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Ex situ genetic conservation of endangered *Vatica guangxiensis* (Dipterocarpaceae) in China

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Abstract

RAPD polymorphisms were applied to check the efficiency of ex situ genetic conservation of endangered *Vatica guangxiensis* X. L. Mo. endemic to southwestern China. Low level of genetic variation was revealed in three remaining natural populations. Twenty random primers, each with 10 base pairs, generated 231 bands with 53.68% being polymorphic, and with an average of 32.46% being polymorphic in each natural population. Strong population differentiation was revealed by AMOVA (analysis of molecular variance) and *Gst* value was 0.3764. The population ML ex situ conserved in the Xishuangbanna Tropical Botanical Garden contained an intermediate genetic variation compared with natural populations, with 30.74% bands being polymorphic. Of the total 231 bands generated in *V. guangxiensis*, 204 bands were also detected in population ML, indicating that 88.31% of the total genetic variations of this species were conserved in ex situ population. If only the alleles with moderate to high frequency ($P > 0.05$) were considered, 204 out of 209 bands (97.61%) occurred in ex situ population ML. RAPD analysis also detected one exclusive band in natural population NS, and five in natural population NP, three of these exclusive bands were generated in every samples of natural population (NP), and other three had moderate to high frequencies. While none of these exclusive bands were detected in ex situ conserved population ML. Our conclusions are that the ex situ conserved population ML contains representative genetic variation to maintain long-term survival and evolutionary process of *V. guangxiensis*, and that more extensive ex situ sampling in natural population NS and NP is needed to conserve more exclusive alleles in ex situ population. The tropical area in the Botanical Garden would play a more important role in the ex situ conservation of rare and endangered plants.

Keywords: Ex situ conservation; Genetic diversity; *Vatica guangxiensis*; Botanical garden

1. Introduction

The conservation of plant diversity is of critical importance, because of the direct benefits to humans that arise from its exploitation in new agricultural and horticultural crops. The development of medical drugs and the pivotal role played by plants are the functions of all natural ecosystems (Maxted et al., 1997). Conservation of plant diversity can be achieved in a number of complementary ways: conservation of whole plant in their native ecosystems, or conservation of samples of a plant's genetic diversity. In recent years attempts have been made to differentiate between ecological and genetic conservation, respectively. While much interest has been focused on developing models for ecosystem and habitat conservation (Forey et al., 1994) and for defining various aspects of genetic conservation (Marshall and Brown, 1975; Yonezawa, 1985; Guarino et al., 1995), less progress has been made in checking the efficiency of genetic conservation that include both in situ and ex situ strategies.

Mass extinction that is primarily due to large-scale habitat destruction caused by human activities. The best remedy to prevent extinction is habitat preservation. But unfortunately, habitat preservation is not an option in many cases. The natural habitat of many species have

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already been completely destroyed, and those of many others have been so reduced in size and so fragmented that the species are in imminent danger of extinction. Even when habitat preservation can be practical, it frequently requires the reintroduction of propagules. In such cases, ex situ conservation is needed to preserve a species that has gone to extinction in nature (Maxted et al., 1997). Ex situ conservation plays the most important role in the conservation of plants especially through botanical gardens.

In order to preserve a species' genetic diversity in captivity, it is obviously necessary to carry over much of that diversity from the natural population into the initial captive population. The existing levels of genetic diversity and the maintenance of these levels of diversity are major issues in conservation biology. Genetic diversity became an issue when Frankel (1974) postulated that genetic variation is essential for the long-term survival of endangered species, and genetic diversity is a critical feature because it contributes directly to the likelihood of persistence and ecological success. If the natural populations become extinct, ex situ conserved population is to maintain the evolutionary process of the endangered species, and will to be released back to nature until habitat restoration. Consequently, the amount of genetic variation holding in the captive population is critical to assure the success of ex situ conservation and subsequent releasing. However, information about the genetic structure of rare plant populations is based almost entirely on data from electrophoretic surveys of soluble enzymes, but these surveys are not expected to reflect the pattern of genetic diversity of the whole genomic DNA. RAPD markers are based on the amplification of unknown DNA sequences using single, short, random oligonucleotide primers, therefore, RAPD polymorphisms are the reflection of variation of the whole genomic DNA, and would be a better parameter to measure the pattern of genetic diversity of the rare and endangered plants.

