GENETIC STRUCTURE AND MATING BEHAVIORS IN *VATICA GUANGXIENSIS*: IMPLICATIONS FOR CONSERVATION AND UTILIZATION

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Abstract. This study investigated the genetic diversity and mating system of 64 parental and offspring individuals of *Vatica guangxiensis*, using SSR molecular markers. The goal was to uncover the genetic structure and mating behaviors, providing a theoretical groundwork for the conservation and utilization of wild *Vatica guangxiensis* resources. The results showed that: (1) *Vatica guangxiensis* had an expected heterozygosity (He) of 0.396, Shannon's information index (I) of 0.641. It indicates that the *Vatica guangxiensis* wild population displays moderate genetic diversity. The maternal individuals (I=0.662) surpass the offspring (I=0.621). This implies that maternal individuals have greater genetic variability when compared to the offspring. (2) The results of the Analysis of Molecular Variance (AMOVA) indicate that the variation of *Vatica guangxiensis* predominantly occurs within populations. The genetic differentiation coefficient (F_{st}) between maternal and offspring individuals was 0.014, The estimated gene flow (N_m) was 17.980, indicating low genetic differentiation, frequent gene exchange and close kinship between maternal and offspring individuals. (3) Both maternal and offspring populations of *Vatica guangxiensis* had high levels of multi-locus and single-locus outcrossing rates. The parentage index (t_m-t_s= -0.110＜0), indicates that the species exhibits a mixed mating system that is mainly characterized by outcrossing but also supplemented by self-fertilization.
Keywords: *genetic diversity, mating system, SSR, outcrossing rate, population genetics*

Introduction

Vatica guangxiensis X. L. Mo is a member of the Dipterocarpaceae family and Vatica genus. This evergreen tree can grow up to 37 meters tall and have a diameter of up to 60 centimeters at breast height. Its trunk is straight and symmetrical, with a fine grain structure. This tropical tree species is exclusive to China and of great value. *Vatica guangxiensis* is among the recently discovered rare and valuable tropical tree species found in Guangxi. The wood of *Vatica guangxiensis* is dense and hard, with excellent resistance to decay. It is therefore highly valued for various applications (Deng et al., 2020). Additionally, the leaves and stems of this species contain a substantive quantity of usable secondary metabolites, which can be utilized in the development of fragrances and medicines (Qin et al., 2011). Currently, *Vatica guangxiensis* is only found in Guangxi Province, China and it prefers a climate characterized by mild winters, not excessively hot summers, and high temperatures throughout the year. Due to climate change and human activities, the habitat of this species has become fragmented, resulting in a substantial reduction in the size of its natural forests. It has been classified as Critically Endangered (CR) on the International Union for Conservation of Nature (IUCN) Red List (Li et al., 2002) and is considered an endangered species in the "Red List of Chinese Plants - Rare and Endangered Plants" (Tan et al., 2016). It has been upgraded to a first-

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class protected wild plant at the national level, emphasizing its significant conservation worth.

Simple sequence repeat (SSR) or Microsatellite molecular markers are repetitive DNA sequences composed of 1-6 nucleotide units, which are randomly distributed in the genomes of prokaryotes and eukaryotes (Liu et al., 2018). These markers possess co-dominant inheritance, low cost, as well as excellent stability and repeatability, making them widely applied in genetic studies of rare and endangered plants (Ahmad et al., 2018). Shang (2017) conducted a study on the genetic diversity of *Vatica mangachapoi* on Hainan Island using SSR molecular marker technology. The research systematically evaluated the current status of genetic resources in natural forests of *Vatica mangachapoi* on Hainan Island, providing a scientific basis for the conservation and utilization of *Vatica mangachapoi* natural forests. Cai et al. (2022), using SSR molecular marker technology, discovered that the proportion of low-frequency alleles in the *Hopea exalata* population is significantly lower compared to non-endangered species in the Dipterocarpaceae family. This finding suggests that the reduction in population size may be responsible for this phenomenon. The study recommends implementing measures such as artificial breeding and translocation to promote the recovery of genetic diversity and evolutionary potential.

The genetic characteristics within or between natural plant populations are significant determinants of the long-term survival of species (Liu et al., 2013). The mating system, a sexual system that impacts the genetic relationship between parental and offspring generations, is critical for the genetic structure of a population (Li et al., 2010). It is essential to consider these factors when studying plant populations to make informed decisions for their conservation. Studying the genetic diversity and mating system of endangered plants can reveal their evolutionary mechanisms and the causes of their endangerment. This approach can help to develop scientifically sound conservation and management measures (Li and Ge, 2006). Research on *Vatica guangxiensis* is currently insufficient, with just a small amount of research conducted on its ecological characteristics, population structure, and conservation measures. There is a limited amount of research on the genetic diversity and mating system of *Vatica guangxiensis*. To address this gap, we utilized SSR molecular marker technology to explore the genetic diversity and mating system of 64 *Vatica guangxiensis* samples, consisting of 8 maternal and 56 offspring individuals, the maternal generation consists of adult plants, while the offspring generation comprises seedlings propagated from their seeds. The objective of this study is to establish a scientific foundation for advanced molecular-assisted breeding, efficient conservation, and optimal utilization of germplasm resources pertaining to *Vatica guangxiensis*.

