Journal of Medicinal Plants Research Vol. 5(13), pp. 2687-2694, 4 July, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

*Full Length Research Paper*

# Genetic diversity among *Salvia miltiorrhiza* Bunge and related species using morphological traits and RAPD markers

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Accepted 6 April, 2011

**Genetic diversity within *Salvia* is the key to the genetic improvement of the medicine plant *Salvia miltiorrhiza*. In the current study, morphological and molecular characteristics were studied in 18 taxa of *Salvia*. A coefficient of variation was based on 18 morphological traits ranging from 17.56 to 115.04%. Among 70 random primers examined, 27 randomly amplified polymorphic DNA (RAPD) primers produced 248 extending and repeatable bands, of which 207 bands (83.47%) were polymorphic. All of the data revealed abundant genetic diversity in the genus of *Salvia*. 18 taxa were clustered into five groups based on the morphological markers or four groups based on RAPD markers. The results showed that there was slighter genetic diversity and narrower genetic backgrounds at the intra-specific level, and that we can easily distinguish *S. miltiorrhiza* from the rest of *Salvia* species by two ways. The differences of the two dendrograms might be introduced by many reasons, such as the gene expression, environment, and gene introgressions. However, Mantel's test indicated correction (r = 0.483) of morphological traits and RAPD markers.**

**Key words:** Cluster analysis, genetic diversity, morphological, randomly amplified polymorphic DNA (RAPD), *Salvia*.

## INTRODUCTION

The genus *Salvia* Linn. represents an enormous and cosmopolitan assemblage of nearly one thousand species (Standley and Williams, 1973). With the utilization of natural resources increasing, the genus *Salvia* has been widely used in the industries of pharmaceutical, food, spices, cosmetic and ornamental areas (Farkas et al., 2005). Some species are commonly used in medical practice, such as *Salvia miltiorrhiza* Bunge. For several decades, the root of *S. miltiorrhiza* has been widely used in clinics in China, Korea, Japan and other Asian countries for the treatment of various microcirculatory disturbance-related diseases, such as cardiovascular disease, cerebrovascular disease, liver dysfunction, renal deficiency and diabetic vascular complication (Han et al., 2008). However the standards in local folk practices contain 36 taxa, which shows a medical value for *Salvia*, such as *Salvia przewalskii*, *Salvia yunnanensis*, *Salvia cavaleriei* var. *simplicifolia* and so on (Wang et al., 2009).

The genetic relationships are complex among *Salvia* species, and their taxonomy, species identification and utilization are also unclear, as cross-pollination, wide distributions and great variations (Hou et al., 2005). In order to develop an efficient resources-using program, it is necessary to understand the genetic diversity and genetic relationships of the various accessions that form

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**Abbreviations: RAPD,** Randomly amplified polymorphic DNA; **PCA,** principal component analysis; **UPGMA**, unweighted pair group method with arithmetic means.

**Table 1.** Materials used in this study.

| Code | Taxon | Location | Status |
|---|---|---|---|
| 1 | *S. omeiana* | Emei, Sichuan Province | Wild |
| 2 | *S. roborowakii* | Hongyuan, Sichuan Province | Wild |
| 3 | *S. tricuspis* | Kangding, Sichuan Province | Wild |
| 4 | *S. przewalskii* | Wolong, Sichuan Province | Wild |
| 5 | *S. brevilabra* | Kangding, Sichuan Province | Wild |
| 6 | *S. cavaleriei* var. *simplelefolia* | Dujiangyan, Sichuan Province | Wild |
| 7 | *S. cynica*. | Tianquan, Sichuan Province | Wild |
| 8 | *S. flava* | Heqing, Yunnan Province | Wild |
| 9 | *S. yunnanensis* | Kunming, Yunnan Province | Wild |
| 10 | *S. evansiana* | Heqing, Yunnan Province | Wild |
| 11 | *S. miltiorrhiza* f. *alba* | Laiwu, Shandong Province | Cultivated |
| 12 | *S. miltiorrhiza* | Baohuashan, Jiangsu Province | Wild |
| 13 | *S. miltiorrhiza* | Nanyang, Henan Province | Wild |
| 14 | *S. miltiorrhiza* | Dabie Mountain, Hubei Province | Wild |
| 15 | *S. miltiorrhiza* | Xian, Shanxi Province | Cultivated |
| 16 | *S. miltiorrhiza* | Shengzhou, Zhejiang Province | Wild |
| 17 | *S. miltiorrhiza* | Botanical garden in Beijing | Cultivated |
| 18 | *S. miltiorrhiza* | Zhongjiang, Sichuan Province | Cultivated |

the *S. miltiorrhiza* genetic resource.

