCTAATGTGACTACTGGTATCCCAGCAAACGCAAACACCATAACCTCCTCGGAGCCA
1      M  A  A  I  Q  A  N  V  T  T  G  I  P  A  N  A  N  T  I  T  S  S  E  P
73    GTGCGTCCGTTGGCCAACTTTCCTCCTTCAGTATGGGGTGATCGCTTCCTTTCATTCTCTCTTGACAAATCG
25     V  R  P  L  A  N  F  P  P  S  V  W  G  D  R  F  L  S  F  S  L  D  K  S
145   GAATTGGAGCGACATGCCATAGCTATGGAGAAGCCGAAGGAGGATTTGAGAAAGTTGATCGTGGATCCAACA
49     E  L  E  R  H  A  I  A  M  E  K  P  K  E  D  L  R  K  L  I  V  D  P  T
217   ATGGATTCAAATGAGAAACTAGGCTTGATTTATTCTGTACATCGTCTCGGTTTGACATATATGTTCATGAAA
73     M  D  S  N  E  K  L  G  L  I  Y  S  V  H  R  L  G  L  T  Y  M  F  M  K
289   GAGATCGAAAGCCAGCTTGACAAGCTTTTCAAGGAGTTTAGCTTGCAAGATTGTGAAGAAGTTGATCTATAC
97     E  I  E  S  Q  L  D  K  L  F  K  E  F  S  L  Q  D  C  E  E  V  D  L  Y
361   ACTATTTCGATTAACTTTCAAGTTTTTCGACACCTTGGTTACAAACTACCTTCTGATGTCTTTAACAAATTC
121    T  I  S  I  N  F  Q  V  F  R  H  L  G  Y  K  L  P  S  D  V  F  N  K  F
433   AAGGATGCTAGCTCGGGTACTTTCAGGGAATCCATTACGAGGGATGTGAAGGGTATGTTGGGCTTATATGAA
145    K  D  A  S  S  G  T  F  R  E  S  I  T  R  D  V  K  G  M  L  G  L  Y  E
505   TCTGCACAGTTGAGAACAAGAGGAGAAAAAGTTCTTGATGAAGCCTCGGTTTTCATCGAAGGTAAACTCAAG
169    S  A  Q  L  R  T  R  G  E  K  V  L  D  E  A  S  V  F  I  E  G  K  L  K
577   AGTGTAGTAAGCACTCTTGAAGGTAACCTTGCACAACAAGTGAAACAATCTTTAAGGAGACCATTTCATCAA
193    S  V  V  S  T  L  E  G  N  L  A  Q  Q  V  K  Q  S  L  R  R  P  F  H  Q
649   GGGATGCCAATGATAGAGGCAAGGCTATATTTCTCTAACTATGAAGAAGAATGTTCCTCACACGACTCGCTA
217    G  M  P  M  I  E  A  R  L  Y  F  S  N  Y  E  E  E  C  S  S  H  D  S  L
721   TTTAAGCTTGCAAAGCTACACTTCAAGTATTTAGAGCTACAGCAAAAAGAAGAACTTAGAATTGTAACAAAG
241    F  K  L  A  K  L  H  F  K  Y  L  E  L  Q  Q  K  E  E  L  R  I  V  T  K
793   TGGTGGAAGGATATGAGGTTTCAGGAGACTACTCCTTATATAAGGGATAGAGTACCAGAGATTTACTTATGG
265    W  W  K  D  M  R  F  Q  E  T  T  P  Y  I  R  D  R  V  P  E  I  Y  L  W
865   ATATTGGGATTATACTTTGAGCCTCGTTACTCCTTGGCAAGAATCATCGCAACAAAAATAACATTGTTTCTC
289    I  L  G  L  Y  F  E  P  R  Y  S  L  A  R  I  I  A  T  K  I  T  L  F  L
937   GTCGTTTTAGATGACACATATGATGCTTATGCTACCATTGAAGAGATTCGCCTTCTAACAGATGCGATAAAC
313    V  V  L  D  D  T  Y  D  A  Y  A  T  I  E  E  I  R  L  L  T  D  A  I  N
