# MOLECULAR CLONING AND CHARACTERIZATION OF BETA-AMYRIN SYNTHASE (*SoAMYS*) GENE FROM *SALVIA OFFICINALIS* PLANT

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Terpenes (terpenoids or isoprenoids) play a key role in primary and secondary metabolism in a variety of organisms. In plants, its biosynthesis is catalyzed by two key pathways mevalonate and non-mevalonate pathways. The genome of garden sage plant (*Salvia officinalis*) contains 65 terpene synthase (*SoTPS)* genes, and only a few genes which related to terpenoids were studied. Here, we demonstrate the functional characterization of beta-amyrin synthase (*SoAMYS*) gene, by introducing it into *Arabidopsis thaliana* and found that *SoAMYS* overexpression improved the flowers formation in transgenic *Arabidopsis* compared to the wild type plants. Metabolic analysis reported that the production of various types of terpenes, especially beta-amyrin triterpene which, were increased and decreased in *SoAMYS* overexpression and wild type lines, respectively. These finding suggesting that *SoAMYS* functions as a beta-amyrin synthase in plant. Our results were further supported using some bioinformatics tools to predict the putative subcellular localization and tissue-specific expression of *SoAMYS*. In context that, *SoAMYS* gene was reported to be localized in plastids with highly expression level in flowers stage 15, pedicels. This is the first report of a gene involved in the beta-amyrin as a triterpene from *S. officinalis* plant.

**Keywords:** *Salvia officinalis*, terpene synthase genes, transgenic *Arabidopsis*, functional characterization

## INTRODUCTION

Terpenes (terpenoids or isoprenoids) are considered from the largest ecophysiological active secondary metabolites with over 40,000 known compounds (Bohlmann et al., 1998; Pott et al., 2019 and Ali et al., 2021).

They play numerous functional roles in all living organisms and plants as hormones [abscisic acid (ABA), brassinosteroids (BRs), strigolactones (SLs), cytokinins (CKs), gibberellic acids (GAs), electron carriers (side chain of plastoquinone), structural components of membranes (phytosterols), and photosynthetic pigments (carotenoids side, chain of phytol)] (Gutensohn et al., 2013; Luck et al., 2020; Ali et al., 2021 and Kildegaard et al., 2021). Moreover, organisms used isoprenoids in defense and communication, such as herbivore repellents and toxins, cantibiotics, ompetitive phytotoxins, and as attractants for pollinators and seed dispersers (Köllner et al., 2004; Tholl et al., 2006 and Korankye et al., 2017). The origin name of isoprenoids structures comes from the terebinth tree (*Pistacia terebinthus*), and the structure of isoprenoids units was illustrated then modified by Degenhardt et al. (2009) and Pott et al., (2019).

*Salvia officinalis* is an annual herb which belong to the Lamiaceae family that widely distributed in Central and South America, East Asia and West Asia, while the remaining salvia species are distributed all the world with over than (>1,000) species. For centuries, the aerial parts and essential oils (EOs) of these species have been used in Chinese medicine as antioxidant, antimicrobial activities, anticancer and antimutagenic (Atsuko and Hiroshi, 2011). In the fifties of the last century, the active compounds in the aerial parts and EOs of the salvia species were identifed as monoterpenes, sesquiterpenes, diterpene and triterpene. The composition of the terpenes in the salvia genus depends on the species or cultivars and type of tissues (Ali et al., 2017 and 2018). In addition, terpene synthase (TPSs) genes from various plant species have been cloned, characterized and identified, then used for metabolic manipulation (Aharoni et al., 2006 and Yu and Utsumi, 2009). For example, cloning of (E)-beta-ocimene synthase from *Arabidopsis thaliana* (Fäldt et al., 2003), myrcene and (E)-beta-ocimene synthase from Snapdragon (Dudareva et al., 2003), amorpha-4,11-diene synthase from *Artemisia annua* (Bertea et al., 2006), α-zingiberene synthase from *Ocimum basilicum* (Davidovich-Rikanati et al., 2008), (E)-β-caryophyllene from rice and maize (Cheng et al., 2007 and Degenhardt et al., 2009). Furthermore, our groups has succeeded in the molecular cloning for (−)-germacrene D synthase (TPS6): (3S)-linalool synthase (LINS), (+)-sabinene synthase (SABS), 1,8-cineole synthase (CINS) and (+)-neomenthol dehydrogenase (NEOD), which encoded by *SoTPS6*, *SoLINS*, *SoSABS*, *SoCINS* and *SoNEOD* genes, respectively (Ali et al., 2017). In Egyptian cultivar of *S. officinalis*, the biosynthesis gene for the triterpenes has not been known in *S. officinalis* plants. Indeed, only the beta-amyrin synthase gene (*SoAMYS*) was elucidated and identified in mint family (Aminfar et al., 2019). This study aimed to clone and functionally characterize *SoAMYS* cDNA from *S. officinalis*. Here, we reported the overexpression (OE) and functional characterization of *SoAMYS* cDNA in *A. thaliana*.

## MATERIALS AND METHODS

### 1. Plant Materials and Sample Collection

Seeds of *S. officinalis* were collected from the Egyptian Desert Gene Bank (EDGB), North Sinai Research Station, Department of Genetic Resources, Desert Research Center, Egypt, and grown at National Research Centre, Cairo, Egypt. For gene cloning, three biological replicates from young and old leaves were sampled from two years- old *S. officinalis* plants. The samples were immediately kept in liquid nitrogen-LN2 and then stored at −80°C for RNA extraction.

### 2. Bioinformatics Analysis for *SoAMYS* Gene

Full-length cDNAs for *SoAMYS* was selected from our RNA-Seq (Ali et al., 2017). Physiochemical properties for *SoAMYS* gene was determined using PROTPARAM website (http://web.expasy.org/protparam). Protein domain was prediction using bioinformatics tools InterPro (https://www.ebi.ac.uk/interpro). Comparative sequence analysis of *SoAMYS* was performed using BLASTX tool against the NCBI-protein database (http://blast.ncbi.nlm.nih.gov). Phylogenetic tree was built using PhyML server with the default parameters of the (http://www.phylogeny.fr) (Dereeper et al., 2008). Putative tissue expression profile from forty-nine Arabidopsis tissues were extracted from RNA-Seq Atlas of Arabidopsis (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi). Putative subcellular localization of *SoAMYS* gene was inferred from their sequence similarity with characterized protein from the *Arabidopsis* Information Resource (https://www.arabidopsis.org/cgi-bin/Blast/TAIRblast.pl). Subcellular localization profile image was built using Cell eFP tool (http://bar.utoronto.ca/cell_efp/cgi-bin/cell_efp.cgi).

### 3. RNA Extraction and the First Strand cDNA Synthesis

Total RNAs was extracted from leaf of *S. officinalis* for *SoAMYS* gene cloning and from *A. thaliana* for semiquantitative RT-PCR (Semi-RT-PCR) using TransZol Reagent (Focus Bioscience, Australia) according to the manufacturer's instructions. First strand cDNA was synthesized with TransScript® First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) according to the manufacturer's instructions (Ali et al., 2017; 2018 and 2021).

