Pak. J. Bot., 50(1): 231-243, 2018.

β-AMYRIN SYNTHASE, ONE OF THE MOST IMPORTANT KEY ENZYMES FOR TRITERPENE SKELETON FORMATION IN HIGHER PLANTS

YONG-SHENG MA[†], RUI YANG[†], SHAN ZHOU, YAN-CHAO YIN, XIAO-DONG ZHANG AND YING LIU[*]

School of Life Science, Beijing University of Chinese Medicine
[†]These authors contributed equally to this paper
[*]Corresponding author's email: liuyliwd@sina.comPhone: +8601084738646; Fax: +8601084738611

Abstract

β-amyrin synthase (*β*-AS) is one of the most important key enzymes involved in mevalonic acid (MVA) pathway. It is a cyclase responsible for cyclization of 2, 3-oxidosqualene into *β*-amyrin, which is defined as an important branch point between primary and secondary metabolism. It has been found in 37higher plant species. In this paper, we obtained 475 DNA sequences, 220 mRNA sequences, and 99 amino acid sequences of *β-AS* registered in NCBI by Oct, 2016, andanalyzed conserved domains and the evolutionary relationships between different species with DNAMAN 6.0.3.99 and MEGA 5.0. In order to get the latest and comprehensive information of *β*-AS, more than 300 papers were searched and 80 of them were reviewed. Pub Med, Web of Science, Science Direct, and Research Gate, were information sources through the search terms of "*β*-amyrin synthase", "biosynthesis", "oxidosqualene cyclases" and their combinations, mainly from year 2010 to 2016. Studies were selected from Science Citation Index journals. All of the references linked to the registered DNA and mRNA sequences in NCBI database were also reviewed. The full-length of *β-AS* DNA sequence ranges from 3900 bp to 8800 bp, and *β-AS* mRNA sequence ranges from 2100 bp to 2900 bp. The bioinformatic analysis and a lot of papers show that Gln-Trp (QW) motifs, Asp-Cys-Thr-Ala-Glu (DCTAE) motif, and Met-Trp-Cys-Tyr-Cys-Arg (MWCYCR) motif, are mainly responsible for its catalytic function. So far, the function of *β-AS* has been verified in 30 species. This paper will lay a foundation for further studies of *β*-AS and other oxidosqualene cyclases for triterpene skeleton formation in higher plants.

Key words: *β*-amyrin synthase, Triterpene, MVA pathway, Bioinformatic analysis, Oxidosqualene cyclase.

Abbreviations: *α*-AS = *α*-amyrin synthase, *β*-AS = *β*-amyrin synthase, CAS = cycloartenol synthase, CNVs = copy number variations, DMAPP = dimethylallyl pyrophosphate, DS = dammarenediol-II synthase, FPP = farnesvl diphosphate, GA = glycyrrhizic acid, InDel = insertion-deletion length polymorphism, IPP = isopentenyl diphosphate, LS = lanosterol synthase, LUS = lupeol synthase, MeJA = methyl jasmonate MJ, MEP = 2-C-methy-D-erythritol-4-phosphate, MVA = mevalonic acid, N-J = Neighbor-Joining, OSCs = oxidosqualene cyclases, SNPs = single nucleotide polymorphisms, SQE = squalene monooxygenase, SQS = squalene synthase

Introduction

Terpenoids are very important secondary metabolites in higher plants, including monoterpene, sesquiterpenes, diterpene, triterpene, and polyterpene. Many of them are important active ingredients (Parveen *et al.*, 2010; Shi *et al.*, 2015; Shi *et al.*, 2016; Basyuni *et al.*, 2007), and possess remarkable pharmacological properties, such as antitumor (Petronelli *et al.*, 2009), anti-HIV (Wei *et al.*, 2008; Kongkum *et al.*, 2013; Callies *et al.*, 2015; Kuo *et al.*, 2009), anti-inflammatory (Aziz *et al.*, 2015; Li *et al.*, 2014; Chen *et al.*, 2014; Liaw *et al.*, 2015; Jung *et al.*, 2005), antibacterial (He *et al.*, 2011; Shai *et al.*, 2008; Wang *et al.*, 2013; Harizon *et al.*, 2015; Lopez *et al.*, 2011), antiplatelet (Shi 2013; Yang *et al.*, 2009; Li *et al.*, 2013), hypocholesterolemic (Wang, 2016; Rezaei-Golmisheh *et al.*, 2015), immune adjuvant (Qiao, 2015), and other activities (Song *et al.*, 2011; Jiang *et al.*, 2008; Wang *et al.*, 2009).

Currently, two biosynthetic pathways of terpenoid have been basically clarified in higher plants, they are mevalonic acid (MVA) pathway in the cytoplasm and 2-C-methy-D-erythritol-4-phosphate (MEP) pathway in the plasmid (Kim *et al.*, 2014; Sando *et al.*, 2008). MEP pathway is responsible for monoterpene and diterpene formation (Dudareva *et al.*, 2005), and MVA pathway is responsible for sterol, sesquiterpenes, and triterpene formation (Yang *et al.*, 2012). In MVA pathway, 2, 3-oxidosqualene is the precursor of terpenes, which can be catalyzed into different terpenes by different 2, 3-oxidosqualene cyclases (OSCs). Plant OSCs provide a number of options for their catalytic control. A number of OSCs have been cloned and their functions have been confirmed, including *β*-amyrin synthase (*β*-AS) from *Panax ginseng*(Yang *et al.*, 2012), *Medicago truncatula* (Iturbe-Ormaetxe *et al.*, 2003), *Glycyrrhiza glabra* (Hayashi *et al.*, 2001), and *Pisum sativum* (Morita *et al.*, 2000), lupeol synthase (LUS) from *Arabidopsis thaliana* (Herrera *et al.*, 1998) and *Withania vinifera* (Dhar *et al.*, 2014), cycloartenol synthase (CAS) from *G. glabra* (Hayashi *et al.*, 2000)and *Costus speciosus* (Kawano *et al.*, 2002), Dammarenediol-II synthase (DS) from *P. ginseng* (Tansakul *et al.*, 2006), lanosterol synthase (LS) from *Siraitia grosvenorii* (Dai *et al.*, 2015), and multifunctional triterpene synthases from *P. sativum*, which catalyze the formation of *α*-amyrin, *β*-amyrin, and lupeol (Morita *et al.*, 2000). These OSCs, including *β*-AS, *α*-AS, LUS, DS, LS, and CAS, finally lead to the production of different triterpene saponins, such as oleanane-type triterpene saponin, bearberry hexane-type triterpene saponin, lupinane-type triterpene saponin, dammarane-type triterpene saponin, lanostane-type triterpene saponin, and sterol (Fig. 1).

β-AS is responsible for the production of oleanane-type triterpene saponin, which is widely present in Leguminosae (Ali *et al.*, 2016), Araliaceae (Gao *et al.*, 2015), Umbelliferae (Wu *et al.*, 2012), and other families (Wang *et al.*, 2011). Among these families, there are many famous medicinal plants, such as *Glycyrrhiza uralensis*, *Astragalus membranaceus*, *Isatis indigotica*, *P. ginseng*, and *Panax quinquefolium*. Oleanane-type triterpene saponin is extensively regarded as the marker active compound in these medicinal plants. For example, glycyrrhizic acid (GA), the

marker compound in *G. uralensis*, possesses antitumor (Li *et al.,* 2014), anti-inflammatory (Pang *et al.,* 2016), antiviral (Baltina *et al.,* 2015), and immune-regulation activities (Asl *et al.,* 2008). Ginsenosides, the most important active components in *P. ginseng* and *P. quinquefolium*, possess immune regulation (Zhang *et al.,* 2015), antitumor (Chen *et al.,* 2008), antiaging (Lee *et al.,* 2012), and antiviral (Song *et al.,* 2014)activities. Oleanolic acid, the most important compound in *Swertia leducii* and *Ligustrum lucidum* Ait., possesses liver protection (Kim *et al.,* 2004), antitumor (Yan *et al.,* 2010), and anti-inflammatory (Bednarczyk-Cwynar *et al.,* 2016) activities. *β*-AS is a key enzyme for the production of these important triterpene saponins. Studies of *β*-AS can help us to deeply parse their biosynthetic pathway and improve their accumulation.

Attracted by the key position of *β*-AS, we conducted a series of studies about *β-AS* gene in *G. uralensis*, one of the most frequently used Chinese herbs. We cloned a 4109 bp *β-AS* full-length DNA sequence (Chen *et al.,* 2013)and a 2289 bp *β-AS* full-length cDNA sequence from *G. uralensis* (Shen *et al.,* 2009), and we also verified its function in *Saccharomyces cerevisiae*. We investigated the influences of co-expression of *β-AS* gene and another functional gene involved in MVA pathway, squalene synthase (*SQS*) gene, in *S. cerevisiae*, and found that the co-expression enhanced the accumulation of *β*-amyrin (Liu *et al.,* 2014). And we also revealed the temporal and spatial specificity of the expression of *β-AS* gene in *G. uralensis*, and found that the root tip was the suitable plant material and May, June, August and September were the right acquisition time (Liu *et al.,* 2012). However, after finishing the above researches about *G. uralensis β-AS* gene, we find there are still so many questions and puzzles to be resolved. For example, single nucleotide polymorphisms (SNPs), insertion-deletion length polymorphism (InDel) and copy number variations (CNVs) are present in *β-AS* genes from *G. uralensis*, but we are not clear how they influence the GA accumulation. And there is a pair of GA epimer, differed only in the C_{18}-H, the formation of which are influenced by *β-AS* gene, but we don't know how it works. So we decide to deeply and comprehensively analyze and recognize the *β-AS* genes in higher plants, and which is the original cause of this paper.

