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High genetic diversity and small genetic variation among populations of *Magnolia wufengensis* (Magnoliaceae), revealed by ISSR and SRAP markers

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ABSTRACT

Background: Genetic diversity and genetic variation of 10 populations and subpopulations of *Magnolia wufengensis*, a new and endangered endemic species, were examined by inter simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) molecular markers. Compared with other endangered endemic Magnolia taxa, *M. wufengensis* holds a relatively high level of genetic variation.
Result: Total genetic diversity was found to be 87.7% for ISSR and 88.0% for SRAP markers. For polymorphic loci (*P*), the effective mean number of alleles (*Ae*) was 1.414 for ISSR markers and 1.458 for SRAP markers, while the mean expected heterozygosity (*H*) was 0.256 using ISSR and 0.291 for SRAP markers. Within-population variation was estimated for *P* as 74.9% using ISSR and 74.6% with SRAP markers; the number of alleles *Ae* was 1.379 with ISSR and 1.397 for SRAP and *H* 0.235 with ISSR and 0.247 for SRAP markers.
Conclusion: The analysis of molecular variation of both ISSR and SRAP marker systems indicated that most genetic variation is within populations, with values of 90.64% and 82.92% respectively. Mantel tests indicated a moderate association between the two marker systems and a low correlation between genetic and geographic distances. High levels of genetic diversity and low levels of population divergence suggest that genetic drift is not currently of great concern for this species. Severe habitat loss and fragmentation, predominantly ascribed to anthropogenic pressures, caused *in-situ* developing restriction of this species. Action for conserving this rare species for its long-term survival should be taken immediately.

1. Introduction

Magnolia wufengensis is under the subgenus *Yulania*, *Magnolia*, found in 2006 by Ma et al. [1,2]. It can be found in Wufeng, southwest in Hubei Province, and neighboring counties in China. Considerable morphological variation, such as a diverse number of tepals *i.e.*, 9, 12, 15, 18 or even 46, different depths of tepal color ranging from purple-red, red to pale red and even paler, close to white, various sizes and widths of tepals and leaves in trees, have been observed during field investigations of this species. Its rich morphological diversity and relatively narrow distribution have a considerable academic value for taxonomy, phylogeny and evolution of Magnolia, and high values as a prospective and promising ornamental tree for horticulture and city landscapes. *MAwuAG* gene, an *AGAMOUS* homologous gene, isolated from the *M. wufengensis* flower, belongs to the clade of *euAG* lineages, an early-diverging C-lineage without the N-terminal extensions [3]. Wu et al. [3] deduced that this species plays an important role in plant evolution history, by proving the ancient structure and function of *AGAMOUS* homeotic genes in *M. wufengensis* rather than in other core eudicots. In Jing et al.'s. [4] studies on function of *AP3* homologs, *MawuAP3_1/2*, in petal and stamen development, this species also revealed its archaic trait [4].

Much work needs to be done with this species, such as classification, introduction and propagation, but the first important thing is its conservation. The preservation of genetic diversity in endangered species is a major goal in conservation planning, since long-term species survival depends on the maintenance of sufficient genetic variability within and among populations to accommodate new selection pressures brought about by environmental change [5]. Understanding the biology and genetic variations within and between populations is essential for the establishment of effective and efficient conservation practices for rare plants [6].

The objectives of our study are the following: 1) to evaluate the level of genetic diversity and to analyze the genetic structure of *M. wufengensis* through inter simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) markers; 2) to

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compare the usefulness, similarities and differences of these two markers in estimating population genetics; and 3) to propose conservation measures for *M. wufengensis* based on its population diversity and genetic variation.

2. Materials and methods

2.1. Plant material and DNA isolation

Given its geography, six representative *M. wufengensis* populations were selected based on field investigations and their distribution in and around Wufeng in Hubei (Fig. 1). The largest population, *i.e.*, Dushuping (DSP), was further divided into five subpopulations on the basis of slope aspect and elevation. A range of 18–33 samples of each population were collected for analysis for a total sample size of 252 (Table 1). During spring blooming, twigs from adult trees, at least 30 to 50 years old, with buds were cut and cultured in 25°C water at our laboratory. Young, 3 to 5 cm long leaves were spread out and selected for DNA isolation.

Genomic DNA of single individuals was extracted following the improved CTAB protocol [7]. The concentration and quality of DNA samples were checked on 0.8% agarose gel by a comparison with lambda DNA standards and with UV spectroscopy. The DNA samples were diluted to approximately 10 ng/μL with a 0.1% TE buffer.

2.2. ISSR analysis

The sequence and codes of 56 ISSR primers followed by NAPS Standard Unit Primers (UBC, Canada) were screened. Of these, 10 primers with clear and polymorphic bands were used for amplification of all 252 accessions from the six populations. The annealing temperature of each primer was optimized by a gradient PCR (Table 2).

Table 1
Center of populations locality and sample size.