### 4. Gene Cloning and Vector Construction

Full length sequence of *SoAMYS* was cloned in our laboratory by PCR machine using short and long specific primers according to RNA-Seq sequence information from our RNA-sequencing of *S. officinalis* leaves (Ali et al., 2017). cDNA from leave was used for the initial PCR amplification using short primers such as *SoAMYS* forward 5′-ATGTGGCGGCTGAAGATTG-3′ and reverse 5′-TCATCTCCTCCATTGCTTTAATACT-3′ with the TaKaRa Ex Taq® DNA Polymerase (TaKaRa, China) using the following PCR conditions: 4 minutes at 96°C followed by 12 seconds at 98°C; 30 seconds at

59°C (Annealing temperatures), 2:30 minutes at 72°C, and then 10 minutes at 72°C. This process was repeated for 30 cycles. The first PCR products was used as a template for the PCR cloning using long primers, such as *SoAMYS* forward 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTGGCGGCTGAAGA-3′ and reverse 5′-GGGGACCACTTTGTACAAGAAAGCTGGGTTCATCTCCTCCATTG-3′ with the TaKaRa Ex Taq® DNA-Polymerase for the Gateway cloning vector. The PCR product was purified and cloned into the pDONR221 vector by BP Clonase (Invitrogen, USA). The constructs pDONR221vectors harbouring target gene sequencing was sequenced, and Gateway LR Clonase enzyme (Invitrogen, USA) was used for recombination the target gene sequencing into the over-expression vector pB2GW7 for *A. thaliana* transformation. The construct vectors containing *SoAMYS* was confirmed by sequencing.

**5. Arabidopsis Transformation Procedure and Preparation of *Agrobacterium* Cultures for Floral-Dip Transformation**

Gateway cloning technology was used for construction of plant transformation vector pB2GW7-*SoAMYS* as described by (Ali et al., 2017; 2018 and 2021). The pB2GW7-*SoAMYS* vector was introduced into *A. tumefaciens* strain GV3101 by direct electroporation method. The transformation procedure was performed using the floral-dip transformation method as described previously (Aharoni et al., 2003; Su-Fang et al., 2014 and Ali et al., 2018) with a few modifications. Transgenic *A. thaliana* lines were generated and examined with RT-PCR for positive transgenic lines, only those containing the *SoAMYS* gene were used for further analysis.

**6. Terpenoid Extraction and Gas Chromatography–Mass Spectrometry (GC–MS) Analysis**

All terpenoid compounds from transgenic *A. thaliana* leave containing *SoAMYS* gene and wild type leaves (control) were extracted and isolated. Leaves of *A. thaliana* lines and a wild type were collected and homogenized to a powder using liquid nitrogen with pestle and a mortar, then the powder was soaked in Amber storage bottles [(20 ml screw-top vials with silicone/PTFE septum lids) (http://www.sigmaaldrich.com)] containing n-hexane as a solvent. After that, Amber storage bottles were incubated in shaking at 37°C and 210 rpm for 70 hours. Afterward, the supernatant solvent was collected by centrifuged at 5,000 rpm for 10 minutes at 4°C, then pipette into glass vials and concentrated to 1.5 ml of concentrated oils under a stream of nitrogen gas with a nitrogen evaporator (Organomation; Toption-China-WD-12). The concentrated oils were transferred to a fresh 1.5 ml crimp vial amber glass, and placed on the auto-sampler of the gas chromatography mass spectrometer (GC-MS) system for GC-MS analysis as described previously by Ali et al. (2017, 2018 and 2021).

**7. Semi-Quantitative RT-PCR Assay**

Semi-quantitative real-time PCR was performed on Eppendorf-PCR system (Mastercycler Nexus PCR Machine from Eppendorf, UK) with a total reaction volume of 25 µl. Gene-specific primer for *At-B-actin* forward 5′-GGCTGAGGCTGATGATATTC-3′ and reverse 5′-CCTTCTGGTTCATCCCAAC -3′ was used as a reference gene with 155 bp, and *SoAMYS* forward 5′-CTGCACCGAGCCAATAAT-3′ and reverse 5′-CCAGCACATCATCTGTAGAC-3′ with 151 bp length, gene involved in beta-amyrin synthase. All primers were designed using the IDTdna website (http://www.idtdna.com/scitools/Applications/RealTimePCR/). The semi-qRT-PCR reaction conditions were as follows: [predenaturation step at 95°C for 4 minutes, 35 cycles of amplification (95°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute), and a final extension step at 72°C for 10 minutes]. Final PCR products density represents the expression levels of both *At-B-actin* and *SoAMYS* genes.

## RESULTS AND DISCUSSION

**1. Bioinformatic Analysis and Sequence Characterization of *SoAMYS* Gene**

The complete ORF of *SoAMYS* gene from *S. officinalis* with 2,481 bp encoded a 827 amino acid protein with a 94.72 kDa of molecular mass and 5.84 of predicted theoretical isoelectric point (pI). Based on the BLASTX analysis (Table 1), the highest homologue sequencing of *SoAMYS* gene is the beta-amyrin sequencing from *Ocimum basilicum* with 91.10% of identity. On the other hand, *SoAMYS* gene sequence has relatively similarity with other homologues sequencing higher than ≧80.66%.

**Table (1).** BLASTX analysis *SoAMYS* was compared with the NCBI protein database for gene identification purposes.

| NCBI Accession | [a]Descriptiona | Organism | *E* value | Identity (%) | Accession length |
|---|---|---|---|---|---|
| AJT60358.1 | Amyrin synthase | *Ocimum basilicum* | 0.0 | 91.10 | 765 |
| KZV51042.1 | Beta-amyrin synthase | *Dorcoceras* | 0.0 | 84.01 | 761 |
| GER42726.1 | Amyrin synthase | *Striga asiatica* | 0.0 | 84.27 | 761 |
| AWM98359.1 | Beta-amyrin synthase | *Osmanthus fragrans* | 0.0 | 83.90 | 762 |
| AEX99665.1 | Amyrin synthase | *Catharanthus roseus* | 0.0 | 83.51 | 762 |
| AFJ19235.1 | Mixed amyrin | *Catharanthus roseus* | 0.0 | 83.38 | 762 |
| AND78515.1 | Amyrin synthase | *Calotropis procera* | 0.0 | 81.58 | 761 |
| BAF63702.1 | Mixed amyrin | *Olea europaea* | 0.0 | 82.98 | 762 |
| QJS40211.1 | Amyrin synthase | *Gymnema sylvestre* | 0.0 | 80.66 | 761 |

[a]Description—homology search using BLASTX.

Putative function of *SoAMYS* gene was initially predicted using bioinformatics tools InterPro (https://www.ebi.ac.uk/interpro/) database and the presence of terpene synthases conserved domain as well as structural homology to known TPSs from Lamiaceae family and other plants (Su-Fang et al., 2014 and Blum et al., 2020). The *SoAMYS* protein with a 827-aa length has protein family membership squalene cyclase domain (IPR018333)

from 1-760 aa (Fig. 1). The squalene cyclase domain is responsible for rearrangement the complex cyclic in each of squalene, squalene cyclase, 2, 3 oxide-squalene and 2, 3-oxidosqualene cyclase, which consider integral membrane proteins that can catalyse a cationic cyclization cascade to produce linear triterpenes compounds (Wendt and Schulz, 1998 and Wendt et al., 2000). Moreover, the enzyme that has this kind of domain can catalyses the cyclization of squalene compounds to squalene-hopene or squalene-diplopterol, which consider a diverse subclass in triterpenoids metabolism (Wendt et al., 1999). Furthermore, *SoAMYS* protein domain has four minor domains, which take shape an alpha-alpha barrel that subjoin with the major domain. These four minor domains are SQ-cyclase-N (Squalene cyclase, N-terminal: IPR032697) from 106 - 364 aa, SQHop-cyclase-N (Squalene-hopene-cyclase N-terminal domain: PF13249) from 106 - 364 aa, SQ_cyclase_C (Squalene cyclase, C-terminal: IPR032696) from 418 - 757 and SQHop_cyclase_C (Squalene-hopene cyclase C-terminal domain:PF13243) from 418 - 757. Also, *SoAMYS* protein sequenc have one terpene synthase, conserved site (IPR002365) from 609 - 623 aa (Hoshino et al., 2004) (Fig. 1). Finally, the protein sequence that contained one or some from these domains considers a member of the terpene synthase family (Köllner et al., 2008). On the other hand, phylogenetic tree analysis showed that *SoAMYS* from *S. officinalis* form a clade homology with amyrin synthase-1 from *Ilex asprella*, and this clade belongs to angiosperm-specific TPS-f which encode to sesquiterpene and triterpenes (Bohlmann et al., 1998 and Danner et al., 2011) (Fig. 2).