In this paper, we obtained 475 DNA sequences, 220 cDNA sequences, and 99 amino acid sequences of *β*-AS registered in NCBI database by Oct, 2016. We analyzed typical *β-AS* DNA sequences in 15 different species from 9 families, mRNA sequences in 37 species from 20 families, and the corresponding amino acid sequences using all kinds of bioinformatics online tools and softwares such as ExPASy Proteomic tools, DNAMAN 6.0.3.99, and MEGA 5.0.The sequence alignment, physicochemical properties, functional domains, and evolutionary relationship were investigated. To get the latest and comprehensive information of *β-AS*, more than 300 papers were searched and 80 of them were reviewed. Pub Med, Web of Science, Science Direct, and Research Gate, were information sources through the search terms of "*β*-amyrinsynthase", "biosynthesis", "OSCs", and their combinations, mainly from year 2010 to 2016. Studies were selected from Science Citation Index journals. All the references linked to the registered DNA and cDNA sequences in NCBI database were also reviewed. Furthermore, the latest technology and the applications of *β-AS* in focused plants were also summarized and discussed. Hopefully, we wish this paper could lay a foundation for further studies of*β*-AS and other OSCs.

Bioinformatics analysis of *β-AS*: The full-length DNA, mRNA, and amino acid sequences of *β-AS* were analyzed using online bioinformatics tools (http://www.ncbi.nlm.nih.gov) with the time restriction of Oct. 2016. The deduction of the amino acid sequences, calculation of theoretical molecular mass and pI, were performed with ExPASy Proteomic tools provided at http://www.expasy.ch/tools/ . Conserved domains in *β*-AS were detected using Conserved Domain Database search tool (CDD) on NCBI server (http://www.ncbi.nlm.nih.gov/structure/cdd/wrpsb.cgi). A multiple alignment of amino acid sequences was performed with DNAMAN 6.0.3.99. Phylogenetic tree was constructed using MEGA 5.0. by Neighbor-Joining (N-J) method and reliability of nodes has been tested with 1000 bootstrap replicates.

Bioinformatic analysis of *β-AS* DNA sequences: *β-AS* DNA sequences of 15 different species from 9 families have been recorded in NCBI. The length of these *β-AS* DNA sequences ranges from 3900 bp to 8800 bp. Using DNAMAN 6.0.3.99 the consistency of them is determined to be 35.86%. The phylogenetic tree (Fig. 2) shows that *Fragaria vesca* L., *Prunus mume* Sieb. *Pyrus bretschneideri* Rehder, *Malus pumila* Mill. from family Rosaceae, and *Camelina sativa* (L.) Crantz, *Arabidopsis thaliana* (L.) Heynh. from family Cruciferae are clustered into one branch. *Lycopersicon esculentum* Mill., *Nicotiana tomentosiformis*, and *Nicotiana sylvestris* from family Solanaceae, are clustered into one branch. And *Glycine max* (L.) Merr. and *Cicer arietinum* L. from family Leguminosae, are clustered into one branch. The N-J tree analysis results are basically in line with genetic relationship, *β-AS* DNA sequences from different families are separated into different branches, respectively.

Bioinformatic analysis of *β-AS* mRNA sequences: *β-AS* mRNA sequences of 37 species from 20 families have been recorded in NCBI. The length of these *β-AS* mRNA sequences ranges from 2100 bp to 2900 bp. The consistency of them is 63.38%. As the N-J tree (Fig. 3) shows, seven species from family Leguminosae including *G. glabra*, *G. uralensis*, *Lotus corniculatus* L. var. japonicus Regel, *Vigna radiata* (L.) Wilczek, *G. max*, *Cicer arietinum* L., and *Pisum sativum* L. are aggregated. *Jatropha curcas* L. and *Euphorbia tirucalli* L. both from family Euphorbiaceae are clustered together. *F. vesca*, *P. mume*, *P. bretschneideri*, and *M. pumila* from family Rosaceae are clustered into one branch. Furthermore, *Aralia elata*, *P. ginseng*, *P. quinquefolius*, and *Panax japonicas* from family Araliaceae are gathered into one branch. *Bupleurum chinense* DC. from family Umbelliferae is also clustered into this branch, which indicates that *B. chinense* DC. has a close relationship with Araliaceae plants. In addition, *Barbarea vulgaris* R. Br., *C. sativa*, and *A. thaliana* from family Cruciferae are clustered together. *Centella asiatica* (L.) Urban from family Umbelliferae and *Bacopa monnieri* (L.) Wettst. from family Scrophulariaceae are gathered together. Except individual examples, the N-J tree analysis results are basically in line with genetic relationship, *β*-AS mRNA sequences from different families are also clustered into different branches.

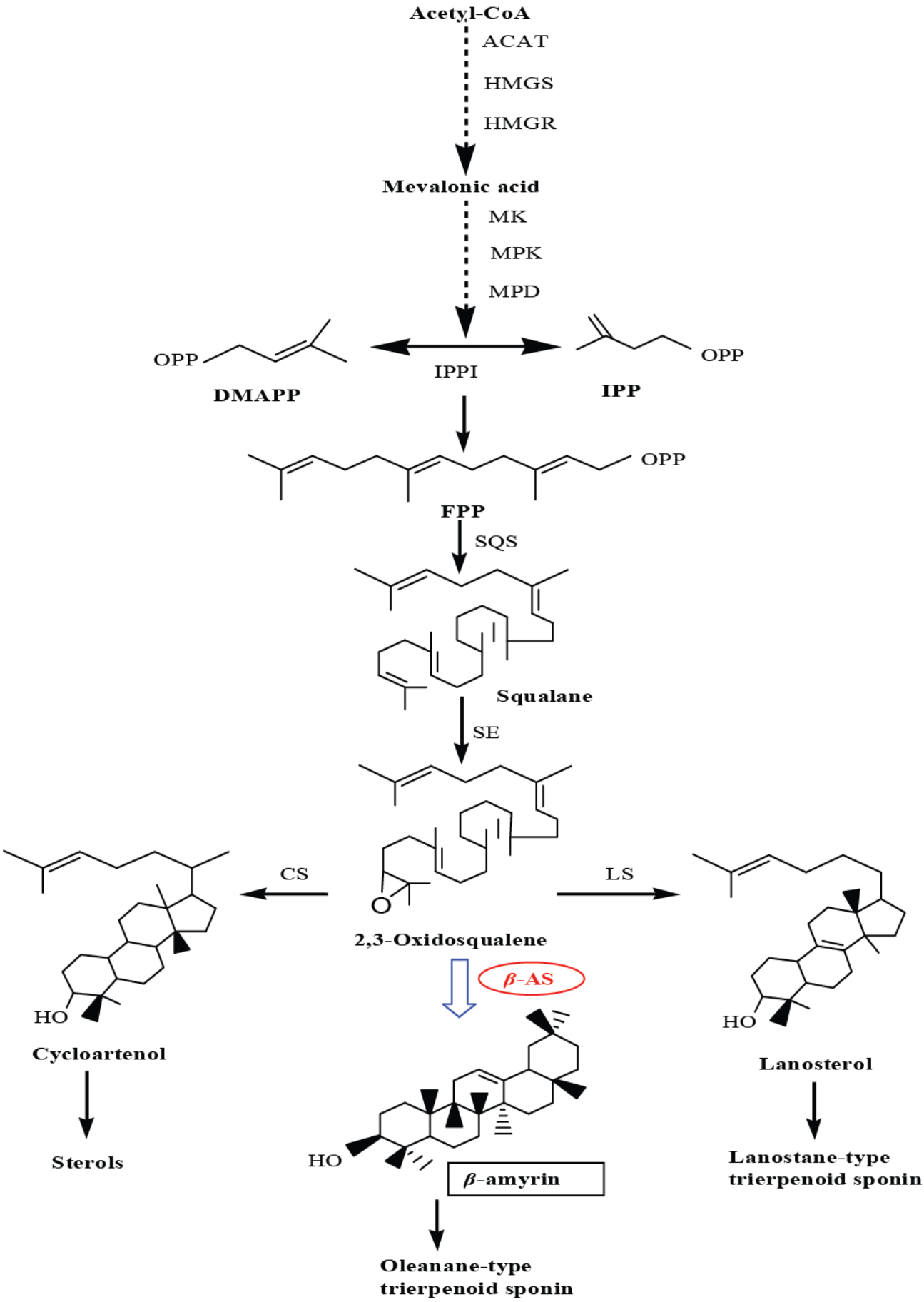

Fig. 1. The MVA pathway for triterpene biosynthesis (*β*-AS is marked in red).

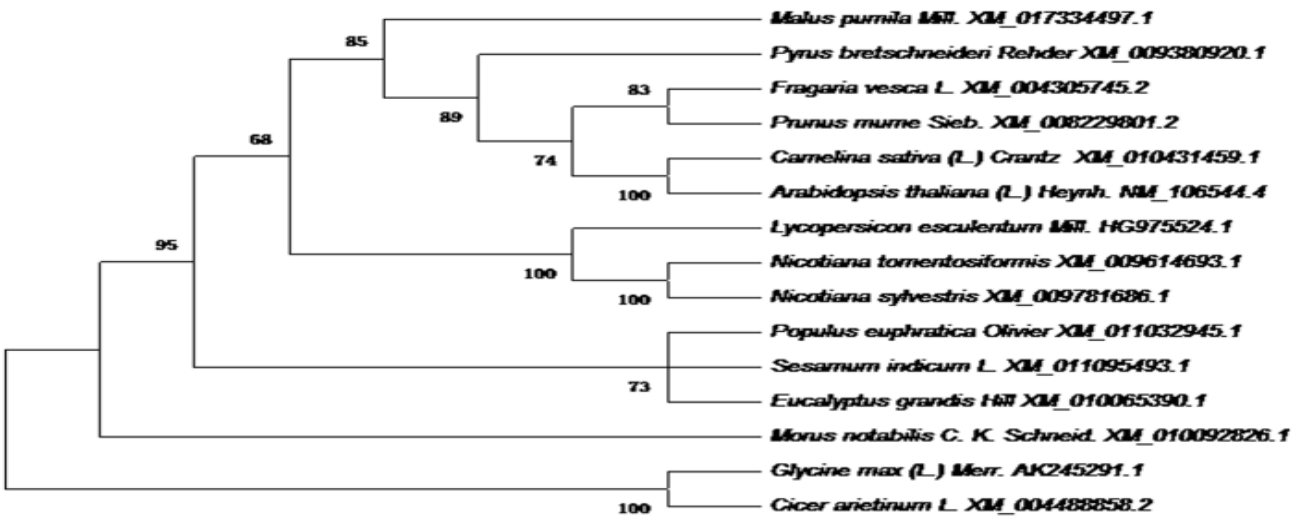

Fig. 2. The cluster tree based on 15 *β-AS* full-length DNA sequences.

