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Original Article

Bioinformatics analysis of the squalene synthase gene and the amino acid sequence in ginseng species

Can Ding[1], Chang-Ling Zhao[2], Zhong-Jian Chen[3], Fu-Gang Wei[3], De-Gong Song[3], Kang-Ru Miao[3]

[1]Department of Tobacco Production, College of Tobacco Sciences, Yunnan Agricultural University, Heilongtan, Kunming, Yunnan, China; [2]Department of Traditional Chinese Medicinal Materials, College of Agronomy and Biotechnology, Yunnan Agricultural University, Heilongtan, Kunming, Yunnan, China; [3]Miaoxiang Sanqi Industrial Corporation Ltd. of Wenshan City, Wenshan, Yunnan, China

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Abstract: The cDNA sequence, their structure, physical properties, signal peptide, hydrophobicity, hydrophilicity, subcellular localization domain of transmembrane domain and evolutionary relationship of encoded amino acid sequences were analyzed in squalene synthase of 9 species of ginseng plant using bioinformatics methods on GenBank. The results showed that the averaged similarity of squalene synthase cDNA sequence structure in Ginseng species was 96.245%, the similarity of the amino acid encoding sequence was 95.5%. The secondary structure prediction results showed that the amino acid sequence of 9 squalene synthase had α helix and random coil as the main components. After the phylogenetic analysis in 9 kinds of ginseng species, we found that they can be divided into two subfamilies. The analysis showed that plants, animals, yeasts belonged to different species, the homology was high within plant species and animal species. By analyzing the ginseng species squalene synthase and their encoding gene bioinformatics features, we can provide the theoretical reference for the squalene synthase gene cloning and the genetic manipulation.

Keywords: Ginseng species, squalene synthase, bioinformatics analysis

Introduction

Triterpenoid saponins is the main active ingredient of panax, ginseng and other important medicinal plants. With the biosynthetic pathway analysis of obtained triterpenoid saponins, we learnt that squalene synthase is at the branch point in the metabolic pathway, it is an important speed-limiting enzymes.

The synthesis pathway of triterpenoid saponins was clear, the saponin biosynthesis pathway was divided into three steps: ① Biosynthesis of active isoprene unit Δ3-isopentenyl pyrophosphate (IPP) and γ, γ-dimethylallyl diphosphate (DMAPP). ② Biosynthesis of triterpenes carbocyclic system. ③ After the complex processes of ring functionalization reactions, complete triterpenoid saponins molecules eventually formed [1]. Mevalonate pathway (MVA) was considered as the key pathway of saponin triterpenoid aglycone synthesis. In MVA, 3 of acetyl-CoA molecules were raw materials, after mevalonate and gradually synthesis of IPP, monoterpenes, diterpenes and other terpenoids would be generated by the IPP polymerization. Two acetyl-CoA molecules were condensed into Acetyl CoA under the catalysis of acetyl CoA C acyl transfer enzymes (AACT) [2]. Acetoacetyl-CoA and acetyl-CoA were condensed into 3-hydroxy-3-methyl-glutaryl coenzyme single A (HMG-CoA) under the action of 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMGS) [3]. HMG-CoA was reduced to mevalonate under 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGR). This reaction was the rate-limiting reaction on the MVA pathway [4]. After naphthalene phosphorylation, decarboxylation and dehydration, the MVA had become isopentenylallyl diphosphate (IPP). IPP isomerization would result in DMAPP, the two isomers combined into GPP, the latter would release pyrophosphate and turn into monoterpenes. IPP and GPP were connected from the head to tail, farnesyl pyrophosphate (FPP) would be generated, FPP would become SQ under the effects of SS. The squalene epoxidase (SQE) would be converted into squalene

Table 1. Squalene synthase cDNA sequence and the encoded amino acid sequence in 9 kinds of Ginseng species

Nucleotide registry number	Protein registry number	cDNA sequence length/bp	Encoding amino acids length/aa
EU502717	ACA66014	1390	415
GU183406	ACZ71037	1329	415
GQ468527	ACV88718	1335	415
AB010148	BAA24289	1476	415
AB115496	BAD08242	1434	415
GU997681	AED99863	1330	415
AM182456	CAJ58418	1497	415
AM182457	CAJ58419	1497	415
DQ186630	ABA29019	1270	415

2,3-oxide(SQ) under the oxidized catalytic conversion [6]. Under the effects of squalen 2,3-oxide cycloartenol cyclase (OSCs) and a series of complex functionalization reactions with redox reactions, complete sterols and triterpenes would be obtained [7]. IPP and FPP were connected to form GGPP, tetraterpenes would form after pyrophosphate removal. GGPP was further connected with more IPP from head to tail to form polyterpenes [5]. From triterpenoid saponin synthesis pathway, we learnt that squalene synthase was at the branch point of metabolic pathway FPP and other metabolic products, it was an important rate-limiting enzyme [8].

