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·**Short Communication**·

A Preliminary Study on Conservation Genetics of Endangered *Vatica guangxiensis* (Dipterocarpaceae)

LI Qiao-Ming[1,2], XU Zai-Fu[1], HE Tian-Hua[3]*

(1. *Xishuangbanna Tropical Botanical Garden, The Chinese Academy of Sciences*, Mengla 666303, China; 2. *Kunming Institute of Botany, The Chinese Academy of Sciences*, Kunming 650204, China; 3. *Laboratory of Systematic and Evolutionary Botany, Institute of Botany, The Chinese Academy of Sciences*, Beijing 100093, China)

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Vatica guangxiensis is a characteristic tree species of tropical rain forest in Southern Yunnan, it is also an important timber tree species[1]. This species has limited number of individuals, only with three natural populations distributed in Nanshahe and Maochaoshan, Mengla County of Southern Yunnan, and Linshaoshan, Napo County of Guangxi, and it was listed as an endangered plant species in China[2]. From 1980s, Xishuangbanna Tropical Botanical Garden, the Chinese Academy of Sciences (XTBG) began to carry out conservation program and research on this species. Since the evolutionary potential of species and populations is determined to a great extent by the level of genetic diversity and the pattern of genetic variation[3], the well understanding of genetic diversity and population genetic structure is a prerequisite for conservation and management of rare and endangered species. Allozyme analysis has ever been applied to detect the genetic diversity of *V. guangxiensis*, but the limited loci could only detect low genetic diversity, which could provide limited information for conservation and management of this species[4]. Therefore, in this study, with an intent to obtain more detailed information of population genetic structure of this endangered plant, the more sensitive random amplified polymorphic DNA (RAPDs) assay[5,6] were applied to analyze the genetic diversity and population genetic structure.

1 Materials and Methods

Samples of *Vatica guangxiensis* X. L. Mo (Dipterocarpaceae) were collected from the three remaining natural populations (NS, NP, NX) and one cultivated population (ML) conserved in the Xishuangbanna Tropical Botanical Garden. The population locations and sample sizes are presented in Table 1. The fresh leaves were dried quickly by using silica gels in field, and stored at room temperature for further use.

Total DNA was isolated according to the protocol of 2 × CTAB[7], and was dissolved in 0.1 × TE (1 mmol/L Tris-HCl (pH 8.0), 0.1 mmol/L EDTA (pH 8.0)) and subjected to PCR amplification after adjusting concentration. Twenty arbitrarily primers that could amplify reproducible and clear DNA bands were selected from 138 primers (Shengong Inc.) for further amplification.

DNA amplification was performed in a Rapidcycler 1818 (Idaho Tech.), programmed for an initial 1 min at 94 ℃, 10 s at 35 ℃, 20 s at 72 ℃ for 2 cycles, followed by 40 cycles of 0 s at 94 ℃, 0 s at 35 ℃, and 1 min at 72 ℃, and ended with 7 min at 72 ℃[8]. Reactions were carried out in a volume of 10 μL containing 50 mmol/L Tris-HCl (pH 8.3), 500 μg/mL BSA, 10% Ficoll, 1 mmol/L Tartrazine, 2 mmol/L $MgCl_2$, 200 μmol/L dNTP, 1 μmol/L primer, 5 ng of DNA template and 0.5 U Taq polymerase. Amplification products were analyzed with electrophoresis (TBE electrophoresis) on 1.5% agrose gel stained with ethidum bromide, and imaged on the Bio-Rad imaging devices (Gel Doc 2000 Gel Documentation System) supported by Quantity One (version 4.2). Molecular weights were estimated using 100 – 3 000 bp DNA Ladder.

The bands were marked by Quantity One software, and the bands were watched at the same position (molecular weights). Amplified fragments were scored for the presence (1) absence (0) of homologous bands and the matrix of the RAPD phenotypes was assembled for the following analysis: Genetic diversity as measured by the percentage of polymorphic bands (*PPB*), Shannon diversity index (*I*), population gene diversity (*Ht*), subpopulation gene diversity (*Hs*), and subpopulation differentiation (*Gst*) by using POPGENE[9]. RAPDistance program[10] was used to calculate Jaccard similarity coefficients for further analysis of AMOVA[11]. With AMOVA we calculated variance components which were partitioned among individuals within populations, among populations within regions (Yunnan and Guangxi), and between regions.

2 Results

Twenty primers were used for amplification in 95 individuals in 4 populations of *V. guangxiensis*. A total of 231 bands ranged from 170 – 1 995 bp were scored, corresponding to 6 – 18 bands per primer. The bands amplified by primer S317, S226, S246, S501 are shown in

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* Author for correspondence.