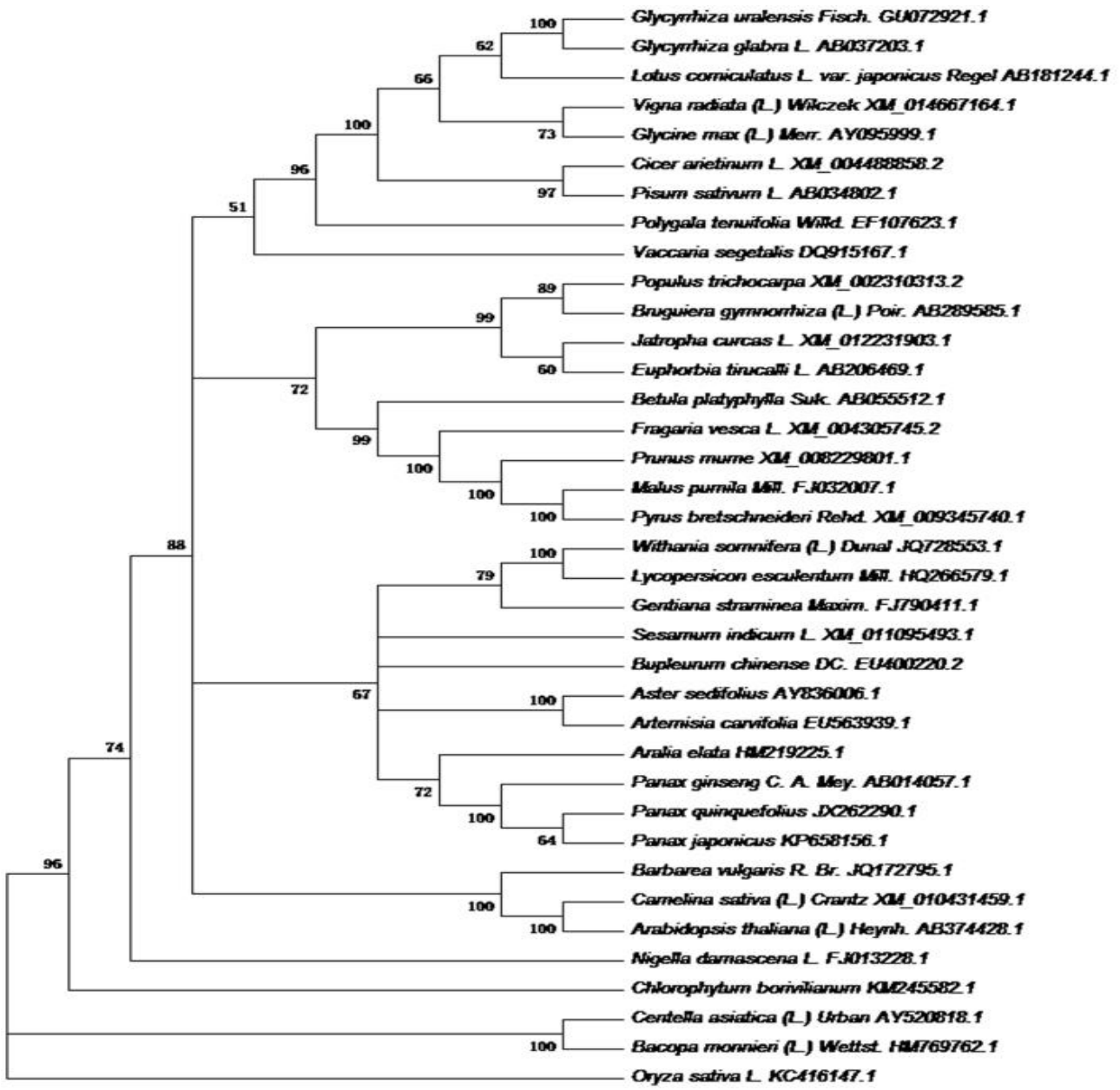

Fig. 3. The cluster tree based on 37 *β-AS* full-length mRNA sequences.

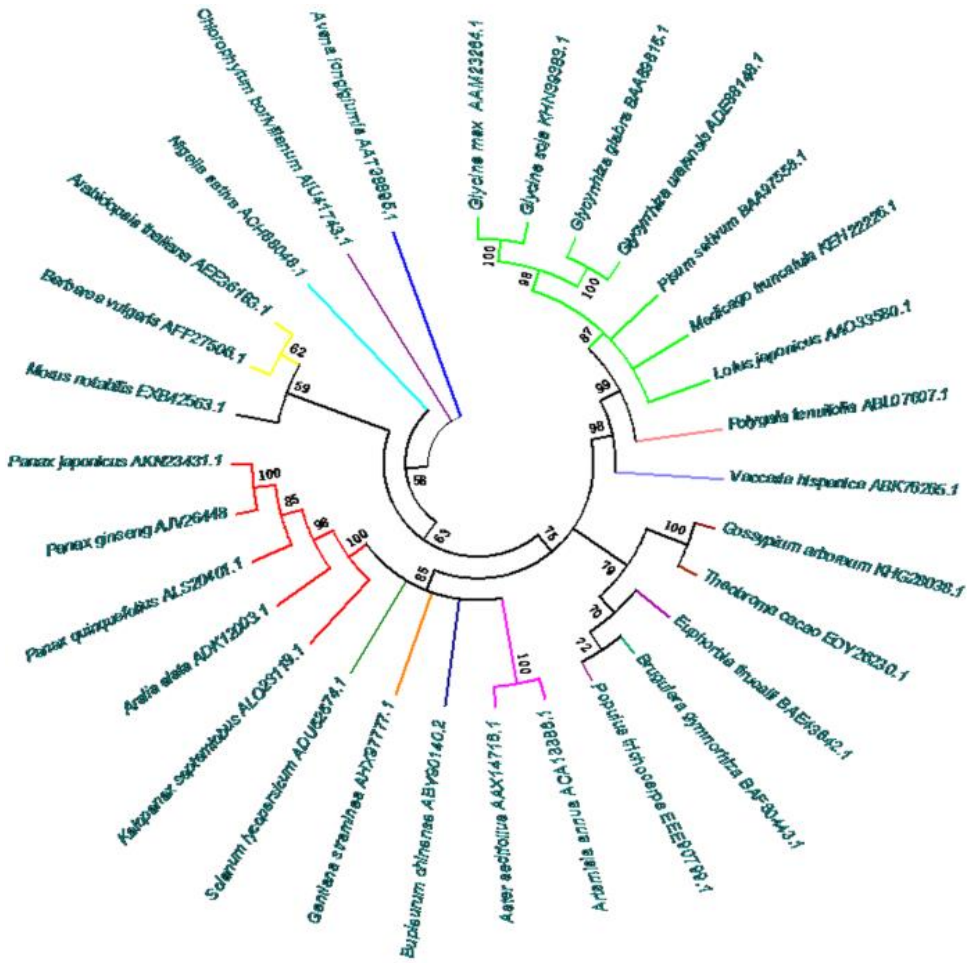

Fig. 4. The N-J tree of*β*-AS amino acid sequences. (Light green represents Leguminosae, pink represents Asteraceae, red represents Araliaceae, yellow represents Cruciferae, and other colors represent different families, respectively.)

Bioinformatics analysis of *β-AS* amino acid sequences: *β*-AS amino acid sequences of 32 species from 29 genera, 20 families have been recorded in NCBI. The similarity of these sequences is 81.62%. The N-J tree is showed in Fig. 4, species from the same family are marked in the same colour. Seven species from family Leguminosae including *G. uralensis*, *G. glabra*, *G. soja*, *G. max*, *P. sativum*, *M. truncatula*, and *L. japonicas* (marked in green) are clustered together. *K. septemlobus*, *A. elata*, *P. ginseng*, *P. quinquefolius*, and *P. japonicas* (marked in red) from family Araliaceae are clustered into the same branch. *Artemisia annua* and *Aster sedifolius* (marked in pink) from family Composite are clustered together. *A. longiglumis* from family gramineae, *C. borivilianum* from family Liliaceae and *N. sativa* from family Ranunculaceae are separated into different branches. N-J tree is consistent with botanical classification status, and accorded with genetic evolution rule.

Physicochemical properties analysis of *β-AS*: The basic physicochemical properties of *β*-AS, including number of amino acid residues, molecular weight, isoelectric point, half-life period, instability parameters, and average hydrophilic coefficient are listed in Table 1. *β*-AS is composed of about 760 amino acid residues with the molecular mass of 87 kDa or so. The isoelectric points range from 5.70 to 6.30. Theoretically, the half-life period is consistent, it is 30 hours in mammalian reticulocytes *In vitro*, and exceed 20 hoursin yeast and 10 hours in *E. coli In vivo.* The average coefficient of hydrophilic indicates that these *β*-AS are consistently hydrophobic.

