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Genetic diversity and phylogeny analysis of 3-hydroxy 3-methylglutaryl-CoA reductase gene (*SmHMGR*) in Danshen (*Salvia miltiorrhiza* Bunge)

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Research Article

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Abstract

HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase, is a major rate-limiting enzyme in mevalonate (MVA) pathway for isoprenoids and subsequent tanshinone biosynthesis in the Chinese traditional bulk herbal medicine Danshen, *Salvia miltiorrhiza*, mainly for cardiovascular disorders. In this paper, the genomic *SmHMGR* genes of 38 cultivated populations of *S. miltiorrhiza* collected in China were for the first time sequenced to reveal the genetic diversity and phylogeny. The *SmHMGR* gene was shown to be intron-free, 1650~1659 bp in complete CDS with the majority being 1656 bp, and two unique populations (W-FJLY-V-1 and W-SCHY-W-1) being 1659 and 1650 bp respectively. A total of 103 SNP variation sites were detected with a variation rate of 6.22%, most of which occurred in *S. miltiorrhiza f. alba* population W-SCHY-W-1; a total of 25 amino acid variation sites were found, of which 19 was in W-SCHY-W-1. The same four populations, W-SCHY-W-1, V-HBAG-V-1, V-JLCC-V-1 and S-NM-V-1 could be discriminated from the remaining 34 by both the SNP fingerprints and the deduced amino acid variation sites. Other or composite DNA markers are needed for better identification. The *SmHMGR* gene of white flower *S. miltiorrhiza* f. alba population W-SCHY-W-1 is especially rich in variations and worthy of further studies. Phylogenetic trees based on both the gene and the deduced amino acid sequences showed a very similar two-clade topological structure. This research enriched the content and the genetic means for the molecular identification, genetic diversity and phylogenetic studies of the cultivated *S. miltiorrhiza* populations, and laid a solid foundation for further related and in-depth investigations.

Introduction

The Chinese traditional herbal medicine, one of the bulky, Danshen, the radix of *Salvia miltiorrhiza* Bunge, is a perennial herb of the genus *Salvia* and the family Labiatae. It cherishes a rich and long history of several thousands of years, and possesses a wide range of pharmacological effects, including eliminating stasis to activate blood, relieving dysmenorrhoea and pain, cleaning the mind and keep free from troubles, cooling blood and relieving pain (Chen *et al.*, 2015; Maione and Mascolo, 2016; Cao *et al.*, 2017). It has been recorded early in *Shennong's Classic of Materia Medica* written in 1488~1505 AD as 'mild cold, non-toxic and bitter taste' and has the function of 'blemish removal, vexation stopping and Qi benefiting'.

Traditionally, the identification and evaluation of Danshen have been based on sources, characters, physicochemical properties and bioassay (Li and Guo, 2010). There have been genetic diversity studies of *S. miltiorrhiza* by various molecular marker techniques, such as random amplified polymorphic DNA (RAPD) (Guo *et al.*, 2002; Shi *et al.*, 2010), amplified fragment length polymorphism (AFLP) (Tang *et al.*, 2006; Wen *et al.*, 2007), inter-simple sequence repeat (ISSR) (Song *et al.*, 2008), ISSR and SRAP (Song *et al.*, 2010), chloroplast simple sequence repeats (SSR) (Wang *et al.*, 2012) and expressed sequence tag-simple sequence repeats (EST-SSR) (Song *et al.*, 2014).

Recently Zhou *et al.* (2021) assessed the wild germplasm diversity for *S. miltiorrhiza* and its related species which provided a fundamental genetic background for the cultivation and molecular breeding of this medicinally important species. Our group revealed the genetic diversity of cultivated Danshen populations by internal transcribed spacers (*ITS*) of the rDNA (Liao *et al.*, 2021) and chloroplast rubisco large subunit (*rbcL*) gene (Feng *et al.*, 2021).