Table 1 Population location and sample size

Population Code	Sample sites	Estimated population size	Sample size
1. NS	Nanshahe, Mengla county, Yunnan 21°30′ N, 101°35′ E, 800 – 1 100 m Alt	100	27
2. NP	Liushaoshan, Napo county, Guangxi 23°07′ N, 105°42′ E, 500 – 600 m Alt.	40	30
3. NX	Maocaoshan, Mengla county, Yunnan 21°37′ N, 101°50′ E, 750 – 1 000 m Alt.	50	10
4. ML	XTBG, Menglun, Mengla county, Yunnan 21°54′ N, 101°18′ E, 600 m Alt.	90	28

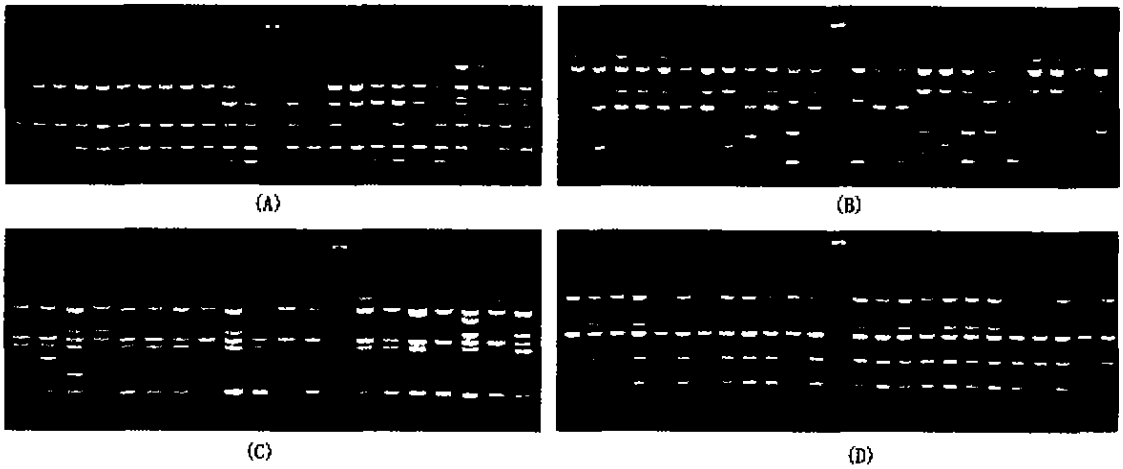

Fig. 1. Amplification products of *Vatica guangxiensis* using primer S317 (A), primer S226 (B), primer S246 (C) and primer S501 (D).

Fig. 1. Of the 231 bands, 124 were polymorphic bands (*PPB*), accounted for 53.68% (Table 2). According to the *PPB*, the amount of genetic variation within these 4 populations was NS > NP > ML > NX (from high to low), and the Shannon diversity index gave another result: NS > ML > NP > NX, which differed slightly from the results of *PPB*. By using POPGENE, the observed number of alleles (*na*), effective number of alleles (*ne*), and Nei's gene diversity index (*h*) were calculated (Table 3). The genetic diversity of population NS was the highest, which was in accordance with the results measured by Shannon diversity index.

V. guangxiensis was grouped into 2 regions, Yunnan (NS、ML、NX) and Guangxi (NP). The analysis by AMOVA implied that the genetic variation within populations (55.09%) was higher than that among populations (44.91%); and the variation among regions (51.18%) was higher than that within regions (48.82%). On the whole, the variation within populations (45.13%) was higher than that among regions (45.08%), and the variation (9.79%) among populations of region was low. The *Gst* of this species was 0.374 6, population *Ht* was 0.164 6, and subpopulation gene *Hs* was 0.103 0. Table 4 shows the genetic distance and genetic identity among populations. The population NS and NX, both located in Mengla County of Yunnan, are close in spatial distance (about 30 km). Accordingly, the genetic identity between these two populations was high (0.962 0), and the genetic distance was 0.038 8. On the contrary, compared with population NP located at Napo County of Guangxi, the spatial distance between NS and NP is about 500 km, the genetic distance was 0.148 0 between population NS and NP, and 0.165 3 between NX and NP, suggesting that geographic isolation has profound effects on population genetic differentiation.

Table 2 RAPD polymorphism of populations of *Vatica guangxiensis*

Populations	N_b	N_{pb}	*PPB* (%)	N_{pb}/p	*I*
NS	213	89	38.53	4.45	0.178 8
NP	201	73	31 60	3.65	0.149 2
NX	199	63	27.27	3 15	0.142 8
ML	204	71	30.74	3.55	0.150 1
Total	231	124	53.68	6.20	0.254 3

N_b, the sum of all bands; N_{pb}, the sum of polymorphic bands; *PPB*, the ratio of polymorphic bands; N_{pb}/p, the ratio of polymorphism per primer; *I*, Shannon diversity index.

Table 3 Statistical analysis of genetic variation for all loci of *Vatica guangxiensis*

Populations	*na*	*ne*	*h*
NS	1.385 3	1.201 8	0.118 0
NP	1 316 0	1.165 8	0 098 3
NX	1.272 7	1 163 7	0.095 5
ML	1.307 4	1.171 8	0 100 1
Total	1.536 8	1.287 8	0.168 6

na, observed number of alleles; *ne*, effective number of alleles; *h*, Nei's gene diversity.

