GENETIC DIVERSITY OF THE CRITICALLY ENDANGERED *PINUS KWANGTUNGENSIS*

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Abstract. To assess the genetic diversity and differentiation of *Pinus kwangtungensis*—a rare and endangered conifer endemic to China—and to identify priority populations for conservation, we conducted a genetic analysis of three wild populations in Guangxi using simple sequence repeat (SSR) markers. Of the 96 primer pairs, seven highly polymorphic pairs were selected, yielding a total of 24 alleles. The mean values for the number of alleles (N_a), effective number of alleles (N_e), Shannon's information index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), and polymorphic information content (PIC) were 3.429, 2.342, 0.788, 0.415, 0.436, and 0.380, respectively. Among the three populations, the Zhongluntun population in Huanjiang Maonan Autonomous County, Hechi (HDLT) exhibited the highest genetic diversity ($I = 0.745$, $H_e = 0.462$), whereas the Antai Township population in Rongshui Miao Autonomous County, Liuzhou (YBS) showed the lowest ($I = 0.447$, $H_e = 0.249$). Analysis of molecular variance (AMOVA) indicated that most of the genetic variation (72%) occurred within individuals. Cluster analysis grouped the HDLT and YBS populations together, with the Jianfulun population in Huanjiang Maonan Autonomous County (HJS) forming a separate clade. Overall, *P. kwangtungensis* populations in Guangxi exhibited relatively low genetic diversity, with HDLT representing a genetic diversity hotspot and a promising source of high-quality germplasm for conservation. These findings provide a scientific basis for the development of effective conservation and management strategies for this species.
Keywords: *Pinus kwangtungensis, microsatellite markers, population structure, genetic differentiation, conservation genetics*

Introduction

Pinus kwangtungensis (family Pinaceae) is an evergreen tree species endemic to China, listed as a second-class nationally protected wild plant and categorized as Near Threatened (NT) in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Tang et al., 2023). Its natural distribution is mainly restricted to southern provinces, including Hunan, Guizhou, Guangdong, Guangxi, and Hainan (Cai et al., 2024). As a phylogenetically primitive and relict species, *P. kwangtungensis* is highly sensitive to habitat conditions, occupying a narrow ecological niche. It primarily occurs in subtropical montane environments at higher elevations with distinct warm and humid climates (Editorial Committee of the Institute of Botany, Chinese Academy of Sciences, 1978). The

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species exhibits low seedling survival and poor natural regeneration capacity, with existing wild populations being highly fragmented and geographically isolated (Wang et al., 2022). In response to accelerated global warming and the northward shift of climatic zones, the lower altitude limit of its distribution has been rising. Populations in the southern subtropical and northern tropical regions have shown upward migration toward mountain summits and, in some cases, local extirpation (Tao et al., 2012). Consequently, systematic genetic studies aimed at evaluating the genetic diversity and population structure of *P. kwangtungensis* are of critical importance for developing effective conservation strategies, expanding population size, and enhancing population quality.

Previous research on *P. kwangtungensis* has largely focused on its distribution patterns (Liu et al., 2025), habitat characteristics (Gong et al., 2022), endangerment mechanisms (Wang et al., 2022), community composition (Shen et al., 2016), and responses to climate change (Cai et al., 2024; Tao et al., 2012). Molecular studies have revealed that *P. kwangtungensis* has undergone glacial expansion and interglacial retreat, which has shaped its genetic structure, resulting in significant genetic differentiation among populations (Tian et al., 2010). Further genetic analyses using novel minisatellite markers in plastid DNA have confirmed clear genetic structure and limited gene flow among populations, indicating the presence of distinct haplotypes (Tian et al., 2008). This underscores the importance of multi-population genetic assessments for informing effective conservation strategies. In the conservation of endangered plants, genetic diversity is a key indicator of population health and conservation value. It serves as a fundamental criterion for evaluating germplasm quality and reflects a species' adaptive potential to environmental changes (Qin et al., 2022). Investigating the genetic diversity of *P. kwangtungensis* is therefore essential for identifying genetically valuable populations and for guiding the development of in situ and ex situ conservation measures, including translocation and reintroduction programs.

Simple sequence repeats (SSR), are molecular markers based on polymorphisms in short tandem repeat sequences. They offer high levels of polymorphism, strong reproducibility, high detection efficiency, and low sample requirements. As such, SSRs have been widely employed in plant genetic diversity assessments, gene mapping, germplasm identification, and evolutionary studies (Tang et al., 2024; Yang et al., 2025). In this study, we applied SSR markers to analyze the genetic diversity and genetic structure of three wild *P. kwangtungensis* populations distributed across different regions of southern China. The objectives were to assess the levels of genetic variation and patterns of population differentiation, thereby providing a scientific foundation for conservation genetics research and the sustainable utilization of the species' germplasm resources.