The evaluation of genetic diversity and phylogenetic relationships among the members would promote the efficient use of genetic variations in the breeding programme (Sikdar et al., 2010). Studies of genetic diversity have employed morphological traits, chromosome characteristics, isozymes and DNA based markers (Ye et al., 2008). Morphological traits were used a lot because it was easy to observe and record. However, few studies were carried out based on the morphological traits of *Salvia*. Xiao et al. (1997) separated some *Salvia* species using numerical taxonomy, but genetic diversity was not determined.

Molecular variability studies can help in the utilization and conservation of germplasm, as they provide a background for the development of better strategies for germplasm maintenance (Viana et al., 2010). Random amplified polymorphic DNA (RAPD) molecular markers have increasingly been used to analyze genetic relationships and genetic variation (Zheng et al., 2009), since it has some advantages, such as quickness and simplicity, low cost, and high veracity. Nevertheless, only a few results have been published in the case of *Salvia*. Most of these were from outside Asia (Guo et al., 2002; Xu et al., 2008; Khalil et al., 2005; Tian et al., 2006).

In this study, morphological traits and RAPD markers were first employed on 18 *Salvia* taxa, which were the most used medically. The objective of this research was to evaluate the genetic diversity of the collection materials by two different approaches, and to estimate the genetic base of these germplasm to be used in future breeding program in China.

## MATERIALS AND METHODS

### Plant materials

A total of 18 *Salvia* taxa (consisting of 10 species,1 variety, 1 form and 7 accessions of *S. miltiorrhiza*) were collected from their main natural growing regions in different provinces of China and then were taken back to be planted in the botanical garden of Sichuan Agriculture University, Yaan, China (Table 1). Samplings were carried out from the early spring until mid-summer. Young leaves were collected from the field-grown plants of the respective lines and stored at -20℃ prior to DNA extraction.

### Morphological traits

Eighteen quantitative and qualitative morphological traits were evaluated across the 18 taxa (Table 2). The experiment consisted of three replications in a complete randomized design and data for each trait were scored for 20 to 50 individual plants of each taxon, with observations being made of the plants reaching full-bloom.

### DNA extraction

Total genomic DNA extraction procedure was based on CTAB method according to Doyle and Doyle (1987). The purity and quantity of genomic DNA was determined spectrophotometrically and confirmed by using 0.8% agarose gel electrophoresis against known concentrations of unrestricted lambda DNA. DNA samples were stored at -20℃ prior to RAPD analysis.

### RAPD

The primers used for RAPD reactions comprised of 27 random primers within the 70 primers based on repeated sequences. The polymerase chain reaction (PCR) was performed in a total reaction