There are two major classes of pharmacologically effective components in Danshen, the hydrophilic salvianolic acids, which are important compounds determining the medicinal

Table 1. The cultivated *S. miltiorrhiza* populations used in this study

No.	Code	Production region	N/E (°)	Source[a]	No.	Code	Production region	N/E (°)	Source[a]
1	V-JLCC-V-1	Changchun, Jilin	44/125	HDSI	20	W-GD-V-2	Guangdong	23/113	TDSI
2	V-ZZY-V-1	Zunyi, Guizhou	26/106	HDSI	21	W-JS-V-2	Jiangsu	34/118	TDSI
3	V-JSSY-V-1	Shuyang Jiangsu	34/118	HDSI	22	S-SDYT-V-1	Yantai, Shandong	36/117	HDSI
4	V-YNLJ-V-1	Lijiang, Yunnan	27/100	HDSI	23	S-HNFC-V-1	Fangcheng, Henan	33/113	HDSI
5	V-GD-V-1	Guangdong	23/113	HDSI	24	S-HNCS-V-1	Changsha, Hunan	28/112	HDSI
6	V-CQ-V-1	Chongqing	30/106	HDSI	25	S-SDJX-V-1	Juxian, Shandong	36/117	HDSI
7	V-SD-V-2	Shandong	36/117	TDSI	26	S-GSJQ-V-1	Jiuquan, Gansu	39/98	HDSI
8	V-GZ-V-2	Guizhou	26/106	TDSI	27	S-NM-V-1	Nemeng	40/111	HDSI
9	V-JS-V-2	Jiangsu	32/119	TDSI	28	S-GX-V-1	Guangxi	24/112	HDSI
10	V-BJ-V-1	Beijing	40/116	FDSI	29	S-HBAG-V-1	Anguo, Hebei	38/115	HDSI
11	V-HBAG-V-1	Anguo, Hebei	38/115	FDSI	30	B-AHQJ-V-1	Quanjiao, Anhui	32/118	HDSI
12	V-GSLX-V-1	Longxi, Gansu	35/104	FDSI	31	B-SCZJ-V-1	Zhongjiang, Sichuan	31/104	HDSI
13	W-HBJM-V-1	Jingmen Hubei	31/112	HDSI	32	B-SD-V-2	Shandong	36/117	TDSI
14	W-SXXA-V-1	Xi′an, Shaanxi	34/109	HDSI	33	B-SC-V-2	Sichuan	30/104	TDSI
15	W-LNSY-V-1	Shenyang, Liaoning	41/123	HDSI	34	B-JS-V-2	Jiangsu	34/118	TDSI
16	W-FJLY-V-1	Luoyuan, Fujian	26/119	HDSI	35	B-GD-V-2	Guangdong	23/113	TDSI
17	W-SD-V-2	Shandong	36/117	TDSI	36	CYSWC-DZH-V-4	Mediterranean	45/30	FDSI
18	W-SC-V-2	Sichuan	30/104	TDSI	37	Q-DZH-V-4	Mediterranean	45/30	FDSI
19	W-GZ-V-2	Guizhou	26/106	TDSI	38	W-SCHY-W-1	Hongyuan, Sichuan	32/102	SC

N/E, North latitude/East longitude.
[a]HDSI, Hengda Seed Industry; TDSI, Tongda Seed Industry; FHSI, Fenghong Seed Industry; SC, Self-Collected.

quality of Danshen (Zhao *et al.*, 2006), and the lipophilic tanshinones (Hung *et al.*, 2016). So far, it seems no genetic diversity has been revealed by the functional genes of *S. miltiorrhiza*, especially those related to tanshinone synthesis pathway. Recently a high-quality reference genome sequence of *S. miltiorrhiza* with the highest level of continuity has been released providing insights into tanshinone synthesis in its red rhizomes, and will facilitate future studies on the elucidation of the secondary metabolic pathway and the genetic improvement of *S. miltiorrhiza* (Song *et al.*, 2020).

HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase (EC1.1.1.34) catalyses HMG-CoA to mevalonate (MVA), the first committing step in the MVA pathway for isoprenoid biosynthesis in plants (Liao *et al.*, 2009). It should be also one of the major regulatory targets for the biosynthesis of tanshinones in *S. miltiorrhiza*.

There was a report showing the structural and functional conservation of the *HMGR* gene between yeast and human (Basson *et al.*, 1988). The *HMGR* gene in *S. miltiorrhiza* has been cloned and characterized (Liao *et al.*, 2009), and the molecular mechanism of *SmHMGR* together with other several related genes in elicitor-induced tanshinone accumulation in *S. miltiorrhiza* hairy root cultures has been studied (Kai *et al.*, 2012).

