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Original Research Article

Cloning and Characterisation of the Gene Encoding Acetyl-CoA *C*-acetyltransferase in *Matricaria chamomilla*

Tingting Tao, Xiaomeng Liu, Jie Chang, Feng Xu*, Yangbo Yin

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

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

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ABSTRACT

Matricaria chamomilla is a widely used medicinal plant with great exploitative value for its sesquiterpenoid. α-bisabolol is sesquiterpenoid and the biosynthesis takes place via the isoprenoid pathway starting with acetyl-CoA. Acetyl-CoA *C*-acetyltransferase (AACT) catalyzes the condensation of two acetyl-CoA to form 4-C compound acetoacetyl-CoA in the mevalonic acid (MVA) pathway, was isolated from young leaves of *M. chamomilla* by RT-PCR for the first time. The full-length cDNA of the *McAACT* (GenBank Accession No. KX925560) was 1888 bp containing a 1227 bp open reading frame (ORF) encong a 408 amino acids. Comparative and bioinformatic analyses revealed that McAACT showed extensive homology with AACTs from other plant species. Phylogenetic tree analysis indicated that McAACT belonged to the plant AACT super family and had the closest relationship with AACT from *Taraxacum koksaghyz*. The isolation and characterization of *McAACT* gene will be helpful to further study the role of *McAACT* gene in the biosynthesis of sesquiterpenoids in *M. Chamomilla*.

Keyword: *Matricaria chamomilla*; *McAACT*; sesquiterpenoids; α-bisabolol

INTRODUCTION

Chamomile (*Matricaria chamomilla* L.) is a widely used traditional medicine that exhibits antiphlogistic and antimicrobial activity [1]. And therefore used for several pharmaceutical and cosmetic applications. The therapeutic values of chamomile come from its essential oil component, α-bisabolol [2]. *M. chamomilla* flowers contain 0.24 to 2.0 percent volatile oil that is blue in color. The two key constituents, α-bisabolol and chamazulene, account for 50-65 oercent of total volatile oil content [3]. The α-bisabolol is a sesquiterpene, and the efficacy of

anti-inflammatory, sterilization, heal ulcers, dissolve gallstones has been proved by pharmacology studies [4]. Moreover, the α-bisabolol has been used as an important cosmetic additive components due to its good skin effect [5,6]. Therefore, the quality and value of chamomile herbs can significantly improved by increase the content of α-bisabolol in *M. Chamomilla*.

Terpenoids (or isoprenoids) are the largest and structurally most diverse group of secondary metabolites, with more than 25000 types of terpenoids have been identified so far in plants [7]. Many terpenoids are important economic chemicals including flavors, pigments, waxes, rubbers, vitamins, taxol, and artemisinin [8]. Terpenoids are synthesized in higher plants through two pathways: the cytosolic mevalonic acid (MVA) pathway, and the plastidic 2-methyl-D-erythritol-4-phosphate (MEP) pathway (Fig. 1) [9].