Materials and methods

Plant materials

In 2022, a total of 32 leaf samples of *P. kwangtungensis* were collected from three wild populations in Guangxi, China: Jianfulun (HJS) and Zhongluntun (HDLT) in Huanjiang Maonan Autonomous County, Hechi City, and Antai Township (YBS) in Rongshui Miao Autonomous County, Liuzhou City (*Table 1*). The locations and distribution of the sampled populations are shown in *Figure 1*. The number of samples collected from each population varied according to population size. Given the endangered status and small population size of *P. kwangtungensis*, sampling was conducted as extensively as possible to ensure representative genetic diversity.

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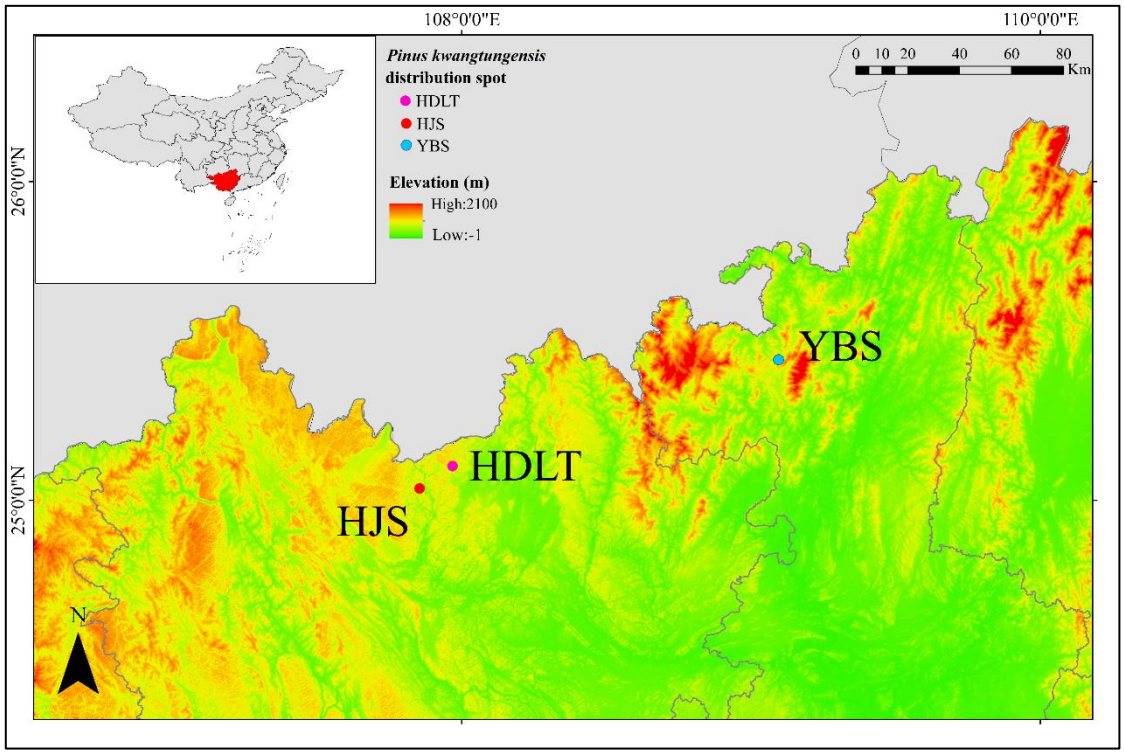

Figure 1. *Locations of the three wild populations of P. kwangtungensis in Guangxi, China*

Table 1. *Collection location information of 3 populations of Pinus kwangtungensis*

Population	Location	Longitude	Latitude	Elevation	The number of samples
HJS	Jianfulun, Huanjiang Maonan Autonomous County, Hechi City, Guangxi Zhuang Autonomous Region, China	25°8′51″	107°55′34″	794	10
HDLT	Zhongluntun, Huanjiang Maonan Autonomous County, Hechi City, Guangxi Zhuang Autonomous Region, China	25°8′48″	107°55′57″	751	7
YBS	Antai Township, Rongshui Miao Autonomous County, Liuzhou City, Guangxi Zhuang Autonomous Region, China	25°26′31″	109°5′43″	167	15

Methods

DNA extraction and quality assessment

Genomic DNA was extracted from needle tissues using a modified cetyltrimethylammonium bromide (CTAB) method. The integrity and purity of the extracted DNA were evaluated by 1% agarose gel electrophoresis, and DNA concentration and purity were quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). DNA samples that met quality standards were stored at −20°C until further analysis.