Materials and Methods

Plant Material Collection

The study area is located on Nazhi Mountain in Pingtan Village, Baihe Township, Napo County, Baise City, Guangxi. It is currently the only discovered wild population of *Vatica guangxiensis* in Guangxi. The distribution coordinates are approximately 105°49'51.56"E longitude and 23°10'19.92"N latitude. This population consists of 9 adult plants. A total of 64 samples were collected in this experiment, including 8 adult plants (maternal individuals) and 54 seedlings (offspring individuals) of *Vatica guangxiensis* from this population. Fresh leaves were collected from each plant and air-dried using

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silica gel for preservation. For each plant, 3-5 healthy leaves free from diseases and pests were selected. The identification of the collected samples was conducted by Dr. Wei Xiao from the Guangxi Institute of Botany and confirmed as *Vatica guangxiensis* (*Figure 1*).

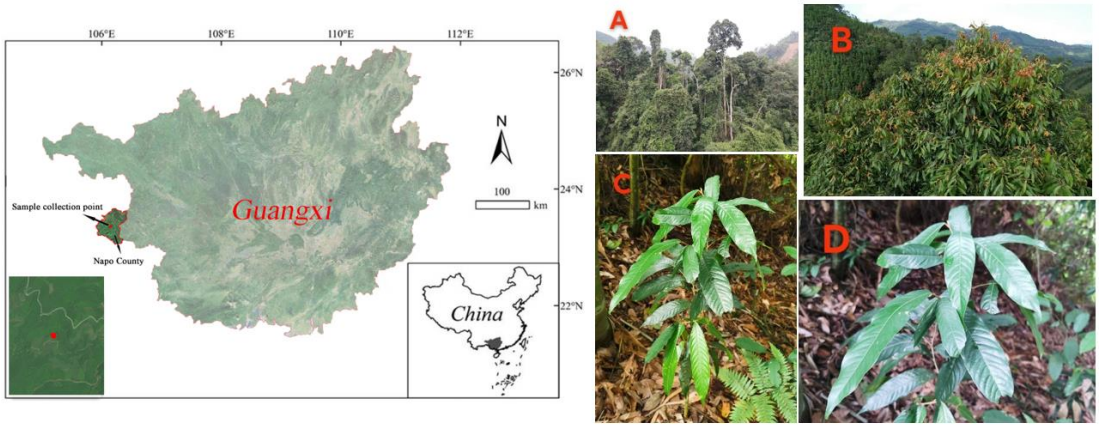

Figure 1. *The maternal generation (A, B) and offspring generation (C, D) of Vatica guangxiensis, and geographic distribution of the samples*

DNA Extraction and PCR Amplification

DNA extraction was performed using the E.Z.N.A. Tissue DNA kit (Omega Bio-Tek), and quality inspection was conducted using 1% agarose gel electrophoresis. Based on simplified genomic sequence analysis, 54 primer pairs were designed for SSR analysis. Eight representative samples were selected for amplification using the 54 primer pairs. The PCR reactions were carried out on a Veriti 384 PCR (Thermo Fisher Scientific) machine, and the primer pairs were validated through screening. A total of four primer pairs with successful amplification and good peak profiles were selected.

The PCR amplification program was set as follows: initial denaturation at 95°C for 5 minutes; denaturation at 95°C for 30 seconds, annealing at a gradient temperature of 62-52°C for 30 seconds, extension at 72°C for 30 seconds, repeated for 10 cycles; denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds, repeated for 25 cycles; final extension at 72°C for 20 minutes, and then stored at 4°C.

Data Analysis

Genetic Diversity and Genetic Differentiation Parameters

GeneAlEx v6.5.1 software was used to calculate the observed number of alleles (Na), effective number of alleles (Ne), Shannon's index (I), observed heterozygosity (Ho), expected heterozygosity (He), and gene flow (N_m) for all loci and for the maternal and offspring individuals of *Vatica guangxiensis*. The genetic differentiation coefficient (F_{st}) among populations and the Wright's fixation index F_1 for polymorphic loci in each population were also calculated. Based on the F_1 value, the theoretical expected outcrossing rate (t_e) for each population was derived as $t_e=(1- F_1)/(1+ F_1)$. Additionally, the polymorphic information content (PIC) for all loci was calculated using PowerMarker v3.25 based on genetic identity and genetic distance.

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Mating System Parameters

The software MLTR was used to calculate the multilocus outcrossing rate (t_m) and single locus outcrossing rate (t_s), the difference between them (t_m-t_s), the multilocus relatedness (r_{pm}), outcrossing rate relatedness (r_t), parental inbreeding coefficient (F), and parental relatedness (r_p) for the maternal and offspring individuals of *Vatica guangxiensis*.

