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Comparison of rDNA ITS sequences and tanshinones between *Salvia miltiorrhiza* populations and *Salvia* species

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ABSTRACT. Danshen is one of the well-known herbs classified as "blood-invigorating" in traditional Chinese herbal medicine. Although *Salvia miltiorrhiza* is listed as the only botanical source of Danshen in the Chinese Pharmacopoeia, more than 20 other *Salvia* species are also used as Danshen in China while about 10 *Salvia* species are employed as non-Danshen. In order to identify *S. miltiorrhiza* and related *Salvia* species, and evaluate their quality, the rDNA ITS regions of ten *Salvia* species and twelve *S. miltiorrhiza* populations were sequenced and compared, and tanshinones (including cryptotanshinone, tanshinone I and tanshinone IIA) were quantitatively determined using the HPLC method. The nucleotide sequences of *Salvia* samples showed obvious diversity, and each *Salvia* species and *S. miltiorrhiza* population were found to have a unique sequence in the ITS region, so that they could be distinguished at the DNA level. Cluster analysis divided the *Salvia* species into two main groups. *Salvia miltiorrhiza* and six other Danshen species had a closer phylogenetic relationship and were differentiated from the non-Danshen species, results which corresponded with their medicinal requirements. The HPLC data showed that tanshinones were present mainly in the roots of *S. miltiorrhiza* and eight other *Salvia* species, including six Danshen species, and the contents were significantly different in each of them. However tanshinones were not detected in the non-Danshen-*S. deserta*, and this chemical variation can distinguish Danshen and non-Danshen species from each other. Although no direct cluster analysis correlation between chemical and DNA data appeared, both types of analysis are important for the quality evaluation of *S. miltiorrhiza* and related *Salvia* species.

Keywords: HPLC; rDNA; ITS; *Salvia miltiorrhiza*; *Salvia*; Tanshinones.

INTRODUCTION

Radix Salviae Miltiorrhizae, commonly known as Danshen in China, is one of the most important traditional Chinese medicines and belongs to the *Salvia* genus. It can stimulate blood circulation to remove blood stasis, relieve restlessness and tranquilize the mind, and is widely used in traditional Chinese medicinal preparation to treat cardio-cerebral vascular diseases (Zhou et al., 2005). The genus *Salvia* L. is composed of 78 species, 24 varieties and eight forma in China. Among them only *S. miltiorrhiza* is listed as a botanical source of Danshen in the Chinese Pharmacopoeia (2005). However, more than 20 other species (including varieties and forma) have been employed as Danshen in local areas in China while about 10 species are described as non-Danshen and are used to reinforce the kidney and lung, relieve toxic heat, and subdue inflammation (Xiao et al., 1997; Guo et al., 2002). This is confusing for consumers because many species of *Salvia* resemble each other in shape, and morphological differentiation is very difficult. Tanshinones, the lipophilic diterpenoids quinines that have been isolated from Danshen, are reported to be the major bioactive components (Kim et al., 2002; Zhou et al., 2006; Fu et al., 2007). Cryptotanshinone, tanshinone I and tanshinone IIA are present in the greatest amounts. In the Chinese Pharmacopoeia, the quantification of *Radix Salviae Miltiorrhizae* is performed by determination of tanshinone IIA with HPLC. However, this is not sufficient to comprehensively evaluate the quality of the herbal medicine because tanshinone IIA is not the sole pharmaceutically active compound.

DNA-based polymorphism assay may offer an alternative method of authenticating this herbal medicine.

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Here, the amplified ITS (internal transcribed spaces) region of ten *Salvia* species, including *S. miltiorrhiza* and six other Danshen species (*S. digitaloides, S. przewalskii, S. flava*, *S. trijuga*, *S. yunnanensis*, *S. bowleyana*), one non-Danshen species (*S. deserta*) and two non-medicinal species (*S. liguliloba*, *S. chienii*) were sequenced and compared to explore the possibility of using the ITS region as a molecular marker to differentiate the *Salvia* species. The contents of cryptotanshinone, tanshinone I and tanshinone IIA were determined to evaluate the chemical variation in *Salvia* species and *S. miltiorrhiza* populations.

MATERIALS AND METHODS

Materials

Fresh plants and dried crude drugs were collected from different regions of China (Table 1) and identified by Dr. Li-Hong Wu and Dr. Hong Xu of the Shanghai University of Traditional Chinese Medicine. Voucher specimens were deposited in the Shanghai University of Traditional Chinese Medicine, Shanghai, China. Cryptotanshinone (purity>97%), tanshinone I (purity>96%) and tanshinone IIA (purity>96%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (P.R. China).

DNA Sequencing

Genomic DNA Extraction. Samples were subjected to DNA isolation with a modified CTAB (cetyltrimethyl ammonium bromide) protocol with additional purification by DNA Purification Kit (Watson Biotechnologies, Inc., Shanghai, China).