1009  AAGTGGGATATTAGTGCTATGGAGCAAATTCCGGAGTATATTAGACCATTTTACAAAATTCTTTAGATGAA
337    K  W  D  I  S  A  M  E  Q  I  P  E  Y  I  R  P  F  Y  K  I  L  L  D  E
1081  TATGCTGAAATTGAGAATATAATGGCTAGAGAAGGGAGAGCAAATACTGTTATTGCTTCAAAAGAAGCTTTT
361    Y  A  E  I  E  N  I  M  A  R  E  G  R  A  N  T  V  I  A  S  K  E  A  F
1153  CAAGACATAGCCAGAGGTTACCTTGAAGAAGCTGAATGGACAAACAATGGATATGTGGCATCATTTCCGGAG
385    Q  D  I  A  R  G  Y  L  E  E  A  E  W  T  N  N  G  Y  V  A  S  F  P  E
1225  TATATGAAGAACGGATTAATCACTTCCGCTTATAATGTCATTTCAAAATCGGCTTTAGTTGGTATGGGTGAG
409    Y  M  K  N  G  L  I  T  S  A  Y  N  V  I  S  K  S  A  L  V  G  M  G  E
1297  ATCGTGAGTGAGGATGCTTTGGCTTGGTATGAAAGTCATCTAAAGACATTGCAAGCTTCCGAGTTGATTTCA
433    I  V  S  E  D  A  L  A  W  Y  E  S  H  L  K  T  L  Q  A  S  E  L  I  S
1369  AGACTTCAAGACGATGTCATGACTTACCAGTTTGAGCGTGAAAGAGGACAATCGGCCACTGGCGTTGATGCA
457    R  L  Q  D  D  V  M  T  Y  Q  F  E  R  E  R  G  Q  S  A  T  G  V  D  A
1441  TTTATCAAGACTTATGGCGTATCAGAAAAGAAAGCTATAGACGAGCTCAAGATCATGATTGAAAATGCATGG
481    F  I  K  T  Y  G  V  S  E  K  K  A  I  D  E  L  K  I  M  I  E  N  A  W
1513  AAAGATATAAACGAAGGATGTCTAAAGCCAAGACAAGTCTCAATGGATTTGCTTGCCCCAATTCTTAACCTT
505    K  D  I  N  E  G  C  L  K  P  R  Q  V  S  M  D  L  L  A  P  I  L  N  L
1585  GCAAGGATGATAGATGTGGTATATAGGTACGACGACGGGTTCACTTTTCCAGGAAAAACTCTCAAAGAGTAT
529    A  R  M  I  D  V  V  Y  R  Y  D  D  G  F  T  F  P  G  K  T  L  K  E  Y
1657  ATCAATCTTTTGTTTGTGGGTTCTCTACCCATGTGA
553    I  N  L  L  F  V  G  S  L  P  M  *
```

Fig. 1: Nucleotide sequence and deducde amino acid sequence of *McTPS3*

Secondly, TPS sequences from sevaral distinct branch-genus clusters. For example, McTPS3 from *M. chamomilia* and other TPSs from asteraceae plants were grouped into a cluster, implying they had a closer genetic relationship.In addition, the TPSs from *Jatropha curcas* (JcJHL), *Populus euphratica* (PeTPS6), *Populus trichocarpa* (PtTPS1 and PtTPS2) also clustered together into another group. Taken together, these result indicated that McTPS3 shared a common evolutionary origin and the conserved sequences motifs with those of the Asteraceae specie TPS.