However, no reports have been seen so far of its genomic *SmHMGR* counterpart, its genetic diversity, and phylogenetic relations in the abundant cultivated *S. miltiorrhiza* germplasm resources.

In order to understand the gene structure, genetic diversity and phylogenetic relations of the genomic *SmHMGR* genes in currently cultivated *S. miltiorrhiza* populations in China, and to lay a basis for its germplasm resources identification and evaluation, the complete genomic *SmHMGR* genes of the 38 cultivated populations of *S. miltiorrhiza* collected in China were for the first time cloned by walking technology with homologous primers, sequenced and the genetic diversity and phylogenetic relations among the populations based on the complete sequences were revealed in this research.

Materials and methods

Plant materials

Thirty-eight cultivated *S. miltiorrhiza* populations purchased mainly from three major seed industries representing more than 30 regions of China were the first field cultivated for morphological observation and confirmation in Southwest University Agricultural Station, Chongqing (N29°/E106°) and then leaves were used as materials for this study (Table 1).

Primer design

Three pairs of walking primers for the cloning of the genomic *SmHMGR* gene based on the reference accession (GU367911.1) were designed with Primer 5 (Table 2). Primer positions in the

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Table 2. Primers designed for the cloning of the *SmHMGR* gene

Primer code	Sequence (5′→3′)	Length (nt)	Annealing temp (Ta)	Ref accession	Position in ref
HMGR-FP1	GGGAGTGAATAATGAAGTATGAGTG	25	57	GU367911.1	1114
HMGR-RP1	TTCAACTCCATCCAGTAACAGCG	23			1970
HMGR-FP2	CTTCTTCTCCGTCGTCTACT	20	57		1425
HMGR-RP2	TGTACTTCATGTGGCTCTTG	20			2928
HMGR-FP3	CAACTACTGCTCCGACAAGAAG	22	56		2397
HMGR-RP3	TACAGAAACCAAAGTGGATCGT	22			3066

Table 3. Basic data of the genomic *SmHMGR* genes of the cultivated *S. miltiorrhiza* populations

No.	Code	Full length (bp)	No. of amino acids coded	GenBank accession	No.	Code	Full length (bp)	No. of amino acids coded	GenBank accession
1	V-JLCC-V-1	1656	551	MW658326	20	W-GD-V-2	1656	551	MW658305
2	V-GZZY-V-1	1656	551	MW658325	21	W-JS-V-2	1656	551	MW658306
3	V-JSSY-V-1	1656	551	MW658322	22	S-SDYT-V-1	1656	551	MW658310
4	V-YNLJ-V-1	1656	551	MW658324	23	S-HNFC-V-1	1656	551	MW658311
5	V-GD-V-1	1656	551	MW658329	24	S-HNCS-V-1	1656	551	MW658312
6	V-CQ-V-1	1656	551	MW658330	25	S-SDJX-V-1	1656	551	MW658336
7	V-SD-V-2	1656	551	MW658328	26	S-GSJQ-V-1	1656	551	MW658332
8	V-GZ-V-2	1656	551	MW658321	27	S-NM-V-1	1656	551	MW658315
9	V-JS-V-2	1656	551	MW658320	28	S-GX-V-1	1656	551	MW658314
10	V-BJ-V-1	1656	551	MW658323	29	S-HBAG-V-1	1656	551	MW658313
11	V-HBAG-V-1	1656	551	MW658327	30	B-AHQJ-V-1	1656	551	MW658331
12	V-GSLX-V-1	1656	551	MW658334	31	B-SCZJ-V-1	1656	551	MW658335
13	W-HBJM-V-1	1656	551	MW658301	32	B-SD-V-2	1656	551	MW658318
14	W-SXXA-V-1	1656	551	MW658302	33	B-SC-V-2	1656	551	MW658316
15	W-LNSY-V-1	1656	551	MW658304	34	B-JS-V-2	1656	551	MW658317
16	W-FJLY-V-1	1659	552	MW658303	35	B-GD-V-2	1656	551	MW658319
17	W-SD-V-2	1656	551	MW658307	36	CYSWC-DZH-V-4	1656	551	MW658333
18	W-SC-V-2	1656	551	MW658308	37	Q-DZH-V-4	1656	551	MW658337
19	W-GZ-V-2	1656	551	MW658309	38	W-SCHY-W-1	1650	549	MW678602

cloned *SmHMGR* gene as that of population W-SCHY-W-1 were given in online Supplementary Fig. S1.