PCR Amplification and Sequencing. The primers used for the amplification of ITS were the P18S forward primer (5′-ATT GAA TGG TCC GGT GAA GTG TTC G-3′) and P26S reverse primer (5′-AAT TCC CCG GTT CGC TCG CCG TTA C-3′). The PCR reactions followed those of a previous report (Xu et al., 2006). The PCR products were purified by a PCR Purification System (Watson, P.R. China) and subcloned into a TA cloning vector pGEM-T (Promega, USA) for sequencing by an ABI PRISM™ 377 Genetic Analyzer (ABI, USA). Each species was tested on two to three specimens from the same collection. Several clones of each PCR product were sequenced to avoid errors introduced by *Taq* DNA polymerase.

Chemical analysis

The sample solution was prepared by ultrasonicating the powders of the dried root using a mixture of chloroform and methanol (8:1) and then analyzed by an Agilent HP1100 liquid chromatograph (Hewlett Packard,

Table 1. Plant used in this study and their accession numbers in Genbank.

No.	Taxon	Subgen.	Locality	Abbreviation	Accession No.	Medicinal parts and uses
1	*S. digitaloides*	*Salvia*	Lijiang, Yunnan	SD-YNLJ	DQ132869	Root used as Danshen
2	*S. przewalskii*	*Salvia*	Lijiang, Yunnan	SP-YNLJ	DQ132862	Root used as Danshen
3	*S. flava*	*Salvia*	Lijiang, Yunnan	SF-YNLJ	DQ132867	Root used as Danshen
4	*S. trijuga*	*Sclarea*	Lijiang, Yunnan	ST-YNLJ	DQ132870	Root used as Danshen
5	*S. yunnanensis*	*Sclarea*	Lijiang, Yunnan	SY-YNLJ	DQ132866	Root used as Danshen
6-1	*S. miltiorrhiza*	*Sclarea*	Shangluo, Shanxi	SM-SXSL	DQ132863	Root used as Danshen
6-2	*S. miltiorrhiza*		Zhongjiang, Sichuan	SM-SCZJ	DQ132864	Root used as Danshen
6-3	*S. miltiorrhiza*		Shanghai	SM-SH	EU591975	Root used as Danshen
6-4	*S. miltiorrhiza*		Pinyi, Shandong	SM-SDPY	EU591972	Root used as Danshen
6-5	*S. miltiorrhiza*		Yuncheng, Shanxi	SM-SXYC		Root used as Danshen
6-6	*S. miltiorrhiza*		Yishui, Shandong	SM-SDYS		Root used as Danshen
6-7	*S. miltiorrhiza*		Sheyang, Jiangsu	SM-JSSY	EU591976	Root used as Danshen
6-8	*S. miltiorrhiza*		Bozhou, Anhui	SM-AHBZ	EU591964	Root used as Danshen
6-9	*S. miltiorrhiza*		Shanxi	SM-SX	EU591974	Root used as Danshen
6-10	*S. miltiorrhiza*		Binhai, Jiangsu	SM-JSBH		Root used as Danshen
6-11	*S. miltiorrhiza*		Shandong	SM-SD	EU591973	Root used as Danshen
6-12	*S. miltiorrhiza*		Rugao, Jiangsu	SM-JSRG		Root used as Danshen
7	*S. bowleyana*	*Sclarea*	Linan, Zhejiang	SB-ZJLA	EU592037	Root used as Danshen
8	*S. deserta*	*Sclarea*	Urumuqi, Xinjiang	SDE-XJWLMQ	DQ132865	Non-Danshen, herb used to cause diuresis, remove toxic heat and resolve phlegm.
9	*S. liguliloba*	*Allagospadonopsia*	Linan, Zhejian	SL-ZJLA	EU592036	Not used as herbal medicine
10	*S. chienii*	*Allagospadonopsia*	Huangshan, Anhui	SC-AHHS	DQ132868	Not used as herbal medicine

Palo Alto, CA, USA) with a Polaris C18 column (5 µm, 250 × 4.6 mm). Elution was with methanol - water (75:25, V:V) at 1 ml /min. The elution was monitored at 270 nm. The three tanshinones were completely separated from the other compounds. The detector response was linear from 3.98×10^{-3} to 5.373×10^{-1} µg of cryptotanshinone (y=4534.32824x+0.2926528, R=0.99999), 4.10×10^{-3} to 5.535×10^{-1} µg of tanshinone I (y=3759.50369x+2.5238072 R=0.99999), and 4.50×10^{-3} to 6.075×10^{-1} µg of tanshinone IIA (y=5040.58974x+5.4577613, R=0.99999) was used for the quantitative data. This method was sensitive and accurate with good reproducibility.