Sequence alignment analysis of *β-AS* amino acid sequences: To have a better understanding of the amino acid sequence characteristics of *β*-AS, 12 representative *β*-AS amino acid sequences from family Leguminosae, Araliaceae, Umbelliferae, Composite, Brassicaceae, and Polygalaceae have been selected for further alignment analysis using DNAMAN 6.0.3.99, since these selected species have attracted much attention depending on their high-level triterpene contents and remarkable pharmacological activities (Chinese Pharmacopoeia Commission, 2015). The consistency of these sequences is 81.57%. Fig. 5 shows the similarity of the 12 sequences. 100%, 75%, 50%-75%, and less than 30% similarity are marked in black, pink, blue, and white, respectively. The similarity between *P. quinquefolius* and *P. japonicas* is highest (98.69%), while the similarity between *A. longiglumis* and *P. quinquefolius* is lowest (49.15%). Using SOPMA, the secondary structure of the above three *β*-AS sequences, *P. quinquefolius*, *P. japonica*, and *A. longiglumis* was investigated. The results are given in Fig. 6. They are all composed of approximate 40% *α*-helices, 17% extended strand, 33% random coil, and 10% *β*-turns.

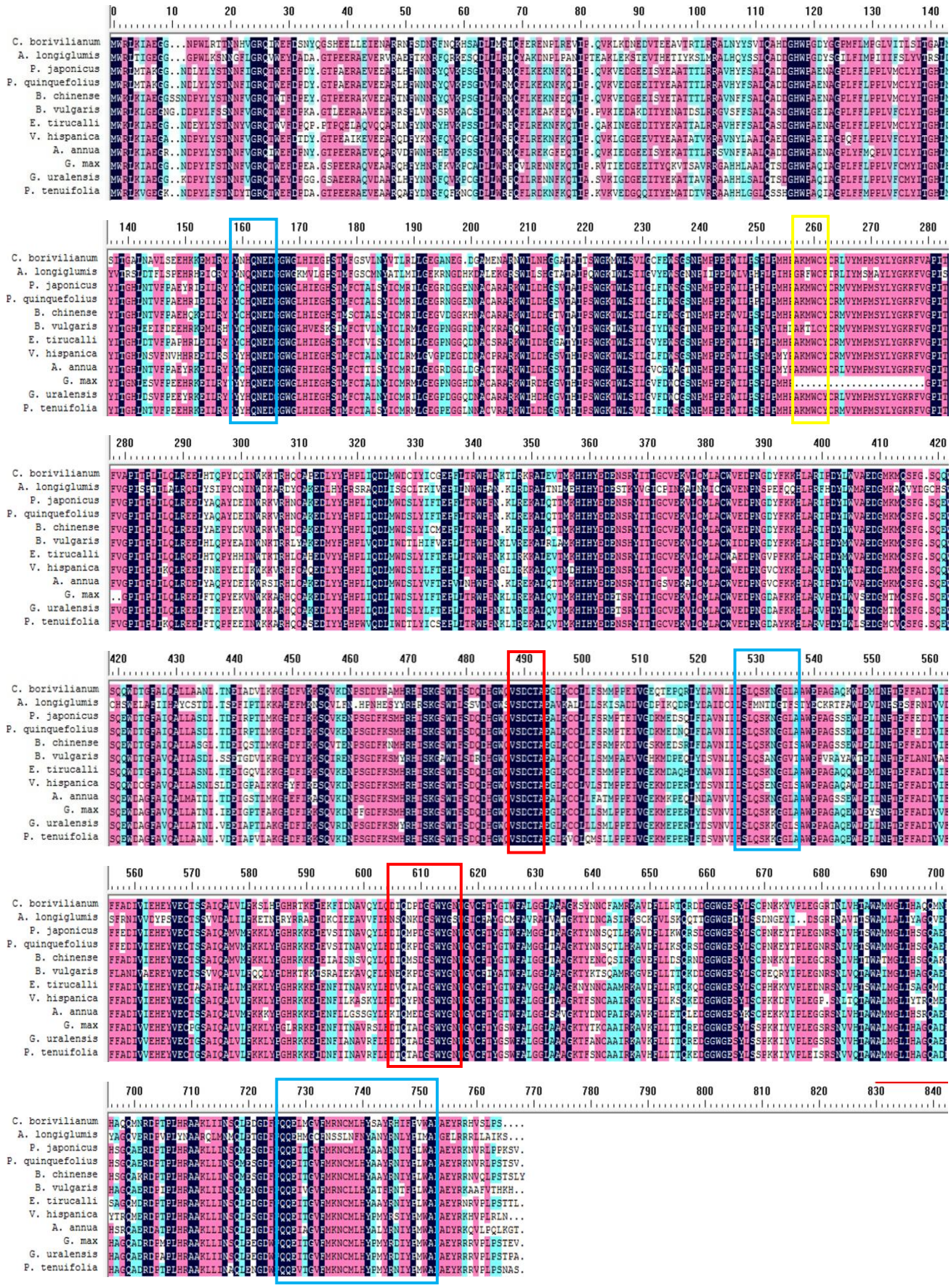

Fig. 5. Analysis of 12 β-AS amino acid sequences. (Black represents the similarity of the amino acid sequences is 100%, pink represents 75%, blue represents 50~75%, and white represents less than 30%. The functional area of β-AS is marked in red. The blue frame showsGln-Trp (QW) motif, the red frame showsAsp-Cys-Thr-Ala-Glu (DCTAE) motif, and the yellow frame shows Met-Trp-Cys-Tyr-Cys-Arg (MWCYCR), respectively.)

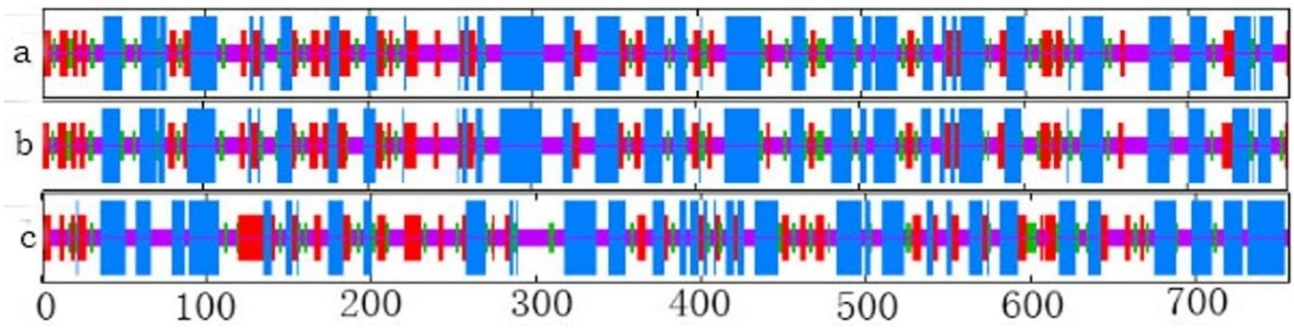

Fig. 6. The prediction of *β*-AS secondary structure. (a, b, and c show the *β*-AS secondary structure of *P. quinquefolius*, *P. japonicas*, and*A. longiglumis*, respectively. Blue represents *α*-helices, red represents extended strand, green represents *β*-turn, and purple represents random coil.).

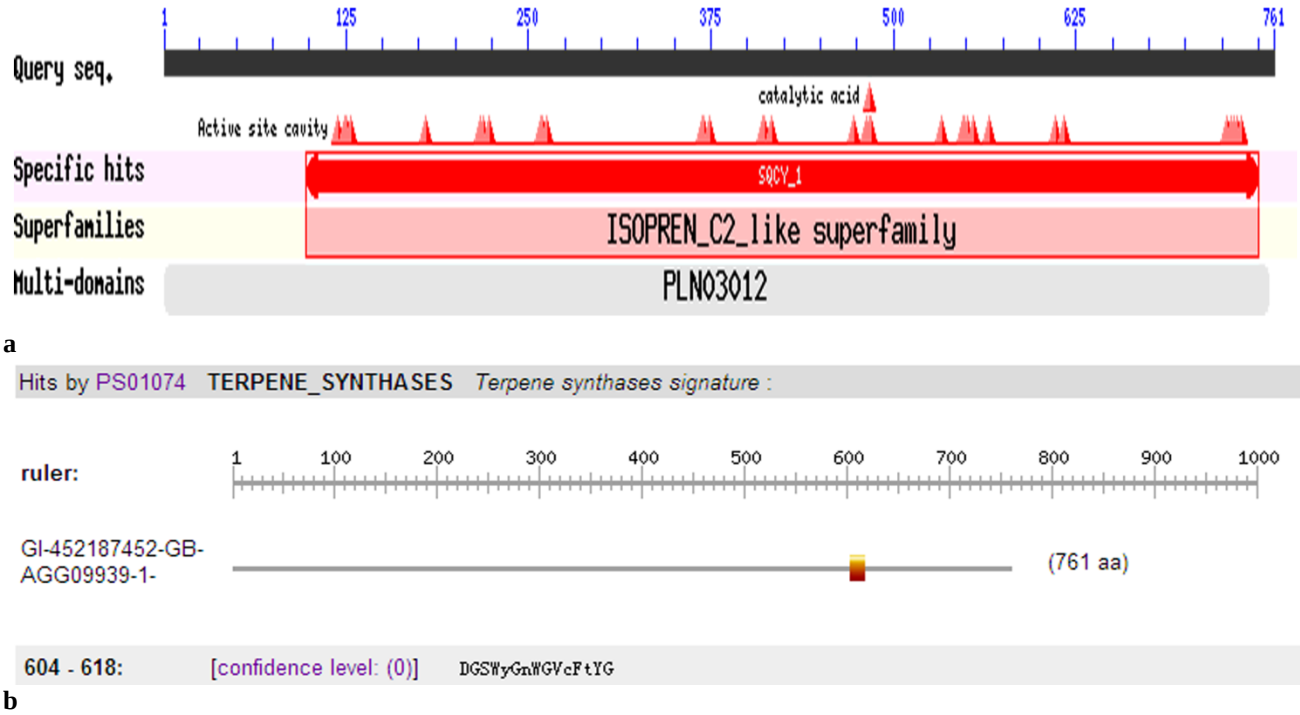

Fig. 7. The prediction of the functional areas of *β*-AS from *P. quinquefolius*. (a is based on NCBI CDD Database and b is based on Protparam.)