Genetic Structure Analysis

Different analyses were employed to describe the genetic structure among populations. Firstly, principal coordinate analysis (PCoA) was used to illustrate the genetic distances between individuals. Secondly, molecular variance analysis (AMOVA) was conducted using GeneAlEx v6.5.1 software to investigate the total genetic variation among and within populations. Additionally, Bayesian clustering analysis was performed on all individuals using STRUCTURE 2.3.4. The analysis was set with K values ranging from 1 to 20, a burn-in period of 10,000, and a Markov Chain Monte Carlo (MCMC) of 100,000. Each K value was run 10 times, and the optimal ΔK value (indicating the best population stratification) was determined using the online tool STRUCTURE HARVESTER. The results based on the optimal K value were plotted.

Phylogenetic Analysis

Nei's genetic distance between pairwise samples was calculated using PowerMarker v3.25. Based on the Nei's genetic distance matrix, an unweighted pair group method with arithmetic mean (UPGMA) dendrogram was constructed using MEGA v6.0, representing the phylogenetic tree of all individuals.

Results

Primer Screening and Polymorphism Analysis

After experimental screening, 4 primer pairs were selected from a pool of 54 primers based on their good peak morphology and rich polymorphism (*Table 1*). These 4 primer pairs were used to amplify a total of 64 DNA samples. As shown in *Table 2*, polymorphism analysis was conducted for the 4 selected allele loci, and a total of 10.00 alleles (Na) were detected among the 64 samples, ranging from 2.00 to 5.00 with an average of 2.50. The effective allele number (Ne) ranged from 1.133 (GXQM092) to 2.567 (GXQM047) with an average of 1.806. The Shannon information index (I) ranged from 0.234 (GXQM092) to 1.089 (GXQM047), with an average of 0.641. The observed heterozygosity (Ho) ranged from 0.125 (GXQM092) to 0.679 (GXQM047), with an average of 0.444. The expected heterozygosity (He) ranged from 0.117 (GXQM092) to 0.608 (GXQM047), with an average of 0.396. The genetic differentiation coefficient (F_{st}) ranged from 0.014 to 0.036, with a maximum of 0.036 (GXQM047) and an average of 0.014. The polymorphic information content (PIC) ranged from 0.1103 (GXQM092) to 0.5147 (GXQM047), with an average of 0.3315. The gene flow (N_m) index ranged from 6.722 (GXQM047) to 305.944 (GXQM095), with an average of 81.542. These results indicate that some of the selected 4 primer pairs exhibit good polymorphism and can be used for subsequent genetic information analysis experiments.

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Table 1. *Information of SSR primer pairs in Vatica guangxiensis*

Primer number	Locus name	Primer sequence
SPE14485	SPE14485_GXQM031_F	GAAGGTGACCAAGTTCATGCTGACTGGTCCTTCTCTCACGC
SPE14486	SPE14486_GXQM031_R	AGCATCCCCATAGCAGTGAC
SPE14487	SPE14487_GXQM047_F	GAAGGTGACCAAGTTCATGCTCCAAATCCAAGGAGCTCAAA
SPE14488	SPE14488_GXQM047_R	CCTGTACACCACCGTCCTCT
SPE14489	SPE14489_GXQM092_F	GAAGGTGACCAAGTTCATGCTGAGCTAGATTGCAAGGCAGG
SPE14490	SPE14490_GXQM092_R	CTGTGGGATAACGGTGGAAC
SPE14491	SPE14491_GXQM095_F	GAAGGTGACCAAGTTCATGCTCAAAGCTCTCCAAAACCTCG
SPE14492	SPE14492_GXQM095_R	CATCATCACCTCCCGAATCT

Table 2. *Polymorphism analysis of 4 pairs of primers*

Locus	Na	Ne	I	Ho	He	F_{st}	PIC	N_m
GXQM031	2.000	1.741	0.612	0.554	0.422	0.018	0.3544	13.500
GXQM047	4.000	2.567	1.089	0.679	0.608	0.036	0.5147	6.722
GXQM092	2.000	1.133	0.234	0.125	0.117	0.000	0.1103	
GXQM095	2.000	1.783	0.631	0.420	0.439	0.001	0.3466	305.944
Mean	2.500	1.806	0.641	0.444	0.396	0.014	0.3315	81.542