DISCUSSION

In this study, a novel *McTPS3* gene was cloned and characterized from *M. chamomilia*. Multiple alignments showed that the deduced *McTPS3* was homologous with other TPS proteins, indicating that McTPS3 was a member of TPS family in *M. chamomilia*. The sesquiterpene were the main pharmaceutically active components in *M. chamomilia* and they played significant roles by possessing anti-inflammatory, vulnerary, deodorant, antimicrobial, anticatarrhal, carminative, sedative, antiseptic and spasmolytic properties [16]. The TPS is a key enzyme in sesquiterpene biosynthetic pathway. There are TPS enzymes that catalyze the formation of just one terpene compound, but there are also many TPS enzymes that have the astonishing capability to synthesize complex product mixtures with high regio- and stereospecificity [17]. Much of the progress achieved in recent years has centered on the structural elucidation of TPS polypeptides and on the identification and biochemical characterization of members of the large *TPS* gene families in a variety of model plants [18]. These discoveries have led to a better understanding of the structural properties of TPS proteins, which drive the reactive mechanisms leading to the formation of multiple products and are the foundation for the molecular evolution analysis of terpene diversity. Since 1992 in tobacco cloned two sesquiterpene synthase genes, Scientists have cloned 200 monoterpene and sesquiterpene synthase gene in 40 kinds of plants [19]. Recently, Irmisch et al. [9] cloned five *TPS* genes from chamomile, and found these *TPS* genes encoding TPS proteases catalyze the synthesis of five kinds of sesquiterpene compound, (*E*)-β-caryophyllene, α-isocomene, β-elemene, (*E*)-β-ocimene, germacrene D respecitively.