Domain structure prediction: The highly conserved motifs of *β*-AS play the key role in substrate binding and protonation. Asp-Cys-Thr-Ala-Glu (DCTAE) implicated in substrate binding motif has been reported to be associated with *β*-amyrin specificity (Vishwakarma *et al.*, 2013). Recently, mutation study of *Euphorbia tirucalli OSC* revealed that DCTAE was a putative initiation site for the polycyclization reaction (Ito *et al.*, 2013). Kushiro *et al*. (Kushiro *et al.*, 1998) illustrated that the tryptophan residue in Met-Trp-Cys-Tyr-Cys-Arg (MWCYCR) and Asp-Cys-Thr-Ala-Glu (DCTAE) motifs of *β*-AS played a significant role in formation of cyclic backbone. Furthermore, Gln-Trp (QW) motifs are indicated by solid bars, whichare properties of the OSC superfamily (Poralla *et al.*, 1994), and may strengthen the structure of the enzyme and stabilize the carbocation intermediates during cyclization. Using Protparam and NCBI conserved domain search tool, it is determined that the fragments from 125th to 735th amino acid residue is the active site cavity of *β*-AS (Fig. 7a), and the fragment from 604th to 618th residues (DGSWYGNWGVCFTYG) is the conserved domain (Fig. 7b).

In Figure 5, DCTAE motif is framed in green, QW motifs are framed in blue, MWCYCR motif is framed in yellow, and the 604th-618th residues are framed in red. DCTAE motif from 491-495 and QW motif from 163-170 are 100% conserved, which confirmed the key positions of these two motifs. In the comparison among *P. quinquefolius*, *P. japonica*, and *A. longiglumis* from 604th to 618th residues, there are three mutation sites, 611th, 614th, and 617th. There are S, I, and A in *A. longiglumis*, while N, V, and T in *P. quinquefolius* and *P. japonica*, respectively. The MWCYCR motif of *P. quinquefolius* and *P. japonica* are same with each other while *A. longiglumis* are quite different. In the QW motifs from 728 to 752, *A. longiglumis* shows the most obvious difference with other species, which is correlated with the genetic distance. Since *A. longiglumis* belong to monocotyledon, while the other 11 species are from dicotyledon.

Table 1. The basic physicochemical properties of *β*-AS amino acid sequences.

species	Accession number	Number of amino acids	Molecular weight	Isoelectric point	Instability parameters	Hydrophilic coefficient	Half-life period
Glycine max	AAM23264.1	739	84603.6	6.10	48.60	-0.342	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Glycyrrhiza uralensis	ADE88148.1	762	87072.7	6.19	47.77	-0.286	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Avena longiglumis	AAT38895.1	757	86858.9	6.12	45.03	-0.336	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Euphorbia tirucalli	BAE43642.1	762	87589.5	6.78	47.35	-0.320	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Artemisia annua	ACA13386.1	761	87503.2	5.87	49.24	-0.331	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Bupleurum chinense	ABY90140.2	764	87791.2	6.02	49.99	-0.374	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Panax quinquefolius	AGG09939.1	761	87775.2	5.92	58.44	-0.368	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Panax japonicus	AKN23431.1	761	87900.4	5.84	49.38	-0.360	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Vaccaria hispanica	ABK76265.1	760	87521.3	5.89	45.91	-0.312	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Barbarea vulgaris	AFF27506.1	762	87503.4	6.29	47.91	-0.271	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)
Polygala tenuifolia	ABL07607.1	762	87343.0	6.21	46.50	-0.308	>10h(E, *in vivo*); 30(m, *in vitro*); >20(y, *in vivo*)

Table 2. The registered *β*-amyrin synthetase sequence information in GenBank.

Family	Species	GenBank accession number	Sequence length
Liliaceae	*Chlorophytum borivilianum*	KM245582.1	2277 bp
Euphorbiaceae	*Jatropha curcas*	XM_012232059.1	2307 bp
Leguminosae	*Lotus japonicus*	AF478455.1	2458 bp
	Glycine max	AY095999.1	2416 bp
	Pisum sativum	AB034802.1	2601 bp
	Glycyrrhiza glabra	AB037203.1	2671 bp
	Glycyrrhiza uralensis	FJ627179.1	2289 bp
	Vigna radiate	XM014667164.1	2647 bp
	Cicer arietinum	XM_004488858.2	2672 bp
Poaceae	*Oryza sativa* Japonica	KC416147.1	2262 bp
Rhizophoraceae	*Bruguiera gymnorhiza*	AB289585.1	2280 bp
Pedaliaceae	*Sesamum orientale* Linn	XM_011095493.1	2573 bp
Betulaceae	*Betula platyphylla*	AB055512.1	2519 bp
Malvaceae	*Gossypium raimondii*	XM_012592029.1	2712 bp
Asteraceae	*Aster ticuatars*	EU330197.1	2286 bp
Gentianaceae	*Gentiana straminea*	FJ790411.1	2286 bp
Ranunculaceae	*Nigella sativa*	FJ013228.1	2430 bp
	Fragaria × *ananassa* Duch.,	XM_004305745.2	2900 bp
	Pyrus bretschneideri Rehd	XM_009345740.1	2418 bp
	Malus x domestica	FJ032007.1	2486 bp
Rosaceae	*Prunus mume*	XM_008229801.1	2824 bp
Solanaceae	*Solanum lycopersicum*	NM_001247675.1	2535 bp
	Withania somnifera	HQ266579.1	2289 bp
	Bupleurum chinense	HQ166837.1	2602 bp
Umbelliferae	*Centella asiatica*	AY520818.1	2562 bp
	Arabidopsis thaliana	AB374428.1	2280bp
	Barbarea vulgaris	JQ172795.1	2289 bp
Cruciferae	*Camelina sativa*	XM_010431459.1	2392 bp
Caryophyllacea	*Vaccaria hispanica*	DQ915167.1	2511 bp
Sterculiaceae	*Theobroma cacao*	XM_007023302.1	2909 bp
	Chinese aralis	HM219225.1	2292 bp
Araliaceae	*Panax ginseng*	AB009030.1	2589 bp
Scrophulariaceae	*Bacopa monnieri*	HM769762.1	2765 bp
Salicaceae	*Populus trichocarpa*	XM_002310313.2	2682 bp
Polygalaceae	*Polygala tenuifolia*	EF107623.1	2934 bp

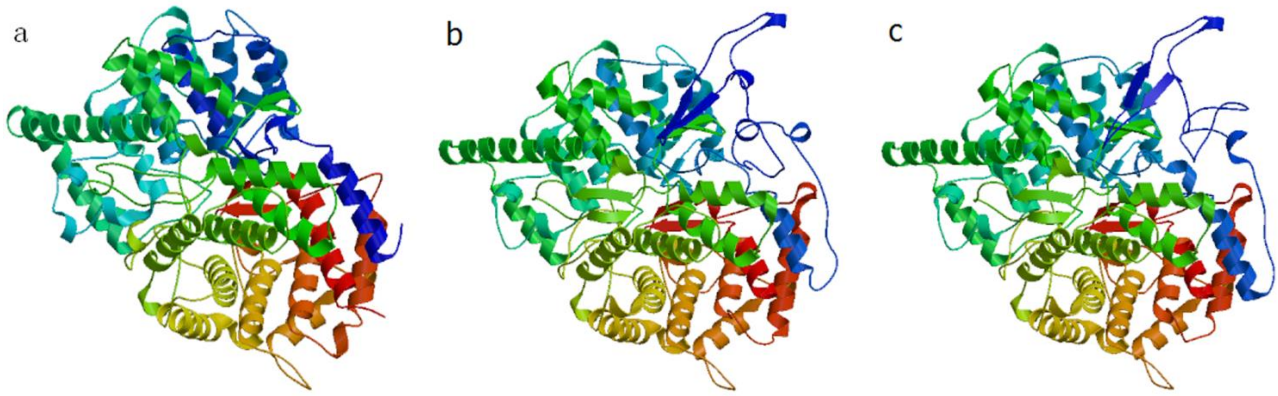

Fig. 8. Three dimensional structure of *β*-AS based on homology modeling. (a, b, and c show the *β*-AS three dimensional structure of *A. longiglumis, P. quinquefolius*,and *P. japonicas*, respectively.)

Transmembrane structure and signal peptide prediction: The transmembrane domain location analysis indicates that *P. quinquefolius* and *P. japonicas* have no transmembrane region, while *A. longiglumis* has two transmembrane region, 117th-141th and 606th-634th amino acid residues. It shows that *β*-AS of *P. japonicas* and *P. quinquefolius* do not cross the membrane, while *β*-AS of *A. longiglumis* is "anchored" to a specific site in the cytoplasmic matrix to perform the catalytic function. Signal peptide analysis indicates that the C-score, S-score and Y-score are the samein *P. quinquefolius* and *P. japonicas*. The highest score of original cleavage site is 0.111 at 47th amino acid residue, the highest score of signal peptide site is 0.134 at 39th amino acid residue, and the highest score of combined cleavage site is 0.111 at 47th amino acid residue. In *A. longiglumis*, the highest score of original cleavage site is 0.111 at 46th amino acid residue, the highest score of signal peptide site is 0.236 at 1st amino acid residue, and the highest score of combined cleavage site is 0.139 at 11th amino acid residue. The above demonstrates that *β*-AS of *P. quinquefolius*, *P. japonicas*, and *A. longiglumis* all have no signal peptides, and they exert their activities in cytoplasm. This inference is consistent with the action site of MVA pathway in cell.

The three-dimensional protein model analysis of *β-AS*: The three-dimensional protein models of *P. quinquefolius*, *P. japonicas*, and *A. longiglumis* were also determined. As shown in Fig. 8, the model of *P. quinquefolius* and *P. japonicas* are quite similar, while *A. longiglumis* showed moderate diversity.