**Table 2.** The morphological classification of characters and basic statistical data of 18 accessions of *Salvia*.

| Trait code | Traits and taxonomic value | Max. | Min. | Mean | S.D. | CV (100%) |
|---|---|---|---|---|---|---|
| PH | Plant height (cm) | 80.67 | 11.00 | 47.85 | 19.57 | 40.89 |
| PL | Petiole length (cm) | 29.56 | 4.25 | 21.10 | 7.47 | 35.40 |
| LL | Leaf length (cm) | 9.66 | 2.83 | 6.20 | 2.02 | 32.51 |
| LW | Leaf width (cm) | 8.45 | 1.37 | 4.23 | 1.82 | 43.00 |
| IL | Inflorescence length (cm) | 3.55 | 1.06 | 2.39 | 0.69 | 28.83 |
| CL | Calyx length (cm) | 1.20 | 0.58 | 0.94 | 0.18 | 18.87 |
| COL | Corolla length (cm) | 11.52 | 1.79 | 6.54 | 2.86 | 43.76 |
| LLW | Leaf length / width | 2.24 | 1.13 | 1.55 | 0.32 | 20.59 |
| PLL | Petiole length / Leaf length | 1.86 | 0.54 | 1.06 | 0.35 | 32.70 |
| CCL | Corolla length / Calyx length | 3.16 | 1.77 | 2.49 | 0.44 | 17.56 |
| NY | Number of years: 1=perennial, 2=annual, 3=biennial | 3.00 | 1.00 | 1.28 | 0.67 | 52.37 |
| LT | Leaf type: 1=simple leaf, 2=compound leaf, 3=both | 3.00 | 1.00 | 1.56 | 0.62 | 39.58 |
| HTL | Haired type of leaf: 1=bristle, 2=vellus, 3=fluff, 4=appressed, 5=seta, 6=none, 7=bristle or none, 8=vellus or none | 4.00 | 1.00 | 2.00 | 0.69 | 34.30 |
| CC | Corolla color: 1=dark purple, 2=lavender, 3=yellow, 4=red | 3.00 | 1.00 | 1.61 | 0.92 | 56.88 |
| OHC | Orbicular hairs in the corolla tube: 1=yes, 2=incompleteness, 3=no | 3.00 | 1.00 | 1.33 | 0.69 | 51.45 |
| SEC | Style extension out of corolla: 1=yes, 2=slightly, 3=no | 3.00 | 1.00 | 1.33 | 0.59 | 44.56 |
| FA | Fertility of anther chamber: 1=yes, 2=no | 1.00 | 0.00 | 0.44 | 0.51 | 115.04 |
| AA | Anther ally situation: 1=whole ally, 2=top ally, 3=bottom ally, 4=separation | 3.00 | 1.00 | 2.56 | 0.62 | 24.09 |

volume of 25.0 µL containing 20 to 40 ng of genomic DNA, 2.5 mM of each type of dNTPs, 1 ul of each primer, 2.5 ul of 10×PCR Buffer, 16.2 ul of $DDH_2O$, 2 ul of 2.5 mM $MgCl_2$, 1.5 U of *ExTaq* DNA polymerase with high fidelity (*TaKaRa* Inc., Dalian, China) and ddwater to the final volume.

PCR was carried out using a PTC-200 thermocycler (MJ Research, Massachusetts, USA). Clear, polymorphic, and reproducible bands were selected and amplified under conditions similar to those used by Curley (2004). The thermal cycling program consisted of one cycle of 91°C for 1 min, 42°C for 15 s, and 72°C for 1 min 10 s, followed by 38 cycles of 91°C for 15 s, 42°C for 15 s, and 72°C for 1 min 10 s, with a final extension step at 72°C for 10 min and then cooling to 4°C at the end of cycling. Reaction products were electrophoresed on 0.8% (w/v) agarose gels stained with ethidium bromide and photographed with an instant camera under UV light.

#### Data analysis

According to the method of Luo and Yang (2010), the morphological data were standardized to be analyzed with Q-mode clustering by SPSS 17.0 software, prior to being used in the calculation of genetic similarity and the Euclidean distance between the different taxa. Principal component analysis (PCA) was performed as well.

In the analysis, morphological traits were taken as the observation indexes and coded using the number of encoding levels (Xu, 1999), by which dualistic traits were labeled as “0” or “1” and ordered multimodal traits were labeled as “1”, “2”, “3” and so on (Table 2). However, the numeric traits were not encoded and the average values were used directly in the next calculation (Kong and Guan, 2008).

The RAPD bands were scored as absent (0) or present (1) if visible, regardless of the relative intensity. The NTSYS-pc software 2.02 (Roholf, 1998) was used to estimate genetic similarities with similarity coefficient values. Cluster analysis was performed by the unweighted pair group method with arithmetic average (UPGMA). To investigate the correlation between the morphological and RAPD data sets, Mantel’s test was done.

## RESULTS

### Morphological traits

Ten quantitative morphological traits were measured and eight qualitative traits were scored in the 18 taxa. From these measurements, the maximum, minimum, mean, standard deviation (S.D.) and coefficient of variation (CV) values across all accessions were calculated for each of the 18 morphological characteristics (Table 2).