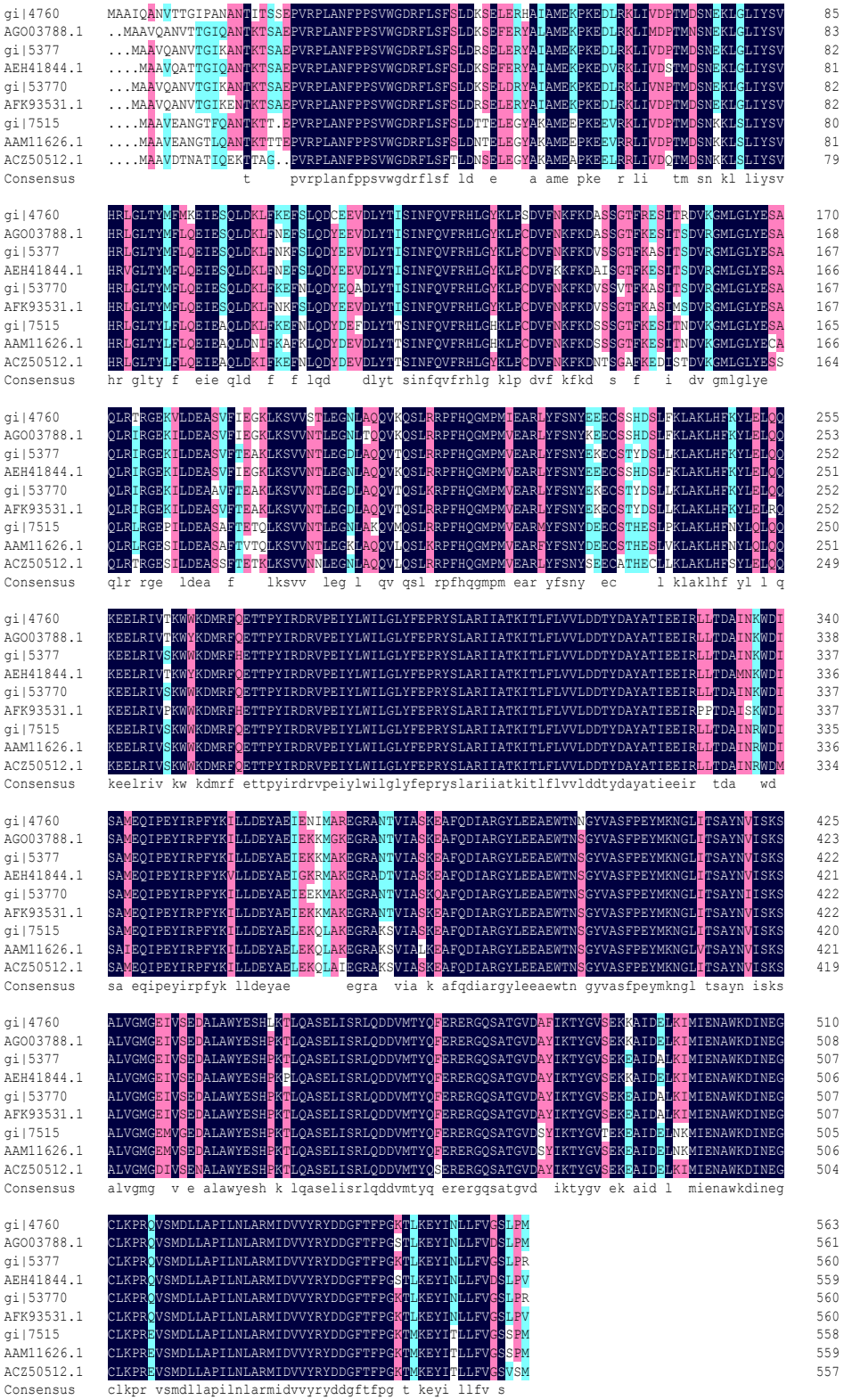

Fig. 2: Sequence multi-alignment of the deduced *McTPS3* protein with other plant TPS protein.
The amino acid sequence of McTPS3 was 93%, 92%, 92%, 91%, 86%, 85%, 85% similarity to TPSs from *Tanacetum cinerariifolium* (GenBank accession no. AGO03788.1), *Artemisia annua* (gi|5377), *Tanacetum parthenium* (AEH41844.1), *Artemisia absinthium* (gi|53770), *Cichorium intybus* (gi|7515), *Lactuca sativa* (AAM11626.1), *Helianthus annuas* (ACZ50512.1), respectively.

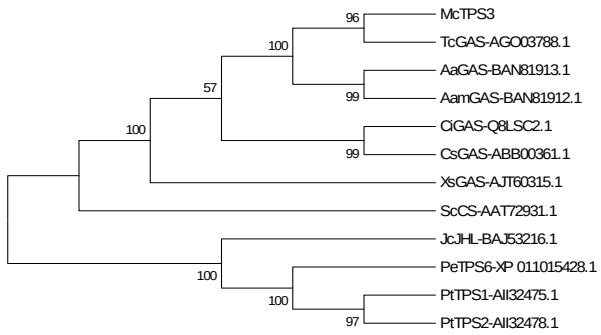

Fig. 3: Phylogenetic tree of the sequences of McTPS3 and TPS proteins from other plants.
The McTPS3 protein sequence was aligned with TPS sequences for TcGAS (*Tanacetum cinerariifolium*), AaGAS (*Artemisia annua*), AamGAS (*Artemisia absinthium*), CiGAS (*Cichorium intybus*), CsGAS (*Crepidiastrum sonchifolium*), XsGAS (*Xanthium strumarium*), ScCS (*Solidago Canadensis*), JcJHL (*Jatropha curcas*), PeTPS6 (*Populus euphratica*), PtTPS1 (*Populus trichocarpa*), PtTPS2 (*Populus trichocarpa*).

However, there are many distinct types of TPSs still need to be isolated and characterized in *M. chamomilia*. Based on cloning and characterization of *McTPS3* gene, overexpression of *McTPS3* gene in *M. chamomilia* to confirm the regulation mechanism of terpene biosythesis is undergoing. Further work need to be carried out on isolatation and function analysis other *TPS* genes from *M. chamomilia.*

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (31400603), the National Training Programs of Innovation and Entrepreneurship for Undergraduates (104892014043), the Natural Science Foundation of Hubei Province (2013CFA039).