Code	Population		Latitude (N)	Longitude (E)	Altitude (m)	Sample size
1	LKY		30°09′30″	110°40′25″	1470	18
2	LZP		30°10′32″	110°35′32″	1690	33
3	NCK		30°15′35″	110°45′14″	1630	27
4	HY		30°17′40″	110°24′12″	1720	23
5	SJY		30°11′14″	110°20′04″	1870	28
6	DSP	Sub1	30°16′48″	110°40′30″	1620	25
7		Sub2	30°16′35″	110°40′45″	1640	23
8		Sub3	30°16′25″	110°40′57″	1680	22
9		Sub4	30°16′31″	110°40′31″	1740	27
10		Sub5	30°16′18″	110°40′25″	2010	26

DSP: Dushuping; HY: Hongyan; LKY: Luokuangyan; LZP: Liziping; NCK: Nanchongkou; SJY: Shuangjianya.

2.3. SRAP analysis

Our SRAP protocol followed the method established by Li and Quiros [8]. Forty eight SRAP primer pair combinations (em1~em6 × me1~me8) were screened by separate PCR products on a 1.5% agarose gel for successful amplification. Ten of these 48 primer pairs produced clear and stable polymorphic bands and labeled as 252 accessions of the six populations (Table 2). The 10 primer pair combinations are em1–me1, em1–me3, em1–me5, em2–me5, em4–me1, em5–me3, em5–me4, em5–me5, em5–me8 and em6–me5. All ISSR and SRAP primers were synthesized by the Sangon Biotech (Shanghai) Co, Ltd.

2.4. Bands scoring and data analysis

Bands were scored by hand. Bands with the same weight were scored as a line according to the weight of the DNA ladder (100 bp).

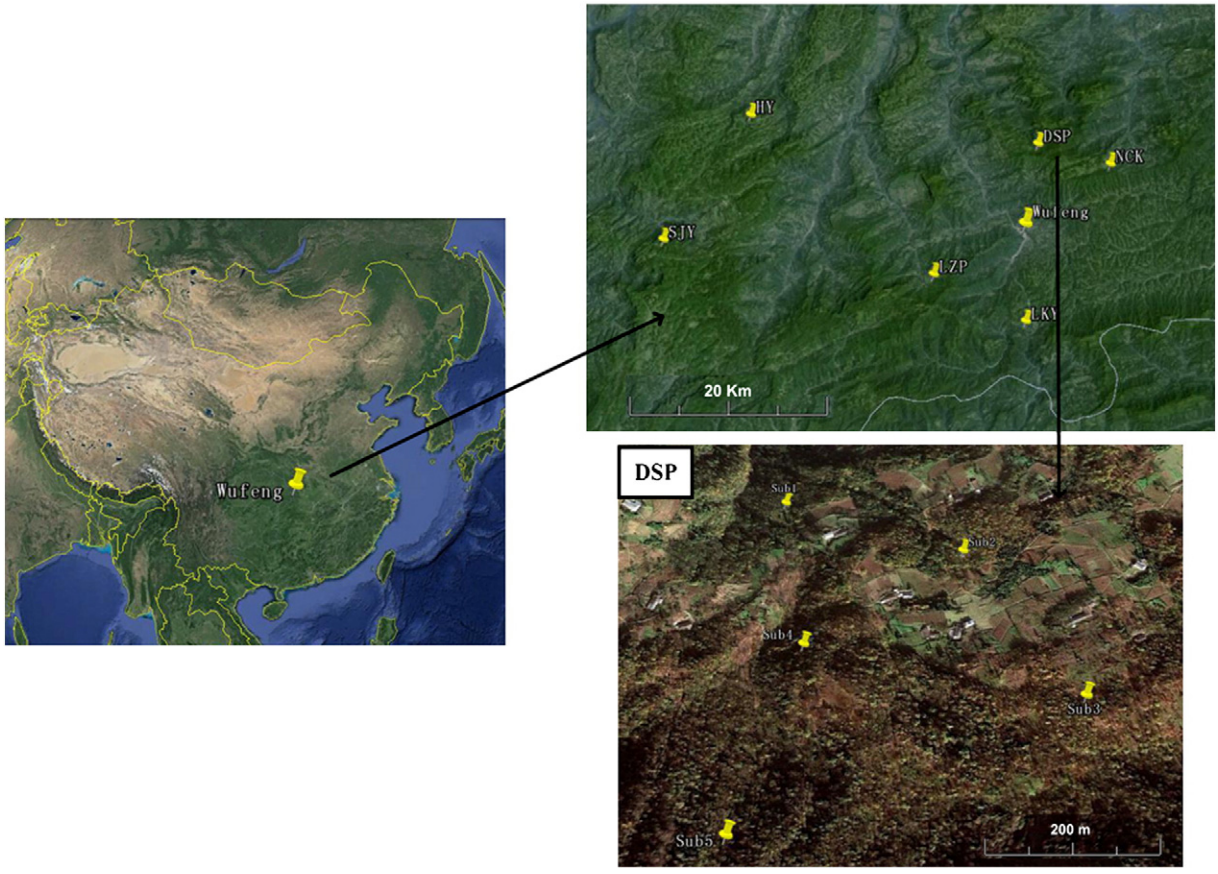

Fig. 1. Maps showing locations of the populations of *M. wufengensis* sampled (2013 Landsat, Digital Global, Mapabc.com, Google, URL: http://www.google.com/earth/).

Table 2
The primers used for ISSR and SRAP amplification.