The research progress of genetic engineering

Gene cloning and functional verification of *β-AS*: Up to date, 60 *β-AS* cDNAs have been cloned from 37 species in 21 families as listed in Table 2. In most studies (Iturbe-Ormaetxe *et al.*, 2003; Kim *et al.*, 2005), the conserved regions of the *β*-AS synthase were used to design specific primers and amplify the target sequences through RT-PCR or RACE methods. The amplified products were cloned into pGEM-T Easy vector and transformed into disarmed DH5*α E. coli* cells. For *B. gymnorrhiza*, *B. platyphlla*, *L. esculentum*, *P. tenuifolia*, and *G. glabra*, the full-length cDNAs were cloned into yeast expression vector pYES2 under the control of the GAL10 promoter, and then obtained plasmids were introduced into a triterpenoid synthase-deficient yeast mutant GIL77, which led to the production of *β*-amyrin (Vishwakarma *et al.*, 2013; Kirby *et al.*, 2008; Hayashi *et al.*, 2001). For *A. apiacea*, *B. vulgaris*, *S. vaccaria*, and *A. thaliana*, the full-length cDNAs were introduced into the high-copy yeast expression vector pESC-URA under control of the GAL10 promoter, and expressed in *S. cerevisiae* (Sun *et al.*, 2013; Wang *et al.*, 2011).

***β-AS* expression studies:** RNA interference was used to analyze the function of *β-AS* involved in ginsenoside biosynthesis, it was found that down-regulation of *β-AS* expression resulted in reducing levels of *β*-amyrin and oleanane-type ginsenoside and increasing level of dammarane-type ginsenoside. Since there are many OSCs led to different triterpene formation, the regulation of related cyclase is also important. Depending on a research conducted by Zhang F (Zhang *et al.*, 2014), the expression levels of SQS, squalene monooxygenase (SQE), and *β*-AS were highly correlated, which suggested that overexpression of coded gene, such as SQS, SQE, and *β*-AS increased the production of triterpene saponin. In another research, two key enzymes involved in sterol pathway in *S. cerevisiae*, HMGR and lanosterol synthase, were manipulated to increase triterpene production. It was found that *β*-amyrin production was improved by 50%, and squalene level had a 12-fold increase, which indicated that a high expression level of LUS and CYS increased the pathway into lupeol and phytosterol synthesis, rather than oleanolic acid synthesis. Therefore, it appeared that an increase of oleanolic acid production required an elevated level of *β*-AS, SQS and SE expression, and a suppression of LUS (Mangas *et al.*, 2008).

Furthermore, the temporal and spatial specificity of the expression of *β-AS* has also been investigated. Iturbe-Ormaetxe I *et al*. found the expression pattern of *β-AS* differed in different plant tissues. *β-AS* gene in different tissues of *M. truncatula* was analyzed, the highest transcript levels was found in the shoot meristem and stem tissue (Iturbe-Ormaetxe *et al.*, 2003). And we also revealed the temporal and spatial specificity of the expression of *β-AS* gene in *G. uralensis*, and found that the root tip was the suitable plant material and May, June, August and September were the right acquisition time (Liu *et al.*, 2012).

Exogenous stimulator regulation: MeJA (methyl jasmonate MJ) was a commonly used chemical inducers for promoting plant cell secondary metabolite biosynthesis. It induced the defense responses and plant protection element (mainly flavonoids and terpenoids) alone or synergistically in plants. As one of the important substances in cell signal transduction system, MeJA is widely involved in the process of plant defense signal transduction and amplification, which induces the expression of anti-reaction products (Mitra & Baldwin, 2014; Wu *et al.,* 2008; Schlogl *et al.,* 2008; Li *et al.,* 2014).

Hayashi H *et al*. (Hayashi *et al.,* 2004) found in the cultured cells, the addition of MeJA up-regulated *β-AS* mRNA expression level and soyasaponin biosynthesis, but down-regulated lupeol synthase expression level. While, the addition of gibberellins down-regulated *β-AS*m RNA expression level. Suzuki & Dixon (2005) explored cell suspension cultures of *M. truncatula* to MeJA, which led to a 50-fold induction of *β*-AS. Their work established *Medicago* cell suspension cultures as a well model for future genomics approaches to explore the regulation of legume secondary metabolism. Also, Confalonieri M *et al*. (Confalonieri *et al.,* 2009) found that *β-AS*mRNA expression level was up-regulated and the accumulation of oleanolic acid increased in *G. scabra* by treatment with MeJA over a period from 6 hours to 10 days (Nasrollahi *et al.,* 2014).

The effect of drought stress on *β-AS* gene expression in *G. glabra* has been studied, and the results showed that drought was conducive to *β-AS* expression, thus contributing to the accumulation of glycyrrhizin (Nasrollahi *et al.,* 2014). Basyuni M *et al*. (Knott & Reynolds, 1990) found salt was beneficial to *β-AS* expression in mangrove plants *Kandelia candel* and *B. gymnorrhiza*. These results enhanced triterpenoids in the adaptation of mangroves to endure salt or water stress.

Transgenic applications: So far, *M. truncatula* can be genetically transformed and regenerated, and which provides a possibility to regulate the content of triterpene saponin in transgenic plants by ectopic expression, overexpression or gene silencing. *Agrobacterium*-mediated transformation was used to introduce a novel *Aster sedifolius β-AS* in *M. truncatula* with the cauliflower mosaic virus 35S promoter, the transgenic plants exhibited greater amounts of triterpenic compounds than control plants.

P. japonicas is a rare Chinese herb containing ginsenosides as its main active ingredient. Rice cannot produce ginsenosides because it lacks a key rate-limiting enzyme, *β*-AS. However, it can produce a secondary metabolite, 2, 3-oxidosqualene, which is a precursor for ginsenoside biosynthesis. *β-AS* gene from *P. japonicas* was transformed into rice cultivar using an *Agrobacterium*-mediated approach, and 68 rice transgenic plants was got. Real-time PCR and Western blotting analyses showed that *β-AS* gene overexpressed in rice. HPLC analysis showed that the concentration of oleanane-type sapogenin oleanolic acid in transgenic rice was 8.3~11.5 mg/100 g dw (Huang *et al.,* 2015).

Discussion

This paper reviewed the research progress of *β*-AS, a key enzyme for triterpene skeleton formation. All of the registered DNA, cDNA, and amino acid sequences of *β*-AS in GenBank have been gathered, the physicochemical, hydrophilic and hydrophobic properties, secondary structures, and functional domains have been investigated. The evolutionary relationships between different species have also been analyzed. In addition, the experimental methods to obtain *β-AS*gene sequences and verify their functions have been summarized. We found that OSCs play a crucial role in terpene formation. There are several kinds of OSCs, such as *β*-AS, *α*-AS, LUS, DS, LS, and CS, which suggest that a high expression level of one OSCs may down-regulate others. Therefore, multi-gene control may be a promising destination for the certain product. It is helpful to improve the content of target products by leading metabolites to the certain direction we need. What's more, both the inside (mRNA sequences) and outside factors (host and production system/conditions, drought stress, and exogenous stimulation) can affect the content of secondary metabolites.

Triterpenes are extensively distributed isoprenoids found in many different kinds of organisms and form the one of main categories of natural products. They have multiple functions in biosystem. Because of diversity of triterpenes constituted in higher plants, it is interesting to confirm how different triterpene synthases controls the product specificity during cyclization of oxidosqualene. In addition, studies have revealed mechanisms that lead to product specificity by comparing the cloning and sequence of several different triterpene synthase cDNAs, such as the folded geometry of the substrate and the end point of the resulting cation.

Furthermore, the improvement of qualitative and quantitative techniques of medicinal plants is the key to the stable supply of crude drugs. An excessive production of triterpenoid saponins in medicinal plants is a solution to this purpose. The introduction and overexpression of the triterpene synthase gene in a suitable host plant should be the primary task to this target.

Competing interests: All authors declare that they have no competing interests.

Acknowledgement

This work was supported by National Science Foundation of China (81503181).

References

Ali, M.M., P. Krishnamurthy and M.H. EI-Hadary. 2016. Identification and expression profiling of a new *β*-amyrin synthase gene (GmBAS3) from soybean. *Russian J. Plant Physiology*, 63(3): 383-390.

Asl, M.N. and H. Hosseinzadeh. 2008. Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Phytother. Res*., 22(6): 709-724.

Aziz, M.A. 2015. Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of Microcos paniculata barks and fruits. *J. Integr. Med*., 13(3): 173-184.

Baltina, L.A., V.V. Zarubaev, I.A. Orshanskaya, A.I. Fairushina, O.I. Kiselev and M.S. Yunusov. 2015. Glycyrrhizic acid derivatives as influenza A/H1N1 virus inhibitors. *Bioorg. Med. Chem. Lett*., 25(8): 1742-1746.

Basyuni, M., H. Oku, E. Tsujimoto, K. Kinjo, S. Baba and K. Takara. 2007. Triterpene synthases from the Okinawan mangrove tribe, Rhizophoraceae. *Febs. J*., 274(19): 5028-5042.

Bednarczykcwynar, B., N. Wachowiak, M. Szulc, E. Kamińska and A. Bogacz. 2016. Strong and long-lasting antinociceptive and anti-inflammatory conjugate of naturally occurring oleanolic acid and aspirin. *Front Pharmacol*., 7: 202-209.

Callies, O., L.M. Bedoya, M. Beltrán, A. Muñoz, P.O. Calderón, A.A. Osorio, I.A. Jiménez and I.L. Bazzocchi. 2015. isolation, structural modification, and HIV inhibition of pentacyclic lupane-type triterpenoids from *Cassine xylocarpa* and *Maytenus cuzcoina*. *J. Nat. Prod*., 78(5): 1045-1055.

Chen, H., Y. Liu and Y. Zhang. 2013. Cloning and characterization of the gene encoding *β*-amyrin synthase in the glycyrrhizic acid biosynthetic pathway in *Glycyrrhiza uralensis*. *Acta Pharmaceutica Sinica B,* 3(6): 416-424.

Chen, I.H., Y.C. Du, T.L. Wang and I.F. Chen. 2014. Anti-inflammatory triterpenoids from the stems of *Microtropis fokienensis*. *Molecules,* 19(4): 4608-4623.