Some important medicine plants, such as ginseng and panax, the triterpenoid substances were the main active ingredients. The triterpenoid synthesis pathway study of these medicinal plants will contribute to the regulation and the biosynthesis of the plant triterpenoids in the future to thereby increase the generation of the active ingredients. Squalene synthase was the key enzyme of triterpenoid synthesis pathway, at present, the squalene synthase cDNA of yeast, human, rat, mouse and a variety of plants were cloned and sequenced. But the squalene synthase gene bioinformatics analysis of Ginseng species were rarely reported. In this study, we used the bioinformatics methods to analyze the squalene synthase cDNA sequence and encoded amino acid sequence in 9 kinds of Ginseng species, we also predicted and analyzed the composition, structure, physicochemical properties, subcellular localization, system attribution and characteristics of functions to provide reference for the squalene synthase cloning and genetic manipulation.

Materials and methods

Materials

The registered, officially published data were collected from National Center for Biotechnology Information (NCBI). The squalene synthase cDNA sequence and encoded amino acid sequence in 9 kinds of Ginseng species with definite source were documented in (**Table 1**).

Methods

The U.S. National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov) was used for line analysis [9, 10]. The composition and properties of squalene synthase cDNA and amino acid sequence were analyzed using ProtParam online analysis. The checking of open reading frame (ORF) was performed with ORF Finder online tool. The search of sequence similarity was completed with Blastn and Blastp [11]. Clustal X1.8 was use for multiple sequence comparison [12]. The SignalP software version 3.0 was used for the signal peptide prediction [14]. The target P1.1Server was used for transit peptide prediction [13]. MITOPROT [prediction of Mitochondrial targeting peptide) was used for mitochondrial transit peptide analysis [15]. Hydrophobic and hydrophilic features were analyzed using Protscale. The software TNHHMM (prediction transmembrane helices in proteins) 2.0 Server was used for transmembrane structure domain [16]. PSORT Prediction [17] can be used in subcellular localization analysis. Clustal X 1.8 [18] and MEGA4 were used to complete the construction of amino acid sequence phylogenetic tree. The sequence alignment was first compared in the Clustal X1.8 software, the phylogenetic tree construction was performed with MEGA4 [19].

Results

Physical and chemical properties of the squalene synthase cDNA sequence structure and the amino acid encoding sequence in Ginseng species

9 of the Ginseng species plants (The initiation codon of squalene synthase cDNA sequences

Table 2. Physical and chemical properties of the squalene synthase cDNA sequence structure and the amino acid encoding sequence in Ginseng species

Nucleotide registry number	EU502717	GU183406	GQ468527	AB010148	AB115496	GU996781	AM182456	AM182457	DQ186630
Length of genes/bp	1390	1329	1335	1476	1434	1330	1497	1497	1270
Open reading frame length/bp	1247	1247	1247	1247	1247	12247	1247	1247	1247
Start site and codon	59ATG	61ATG	67ATG	87ATG	46ATG	49ATG	1ATG	1ATG	11ATG
Stop site and codon	1306	1308	1314	1334	1293	1296	1248	1248	1258
Number of encoded amino acids	415	415	415	415	415	415	415	415	415
Relative molecular mass	47015.5	47120.5	47129.5	47055.5	47055.5	47128.5	47097.4	47061.4	47166.6
Theoretical isoelectric point values	6.36	6.07	6.19	6.36	6.36	6.24	6.19	5.96	6.5
Positively charged residues	50	50	50	50	50	52	50	50	51
Negatively charged residues	46	44	45	46	46	47	45	43	48
Total number of atoms	6623	6619	6624	6623	6623	6631	6623	6598	6636
Extinction coefficient	1.019	1.084	1.081	1.019	1.019	1.018	1.081	1.085	1.017
Half life period	30h	30h	30h	30h	30h	30h	30h	30h	30h
Instability coefficient	41.91	39.65	39.59	42.38	42.38	41.34	38.33	37.95	41.05
Fat coefficient	94.27	96.12	96.12	94.51	94.51	94.15	96.82	94.24	93.57
Overall average hydrophilicity	-0.055	-0.028	-0.046	-0.052	-0.052	-0.062	-0.04	-0.011	-0.084