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Primer synthesis and screening

Based on reference genome sequence analysis in the NCBI database (https://www.ncbi.nlm.nih.gov/), 96 SSR primer pairs were designed and synthesized. Six samples (two from each population) were randomly selected for the initial screening of all primers. PCR amplifications were performed using a Veriti 384 thermal cycler with the following program: initial denaturation at 95°C for 5 min; 10 cycles of touchdown PCR (denaturation at 95°C for 30 s, annealing from 62°C to 52°C for 30 s, extension at 72°C for 30 s); 25 standard cycles (denaturation at 95°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 30 s); and a final extension at 72°C for 20 min. PCR products were stored at 4°C until analysis. Amplification products were resolved by fluorescent capillary electrophoresis, and peak profiles were analyzed using GeneMarker software. Primer pairs were evaluated according to their polymorphic information content (PIC) and amplification quality, and screening was performed from high to low polymorphism. Primers with higher PIC values and clear, reproducible amplification bands were prioritized. Eight primer pairs showing high polymorphism and good amplification quality were retained. A second round of screening was performed using the same six samples and identical PCR conditions, resulting in the selection of seven primer pairs with high stability, specificity, and polymorphism (*Table 2*).

Table 2. *SSR primer information*

Locus	Repeat unit	Upstream primer (5′-3′)	Downstream primer (5′-3′)	Target fragments
HNWZS026	(TGTGC)4	TTTCACGATGAATTGGACGA	TGTGTCCACACACAGCACAG	107
HNWZS036	(CT)6	CACTTTGGCTTGCCAAGATAC	TCAAACTTGAGAAAGAGAGAGTTGG	100
HNWZS056	(GT)7	CAAATTGGTCAAGCCCTGTT	ACCTAAGCGGGGAGGATAAA	117
HNWZS060	(TCTCCA)5	CCTTCTTGCTCTCACCCTTG	ACATTTGGGCCTTCACTACG	107
HNWZS073	(AT)7	AAAGCCCTGAATCAGTGTGG	GATAATGTAAGGCTCCCGCA	123
HNWZS075	(AC)7	TGAATGCTTCGAGACACACAT	TTTTTCCCATGGATTCCAAA	107
HNWZS088	(AT)8	CCAGAAGGATTCACGTCACA	GCAAACCACTGCCTATGGAT	106

PCR amplification and agarose gel electrophoresis

The seven selected SSR primer pairs were used to amplify genomic DNA from all 32 samples. Each 10 μL PCR reaction contained 5.0 μL of 2× Taq PCR Master Mix, approximately 20 ng of template DNA (1.0 μL), 0.5 μL each of forward and reverse primers (10 pmol/μL), and 3.0 μL of ddH_2O. The PCR program was identical to that used during primer screening. Amplification products were analyzed by both fluorescent capillary electrophoresis and 1% agarose gel electrophoresis, and capillary separation was performed on an ABI 3730XL DNA Analyzer following the manufacturer's protocol.

Data analysis

Raw data processing

Raw .fsa files from the ABI 3730XL were organized by locus and imported into GeneMarker for allele calling. Genotype data were exported in Excel format, and electropherogram peak profiles were saved as PDF files.

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Genetic diversity analysis

Genetic diversity parameters, including the number of alleles (N_a), effective number of alleles (N_e), Shannon's information index (I), polymorphic information content (PIC), observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{is}), were calculated using GenAlEx version 6.501 and PowerMarker.

Population genetic structure analysis

Genetic distances among populations were calculated using PowerMarker, and cluster analysis was performed with the unweighted pair group method with arithmetic mean (UPGMA). A circular dendrogram was generated to visualize clustering patterns. Population structure was inferred with STRUCTURE version 2.3.4, testing K values from 1 to 20, with 20 iterations per K. The burn-in period was set to 10,000 steps, followed by 100,000 Markov chain Monte Carlo (MCMC) iterations. The optimal K value (ΔK) was determined using STRUCTURE HARVESTER. The final results were aligned and visualized using CLUMPP and DISTRUCT to reveal genetic composition and admixture patterns among populations.

Analysis of molecular variance (AMOVA) and gene flow estimation

Based on the results of the population structure analysis, AMOVA was performed in GenAlEx version 6.501 to partition genetic variation within and among populations and to assess statistical significance. The fixation index (F_{st}) and gene flow (N_m) were calculated, with Nm estimated using Wright's (1931) formula: $N_m = 0.25(1-F_{st})/F_{st}$.