CONFLICT OF INTEREST STATEMENT

None Declared

REFERENCES

1. Tholl D. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism, Current Opinion in Plant Biology 2006; 9: 297–304.
2. Degenhardt J, Gershenzon J, Baldwin I T et al. Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies. Current Opinion in Biotechnology 2003; 14: 169–176
3. Gershenzon J, Dudareva N. The function of terpene natural products in the natural world. Nature Chemical Biology 2007; 3: 408–414.
4. Kumar S, Das M, Singh A et al. Composition of the essential oils of the flowers, shoots and roots of two cultivars of *Chamomilla recutita*. Journal of Medicinal and Aromatic Plant Sciences 2001; 23: 617–623.
5. Pothke W, Bulin P . Phytochemical study of a new hybrid chamomile variety. II. Essential oil. Pharm Zentralle 1969; 108: 813–823.
6. Isaac O. Pharmacological investigations with compounds of *Chamomilla*. Planta Med 1979; 35: 118–124.
7. Salamon I. Chamomile: a medicinal plant. J Herbs Spices Med Plants 1992; 10: 1-4.
8. Schilcher H, Imming P, Goeters S. Active Chemical Constituents of *Matricaria chamomilla* L. Syn. *Chamomilla recutita* (L.) Rauschert. In: Chamomile-Industrial Profiles (Franke R and Schilcher H, eds). Boca Raton: CRC Press; 2005, p 55–76.
9. Irmisch S, Krause S T, Kunert G et al. The organ-specific expression of terpene synthase genes contributes to the terpene hydrocarbon composition of chamomile essential oils. BMC Plant Biology 2012; 12: 84.
10. Verpoorte R, Van Der Heijden R, Ten Hoopen H J G et al. Metabolic engineering

of plant secondary metabolite pathways for the production of fine chemicals. Biotechnology Letters 1999; 21: 467–479.
11. Verpoorte R, Memelink J. Engineering secondary metabolite production in plants. Current Opinion in Biotechnology 2002; 13:181–187.
12. Oksman-Caldentey K M, Inzé D. Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. Trends in Plant Science 2004; 9: 433–440.
13. Son Y J, Kwon M, Ro D K et al. Enantioselective microbial synthesis of the indigenous natural product (Minus Sign)-alpha-bisabolol by a sesquiterpene synthase from chamomile (*Matricaria recutita*). Biochemical J 2014; 463(2): 239-248.
14. Kumar S, Tamura K, Nei M. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 2004; 5: 150–163.
15. Keeling C I, Weisshaar S, Ralph S G et al. Transcriptome mining, functional characterization, and phylogeny of a large terpene synthase gene family in spruce (*Picea* spp.). BMC Plant Biolo 2011; 11: 43.
16. Muri K, Panchal M, Gajera V et al. Pharmacological properties of *Matricaria recutita*: a review. Pharmacologia 2012; 3: 348-351.
17. Falara V, Akhtar T A, Nguyen T T et al. The tomato terpene synthase gene family. Plant Physiology 2011; 157: 770–789.
18. Köllner T G, Schnee C, Gershenzon J et al. The variability of sesquiterpenes emitted from two *Zea mays* cultivars is controlled by allelic variation of two terpene synthase genes encoding stereoselective multiple product enzymes. Plant Cell 2004; 16: 1115–1131.
19. Aubourq S, Lechamy A, Bohlmann J. Genomic analysis of the terpenoid synthase (*AtTPS*) gene family of *Arabidopsis thaliana*. Mol Genet Genomics 2002; 267: 730–745.

Cite this article as:
Xiaomeng Liu, Li Zhu, Tingting Tao, Qiling Song, Weiwei Zhang, Qiangwen Chen, Feng Xu. Cloning and Sequence Analysis of a Terpene Synthase gene (McTPS3) from *Matricaria chamomilia*. J Pharm Chem Biol Sci 2015; 3(2):272-278