Table 4 Nei's unbiased measures of genetic identity and genetic distance (POPGENE)

Populations	NS	NP	NX	ML
NS	****	0 862 4	0.962 0	0.974 4
NP	0.148 0	****	0.847 6	0.857 8
NX	0.038 8	0 165 3	****	0.963 1
ML	0.026 0	0.153 4	0.037 6	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

3 Discussion

RAPD analysis revealed low level of genetic diversity and high level of population differentiation in the endangered *V. guangxiensis*, which was consistent with the results from allozyme analysis[4], however RAPD detected more detailed data on the amount and distribution of genetic diversity and population genetic structure than the latter. The population genetic structure of a species was the result of long-term evolution, and has close link to its evolutionary history, geographic distribution, life history, etc. Xu and Yu[12] and Zhu[13] suggested that the dipterocarps originated from the old Gangwana Continent in early Tertiary, and the Guyita Continent was the diversity center of this family. The current *V. guangxiensis* is only distributed in Mengla County, South of Yunnan, and Napo County, Southwest of Guangxi, which are the northern margins of the range of dipterocarp, therefore, its current distribution pattern could be the results of the glacial migration. In the Quaternary, as the glacials appeared on the globe, some thermophilous plants had lived through an unfavourable period in "refuge", and some others migrated to the south. When the temperature elevated in the interglacial, the plants migrated to the North and the regions of high elevation gradually. After many repeats, there remained some groups, or some new groups derived from these remains. As a result, it is reasonable that the low leveled genetic diversity of *V. guangxiensis* could be attributed to the effect of the population bottleneck resulting from the advance and retreat of glacial in its evolutionary history. On the other hand, geographic isolation of small populations and genetic drift enlarged the genetic differentiation among populations. Moreover, the gravity dispersal of seeds of *V. guangxiensis* also partly contributed to the high subpopulation differentiation. Considering its big fruits and tropical static wind climate, the dispersal distance was so limited that the high genetic relatives of neighbor plants and result, and selfing frequently occurred in population, which contributed to the high genetic differentiation among populations.

The information of population genetic diversity is of critical importance for conservation and management of rare and endangered plants, including assessment of the conservation value and state of special populations, *ex situ* collection and conservation[14-16]. Because of the low level of genetic diversity and high population differentiation in *V. guangxiensis*, promoting gene flow (via seed and seedling) among populations would be practical efforts of conservation. Furthermore, the results also have special implication for the *ex situ* conservation of this endangered plant. The cultivated population (ML) did not contain the highest genetic diversity, indicating that this population could not represent the whole genetic variation of this species. Thus, more extensive *ex situ* collections are needed to conserve the whole genetic variation of this species. Considering the high genetic variation within populations and among populations of *V. guangxiensis*, *ex situ* collection should sample enough individuals, and take all populations into account. The results showed that the genetic diversity of cultivated population ML was lower than that of population NS and NP (*PPB*), consequently, more emphasis should be given to these two populations in *ex situ* collection afterwards. The results also showed that cultivated population ML had high genetic similarity to population NS, but had high differentiation to population NP, therefore, in order to conserve the more genetic diversity of *V. guangxiensis*, we need to give more attentions to population NP in whenever *in situ* conservation of original habitat and collecting samples supplementally for *ex situ* conservation.

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濒危植物版纳青梅保护遗传学研究初报

李巧明[1,2] 许再富[1] 何田华[3*]

(1 中国科学院西双版纳热带植物园, 勐腊 666303; 2. 中国科学院昆明植物研究所, 昆明 650204;
3. 中国科学院北京植物研究所系统与进化植物学开放研究实验室, 北京 100093)

摘要: 运用 20 个 10 碱基随机引物,对中国龙脑香科(Dipterocarpaceae)特有的珍稀濒危植物版纳青梅(*Vatica guangxiensis* X. L. Mo)进行了 RAPD 多态性分析。3 个自然居群和 1 个迁地保护居群(分布于云南和广西)共扩增出 231 个位点,多态位点所占比例(*PPB*)为 53.68%;观察等位基因数 *na* = 1.536 8,有效等位基因数 *ne* = 1.287 8,Nei 基因多样性指数 *h* 为 0.168 6,居群内的遗传多样性水平较低。基于 AMOVA 和 POPGENE 的结果均表明居群内的遗传变异大于居群间的遗传变异。居群内的遗传变异为 55.09%,居群间的变异为 44.91% (AMOVA);基因分化系数 *Gst* 为 0.374 6 (POPGENE),表明居群间存在高水平的遗传分化。研究结果对该濒危植物的保护有重要意义。考虑到低水平的遗传多样性和高水平的居群分化,通过居群间种子和幼苗的交换来促进基因流是可行的保护方案。迁地保护居群(ML)不具最高的遗传多样性,表明为了保护此濒危物种的全部遗传变异,需要进一步采集更多个体补充到迁地保护居群中。

关键词: 版纳青梅; RAPD; 遗传多样性; 保护生物学

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* 通讯作者。

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