

## RAPD fingerprint construction and genetic similarity of *Mesona chinensis* (Lamiaceae) in China

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**ABSTRACT.** *Mesona chinensis* is an economically important agricultural crop, primarily cultivated for making grass jelly. It was originally discovered in South China. We examined 18 cultivars, including cultivars from Guangdong, Fujian, and Guangxi, China, Taiwan, and Indonesia, and a hybrid (a cross between cultivars from Indonesia and Guangdong), based on RAPD markers. The genetic similarity coefficient was calculated by NTSYS 2.10 and the clustering analysis was made by UPGMA. PCR amplification with 10 primers produced 163 bands; 94% of the amplified loci were polymorphic. The primers S208, S206, and S253 could completely distinguish all 19 samples by constructing a DNA fingerprint. Cluster analysis divided the 19 cultivars into five groups, with an overall genetic similarity coefficient of 0.68. Correlations were found among regional distributions, parental sources, and RAPD markers, demonstrating the rich genetic diversity of these 19 cultivars of *M. chinensis*. This study provides useful information for the classification, identification, and breeding of *M. chinensis*.

**Key words:** *Mesona chinensis*; RAPD fingerprint; Genetic similarity

## INTRODUCTION

*Mesona chinensis*, a medicinal and edible plant native to South China, India, and Malaysia, is one of the dry overground members of the genus *Mesona* in the family Lamiaceae (Zhao et al., 2011). It has strong adaptability and grows well with adequate water and shade. The plant has a sweet flavor and can lower body temperature, so traditional Chinese doctors use it to cure heatstroke and fever (Liu and Fang, 1998). In modern medicine, it can be used to treat diseases such as heatstroke, diabetes, hypertension, and acute nephritis (Shen et al., 2000). *M. chinensis* is also rich in flavones, polysaccharides, polyphenols, pigments, and amino acids, making it useful as a raw material for traditional herbal medicine, health foods, plant beverages, and daily supplements (Liu and Chen, 2004; Huang et al., 2012).

*M. chinensis* Benth., *M. parviflora* Benth., and *M. procumbens* Hemsl. are known to grow in China (Wu and Wang, 2009), but the genetic resources of *M. chinensis* are disordered, and the pedigrees of local resources remain undefined. Identifying the genetic relationships among these species using geographic origin and morphological differences is difficult. Therefore, the use of molecular techniques in further studies of genetic identification is essential.

At present, the genetic relationships between medical plants are mainly established using DNA molecular marker techniques including random amplified polymorphic DNA (RAPD) (Arif et al., 2010), amplified fragment length polymorphism (Ghosh et al., 2011), simple sequence repeat (Xu et al., 2011), DNA barcoding (Guo et al., 2011), and so on. However, each approach has shortages and boundedness. Amplified fragment length polymorphism technology is complicated and expensive and requires DNA and incision enzymes. Simple sequence repeat techniques should be used for species for which DNA information is known; its accuracy is influenced by sequence stitching and primer design software. DNA barcode techniques are costly and so complicated that the outcome of such analyses and their displays are indirect. Comparatively, RAPD techniques have many advantages, such as low DNA requirements and the capability to use primers without genus specificity. RAPD analysis can help to construct DNA fingerprints of species for which researchers have carried out no previous molecular studies (Hammad and Qari, 2010). In brief, RAPD technique can assist in the direct analysis of the polymorphisms of medicinal plant and determination of particular DNA markers. Therefore, it can be used to distinguish genetic specificity among plant populations and to develop means of identifying crude drugs from appearance to gene (Zhu et al., 2011). RAPD techniques have been used to identify many medicinal plants, such as *Dendrobium officinale* (Ding et al., 2009), *Trollius* accessions (Li and Ding, 2010), and *Dalbergia sissoo* (Wang et al., 2011).