On one hand, *S. omeiana* produced the highest plant height, and *S. miltiorrhiza* from Zhejiang province had the highest inflorescence length. The highest mean leaf length, leaf width and calyx length belonged to *Salvia cynica*, and the highest petiole length and corolla length belonged to *Salvia flava*. *Salvia tricuspis* produced the

lowest petiole length, and *S. cavaleriei* var. *simplelefolia* had the lowest calyx and corolla length. The lowest leaf length and leaf width belong to *S. yunnanensis*, and the lowest plant height and inflorescence length belong to *Salvia evansiana*. On the other hand, the qualitative traits were also diverse, especially the haired type of leaf coming up to eight types.

There were significant differences among the samplings for all evaluated morphological traits. The CV values were more than 100% for fertility of anther chamber and about 50% for the multiple-state characteristics of number of years, corolla color and orbicular hairs in the corolla tube. The CV values for 9 of the quantitative traits also indicated a high level of variation (that is, >30%), namely plant height, petiole length, leaf length, leaf width, corolla length, petiole length / leaf length, leaf type, haired type of leaf and style extension out of corolla. By contrast, low levels of variation among the accessions were found for the traits of calyx length and corolla length / calyx length.

The standardized morphological data was used to calculate the Euclidean distances between the populations and a dendrogram was constructed from these values (Figure 1A).

Cluster analysis divided these taxa into five groups according to the level of combination line. Cluster I consisted of all *S. miltiorrhiza* and its variety from different provinces and *S. cavaleriei* var. *simplelefolia*. *S. yunnanensis* stood alone forming cluster II. Cluster III was composed of *S. przewalskii, Salvia brevilabra, S. omeiana, S. cynica* and *S. flava*. We found *Salvia evansiana* alone formed cluster IV and cluster V consisted of *Salvia roborowakii* and *S. tricuspis*.

PCA was carried out using the morphological data for describing the relations of various targets by a few factors. The results indicated that the closest relationships were between *S. omeiana* and *S. cynica*, and also between *S. miltiorrhiza* from different provinces. It was similar to the results obtained from the cluster analysis (Figure 2). The eigen values obtained for the first two principal component scores indicated that they could provide a good description of the data, cumulatively accounting for 54.568% of the standardized variance. The analysis of eigen vectors provides information about the nature of the traits responsible for the separations according to the first two principal components. The PC1 data set could account for 35.280% of the total variation, and the traits of fertility of anther chamber, corolla color and style extension out of corolla associated positively to the PC1, whereas leaf type and anther ally situation were negatively associated. Thus, PC1 was related to floral characters.

PC2 accounted for 19.287% of the total morphological variability and it was mainly attributable to the quantitative differences of plant height, petiole length, leaf length, leaf width and inflorescence length. They were associated positively with PC2. Thus, PC2 was strongly related to plant size.

**RAPD analysis**

Of the 70 total primers screened in the treatments, 27 produced discrete and reproducible amplified DNA fragments those generated 248 fragments on all samplings which are listed in (Table 3). Among them 207 fragments showed polymorphism in two or more populations. The percentage of polymorphic bands of the 27 RAPD primers varied from 57.14% (AN9) to 100% (S19 et al.), with an average value of 83.47%. The number of polymorphic fragments generated by each primer also varied from 3 (A12) to 14 (AS6), with an average of 12.18 fragments. The results showed complicated genetic background in *Salvia*, and there is great genetic diversity among these materials. The RAPD amplification profile produced by primer AS6 (GGCGCGTTAG) was illustrated in (Figure 3).

The calculation of similarity coefficient was based on 207 RAPD polymorphic bands, and the amplitude variations were from 0.384 to 0.807. The similarity matrix showed that the highest similarity was for. *S. cavaleriei* var. *simplelefolia* and *S. yunnanensis*. The lowest similarity was between *S. cavaleriei* var. *simplelefolia* and *S. flava*. In general, similarity coefficients were higher between *S. miltiorrhiza* from different provinces compared with those between different taxa.