Data Analysis

The sequences as well as the content of the tanshinones were subjected to similarity matrix and cluster analyses using the Clustal W programs (Multiple sequence alignment programs, version 1.7), Molecular and Evolutionary Genetic Analysis (MEGA, version 3.1), and Numerical Taxonomy and Multivariate Analysis System program package for PC (NTSYS-pc, version 2.1). The sequences were aligned and compared using the Clustal W programs, and the sequence divergence was analyzed using the MEGA2 programs. The genetic dendrogram was constructed by the Neighbor Joining (NJ) tree construction method (Nei, 1987) using the MEGA2 programs.

The similarity index of the content of tanshinones was constructed using similarity for interval data with Euclidean distance [The Euclidean distance between two points P= (p_1, p_2,……, p_n) and Q= (q_1, q_2….., q_n) is defined as $\sqrt{(p_1-q_1)^2+(p_2-q_2)^2+.....+(p_n-q_n)^2}=\sqrt{\sum_{i=1}^{n}(p_i-q_i)^2}$].

The chemical dendrogram was constructed by applying the unweighted pair group method with arithmetic averages (UPGMA) using NTSYS-pc programs. A Mantel test with 1000 permutations was conducted by NTSYS-pc to compare the chemical and genetic similarity matrices.

RESULTS

Sequence analysis

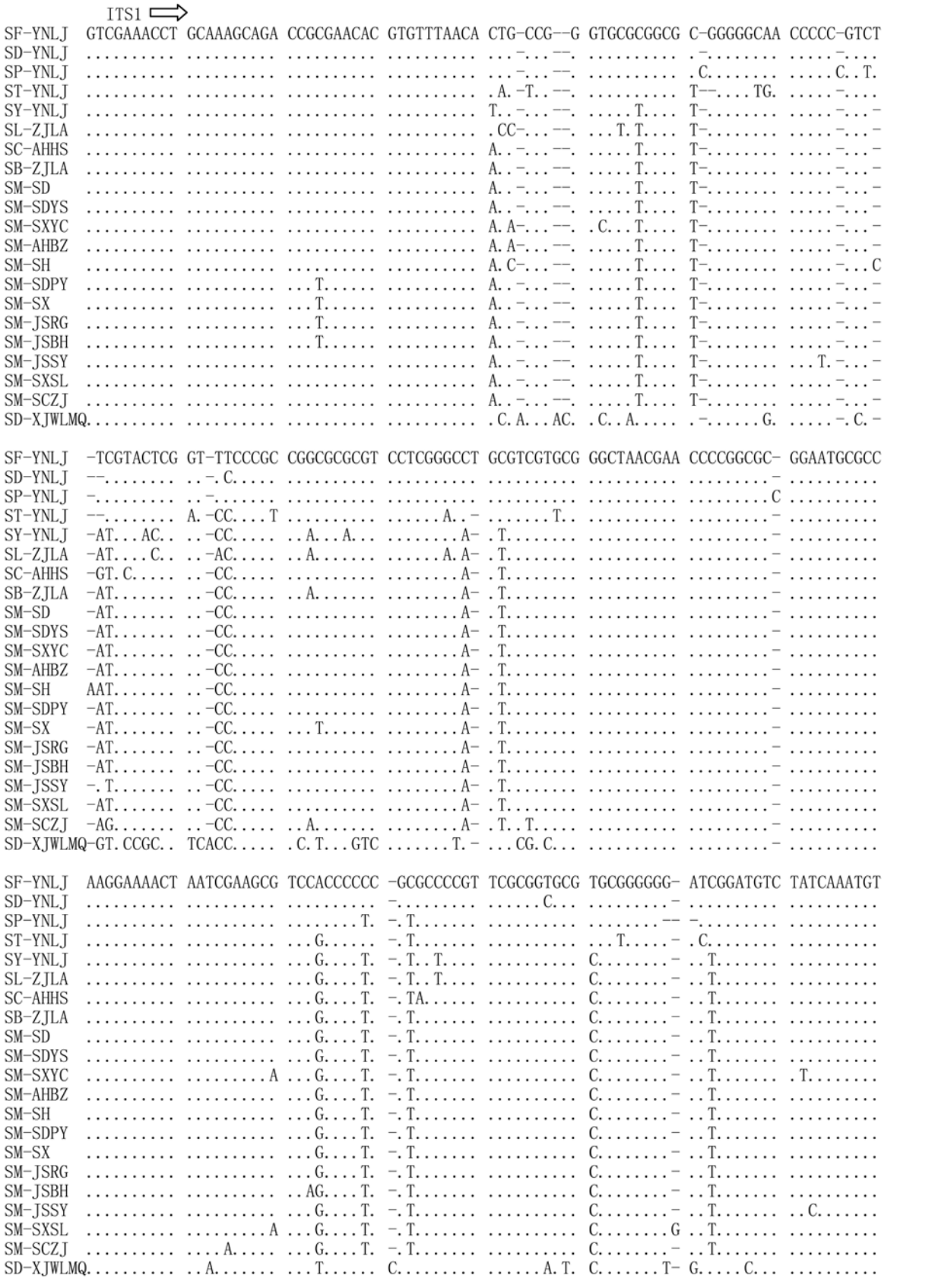
The ten *Salvia* species, including twelve *S. miltiorrhiza* populations, were tested in this study. An approximately 700 bp fragment—including the end of the l8S rDNA, the beginning of the 26S rDNA, and the ITS1-5.8S rDNA-ITS2 regions in their entirety—was specifically amplified and sequenced for all samples. All specimens of the same species from the identical collection place displayed identical sequences. Alignment of these sequences showed that the 18S, 26S and 5.8S rDNA were highly conserved. The ITS1 and ITS2 regions were more variable with the inter-specific sequence divergence ranging from 1.33% to 19.4% in *Salvia* species, and from 0.22% to 3.17% in *S. miltiorrhiza* populations (Figure 1, Table 2). It is clear that genetic divergence in the rDNA ITS region exists among *Salvia* samples, and each *Salvia* species and the population of *S. miltiorrhiza* was found to have a unique sequence in its ITS region, so its members can be easily distinguished at the DNA level.