Results and analysis

Polymorphism analysis of SSR primers

Seven pairs of SSR primers were used to analyze polymorphisms among 32 *P. kwangtungensis* individuals (*Table 3*). A total of 24 alleles (N_a) were detected, with an average of 3.429 alleles per primer pair. Primer HNWZS088 yielded the highest number of alleles ($N_a = 10.000$), significantly greater than that of other primers ($P < 0.01$). The total number of effective alleles (N_e) was 16.395, ranging from 1.118 to 6.474, with an average of 2.342; the highest N_e was also observed for HNWZS088. Shannon's information index (I) ranged from 0.216 to 2.002 (mean = 0.788), again with the highest value in HNWZS088. Observed heterozygosity (H_o) ranged from 0.094 to 0.875 (mean = 0.415), with HNWZS060 showing the highest H_o. Expected heterozygosity (H_e) varied from 0.106 to 0.846 (mean = 0.436), with the highest value in HNWZS088. Polymorphic information content (PIC) ranged from 0.100 to 0.827 (mean = 0.380), also highest in HNWZS088. Among the seven primers, three (HNWZS026, HNWZS056, HNWZS073) exhibited no significant polymorphism, whereas the others showed varying levels of significance. Overall, HNWZS088 demonstrated the highest polymorphism and is suitable for further genetic diversity analysis in *P. kwangtungensis*.

Genetic diversity analysis of populations

Genetic diversity among populations

Genetic diversity parameters for the three wild populations are shown in *Table 4*. N_a ranged from 2.000 to 2.571 (mean = 2.286), with the highest in the HDLT population

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(2.571) and the lowest in HJS (2.000). N_e ranged from 1.636 to 2.112 (mean = 1.857), with HDLT highest (2.112) and HJS lowest (1.636). I ranged from 0.447 to 0.745 (mean = 0.565), with HDLT highest (0.745) and YBS lowest (0.447). H_o ranged from 0.305 to 0.518 (mean = 0.437), with the highest in HJS (0.518) and the lowest in YBS (0.305). H_e ranged from 0.249 to 0.462 (mean = 0.347), with HDLT highest (0.462) and YBS lowest (0.249). Overall, HDLT exhibited the highest diversity levels across N_a, N_e, I, and H_e, while YBS showed the lowest values for I, H_o, and H_e. Although HJS had relatively low N_a and N_e, its high H_o suggests potential conservation value.

Table 3. *Polymorphism of 7 pairs of SSR primers*

Locus	N_a	N_e	I	H_o	H_e	PIC	P value	Sig.
HNWZS026	3.000	1.408	0.553	0.333	0.290	0.267	0.753	ns
HNWZS036	2.000	1.909	0.669	0.094	0.476	0.363	0.000	***
HNWZS056	2.000	1.52	0.526	0.375	0.342	0.284	0.583	ns
HNWZS060	2.000	1.97	0.685	0.875	0.492	0.371	0.000	***
HNWZS073	2.000	1.118	0.216	0.111	0.106	0.100	0.760	ns
HNWZS075	3.000	1.996	0.865	0.3	0.499	0.448	0.023	*
HNWZS088	10.000	6.474	2.002	0.815	0.846	0.827	0.004	**
Mean	3.429	2.342	0.788	0.415	0.436	0.380		
St Dev	2.936	1.851	0.571	0.313	0.229			

Sig.: Significance (ns: not significant, the group conforms to HWE; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$)

Table 4. *The genetic diversity parameters of three populations of Pinus kwangtungensis*

Population	N_a	N_e	I	H_o	H_e	F
HDLT	2.571 ± 0.571	2.112 ± 0.399	0.745 ± 0.151	0.490 ± 0.087	0.462 ± 0.059	-0.101 ± 0.171
HJS	2.000 ± 0.378	1.636 ± 0.184	0.503 ± 0.142	0.518 ± 0.155	0.330 ± 0.089	-0.552 ± 0.147
YBS	2.286 ± 0.837	1.823 ± 0.548	0.447 ± 0.245	0.305 ± 0.162	0.249 ± 0.124	-0.212 ± 0.180
Mean	2.286 ± 0.346	1.857 ± 0.226	0.565 ± 0.106	0.437 ± 0.079	0.347 ± 0.056	-0.274 ± 0.099

Genetic differentiation among populations

AMOVA (*Fig. 2*) indicated that 28% of genetic variation was among populations, 2% was among individuals within populations, and 70% occurred within individuals, suggesting that most genetic variation resides at the individual level, and differentiation among individuals exceeds that among populations. Analysis of N_m (above diagonal) and F_{st} (below diagonal) values (*Table 5*) showed the greatest gene flow between YBS and HDLT ($N_m = 1.918$), corresponding to the lowest genetic differentiation ($F_{st} = 0.115$). The lowest gene flow occurred between HJS and YBS ($N_m = 0.552$), which also showed the highest genetic differentiation ($F_{st} = 0.312$). Gene flow between HDLT and HJS was 1.382 ($F_{st} = 0.153$). These results indicate that there is a certain degree of gene exchange among the three populations, but overall they still exhibit varying levels of genetic differentiation.