Extraction of genomic DNA

Qiagen DNeasy Plant Mini Kit (Multi Sciences, Hangzhou, China) was used for the extraction of total leaf genomic DNAs of the 38 cultivated *S. miltiorrhiza* populations according to the manufacturer's instruction. The purity and quantity of the isolated DNAs were evaluated by agarose gel electrophoresis followed by Goldview staining and determination of the purity ratios of A260/A280, using Shimadzu UV mini-1240 UV-VIS spectrophotometer (Shimadzu, Japan). The purified DNAs were dissolved in 10 mmol/l Tris-HCl buffer and stored at − 70 °C.

PCR amplification and product sequencing

About 1.0 µg of the genomic DNAs of the 38 cultivated *S. miltiorrhiza* populations were amplified in three segments with primer pairs HMGR-FP1/HMGR-RP1, HMGR-FP2/HMGR-RP2 and HMGR-FP3/HMGR-RP3 respectively in a reaction mixture of 50 µl: 1.1 × T3 Super PCR Mix 36.0~44 µl and 10 µmol/l primers each 2.0 µl (final concentration 0.4 µM). PCR amplification was run in Biometra TGRADIENT thermocycler (Biometra GmbH, Germany) with the following programme: initial-denaturation at 98 °C for 3 min followed by 35 cycles of denaturation at 98 °C for

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10 s, annealing at 57/57/56 °C for 10 s and elongation at 72 °C for 5∼15 s and a final extension step at 72 °C for 2 min.

The amplified products were electrophoresed in 1% agarose gel and visualized with Goldview staining; and after recovery and purification, bidirectionally sequenced by dideoxy chain termination with ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, USA).

Sequence data processing

The BLAST confirmed three segments of the *SmHMGR* gene of the 38 cultivated populations of *S. miltiorrhiza* were spliced with Vector NTI Advance 11 (Lu and Moriyama, 2004). The complete sequences were manually checked to ensure the quality of sequences. The nucleotide variation sites of *SmHMGR* gene sequences of the 38 cultivated populations of *S. miltiorrhiza* were identified by Vector NTI Advance 11, and manually checked and confirmed. And finally, MEGA X (Kumar *et al.*, 2018) was used for phylogenetic tree construction by Neighbour-Joining (NJ).

Results and analyses

Genetic diversity analysis of the SmHMGR genes

Basic structural features of the SmHMGR genes of the cultivated S. miltiorrhiza populations

The confirmed complete genomic *SmHMGR* sequences were submitted to GenBank (Table 3). No introns were found in all the *SmHMGR* sequences. The genomic full length *SmHMGR* genes of the majority *S. miltiorrhiza* populations (36 populations) were 1656 bp, encoding 551 amino acids, while 2 populations, W-FJLY-V-1 and W-SCHY-W-1 were 1659 and 1650 bp encoding 552 and 549 amino acids respectively (Table 3).

Nucleotide variation of the genomic SmHMGR *genes*

There were 103 SNP variation sites of the *SmHMGR* gene in 38 populations, with a variation rate of 6.22%, among which, 56 transitions, 35 transversions and 12 insertions/deletions. Most variations (96) occurred in W-SCHY-W-1, followed by five in S-NM-V-1, two in V-JLCC-V-1 and one in V-HBAG-V-1. The remaining 34 populations were in consensus. Four populations, V-JLCC-V-1, V-HBAG-V-1, W-SCHY-W-1 and S-NM-V-1, could be discriminated from the remaining 34 by SNP fingerprints (Table 4). The morphology of seed, flower and root of the four unique Danshen populations is shown in online Supplementary Fig. S2.

Variations of the deduced amino acid sequences of SmHMGR *genes*

The deduced amino acid sequences of *SmHMGR* genes of the 38 *S. miltiorrhiza* populations all shared the two putative HMG CoA binding sites (EMPVGYVQIP/ TTEGCLVA) and the two NAD (P)H binding sites (DAMGMNM/GTVGGGT) as reported by Liao *et al.* (2009), indicating its high conservation. However, the stop codons were all found to be TAA rather than TGA as reported by Liao *et al.* (2009).