Genetic Diversity

The genetic diversity information of the maternal and offspring populations of *Vatica guangxiensis* is presented in *Table 3*. The average number of alleles (Na) in the maternal population is higher than that in the offspring population, with values of 2.750 and an average of 2.500. The effective allele number (Ne) ranges from 1.817 (maternal) to 1.795 (offspring), with an average of 1.806. The maternal population exhibits higher Shannon information index (I) values, with a value of 0.662 and an average of 0.641. The observed heterozygosity (Ho) is lower in the maternal population (0.406) compared to the offspring population (0.482), with an average of 0.444. The expected heterozygosity (He) in the offspring population is higher than that in the maternal population, with values of 0.402 and 0.391, and an average of 0.396. The unbiased expected heterozygosity (UHe) ranges from 0.417 (maternal) to 0.406 (offspring), with an average of 0.411. The polymorphic percentage of loci (PPB) is 100% for both the maternal and offspring populations, indicating a rich genetic diversity in *Vatica guangxiensis* populations. The fixation index (F_1) is negative for both populations, with the maternal population (-0.062) showing a higher value than the offspring population (-0.168), with an average of -0.115. This suggests the presence of an excess of heterozygotes in the maternal and offspring populations. The expected outcrossing rate (t_e) in the offspring population is higher than in the maternal population, with values of 1.403 and 1.132, and an average of 1.268. The observed heterozygosity in both the maternal and offspring populations is higher than the expected heterozygosity. The small differences between the parameters of the maternal and offspring populations indicate a close genetic similarity in *Vatica guangxiensis* populations.

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Table 3. *Genetic diversity levels of Vatica guangxiensis maternal generation and offspring based on 4 pairs of SSR primers*

Population	Na	Ne	I	Ho	He	UHe	PPB	F_1	t_e
Maternal	2.750	1.817	0.662	0.406	0.391	0.417	100.00%	-0.062	1.132
offspring	2.250	1.795	0.621	0.482	0.402	0.406	100.00%	-0.168	1.403
Mean	2.500	1.806	0.641	0.444	0.396	0.411	100.00%	-0.115	1.268

Genetic Differentiation and Variation Distribution

AMOVA molecular variance analysis (*Table 4*) reveals that the variation percentage among populations of *Vatica guangxiensis* is only 1%, while the within-population variation percentage accounts for 99%. Individual-level variation contributes 100% to the within-population variation, whereas the variation between individuals accounts for 0% of the within-population variation. This indicates that the majority of genetic differentiation occurs within the maternal and offspring populations of *Vatica guangxiensis*, with individual-level genetic differentiation playing a dominant role, while genetic exchange between individuals and between maternal and offspring populations is limited. According to *Table 5*, the genetic differentiation coefficient (F_{st}) between the maternal and offspring populations is 0.014, indicating a low level of genetic differentiation. The gene flow (N_m) is 17.980, indicating a relatively high level of gene flow. *Table 6* shows that the genetic concordance between the maternal and offspring populations of *Vatica guangxiensis* is 0.975, which is close to 1, indicating a close genetic relationship between the maternal and offspring populations. Nei's genetic distance is only 0.026, suggesting a low level of genetic differentiation between the maternal and offspring populations.

Table 4. *AMOVA analysis of Vatica guangxiensis population*

Source of variation	df	SS	MS	Variance component	Percentage of variation
Among population	1	0.862	0.862	0.007	1%
Among Individuals	62	42.107	0.679	0.000	0%
Within Individuals	64	60.500	0.945	0.945	99%
Total	127	103.469		0.952	100%

Table 5. *Genetic differentiation coefficient (F_{st}, below the diagonal) and gene flow (N_m, above the diagonal) among maternal generation and offspring of Vatica guangxiensis*

Population	Maternal	offspring
Maternal	***	17.980
offspring	0.014	***

Table 6. *Nei's genetic distance (below the diagonal) and genetic identity (above the diagonal) among maternal generation and offspring of Vatica guangxiensis*

Population	Maternal	offspring
Maternal	***	0.975
offspring	0.026	***

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Genetic Structure

To uncover the genetic structure of the maternal and offspring populations of *Vatica guangxiensis*, a preliminary analysis of their genetic structure was conducted using the Bayesian clustering method in the software program STRUCTURE (*Figure 2*). The results indicate that among K values ranging from 1 to 20, the maximum value of △K is obtained when K equals 14, suggesting that the 64 germplasm samples of *Vatica guangxiensis*, including maternal and offspring generations, were classified into 14 subgroups denoted as G1-G14. As shown in *Figure 3*, with maternal samples (1-8) and offspring samples (9-64) on the X-axis and the corresponding Q-values on the Y-axis, the genetic distribution of the 14 subgroups was found to be evenly distributed within the gene pool. The average gene flow (N_m) value of 17.980, combined with the analysis of genetic diversity indices, indicates a strong gene flow between the maternal and offspring populations of *Vatica guangxiensis*, which is consistent with the results of the population structure analysis.