A dendrogram based on UPGMA analysis of the RAPD data was shown in (Figure 1B). According to this analysis, the 18 taxa were grouped as four major clusters at a similarity level of 62%. Cluster I consisted of *S. omeiana*, *S. roborowakii*, *S. tricuspis*, *S. cavaleriei* var. *simplelefolia* and *S. yunnanensis*. Cluster II consisted of all *S. miltiorrhiza* and its variety from different provinces. Cluster III was made up of *S. przewalskii*, *S. brevilabra*, *S. evansiana* and *S. flava*, and cluster IV consisted of *S. cynica* alone. At a narrower threshold of genetic similarity, RAPD analysis indicated a close relationship of *S. cavaleriei* var. *simplelefolia* with *S. yunnanensis*, and between *S. brevilabra* and *S. evansiana*.

**Comparison between morphological traits and RAPD markers**

The genetic diversities between the 18 taxa were calculated according to the two assessment methods, namely, morphological traits and the RAPD molecular markers. RAPD analysis divided the samples into four groups compared to five groups by morphological clustering. All *S. miltiorrhiza* and its variety from different provinces clustered in one group by both of the two methods. Although *S. cavaleriei* var. *simplelefolia* and *S. yunnanensis* grouped into one cluster which indicated a close relationship based on molecular data, morphological traits grouped them into separate clusters. The same conditions were for *S. omeiana* and *S. roborowakii,* and between *S. cynica* and *S. flava*. Matrices