**2. Putative Tissue Expression Pattern and Subcellular Localizations of *SoAMYS* Gene**

The putative expression patterns of *SoAMYS* gene of *S. officinalis* were uncovered based on their higher similarity with *AT1G78950/TPS-BAS* gene from *A. thaliana*, and by transcript analysis across forty-nine *Arabidopsis* tissues. Interestingly, we observed the highest expression levels of this gene in flowers stage 15, pedicels, seeds stage 3 w/ siliques, flower stage 15, stamen and flower stage 15, carpels (Table 2 and Fig. 3a and b). These results are nearly agree with Liu et al. (2014) and Ali et al. (2021), who reported the higher expression levels of some terpene syntheses genes such as, *GmTPS21, SoHUMS, SoLINS2, SoNEOD, SgTPSV, SgFARD* and *SgGERIS* from *Glysin max, S. officinalis* and *S. guaranitica* were detected in roots and seeds. Moreover, *SoAMYS* was reported to be localized with higher expression levels in the ("cytosol":8, "mitochondrion":4, "nucleus":4, "plastid":2, "plasma membrane":2, "peroxisome":2, "golgi":2) (Fig. 3c). These results are in line with Taniguchi et al. (2014), Chen et al. (2018), Ali et al. (2021) and Wang et al. (2022). They reported that most of TPSs genes were targeted to the cytosol or other cell organelles such as plastid, mitochondrion and nucleus.

### 3. Molecular Analysis of Beta-Amyrin Synthase (*SoAMYS*) Gene in Transgenic *A. thaliana* Leaves

To assess the function of *SoAMYS*, we are generating transgenic *A. thaliana* Columbia-0 (Col-0). Overexpression of *SoAMYS* in *A. thaliana* was accomplished using *Agrobacterium tumefaciens* strain GV101 harboring the overexpression vector pB2GW7-*SoAMYS*. More than ten BASTA-resistant transgenic lines from *A. thaliana* plants were successfully generated. These lines are characterized by long hypocotyls, small green leaves, and long flowering stems (Fig. 4a). While the wild types lines are characterized by short hypocotyls, big bleached out leaves, without flowering stems formation (Fig.4a). The putative transformants were further confirmed and verified using semi-quantitative RT-PCR of the plant genomic cDNA. After forty days from plants growth, matuer leaves from transgenic and wild type plants were sampled for RNA isolation and cDNA syntheses. All the putative transformants showed high expression of the *SoAMYS* gene by the amplification of a distinct band at 151 bp, which was absent in the wild type plants (Fig. 4b). This result certain the existence of the *SoAMYS* gene in the positive transgenic plants. Three of the transgenic lines, designated as *OE-SoAMYS-1*, *OE-SoAMYS-2* and *OE-SoAMYS-3*, were selected for further analysis. Meanwhile, from the observation for morphological properties, we found the wild type plants delayed in growth and flowering than the transgenic plants (Fig. 4a and b). In context, the obtained findings are in line with our previous works Ali et al. (2017 and 2018) that reported the overexpression of genes that involved in the terpenoid biosynthesis, such as *SoLINS, SoNEOD, SoTPS6, SoSABS, SoCINS, SgGPS, SgFPPS* and *SgLINS* from *S. officinalis* and *S. guaranitica* in *Nicotiana tabacum* and *A. thaliana*, also resulted in delayed growth and flowering formation in wild type plants compared to the transgenic plants.

### 4. Overexpression of *SoAMYS* Gene Led to Changes the Levels of Terpenoids in Transgenic *A. thaliana* Leaves

Total terpenoids were extracted from transgenic and wild type of *A. thaliana* leaves with hexane and analyzed by GC-MS to identify the end product that produced by transformation with the *SoAMYS* gene. Different peaks areas (% peak area) represent various types and amounts of mono-, sesqui- and triterpene compounds. The names of terpenoids compounds from transgenic and wild type *A. thaliana* were identified by comparing their mass spectra with various mass spectra libraries and published references. Diverse types and amounts of mono-, sesqui- and triterpenes were observed under the effect of overexpression of *SoAMYS* genes in transgenic *A. thaliana* plants, especially the major peak that detect at the retention time of 32.273 as shown in Table (3) and (Fig. 5). The previous peak was characterized as beta-amyrin compound depending on their closest mass spectra from various libraries (e.g. Wiley GC/MS Library (10th Edition) (Wiley, New York, NY, USA), Volatile Organic Compounds (VOC) Analysis S/W software, and the

NIST Library (2014 edition)). The production of beta-amyrin by *SoAMYS* was in agreement with the findings from Su-Fang et al. (2014) and Ali et al. (2017 and 2018). These results also showed that the overexpression of terpene synthese genes introduced by Su-Fang et al. (2014) and Ali et al. (2017 and 2018), does not affect the product specificity of *SoAMYS* in producing beta-amyrin. Having obtained the similar terpene products in both *N. tabacum* and *A. thaliana*, we have showed that *SoAMYS* was responsible for the production of beta-amyrin as a triterpene and other sesquiterpene through the pathway of sesquiterpenoid and triterpenoid biosynthesis (Ro et al., 2006 and Wang et al., 2016 ).

