INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20F–042/2020/24–3–413–419
DOI: 10.17957/IJAB/15.1453
http://www.fspublishers.org

Full Length Article

Genetic Diversity among the Species of *Michelia* in China using ISSR

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Received 27 February 2020; Accepted 12 March 2020; Published 11 July 2020

Abstract

Michelia as an evergreen tree or shrub contains 80 species in the world out of which nearly 70 species could be found in China. In this study, Inter-simple Sequence Repeat Amplification was used to analyze the genetic diversity of 48 species of *Michelia*. A total of 151 loci were detected in 10 primers, and all were polymorphic, showing abundant polymorphism. According to the analysis of PopGen32 software package, for these 48 varieties, the Nei's gene diversity index (*H*), Shannon diversity index (*I*), coefficient gene differentiation (*Gst*) and gene flow (*Nm*) were 0.3261, 0.4946, 0.4838 and 0.5336, respectively. There was extensive genetic diversity among them. UPGMA clustering method divided these 48 species into seven categories, the clustering results support Nooteboom and Chen (2000) system's classification of *Michelia*. We suggest preserving the *M. sphaerantha* in the allotrope group. © 2020 Friends Science Publishers

Keywords: Magnoliaceae; *Michelia*; ISSR; Genetic diversity; Fingerprint

Introduction

Michelia, a kind of evergreen tree or shrub, is the sec largest genus in the Magnoliaceae family. The genus has about 80 species, mainly distributed in the tropical, subtropical and temperate regions of Asia. Nearly 70 species could be found in China, one of the countries with the most abundant germplasm resources of *Michelia*, and mainly produced in the southwest to the east (Liu and Wu 1988; Liu *et al.* 1995; Liu 2004). The subdivision of Magnoliaceae has always been a hot spot in taxonomic research (Wang 2000; Sun and Zhou 2004), the main difference is that there are many overlaps in the internal structure and external morphology of the genera of Magnoliaceae family and they are in constant differentiation. By means of palynology (Xu 1999), morphological anatomy (Cai and Hu 2000; Bao *et al.* 2002), cell biology (Meng *et al.* 2006), molecular biology (Azuma *et al.* 2000; Shi *et al.* 2000) and chemical analysis (Kumar *et al.* 2012), scholars at home and abroad are constantly looking for classification evidence for the family of Magnoliaceae and its genera. As the sec largest genus of Magnoliaceae, the interspecific and even intraspecific phenotypic variations of *Michelia* are extremely abundant, which has prompted the genus *Michelia* a top priority in the systematic classification (Zhang 2007). Due to the long-term introduction and hybridization, the genetic background of Magnoliaceae plant varieties is fuzzy, and the relationship between varieties is difficult to define (Zhang *et al.* 2018). In addition, the environment is one of the factors that cause the differences in phenotypic traits. It is difficult to use traditional classification methods to identify species, which is not only inefficient but also unreliable (Fowler *et al.* 1988). Therefore, it is particularly important to find a new basis for classification at DNA level.

ISSR marker is one of the commonly used molecular markers, which has the characteristics of low cost, high stability, simple operation and high repeatability, and it has been widely used in the research of identification of plant germplasm resources (Zietkiewicz *et al.* 1994; Zhu *et al.* 2010). ISSR analysis of genus *Phoebe* germplasm resources (Li *et al.* 2018) showed that its genetic diversity was high, among which *Phoebe bournei* was closely related to *P. zhennan*. Therefore, it was suggested that they should be merged. Zhang (2013) conducted ISSR analysis on the genetic diversity of germplasm resources of *Polygonatum* in Anhui province. Fourteen plants of *Polygonatum* were grouped into four subgroups, and the genetic diversity and genetic structure of the populations were analyzed. Zhang *et al.* (2015) carried out ISSR analysis on 56 *Osmanthus* varieties, and the experimental results showed that the genetic diversity among these varieties was rich, and the variation among the groups was greater than that within a