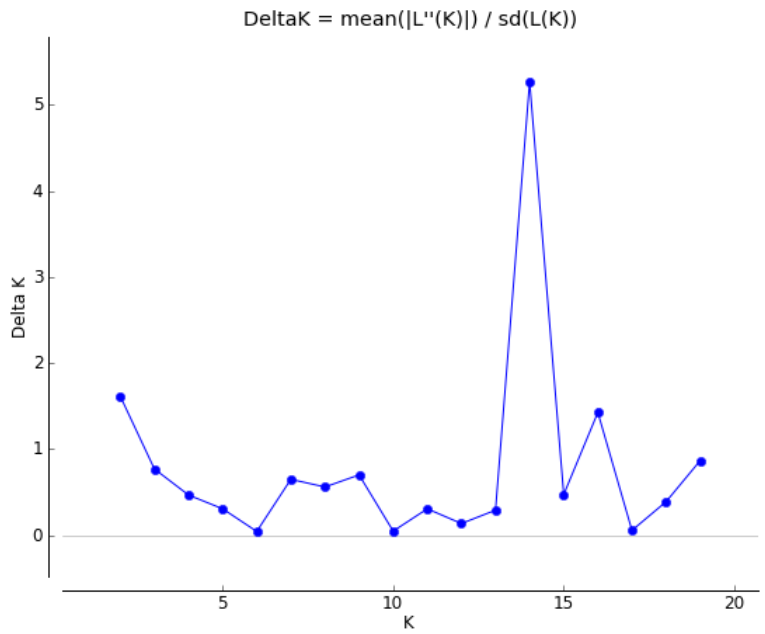

Figure 2. *Analysis of the population structure of the maternal generation and offspring of Vatica guangxiensis ΔK-value distribution*

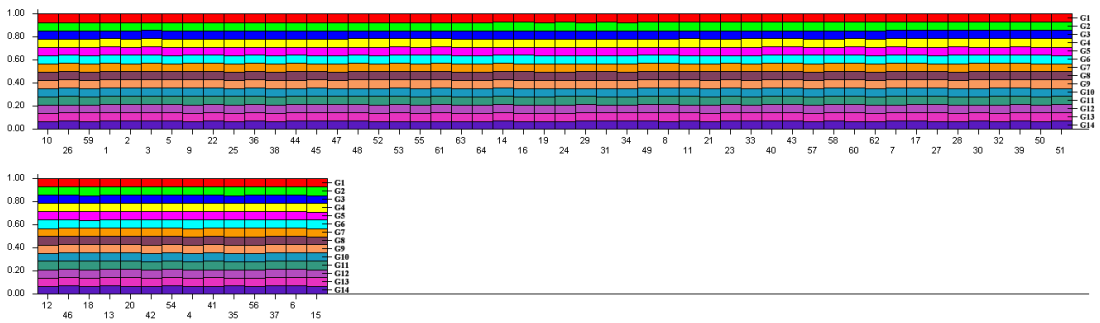

Figure 3. *Genetic structure of the maternal generation and offspring of Vatica guangxiensis*

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Cluster Analysis

Cluster analysis of the germplasm resources of 64 maternal and offspring samples of *Vatica guangxiensis* was performed using Nei's genetic distance and the UPGMA method. The results shown in *Figure 4* indicate that three maternal samples and 27 offspring samples form one cluster (Cluster I), while five maternal samples and 29 offspring samples form another cluster (Cluster II). Furthermore, both Cluster I and Cluster II can be further divided into two subclusters, namely I-1, I-2, II-1, and II-2. Cluster I-1 includes three maternal samples and 25 offspring samples, Cluster I-2 includes two offspring samples, Cluster II-1 includes four maternal samples and 17 offspring samples, and Cluster II-2 includes one maternal sample and 12 offspring samples. Principal Coordinate Analysis (PCoA) was employed to analyze the genetic distance of the 64 maternal and offspring germplasm samples of *Vatica guangxiensis*. As shown in *Figure 5*, the PCoA results demonstrate that the genetic distances between the maternal and offspring populations of *Vatica guangxiensis* are relatively close. This indicates that the results of the cluster analysis and the PCoA analysis are consistent.