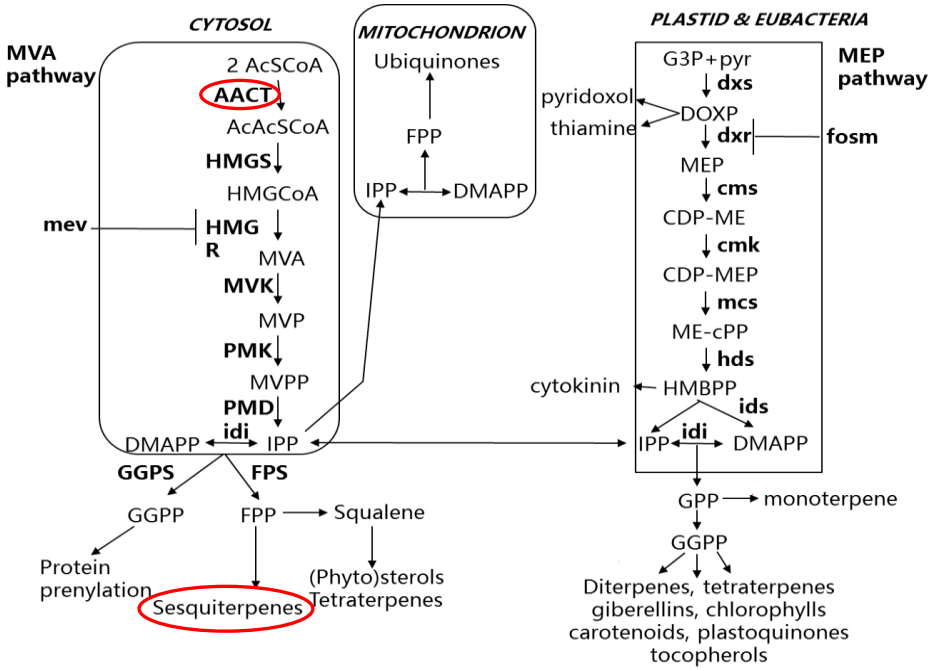

Fig. 1: Isoprenoid biosynthetic MVA and pathway in the plants

The MVA pathway starts with the two-carbon compound acetyl CoA, which condenses into the 4-C compound acetoacetyl-CoA catalyzed by acetyl-CoA acetyltransferase (AACT) [10]. AACT belongs to the thiolase family that has two conserved cysteine residues, which is important for thiolase activity. The first one is located in the N-terminal section of the enzymes and involved in the formation of an acetyl-enzyme intermediate; the second is located in the C-terminal extremity and it is the active site involved in deprotonation in the condensation reaction. Acetoacetyl-CoA is an important starting molecule for biosynthesis of various metabolites [11,12]. In view of its significance in isoprenoid metabolism, genes encoding AACTs have been isolated and extensively characterized from many plants including *Orchid Hybrid Vanda* [13], *Bacopa monniera* [14] and *Helianthus annuus* [15]. Here, we report for the first time the cloning and characterization of a new functional *AACT* gene from *M. chamomilla*.

MATERIAL AND METHODS

Plants materials

M. chamomilla was grown in a phytochamber maintained with a 16 h light/8 h dark photoperiod at 23 ℃. The leaves was harvested from 8-week-old *M. Chamomilla* and quickly frozen in liquid nitrogen and preserved at -80 ℃ prior to RNA extraction. 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).

RNA extraction and isolation of *McAACT*

Total RNA was isolated from *M. chamomilla* using the TaKaRa MiniBEST Plant RNA Extraction kit (Dalian, China) according to the manufacturer's instructions. The concentration and quality of the RNA were measured via spectrophotometry and agarose gel electrophoresis. The primers McAACTR (5'-ATGGCCCCAGCAGCTGTAGT-3') and McAACTF (5'-TTTCGTTGTGGAACTTCTG-3') were designed and synthesized according to the gene annotation of *M. chamomilla* in the transcriptome database. The full-length cDNA sequence of *McAACT* was amplified with the one-step RT-PCR kit (Dalian TaKaRa, Dalian, China) under the following condition: 94℃ for 3 min; 33 cycles of amplification at 94℃ for 20 s, 52℃ for 40 s, and 72℃ for 60 s; and a final extension at 72℃ for 7 min. The PCR products were purified, ligated into the pMD18-T vector (Dalian TaKaRa), and then cloned into the *E. coli* strain DH5a before sequencing.

Bioinformatics analysis and molecular evolution analyses

The obtained nucleotide sequence and deduced amino acid sequence were compared by a BLAST database search (http://www.ncbi.nlm.nih.gov). The molecular weight and isoelectric point (pI) of the deduced McAACT protein were computed with the Compute pI/Mw tool (http://web.expasy.org). Multiple sequence alignment was performed with the Vector NTI suite 10.0 program (Invitrogen, Paisley, UK). A phylogenetic tree was constructed with CLUSTALX 2.0 and MEGA 6.0 software [16]. The numbers of nodes represent the percentage of boot strap value obtained from 1000 sampling.

RESULTS

Cloning of the full-length cDNA of the *McAACT*

Using one-step RT-PCR, the full-length cDNA sequence of *McAACT* gene (GenBank Accession No. KX925560) of *M. chamomilla* was finally obtained. It was 1888 bp and contaied a 1227-bp open reading frame (ORF) encoding a 408-amino-acid protein (Fig. 2). The nucleotide sequence of *McAACT* was 87.0%, 79.0%, 79.0%, 78.0%, 77.0% and 76.0% identical to those of the *AACT* genes from *Taraxacum koksaghyz*, *Prunus persica*, *Hevea brasiliensis*, *Elaeis guineensis*, *Solanum tuberosum* and *Sesamum indicum*, respectively (Table 1). The results indicate that the gene we cloned is a member of the *AACT* gene family.