To cite this paper: Jiaojie L, P Jiqing, W Yi, W Yaling, L Tianzhu, M Bin, X Chao, Y Liangqian, X Qiaoying, W Hongyong, C Jiwu (2020). Genetic diversity among the species of *Michelia* in China using ISSR. *Intl J Agric Biol* 24:413–419

group. Lu *et al.* (2017) studied the genetic diversity of 24 ancient *Litchi* resources based on ISSR molecular markers, and the results showed that the genetic distance between the germplasm of ancient *Litchi* was wide, which could be used as an alternative in breeding program. Hao *et al.* (2019) used ISSR technology to analyze the genetic diversity of 44 materials from 5 wild populations of *Acer pentaphyllum*, an endangered endemic wild plant in Sichuan, China. Their results showed that the genetic diversity of *Acer pentaphyllum* at the population level is low and medium, and the level of intraspecific genetic diversity is low, so attention should be paid to the protection of this endangered plant.

In this study, ISSR molecular marker technology was used to detect the 48 species of *Michelia*, to analyze the genetic differences between *Michelia* species and to carry out interspecific clustering analysis, in order to provide help for the genetic breeding and genetic linkage map research of *Michelia*.

Materials and Methods

Plant materials

A total of 48 plant materials were collected from the campus of South Central University of Forestry and Technology, Changsha, Hunan province, Xinning Langshan Academy of Rare Plant, Hunan province, and South China Botanical Garden, Guangzhou, Guangdong province in China. Plant leaves with robust growth, free from diseases, insects and microbial contamination were randomly selected. After scrubbed by gauze, they were put into the preservation bags, numbered and stored in -70ºC ultra-low temperature refrigerator for later use. The number and name of each experimental material are shown (Table 1).

ISSR analysis

The experiment was carried out in the laboratory of Central South University of Forestry and Technology from July to October, 2018. The primer sequence used in the experiment was derived from the ninth set of ISSR primer sequence published by the University of Columbia, Canada, which was synthesized by GenScript USA Inc., and the primer number and sequence were screened (Table 2). Plant genome DNA extraction kit (Tiangen Biotech Co. Ltd. Beijing) and modified CTAB method were used to extract the total DNA. The quality and concentration of nucleic acid in the extract were detected by UV spectrophotometer, diluted with ddH_2O to 35 ~ 50 ng/ L, and stored at -20°C for later use. ISSR-PCR amplification reaction system was optimized and established on the basis of reference plant reaction system. The optimum reaction system was (20 μL): 10 PCR Buffer 2.0 μL, DNA 30 ng, Mg^{2+} 2.0 mmol/L, ISSR primer 0.6 mol/L, dNTPs 0.2 mmol/L, Taq DNA polymerase 1.5 U. Finally, the system was replenished with ddH_2O to 20 μL. The amplification reaction program was: pre-denaturation at 94ºC for 5 min, denaturation at 94ºC for 30 s, annealing for 45 s, extension at 72ºC for 90 s, a total of 36 cycles, total extension at 72ºC for 420 s, PCR amplification products preserved at 4ºC. Agarose gel electrophoresis was used for detection, and the results were imaged and photographed by gel imaging system.

Data analysis

Using 200 bp ladder DNA marker as reference, and the position and size of the amplified bands in all sample maps were determined according to the electrophoretogram. After statistical analysis of the amplified bands, the clear and recognizable bands were denoted as "1", and the ambiguous or no bands were denoted as "0". Besides, the bands were sorted by fragment size to form the original matrix, and the original matrix was modified according to the Hardy-Weinberg equilibrium. Pop Gen32 software package was used to calculate and analyze the modified data matrix, and MEGA6 software was used for cluster analysis of *Michelia.*

Results

Polymorphism analysis of amplified products

Ten ISSR primers were used to carry out ISSR-PCR amplification on 48 species of *Michelia* (Fig. 1), and clear bands of all plants were amplified. A total of 151 bands with a size between 150 and 1300 bp were amplified, among which 151 were polymorphic bands and the polymorphic rate was 100%. The polymorphic bands detected by each pair of primers ranged from 11 to 17 (Table 2), and the average number of polymorphic bits was 15.1. The amplification bands of primer UBC844 and primer UBC895 were the most, for 17, and the amplification bands of primer UBC810 were the least, for 11. The results showed that there are many polymorphic bands in plants of *Michelia* and the genetic variation was abundant, and they had a strong adaptability to environmental variation.