ISSR primer	Sequence (5′–3′)	Annealing temperature (°C)	SRAP primer	Sequence (5′–3′)
817	$(CA)_8A$	58.6	em1	GACTGCGTACGAATTAAT
818	$(CA)_8G$	58.6	em2	GACTGCGTACGAATTTGC
826	$(AC)_8C$	62.0	em4	GACTGCGTACGAATTTGA
835	$(AG)_8YC$	58.5	em5	GACTGCGTACGAATTAAC
836	$(AG)_8YA$	59.0	em6	GACTGCGTACGAATTGCA
841	$(GA)_8YC$	51.4	me1	TGAGTCCAAACCGGATA
848	$(CA)_8RG$	56.2	me3	TGAGTCCAAACCGGAAT
855	$(AC)_8YT$	58.5	me4	TGAGTCCAAACCGGACC
857	$(AC)_8YG$	56.2	me5	TGAGTCCAAACCGGAAG
873	$(GACA)_4$	52.5	me8	TGAGTCCAAACCGGTGC

Y = Pyrimidine (C or T); R = Pyrimidine (A or G).

Both ISSR and SRAP scores were recorded as either present (1) or absent (0); bands with the same molecular weight were considered to be allelic, while weak or ambiguous bands were excluded from the analysis. A binary data matrix was compiled for each primer set. Most of the following analyses were carried out based on adjoined matrix data.

Genetic diversity was described by three molecular diversity indices: (1) the number (percentage) of polymorphic loci (*P*) at the 99% criterion; (2) the mean expected heterozygosity (*H*), for a Hardy–Weinberg equilibrium; and (3) the effective mean number of alleles (*Ae*). The three parameters and Nei's standard genetic distance (*D*) [9] were calculated by PopGen32 [10]. Phylogeny trees, based on UPGMA (Unweighted Pair Group Method with Arithmetic averages), were constructed using NTSYSpc 2.11f [11]. One thousand permutations were performed to evaluate the robustness of the groupings by bootstrapping. Genetic structures, genetic variation in three hierarchies (population, subpopulation and individuals), were inferred from AMOVA (Analysis of Molecular Variation) using Arlequin 3.1 [12]. The F_{ST} (Fixation index) was calculated with the formula [Equation 1]:

$$F_{ST} = \frac{\left(\sigma_a^2 + \sigma_b^2\right)}{\sigma_\tau^2} \quad \text{[Equation 1]}$$

where $\sigma_a{}^2$ is the expected variance among populations, $\sigma_b{}^2$ the expected variance among subpopulations and σ_τ^2 the sum of the two expected variances. The 1000 permutations were replicated for a 0.05 significance test. Gene flow (*Nm*) was calculated as [Equation 2] [13]:

$$Nm = \frac{(1 - F_{ST})}{4F_{ST}}. \quad \text{[Equation 2]}$$

The correlation of ISSR and SRAP markers was assessed by calculating the coefficient of genetic diversity indices *P*, *H* and *Ae*, as well as by Nei's standard genetic distance matrix Mantel test [14] using the NTSYS-pc program. The correlation between genetic distances and geographical distance of populations was also evaluated by Mantel tests. The significance of the correlation coefficients was tested by 1000 permuted samples.

3. Results

3.1. Gene diversity analysis

Genetic diversity was described for the three parameters *P*, *Ae* and *H* on two hierarchical levels of species and population. Based on the 252 trees, the 10 ISSR primers yielded 421 polymorphic bands out of a total of 480 clearly scorable fragments with *P* of 87.7% (Table 3). The number of scored bands per ISSR primer varied from 57 for primer 826 to 39 for primer 841, with a mean of 48 bands per test primer. The selected 10 SRAP primer combinations resulted in a total of 415 reproducible fragments. Among these 415 bands, 365 loci were polymorphic, for which the *P* value was 88.0%. The number of bands ranged from 27 for em4–me1 to 55 for em5–me5 per SRAP primer pair.

Comparing the values of *P*, *Ae* and *H* between the ISSR and SRAP marker systems on a population level and T-tested through SPSS Statistic Version 21, population Luokuangyan (LKY) showed significantly low variation within the population ($P < 0.05$). Based only on the *H* value, populations Shuangjianya (SJY) and Hongyan (HY) also showed a slightly lower genetic diversity. Within population DSP, the ISSR system indicated that Sub1 has the smallest values for *P*, *Ae* and *H*, which infers a relatively low genetic variation in Sub1. Sub4 with the highest value for *P* and *Ae* and Sub5 with the highest *H* denote a higher genetic diversity in these two subpopulations. The SRAP marker system shows that Sub3 has the highest diversity with maximum *P*, *Ae* and *H* values, with Sub4 the smallest values for *P* and *Ae*. Sub1 with the smallest *H* value, infers low diversity in these two populations (Table 3).

3.2. Analysis of genetic differentiation

AMOVA for the ISSR shows that genetic variation within populations accounted for 90.6% of the total variation, while the variation among populations was 1.08% and among subpopulations 8.28%. Similarly, with the SRAP, 82.9% was ascribed to within-populations, 4.23% to among-populations and 12.9% to subpopulations (Table 4). Both ISSR and SRAP markers indicated that most variation in population genetics of *M. wufengensis* occurs within populations. The FST and *Nm* assessed by ISSR and SRAP were 0.0936, 2.42 and 0.171, 1.21, respectively.

3.3. Genetic distance and cluster analysis

Nei's standard genetic distances (*D*) between pairs of populations or subpopulations with ISSR markers varied from 0.025 to 0.053 (Table 5).