Chen, J., H. Peng, X. Ou-Yang and X. He. 2008. Research on the antitumor effect of ginsenoside Rg3 in B16 melanoma cells. *Melanoma Res*., 18(5): 322-329.

Chinese Pharmacopoeia Commission. 2015. Pharmacopoeia of the People's Republic of China. Part I. Beijing: China Medical Science Press, 86-87.

Confalonieri, M., M. Cammareri, E. Biazzi, P. Pecchia and M. Fevereiro. 2009. Enhanced triterpene saponin biosynthesis and root nodulation in transgenic barrel medic (*Medicago truncatula* Gaertn) expressing a novel beta-amyrin synthase (AsOXA1) gene. *Plant Biotechnol J*., 7(2): 172-182.

Dai, L., C. Liu, Y. Zhu, J. Zhang and Y. Men. 2015. Functional characterization of cucurbitadienol synthase and triterpene glycosyltransferase involved in biosynthesis of mogrosides from *Siraitia grosvenorii*. *Plant Cell Physiol*., 56(6): 1172-1182.

Dhar, N., S. Rana, S. Razdan, W.W. Bhat and A. Hussain. 2014. Cloning and functional characterization of three branch point oxidosqualene cyclases from *Withania somnifera* (L.) dunal. *J.Biol. Chem.*, 289(24): 17249-17267.

Dudareva, N., S. Andersson, I. Orlova, N. Gatto and M. Reichelt. 2005. The nonmevalonate pathway supports both monoterpene and sesquiterpene formation in snapdragon flowers. *Proc. Natl. Acad. Sci*., 102(3): 933-938.

Gao, K., S.R. Wu and L. Wang. 2015. Cloning and analysis of *β*-amyrin synthase gene in Bupleurum chinense. *Genes & Genomics*, 37(9): 767-774.

Harizon, B. Pujiastuti, D. Kurnia, D. Sumiarsa, Y. Shiono and U. Supratman. 2015. Antibacterial triterpenoids from the bark of *Sonneratia alba* (Lythraceae). *Nat Prod Commun*., 10(2): 277-280.

Hayashi, H., N. Hiraoka, Y. Ikeshiro, T. Kushiro and M. Morita. 2000. Molecular cloning and characterization of a cDNA for *Glycyrrhiza glabra* cycloartenol synthase. *Biol. Pharm. Bull*., 23(2): 231-234.

Hayashi, H., P. Huang, A. Kirakosyan, K. Inoue and N. Hiraoka. 2001. Cloning and characterization of a cDNA encoding beta-amyrin synthase involved in glycyrrhizin and soyasaponin biosyntheses in licorice. *Biol. Pharm. Bull*., 24(8): 912-916.

Hayashi, H., P.S. Huang, M. Obinata, K. Inoue and M. Shibuya. 2004. Differential expression of three oxidosqualene cyclase mRNAs in *Glycyrrhiza glabra*. *Biol. Pharm. Bull*., 27(7): 1086-1092.

He, X.F., X.N. Wang, S. Yin, L. Dong and J.M. Yue. 2011. Ring A-seco triterpenoids with antibacterial activity from Dysoxylum hainanense. *Bioorg. Med. Chem. Lett*., 21(1): 125-129.

Herrera, J.B., B. Bartel, W.K. Wilson and S.P. Matsuda. 1998. Cloning and characterization of the *Arabidopsis thaliana* lupeol synthase gene. *Phytochemistry*, 49(7): 1905-1911.

Huang, Z., J. Lin, Z. Cheng, M. Xu and M. Guo. 2015. Production of oleanane-type sapogenin in transgenic rice via expression of beta-amyrin synthase gene from *Panax japonicus* C.A. Mey. *Bmc. Biotechnol*., 15(1): 1-8.

Ito, R., Y. Masukawa and T. Hoshino. 2013. Purification, kinetics, inhibitors and CD for recombinant beta-amyrin synthase from *Euphorbia tirucalli* L. and functional analysis of the DCTA motif, which is highly conserved among oxidosqualene cyclases. *Febs. J*.,280(5): 1267-1280.

Iturbe-Ormaetxe, I., K. Haralampidis, K. Papadopoulou and A.E. Osbourn. 2003. Molecular cloning and characterization of triterpene synthases from *Medicago truncatula* and *Lotus japonicus*. *Plant Mol. Biol*., 51(5): 731-743.

Jiang, R.W., A.L. Lane, L. Mylacraine, K.I. Hardcastle and C.R. Fairchild. 2008. Structures and absolute configurations of sulfate-conjugated triterpenoids including an antifungal chemical defense of the green macroalga *Tydemania expeditionis*. *J. Nat. Prod.*, 71(9): 1616-1619.

Jung, H.J., J.H. Nam, J. Choi, K.T. Lee and H.J. Park. 2005. 19Alpha-hydroxyursane-type triterpenoids: Antinociceptive anti-inflammatory principles of the roots of *Rosa rugosa*. *Biol. Pharm. Bull*., 28(1): 101-104.

Kawano, N., K. Ichinose and Y. Ebizuka. 2002. Molecular cloning and functional expression of cDNAs encoding oxidosqualene cyclases from Costus speciosus. *Biol. Pharm. Bull*., 25(4): 477-482.

Kim, K.A., J.S. Lee, H.J. Park, J.W. Kim, C.J. Kim, I.S. Shim, N.J. Kim, S.M. Han and S. Lim. 2004. Inhibition of cytochrome P450 activities by oleanolic acid and ursolic acid in human liver microsomes. *Life Sci*., 74(22): 2769-2779.

Kim, O.T., M.Y. Kim, S.M. Huh, D.G. Bai, J.C. Ahn and B. Hwang. 2005. Cloning of a cDNA probably encoding oxidosqualene cyclase associated with asiaticoside biosynthesis from *Centella asiatica* (L.) Urban. *Plant Cell Rep*., 24(5): 304-311.

Kim, Y.K., Y.B. Kim and J.K. Kim. 2014. Molecular cloning and characterization of mevalonic acid (MVA) pathway genes and triterpene accumulation in *Panax ginseng*. *J. Korean Society for Applied BioChem*., 57(3): 289-295.

Kirby, J., D.W. Romanini, E.M. Paradise and J.D. Keasling. 2008. Engineering triterpene production in *Saccharomyces cerevisiae*-beta-amyrin synthase from *Artemisia annua*. *Febs J*., 275(8): 1852-1859.

Knott, C. and F. Reynolds. 1990. Therapeutic drug monitoring in pregnancy: Rationale and current status. *Clin. Pharmacokinet*, 19(6): 425-433.

Kongkum, N., P. Tuchinda, M. Pohmakotr, V. Reutrakul and P. Piyachaturawat. 2013. Cytotoxic, antitopoisomerase IIalpha, and anti-HIV-1 activities of triterpenoids isolated from leaves and twigs of *Gardenia carinata*. *J. Nat. Prod.*, 76(4): 530-537.

Kuo, R.Y., K. Qian, S.L. Morris-Natschke and K.H. Lee. 2009. Plant-derived triterpenoids and analogues as antitumor and anti-HIV agents. *Nat. Prod. Rep*., 26(10): 1321-1344.

Kushiro, T., M. Shibuya and Y. Ebizuka. 1998. Beta-amyrin synthase-cloning of oxidosqualene cyclase that catalyzes the formation of the most popular triterpene among higher plants. *Eur. J. Biochem*., 256(1): 238-244.

Lee, H.S., M.R. Kim, Y. Park, H.J. Park, U.J. Chang, S.Y. Kim and H.J. Suh. 2012. Fermenting red ginseng enhances its safety and efficacy as a novel skin care anti-aging ingredient: *In vitro* and animal study. *J. Med. Food*, 15(11): 1015-1023.

Li, C.T., H.B. Wang and B.J. Xu. 2013. A comparative study on anticoagulant activities of three Chinese herbal medicines from the genus Panax and anticoagulant activities of ginsenosides Rg1 and Rg2. *Pharm. Biol*., 51(8): 1077-1080.

Li, C., F. Chen and Y. Zhang. 2014. GA3 and other signal regulators (MeJA and IAA) improve xanthumin biosynthesis in different manners in *Xanthium strumarium* L. *Molecules*, 19(9): 12898-12908.

Li, J.Y., H.Y. Cao, P. Liu, G.H. Cheng and M.Y. Sun. 2014. Glycyrrhizic acid in the treatment of liver diseases: Literature review. *Biomed. Res. Int.,* 2014(8): 872139-872154.

Li, M.M., X.Q. Su, J. Sun, Y.F. Gu, Z. Huang, K.W. Zeng, Q. Zhang, Y.F. Zhao, D. Ferreira, J.K. Zjawiony, J. Li and P.F. Tu. 2014. Anti-inflammatory ursane- and oleanane-type triterpenoids from *Vitex negundo* var. *cannabifolia*. *J. Nat. Prod.*, 77(10): 2248-2254.

Liaw, C.C., H.C. Huang, P.C. Hsiao, L.J. Zhang and Z.H. Lin. 2015. 5beta, 19-epoxycucurbitane triterpenoids from Momordica charantia and their anti-inflammatory and cytotoxic activity. *Planta. Med*., 81(1): 62-70.

Liu, Y. and C.S. Liu. 2012. Study on the spatial and temporal expression of beta-*AS* gene of *Glycyrriza uralensis*. *Zhong Yao Cai*., 35(4): 528-531.

Liu, Y., H.H. Chen, H. Wen, Y. Gao, L.Q. Wang and C.S. Liu. 2014. Enhancing the accumulation of beta-amyrin in *Saccharomyces cerevisiae* by co-expression of *Glycyrrhiza uralensis* squalene synthase 1 and beta-amyrin synthase genes. *Acta Pharmaceutica Sinica*., 49(5): 734-741.

Lopez, M.R., L. de Leon and L. Moujir. 2011. Antibacterial properties of phenolic triterpenoids against *Staphylococcus epidermidis*. *Planta Med*., 77(07): 726-729.