Based on the ITS1 and ITS2 sequence from *Salvia* species, a NJ phylogenetic tree was constructed (Figure 2). The *Salvia* taxon clustered naturally into two separate groups. *Salvia miltiorrhiza*, the six other Danshen species, and the two non-medicinal species clustered together, had a closer phylogenetic relationship, and were differentiated from non-Danshen species. All members of *S. miltiorrhiza* from different populations clustered together. Based on the classification of *Salvia* in the Florae Repubicae Sinicae (1977), the 10 studied *Salvia* species should be grouped under three subgenus: *Salvia*, *Sclarea* and *Allagospadonopsia*. The polygenetic tree of *Salvia* species deduced from the ITS region, however, does not totally match the classification based on morphological characters. The five species of *Sclarea*, *S. trijuga, S. yunnanensis, S. miltiorrhiza*, *S. bowleyana*, and *S. deserta* were separated into different clades, as were *S. liguliloba* and *S. chienii* of *Allagospadonopsia*. The three Subgen. *Salvia* species considered, *S. digitaloides, S. przewalskii*, and *S. flava* formed a strongly supported monophyletic group.

Chemical analysis

The HPLC method was established for the simultaneous determination of tanshinones in *Salvia* samples. Table 3 shows the summary results. Figure 3 shows the HPLC chromatogram of *S. miltiorrhiza* from Shangluo, Shanxi (SM-SXSL). It was noticed that tanshinones were present mainly in the extracts from the dried roots of *S. miltiorrhiza* and eight other *Salvia* species, including six Danshen species and two non-medicinal species, and the contents of the tanshinones were significantly different with the highest in *S. przewalskii* and the lowest in *S. miltiorrhiza* from Sheyang, Jiangsu province (SM-JSSY). The contents of tanshinone IIA met the standard of the Chinese Pharmacopoeia (>2 mg/g) in only four *S. miltiorrhiza* populations (SM-SDPY, SM-SDYS, SM-SD, SM-SX) and four other Danshen species (SD-YNLJ, SP-YNLJ, ST-YNLJ, SY-YNLJ). The results suggest that the quality of Danshen is not only relevant to the different species, but also to the distribution of the species. Tanshinones were not detected in non-Danshen species, according to this chemical variation, Danshen species and Non-Danshen can be distinguished from each other.

Based on the chemical similarity matrices, a dendrogram was constructed by applying the UPGMA method (Figure 4). Cluster analysis divided all the samples into two main groups according to the content of one of the tanshinones. The SP-YNLJ sample, characterized by a relatively high content of tanshinone IIA (14.08 mg/g), was the first main group. The second main group was divided into two subgroups: (1) the SM-SD group, with a high content of cryptotanshinone (7.021 mg/g) and (2) a second subgroup that was divided into three further subgroups:


ITS1
SF-YNLJ GTCGAAACCT GCAAAGCAGA CCGCGAACAC GTGTTTAACA CTG-CCG--G GTGCGCGGCG C-GGGGGCAA CCCCC-GTCT
SD-YNLJ
SP-YNLJ
ST-YNLJ
SY-YNLJ
SL-ZJLA
SC-AHHS
SB-ZJLA
SM-SD
SM-SDYS
SM-SXYC
SM-AHBZ
SM-SH
SM-SDPY
SM-SX
SM-JSRG
SM-JSBH
SM-JSSY
SM-SXSL
SM-SCZJ
SD-XJWLMQ
SF-YNLJ -TCGTACTCG GT-TTCCCGC CGGCGCGCGT CCTCGGGCCT GCGTCGTGCG GGCTAACGAA CCCCGGCGC- GGAATGCGCC
SD-YNLJ
SP-YNLJ
ST-YNLJ
SY-YNLJ
SL-ZJLA
SC-AHHS
SB-ZJLA
SM-SD
SM-SDYS
SM-SXYC
SM-AHBZ
SM-SH
SM-SDPY
SM-SX
SM-JSRG
SM-JSBH
SM-JSSY
SM-SXSL
SM-SCZJ
SD-XJWLMQ
SF-YNLJ AAGGAAAACT AATCGAAGCG TCCACCCCCC -GCGCCCCGT TCGCGGTGCG TGCGGGGGG- ATCGGATGTC TATCAAATGT
SD-YNLJ
SP-YNLJ
ST-YNLJ
SY-YNLJ
SL-ZJLA
SC-AHHS
SB-ZJLA
SM-SD
SM-SDYS
SM-SXYC
SM-AHBZ
SM-SH
SM-SDPY
SM-SX
SM-JSRG
SM-JSBH
SM-JSSY
SM-SXSL
SM-SCZJ
SD-XJWLMQ