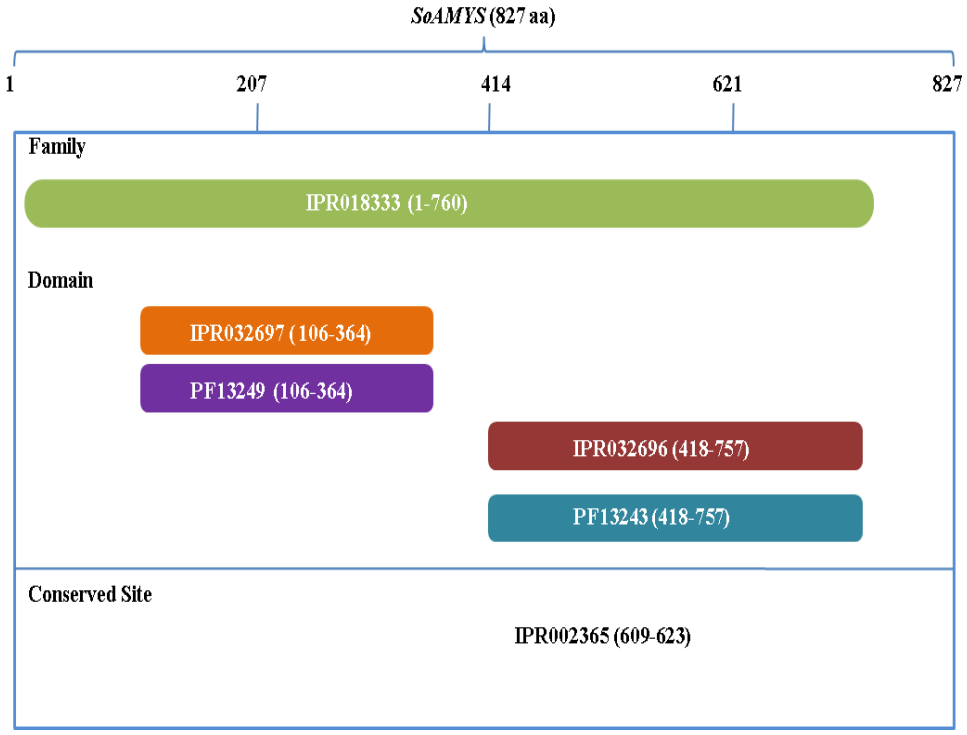


**Fig. (1).** Putative domain analysis for *SoAMYS* using the InterPro protein sequence analysis & classification (https://www.ebi.ac.uk/interpro/) database. *SoAMYS* protein sequence have one protein family membership squalene cyclase domain (IPR018333), four minor domains SQ_cyclase_N (Squalene cyclase, N-terminal: IPR032697, SQHop_cyclase_N (Squalene-hopene cyclase N-terminal domain: PF13249, SQ_cyclase_C (Squalene cyclase, C-terminal: IPR032696) and SQHop_cyclase_C (Squalene-hopene cyclase C-terminal domain:PF13243) and one terpene synthase, conserved site (IPR002365).


**Fig. (2).** Phylogenetic tree of *SoAMYS* with selected terpene synthases fromother plants. Seven previously identified TPS subfamilies (Tps-a to Tps-g) were chosen based on Bohlmann et al. (1998) and Danner et al. (2011). The alignment was performed using the PhyML server. The numbers indicated are the actual bootstrap values of the branches.

**Table (2).** Putative tissue expression levels of *SoAMYS* (AT1G78950) gene based on *Arabidopsis* gene expression.

| N | Tissue | Expression Level | Standard Deviation |
|---|---|---|---|
| 1 | Dry seed | 5.07 | 1.97 |
| 2 | Imbibed seed, 24 h | 7.56 | 1.44 |
| 3 | 1st Node | 131.93 | 9.73 |
| 4 | Flower Stage 12, Stamens | 25.93 | 2.32 |
| 5 | Cauline Leaf | 11.05 | 1.60 |
| 6 | Cotyledon | 13.48 | 2.17 |
| 7 | Root | 13.53 | 0.94 |
| 8 | Entire Rosette After Transition to Flowering | 13.21 | 0.75 |
| 9 | Flower Stage 9 | 16.21 | 3.12 |
| 10 | Flower Stage 10/11 | 40.94 | 1.45 |
| 11 | Flower Stage 12 | 64.28 | 6.88 |
| 12 | Flower Stage 15 | 184.8 | 15.32 |
| 13 | Flower Stage 12, Carpels | 33.36 | 1.23 |
| 14 | Flower Stage 12, Petals | 67.05 | 2.30 |
| 15 | Flower Stage 12, Sepals | 116.86 | 6.09 |
| 16 | Flower Stage 15, Carpels | 229.25 | 17.18 |
| 17 | Flower Stage 15, Petals | 118.70 | 9.17 |
| 18 | Flower Stage 15, Sepals | 11.46 | 0.79 |
| 19 | Flower Stage 15, Stamen | 280.05 | 19.32 |
| 20 | Flowers Stage 15, Pedicels | 773.78 | 8.61 |
| 21 | Leaf 1 + 2 | 17.03 | 1.75 |
| 22 | Leaf 7, Petiole | 10.78 | 2.72 |
| 23 | Leaf 7, Distal Half | 11.93 | 0.30 |
| 24 | Leaf 7, Proximal Half | 12.55 | 3.29 |
| 25 | Hypocotyl | 30.48 | 1.94 |
| 26 | Root | 26.25 | 2.95 |
| 27 | Rosette Leaf 2 | 11.81 | 0.74 |
| 28 | Rosette Leaf 4 | 12.81 | 1.93 |
| 29 | Rosette Leaf 6 | 14.96 | 2.50 |
| 30 | Rosette Leaf 8 | 13.31 | 2.31 |
| 31 | Rosette Leaf 10 | 14.85 | 1.96 |
| 32 | Rosette Leaf 12 | 13.85 | 3.45 |
| 33 | Senescing Leaf | 10.55 | 0.86 |
| 34 | Shoot Apex, Inflorescence | 17.41 | 1.36 |
| 35 | Shoot Apex, Transition | 15.91 | 4.02 |
| 36 | Shoot Apex, Vegetative | 18.86 | 2.27 |
| 37 | Stem, 2nd Internode | 92.80 | 10.37 |
| 38 | Mature Pollen | 20.90 | 1.05 |
| 39 | Seeds Stage 3 w/ Siliques | 551.56 | 38.2 |
| 40 | Seeds Stage 4 w/ Siliques | 28.68 | 2.53 |
| 41 | Seeds Stage 5 w/ Siliques | 12.15 | 1.20 |
| 42 | Seeds Stage 6 w/o Siliques | 16.83 | 1.14 |
| 43 | Seeds Stage 7 w/o Siliques | 17.68 | 2.28 |
| 44 | Seeds Stage 8 w/o Siliques | 51.15 | 6.47 |
| 45 | Seeds Stage 9 w/o Siliques | 22.04 | 6.08 |
| 46 | Seeds Stage 10 w/o Siliques | 31.05 | 5.71 |
| 47 | Vegetative Rosette | 17.23 | 1.59 |