A total of 25 amino acid variation sites were detected: 10 were similar substitutions, 11 somewhat dissimilar substitutions, 3 deletion/insertions and one extra in W-SCHY-W-1 in which a

Table 4. Nucleotide variation sites of *SmHMGR* sequences of *S. miltiorrhiza* populations

Site	8	35	37	41	321	324	354	358	369	372	373	374	375	376	377	387	388	389	390	402	418	432	457	472	484	498	499	516	549	580	582	606	609	627	636
Population																																			
V-JLCC-V-1	**A**	C	**G**	C	G	A	A	G	C	C	G	C	G	C	C	G	G	C	G	G	C	C	T	C	G	C	G	G	C	T	A	C	C	C	G
V-HBAG-V-1	T	**G**	A	**G**	G	A	A	G	C	C	G	C	G	C	C	G	G	C	G	G	C	C	T	C	G	C	G	G	C	T	A	C	C	C	G
W-SCHY-W-1	T	C	A	C	**C**	**G**	**C**	**A**	**A**	**/**	**/**	**/**	**/**	**/**	**T**	**/**	**/**	**/**	**/**	**C**	**A**	**G**	**G**	**T**	**C**	**T**	**A**	**A**	**T**	**C**	**G**	**G**	**A**	**G**	**T**
Remaining 35	T	C	A	C	G	A	A	G	C	C	G	C	G	C	C	G	G	C	G	G	C	C	T	C	G	C	G	G	C	T	A	C	C	C	G
Site	639	643	648	654	657	658	660	663	669	672	711	744	774	783	810	846	864	871	873	918	949	966	981	1014	1068	1092	1101	1104	1134	1144	1151	1161	1182	1188	1194
Population																																			
W-SCHY-W-1	**G**	**A**	**G**	**C**	**G**	**A**	**C**	**G**	**G**	**C**	**A**	**G**	**A**	**T**	**C**	**C**	**C**	**T**	**G**	**G**	**C**	**A**	**C**	**C**	**T**	**G**	**G**	**G**	**C**	**T**	**T**	**G**	**C**
Remaining 37	C	C	A	T	A	G	T	A	C	A	C	C	G	C	G	T	G	C	C	A	A	G	G	C	C	G	G	C	C	A	A	G	G	C	T
Site	1197	1239	1245	1254	1257	1278	1284	1293	1302	1320	1326	1327	1329	1365	1392	1404	1410	1416	1428	1458	1470	1503	1515	1544	1561	1563	1585	1587	1603	1650	1651	1652	1653		
Population																																			
S-NM-V-1	G	C	C	G	G	C	C	C	C	G	T	A	C	C	G	G	C	T	C	G	G	C	G	G	C	C	T	C	**C**	**G**	/	/	/		
W-SCHY-W-1	**C**	**G**	**A**	**T**	**C**	**T**	**A**	**G**	**G**	**A**	**C**	**G**	**G**	**G**	**A**	**A**	**T**	**C**	**A**	**A**	**T**	**T**	**C**	**T**	**T**	**G**	G	**G**	G	**A**	**T**	**C**	**A**		
Remaining 36	G	C	C	G	G	C	C	C	C	G	T	A	C	C	G	G	C	T	C	G	G	C	G	G	C	C	G	C	G	G	/	/	/		

SNP variations were shown in bold type; '/': deletions

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stop codon was mutated to a sense codon for threonine; and among which 19 were in W-SCHY-W-1, two in each of S-NM-V-1, V-HBAG-V-1 and V-JLCC-V-1. All the amino acid variations occurred outside the conserved sites.

Four populations i.e.W-SCHY-W-1, S-NM-V-1, V-HBAG-V-1 and V-JLCC-V-1, could be discriminated from the remaining 34 populations by the amino acid variation sites of the *SmHMGR* gene (Table 5).