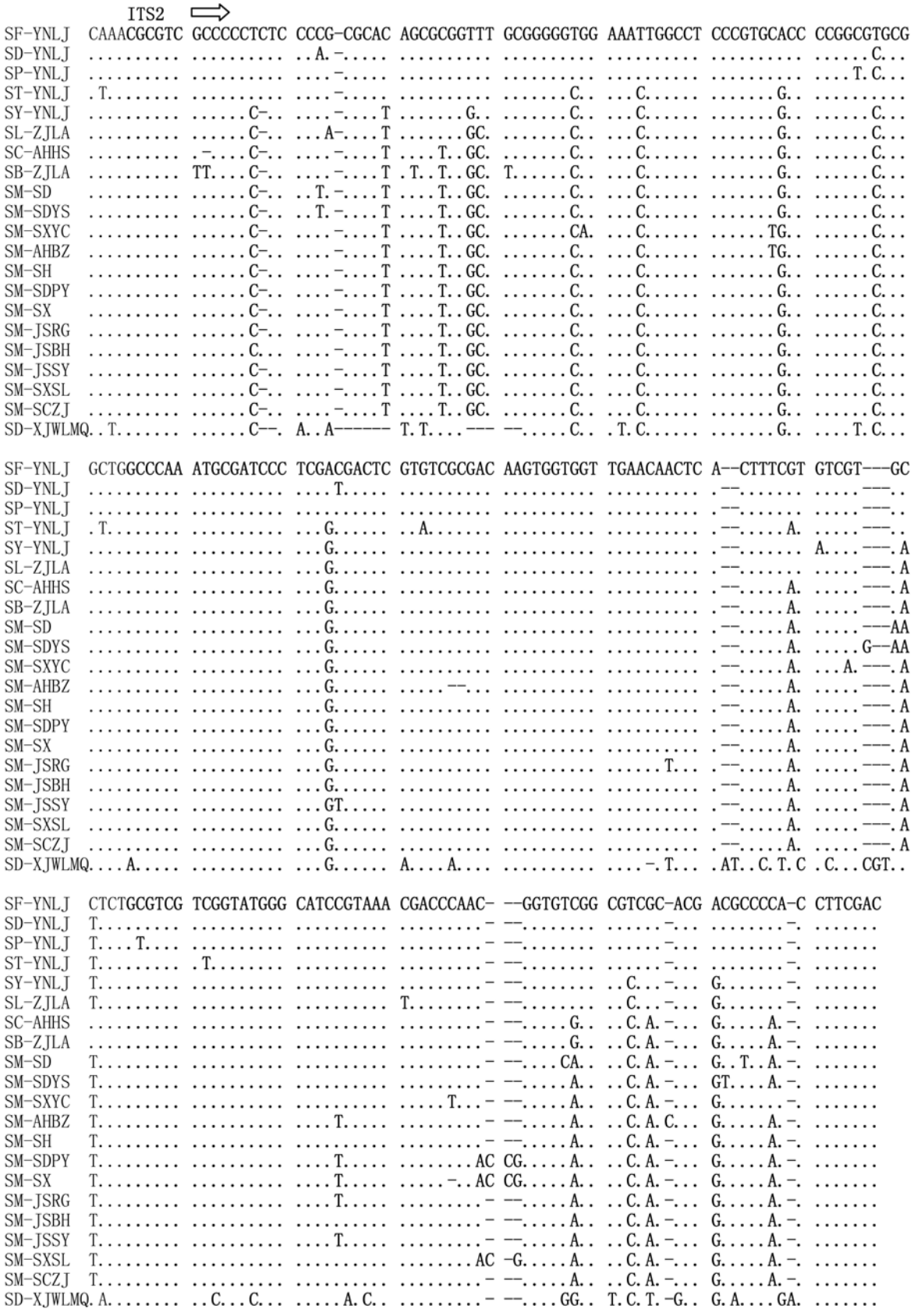


Figure 1. Alignments of ITS 1 and ITS 2 sequences of *Salvia* species, ITS 1 corresponds to position 1~244 bp; ITS 2: 245~478 (Dots indicate identical nucleotide with SF-YNLJ; hyphens indicate alignment gap).