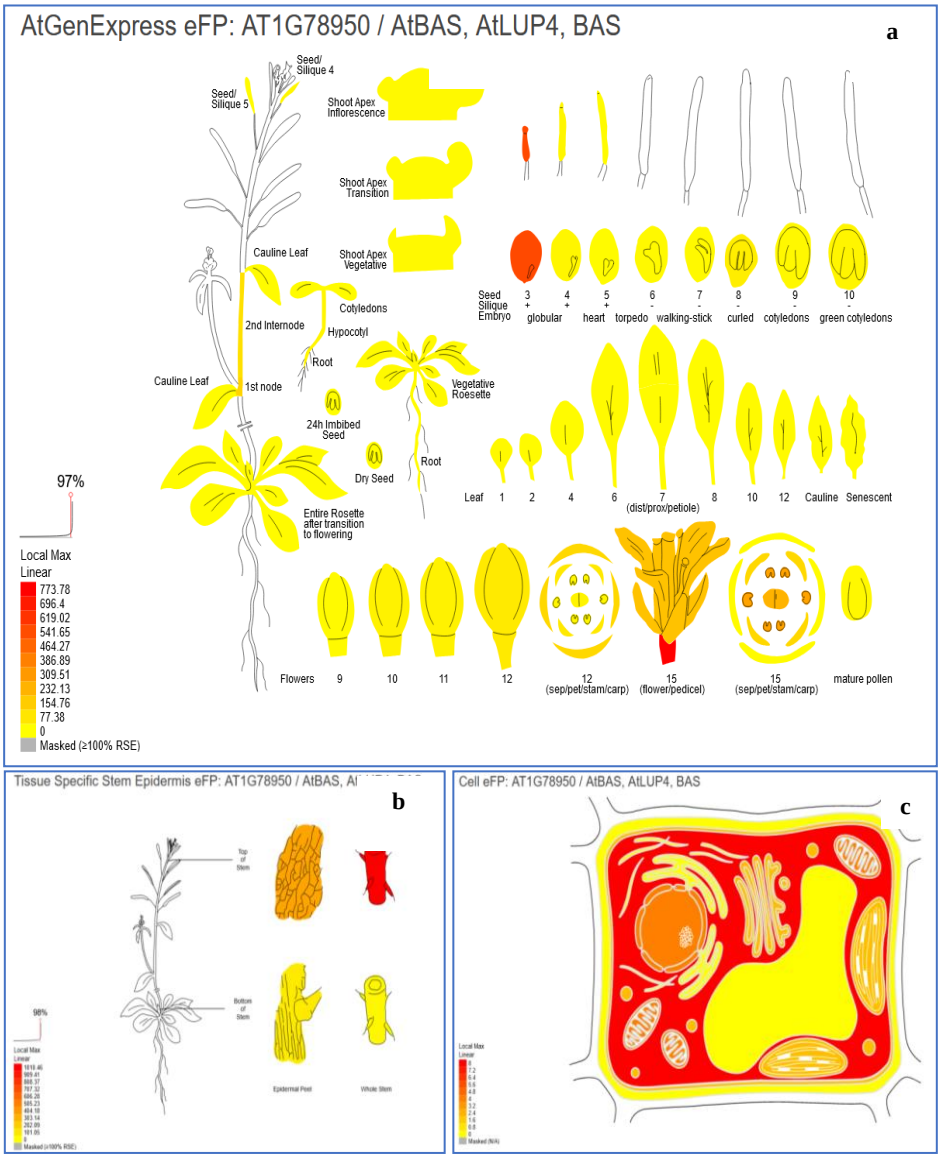


**Fig. (3)**. Visualization the putative an "electronic fluorescent pictograph" browsers for exploring the putative tissue expression and cell localization of *SoAMYS* (AT1G78950) gene, based on Arabidopsis gene expression and protein localization at different tissues and cell organs. **a.** Expression data at different tissues from seedling to flowering stages. **b.** Expression data of tissue specific stem epidermis at top and bottom. **c.** Expression data at different cell organs. The blue arrow points the expression scale (the more intense red color, the more gene expression).

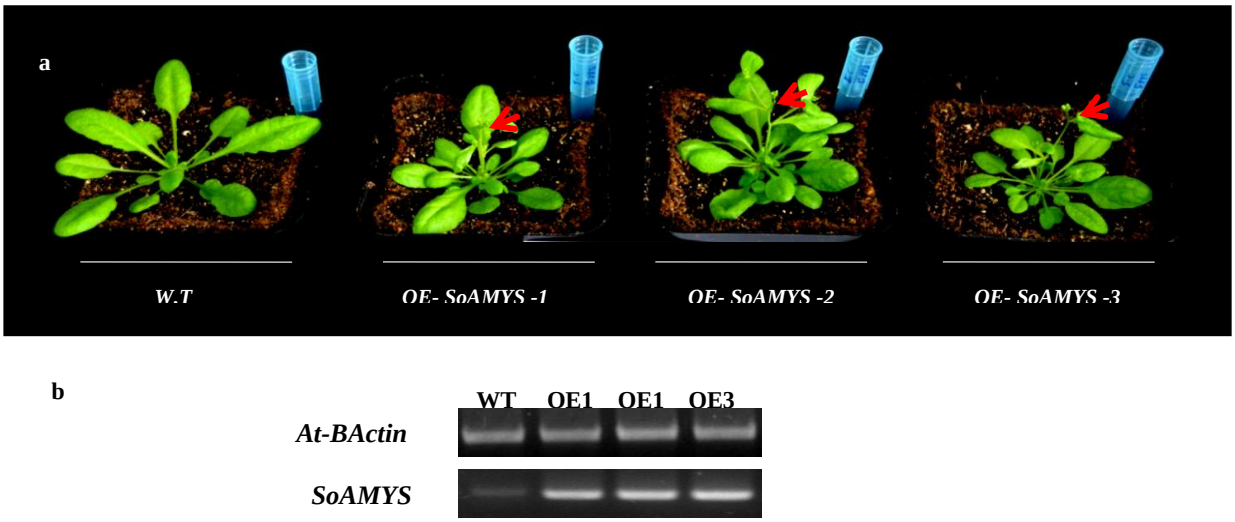


**Fig. (4).** Overexpression of *SoAMYS* gene in transgenic *Arabidopsis*. **a.** Comparison of the phenotypes of the transgenic *A. thaliana* and wild type *A. thaliana*. The red arrow (↘) indicates the flowers at the transgenic *A. thaliana*. **b.** Semiquantitative RT-PCR to confirm the expression of terpenoid genes.

**Table (3).** The major terpenoid compositions in transgenic *A. thaliana* leave over-expressing of *SoAMYS*.

| N | Compound name | R.T (min.) | Formula | Molecular mass (g mol$^{-1}$) | Type of terpene | % Peak area | |
|---|---|---|---|---|---|---|---|
| | | | | | | *at W.T* | *SoAMYS* |
| 1 | Benzeneethanol, α,β-dimethyl- | 5 | $C_{10}H_{14}O$ | 150.2176 | Mono | | 0.17 |
| 2 | Benzene, (azidomethyl)- | 5.688 | $C_7H_7N_3$ | 133.1506 | | | 0.18 |
| 3 | α-Pinene | 5.825 | $C_{10}H_{16}$ | 136.2340 | Mono | 9.29 | |
| 4 | 2-(4-Hydroxybutyl)cyclohexanol | 7.810 | $C_{10}H_{20}O_2$ | 172.2600 | | 2.79 | |
| 5 | Cyclohexasiloxane, dodecamethyl- | 24.010 | $C_{12}H_{36}O_6Si_6$ | 444.9236 | | 4.08 | |
| 6 | Cyclohexasiloxane, dodecamethyl- | 25.790 | $C_{12}H_{36}O_6Si_6$ | 444.9236 | | | 0.25 |
| 7 | α-Copaene | 28.625 | $C_{15}H_{24}$ | 204.3511 | Sesquit | | 1.63 |
| 8 | β-Elemene, (-) | 29.250 | $C_{15}H_{24}$ | 204.3511 | Sesquit | | 14.14 |
| 9 | (Z)-β-Elemene | 29.620 | $C_{15}H_{24}$ | 204.3511 | Sesquit | 4.39 | |
| 10 | 9-epi-Caryophyllene | 30.270 | $C_{15}H_{24}$ | 204.3511 | Sesquit | | 4.47 |
| 11 | α-Humulene | 31.288 | $C_{15}H_{24}$ | 204.3511 | sesquit | | 0.64 |
| 12 | Beta-amyrin | 32.273 | $C_{30}H_{50}O$ | 426.7174 | Triterpe | | 39.73 |
| 13 | Cyclooctasiloxane, hexadecamethyl- | 33.128 | $C_{16}H_{48}O_8Si_8$ | 593.2315 | | | 6.33 |
| 14 | δ-Amorphene | 33.473 | $C_{15}H_{24}$ | 204.3511 | sesquit | | 2.06 |
| 15 | σ-Cadinene | 34.483 | $C_{15}H_{24}$ | 204.3511 | sesquit | 3.93 | |
| 16 | (-)-.beta.-Bourbonene | 35.278 | $C_{15}H_{24}$ | 204.3511 | Sesquit | | 1.78 |
| 17 | Cyclooctasiloxane, hexadecamethyl- | 36.615 | $C_{16}H_{48}O_8Si_8$ | 593.2315 | | | 0.43 |
| 18 | Cedrol | 37.215 | $C_{15}H_{26}O$ | 222.3663 | Sesquit | | 1.34 |
| 19 | Caryophyllene oxide | 38.248 | $C_{15}H_{24}O$ | 220.3505 | Sesquit | | 0.86 |
| 20 | Caryophyllene oxide | 38.248 | $C_{15}H_{24}O$ | 220.3505 | Sesquit | 1.85 | |
| 21 | Hexa-hydro-farnesol | 39.278 | $C_{15}H_{32}O$ | 228.4140 | | | 0.47 |
| 22 | Pentadecanal- | 41.768 | $C_{15}H_{30}O$ | 226.3981 | | | 0.38 |
| 23 | Butane, 1,4-dichloro-2,3-bis(trimethylsiloxy)- | 42.333 | $C_{10}H_{24}Cl_2O_2Si_2$ | 303.3700 | | 2.02 | |
| 24 | 2-Pentadecanone, 6,10,14-trimethyl- | 42.370 | $C_{18}H_{36}O$ | 268.4778 | | | 0.81 |