Table 1: Nucleotide sequence of *McAACT* similarity to the *AACT* genes from other plant species

Species	Accession No.	Homology (%)
Taraxacum kok-saghyz	KT899402.1	87
Prunus persica	XM_007215406.1	79
Hevea brasiliensis	AB294687.1	79
Elaeis guineensis	XM_010944301.1	78
Solanum tuberosum	XM_015313520.1	77
Sesamum indicum	XM_011087108.1	76

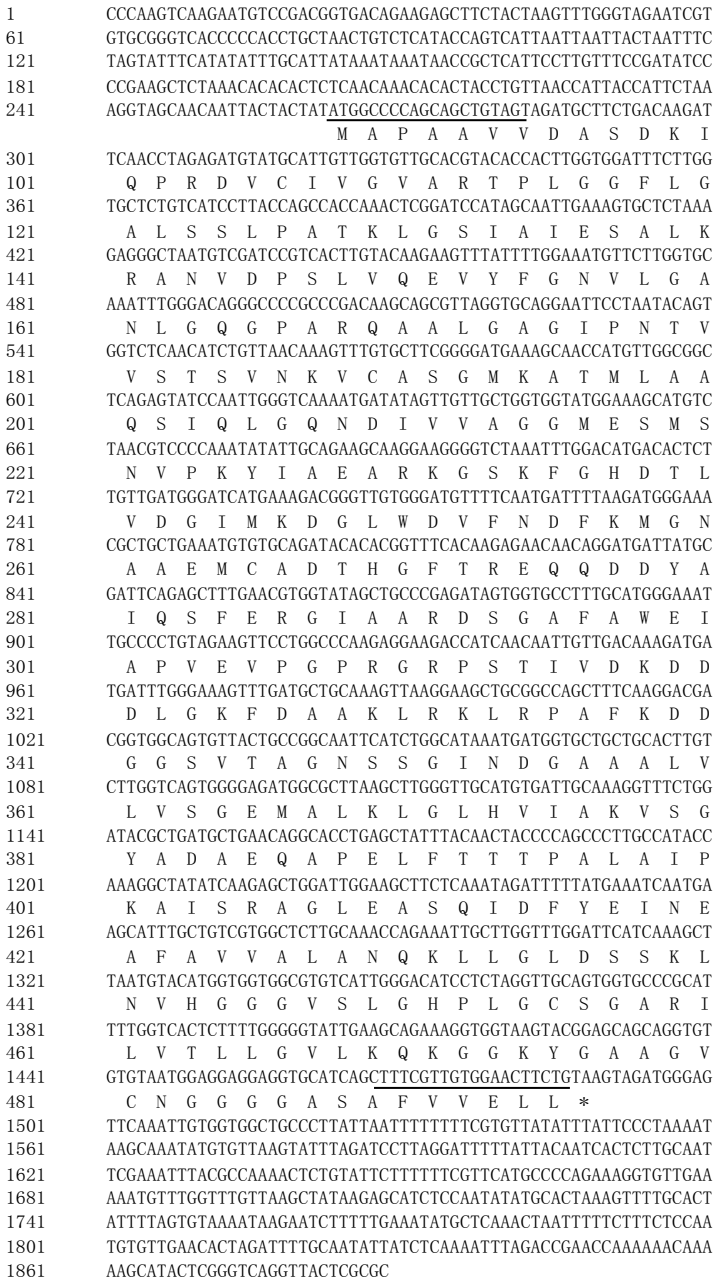

Fig. 2: The nucleotide acid sequence and deduced amino acid sequence of *McAACT*. The underline for specific primers McAACTR and McAACTF

Characterization of the McAACT protein

By using the software of Compute pI/Mw Tool, the calculated isoelectric point (pI) and molecular weight of the McAACT were predicted to be 5.81 and 41.9 kDa, respectively. The blastp results analysis demonstrated that McAACT exhibited high similarity to AACT protein from other plants, including *T. koksaghyz* (93%), *Ziziphus jujuba* (83%), *Camellia oleifera* (83%), *Nelumbo nucifera* (82%), *P. persica* (82%), *Malus domestica* (81%) and *Panax notoginseng* (81%) (Fig. 3). Multi-align analysis of McAACT also confirmed structural and functional conservation compared to other plant AACTs.