Table 2. The percent of sequence divergence of ITS sequences of 21 samples in *Salvia* species with gaps deleted in each pairwise comparison of sequences (%).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	1.33																			
3	1.57	2.02																		
4	5.80	6.28	6.57																	
5	7.54	7.30	7.32	8.56																
6	8.29	8.05	8.07	8.80	2.71															
7	7.78	8.05	8.07	8.81	3.88	4.59														
8	8.49	8.77	8.79	9.53	3.86	4.56	1.57													
9	8.53	8.04	8.31	9.05	3.87	4.58	2.03	2.48												
10	8.27	7.78	8.05	8.79	3.64	4.34	1.80	2.25	0.67											
11	9.31	9.07	9.09	9.83	4.59	5.06	3.18	3.63	2.94	2.71										
12	8.31	8.07	8.09	8.83	3.65	4.12	1.81	2.26	1.58	1.35	1.80									
13	7.98	7.75	7.77	8.50	3.16	3.40	1.34	1.79	1.11	0.89	2.02	0.67								
14	8.26	8.02	8.04	8.77	3.63	4.33	1.80	2.24	1.57	1.34	2.71	0.89	0.89							
15	8.53	8.30	8.32	9.05	3.87	4.58	2.03	2.48	1.80	1.57	2.71	1.12	1.12	0.22						
16	8.26	8.02	8.04	8.77	3.63	4.33	1.80	2.24	1.57	1.34	2.71	0.89	0.89	0.44	0.67					
17	7.98	7.75	7.77	8.50	3.40	4.10	1.57	2.02	1.34	1.11	2.48	1.12	0.67	0.67	0.89	0.67				
18	8.29	7.78	8.07	9.05	4.10	4.81	2.03	2.70	2.02	1.79	3.17	1.35	1.34	1.34	1.57	1.34	1.56			
19	8.00	7.77	7.79	8.52	3.40	4.10	1.57	2.02	1.34	1.11	2.02	1.12	0.67	0.66	0.89	1.11	0.89	1.56		
20	8.26	8.02	8.04	8.77	3.40	4.10	2.03	2.02	1.79	1.57	2.94	1.57	1.11	1.56	1.80	1.56	1.34	2.02	1.34	
21	17.3	17.3	17.7	19.4	17.6	17.5	16.6	18.1	18.4	18.7	19.0	18.2	17.8	18.4	18.1	17.8	18.1	18.7	18.1	18.4

1, SF-YNLJ; 2, SD-YNLJ; 3, SP-YNLJ; 4, ST-YNLJ; 5, SY-YNLJ; 6, SL-ZJLA, 7, SC-AHHS; 8, SB-ZJLA; 9, SM-SD; 10, SM-SDYS; 11, SM-SXYC; 12, SM-AHBZ; 13, SM-SH; 14, SM-SDPY; 15, SM-SX; 16, SM-JSRG; 17, SM-JSBH; 18, SM-JSSY; 19, SM-SXSL; 20, SM-SCZJ; 21, SDE-XJWLMQ.

(I) The four *S. miltiorrhiza* populations (SM-SXSL, SM-SDPY, SM-SD, SM-SX) were clustered together in a cryptotanshinone group (2.915~3.322 mg/g); (II) the seven other *S. miltiorrhiza* populations and five *Salvia* species (SF-YNLJ, SB-ZJLA, SC-AHHS, SL-ZJLA, SDE-XJWLMU), characterized by low concentrations of three tanshinones, were clustered together; and (III) the tanshinone IIA group (5.368~6.427 mg/g), in which three *Salvia* species (ST-YNLJ, SD-YNLJ, SY-YNLJ) were clustered. Three *S. miltiorrhiza* populations from Jiangsu Province were clustered in the same subgroup with a relatively low content of three tanshinones. Two of three Shandong populations, SM-SDPY and SM-SDYS were clustered into the cryptotanshinone group, and the third Shandong population, SM-SD was clustered into the cryptotanshinone dominant group. The Shanxi populations also clustered into the separate subgroup. In addition, the tanshinones were obviously found at higher concentrations in four Danshen species (ST-YNLJ, SD-YNLJ, SY-YNLJ, SP-YNLJ) than those in *S. miltiorrhiza*.