Vatica guangxiensis X. L. Mo is an endangered dipterocarp endemic to southwestern China, with only three remaining natural populations distributed in Nanshahe, Maocaoshan, Mengla County of Yunnan Province and Liushaoshan, Napo County of Guangxi Autonomous Region (Fig. 1). Each population has a limited number of individuals with no more than 100 adults and a few juveniles (Tao and Zhang, 1983). According to its current situation, this species was listed as an endangered plant in the Chinese Plant Red Book (Fu, 1992). From the 1980s, Xishuangbanna Tropical Botanical Garden (XTBG), the Chinese Academy of Sciences began to carry out conservation of this species. On one hand, the distribution ranges of *V. guangxiensis* were incorporated into the National Natural Reserve to conserve its native ecosystem. On the other hand, studies on its biological characteristics of seed and seedling

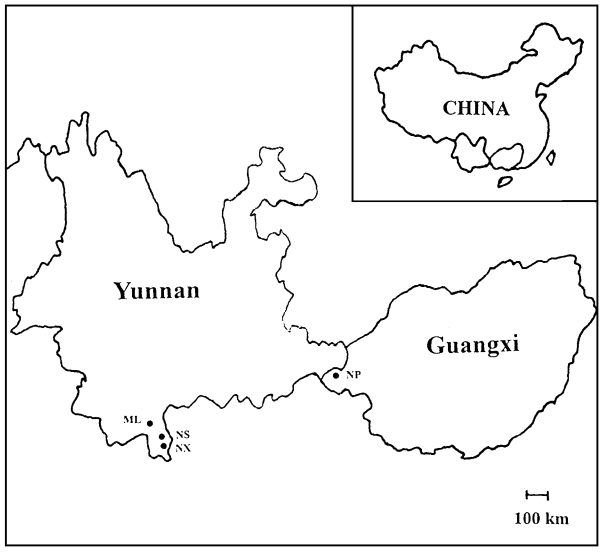

Fig. 1. Population locations of *Vatica guangxiensis* (NS: 21°30′ N, 101°35′ E, 800–1100 m Alt.; NP: 23°07′ N, 105°42′ E, 500–600 m Alt.; NX: 21°37′ N, 101°50′ E, 750–1000 m Alt.; ML: 21°54′ N, 101°18′ E, 600 m Alt.).

were conducted, and more than 200 seeds and 50 seedlings were transplanted into the ex situ conservation area in XTBG. Main climate conditions of native habitat and those of ex situ conservation site are very similar (Table 1). In fact, the Botanical Garden is only decades of kilometers away from the main range of distribution of *V. guangxiensis*. Consequently, the habitat conditions in the Botanical Garden are suitable for ex situ conservation of this endangered plant. Up to 2000, about 90 individuals survived in ex situ conservation site (Ma et al., 1996).

In this work, we detected the genetic diversity of three remaining natural populations (Population NS, NP, NX) and one captive population (ML) of endangered *V. guangxiensis* by using RAPD polymorphism, with an intent to check whether captive population conserved representative genetic variation of natural populations, and to shed light on the sampling strategy for ex situ conservation of *V. guangxiensis*.

2. Materials and methods

2.1. Plant material and DNA extraction

Ninety-five individuals of *V. guangxiensis* were collected from three remaining natural populations (Population NS, NP and NX) and one captive population (ML) ex situ conserved in XTBG (Table 2). Population locations were simply showed in Fig. 1. The fresh leaves were dried quickly by using silica gels. Total DNA was isolated according to the protocol of Doyle and Doyle (1990). Total DNA was solvated in 0.1×TE for further use.

Table 1
Comparison of climate and soil condition between native habitat and ex situ conservation site of *Vatica guangxiensis*

Location	Elevation (m)	T_{mean} (°C)	T_{min} (°C)	T_{max} (°C)	P (mm)	H_r (%)	D_f	Soil
Mengla	632	20.9	5.4	38.1	1532	85%	153	Lateritic red soil
Garden	580	21.5	5.0	40.0	1500	84%	130	Laterite

T_{mean}, annual main temperature; T_{min}, extreme low temperature; T_{max}, extreme high temperature; P, annual precipitation; H_r, relative humidity; D_f, fogging day.