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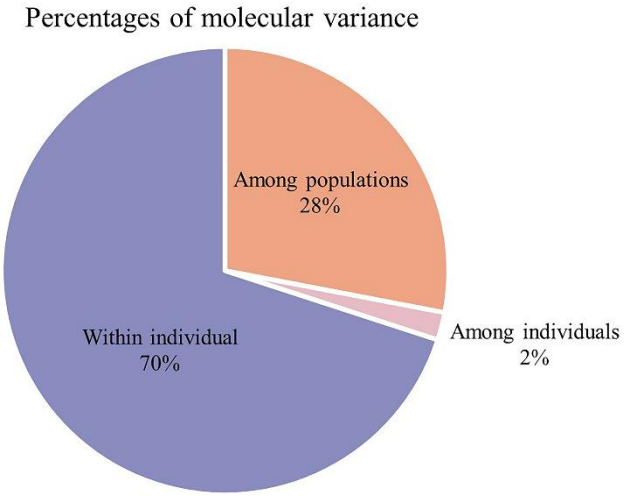

Figure 2. *Analysis of molecular variance among populations of Pinus kwangtungensis*

Table 5. *Gene flow between populations (upper triangle) and coefficient of genetic differentiation (lower triangle) of Pinus kwangtungensis*

Population	HDLT	HJS	YBS
HDLT	-	1.382	1.918
HJS	0.153	-	0.552
YBS	0.115	0.312	-

Population genetic structure

Bayesian clustering analysis using Structure revealed that Delta *K* was maximal at *K* = 2 (*Fig. 3*), indicating two distinct genetic clusters among the 32 individuals from the three populations (*Fig. 4*). Most individuals belonged predominantly to a single genetic cluster, with only a few showing admixture, suggesting limited gene flow among populations but evidence of some genetic introgression.

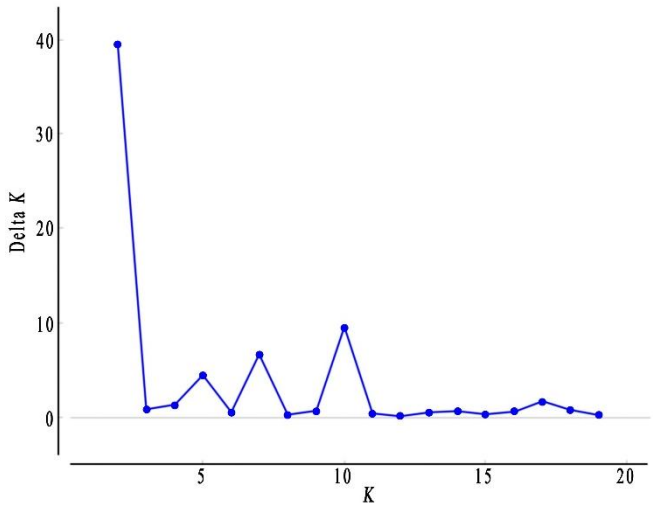

Figure 3. *Trend of the rational groups number K and estimated value Delta K*

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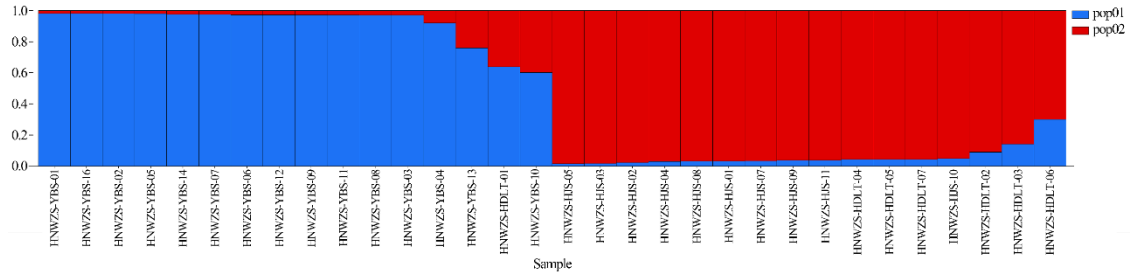

Figure 4. *Structure analysis of Pinus kwangtungensis (K = 2)*

Genetic distance and cluster analysis

The smallest geographic distance was between HDLT and HJS, while the largest was between HDLT and YBS. In contrast, genetic distance was smallest between HDLT and YBS, and largest between YBS and HJS (*Table 6*). Mantel tests showed no significant correlation between geographic and genetic distances ($P = 0.667 > 0.05$; *Fig. 5*). UPGMA clustering (*Fig. 6*) grouped YBS and HDLT into one cluster, while HJS formed a separate cluster. At the individual level (*Fig. 7*), the clustering pattern matched that of populations, with no evidence of intermixing between groups. Within clusters, individuals showed some degree of variation, reflecting within-population genetic diversity.