Table 3
Genetic diversity of populations analyzed by ISSR and SRAP data.

Population	ISSR			SRAP		
	P	*Ae*	*H*	*P*	*Ae*	*H*
LKY	345 (71.9%)	1.375	0.241	297 (71.6%)	1.361	0.241
LZP	374 (77.9%)	1.395	0.248	322 (77.6%)	1.385	0.250
NCK	367 (76.5%)	1.386	0.239	323 (77.8%)	1.405	0.258
HY	350 (72.9%)	1.369	0.227	299 (72.0%)	1.383	0.236
SJY	363 (75.6%)	1.359	0.222	328 (79.0%)	1.418	0.253
DSP	412 (85.8%)	1.411	0.250	355 (85.5%)	1.442	0.263
Sub1	352 (73.3%)	1.357	0.218	308 (74.2%)	1.373	0.236
Sub2	360 (75.0%)	1.377	0.232	303 (73.0%)	1.385	0.244
Sub3	353 (73.5%)	1.388	0.237	314 (75.7%)	1.429	0.266
Sub4	368 (76.7%)	1.389	0.237	298 (71.8%)	1.429	0.238
Sub5	363 (75.6%)	1.393	0.244	303 (73.0%)	1.403	0.248
Mean	359.5 (74.9%)	1.379	0.235	309.5 (74.6%)	1.397	0.247
Total	421 (87.7%)	1.414	0.256	365 (88.0%)	1.458	0.291

P (%): number (percentage) of polymorphic loci; *Ae*: mean effective number of alleles; *H*: mean expected heterozygosity; DSP: Dushuping; HY: Hongyan; LKY: Luokuangyan; LZP: Liziping; NCK: Nanchongkou; SJY: Shuangjianya.

Table 4
Analysis of molecular variance (AMOVA) based on ISSR and SRAP data.

	Source of variation	Degree of freedom	Sum of Squares (10^2)	Variance components	Percentage of variation	Fixation index (F_{ST})	Gene flow (Nm)
ISSR	Among populations	5	10.6	0.672*	1.08	0.0936	2.42
	Among subpopulations within populations	4	7.31	5.15*	8.28		
	Within populations	242	136	56.4*	90.6		
	Total	251	154	62.2			
SRAP	Among populations	5	17.4	2.61*	4.23	0.171	1.21
	Among subpopulations within populations	4	9.85	7.94*	12.9		
	Within populations	242	124	51.2*	82.9		
	Total	251	151	61.8			

* $P < 0.05$.

The smallest distance was between DSP Sub1 and Sub2 and the largest between populations LKY and the subpopulation Sub3 of DSP. For the SRAP analysis, pairwise *D* values were from 0.041 (DSP Sub4 and Sub5) to 0.122 (populations LKY and DSP Sub1). The UPGMA dendrogram (Fig. 2a) based on pairwise genetic distances with the ISSR indicates that the tree is composed of two main branches, one clustered around the five subpopulations of DSP and the other contains the remaining five populations. However, the Nei's standard genetic distance for ISSR and SRAP are measured in different scales and relationship among all populations demonstrated by ISSR markers is much closer than by SRAP markers. The ISSR Fig. 2a shows that Liziping (LZP) and Nanchongkou (NCK) were grouped with SJY, HY, and LKY, but subpopulations of DSP were clustered firstly as Sub1 and Sub2, Sub4 and Sub5, and then with Sub3 together. Meanwhile, the SRAP Fig. 2b shows that the tree is formed by four main branches. The first branch consists of populations LKY and LZP, the second of NCK, SJY and HY, the third branch grouped the three DSP subpopulations Sub1 and Sub2, and the fourth branch consists of Sub3, Sub4 and Sub5. The analytical results of ISSR and SRAP systems showed a close relationship between populations LKY and LZP. Comparing tree a with tree b, the cluster trend of both trees is similar, but the greatest differentiation consists in the fact that Nei's standard genetic distance in tree b shows a closer relationship of all the populations than in tree a.

3.4. Correlation of ISSR and SRAP markers

The association of the genetic diversity parameters *P*, *H* and *Ae* was evaluated by EXCEL; the correlation coefficients of *P*, *H* and *Ae* between ISSR and SRAP markers were 0.9126, 0.6492 and 0.6688, respectively. The correlation coefficient evaluated by the Mantel test of the two genetic matrices from ISSR and SRAP markers was 0.72 ($P < 0.001$), which indicates a poor fit of the two matrices and infers a low correlation of the two markers. As well, the Mantel correlation between genetic and geographical distances was low ($r = 0.29$, $p = 0.10$) for ISSRs and for SRAPs ($r = 0.26$, $p = 0.15$) (Fig. 3).

4. Discussion

4.1. Relationship among different molecular markers

When two or more types of molecular marker systems are used to evaluate genetic diversity of wild or domestic populations with the same accessions, the correspondence between marker systems might be an interesting matter for studies. Various authors provide different answers, ranging from harmony to no correlation in the relationships. The genetic similarities between SSR and AFLP markers of durum wheat were defined by a correlation coefficient $r = 0.81$ [15]. Correlation between ISSR and RAPD similarity matrices of cashew was calculated as $r = 0.63$, but AFLP displayed no linear association with RAPD ($r = 0.01$) and ISSR ($r = 0.11$) of cashew [16]. And in this research, there's a poor fit of the two matrices ($r = 0.72$, $P < 0.001$) and infers a low correlation of the two markers, due to the few sample we could get from the small natural populations. It should also be considered that these marker systems and primers anchor at different sites in the genome. It is expected that a greater number of markers will provide for more precise estimates of genetic relationships, but the distribution of these markers over the genome is equally important. Diversity studies based on a set of markers with poor coverage of the genome can give a different classification of the germplasm [17].