Phylogenetic analyses of SmHMGR *gene and the deduced amino acid sequences*

Phylogenetic trees based on the gene and the deduced amino acid sequences with the reference accession (GU367911.1) as the outgroup, both showed a very similar two-clade topological structure. The phylogenetic tree based on *SmHMGR* gene sequences showed that population W-SCHY-W-1 stands alone, and the other 37 populations clusters in another clade, which is subdivided into two subclades, with population V-JLCC-V-1 alone and the other 36 in another (Fig. 1A). Phylogenetic tree based on the deduced amino acid sequences showed that population W-SCHY-W-1 stands alone, and the other 37 populations clusters in another clade, which is subdivided into two subclades, with population V-HBAG-V-1 alone and the other 36 in another (Fig. 1B).

Conclusion and discussion

This study for the first time clarified the structure of the full length genomic *SmHMGR* genes for the majority *S. miltiorrhiza* populations: without intron, about 1656 bp in length and encoding about 551 amino acids, slightly different from the full length HMGR cDNA gene of *S. miltiorrhiza* as reported by Liao *et al.* (2009) (EU680958) which contained 1695 bp open reading frame (ORF) encoding 565 amino acids. Two unique populations W-SCHY-W-1 and W-FJLY-V-1 were found to be 1650 and 1659 bp in full length and encode 549 and 552 amino acids respectively.

The same four populations could be discriminated by both the nucleotide and the deduced amino acid sequence fingerprints of the *SmHMGR* gene.

The intronless nature of the *SmHMGR* gene might suggest its primitive nature. And the fact that all the stop codons found in the cloned *SmHMGR* genes were TAA rather than TGA as reported by Liao *et al.* (2009) might indicate the preference of stop codons in different populations. It would be very interesting to further study the evolution of introns and stop codon preference of the *HMGR* gene family.

Extensive nucleotide and deduced amino acid variations were found in the white flower *S. miltiorrhiza* Bge. f. alba population W-SCHY-W-1. It was reported that two more bioactive ingredients namely tetrahydrotanshiquinone and tanshinaldehyde were detected in *S. miltiorrhiza* Bge. f. alba (Li *et al.*, 1991). Hang *et al.* (2008) found that most plant parts of *S. miltiorrhiza* Bge. f. alba has higher contents of bioactives than in *S. miltiorrhiza* Bge. There was no difference in the contents of tanshinone IIA and salvianolic acid B between *S. miltiorrhiza* Bge and *S. miltiorrhiza* Bge. f. alba, as revealed by HPLC (Shao *et al.*, 2009). The most recent integrated analysis of transcriptomics and metabolomics showed that certain anthocyanins were mainly responsible for the purple flower colour of *S. miltiorrhiza*. Low expression of the anthocyanin synthesis genes decreased the

Table 5. Amino acid variations encoded by *SmHMGR* genes of *S. miltiorrhiza* populations

Site Population	3	12	13	14	107	120	125	126	127	128	129	130	140	153	162	167	220	382	384	413	443	529	535	551	552
S-NM-V-1	M	A	K	A	E	A	A	P	C	K	T	A	L	S	G	V	V	I	K	I	I	**S**	T	T	*
V-HBAG-V-1	M	**G**	K	**G**	E	A	A	P	C	K	T	A	L	S	G	V	V	I	K	I	I	A	S	T	*
V-JLCC-V-1	**K**	A	**E**	A	E	A	A	P	C	K	T	A	L	S	G	V	V	I	K	I	I	A	S	T	*
W-SCHY-W-1	M	A	K	A	**D**	**T**	**C**	**/**	**/**	**/**	**K**	**T**	**I**	**A**	**R**	**I**	**I**	**V**	**I**	**M**	**V**	A	S	**S**	**T**	*
Remaining 34	M	A	K	A	E	A	A	P	C	K	T	A	L	S	G	V	V	I	K	I	I	A	S	T	*

Amino acid variation sites were shown in bold type; '/' represents deletions; and '*' represents stop codons.