Construction and analysis of system tree

Through cluster analysis, 48 species of *Michelia* could be divided into 7 branches (Fig. 1); these were: Group I (17 species), Group II (6 species), Group III (3 species), Group IV (13 species), Group V (7 species), Group VI (1 species) and Group VII (1 species), the varieties of each group are shown (Fig. 2).

Group I was a complex group, which could be subdivided into five subgroups. Subgroup I for *M. martinii*, *M. microcarpa* and *M. opipara*, Subgroup II comprised *M. guangxiensis*, *M. skinneriana*, *M. macclurei*, *M. xiangnanensis* and *M. longipedunculata*, Subgroups III for the *M. lacei* and *M. figo*; Subgroup IV consisted of *M. maudiae*, *M. xinningia*, *M. alba* and *M. elegans* and Subgroup V contained *M. szechuanica*, *M. wilsonii* and

Table 1: No. and names of plant materials (CSUFT: Central South University of Forestry and Technology; XLARP: Xinning Langshan Academy of Rare Plant; SCBG: South China Botanical Garden)

Clone name	Collection sites	Introduction the year
M. szechuanica	CSUFT	2006
M. foveolata	CSUFT	2006
M. floribunda	CSUFT	2006
M. longipedunculata	XLARP	1982
M. xiangnanensis	XLARP	1979
M. foveolata var. *cinerascens*	CSUFT	2006
M. wilsonii	CSUFT	2006
M. opipara	SCBG	1991
M. lacei	SCBG	2003
M. fujianensis	SCBG	1999
M. macclurei	CSUFT	2007
M. maudiae	CSUFT	2005
M. skinneriana	CSUFT	2008
M. yunnanensis	CSUFT	2006
M. virensipetala	XLARP	1985
M. foveolata var. *xiangnanensis*	XLARP	1975
M. fulgens	XLARP	1984
M. champaca	SCBG	1976
M. sirindhorniae	SCBG	2003
M. alba	SCBG	1990
M. elegans	SCBG	1983
M. xinningia	SCBG	1984
M. xanthantha	XLARP	1985
M. rufivillosa	SCBG	2000
M. shiluensis	CSUFT	2006
M. compressa	CSUFT	2007
M. sphaerantha	CSUFT	2006
M. macclurei var. *sublanea*	XLARP	1982
M. flaviflora	SCBG	2005
M. guangxiensis	SCBG	1986
M. cavaleriei	CSUFT	2006
M. martinii	CSUFT	2005
M. platypetala	CSUFT	2005
M. chartacea	CSUFT	2006
M. fulva	SCBG	1991
M. doltsopa	SCBG	1999
M. chapensis	CSUFT	2003
M. balansae	CSUFT	2006
M. longistamina	XLARP	1986
M. hedyosperma	SCBG	1984
M. gushanensis	CSUFT	2005
M. gigantea	CSUFT	2006
M. microcarpa	SCBG	2003
M. mediocris	XLARP	1982
M. figo	CSUFT	2004
M. zhejiangensis	XLARP	1983
M. coriacea	XLARP	2001
M. fadouensis	XLARP	2001