Table 3. Similarity of the squalene synthase cDNA sequence structure and the amino acid encoding sequence in Ginseng species

Registry Number (nucleotide/protein)	Nucleotide similarity	Amino acid similarity
EU502717/ACA66014	98%	98%
GU183406/ACZ71037	94%	93%
GQ468527/ACZ88718	94%	93%
AB010148/BAA24289	99%	98%
AB115496/BAD08242	99%	98%
GU997681/AED99863	99%	99%
AM182456/CAJ58418	94%	93%
AM182457/CAJ58419	93%	92%

Table 4. Possibility of the potential transit peptide in squalene synthase sequence

Protein registry number	Reliability			
	Chloroplast transit peptide	Mitochondrial target peptide	Signal peptide of secretory pathway	Reliability prediction
ACA66014	0.065	0.236	0.062	3
ACZ71037	0.073	0.187	0.073	3
ACV88718	0.069	0.179	0.067	3
BAA24289	0.065	0.236	0.062	3
BAD08242	0.065	0.236	0.062	3
AED99863	0.063	0.223	0.064	3
CAJ58418	0.069	0.179	0.067	3
CAJ58419	0.078	0.187	0.078	3
ABA29019	0.063	0.223	0.064	3

was ATG, the stop codons were TAA, TAG, TGA, **Table 2**) had the same open reading frame and the number of amino acids. From the relative molecular analysis, the DQ186630 had the largest relative molecular mass, EU502701 had the smallest relative molecular mass. In theoretical isoelectric point value analysis, AM182457 was the minimum, DQ186630 was the maximum. Residues negative charge, total atoms and extinction coefficient were basically the same. The half-life period was exactly the same. According to stability coefficients GU183406, GQ468527, AM182456, AM182457 were stabilize proteins, the remaining were non-stable protein.

Similarity of the squalene synthase cDNA sequence structure and the amino acid encoding sequence in Ginseng species

The squalene synthase cDNA sequence structure and the amino acid encoding sequence in Ginseng species had high levels of similarity. Analysis from (**Table 3**) showed that the averaged similarity of squalene synthase cDNA sequence structure in Ginseng species was 96.245%, the similarity of the amino acid encoding sequence was 95.5%. Squalene synthase may have particular base preference and codon degeneracy in different plants.

Signal peptide, transit peptide, hydrophobicity/hydrophilicity, transmembrane domain, and subcellular localization features of squalene synthase

Characteristics of the signal peptide and the transit peptide: Signal peptide was the N-terminal sequence of 15 to 30 amino acids in the synthesis of a precursor form of secreted proteins and membrane proteins [20]. In protein synthesis, this precursor polypeptide used the sequence to form membrane-bound ribosomes, the peptide chain were extended through the membrane. Membrane peptidase would remove the signal peptide through the membrane. Transit peptide would induce the newly synthesized peptides into the organelle. In addition to cell signaling proteins, various intrinsic proteins used transit peptides to reach organelles. The signal peptide was a part of the transit peptide which was located at one amino acid sequence near the N-terminus. The transit peptide function required the presence of a signal peptide [21]. According to (**Table 4**), the prediction probability of ACA66014.1 was 3 which meant that the possibility was between 0.6 and 0.8. The reliability of the chloroplast transit peptide was only 0.065, the reliability of secretory pathway signal peptide was 0.062, these two values were too low. So there can be no chloroplast transit peptide or signal peptide of secretion pathway. The target peptide reliable value of mitochondrial was 0.236, it was possible to exist. The remaining eight species of plants were similar with ACA66014.1.