4.2. Genetic diversity and differentiation of M. wufengensis populations

Both early reviews from allozyme data [6,18] and more recent reviews from nuclear DNA markers STMS, RAPD, AFLP and ISSR [19] revealed that plant species with restricted geographic ranges maintain less genetic diversity than more widespread species. The mean of within-population gene diversity (*Hpop*), derived from estimates of

Table 5
Nei's standard genetic distance between pairs of population and subpopulation detected by ISSR and SRAP markers.

population	LKY	LZP	NCK	HY	SJY	Sub1	Sub2	Sub3	Sub4	Sub5
LKY	****	0.046	0.064	0.100	0.106	0.122	0.114	0.091	0.108	0.120
LZP	0.029	****	0.046	0.081	0.074	0.096	0.097	0.072	0.080	0.084
NCK	0.038	0.028	****	0.045	0.051	0.080	0.082	0.069	0.081	0.082
HY	0.043	0.034	0.031	****	0.052	0.098	0.090	0.094	0.106	0.103
SJY	0.040	0.032	0.032	0.033	****	0.089	0.093	0.074	0.092	0.093
Sub1	0.043	0.039	0.036	0.043	0.040	****	0.047	0.055	0.080	0.079
Sub2	0.044	0.037	0.040	0.044	0.047	0.025	****	0.054	0.091	0.099
Sub3	0.053	0.039	0.034	0.051	0.041	0.034	0.037	****	0.048	0.058
Sub4	0.041	0.038	0.037	0.042	0.041	0.036	0.034	0.034	****	0.041
Sub5	0.052	0.041	0.040	0.050	0.046	0.044	0.040	0.040	0.031	****

Nei's genetic distance matrices detected by ISSR (below diagonal) and SRAP (above diagonal). DSP: Dushuping; HY: Hongyan; LKY: Luokuangyan; LZP: Liziping; NCK: Nanchongkou; SJY: Shuangjianya.
**** : showed the diagonal, on which the Nei's genetic distance was zero.

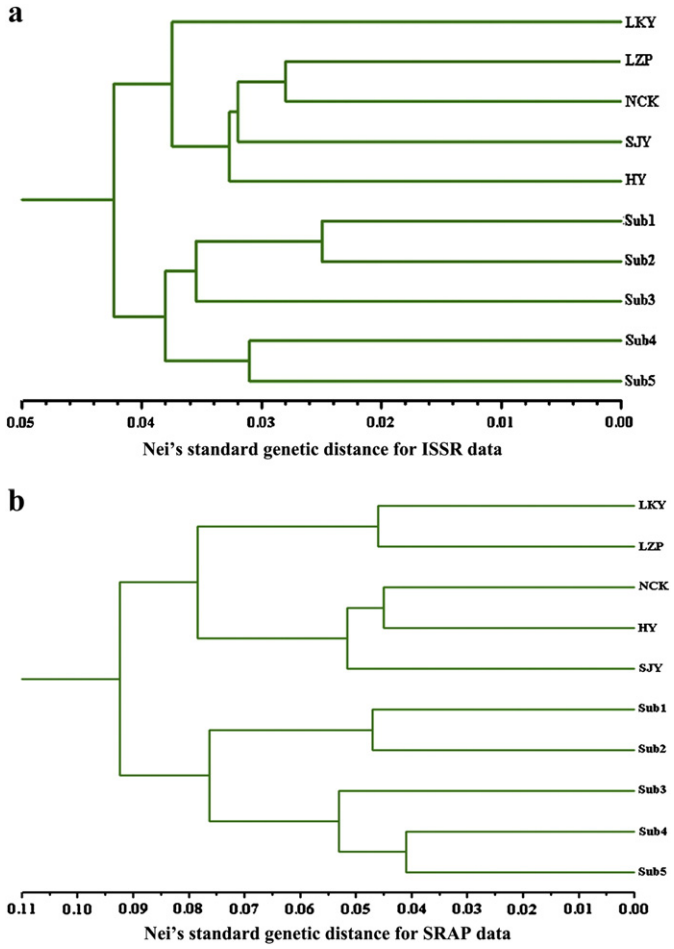

Fig. 2. UPGMA dendrogram of 10 populations and subpopulations based on Nei's standard genetic distance. (a) Tree built using ISSR data. (b) Tree built using SRAP data. Bootstrap values are indicated as 5% of 1000 replicates. DSP: Dushuping; HY: Hongyan; LKY: Luokuangyan; LZP: Liziping; NCK: Nanchongkou; SJY: Shuangjianya.

dominant markers, was 0.20 for endemic species [19]. As an endemic tree, the *H* value of *M. wufengensis* within-population was 0.254 for ISSR and 0.275 for SRAP. These high *H* values would indicate that *M. wufengensis* populations have relatively high levels of genetic variation as an endemic species. Comparisons with gene diversity of *Magnolia officinalis* (*P*: 49.8%; *H*: 0.194; *Ae*: 1.33) [20] and nine other Chinese endemic or nearly endangered plant species at population level (*P*: 12.30%–87.01%; *H*: 0.03–0.32; *Ae*: 1.032–1.401) detected by ISSR markers [21], confirm high genetic diversity in *M. wufengensis* populations. This results also supported by AFLP analyses of this species (*P*: 95.51%; *H*: 0.211 ± 0.028) [22]. Moreover, gene flows of *M. wufengensis* among populations (Nm: ISSR 2.42) are also higher than of some endangered species [20,21].