M. foveolata var. *Cinerascens*. The genetic distance between *M. alba* and *M. elegans* was 0.2637, and that between *M. xiangnanensis* and *M. longipedunculata* was 0.3445, indicating that they were closely related. Group II contained *M. virensipetala*, *M. rufivillosa*, *M. doltsopa*, *M. gigantea*, *M. mediocris*, and *M. coriacea.* The genetic distance between *M. doltsopa* and *M. mediocris* was 0.3538, which was the closest genetic relationship. Group III contained *M. cavaleriei*, *M. fulgens*, *M. foveolata* var. *Xiangnanensis*. There were relatively more *M.* germplasm in Group IV, which could be divided into two subgroups. Subgroup I for the *M. fulva*, *M. balansae*, *M. macclurei* var. *sublanea*, *M. flaviflora* and *M. yunnanensis*, Subgroup II for the *M. chartacea*, *M. platypetala*, *M. compressa*, *M. shiluensis*, *M. fujianensis*, *M. zhejiangensis*, *M. floribunda* and *M. foveolata.* Group V contained *M. champaca*, *M. sirindhorniae*, *M. xanthantha*, *M. chapensis*, *M. longistamina*, *M. gushanensis* and *M. fadouensis.* The genetic distance between *M. longistamina* and *M. xanthantha* was 0.3826, which was the closest genetic relationship. Both Group VI and VII had only one species, respectively was *M. sphaerantha* (0.4121 ~ 0.7832) and *M. hypolampra* (0.4529 ~ 0.7268), and they were more distantly related to all the other plants of *Michelia.*

Analysis of genetic diversity between groups

The genetic diversity of 48 species of *Michelia* in 7 groups was calculated by Pop Gen 32 software (Table 3). The number of polymorphic loci of Group I was 137, accounting for 90.73%; The number of polymorphic loci of Group II was 116, accounting for 76.82%; The number of polymorphic loci of Group III was 77, accounting for 50.99%; The number of polymorphic loci of Group IV was 143, accounting for 94.7%; The number of polymorphic loci of Group V was 126, accounting for 83.44%. A total of 151 loci were detected in a total of 48 individuals, among which 151 were polymorphic loci. It can be seen that the percentage of polymorphic band at the total level of *Michelia* was as high as 100%, which had a high genetic diversity, but the percentage of polymorphic loci varied greatly among groups. Group IV had the highest percentage of polymorphic loci, for 94.7%, which meant that the genetic variation of Group IV was high, the genetic basis was best, the gene exchange was frequent, and the ability to adapt to the environment was the strongest. At the same time, the success rate of intergroup crossbreeding was relatively high, which is beneficial to the development of genus crossbreeding. The total level of Nei's genetic diversity index (*H*) was 0.3261, Shannon diversity index (*I*) was 0.4946, the observed allele number (*Na*) was 2.0000, and the efficient allelic number (*Ne*) was 1.5424 in the 48 samples. These results indicated that the 48 species of *Michelia* have rich genetic diversity at the species level.

Analysis of genetic differentiation between groups

The total genetic diversity, intra-population genetic diversity, inter-population genetic diversity, coefficient gene differentiation and gene flow of 48 species in seven groups were analyzed, and the results were shown (Table 4). The total population genetic diversity (*Ht*) of the 48 samples was 0.3630, the intra-population genetic diversity (*Hs*) was 0.1874, and the inter-population genetic diversity was 0.1756. At the species level, 51.62% of the genetic variation existed intra the population, while 48.38% of the genetic variation existed inter the population. The coefficient gene

Table 2: Amplification sites of ISSR primers

No.	Sequence	Expand the strip	Polymorphic band	Polymorphism ratio
810	GAGAGAGAGAGAGAGAT	11	11	100%
817	CACACACACACACACAA	13	13	100%
818	CACACACACACACACAG	16	16	100%
836	AGAGAGAGAGAGAGAGYA	16	16	100%
843	CTCTCTCTCTCTCTCTRA	15	15	100%
844	CTCTCTCTCTCTCTCTRC	17	17	100%
845	CTCTCTCTCTCTCTCTRG	14	14	100%
848	CACACACACACACACARG	16	16	100%
876	GATAGATAGACAGACA	16	16	100%
895	AGAGTTGGTAGCTCTTGATC	17	17	100%
Total		151	151	100%