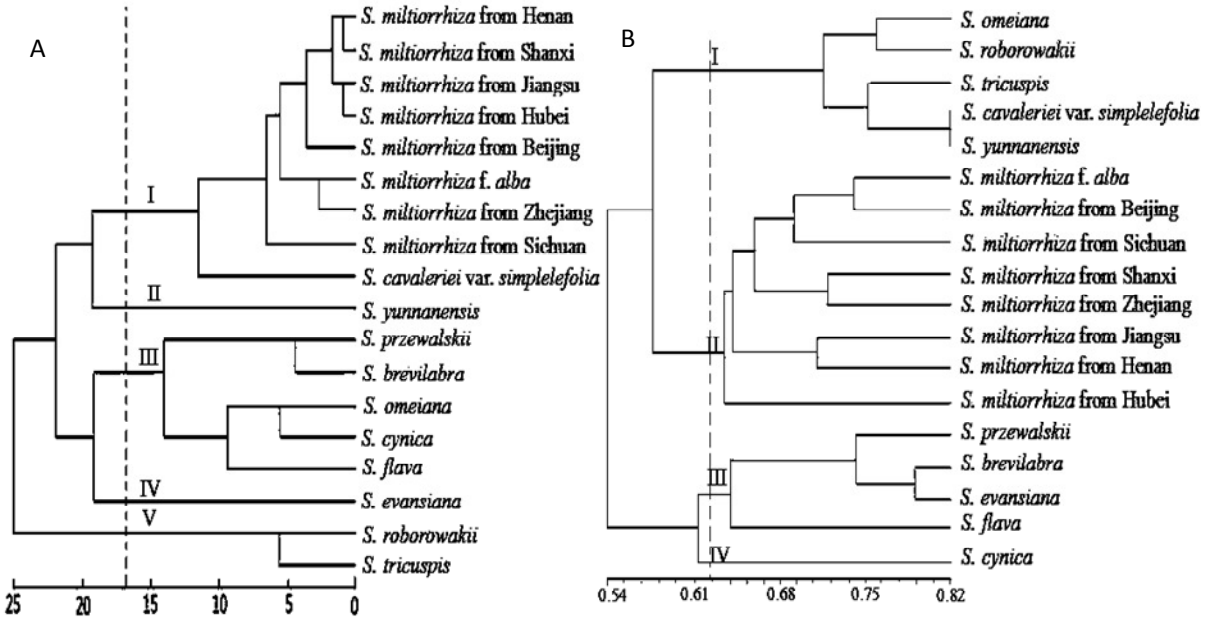


**Figure 1.** Cluster analysis of 18 *Salvia* accessions based on morphological traits (A) and RAPD data (B).

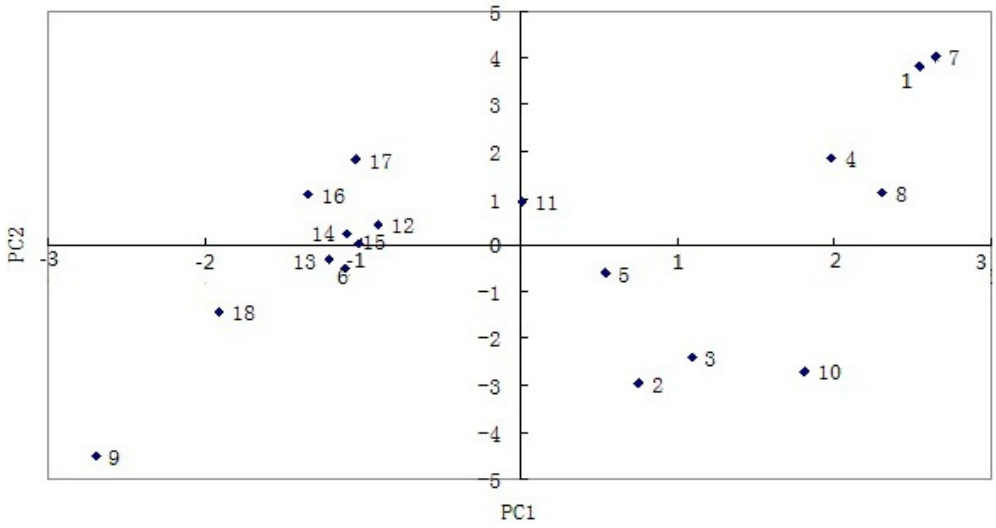


**Figure 2.** Two-dimensional graph based on PCA using morphological data. The two axes represent the first two principal components (PC) that accounted for 54.568% of the total variation. Refer to Table 1 for 18 taxa. Scale bar represents rescaled principal component values.

were constructed from these two respective genetic similarity coefficient and their correlations assessed according to the Mantel test by NTSYS software. The correlation coefficient between the morphological and RAPD matrices was calculated to be 0.483.

## DISCUSSION

In our study two different techniques, namely scoring morphological traits and amplifying RAPD-molecular markers were evaluated for the genetic characterization of 18 taxa of *Salvia*. The results revealed abundant genetic diversities in the genus of *Salvia*. The average coefficient of variation values for 18 morphological traits was 40.69%, and the minimum and maximum were 17.56 and 115.04% respectively. The results of RAPD analysis showed that the mean number of polymorphic bands was 12.18 and the mean polymorphic was 83.47%. However, the cluster results based on both methods showed that the genetic

**Table 3.** List of primers, their sequences and the number of amplification bands.

| Primers | Sequence 5'-3' | Total no. of bands | No. of polymorphic bands | Polymorphic (%) | Primers | Sequence 5'-3' | Total no. of bands | No. of polymorphic bands | Polymorphic (%) |
|---|---|---|---|---|---|---|---|---|---|
| A12 | TCGGCGATAG | 4 | 3 | 75.00 | T7 | GGCAGGCTGT | 11 | 10 | 90.91 |
| O6 | CCACGGGAAG | 17 | 12 | 70.59 | AN9 | GGGGGAGATG | 7 | 4 | 57.14 |
| AD3 | TCTCGCCTAC | 13 | 11 | 84.62 | AS6 | GGCGCGTTAG | 14 | 14 | 100.00 |
| AI14 | TGGTGCACTC | 12 | 9 | 75.00 | BE19 | AGGCCAACAG | 7 | 7 | 100.00 |
| AY18 | ACCCCAACCA | 11 | 8 | 72.73 | S4 | CACCCCCTTG | 7 | 7 | 100.00 |
| S1 | CTACTGCGCT | 7 | 7 | 100.00 | S6 | GATACCTCGG | 6 | 6 | 100.00 |
| S5 | TTTGGGGCCT | 8 | 6 | 75.00 | S8 | TTCAGGGTGG | 6 | 5 | 83.33 |
| S7 | TCCGATGCTG | 11 | 11 | 100.00 | S11 | AGTCGGGTGG | 8 | 7 | 87.50 |
| S10 | ACCGTTCCAG | 10 | 10 | 100.00 | S14 | AAAGGGGTCC | 10 | 9 | 90.00 |
| S12 | CTGGGTGAGT | 10 | 8 | 80.00 | S16 | AGGGGGTTCC | 8 | 5 | 62.50 |
| S15 | CAGTTCACGG | 9 | 9 | 100.00 | S18 | CTGGCGAACT | 8 | 7 | 87.50 |
| S17 | TGGGGACCAC | 8 | 7 | 87.50 | S19 | GAGTCAGCAG | 6 | 6 | 100.00 |
| S20 | TCTGGACGGA | 9 | 6 | 66.67 | | | | | |
| L18 | ACCACCCACC | 12 | 10 | 83.33 | Total | | 248 | 207 | -- |
| M6 | CTGGGCAACT | 9 | 7 | 77.78 | Mean | | 14.59 | 12.18 | 83.47 |