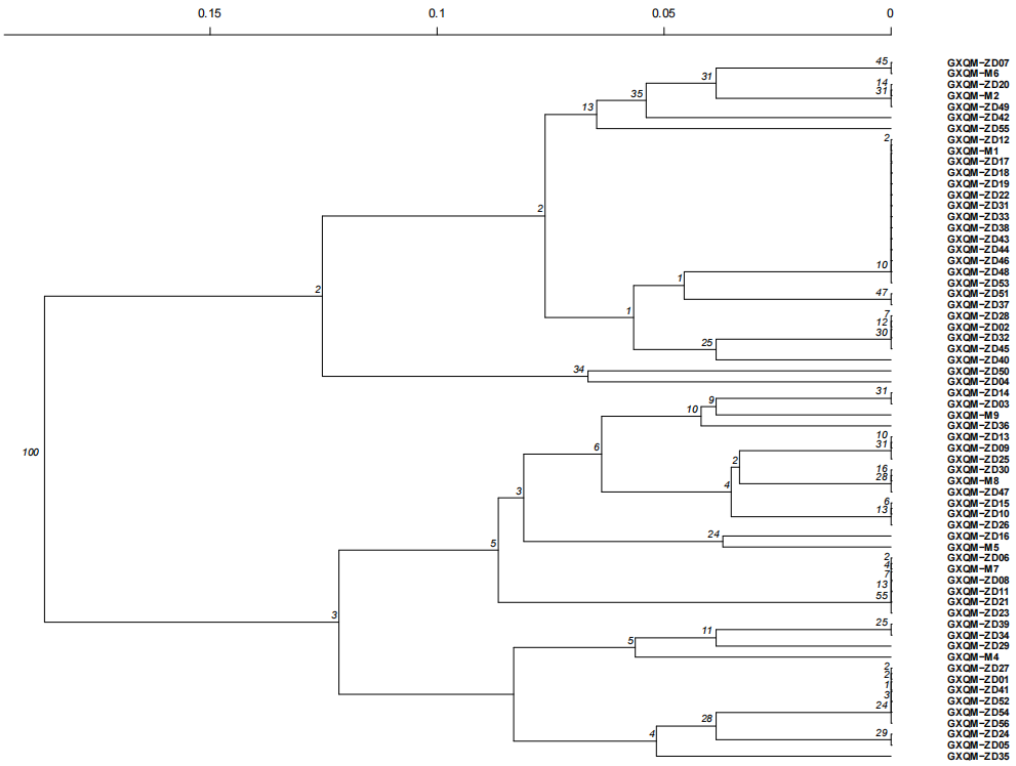

Figure 4. *Evolutionary tree of genetic relationships between germplasm resources of the maternal generation and offspring of Vatica guangxiensis*

Mating System

The mating system of the collected 64 maternal and offspring samples of *Vatica guangxiensis* was investigated using SSR molecular markers. The samples consist of 8 maternal samples and 56 offspring samples, with each maternal sample paired with 7 offspring samples, forming a total of 8 families. According to *Table 7*, both the multilocus outcrossing rate (t_m) and the single-locus outcrossing rate (t_s) of the maternal and

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offspring populations of *Vatica guangxiensis* are at a relatively high level, with values of 1.090 and 1.200, respectively. The difference between t_m and t_s reflects the degree of inbreeding, where t_m is smaller than t_s, t_m-t_s=-0.110 < 0. The correlation coefficient (r_t) represents the correlation of outcrossing rates among families, and its value of 0.997 is at a high level. The multilocus correlation coefficient (r_{pm}) of 0.025 is greater than the single-locus correlation coefficient (r_{ps}) of -0.030, r_{ps}-r_{pm}=-0.056 < 0. The inbreeding coefficient (F), which is a measure of population inbreeding, has a value of -0.200 < 0. The parental correlation (r_p) is -0.891 < 0.

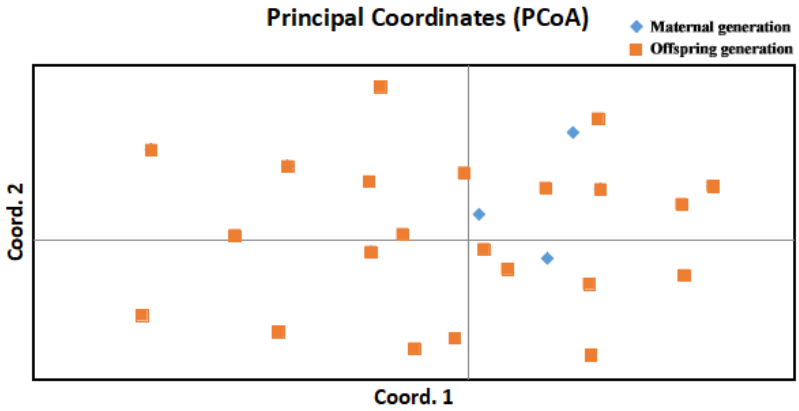

Figure 5. *Principal coordinate analysis of the maternal generation and offspring population of Vatica guangxiensis (PCoA)*

Table 7. *The mating system parameters of the maternal generation and offspring population of Vatica guangxiensis*

Parameter	Species level
The number of families	8
The number of parent individuals	8
The number of offsprings	56
t_m	1.090
t_s	1.200
t_m-t_s	-0.110
r_t	0.997
r_{pm}	0.025
r_{ps}	-0.030
r_{ps}-r_{pm}	-0.056
F	-0.200
r_p	-0.891

Discussion

The genetic diversity of plants is a direct reflection of their genetic evolution and their ability to adapt to ecological environments. Lower levels of genetic diversity indicate poorer evolutionary capability and weaker environmental adaptability, ultimately increasing the risk of species endangerment (Hughes et al., 2008; Haloin and Strauss, 2008; Vandewoestijne et al., 2008). In this study, based on SSR molecular markers, we explored the genetic differentiation, genetic structure, genetic diversity, and mating system between maternal and offspring populations of *Vatica guangxiensis* at the