Recently, changes in the *M. chinensis* germplasm have occurred owing to cultivar selection and natural evolution. Therefore, analyzing the diverse genetic resources of *M. chinensis* at the molecular level is necessary. Until now, we have found only one related molecular research in the literature in which the genomic DNA of *M. chinensis* is extracted from the 18 cultivars and one hybrid and analyzed (Guan et al., 2010). Additional investigations are still needed. The current study of various producing areas and populations of *M. chinensis* provides a concentrated resource through the construction of RAPD fingerprints, which offer a theoretical basis for classification, identification, and breeding.

## MATERIAL AND METHODS

### Plant material

A total of 19 *M. chinensis* samples were collected from Guangdong (China), Fujian (China), Guangxi (China), Taiwan (China), and Indonesia. The samples included one hybrid (Table 1).

**Table 1.** Source of materials.

Code	Source	Collecting date
N1	Zengcheng City in Guangdong Province, China	September 14, 2009
N2	Yangchun City in Guangdong Province, China	January 2, 2010
N3	Pingyuan County in Guangdong Province, China	September 24, 2009
N4	Chaoyang District of Shantou City in Guangdong Province, China	September 24, 2009
N5	Fenghuang Mountain of Chaozhou City in Guangdong Province, China	January 2, 2010
N6	Yongan City in Fujian Province, China	September 26, 2009
N7	Yongding County in Fujian Province, China	December 26, 2009
N8	Xintian Mountain at Xinluo District of Longyan City in Fujian Province, China	September 26, 2009
N9	Xintian Village at Xinluo District of Longyan City in Fujian Province, China	January 2, 2010
N10	Loumian Village at Xiaba Country of Wuping County in Fujian Province, China	September 14, 2009
N11	Xiaba Village at Xiaba Country of Wuping County in Fujian Province, China	December 26, 2009
N12	Pingchuan Town of Wuping County in Fujian Province, China	December 26, 2009
N13	Yanqian Town of Wuping County in Fujian Province, China	December 26, 2009
N14	Shifang Town of Wuping County in Fujian Province, China	December 26, 2009
N15	Zhongbao Town of Wuping County in Fujian Province, China	December 26, 2009
N16	Lingshan County in Guangxi Province, China	October 24, 2009
N17	Guanxi Town of Xinzhu County in Taiwan Province, China	December 26, 2009
N18	Indonesia	January 2, 2010
N19	Hybrid cross Indonesia and Chaoyang District of Shantou City in Guangdong Province	October 24, 2009

### RAPD analysis

A modified cetyltrimethylammonium bromide method (Guan et al., 2010) was used to extract the genomic DNA from fresh leaves of 19 *M. chinensis* samples. The clear and repeatable bands were screened using random primers with mixed genomic DNA. Then, RAPD amplifications of 19 *M. chinensis* samples were performed three times.

The amplification system (20  $\mu$ L) was as follows: 2  $\mu$ L 10X buffer solution, 1.5  $\mu$ L 25 mM  $Mg^{2+}$ , 1.6  $\mu$ L 2.5 mM deoxyribonucleotide triphosphate, 1  $\mu$ L 50 ng/ $\mu$ L DNA, 1  $\mu$ L 20  $\mu$ M primer, 0.2  $\mu$ L 5 U/ $\mu$ L Taq enzyme, and double-distilled  $H_2O$ . All reagents were purchased from TAKARA Company (Dalian, China), and the reaction was carried out using a DNA Engine polymerase chain reaction (PCR) instrument (BIO-RAD Co., USA). The amplification procedure was as follows: samples were denatured at 94°C for 5 min, then at 94°C for 30 s. They were renatured at 36°C for 45 s and then extended at 72°C for 1 min. The entire procedure was repeated 40 times. The samples were then extended at 72°C for 10 min and preserved at 4°C.

The PCR products were separated using 1.5% agarose gel electrophoresis in 1X Tris-acetate-ethylenediaminetetraacetic acid buffer for 30 to 60 min at 120 V and photographed with a Gel Doc-It imaging system (UVP, Bio Doc Co., USA).