Table 6. *Genetic distances (upper triangle) and geographic distances (lower triangle, in km) among three Pinus kwangtungensis populations*

Population	HDLT	HJS	YBS
HDLT	-	0.202	0.197
HJS	0.646	-	0.321
YBS	122.192	121.573	-

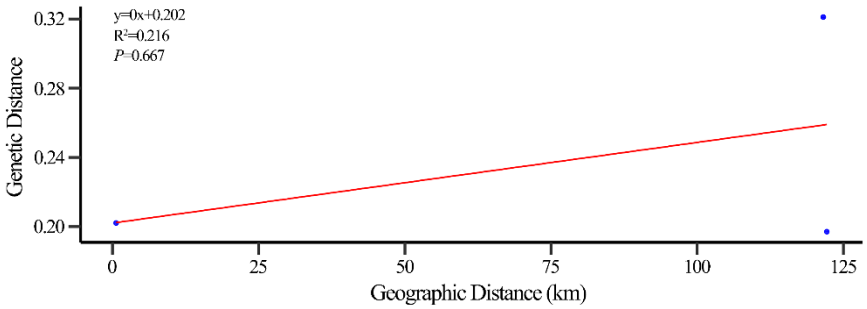

Figure 5. *Mantel test between geographic and genetic distances among three Pinus kwangtungensis populations*

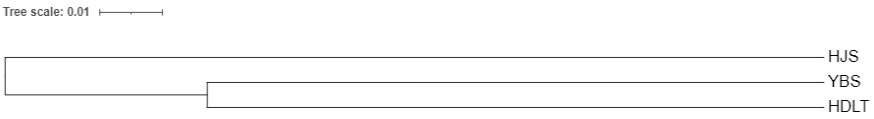

Figure 6. *UPGMA clustering results of the three populations*

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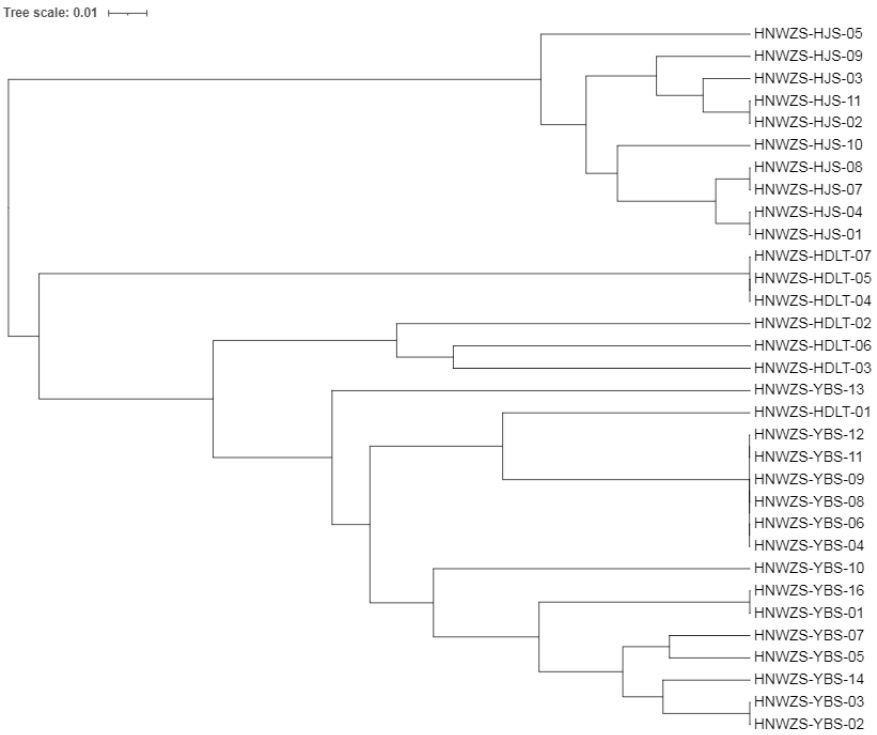

Figure 7. *UPGMA clustering results of 32 samples were obtained*

Principal coordinate analysis (PCoA) further illustrated the genetic relationships (*Fig. 8*). PC1 and PC2 explained 35.64% and 16.50% of the total variation, respectively. YBS individuals were tightly clustered in the negative PC1 axis (high genetic uniformity), HJS individuals were widely dispersed along the positive PC1 axis (greater variation), and the two were clearly separated. HDLT individuals occupied an intermediate position, with partial overlap with both YBS and HJS, suggesting it may harbor both genetic types or reflect historical gene flow. Overall, YBS and HJS were the most genetically differentiated, with HDLT representing an intermediate type exhibiting some connectivity.