The reason for *M. wufengensis* to have high genetic diversity is perhaps its evolutionary history and provenances. Studies on widespread, endangered or endemic species have proved that abundant genetic variation and diversity characteristics of a species are not directly correlated to individual numbers, but inherited from their ancestors and parents, so that their seedlings have high genetic diversity at species level [20], which could be especially maintained after polyploidization even at small population scale [23]. Magnolias are thought to be among the earliest flowering plants in evolutionary history [24]. The Wulingshan region, where *M. wufengensis* is found, is the intersection region of East-Asian flora, for not only is it an original and complex terrain for many ancient species, but also a gathering area for plant species as well as a channel (the Wulingshan Corridor) of migration routes for East-Asian plant elements [25]. From a point of view of its various morphologies of flowers and leaves remaining among existing individuals, we infer that polyploids give the foundation of such an abundant genetic diversity derived from its ancestors even though surrounded by inbreeding disadvantages [26].

The percentage of total genetic variation among populations (FST) was 9.36% for ISSR and 17.08% for SRAP, which is low for the mean FST values (25%) found for 52 endemic plants [25]. Like other Magnolias, *M. wufengensis* is a long-living species and the accessions in this study are all adult trees, at least 30 to 50 years old, located in natural woods and proved by local residents. For rare species, it is of greater concern when gene flow estimates are high since they may reflect previous intermingling of populations and should not be interpreted as indicating the present state of population isolation [27]. In our study, the high estimated Nm values between populations, plus high levels of genetic diversity within geographical regions, suggest a likely migrant-pool migratory model [28], which describes a migratory pattern with colonists recruited from a random sample of previously existing populations. This model is usually associated with glaciation

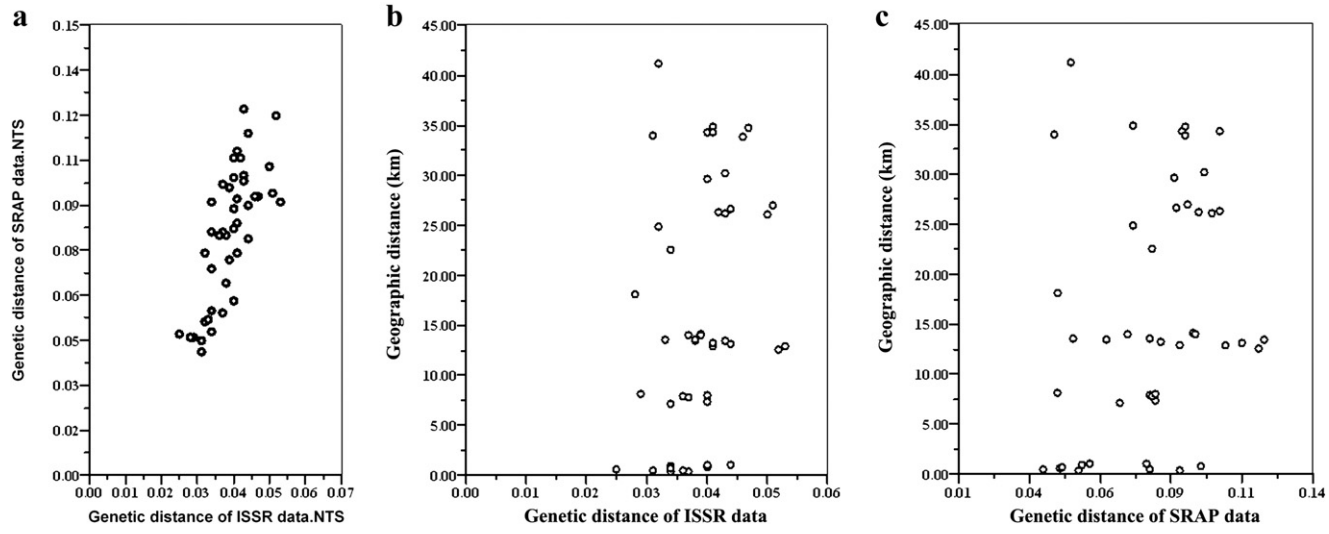

Fig. 3. The consistence Mantel test for ISSR and SRAP data and the correlation of genetic distance with geography distance. (a) Correlation of ISSR and SRAP data. (b) Correlation of population genetic distance for ISSR data and geographic distance. (c) Correlation of population genetic distance for SRAP data and geographic distance.

or vicarious events [29,30,31]. Meanwhile, the High Relief Climate Oscillation (HIRCO) model gives another perspective on source of genetic diversity of magnolias [26].