Of the 2000 permutations, the Mantel test, conducted

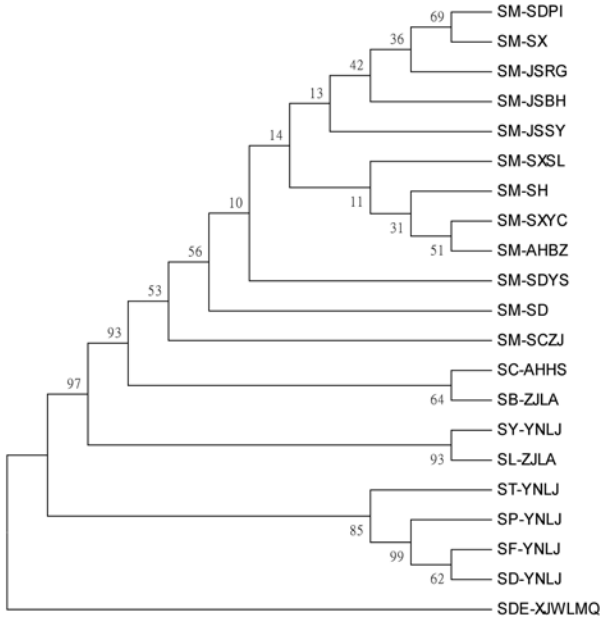


Figure 2. NJ phylogenetic tree based on ITS1+ITS2 sequences from *Salvia* species (Bootstrap values are shown above the branches).

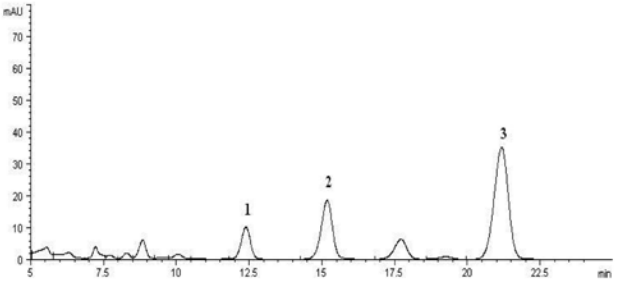


Figure 3. HPLC chromatogram of *S. miltiorrhiza* from Shangluo, Shanxi (SM-SXSL). 1: cryptotanshinone; 2: tanshinone I; 3: tanshinone IIA.

to analyze the relation between the genetic and chemical content matrices, indicated a low correlation (R= 0.19760) with no significant relation between the matrices (P= 0.8571).

DISCUSSION

Owing to the use of many *Salvia* species going under the name Danshen, and of some species as non-Danshen in China, it is necessary to authenticate the medicinal *Salvia* species to insure its safe clinical application. However, the traditional method of visual inspection is insufficient to distinguish among the various *Salvia* species. Unlike other traditional methods of authentication, the DNA sequencing procedure is based on genotype rather than phenotype, so the authentication is reliable, reproducible, and unaffected by the physical form or physiological condition of the drug sample. In angiosperm, ITS1 and ITS2 are rapidly evolving regions of nuclear ribosomal RNA genes (rDNA), and they are variable in different species. Such sequences are proposed to be useful in identification of both the medicinal plant and the botanical origins of the crude medicine (Zhao et al., 2001; Lau et al., 2001; Chen et al., 2002; Shiba et al., 2006; Xu et al., 2006). This is true even though the commercial crude drugs, which are invariably sun-dried and stored for long periods, and their genomic DNA have the potential to degrade. It is probably to amplify the ITS regions of the multi-copy rDNA repeat from their DNA samples. In this study, the ITS region was successfully amplified and sequenced, whether the template DNA used was isolated from fresh leaves or dried root. The sequence divergence between the ITS regions in *Salvia* species ranged from 1.33% to 19.4% and populations of *S. miltiorrhiza* ranged from 0.22% to 3.17%. Therefore, ITS regions could be adopted as a molecular marker for differentiating *Salvia* species from one another and also populations of *S. miltiorrhiza* from each other. Cluster analysis in the study divided the *Salvia* species into two main groups according to ITS sequence. *S. miltiorrhiza* and six other Danshen species were clustered together and separated from non-Danshen species, which corresponded to differing medicinal requirements. However, the sequences did not segregate the ten *salvia* species into the groups estab-

Table 3. Contents of tanshinones in the root of *Salvia* species.

Voucher	Cryptotanshinone (mg/g)	Tanshinone I (mg/g)	Tanshinone IIA (mg/g)
SM-SCZJ	1.098	0.133	0.246
SM-SDPY	3.322	1.276	2.968
SM-SXYC	1.348	0.407	0.810
SM-SDYS	3.140	1.067	2.502
SM-JSSY	0.404	0.104	0.111
SM-AHBZ	1.125	0.397	0.598
SM-SX	3.019	1.053	2.219
SM-JSBH	0.564	0.157	0.197
SM-SD	7.021	2.862	5.181
SM-JSRG	0.566	0.204	0.253
SM-SXSL	2.915±0.112	0.610±0.021	0.335±0.012
SM-SH	0.819±0.024	0.124±0.004	1.114±0.040
SP-YNLJ	6.428±0.186	1.590±0.049	14.08±0.309
SF-YNLJ	0.606±0.025	0.220±0.010	1.254±0.035
ST-YNLJ	1.581±0.059	1.575±0.055	6.427±0.221
SY-YNLJ	1.289±0.044	0.708±0.027	5.368±0.168
SD-YNLJ	1.214±0.033	1.587±0.043	6.218±0.210
SC-AHHS	0.115±0.003	0.121±0.004	0.916±0.034
SL-ZJLA	0.235±0.010	0.102±0.003	0.354±0.011
SB-ZJLA	0.724±0.016	0.522±0.017	0.837±0.035
SDE-XJWLMU	ND	ND	ND

ND: not detected. Content: mean value (n=2) or mean value± SD (n=3).