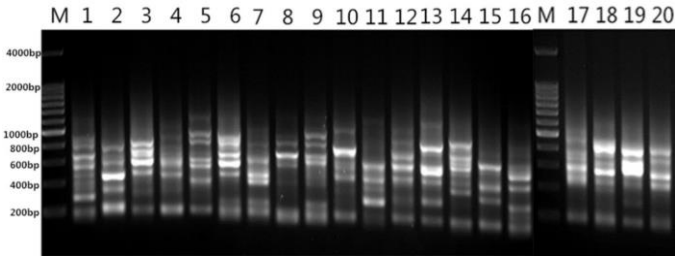

Fig. 1: ISSR amplification electrophoretogram of some samples with primer UBC844

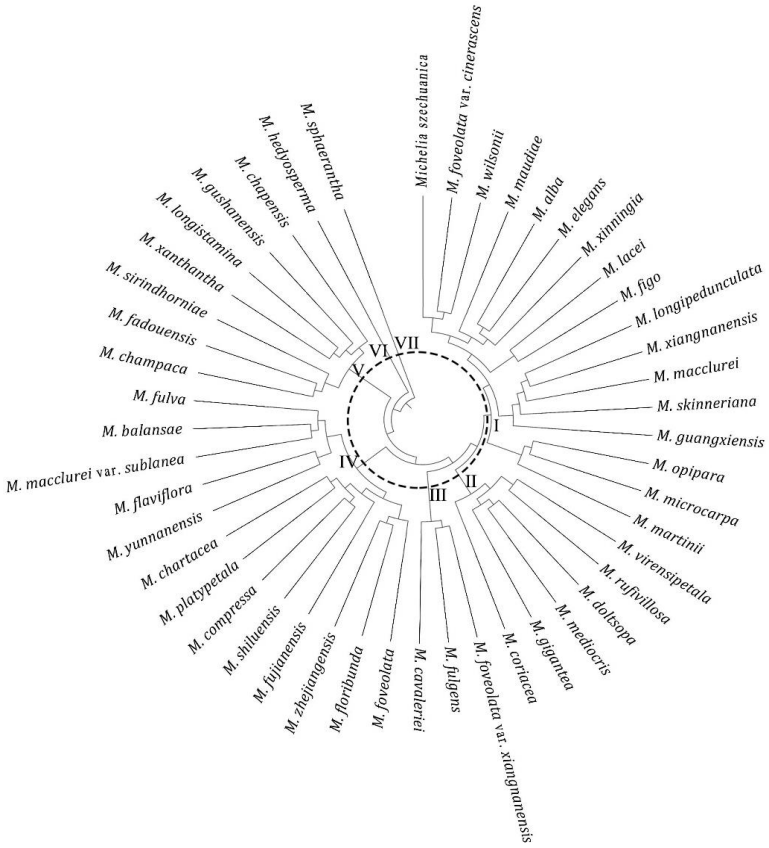

Fig. 2: UPGMA cluster map of 48 species of *Michelia*

differentiation (*Gst*) between each population was 0.4838, indicating that there was some genetic differentiation among the seven groups. The gene exchange in the population of the *Michelia* was frequent, which is beneficial to the research of intra-population crossbreeding technology and the cultivation of new plant varieties. The gene flow (*Nm*)

between groups was 0.5336, less than 1, indicating that the gene flow among the populations of the *Michelia* was large, and genetic drift would occur, leading to genetic differentiation within the genus.