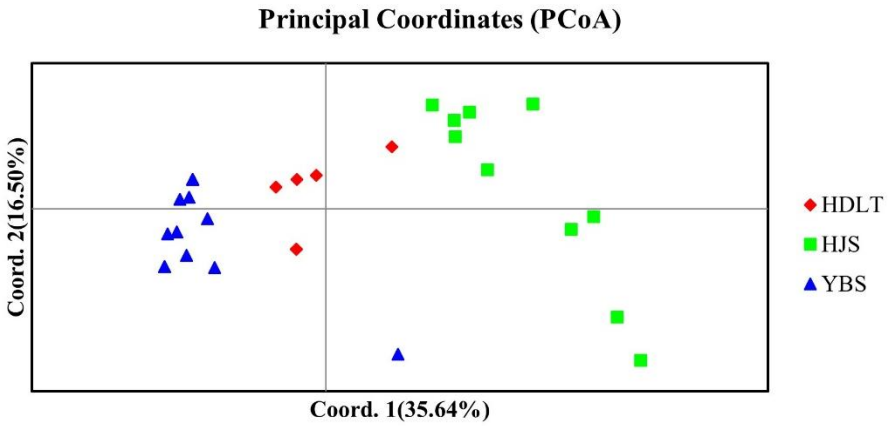

Figure 8. *Principal component analysis of 32 samples. Overlapping points occur due to highly similar genetic profiles, resulting in fewer visible points*

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Discussion

Genetic diversity of Pinus kwangtungensis

Genetic diversity, a key component of biodiversity, reflects a species' capacity to adapt to environmental change. SSR analysis enables direct assessment of genetic variation at the DNA level, providing theoretical support for the utilization and conservation of germplasm resources (Wang et al., 2025; Wen et al., 2025). In SSR marker studies, polymorphic information content (PIC) serves as an index of genetic variability. Generally, PIC > 0.5 indicates high polymorphism, reflecting rich genetic information and suitability for diversity studies; 0.25 < PIC ≤ 0.5 denotes moderate polymorphism; and PIC ≤ 0.25 indicates low polymorphism with limited discriminatory power (Xiao et al., 2014). In this study, seven primer pairs were selected from 96 candidates for genetic diversity analysis of three *P. kwangtungensis* populations comprising 32 specimens. Among them, one primer pair (HNWZS088) exhibited high polymorphism (PIC > 0.5), five primer pairs (HNWZS026, HNWZS036, HNWZS056, HNWZS060, HNWZS075) showed moderate polymorphism (0.25 < PIC ≤ 0.5), and one primer pair (HNWZS073) displayed low polymorphism (PIC ≤ 0.25). The average PIC across all loci was 0.380 (>0.25), indicating an overall moderate level of polymorphism, sufficient for reliable genetic diversity assessment.

In general, plant species with broad distribution ranges and large population sizes tend to exhibit high genetic diversity, enhancing their adaptive capacity and evolutionary potential, which in turn promotes population stability and expansion. Conversely, endangered or geographically restricted species often show low genetic diversity due to small population sizes and limited gene flow, reducing adaptability and constraining survival and dispersal (Yang et al., 2025; Wang et al., 2024). In *P. kwangtungensis*, the mean Shannon's information index (I) was 0.565 and the mean expected heterozygosity (H_e) was 0.347—significantly lower than in widespread *Pinus* species, such as *P. armandii* (I = 1.122, H_e = 0.605) (Zhu, 2016), and slightly lower than *P. massoniana* (I = 0.810, H_e = 0.445), *P. densata* (I = 0.825, H_e = 0.471), and *P. yunnanensis* (I = 0.809, H_e = 0.430) (Xu et al., 2017). Compared with some endangered *Pinus* species, *P. kwangtungensis* had higher diversity than *P. bungeana* (I = 0.313, H_e = 0.199) (Zhao et al., 2014) but slightly lower than *P. koraiensis* (I = 0.665, H_e = 0.381) (Shang et al., 2025). These results suggest that although *P. kwangtungensis* has not reached the critically low genetic diversity typical of severely endangered species, its relatively low diversity may still limit its capacity to withstand environmental stress and maintain population stability.