## Data analysis

All gel bands in the RAPD fingerprints were scored as present (1) or absent or undistinguishable (0) in each genotype for each primer. The number of the fingerprints was determined with number strings including 1 and 0 from top to bottom. The genetic similarity coefficient between materials was calculated using the NTSYS 2.10 software, and a dendrogram was constructed with the unweighted pair-group method with arithmetic average.

## RESULTS

### Screening of RAPD primers and analysis of genotype polymorphism

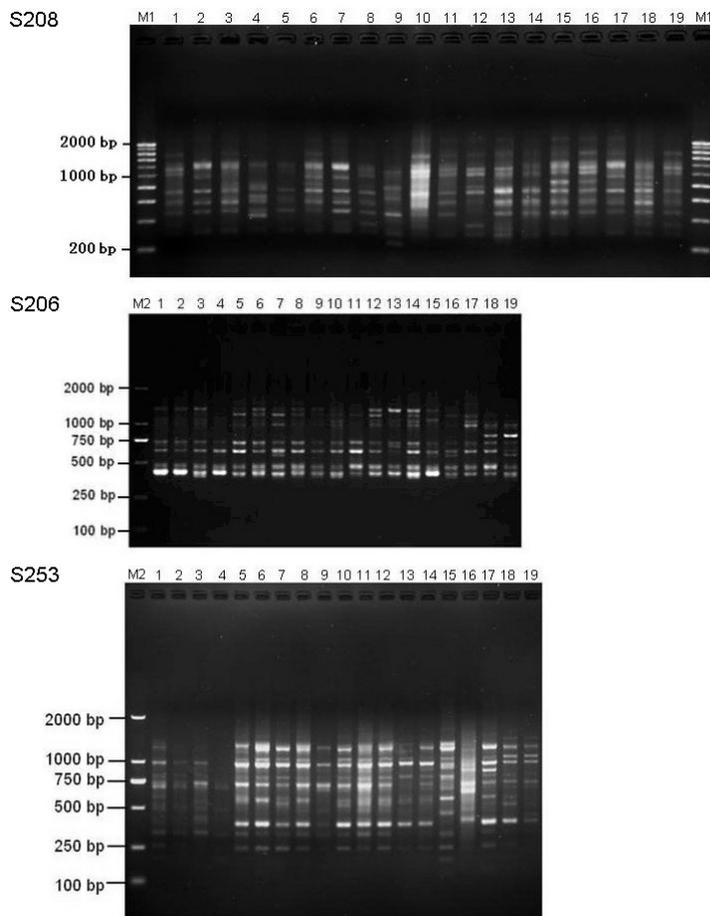
Ten primers with good polymorphism and high repeatability from 100 10-bp primers were amplified from 19 *M. chinensis* samples. A total of 163 bands were observed at 12-24 amplification loci on 19 electrophoretic gels, in which the lowest number of polymorphic bands was 12 from primers S207 and S252 and the most was 24 from primer S206 (Table 2). One hundred and fifty-three bands were polymorphic and the average polymorphic rate was 96.65% among 10 primers, implying rich genetic polymorphism among 19 *M. chinensis* samples.

**Table 2.** RAPD primer data and the percentage of polymorphic bands.

Primer	Sequence (5'-3')	Amount of DNA bands	Amount of polymorphic bands	Polymorphic rate (%)
S202	GGA GAG ACTC	15	14	93.33
S205	GGG TTT GGCA	16	15	93.75
S206	CAA GGG CAGA	24	24	100.0
S207	GGC AGG CTGT	13	12	92.31
S208	AAC GGC GACA	18	17	94.44
S215	GGA TGC CACT	14	13	92.86
S238	TGG TGG CGTT	19	19	100.0
S252	TCA CCA GCCA	12	12	100.0
S253	GGC TGG TTCC	17	13	76.47
S377	CCC AGC TGTG	15	14	93.33
Total		163	153	
Average		16.30	15.30	93.65

### Fingerprint construction

Figure 1 shows the amplification results from partial 10-bp random primers (S208, S206, and S253) to 19 genomic DNA samples. Seventeen polymorphic bands amplified by primer S208 distinguished the 19 samples completely. Primer S206 distinguished only 17 samples but distinguished all samples when paired with primer S253. Therefore, the DNA fingerprint of the 19 *M. chinensis* samples provides molecular proof for the identification of the *M. chinensis* germplasm.