4.3. Implications for conservation

Maintenance of genetic variation is one of the major objectives for conserving endangered and threatened species [32]. Knowledge of genetic variation between and within populations provides essential information in the formulation of appropriate management strategies directed towards their conservation [33]. From the results obtained in this study it is possible to draw inferences on the conservation of *M. wufengensis*. Given that the genetic structure of *M. wufengensis* has high genetic diversity, high gene flow and low population divergence suggest that genetic drift is not currently of great concern for this species. However, during the past few decades, most habitats have been exploited for mono-culture plantations. This tree has been over-exploited for timber and ornamental use, severely threatening this species, even though the medicine use may probably helped the survival in past time. Many trees are now scattered along roadsides where habitats are accessible. Furthermore, according to the finding of pollen flow and effects of the population structure of *Magnolia stellata*, population fragmentation is associated with population size decreasing and increasing geographic separation of the populations, which reduces its reproductive success rate [34]. The size of present populations makes the species susceptible to the loss of genetic polymorphisms, which could be reduced by the effects of random genetic drift and inbreeding.

Theoretical population genetics predicts that large populations tend to maintain high allelic diversity [35,36]. In addition, high levels of genetic diversity also suggest that the polymorphism of the present isolated and fragmented populations is probably decreased in spite of deriving from ancestors surviving from paleoclimate and the HIRCO with at least one polyploidization [23,26,37]. Action for conserving this rare species for its long-term survival should be taken immediately. And the concentration of high levels of genetic diversity should be taken into consideration for the delimitation of conservation areas containing diverse genetic plant reservoirs. Among the investigated populations, the DSP population is the largest, with about 2000 trees. It should be given the highest priority for *in-situ* conservation in order to expand its range and enlarge its size. Other populations, including their habitats and plants, should be protected properly as well [34].

Furthermore, *ex-situ* conservation should be implemented as soon as possible. For a sampling strategy, the DSP population should be sufficient, considering its genetic diversity which is higher than that of the other populations. Because the molecular investigations can solve the morphological problems, the construction of the core collection of this species [38] will take the selected accessions in this research as a subset that represents most of the total genetic variability.

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Author contribution

Proposed the theoretical frame: LM; Conceived and designed the experiments: FC; Software development: SH; Contribute reagents/materials/analysis tools: LM, FC; Wrote the paper: LC, SH; Performed the experiments: LC; Analyzed the data: LC.

References

[1] Ma LY, Wang LR, He SC, Liu X, Wang XQ. A new species of *Magnolia* (Magnoliaceae) from Hubei, China. Bull Bot Res 2006;26:4–7. http://dx.doi.org/10.3969/j.issn.1673-5102.2006.01.003.

[2] Ma LY, Wang LR, He SC, Liu X, Wang XQ. A new variety of *Magnolia* (Magnoliaceae) from Hubei, China. Bull Bot Res 2006;26:516–9. http://dx.doi.org/10.3969/j.issn.1673-5102.2006.05.002.

[3] Wu WT, Chen FJ, Jing DL, Liu Z, Ma L. Isolation and characterization of an agamous-like gene from *Magnolia wufengensis* (Magnoliaceae). Plant Mol Biol Rep 2012;30:690–8. http://dx.doi.org/10.1007/s11105-011-0385-3.

[4] Jing DL, Liu ZX, Zhang B, Ma J, Han YY, Chen FJ. Two ancestral *APETALA3* homologs from the basal angiosperm *Magnolia wufengensis* (Magnoliaceae) can affect flower development of *Arabidopsis*. Gene 2014;537:100–7. http://dx.doi.org/10.1016/j.gene.2013.11.076.

[5] Barrett SCH, Kohn JR. Genetic and evolutionary consequences of small population size in plants: Implications for conservation. In: Falk DA, Holsinger KE, editors. Genetics and conservation of rare plants. New York: Oxford University Press; 1991. p. 3–35.

[6] Hamrick JL, Godt MJW. Effects of life history traits on genetic diversity in plant species. Philos Trans R Soc Lond B Biol Sci 1996;351:1291–8. http://dx.doi.org/10.1098/rstb.1996.0112.

[7] Porebski S, Bailey LG, Baum BR. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep 1997;15:8–15. http://dx.doi.org/10.1007/BF02772108.

[8] Li G, Quiros CF. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. Theor Appl Genet 2001;103:455–61. http://dx.doi.org/10.1007/s001220100570.

[9] Nei M. Genetic distance between populations. Am Nat 1972;106:283–92. http://dx.doi.org/10.1086/282771.

[10] Yeh FC, Yang RC, Boyle T. Popgene: Microsoft Windows based freeware for population genetic analysis. URL: http://www.ualberta.ca/~fyeh/popgene_download.html; 2007.

[11] Rohlf FJ. NTSYSpc numerical taxonomy and multivariate analysis system version 2.0 user guide. URL: http://www.exetersoftware.com/cat/ntsyspc/ntsyspc.html; 2007.

[12] Excoffier L, Laval G, Schneider S. Arlequin ver 3.1: An integrated software package for population genetics data analysis. URL: http://cmpg.unibe.ch/software/arlequin3; 2006.

[13] Slatkin M, Barton NH. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 1989;43:1349–68. http://dx.doi.org/10.2307/2409452.

[14] Mantel N. The detection of disease clustering and a generalized regression approach. Cancer Res 1967;27:209–20.

[15] Maccaferri M, Stefanelli S, Rotondo F, Tuberosa R, Sanguineti MC. Relationships among durum wheat accessions. I. Comparative analysis of SSR, AFLP, and phenotypic data. Genome 2007;50:373–84. http://dx.doi.org/10.1139/g06-151.