Among the three wild populations studied, the HDLT population exhibited the highest genetic diversity, indicating a relative advantage in maintaining genetic variation. This population could serve as a valuable germplasm source for future conservation, artificial introduction, and genetic improvement programs. In contrast, the HJS and YBS populations exhibited lower diversity, warranting priority attention in future management, including both in situ and ex situ conservation measures to prevent further genetic erosion or localized extinction.

Genetic structure and differentiation of Pinus kwangtungensis

The fixation index (F_{st}) is commonly used to quantify population differentiation, with F_{st} < 0.05 indicating negligible differentiation, 0.05–0.15 indicating moderate differentiation, and 0.15–0.25 indicating high differentiation (Balloux and Lugon-

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Moulin, 2002). In *P. kwangtungensis*, F_{st} values among the three populations ranged from 0.115 to 0.312 (mean = 0.193), indicating substantial genetic differentiation, with 19.3% of the total genetic variation occurring among populations. Analysis of molecular variance (AMOVA) confirmed these results, revealing that 70% of variation was within individuals, 2% was among individuals within populations, and the majority of genetic diversity was maintained at the individual level.

Gene flow (N_m) is a major factor influencing the distribution of genetic variation within and among populations. Higher N_m values are associated with reduced interpopulation differentiation, as gene flow can counteract the effects of genetic drift. When $N_m > 1$, gene flow is generally sufficient to offset drift, whereas $N_m < 1$ indicates that drift becomes the dominant driver of differentiation (Lin, 2018; Li et al., 2012). In this study, N_m ranged from 0.552 to 1.918 (mean = 1.284), indicating an overall moderate-to-high level of gene flow among populations. Geographically, the HDLT and HJS populations are relatively close, with $N_m = 1.382$, suggesting frequent exchange. Interestingly, HDLT and YBS—despite being farther apart—exhibited the highest N_m (1.918), possibly due to long-distance pollen dispersal via wind. In contrast, YBS and HJS, also geographically distant, had the lowest N_m (0.552), indicating weak genetic exchange and potential genetic isolation. This suggests that geographic distance alone does not determine gene flow intensity; factors such as pollination mode, prevailing winds, and topographic barriers may also play important roles (McRae, 2006; Kling and Ackerly, 2021). Similar patterns have been reported in *P. taiwanensis*, where wind-mediated pollination maintained high gene flow despite geographic isolation, thereby preventing significant genetic differentiation (Luo et al., 2022). Studies on *P. yunnanensis* reached comparable conclusions (Xu et al., 2016). However, this contrasts with findings in *Cathaya argyrophylla*, where geographic isolation substantially limited interpopulation gene exchange (Yang et al., 2025).

The Structure analysis and cluster analysis (UPGMA) of *P. kwangtungensis* populations clearly grouped the three populations into two main clusters: YBS and HDLT formed one cluster, while HJS stood alone. Although principal component analysis (PCA) separated the samples into three groups, this primarily reflects subtle genetic differences among individuals within the HDLT population. Overall, the main genetic divergence remains between YBS–HDLT and HJS, supporting the conclusion that the three sampling locations can be effectively categorized into two genetic clusters. This clustering pattern did not align with a clear geographic distribution. Consequently, conservation of *P. kwangtungensis* should emphasize in situ protection of the YBS and HJS populations, with artificial interventions—such as assisted migration or pollen introduction—used when necessary to enhance connectivity. The HDLT population should be prioritized as a genetic reservoir to maintain the species' overall genetic stability and evolutionary potential.

Conclusions

Habitat protection is one of the most effective strategies for prolonging the lifespan of endangered plants and restoring wild populations (Deng et al., 2023). Pinus kwangtungensis, a rare and endemic five-needle pine in China, has a narrow geographic range, weak natural regeneration, and specialized habitat requirements, making it of high conservation value. To assess its genetic diversity and inform conservation strategies, we analyzed the genetic diversity and structure of three wild populations in Guangxi using

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SSR markers. The HDLT population had the highest genetic diversity and should be given priority for conservation. Given the lower diversity in HJS and YBS, and the weak gene flow between them, we recommend establishing small-scale reserves at each site for in situ conservation to maintain genetic stability. Dynamic monitoring of these two small populations should be implemented to track demographic changes and survival probabilities. For ex situ conservation, priority should be given to collecting germplasm from HDLT and YBS for systematic introduction and breeding trials to enhance genetic diversity in cultivated stocks. Considering the scarcity of *P. kwangtungensis* seed sources and its limited natural regeneration, further studies should focus on artificial pollination, seedling propagation, biological characteristics, and cultivation management to improve propagation efficiency. In addition, both in situ and ex situ reintroduction trials should be gradually implemented to expand population size and connectivity, thereby enhancing genetic diversity and environmental adaptability, ensuring the long-term survival and sustainable utilization of *P. kwangtungensis*.

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