**Figure 1.** RAPD electrophoresis patterns of the primers S208, S206 and S253. Lane M1 = 200-bp DNA ladder; lane M2 = DL2000 DNA marker; lanes 1-19 are identified in Table 1.

### Analysis of genetic similarity

According to the 163 bands amplified by the 10 primers described above, a similarity coefficient among the 19 samples was calculated using qualitative data with the similarity function in NTSYS 2.10. The value among the 19 samples ranged from 0.509 to 0.883 (Table 3), in which the largest value (implying the closest genetic relationship) was 0.883 between sample N12 (Pingchuan Town, Wuping County, Fujian Province, China) and sample N14 (Shifang Town, Wuping County, Fujian Province, China), whereas the lowest value (revealing the farthest genetic relationship) was 0.509, between N6 (Yongan City, Fujian Province, China) and N16 (Lingshan County, Guangxi Province, China). Table 4 shows the similarity coefficient between five regions ranging from 0.534 to 0.773, in which the largest value was 0.773 between the Indonesia sample (N18) and the hybrid (N19), and the lowest value was 0.534 between the Guangxi Province (China) and Indonesia samples.

Table 3. Genetic similarities of 19 samples of *Mexona chinensis* Benth.

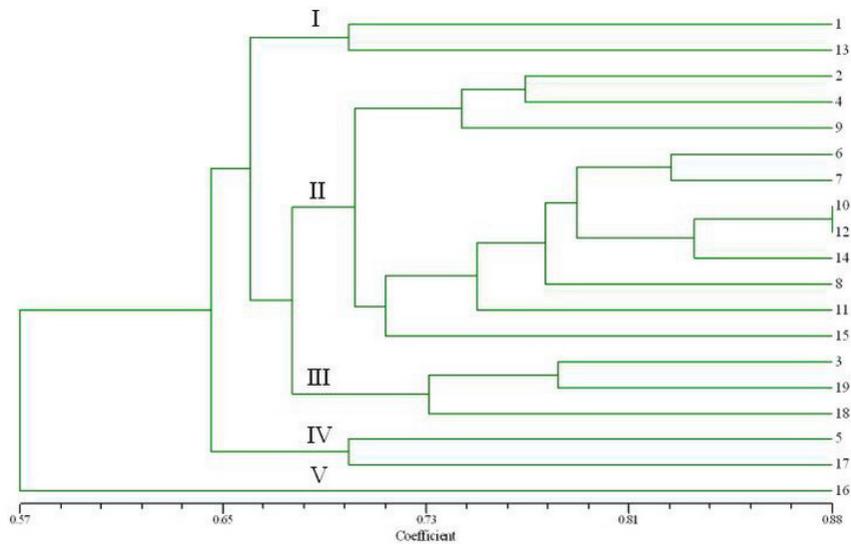
	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	N17	N18	N19	
N1	1.000																			
N2	0.730	1.000																		
N3	0.749	0.749	1.000																	
N4	0.620	0.767	0.638	1.000																
N5	0.607	0.644	0.614	0.546	1.000															
N6	0.681	0.755	0.675	0.693	0.681	1.000														
N7	0.638	0.810	0.706	0.712	0.675	0.822	1.000													
N8	0.669	0.730	0.675	0.669	0.742	0.755	0.773	1.000												
N9	0.693	0.730	0.724	0.644	0.718	0.620	0.693	0.749	1.000											
N10	0.607	0.681	0.687	0.571	0.730	0.706	0.749	0.767	0.706	1.000										
N11	0.687	0.736	0.742	0.712	0.675	0.810	0.779	0.785	0.687	0.883	1.000									
N12	0.699	0.650	0.620	0.577	0.699	0.712	0.632	0.687	0.564	0.699	0.589	0.681	1.000							
N13	0.718	0.755	0.736	0.718	0.693	0.816	0.761	0.767	0.681	0.804	0.693	0.859	0.724	1.000						
N14	0.675	0.663	0.607	0.749	0.638	0.712	0.693	0.761	0.601	0.712	0.626	0.742	0.589	0.749	1.000					
N15	0.558	0.558	0.601	0.522	0.620	0.509	0.552	0.558	0.632	0.632	0.632	0.577	0.589	0.607	0.515	1.000				
N16	0.564	0.650	0.656	0.540	0.699	0.749	0.669	0.601	0.601	0.736	0.699	0.718	0.656	0.675	0.571	0.577	1.000			
N17	0.644	0.730	0.687	0.656	0.583	0.718	0.773	0.706	0.706	0.718	0.656	0.724	0.601	0.669	0.601	0.534	0.663	1.000		
N18	0.699	0.675	0.779	0.614	0.515	0.638	0.656	0.626	0.650	0.663	0.601	0.669	0.620	0.699	0.558	0.564	0.583	0.773	1.000	
N19																				1.000