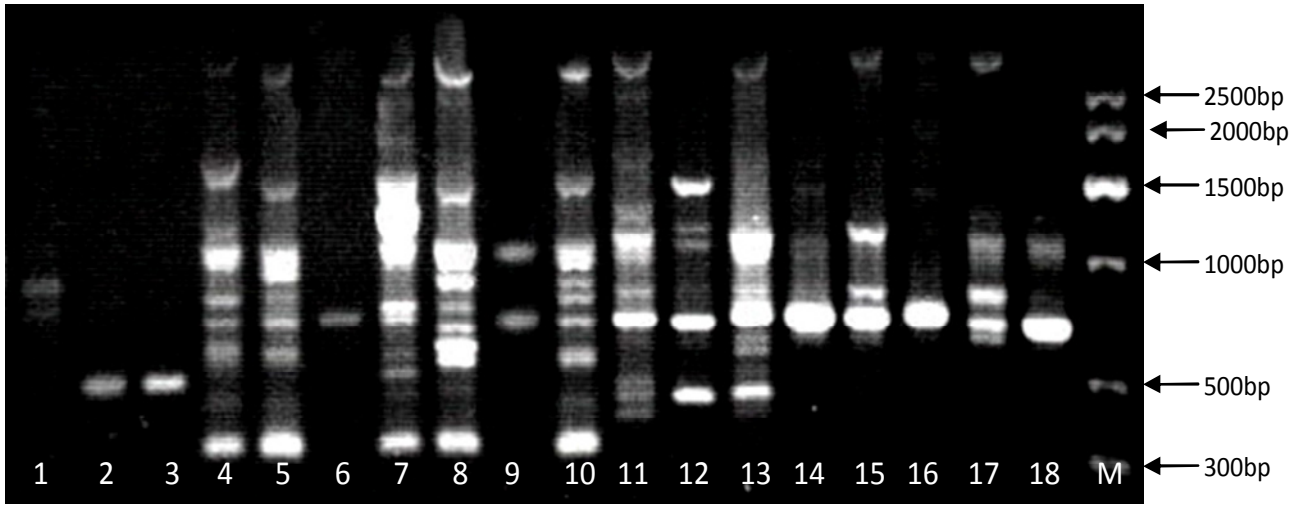


**Figure 3.** RAPD bands amplified with the arbitrary decamer primer AS6 using DNA of 18 *Salvia* samples. The numbers 1-18 correspond to samples from the 18 taxa of *Salvia* (accession codes as listed in Table 1); M shows the DNA markers DL 2000.

diversities mainly embodied among inter-species, and there were slighter genetic diversities and narrower genetic backgrounds at the intra-specific level, such as *S. miltiorrhiza*. To some extent, in the creative exploitations and cross breeding for the germplasm resources, it can be tried to combine *S. miltiorrhiza* and its related species to broaden genetic backgrounds of the breeding materials and create more fruitful genetic variations.