Hydrophobic and absorbent predictions of protein: Hydrophobic and absorbent predictions of

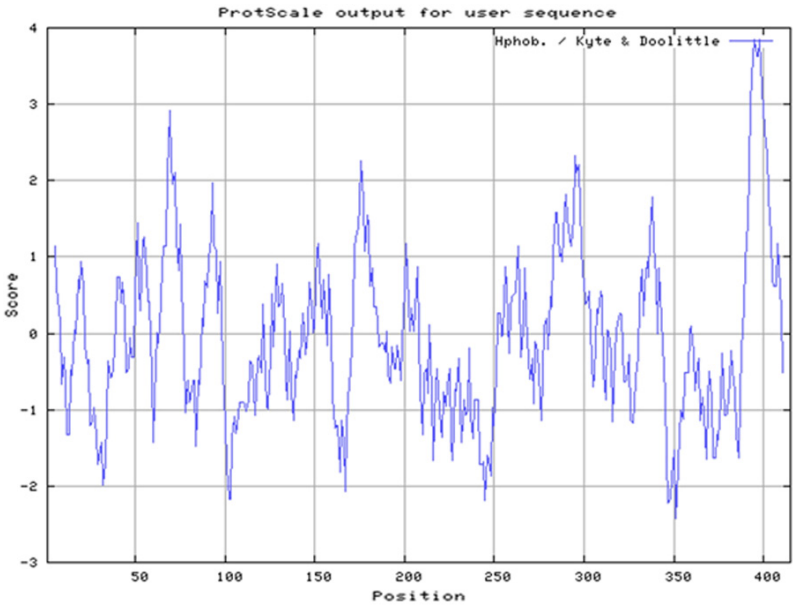

Figure 1. Hydrophobic and absorbent predictions of ACA66014.

Table 5. Subcellular localization of squalene synthase proteins

Protein registry number	Degree of certainty				
	Chloroplast thylakoid membrane	Golgi bodies	Plasma membrane	Endoplasmic reticulum	Microfossils
ACA66014	0.502	0.4	0.64	0.3	
ACZ71037	0.491	—	0.73	0.2	0.31
ACV88718	0.485	—	0.73	0.2	0.326
BAA24289	0.502	0.4	0.64	0.3	—
BAD08242	0.502	0.4	0.64	0.3	—
AED99863	0.502	0.4	0.64	0.3	—
CAJ58418	0.485	—	0.73	0.2	0.326
CAJ58419	0.485	—	0.73	0.2	0.362
ABA29019	0.502	0.4	0.64	0.3	—

protein were the necessary process of protein secondary structure prediction and functional domain dividing. According Protscale application analysis, the section 350 Arg of squalene synthase polypeptide chain in ACA66014 had the lowest hydrophilic score of -4.5 meaning that it had the strongest hydrophilic ability. Ile protein in section 390 had the most hydrophobic ability with the score of 4.5. From the whole peptide chain, the hydrophilic amino acids were distributed evenly, the number was larger than the number of hydrophobic amino acids (**Figure 1**). The entire polypeptide chain of ACA66014 presented the hydrophilic feature, it had no significant hydrophobic regions. They all belonged to the hydrophilic proteins. After the squalene synthase protein analysis in the remaining eight kinds of Ginseng species plants using protscale, their hydrophobicity/absorption were alike with the ACA66-014.

Subcellular localization features of squalene synthase in Ginseng species: With PSORT Prediction, the subcellular localization of 9 kinds of Ginseng species squalene synthase protein, we learnt that the all known squalene synthase were located in the membrane structure. The possibility of ACA66014 squalene synthase located in the plasma membrane was 0.64 which was significantly greater than possibilities in the chloroplast thylakoid membrane, Golgi membrane and endoplasmic reticulum. So the squalene synthase was mostly likely to be located in the plasma membrane. The remaining eight kinds of Ginseng species squalene synthase were similar with ACA66014. The probability of locating in the plasma membrane was the maximum (**Table 5**).

Characteristics of transmembrane domains: The transmembrane domains were the main combination location of membrane protein and membrane lipids. With the HNN SECONDARY STRUTURE PREDICTION METHOD analysis, the secondary structure of squalene synthase were α helix, extended chain structure and random coils, the proportion of three forms were shown in (**Table 6**). The analysis showed that in this nine squalene synthase proteins, the percentage of 3 kinds of secondary structure were as follows: α helix > random coil > extended chain structure. According to analysis of TMHMM posterior probabilities for sequence (**Figure 2**), ACA66014.1 had two transmembrane domains, the protein N-terminal was located outside of the membrane. TMHMM analysis results of the remaining eight kinds of squalene synthase were similar with ACA66014.