Table 2
Sampling sites and sampling size in each of population of *Vatica guangxiensis*

Population	Sampling site	Estimated population size	Sampling size
NS	Nanshahe, Mengla county, Yunnan province 21°30′ N, 101°35′ E, 800–1100 m Alt.	100	27
NP	Liushaoshan, Napo county, Guangxi Autonomous Region 23°07′ N, 105°42′ E, 500–600 m Alt.	40	30
NX	Maocaoshan, Mengla county, Yunnan province 21°37′ N, 101°50′ E, 750–1000 m Alt.	50	10
ML	XTBG, Mengla county, Yunnan province 21°54′ N, 101°38′ E, 600 m Alt.	90	28

XTBG, Xishuangbanna Tropical Botanical Garden.

2.2. RAPD PCR amplification

Twenty arbitrary primers (for primers and their sequences, please contact Qiaoming Li) that can obtain reproducible and clear amplification products were selected from 138 primers (Shengong Inc.) and then employed in the amplification. DNA amplification was performed in a Rapidcycler 1818 (Idaho Tech.), programmed for an initial 1 min at 94 °C, 10 s at 35 °C, 20 s at 72 °C for 2 cycles, followed by 40 cycles of 0 s at 94 °C, 0 s at 35 °C, and 1 min at 72 °C, and ended with 7 min at 72 °C. 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. Negative controls were also employed in the experiment. Amplification products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide, and imaged by the Bio-Rad imaging devices (Gel Doc 2000 Gel Documentation System) supported by Quantity One (version 4.2). Molecular weights were estimated using 100–3000 bp DNA Ladder.

2.3. Data analysis

RAPD is a kind of dominant marker. Therefore, amplified fragments were scored for the presence (1) and absence (0) of homologous bands, and the matrix of the RAPD phenotypes was assembled for the following analysis: genetic diversity was measured by the percentage of polymorphic bands (PPB), Shannon index of diversity (I) and coefficient of gene differentiation (Gst) with POPGENE computer program (Yeh and Yang, 1999). RAPDistance computer program (Armstrong et al., 1994) was used to calculate Jaccard similarity coefficients for the further analysis of genetic variation component that was partitioned among individuals within populations and among populations within regions by using analysis of molecule variance (AMOVA; Excoffier, 1993). Subsequently, the amount of genetic diversity were compared between natural populations (NS, NP, NX) and cultivated population (ML) to assess the efficiency of ex situ conservation.

3. Results

Table 3 summarized the genetic diversity of the four populations of *V. guangxiensis* revealed by RAPD polymorphism. Using 20 primers, each with 10 base pairs, a total of 231 bands ranging from 170 to 1995 bp were generated, corresponding to an 11.5 bands per primer. Of them, 53.68% (124 in total) were polymorphic among four populations with 95 individuals. Among three natural populations, population NS contained the highest genetic diversity, though only 38.53% bands were polymorphic, and the Shannon diversity index was 0.1788. The ex situ population ML have intermediate genetic variation compared with three natural populations, with 30.74% bands being polymorphic. The percentage of polymorphic bands in population ML was lower than that in population NS, but higher than those in population NP and NX. AMOVA revealed that 55.09% of genetic variation were distributed within populations, and as high as

Table 3
Genetic diversity in four populations of *Vatica guangxiensis* revealed by RAPDs polymorphisms

Population	*N*	*Np*	PPB (%)	*I*
NS	213	89	38.53	0.1788
NP	201	73	31.60	0.1492
NX	199	63	27.27	0.1428
ML	204	71	30.74	0.1501
Total	231	124	53.68	0.2543

N, number of amplified bands; *Np*, number of polymorphic bands; PPB, percentage of polymorphic bands; *I*, Shannon diversity index.

44.91% existed among populations, indicating high genetic differentiation among populations of *V. guangxiensis*, which was also confirmed by high *Gst* value (0.3746).