Mangas, S., E. Moyano, L. Osuna, R.M. Cusido, M. Bonfill and J. Palazon. 2008. Triterpenoid saponin content and the expression level of some related genes in calli of Centella asiatica. *Biotechnol. Lett*., 30(10): 1853-1859.

Mitra, S. and I.T. Baldwin. 2014. RuBPCase activase (RCA) mediates growth-defense trade-offs: Silencing RCA redirects jasmonic acid (JA) flux from JA-isoleucine to methyl jasmonate (MeJA) to attenuate induced defense responses in *Nicotiana attenuata*. *New Phytol*., 201(4): 1385-1395.

Morita, M., M. Shibuya, T. Kushiro, K. Masuda and Y. Ebizuka. 2000. Molecular cloning and functional expression of triterpene synthases from pea (*Pisum sativum*) new alpha-amyrin-producing enzyme is a multifunctional triterpene synthase. *Eur. J. Biochem*., 267(12): 3453-3460.

Nasrollahi, V., A. Mirzaie-asl, K. Piri, S. Nazeri and R. Mehrabi. 2014. The effect of drought stress on the expression of key genes involved in the biosynthesis of triterpenoid saponins in liquorice (*Glycyrrhiza glabra*). *Phytochemistry*, 103(10): 32-37.

Pang, H., T. Huang, J. Song, D. Li, Y. Zhao and X. Ma. 2016. Inhibiting HMGB1 with glycyrrhizic acid protects brain injury after DAI via its anti-inflammatory effect. *Mediators Inflamm*, 2016(3): 456-465.

Parveen, R., M.A. Azmi and R.M. Tariq. 2010. Determination of antifungal activity of Cedrus deodara root oil and its compounds against *Candida albicans* and *Aspergillus fumigatus*. *Pak. J. Bot*., 42(5): 3645-3649.

Petronelli, A., G. Pannitteri and U. Testa. 2009. Triterpenoids as new promising anticancer drugs. *Anticancer Drugs*, 20(10): 880-892.

Poralla, K., A. Hewelt, G.D. Prestwich, I. Abe, I. Reipen and G. Sprenger. 1994. A specific amino acid repeat in squalene and oxidosqualene cyclases. *Trends Biochem. Sci*., 19(4): 157-158.

Qiao, X., Q. Wang, S. Ji, Y. Huang, K.D. Liu and M. Ye. 2015. Metabolites identification and multi-component pharmacokinetics of ergostane and lanostane triterpenoids in the anticancer mushroom *Antrodia cinnamomea*. *J. Pharm. Biomed. Anal*., 111(4): 266-276.

Rezaei-Golmisheh, A., H. Malekinejad, S. Asri-Rezaei, A.A. Farshid and P. Akbari. 2015. Hawthorn ethanolic extracts with triterpenoids and flavonoids exert hepatoprotective effects and suppress the hypercholesterolemia-induced oxidative stress in rats. *Iran J. Basic. Med. Sci*., 18(7): 691-699.

Sando, T., S. Takeno, N. Watanabe, H. Okumoto and T. Kuzuyama. 2008. Cloning and characterization of the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway genes of a natural-rubber producing plant, Hevea brasiliensis. *Biosci Biotechnol. Biochem*., 72(11): 2903-2917.

Schlogl, P.S., F.T.S. Nogueira, R. Drummond, J.M. Felix and M. Menossi. 2008. Identification of new ABA- and MEJA-activated sugarcane *bZIP* genes by data mining in the SUCEST database. *Plant Cell Rep*., 27(2): 335-345.

Shai, L.J., L.J. McGaw, M.A. Aderogba, L.K. Mdee and J.N. Eloff. 2008. Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm. leaves. *J. Ethnopharmacol*, 119(2): 238-244.

Shen, Z., C. Liu and X. Wang. 2009. Cloning and characterization of open reading frame encoding beta-amyrin synthase in *Glycyrrhiza uralensis*. *Zhongguo Zhong Yao Za Zhi*., 34(19): 2438-2440.

Shi, W., H.W. Liu, X. Guo, L. Hou and J.M. Gao. 2016. Triterpenoids from the stems of *Schisandra grandiflora* and their biological activity. *J. Asian Nat. Prod. Res*., 18(7): 711-718.

Shi, Y.M., X.B. Wang, X.N. Li, X. Luo, Z.Y. Shen, Y.P. Wang, W.L. Xiao and H.D. Sun. 2013. Lancolides, antiplatelet aggregation nortriterpenoids with tricyclo [6.3.0.0 (2,11)] undecane-bridged system from *Schisandra lancifolia*. *Org. Lett*., 15(19): 5068-5071.

Shi, Y.M., W.L. Xiao, J.X. Pu and H.D. Sun. 2015. Triterpenoids from the Schisandraceae family: An update. *Nat. Prod. Rep*., 25(5): 367-410.

Song, J.H., H.J. Choi, H.H. Song, E.H. Hong, B.R. Lee, S.R. Oh, K. Choi, S.G. Yeo, Y.P. Lee, S. Cho and H.J. Ko. 2014. Antiviral activity of ginsenosides against coxsackievirus B3, enterovirus 71, and human rhinovirus 3. *J. Ginseng. Res*., 38(3): 173-179.

Song, Q.Y., W.Y. Qi, Z.M. Li, J. Zhao, J.J. Chen and K. Gao. 2011. Antifungal activities of triterpenoids from the roots of *Astilbe myriantha* Diels. *Food Chem*., 128(2): 495-499.

Sun, J., X. Xu, Z. Xue, J.H. Snyder and X. Qi. 2013. Functional analysis of a rice oxidosqualene cyclase through total gene synthesis. *Mol. Plant*, 6(5): 1726-1729.

Suzuki, H. and R.A. Dixon. 2005. Methyl jasmonate and yeast elicitor induce differential transcriptional and metabolic re-programming in cell suspension cultures of the model legume *Medicago truncatula*. *Planta*, 220(220): 696-707.

Tansakul, P., M. Shibuya, T. Kushiro and Y. Ebizuka. 2006. Dammarenediol-II synthase, the first dedicated enzyme for ginsenoside biosynthesis, in *Panax ginseng*. *Febs Lett*., 580(22): 5143-5149.

Vishwakarma, R.K., P. Sonawane, S. Singh, Kumari, U., Ruby and B.M. Khan. 2013. Molecular characterization and differential expression studies of an oxidosqualene cyclase (OSC) gene of Brahmi (*Bacopa monniera*). *Physiol. Mol. Biol. Plants*, 19(4): 547-553.

Wang, J., J. Ji, Z. Song, W. Zhang, X. He, F. Li, C. Zhang, C. Guo, C. Wang and C. Yuan. 2016. Hypocholesterolemic effect of emodin by simultaneous determination of *In vitro* and *In vivo* bile salts binding. *Fitoterapia*, 110(7): 116-122.

Wang, X.N., H.J. Zhang, D.M. Ren, M. Ji, W.T. Yu and H.X. Lou. 2009. Lobarialides A-C, antifungal triterpenoids from the lichen Lobaria kurokawae. *Chem. Biodivers*, 6(5): 746-753.

Wang, X.Y., G.H. Tang, C.M. Yuan, Y. Zhang, T. Zou, C. Yu, Q. Zhao, X.J. Hao and H.P. He. 2013. Aphagrandinoids A-D, cycloartane triterpenoids with antibacterial activities from *Aphanamixis grandifolia*. *Fitoterapia*, 85(1): 64-68.

Wang, Z., O. Guhling, R. Yao, F. Li, T.H. Yeats, J.K. Rose and R. Jetter. 2011. Two oxidosqualene cyclases responsible for biosynthesis of tomato fruit cuticular triterpenoids. *Plant Physiol*., 155(1): 540-552.

Wei, Y., C.M. Ma, D.Y. Chen and M. Hattori. 2008. Anti-HIV-1 protease triterpenoids from *Stauntonia obovatifoliola* Hayata subsp. intermedia. *Phytochemistry*, 69(9): 1875-1879.

Wu, J., L. Wang and I.T. Baldwin. 2008. Methyl jasmonate-elicited herbivore resistance: Does MeJA function as a signal without being hydrolyzed to JA? *Planta*, 227(5): 1161-1168.

Wu, Y., H.D. Zou, H. Cheng, C.Y. Zhao, L.F. Sun, S.Z. Su, S.P. Li and Y.P. Yuan. 2012. Cloning and characterization of a beta-amyrin synthase gene from the medicinal tree *Aralia elata* (Araliaceae). *Genet. Mol. Res.*, 11(3): 2301-2314.

Yan, S. L., C.Y. Huang, S.T. Wu and M.C. Yin. 2010. Oleanolic acid and ursolic acid induce apoptosis in four human liver cancer cell lines. *Toxicol in Vitro*, 24(3): 842-848.

Yang, C., Q. An, Z. Xiong, Y. Song, K. Yu and F. Li. 2009. Triterpenes from Acanthopanax sessiliflorus fruits and their antiplatelet aggregation activities. *Planta Med*., 75(06): 656-659.

Yang, D., X. Du, X. Liang, R. Han, Z. Liang, Y. Liu, F. Liu and J. Zhao. 2012. Different roles of the mevalonate and methylerythritol phosphate pathways in cell growth and tanshinone production of Salvia miltiorrhiza hairy roots. *Plos One*, 7(11): e46797.

Zhang, F., X. Li, Z. Li, X. Xu, B. Peng, X. Qin and G. Du. 2014. UPLC/Q-TOF MS-based metabolomics and qRT-PCR in enzyme gene screening with key role in triterpenoid saponin biosynthesis of *Polygala tenuifolia*. *Plos One*, 9(8): e105765.

Zhang, W., Y. Zhang, X. Ma and Y. Chen. 2015. Effects of acupuncturing Pishu combined with Ginsenoside Rg3 on the immune function of rats with chronic fatigue. *Int. J. Clin. Exp. Med*., 8(11): 19022-19029.

(Received for publication 27 February 2018)