[16] Archak S, Gaikwad AB, Gautam D, Rao EVVB, Swamy KRM, Karihaloo JL. Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. Genome 2003; 46:362–9. http://dx.doi.org/10.1139/G03-016.

[17] Menz MA, Klein RR, Unruh NC, Rooney WL, Klein PE, Mullet JE. Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. Crop Sci 2004;44:1236–44. http://dx.doi.org/10.2135/cropsci2004.1236.

[18] Hamrick JL, Godt MJW. Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, editors. Plant population genetics, breeding and germplasm resources. Sunderland: Sinauer; 1990. p. 43–63.

[19] Nybom H. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 2004;13:1143–55. http://dx.doi.org/10.1111/j.1365-294X.2004.02141.x.

[20] Yu HH, Yan ZL, Sun B, Liu RN. Genetic diversity and relationship of endangered plant *Magnolia officinalis* (Magnoliaceae) assessed with ISSR polymorphisms. Biochem Syst Ecol 2011;39:71–8. http://dx.doi.org/10.1016/j.bse.2010.12.003.

[21] Chen JQ, Ci XQ, Li QM, Li J. Genetic diversity of *Litsea szemaois*, an endangered species endemic to China, detected by inter-simple sequence repeat (ISSR). Biodivers Sci 2006;14:410–20.

[22] He SC, Ma LY, Chen FJ. Genetic diversity of *Magnolia wufengensis* based on AFLP. Acta Bot Boreali-Occident Sin 2007;27:2421–8. http://dx.doi.org/10.3321/j.issn:1000-4025.2007.12.011.

[23] Soltis DE, Soltis PS. Polyploidy: Recurrent formation and genome evolution. Trends Ecol Evol 1999;14:348–52. http://dx.doi.org/10.1016/S0169-5347(99)01638-9.
[24] Cronquist A. An integrated system of classification of flowering plant. New York: Columbia University Press; 1981.
[25] Chen GX, Liao WB, Ao CQ, Liu WQ, Zhang HD. Studies on character and feature of seed plants flora of Wulingshan region. Bull Bot Res 2002;22:98–120. http://dx.doi.org/10.3969/j.issn.1673-5102.2002.01.024.
[26] Hebda RJ, Irving E. On the origin and distribution of magnolias: Tectonics, DNA and climate change. In: Channell JET, Kent DV, Lowrie W, Meert JG, editors. Timescales of the Paleomagnetic Field. Washington, D.C.: American Geophysical Union; 2004. http://dx.doi.org/10.1029/145GM04.
[27] Godt MJW, Caplow F, Hamrick JL. Allozyme diversity in the federally threatened golden paintbrush, *Castilleya levisecta* (Scrophulariaceae). Conserv Genet 2005;6: 87–99. http://dx.doi.org/10.1007/s10592-004-7746-5.
[28] Wade MJ, Mccauley DE. Extinction and recolonization: Their effects on the genetic differentiation of local populations. Evolution 1988;42:995–1005. http://dx.doi.org/10.2307/2408915.
[29] Hewitt GM. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linn Soc 1996;58:247–76. http://dx.doi.org/10.1111/j.1095-8312.1996.tb01434.x.
[30] Huang S, Chiang YC, Schaal BA, Chou CH, Chiang TY. Organelle DNA phylogeography of *Cycas taitungensis*, a relict species in Taiwan. Mol Ecol 2001;10:2669–81. http://dx.doi.org/10.1046/j.0962-1083.2001.01395.x.
[31] Chiang TY, Schaal BA. Phylogeography of plants in Taiwan and the Ryukyu Archipielago. Taxonomy 2006;51:31–41. http://dx.doi.org/10.2307/25065526.
[32] Avise JC, Hamrick JL. Conservation genetics: Case histories from nature. New York: Chapman and Hall; 1996 1–15.
[33] Milligan BG, Leebens-Mack J, Strand AE. Conservation genetics: Beyond the maintenance of marker diversity. Mol Ecol 1994;3:423–35. http://dx.doi.org/10.1111/j.1365-294X.1994.tb00082.x.
[34] Setsuko S, Nagamitsu T, Tomaru N. Pollen flow and effects of population structure on selfing rates and female and male reproductive success in fragmented *Magnolia stellata* populations. BMC Ecol 2013;13:10–22. http://dx.doi.org/10.1186/1472-6785-13-10.
[35] Hedrick PW. Genetics of populations. Boston: Jones and Bartlett; 2011 187–246.
[36] Ellstrand NC, Elam DR. Population genetic consequences of small population size: Implications for plant conservation. Annu Rev Ecol Syst 1993;24:217–42. http://dx.doi.org/10.1146/annurev.es.24.110193.001245.
[37] Kang YX, Ejder E. *Magnolia sprengeri* Pamp.: Morphological variation and geographical distribution. Plant Biosyst 2011;145:906–23. http://dx.doi.org/10.1080/11263504.2011.633352.
[38] Bai CK, Wen MM, Zhang LJ, Li GH. Genetic diversity and sampling strategy of *Scutellaria baicalensis* germplasm resource based on ISSR. Genet Resour Crop Evol 2013;60:1673–85. http://dx.doi.org/10.1007/s10722-012-9949-9.