**Table (3). Cont.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 | Bisphenol A | 43.745 | $C_{15}H_{16}O_2$ | 228.2863 | | | 0.98 |
| 26 | 1H-Inden-1-one, 2,3-dihydro-4,7-dimethyl- | 44.948 | $C_{11}H_{12}O$ | 160.2100 | | | 0.47 |
| 27 | Heneicosane | 46.407 | $C_{21}H_{44}$ | 296.5741 | | | 0.41 |
| 28 | Chrysene, 1,2,3,4,4a,7,8,9,10,11,12,12a-dodecahydro- | 47.438 | $C_{18}H_{24}$ | 240.3832 | | | 3.69 |
| 29 | 1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,10a-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-(1.alpha.,4a.beta., | 47.782 | $C_{21}H_{28}O_2$ | 312.4500 | | | 0.68 |
| 30 | Octanal, 2-(phenylmethylene)- | 48.470 | $C_{15}H_{20}O$ | 216.3187 | | | 1.52 |
| 31 | Cyclononasiloxane, octadecamethyl- | 48.773 | $C_{18}H_{54}O_9Si_9$ | 667.3855 | | 2.58 | |
| 32 | Docosane | 50.875 | $C_{22}H_{46}$ | 310.6006 | | | 0.50 |
| 33 | 1-Monolinoleoylglycerol trimethylsilyl ether | 51.797 | $C_{27}H_{54}O_4Si_2$ | 498.8863 | | 3.36 | |
| 34 | 4H-1-Benzopyran-4-one, 5,6,7-trimethoxy-2-(4-methoxyphenyl)- | 53.023 | $C_{19}H_{18}O_6$ | 342.3426 | | | 0.77 |
| 35 | Pentadecane | 53.968 | $C_{15}H_{32}$ | 212.4146 | | | 0.67 |
| 36 | δ-Amylvalerolactone | 55.428 | $C_{10}H_{18}O_2$ | 170.2487 | | | 0.23 |
| 37 | 2(1H)-Phenanthrenone, 3,4,4a,9,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)- | 56.433 | $C_{20}H_{28}O_2$ | 300.4400 | | | 3.35 |
| 38 | 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)- | 57.185 | $C_{22}H_{41}ClO_3$ | 389.0120 | | 1.95 | |
| 39 | Diethylmalonic acid, monochloride, pentadecyl ester | 58.005 | $C_{26}H_{54}$ | 366.7070 | | | 0.73 |
| 40 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | 58.513 | $C_{21}H_{44}$ | 296.5741 | | 2.90 | |
| 41 | 5,5-Diethylheptadecane | 59.98 | $C_{15}H_{30}O_2$ | 242.4000 | | | 0.21 |
| 42 | Oxirane, [(dodecyloxy)methyl]- | 61.815 | $C_{24}H_{46}O_4$ | 398.6000 | | 3.19 | |
| 43 | Oxalic acid, heptadecyl hexyl ester | 62.815 | $C_{21}H_{44}$ | 296.5741 | | | 0.7 |
| 44 | Heneicosane | 63.933 | $C_{24}H_{38}O_4$ | 390.5561 | | | 0.32 |
| 45 | Di-n-octyl phthalate | 65.737 | $C_{10}H_{16}O_2$ | 168.2328 | Mono | | 0.21 |
| 46 | α-Limonene diepoxide | 68.228 | $C_{18}H_{38}$ | 254.4943 | | | 0.45 |
| 47 | Octadecane | 68.485 | $C_{22}H_{46}$ | 310.6006 | | 23.75 | |
| 48 | 6,6-Diethylhoctadecane | 69.088 | $C_{30}H_{50}O_2$ | 442.7168 | Triterpe | | 0.76 |
| 49 | Betulin | 72.570 | $C_{12}H_{24}$ | 168.3200 | | | |
| 50 | 2-Undecene, 6-methyl-, (Z)- | 72.600 | $C_{27}H_{54}$ | 378.7177 | | 20.87 | |
| 51 | Cyclohexane, (1-decylundecyl)- | 73.985 | $C_{21}H_{44}$ | 296.5741 | | | 3.58 |
| 52 | Heneicosane | 74.350 | $C_{15}H_{26}O_2$ | 238.3657 | Sesquit | 6.58 | |
| 53 | Geranyl isovalerate | 77.420 | $C_{15}H_{26}$ | 206.3669 | Sesquit | | 0.28 |
| 54 | Isoledane | 57.185 | $C_{22}H_{41}ClO_3$ | 389.0120 | | 1.95 | |
| | Total % of monoterpene | | | | | 9.29 | 0.38 |
| | Total % of sesquiterpene | | | | | 16.75 | 27.20 |
| | Total % of triterpene | | | | | | 40.49 |