Genetic distance and genetic consistency

Genetic distance and genetic consistency are important indicators to evaluate the degree of genetic differentiation and distance of genetic relationship between populations. The smaller the genetic distance is, the closer it is to "0", and the higher the genetic consistency is, the closer it is to "1", indicating that the smaller the genetic differentiation degree between the populations is, the closer the genetic relationship will be. On the contrary, the greater the degree of genetic differentiation between the populations is, the farther the genetic relationship will be. The genetic distance of Nei's between 7 groups of *Michelia* ranged from 0.0287 to 0.5865, with an average value of 0.2635, and the genetic consistency ranged from 0.5563 to 0.9717, with an average value of 0.7814 (Table 5). In the seven groups, Group I and Group IV had the highest genetic consistent degree, and the lowest was Group VI and Group VII. Similarly, Group I and Group IV had the closest genetic distance, and Group VI and Group VII had the farthest genetic distance. *M. alba* and *M. elegans* in Group I have the closest genetic consistency over the 48 species, for 0.7682. And the lowest ones were *M. szechuanica* and *M. sphaerantha* in Group I, for just 0.457. *M. szechuanica* were found in western Hubei, southern Sichuan and southeast, northern Guizhou, northeast Yunnan, born in the mountain forest at an altitude of 1300 ~ 1600 meters. *M. sphaerantha* were found in Yunnan, where they were found in forests at an altitude of 1300 ~ 1600 meters, and the two species were also very different in form. *M. szechuanica*'s leaves are slender, narrowly obovate, and the color of the perianth is canary yellow. Besides, the aggregate fruit is small, and follicles are compressed into spheroidicity as they mature. However, *M. sphaerantha*'s leaves are round and long, obovate-oblong, and the color of the perianth is white. Besides, the aggregate fruit is big, and follicles are compressed into oval as they mature.

Cluster analysis among groups

Using PopGen32 packages to carry out UPGMA cluster analysis, the results show that the seven groups could be grouped into three major categories. Group I to V could be grouped into one major category, and then be divided into three small classes. Group I, Group IV and Group V could be grouped into a small class, Group II and Group III could also be grouped into one. Both Group VI and Group VII could be grouped into one major category, respectively (Fig. 3).

Table 3: Genetic diversity of 48 species of *Michelia* (*PPB*: Percentage of polymorphic bands; *Na*: The number of observational allele; *Ne*: Effective number of alleles; *H*: Genetic diversity; *I*: Shannon's index of genetic diversity)

Group	Sample size	Polymorphic bit count	*PPB*	*Na*	*Ne*	*H*	*I*
I	17	137	90.73%	1.9073	1.4564	0.2791	0.4285
II	6	116	76.82%	1.7682	1.4522	0.2663	0.4004
III	3	77	50.99%	1.5099	1.3330	0.1940	0.2878
IV	13	143	94.70%	1.9470	1.4797	0.2910	0.4463
V	7	126	83.44%	1.8344	1.4696	0.2813	0.4260
VI	1	-	-	-	-	-	-
VII	1	-	-	-	-	-	-
Total	48	151	100%	2.0000	1.5424	0.3261	0.4946

Table 4: Genetic differentiation coefficients of 48 species in 7 groups of *Michelia* (*Ht*: the total population genetic diversity; *Hs*: the intra-population genetic diversity; *Gst*: the coefficient gene differentiation; *Nm*: the gene flow)

Ht	*Hs*	The inter-population genetic diversity	*Gst*	*Nm*
0.3630	0.1874	0.1756	0.4838	0.5336

Table 5: Genetic distance and genetic consistency among 7 groups

Group	I	II	III	IV	V	VI	VII
I	--	0.9459	0.8991	0.9717	0.9568	0.6665	0.6615
II	0.0556	--	0.8801	0.9425	0.9268	0.6778	0.6744
III	0.1063	0.1277	--	0.893	0.8808	0.5965	0.6187
IV	0.0287	0.0593	0.1131	--	0.9561	0.6796	0.685
V	0.0442	0.0761	0.1269	0.0449	--	0.6591	0.6805
VI	0.4057	0.3889	0.5167	0.3862	0.4169	--	0.5563
VII	0.4133	0.3939	0.4802	0.3783	0.3849	0.5865	--