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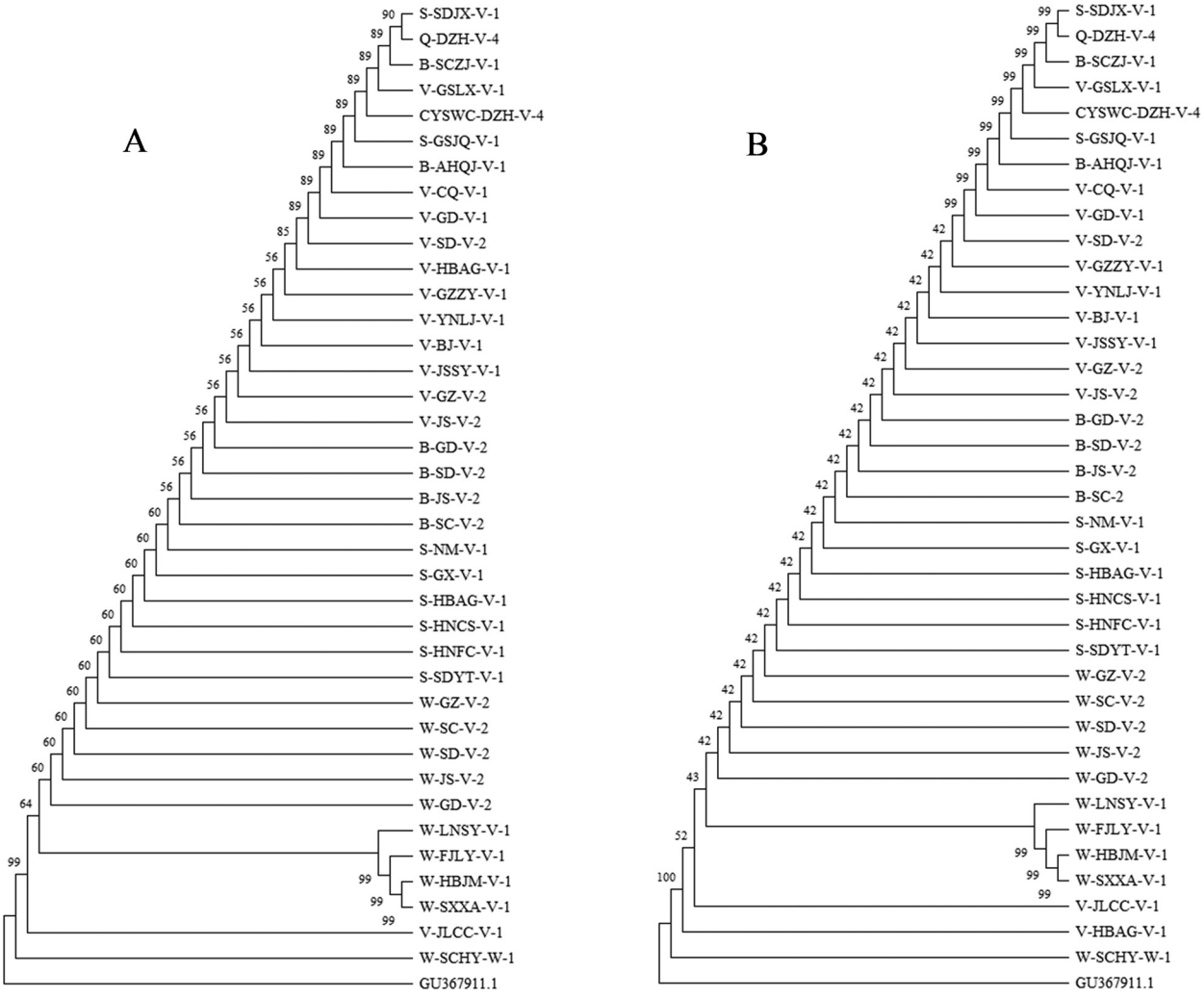

Fig. 1. Phylogenetic trees based on *SmHMGR* gene (A) and the deduced amino acid sequences (B).

anthocyanin content but enhanced the accumulation of flavonoids in *S. miltiorrhiza* Bge. f. alba (Jiang *et al.*, 2020).

Compared with the purple flower majority, *S. miltiorrhiza* Bge. f. alba is generally of higher medicinal value. We suggest it will be worthy while to further investigate the genetic and expressional differences between *S. miltiorrhiza* Bge and *S. miltiorrhiza* Bge. f. alba as regard to the genes involved in the salvianolic acid and the tanshinone biosynthetic pathways.

This study laid a solid basis for the identification and evaluation of the cultivated *S. miltiorrhiza* germplasm resources, and for further comparative *SmHMGR* gene expression studies in relation to the contents of pharmacological ingredients among the currently cultivated *S. miltiorrhiza* populations.

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