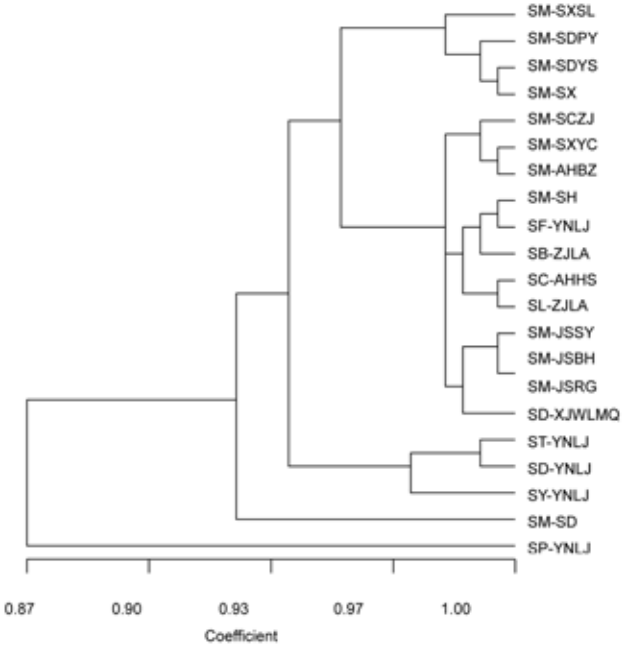


Figure 4. Dendrogram showing Euclidean distance based on tanshinones percentages of *Salvia* species using UPGMA cluster analysis.

lished by morphological classification.

Chemical analysis showed high phenotypic variability among the *Salvia* species and *S. miltiorrhiza* populations. *S. miltiorrhiza*, six other Danshen species, and two non-medicinal species closely resemble each other chemically and are distinct from non-Danshen species. The tanshinone content divided the *Salvia* samples into two distinct clusters, which are not in agreement with their geographic origin or morphological classification. The Mantel test, used to compare tanshinone content to the ITS sequence matrices, indicated a low, non-significant relation between the matrices. The marked variations in the chemical characteristics among the samples result not only from genetic factors, but also from environmental factors. Additionally, medicinal plants are processed for use as crude drugs, causing some chemical constituents to change, so it will be important to analyze the chemical composition combined with some other non-genetic factor in the future. However, the chemical variability found among the *Salvia miltiorrhiza* populations on the one hand and the ITS sequence differentiation on the other may contribute to the wide distribution of *S. miltiorrhiza* in China. Therefore, it is necessary to preserve the good population resources of *S. miltiorrhiza* and construct a good agriculture product (GAP) system to produce high quality Danshen crude drugs at the current stage. Chemical analysis also showed that the content of tanshinones in the roots of some other Danshen species are higher than in *S. miltiorrhiza,* which might explain the reasoning behind their use as Danshen. Further, their medicinal use could be validated through future pharmacological research.

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丹參及其近緣種 rDNA ITS 序列與丹參酮含量的比較分析

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中藥丹參是著名的活血化瘀類藥，鼠尾草屬丹參（*Salvia miltiorrhiza*）是中國藥典收載的其唯一基源植物，但是該屬 20 餘種在中國很多地方也做丹參類藥用，此外尚有 10 餘種該屬植物做非丹參類藥用。為了準確的鑒別與評價丹參及其相關的鼠尾草屬植物，本文採用 DNA 序列與 HPLC 分析技術對丹參及同屬 9 種的 rDNA ITS 序列與隱丹參酮、丹參酮 I 和丹參酮 IIA 的含量進行了比較研究。序列分析表明丹參與同屬相關植物以及丹參居群在 ITS 區表現出豐富的多樣性，根據序列差異可以將它們在 DNA 水準上準確鑒別 ； 遺傳聚類分析將丹參類與非丹參類分為兩個類群，表現出與藥用習慣的一致性。HPLC 分析表明，丹參酮主要分佈於丹參與 6 種民間藥用丹參中，其含量在它們之間以及不同產地的丹參間差異明顯 ； 在非丹參類藥用植物－新疆鼠尾草中沒有檢測到丹參酮，據此可將它與丹參類區分 ； 化學分析聚類與遺傳聚類結果沒有明顯的相關性，但是兩種方法的研究結果可共同為丹參及同屬藥用植物的品質評價提供科學依據。

關鍵詞 ：HPLC；rDNA；ITS； 丹參 ； 鼠尾草屬 ； 丹參酮。