According to the dendrograms based on morphological traits and amplifying RAPD-molecular marker, all accessions of *S. miltiorrhiza* were clustered in one group and showed obvious differences in both morphological traits and heredity compared with other taxa in *Salvia*. We can also distinguish *S. miltiorrhiza* obviously from the other *Salvia* species by these two ways. Based on the RAPD analysis the Hubei population of *S. miltiorrhiza*, further relationships with the others could be seen. What's more, according to morphological traits, the Sichuan population showed the further relationships compared with the others among the different accessions of *S. miltiorrhiza.* As there is a long history of cultivating in Sichuan, there were no wild samples. So we can not be sure whether the genetic variations came from the cultivated choosing, or the long time of cultivation that made the genetic diversity change.

As we know, *S. miltiorrhiza* is regarded as an important traditional Chinese herbal medicine with a history of more than 2,000 years, and it could deal with many coronary heart diseases, particularly angina pectoris and myocardial infarction. With the decline of the herb's resources, finding, identifying and evaluating the new germplasm resources from the closely related taxa in *Salvia* are necessary (Xiao et al., 1999). According to our study, the result is similar to the traditional classification. The one which has the closest relationships with *S. miltiorrhiza* is *S. cavaleriei* var. *simplelefolia,* and it has medical values in hematemesis, bleeding wound, bloody dysentery etc. (Yang et al., 2008). Although there were few researches on this taxon, it needs more evidence to prove whether *S. cavaleriei* var. *simplelefolia* has the same functions as *S. miltiorrhiza*, which is a good potential germplasm resource. In recent years more and more studies on the chemical compositions were carried out on *S. yunnanensis*, by which researchers found many compounds which were similar to those in *S. miltiorrhiza*, and many researches showed that the contents of the active components in *S. miltiorrhiza*, such as total tanshinone and cryptotanshinone, sometimes were less than those in *S. yunnanensis* which was recorded in literature documenting the drug standards of Yunnan (Qian et al., 2002; Shi et al., 2006). Actually, according to the information in our study, *S. yunnanensis* which has close relationship with *S. miltiorrhiza*, is one of the good germplasm resources.

As it is a long period for identification many morphological characters which are subject to environmental effects, may lead the variation to be interacted by heredity and environment (Bai, 2007). In some species environmental effects may be greater and the morphological traits can not be completely used for the assessment of genetic diversity (Li et al. 2004). Molecular biology techniques are commonly applied to analyze the genetic diversity of living organisms and RAPD is one of the simplest and most common methods used in the determination of genetic similarity and diversity (Przyborowski and Sulima, 2010). It has been successfully applied to investigate many wild and cultivated plant species (Kim et al., 2008; Bhutta et al., 2006; Liang, 2010). However differences in results obtained in groupings by using RAPD markers and morphological characters have been reported in other plants, for example, banana (Uma et al., 2004), pomegranate (Zamani et al., 2007) and Persian shallot (Ebrahimia et al., 2009). In our study the significant positive correlations ($r = 0.483$) between the matrices obtained from the molecular genomic markers and the morphological traits matrices also indicate that morphological characters can provide a useful measure of genetic diversity amongst *Salvia*.

To the best of our knowledge, this is the first report for evaluating genetic diversity by using morphological traits and RAPD marker simultaneously on *S. miltiorrhiza* and related species. However, there are some differences between the dendrograms based on morphological and RAPD analysis. For example, *S. omeiana* and *S. roborowakii* which had high similarities were clustered into one group in the RAPD morphology, but the results in morphology indicated the two formers had further relationships with each other (Figure 1). The differences in parts of the results from the two methods may have three reasons. First, as there are a series of complex intermediate links between the phenotype (morphology) and genotype (molecular) such as gene expression, regulation, individual development. It is difficult to get the same results exactly by using these two different ways. Secondly, concerning the conditions of the growing place of these plants, it can be proposed that the original data obtained from the morphological characters may change with the environment. On the other hand, in the long time of natural selection, there are gene introgressions among the *Salvia* species. With the continuous improvement of RAPD technology, and combining it with the traditional classification, cytology and chemical components, it will provide more information for the classification and genetic diversity of *Salvia* species.

## ACKNOWLEDGEMENTS

This work was financially supported by the Youth Scientific and Technological Foundation of Sichuan Province, China (08ZQ026-034), the Science and Technology Bureau of Sichuan Province, China (2008FZ0148, 2008SZ0074) and the Education office of

Sichuan Province, China (09ZA067).

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