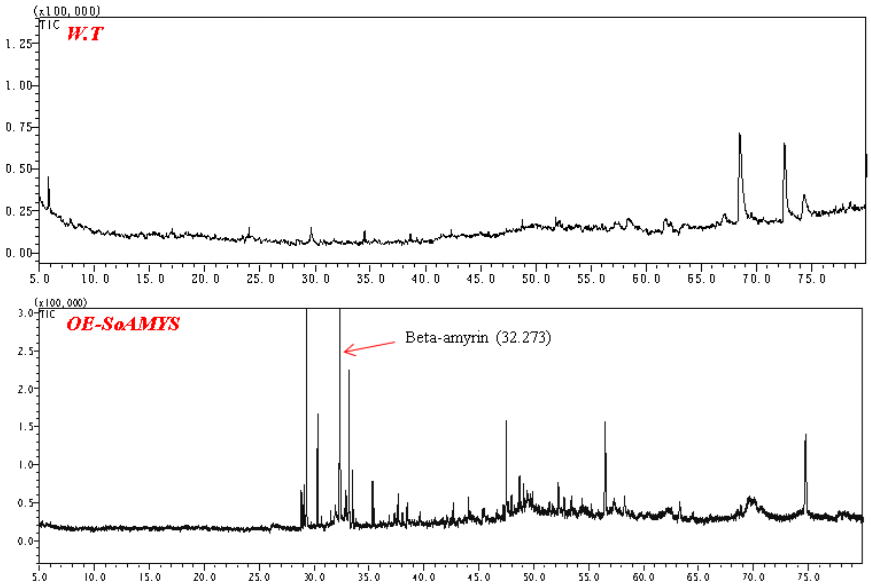


**Fig. (5).** Typical GC-MS mass spectrographs for terpenoids from leaf of *A. thaliana* plants.

## CONCLUSION

In this study, we cloned and functionally characterized one of the scarcely expressed triterpene synthase (*SoAMYS*), which is responsible for the production of beta-amyrin in *S. officinalis*. Also, we applied transgenic technology by transformed and expressed this gene into *A. thaliana* plants. Positive growth acceleration was clearly spotted in the transgenic lines *OE-SoAMYS-1*, *OE-SoAMYS-2* and *OE-SoAMYS-3*. These previous transgenic lines showed a high expression of *SoAMYS* gene, which related with the higher production of beta-amyrin. The success in overexpression of *SoAMYS* gene and production of beta-amyrin in transgenic *A. thaliana* plants, reverse the effectiveness of *A. thaliana* plant as a model system in synthesizing the same product through the same pathway. *SoAMYS* protein exhibits a strong sequence similarity to other triterpene synthases, it is clustered under TPS-f subfamily that contained one major squalene cyclase domain and four minor domains that are inserted into this major domain. These results will provide a foundation for understanding the function of *SoAMYS* gene and its regulatory mechanisms in triterpenoid synthesis in *S. officinalis*.

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## REFERENCES

Aharoni, A., A.P. Giri, S. Deuerlein, F. Griepink, W.J. de Kogel, F.W. Verstappen, H.A. Verhoeven, M.A. Jongsma, W. Schwab and H.J. Bouwmeester (2003). Terpenoid metabolism in wild-type and transgenic *Arabidopsis* plants. Plant Cell, 15 (12): 2866-2884.

Aharoni, A., M.A. Jongsma, T.Y. Kim, M.B. Ri, A.P. Giri, F.W.A. Verstappen, W. Schwab and H.J. Bouwmeester (2006). Metabolic engineering of terpenoid biosynthesis in plants. Phytochem. Rev., 5: 49–58.

Ali, M., P. Li, G. She, D. Chen, X. Wan and J. Zhao (2017) Transcriptome and metabolite analyses reveal the complex metabolic genes involved in volatile terpenoid biosynthesis in garden sage (*Salvia officinalis*). Sci. Rep., 7 (1): 16074.

Ali, M., R.M. Hussain, N.U. Rehman, G. She., P. Li, X. Wan, L. Guo and J. Zhao (2018). *De novo* transcriptome sequencing and metabolite profiling analyses reveal the complex metabolic genes involved in the terpenoid biosynthesis in blue anise sage (*Salvia guaranitica* L.). DNA Res., 25 (6): 597–617.

Ali, M., L. Miao, Q. Hou, D.B. Darwish, S.S, Alrdahe, A. Ali, A.V. Benedito, M. Tadege, X. Wang and Z. Zhao (2021). Overexpression of terpenoid biosynthesis genes from Garden Sage (*Salvia officinalis*) modulates *Rhizobia* interaction and nodulation in Soybean. Front. Plant Sci., 12: 783269.

Aminfar, Z., B. Rabiei, M. Tohidfar and M.H. Mirjalili (2019). Identification of key genes involved in the biosynthesis of triterpenic acids in the mint family. Sci. Rep., 9 (1): 15826.

Atsuko, T. and O. Hiroshi (2011). Phylogenetic relationships among subgenera, species, and varieties of Japanese *Salvia* L. (Lamiaceae). J. Plant Res., 124: 245–252.

Bertea, C.M., A. Voster, F.W. Verstappen, M. Maffei, J. Beekwilder and H.J. Bouwmeester (2006). Isoprenoid biosynthesis in *Artemisia annua*: cloning and heterologous expression of a germacrene A synthase from a glandular trichome cDNA library. Arch. Biochem. Biophys., 448 (1-2): 3–12.

Blum, M., H. Chang, S. Chuguransky, T. Grego, S. Kandasaamy, A. Mitchell, G. Nuka, T. Paysan-Lafosse, M. Qureshi, S. Raj, L. Richardson, G.A. Salazar, L. Williams, P. Bork, A. Bridge, J. Gough, D.H. Haft, I. Letunic, A. Marchler-Bauer, H. Mi, D.A. Natale, M. Necci, C.A. Orengo, A.P. Pandurangan, C. Rivoire, C.J. A. Sigrist, I. Sillitoe, N.

Thanki, P.D. Thomas, S.C.E. Tosatto, C.H. Wu, A. Bateman and R.D. Finn (2020). The InterPro protein families and domains database: 20 years on. Nucleic Acids Res., doi: 10.1093/nar/gkaa977.

Bohlmann, J., G. Meyer-Gaueand and R. Croteau (1998). Plant terpenoid synthases:molecular biology and phylogenetic analysis. Proc. Natl. Acad. Sci., 95 (8): 4126-4133.

Chen, X., H. Chen, J.S. Yuan, T.G. Köllner, Y. Chen, Y. Guo, X. Zhuang, X. Chen, Y.J. Zhang, J. Fu, A. Nebenführ, Z. Guo and F. Chen (2018). The rice terpene synthase gene *OsTPS19* functions as an (S)-limonene synthase in planta, and its overexpression leads to enhanced resistance to the blast fungus *Magnaporthe oryzae*. Plant Biotechnol. J., 16 (10): 1778-1787.

Cheng, A.X., C.Y. Xiang, J.X. Li, C.Q. Yang, W.L. Hu, L.J. Wang, Y.G. Lou and X.Y. Chen (2007). The rice (E)-beta-caryophyllene synthase (*OsTPS3*) accounts for the major inducible volatile sesquiterpenes. Phytochemistry, 68: 1632–1641.

Danner, H., G.A. Boeckler, S. Irmisch, J.S. Yuan, F. Chen, J. Gershenzon, S.B. Unsicker and T.G .Köllner (2011). Four terpene synthases produce major compounds of the gypsy moth feedinginduced volatile blend of *Populus trichocarpa*. Phytochemistry, 72 (9): 897–908.

Davidovich-Rikanati, R., E. Lewinsohn, E. Bar, Y. Iijima, E. Pichersky and Y. Sitrit (2008). Overexpression of the lemon basil alpha-zingiberene synthase gene increases both mono and sesquiterpene contents in tomato fruit. Plant J., 56: 228–238.

Degenhardt, J., T. G. Köllner and J. Gershenzon (2009). Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. Phytochemistry, 70: 1621–1637.

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J.M. Claverie and O. Gascuel (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res., 36: W465–W469.

Dudareva, N., D. Martin, C.M. Kish, N. Kolosova, N. Gorenstein, J. Fäldt, B. Miller and J. Bohlmann (2003). (E)-beta-ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: function and expression of three terpene synthase genes of a new terpene synthase subfamily. Plant Cell, 15 (5): 1227–1241.

Fäldt, J., G. Arimura, J. Gershenzon, J. Takabayashi and J. Bohlmann (2003). Functional identification of *AtTPS03* as (E)-beta-ocimene synthase: a monoterpene synthase catalyzing jasmonate- and wound-induced volatile formation in *Arabidopsis thaliana*. Planta, 216 (5): 745–751.