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molecular level. These findings provide a reference for the breeding and conservation of superior germplasm resources of *Vatica guangxiensis*. The genetic diversity analysis of the 64 *Vatica guangxiensis* samples revealed an average polymorphic information content (PIC) of 0.3315, indicating high levels of polymorphism. This suggests that the four loci exhibit rich genetic diversity and compared to traditional SSR primer development, SSR markers are more rapid and convenient, making them suitable for studying and identifying the genetic diversity of *Vatica guangxiensis* germplasm resources (Li et al., 2020). Overall, the average Shannon information index (I) of *Vatica guangxiensis* was 0.641, the average expected heterozygosity (He) was 0.396, and the average percentage of polymorphic loci (PPB) was 100%. These results indicate a high level of genetic diversity. However, compared to the genetic diversity of the Hainan *Vatica mangachapoi* population studied by Shang Shuaibin (2017) (I=1.416, He=0.698), the genetic diversity of *Vatica guangxiensis* is lower than that. On the other hand, it is higher than the genetic diversity of the *Vatica xishuangbannaensis* population studied by Li et al. (2001), where the average expected heterozygosity (He) was 0.085. It is also lower than the genetic diversity of the wingless slope barrier (a member of the Dipterocarpaceae family) studied by Ying et al. (2022) (I=1.035, He=0.599). It is generally believed that the genetic diversity of rare, endangered, and locally-specific plant species is lower (Wang et al., 2020). This is mainly influenced by factors such as plant breeding systems, geographic distribution, and human impacts (Wu et al., 2019). The lower genetic diversity of *Vatica guangxiensis* compared to non-endangered plant populations such as Hainan *Vatica mangachapoi* may be due to the smaller population size and habitat destruction caused by human activities (Xiao et al., 2023). It may also be similar to the case of *Shorea robusta* in the Dipterocarpaceae family, where global climate change has resulted in rapid reduction of the distribution range, leading to bottleneck effects and genetic drift (Zhu and Tan, 2023).

The average number of allele per locus (Na), effective number of alleles (Ne), Shannon's information index (I), and unbiased expected heterozygosity (UHe) of the maternal population of *Vatica guangxiensis* are higher than those of the offspring population. On the other hand, the observed heterozygosity (Ho) and expected heterozygosity (He) of the offspring population are slightly higher than those of the maternal population, indicating that the maternal population exhibits higher genetic diversity than the offspring population. This result can be explained by a phenomenon similar to that observed in the wingless slope barrier of the Dipterocarpaceae family, where the offspring population experiences a population bottleneck and requires human intervention in their growth environment to overcome it (Cai et al., 2022). Additionally, this finding aligns with the discoveries made by Zhu et al. (2023) regarding the genetic diversity changes in *Vatica guangxiensis*. They reported a continuous decrease in Ne throughout the population's history and a reduction in genetic diversity during the natural regeneration process. *Vatica guangxiensis* is likely to be at risk of extinction due to its decreasing genetic diversity. Fortunately, in comparison to the findings of this study, the observed heterozygosity (Ho), expected heterozygosity (He), and effective number of alleles (Ne) of the maternal and offspring populations of *Vatica guangxiensis* are relatively high. This may be attributed to the fact that *Vatica guangxiensis* grows in a protected area with gradually improving living conditions, as well as the increased attention and conservation efforts from the society and researchers. It indicates that our conservation efforts for *Vatica guangxiensis* have shown initial success, but there is still a long way to go.

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Genetic differentiation explains the proportion of genetic variation within and between populations, serving as an important basis for the conservation of germplasm diversity and genetic improvement (Wang et al., 2022). According to the results of AMOVA molecular variance analysis, only 1% of the genetic variation occurred between populations of the *Vatica guangxiensis* species, while 99% of the genetic variation occurred within populations. Moreover, all of the genetic variation within populations was found to be located within individuals, indicating that genetic gene flow primarily occurs within individuals of the *Vatica guangxiensis* population. Similar results were observed for the *Shorea robusta* genus (Cardillo and Bernal, 2006). This outcome may be attributed to genetic mutations within individuals or the influence of natural selection. Previous studies have reported that F_{st} values ranging from 0 to 0.05 indicate weak differentiation between subpopulations, while F_{st} values ranging from 0.05 to 0.15 indicate moderate differentiation. F_{st} values ranging from 0.15 to 0.25 indicate high differentiation (Dong et al., 2019). The genetic differentiation coefficient, F_{st}, between the maternal and offspring populations of the *Vatica guangxiensis* species was calculated as 0.014, suggesting weak differentiation between these subpopulations, which can be considered negligible. A larger N_m value between populations indicates a more uniform distribution, as a larger N_m value homogenizes the populations and effectively inhibits differentiation between them (Wei et al., 2023). According to Govindaraju's categorization of N_m levels (Gamba and Muchhala, 2023), the N_m value between the maternal and offspring populations of *Vatica guangxiensis* was determined to be 17.980. This indicates a high level of gene flow between the maternal and offspring populations, thereby reducing the genetic differentiation between them. It indirectly demonstrates the high genetic diversity of *Vatica guangxiensis*, which is consistent with the conclusions drawn from the molecular variance analysis. Furthermore, the genetic structure analysis using Principal Coordinate Analysis (PCoA) and Bayesian clustering reaffirmed that there is no apparent genetic differentiation between the maternal and offspring populations of *Vatica guangxiensis*, indicating frequent gene flow. The underlying reason for this outcome may be attributed to the fact that *Vatica guangxiensis* is uniformly distributed within the protected area of Napo County in Guangxi, where it receives adequate protection. However, due to the limited distribution area, it promotes gene flow to some extent among individuals. Conversely, when there are greater geographical distances or geographical barriers such as mountains and rivers between individuals or populations, it hinders genetic exchange (Yi et al., 2018).