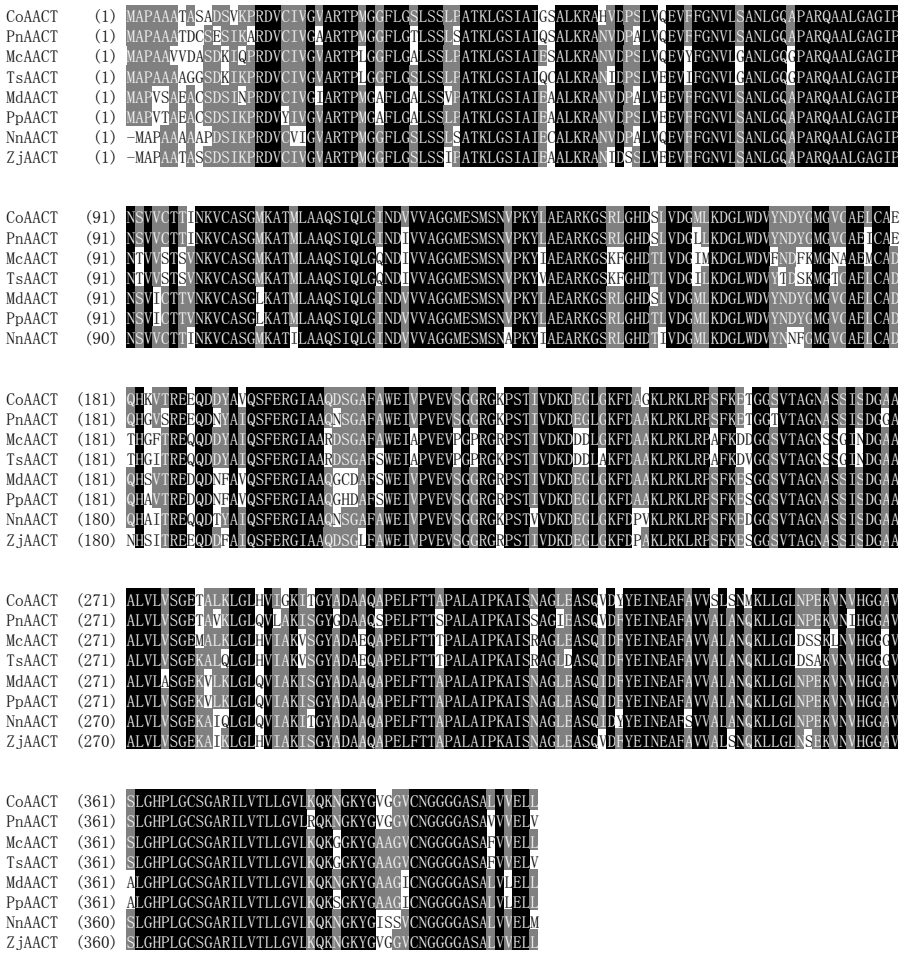

Fig. 3: Multiple alignment of McAACT with the following other plant AACTs: *Taraxacum koksaghyz*: TsAACT (AMB19691.1); *Prunus persica*: PpAACT (XP_007215468.1); *Malus domestica*: MdAACT (XP_008365553.1); *Ziziphus jujuba*: ZjAACT (XP_015880842.1); *Nelumbo nucifera*: NnAACT (XP_010267979.1); *Panax notoginseng*: PnAACT (AIK21787.1); *Camellia oleifera*: CoAACT (ADD10719.1). The completely identical amino acids are indicated with white foreground and black background. The conserved amino acids are indicated with white foreground and grey background. Non-similar amino acids are indicated with black foreground and white background

Molecular evolution analysis

The phylogenetic tree was constructed according to the deduced amino acid sequence of McAACT and other *AACT* genes from different hosts, including Dicotyledoneae, Monocotyledoneae and Algae to investigate the evolutionary relationships among different AACTs (Fig. 4). The McAACT protein had the closest relationship to TsAACT of *T. Koksaghyz* Asteraceae. The tree revealed that McAACT shares a common evolutionary with other plant AACT proteins based on conserved structure and sequence characteristics, such as amino acid homologies and conserved motifs, respectively.