Of the total 231 bands, 204 bands were amplified in the ex situ population ML, which suggested that 88.31% of the total genetic variation were conserved in population ML. Among the total of 231 bands, 209 had frequencies greater than 0.05, and 204 were detected in population ML, indicating that 97.61% of the alleles with moderate to high frequency ($P > 0.05$) were conserved in ex situ population ML. In the population NS, 213 bands were generated, with 204 had frequencies greater than 0.05, and 200 occurred in the population ML, implied that 93.90% of the total population genetic variation and 98.04% of alleles with moderate to high frequency ($P > 0.05$) were conserved in ex situ population ML. In the other two natural populations, both percentages were greater than 90% (Table 4). As to low-frequency alleles ($P < 0.05$), population NS and NP each had nine bands, three and five bands occurred in population ML, respectively, indicating that about 44.44% of the rare loci were also conserved in population ML (Table 4).

RAPD analysis revealed exclusive bands in population NS and NP (Table 5). Primer S323 generated a unique band in population NS, with a frequency of 0.3928. In population NP, primer S273, S352, S382 generated an exclusive band with frequency of 1.000, respectively; Primer S501 generated two unique bands in this population, with frequency of 0.5000 and 0.8667, respectively. All these exclusive bands were not detected in the ex situ population ML.

4. Discussion

RAPDs polymorphisms revealed a low level of genetic diversity in endangered *V. guangxiensis*, only with an average of 32.46% RAPD bands being polymorphic in three natural populations. Strong population differentiation was demonstrated in this species and the *Gst* value being 0.3746. Because we lack the knowledge of evolutionary history, reproductive characteristics and the mating system of *V. guangxiensis*, it is difficult to explain the low level of genetic diversity and strong population differentiation, which apparently needs further study. The ex situ conserved population ML holds an intermediate level of genetic diversity compared with three natural populations and conserved 88.31% of the total genetic variation of the species. If only considering the alleles with moderate to high frequency ($P > 0.05$), population ML holds 97.61% of the total genetic variations of the species.

It is hoped that captive populations can ultimately be released into preserved or restored habitat. Hence, the goal of such conservation programmes is to maintain the species in captivity until habitat restoration allows its release back to nature. Nevertheless, such restoration could take decades or even centuries. Therefore, captive populations must be managed as a long-term, multigenerational breeding programme. There has been general recognition in recent years that the genetic variation present in a species is a valuable biological resource. Moreover, the restored environments will undoubtedly differ from the original habitats and communities. It is therefore critical that the released populations have sufficient genetic variability to provide adaptive flexibility in an uncertain future (Templeton, 1982, 1991). Ideally, populations of the target taxon that contain the maximum amount of genetic diversity in the minimum number of populations will be identified. Commonly, there will be too much diversity in plant species to conserve all their alleles. It is important to conserve the range of diversity that best reflects the total genetic diversity of the species.

Assuming that a population's short-term viability has been assured, its long-term viability will probably depend, in part, on the amount of genetic variability it retains. But the absolute level of genetic variability is not worrisome for conservation biology. Some suggested that all genetic variation within a species should be captured (Hawkes, 1976, 1987), but as Brown and Briggs (1991) pointed out, this is unrealistic, and it is also unnecessary. First, many low-frequency alleles are unconditionally deleterious and are maintained only as a result of recurrent mutation, and many low-frequency alleles might actually contribute to genotypes that lower the average viability of individuals. Second, most adaptively significant variation is contained in alleles found in a moderate to high-frequency. Third, low-frequency alleles are likely to be lost in just a few generations. In short, maintenance of long-term population viability requires attempts to preserve a representative sample of moderate to high-frequency alleles, whether the population is managed in its natural habitat or samples are collected for off-site preservation (Templeton, 1991). Moreover, the sampling for ex situ conservation of endangered species is aimed primarily at the preservation

Table 4
Genetic diversity conserved in ex situ population (ML)