Gutensohn, M., I. Orlova, T.T. Nguyen, R. Davidovich-Rikanati, M.G. Ferruzzi, Y. Sitrit, E. Lewinsohn, E. Pichersky and N. Dudareva

(2013). Cytosolic monoterpene biosynthesis is supported by plastid-generated geranyl diphosphate substrate in transgenic tomato fruits. Plant J., 75 (3): 351–363.

Hoshino, T., K. Shimizu and T. Sato (2004). Deletion of the Gly600 residue of *Alicyclobacillus acidocaldarius* squalene cyclase alters the substrate specificity into that of the eukaryotic-type cyclase specific to (3S)-2,3-oxidosqualene. Angew. Chem. Int. Ed. Engl., 43 (48): 6700-6703.

Kildegaard, K.R., J.A. Arnesen, B. Adiego-Pérez, D. Rago, M. Kristensen, A.K. Klitgaard, E.H. Hansen, J. Hansen and I. Borodina (2021). Tailored biosynthesis of gibberellin plant hormones in yeast. Metab. Eng., 66: 1–11.

Köllner, T.G., C. Schnee, J. Gershenzon and J. Degenhardt (2004). 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, 16 (5): 1115-11131.

Köllner, T.G., M. Held, C. Lenk, I. Hiltpold, T.C. Turlings, J. Gershenzon and J. Degenhardt (2008). A maize (E)-beta-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. Plant Cell, 20 (2): 482-494.

Korankye, A.E., R. Lada, S. Asiedu and C. Claude (2017). Plant senescence: the role of volatile terpene compounds (VTCs). Am. J. Plant Sci., 8: 3120-3139.

Liu, J., F. Huang, X. Wang, M. Zhang, R. Zheng, J. Wang and D. Yu (2014). Genome-wide analysis of terpene synthases in soybean: functional characterization of *GmTPS3*. Gene, 544 (1): 83–92.

Luck, K., X. Chen, A.M. Norris, F. Chen, J. Gershenzon and T.G. Köllner (2020). The reconstruction and biochemical characterization of ancestral genes furnish insights into the evolution of terpene synthase function in the Poaceae. Plant Mol. Biol., 104 (1-2): 203-215.

Pott, D.M., S. Osorio and J.G. Vallarino (2019). From central to specialized metabolism: an overview of some secondary compounds derived from the primary metabolism for their role in conferring nutritional and organoleptic characteristics to fruit. Front. Plant Sci., 10: 835.

Ro, D., J. Ehlting, C. Keeling, R. Lin, N. Mattheus and J. Bohlmann (2006). Microarray expression profiling and functional characterization of *AtTPS* genes: Duplicated *Arabidopsis thaliana* sesquiterpene synthase genes *At4g13280* and *At4g13300* encode root-specific and wound-inducible (Z)-Y-bisabolene synthases. Arch. Biochem. Biophys., 448 (1-2): 104-116.

Su-Fang, E., M. Zeti-Azura, O. Roohaida, A.S. Noor, I. Ismanizan and Z. Zamri (2014). Functional characterization of sesquiterpene synthase from *Polygonum minus*. Sci. World J., 10.1155/2014/840592.

Taniguchi, S., S. Miyoshi, D. Tamaoki, S. Yamada, K. Tanaka, Y. Uji, S. Tanaka, K. Akimitsu and K. Gomi (2014). Isolation of jasmonate-induced sesquiterpene synthase of rice: product of which has an antifungal activity against *Magnaporthe oryzae*. J. Plant Physiol., 171: 625–632.

Tholl, D., W. Boland, A. Hansel, F. Loreto, U.S. Röse and J.P. Schnitzler (2006). Practical approaches to plant volatile analysis. Plant J., 45: 540–560.

Wang, Q., M. Jia, J.H. Huh, A. Muchlinski, R.J. Peters and D. Tholl (2016). Identification of a dolabellane type diterpene synthase and other root-expressed diterpene synthases in *Arabidopsis*. Front. Plant Sci., *7*: 1761.

Wang, Y., Q. Yang, Y. Zhu, L. Zhao, P. Ju, G. Wang, C. Zhou, C. Zhu, H. Jia, Y. Jiao, H. Jia and Z. Gao (2022). *MrTPS3* and *MrTPS20* are responsible for β-caryophyllene and α-pinene production, respectively, in Red Bayberry *(Morella rubra*). Front. Plant Sci., 12: 798086.

Wendt, K.U. and G.E. Schulz (1998). Isoprenoid biosynthesis: manifold chemistry catalyzed by similar enzymes. Structure (London, England: 1993), 6 (2): 127-133.

Wendt, K.U., A. Lenhart and G.E. Schulz (1999). The structure of the membrane protein squalene-hopene cyclase at 2.0 A resolution. J. Mol. Biol., 286 (1): 175-187.

Wendt, K.U., G.E. Schulz, E.J. Corey and D.R. Liu (2000). Enzyme mechanisms for polycyclic triterpene formation. Angew Chem. Int. Ed. Engl., 39 (16): 2812-2833.

Yu, F. and R. Utsumi (2009). Diversity, regulation, and genetic manipulation of plant mono-and sesquiterpenoid biosynthesis. Cell Mol. Life Sci., 66: 3043–3052.

# الإستنساخ الجزيئي والتوصيف لجين (*SoAMYS*) beta-amyrin synthase من نبات المريمية

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تلعب التربينات (terpenoids or isoprenoids) دورًا رئيسيًا في الأيض الأولي والثانوي في مجموعة متنوعة من الكائنات الحية، ففى النباتات يتم تحفيز التخليق الحيوي للتربينات من خلال مسارين رئيسيين هما مسار الmevalonate (MVA) وكذلك مسار ال non-mevalonate ويحتوى جينوم نبات المريمية (*Salvia officinalis*) على خمسة وستين جينًا من جينات التخليق الحيوي للتربين *SoTPS* ولم يتم دراسة سوى عدد قليل من هذه الجينات سابقة الذكر ودورها فى التخليق الحيوي للتربين. فى هذه الدراسة تم إجراء التوصيف الوظيفي لجين *SoAMYS* وذلك من خلال نقله إلى نبات الأرابيدوبسيس (*Arabidopsis thaliana*) ووجد أن الزيادة المفرطة في تعبيرهذا الجين *SoAMYS* أدت إلى تحسين تكون الأزهار على نبات الأرابيدوبسيس المعدل وراثيًا مقارنة بالنباتات البرية. كما أظهر التحليل الأيضي كذلك إنتاج أنواع مختلفة من التربين، وخاصة ترايتيربين بيتا أميرين (beta-amyrin) والذى أظهر زيادة في السلالات ذات التعبير العالي من جين *SoAMYS* وانخفاضًا فى النباتات البرية، مما يشير إلى أن جين ال *SoAMYS* يعمل على تخليق البيتا أميرين سينسيز في النبات. وقد تم دعم هذه النتيجة بشكل أكبر باستخدام أدوات المعلوماتية الحيوية للتنبؤ بموقع الجين المفترض داخل الخلية وكذلك التعبير المفترض الخاص بهذا الجين *SoAMYS* داخل الأنسجة المختلفة. وفى نفس السياق وجد أن هذا الجين *SoAMYS* يقع داخل السيتوبلازم وأن أعلى تعبير لهذا الجين يكون في مرحلة تطور الزهرة (flowers stage 15, Pedicels). ويعتبر هذا هو التقرير الأول عن عزل وتوصيف جين متعلق بأنتاج مركب البيتا أميرين (beta-amyrin) باعتباره ترايتيربين (triterpene) من نبات المريمية *S. officinalis*.