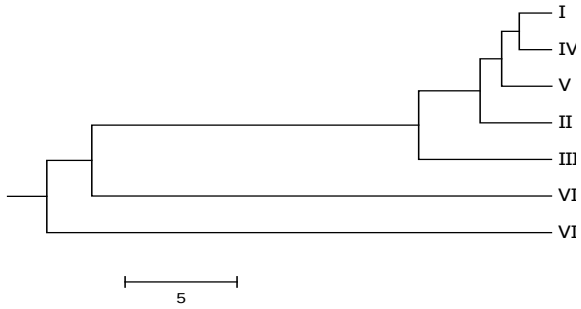

Fig. 3: UPGMA cluster graph of 7 groups

Discussion

The accurate evaluation of genetic diversity is helpful to the analysis of its evolutionary potential and prediction of its future destiny, as well as the premise of the survival and evolution of organisms, which is of important guiding significance in the protection and utilization of species germplasm resources (Chen *et al.* 2017). In recent years, ISSR markers had been used to study the relationship between populations of *Michelia.* The results of this study showed that the polymorphism ratio of 10 primers in 48 species of *Michelia* was 100%, which was higher than the

result of the study by Wen Qiang et al. that the polymorphism percentage of ISSR primers in 13 germplasm of *Michelia* was 90.16% (Wen *et al.* 2014). Li (2013) showed that ISSR primer polymorphism in 21 evergreen species of Magnoliaceae was 99.04%, which was similar to the results of this study. Huang (2007) showed that the polymorphism ratio of ISSR primers in 20 plants of six genera in Magnoliaceae was 100%, which was basically consistent with this study. ISSR markers can fully reveal the genetic differences between different populations of *Michelia*. Similar to the research results of Li (2013) and Huang (2007), the results indicated that there was a relatively rich genetic diversity between populations or individuals of *Michelia*, which provided theoretical evidence for further understanding of the genetic background and protection, development and utilization of germplasm resources of *Michelia.*

Factors that affect the genetic differentiation of plant populations includes evolutionary history, mutation, recombination, genetic drift, breeding system, gene flow and natural selection (Slatkin 1987; Li *et al.* 1994; Schaal *et al*. 1998). Based on RAPD, Jiang *et al.* (2005) analyzed the genetic diversity and differentiation degree of 6 populations of *M. chapensis*. POPGENE analysis showed that Nei's gene diversity (*He*) and Shannon phenotypic index (*I*) of *M. chapensis* were 0.3255 and 0.4751 respectively, which showed a higher level of genetic diversity compared with other plants. The coefficient gene differentiation (*Gst*) of 6 populations of *M. chapensis* was 0.2226, which indicated that the genetic variation within populations was bigger than that between populations. Hamrick et al. believed that if *Nm*>1, gene flow would be sufficient to resist the differentiation between populations caused by genetic drift. If *Nm*< 1, it would not be enough to resist the inter-group genetic differentiation caused by genetic drift in the population, and drift would become the dominant factor of population genetic structure (Hamrick 1989). The coefficient gene differentiation (*Gst*) of 48 samples in the present study was 0.4838, and the gene flow between groups (*Nm*) was 0.5336, less than 1, indicating that there is a large gene flow between populations of *Michelia*, and preventing genetic drift may cause intra-genus genetic differentiation. At the same time, it also indicated that there is a certain degree of genes differentiation among the seven groups, and the gene exchange within *Michelia* was relatively frequent, which is conducive to the research on hybrid breeding technology of plants in the population and the cultivation of new plant varieties.