Population	N	N ($P>0.05$)	N ($P<0.05$)	Nc	Nc ($P<0.05$)	Gc (%)	Gc (%; $P>0.05$)	Gc (%; $P<0.05$)
NS	213	204	9	200	3	93.90	98.04	33.33
NP	201	192	9	181	5	90.05	94.27	55.55
NX	199	199	0	190	–	95.48	95.48	–
ML	204	–	–	–	–	–	–	–
Mean	204	198	–	190	–	93.14	95.96	44.44
Total	231	209	18	204	8	88.31	97.61	44.44

N, number of amplified bands; N ($P>0.05$), number of bands amplified with a frequency greater than 0.05; N ($P<0.05$), number of bands amplified with a frequency lower than 0.05; Nc, number of the same bands of natural population amplified in population ML; Nc, ($P<0.05$), number of low-frequency bands of natural population amplified in population ML; Gc, percentage of genetic variation conserved in population ML (for all bands); Gc ($P>0.05$), percentage of genetic variation conserved in population ML (for the bands with a frequency greater than 0.05); Gc (%; $P<0.05$), percentage of genetic variation conserved in population ML (for the bands with a frequency lower than 0.05).

Table 5
The probability of exclusive bands of three natural populations occurring in the ex situ population (ML)

Population	Exclusive band (frequency)	Frequency occurring in ML
NS	S323-01 (0.3928)	0
NP	S273-06 (1.000)	0
	S352-01 (1.000)	0
	S382-18 (1.000)	0
	S501-04 (0.5000)	0
	S501-06 (0.8667)	0
NX	No exclusive band	–

of the species, principally from threats due to humans. Rare alleles may have some place in plant breeding, but they are likely to have far less a role in conserving endangered species. The current-local rarity of such alleles implied their insignificant contribution to present adaptation.

Marshall and Brown (1975) suggested that the objective is to conserve the plants that will contain 95% of all the alleles at a random locus occurring in the target population with a frequency greater than 0.05. According to Marshall and Brown's view, in our study, ex situ population ML conserved enough genetic variation to sustain long-term survival and could meet the needs of future release.

However, RAPDs analysis also revealed exclusive bands in natural populations of *V. guangxiensis*, while these bands were not detected in ex situ population ML. It is worth noting that all of these bands occurred in natural populations with moderate to high frequency, three out of six even occurred in every individual of the natural populations. The populations that are distinct in their DNA traits should have a high conservation status (Avise, 1989; Dizon et al., 1992). Consequently, in order to adapt future restoration environment, more extensive sampling from population NS and NP is needed for ex situ population to contain the exclusive traits.

In recent years, Botanical Gardens have played important role in ex situ conservation of rare and endangered plants (Maunder, 1994a,b). Botanical Gardens have advantages for ex situ conservation: freedom to focus on wild plants and non-economic plants, and easy public access for conservation education (Maxted et al., 1997). While there are two main disadvantages to Botanical Garden conservation. The first is that the number of species that can be genetically conserved in a Botanical Garden will always be limited because of the available space for growing plants. The majority of Botanical Gardens are located in urban areas of temperate countries. At their present site, expansion would be prohibitively expensive. The majority of botanical diversity is located in tropical countries, so there is a need to keep the species included in expensive greenhouses, which will also necessarily limit the space available. The second disadvantage is related to the first: only a very few individuals can be cultivated of each species, thus neglecting the need to conserve the range of genetic diversity.

Xishuangbanna Tropical Botanical Garden, the Chinese Academy of Sciences is one of the few Botanical Gardens located in the tropical area, its geographic location and large area of ex situ conservation site would overcome the two main disadvantages of the Botanical Garden conservation. Rare and endangered plant diversity from tropical and subtropical areas can be conserved in this Botanical Garden.

Although many plant species are being rescued by ex situ methods, and reintroductions, the single most important way to conserve a plant species is through the protection of the habitat in which it lives. This conserves the associated animals upon which it may depend for pollination and dispersal of its diaspores and also the animals, particularly insects, that might depend upon the plant species. As a result, the Tropical Botanical Garden is more likely to provide the similar habitat of a native ecosystem for rare and endangered plants. In fact, besides about 90 individuals of endangered *V. guangxiensis*, there are other five rare dipterocarps

of China conserved in the Xishuangbanna Tropical Botanical Garden.

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