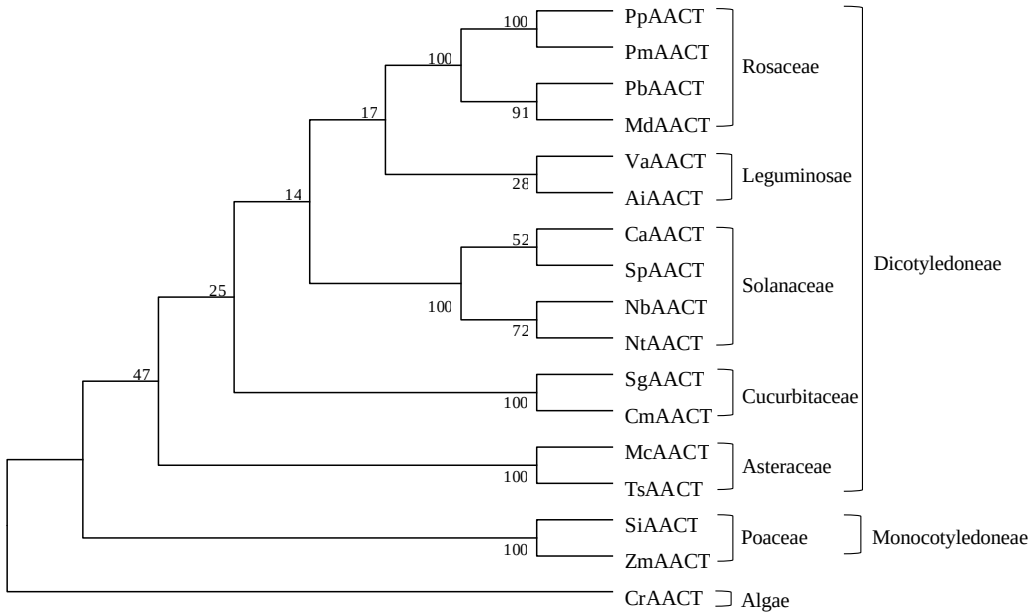

Fig. 4: Phylogenetic tree of AACT from different species using the neighbour-joining method.
The species, protein names anGenBank accession number are as following: *Matricaria chamomilla*: McAACT; *Prunus persica*: PpAACT (XP_007215468.1); *Prunus mume*: PmAACT (XP_008229933.1); *Pyrus x bretschneideri*: PbAACT (XP_009361536.1); *Malus domestic*a: MdAACT (XP_008365553.1); *Vigna angularis*: VaAACT (XP_017429943.1); *Arachis ipaensis*: AiAACT (XP_016166329.1); *Capsicum annuum*: CaAACT (XP_016580453.1); *Solanum pennellii*: SpAACT (XP_004243582.1); *Nicotiana benthamiana*: NbAACT (BAR94039.1); *Nicotiana tabacum*: NtAACT (XP_009590926.1); *Siraitia grosvenorii*: SgAACT (AEM42969.1); *Cucumis melo*: CmAACT (XP_008462041.1); *Taraxacum koksaghyz*: TsAACT (AMB19691.1); *Setaria italica*: SiAACT (XP_004977187.1); *Zea mays*: ZmAACT (NP_001266315.2); *Chlamydomonas reinhardtii*: CrAACT (XP_001694888.1)

DISCUSSION

In recent years, there has been a remarkable progress in the understanding of the molecular regulation of sesquiterpenoid biosynthesis in plant [17,18]. Sesquiterpenoid is among the most prevalent and biologically significant classes of secondary metabolites in Asteraceae; it has been the subject of a number of studies [19]. Acetyl-CoA C-acetyltransferase (AACT) catalyzes the condensation of two acetyl-CoA to form 4-C compound acetoacetyl-CoA in the mevalonic acid (MVA) pathway [20]. Here, we attempted to examine the molecular biology of sesquiterpenoid biosynthesis pathways in *M. chamomilla* by cloning and characterization of full-length cDNA of *McAACT* gene.

Chamomile (*Matricaria chamomilla L*.) is a widely used medicinal plant with great exploitative value for its sesquiterpenoid [21]. The α-bisabolol as a sesquiterpene compound, which the precursor of the substrate from the MVA pathway intermediate FPP [22]. As the first step of MVA pathway, AACT plays a fundamental role in carbon skeleton assembly patterns in pathway of sesquiterpene biosynthesis [23]. In this study, a 1888 bp full-length cDNA of the *McAACT* gene was isolated from *M. chamomilla*. *The McAACT* gene encodes a 408 amino acid protein. Our multiple alignments showed that the deduced McAACT sequence exhibited high similarity to HMGS proteins from other plants. The phylogenetic tree indicated that McAACT has a distinct and ancient relationship with the AACTs from other Asteraceae plants.

In conclusion, The cloning and characterization analysis of *McAACT* will help in elucidating its role in the sesquiterpenoid biosynthesis pathway. In this present investigation, we have successfully cloned and characterized the gene encoding *AACT* involved in the biosynthesis of α-bisabolol, known as sesquiterpenoids in *M. Chamomilla*. Meanwhile, *McAACT* is a first 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. Further work needs to be done to find the unknown gene involved in sesquiterpenoid biosynthesis pathway.

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CONFLICT OF INTEREST STATEMENT

Authors declare that they have no conflict of interest.

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