Table 6. Secondary structure analysis of squalene synthase

Protein registry number	Ahelix number	α helix ratio/%	Extended chain structure number	Extended chain structure ratio /%	Random coil number	Random coil ratio /%
ACA66014	265	63.86	29	6.99	121	29.16
ACZ71037	256	61.69	41	9.88	118	28.43
ACV88718	267	64.34	26	6.27	122	29.40
BAA24289	270	65.06	29	6.99	116	27.95
BAD08242	270	65.06	29	6.99	116	27.95
AED99863	276	66.51	24	5.78	115	27.71
CAJ58418	265	63.86	26	6.27	124	29.88
CAJ58417	253	60.96	40	9.64	122	29.40
ABA29019	272	65.54	24	5.78	119	28.67

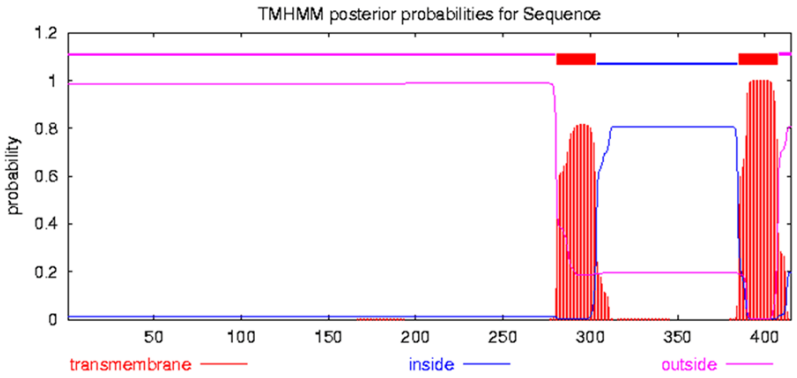

Figure 2. ACA66014.1 transmembrane domains prediction.

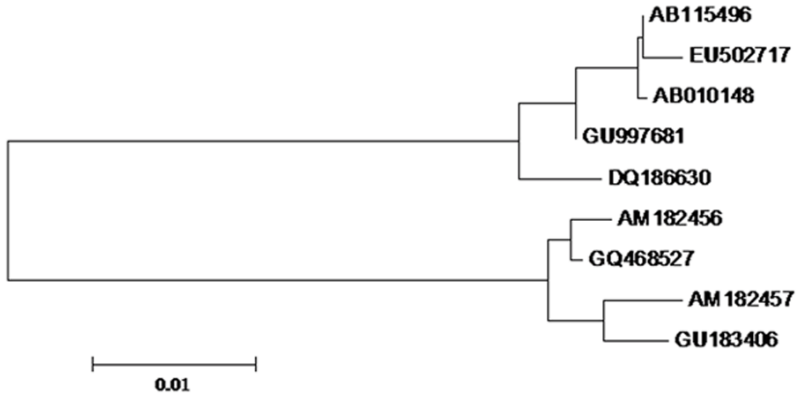

Figure 3. Phylogenetic tree analysis of squalene system in 9 species of ginseng plant.

Phylogenetic analysis of squalene synthase system

After the phylogenetic tree analysis of squalene synthase in 9 individual Ginseng species (**Figure 3**), they can be divided into two groups, AB115496, EU502717, AB010148, GU997681, DQ186630 were in the first group. AM182456, GQ468527, AM182457, GU183406 were in the second groups. After the phylogenetic tree analysis of saccharomyces cerevisiae (HM012838), ginseng (EU502717, GU183406, GQ468527, AB010148, AB115496), American ginseng (GU997681, AM182456, AM182457), San QI (DQ186630), Arabidopsis (D29017), tobacco (U60057), human (X69141), mice (D29016), Ganoderma (DQ494674), potatoes (AB022599), tomato (NM001247787), scutellaria baicalensis (HQ829974), psammosilene tunicoides (EF585250), zhiyuan (DQ672339), artemisinin (AF302464) (**Figure 4**). The analysis showed that plants, animals, yeasts belonged to different species, the homology was high within plant species and animal species.