It is difficult to distinguish the species or varieties of *Michelia* according to morphology because of the rich genetic variation between species during the long-term artificial culture selection. At present, there are two classification systems: Liu Yuhu system (Liu *et al.* 1996) and Nooteboom and Chen system (Nooteboom 2000). In this study, ISSR molecular marker technology was used to preliminarily analyze the phylogenetic relationships of 48 related species of *Michelia*. Group I to V were clustered as a broad category, Group VI and Group VII were respectively divided into a category, and the genetic distance between seven groups ranged from 0.2637 to 0.7832. In Group I, *M. alba* (Sect. Michelia) and *M. elegans* (Sect. Anisochlamys) had the closest genetic relations, and the interspecific genetic distance was 0.2637. In Group II, *M. doltsopa* (Sect. Michelia) and *M. mediocris* (Sect. Anisochlamys) had the closest genetic relations, and the interspecific genetic distance was 0.3538. In Group IV, *M. balansae* (Sect. Dichlamys) and *M. mediocris* (Sect. Micheliopsis) had the closest genetic relations, and the interspecific genetic distance was 0.474. In Group V, *M. longistamina* (Sect. Dichlamys) and *M. mediocris* (Sect. Dichlamys) had the closest genetic relations, and the interspecific genetic distance was 0.3826. Group VI and Group VII had only one species, respectively *M. sphaerantha* (Sect. Anisochlamys) and *M. hedyosperma* (Sect. Anisochlamys), and they were distantly related to all other *Michelia* species. This study supported the classification method of Nooteboom and Chen system (2000), and a large number of *Michelia* species were merged. Most species of Sect. Anisochlamys in Subgen. Metamichelia should be merged with Sect. Michelia; some species in Sect. Dichlamys and Sect. Micheliopsis should be merged and *M. hedyosperma* of Sect. Anisochlamys should be reserved (Nooteboom 2000). At the same time, this study suggested that *M. sphaerantha* in the Sect. Anisochlamys should be reserved.

Although the 48 species of *Michelia* have high genetic diversity at the species level (PPB=100%), among them including two endangered species, *M. wilsonii* and *M. hedyosperma*, recorded in the Chinese Plant Red Book, with extremely limited distribution and population. Studies have shown that rare species or endemic species could maintain a high level of genetic variation (Cosner and Crawford 1994). Besides, endangered species did not mean a decrease in the level of genetic variation, and different types of endangered plants did not show genetic decline (Schwartz 1985; Wang and Hu 1996). Therefore, the protection of *M. wilsonii* and *M. hedyosperma* should take comprehensive protection measures to slow down or restrain the decline trend of population groups instead of single means. *M. wilsonii* is an ancient and endangered Magnoliaceae plant, which was relatively less affected by the Quaternary glaciation, and is one of the preserved ancient, unique and rare plants. Its specific evolutionary history determined that its distribution is narrow and its ecological requirements are relatively strict, and it only lives in broad-leaved forests with an altitude of 900 ~ 1,700 m. Therefore, it is very important to maintain a stable environment for the growth and reproduction of *M. wilsonii* population, and it is necessary to take effective measures to protect the existing population in situ, and to find appropriate methods to rapidly expand the population, reduce the rate of gene loss, and investigate the random factors that affect the small population. However, the resources of *M. hedyosperma* in China is rare, and there are

only dozens of wild tree species scattered in Xishuangbanna. In recent years, people's unreasonable exploitation of tropical forests had led to the deterioration of its habitats and the difficulty in natural renewal, which put it in danger of extinction. According to the introduction report of Guangxi, it could endure the low temperature (-5.5 ~ -6ºC) without being damaged by freezing (Wang *et al.* 1994). Therefore, the introduction and cultivation of *M. hedyosperma* can be carried out in most areas of subtropical region of China, and the scope of introduction and cultivation can be expanded. Only in this way can the number of species be expanded and germplasm resources be continued and developed.

Conclusion

UPGMA clustering method divided these 48 species into seven categories. The clustering data supported Nooteboom and Chen (2000) system's classification of *Michelia*. We suggested preserving the *M. sphaerantha* in the allotrope group.

Acknowledgements

We are very grateful for the plant materials provided by South China Botanical Garden and Langshan Academy of Rare Plant. In addition, This work was financially supported by the Youth Scientific Research Foundation, Central South University of Forestry and Technology (201501030324, QJ2014006A); Central Finance Project Fund of Forestry Science and Technology (2017XT002); National Natural Science Foundation of China (31570631).

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