The mating system is closely associated with genetic diversity as it not only affects population size and genotype distribution but also determines the genetic differentiation and structure of populations. Its core focus is to investigate the outcrossing rates between maternal and offspring lineages (Tang, 2014). The mating system of plants in the Dipterocarpaceae family, including *Vatica guangxiensis*, is generally characterized by outcrossing or long-distance mating, and these outcrossing plants exhibit high levels of genetic diversity within populations (Fukue et al., 2007; Masuda et al., 2013; Guo et al., 2017). However, a large amount of data indicates that the population of *Vatica guangxiensis* has entered a vortex of extinction. In the case of *Vatica guangxiensis*, both the multi-locus outcrossing rate (t_m) and the single-locus outcrossing rate (t_s) are at relatively high levels, with a parental inbreeding coefficient (t_m-t_s) of $-0.110 < 0$, indicating a low level of self-fertilization and a significant advantage of outcrossing in the maternal and offspring populations. The high outcrossing rates may be attributed to the fact that *Vatica guangxiensis* populations are primarily cultivated within protected

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areas, leading to a concentrated distribution that promotes genetic connections among different genotypes, thus increasing the level of outcrossing. This finding is consistent with previous research that found a positive correlation between plant outcrossing rates and distribution density. The correlation coefficient of outcrossing rates (r_t) is relatively high, while the multi-locus correlation coefficient (r_{pm}) is low, indicating a low likelihood of inbreeding in the maternal and offspring populations of *Vatica guangxiensis*. However, the significant difference between r_t and r_{pm} suggests the presence of subpopulation structure within the maternal and offspring populations. The value of r_{ps}-r_{pm}, reflecting the relationship between parental relatedness and mating population structure, is -0.056, indicating the absence of selective pressure favoring inbreeding. The average fixation index F_1 is $-0.115 < 0$, indicating that outcrossing produces a greater number of heterozygous individuals compared to random mating, which is consistent with the results of the parental inbreeding coefficient (F).

Conclusion

Compared to traditional methods, this study employs SSR molecular marker technology, which provides highly polymorphic molecular markers for describing and evaluating the genetic diversity between maternal and offspring populations of *Vatica guangxiensis*. By analyzing the polymorphism of SSR markers, it determines the extent of genetic variation, genetic structure, and the genetic relationship among populations. This study contributes important genetic background information for the future construction of a genetic diversity database and conservation strategies for *Vatica guangxiensis* germplasm resources. The mating system of *Vatica guangxiensis* populations demonstrates a high level of outcrossing, with a mixed mating type where outcrossing is the primary mode and self-fertilization is secondary. However, the overall genetic diversity of *Vatica guangxiensis* populations is only at a moderate level, with slightly higher genetic diversity observed in the maternal lineages compared to the offspring. This result is likely associated with factors such as small population size, narrow ecological adaptation range, and human disturbances. Although the genetic diversity of *Vatica guangxiensis* populations is not high, the high levels of gene flow among individuals or populations ($N_m > 1$), low genetic differentiation ($F_{st} < 0.05$), and high outcrossing rates indicate that the population of *Vatica guangxiensis* is on the edge of survival struggle. Due to the rarity of individuals and populations, there is a strong desire to overcome the disadvantage of low genetic differentiation, highlighting the urgent need for intervention through artificial means to restore the potential for high levels of genetic diversity in the maternal and offspring populations of *Vatica guangxiensis*. It is recommended that in-situ conservation efforts for *Vatica guangxiensis* populations should actively organize artificial pollination while ensuring the integrity of their habitats, thus enhancing communication among individuals. Consideration should be given to a combination of ex-situ and in-situ conservation methods. During ex-situ conservation, planting should occur within a closer range to other *Vatica mangachapoi* varieties, which will help enhance outcrossing rates and gene recombination, thereby improving genetic diversity and survival capacity. Priority should be given to the protection of maternal populations with high genetic diversity, and techniques such as tissue culture and rapid propagation should be utilized to protect and reintroduce the species, thereby preserving the genetic diversity of *Vatica guangxiensis* and promoting population regeneration.

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