Discussion

Basic framework of synthesis pathway of biological plant triterpenoid saponins was gradually clear, the use of genetic engineering for the pathway regulation had achieved desired results. The cloning and preliminary expression of one of the key enzymes namely as squalene synthase gene in triterpenoid saponins synthesis pathway had been realized at the molecular level. At the same time, the plant SS gene regulation studies had made some progress. The experimental results of Therlfall, etc [22] showed that the tobacco cal-

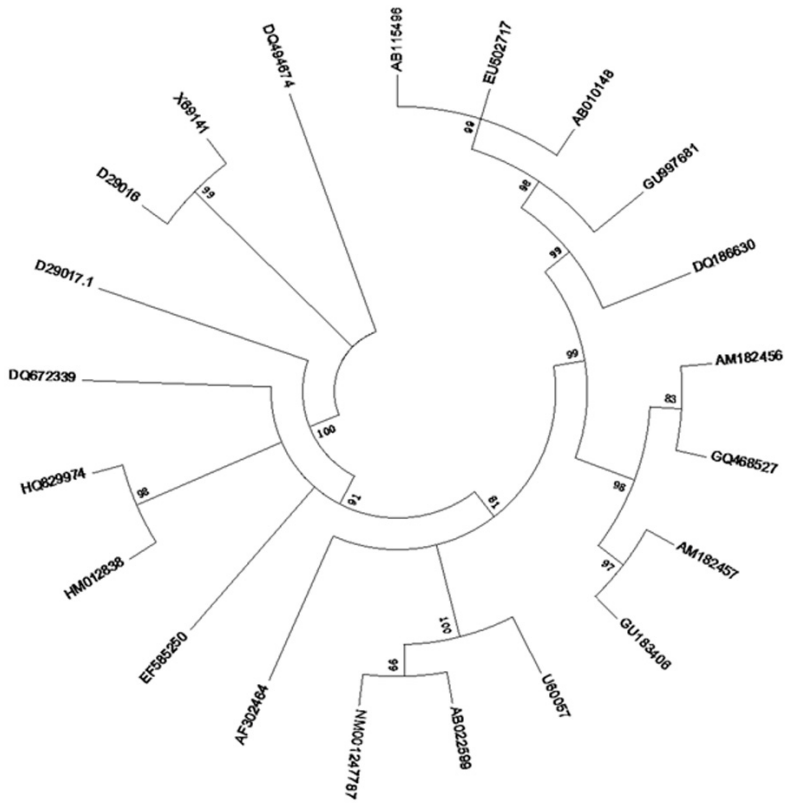

Figure 4. Phylogenetic tree analysis of squalene synthase system in different species.

lus under fungal elicitors would reduce the sterol biosynthesis and accumulation and the SS activity simultaneously in calli. Devarenne found that in tobacco cells treated with the elicitor, SS showed no change in the mDNA levels. It indicated that SS gene regulation was likely to occur in multiple levels from transcription to post-translational processing [23]. However, studies still had some problems, the identification of key genes and cloning were performed in narrow range of species, and it was more common in model organism research. The related enzymes of triterpenoid saponins in synthetic pathway were limited in content and showed low activity, this will bring great difficulties to chemical detection, purification and related enzyme activity assay of terpenoids.

In this study, the ginseng species squalene synthase gene analysis was performed with bioinformatics. The nucleotide sequence of ginseng species squalene synthase showed an average similarity of 96.245%, the average similarity of amino acids was 95.5%. The secondary structure prediction results showed that the amino acid sequence of 9 squalene synthase had α helix and random coil as the main components. After the phylogenetic analysis in 9 kinds of ginseng species, we found that they can be divided into two subfamilies: AB115496, EU502717, AB010148, GU997681, DQ-186630 were in Group 1; AM182456, GQ468527, AM-182457, GU183406 were in Group 2. The analysis showed that plants, animals, yeasts belonged to different species, the homology was high within plant species and animal species.

The bioinformatics analysis of squalene synthase can provide the reference for squalene synthase cloning. The relationship between the level of expression of squalene synthase gene and saponin levels can be further analyzed. For example, it needed further study whether the squalene synthase gene expression would directly result in saponin content increase. Furthermore we can use the genetic engineering technology to effectively alleviate the insufficient resources such as triterpenoid saponins and other medicinal plant insufficiency. It will provide basis for mass production of triterpenoid saponins.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chang-Ling Zhao, College of Agronomy and Biotechnology, Yunnan Agricultural University, Heilongtan, Kunming, Yunnan, China. Tel: +86-0871-65227213; Fax: +86-0871-65227730; E-mail: zhaoplumblossom7@163.com

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