**Table 4.** Genetic similarities of different regions of *Mesona chinensis* Benth.

Region	Guangdong	Fujian	Guangxi	Taiwan	Indonesia	Indonesia-Guangdong (Hybrid)
Guangdong	1.000					
Fujian	0.691	1.000				
Guangxi	0.572	0.580	1.000			
Taiwan	0.622	0.668	0.577	1.000		
Indonesia	0.660	0.687	0.534	0.663	1.000	
Indonesia-Guangdong (Hybrid)	0.656	0.638	0.564	0.583	0.773	1.000

**Cluster analysis**

Figure 2 demonstrates that the 19 *M. chinensis* samples were distinctly clustered into five groups with a similarity coefficient cutoff of 0.68: N1 and N13 in the first group; N3, N18, and N19 in the third group; N5 and N17 in the fourth group; N16 in the fifth group, and the rest in the second group.



**Figure 2.** Dendrogram of UPGMA cluster analysis on 19 samples of *Mesona chinensis* Benth.

**DISCUSSION**

The RAPD amplification results of 19 *M. chinensis* samples demonstrated a high polymorphic rate, implying rich genetic diversity and strong environmental adaptability in this species, which agrees with the growth features of *M. chinensis* and its genetic trait of wide distribution (Wang et al., 2010). In the present study, primer S208 had a distinctive DNA fingerprint that could completely distinguish all of the samples, as did primer S206 combined with primer S253. Therefore, it is feasible to use an RAPD technique to construct an *M. chinensis* DNA fingerprint, which can provide molecular evidence for the identification and further exploitation of *M. chinensis*.

To a certain extent, the genetic similarity coefficient reflects the degree of genetic variation and genetic basis between samples. Our RAPD investigation disclosed that the average value in 19 *M. chinensis* samples was 0.711, demonstrating rich genetic diversity. This diversity is not only the molecular basis of rich morphological character in *M. chinensis* but also the root of genetic evolution in plants.

In cluster analysis, the RAPD divisions of *M. chinensis* had a certain relationship with geographical distribution and parental source. First, the samples with close geographical distribution mostly clustered together. For example, the samples from Guangdong Province (except N1, N3, and N5) and Fujian Province (except N13) were clustered in the second group, in which both N10 and N12 from Wuping County (Fujian Province, China) first clustered together, and the sample from Guangxi Province (China) clustered solely in the fifth group, which implied a greater genetic specificity. Second, N18 and N19, which had a genetic relationship from Indonesia, clustered in the third group. However, N4 and N19 did not cluster, likely owing to the boundedness of the molecular marker technique, the results of which could not conform completely to the actual situation, and the segregation of character after hybridization, in which the genotypes of filial generation were so dispersive that they could not cluster (Zhang et al., 2009). In a word, the results of RAPD markers can provide certain reference values, such as distinguishing *M. chinensis* resources and judging genetic relationships. This molecular marker technique is an effective method for the identification of the germplasm of *M. chinensis*, which plays an important role in resolving the confused sources of this species.

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