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Genetic diversity and population structure of *Dalbergia cochinchinensis* Pierre ex Laness. in Vietnam's Central Highlands using inter-simple sequence repeat markers

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Abstract:

***Dalbergia cochinchinensis* is classified as a critically endangered (CR) species according to the 2022 IUCN Red List [1]. This study aims to assess the genetic diversity of *D. cochinchinensis* populations in Kon Tum, Gia Lai, and Dak Lak, located in the Central Highlands of Vietnam, using the ISSR method. In addition, it seeks to support the conservation and propagation of *D. cochinchinensis*. The amplified products varied in size from 150 to 3,500 bp. Out of 101 loci examined, 93 (92.08%) were found to be polymorphic, while 8 (7.92%) were monomorphic. Most primers exhibited polymorphic loci ranging from 6 to 16. ISSR analysis revealed genetic identity levels among the three populations ranging from 0.749 (Dak Lak and Kon Tum) to 0.896 (Gia Lai and Kon Tum). The total genetic diversity of the species (H_T) was calculated to be 0.261, with the average heterozygosity of the populations (H_S) being 0.166. Additionally, genetic variation within the three populations of *D. cochinchinensis* accounted for the majority of the total genetic variation (G_{ST}=0.361 and F_{ST}=0.432), indicating significant diversity. These findings provide important insights into the genetic structure of *D. cochinchinensis*, which can guide conservation and propagation efforts both in Indochina and globally.**

Keywords: **conservation, critically endangered plant, *Dalbergia cochinchinensis*, genetic diversity, ISSR markers, population structure.**

Classification numbers: **3.4, 3.5**

1. Introduction

The scientific designation for this plant is *Dalbergia cochinchinensis* Pierre ex Laness., commonly referred to as Mai Ka Young in Laos, Payoong in Thailand, and Thailand or Siamese rosewood in Cambodia. It belongs to the genus *Dalbergia* of the family *Fabaceae*. According to J. Dorthe (2000) [2], *D. cochinchinensis* is a species with diverse morphological traits. A mature tree reaches a height of 25-30 m and a trunk diameter ranging from 60 to 120 cm (Fig. 1). Its bark is yellow-brown, characterised by cracks and peeling. The heartwood varies from red to black in colour and emits a distinctive fragrance. Notably, the tree tends to develop buttresses, which are of research interest for improving wood quality in production practices. The compound leaves are pinnately or nearly opposite, comprising 3 to 4 pairs of leaflets along with one terminal leaflet. These green leaflets are ovate or elliptical with smooth margins. The white flowers, which form clusters with bracts attached, are roughly 15-20 cm in length [3]. The fruits are flat and smooth-skinned, maturing from yellow to brown, and measure 5-6 cm long and about 1 cm wide. Each pod typically contains 1 to 3 kidney-shaped, reddish-brown seeds. *D. cochinchinensis* flowers between September and November, with fruits ripening at the onset of the dry season (December - January). Despite leaf shedding during this period, the species produces new buds and demonstrates robust regeneration through both seeds and shoots [2].

D. cochinchinensis naturally inhabits several regions across Vietnam, Laos, Cambodia, and Thailand. It is characterised as an indigenous species exclusive to

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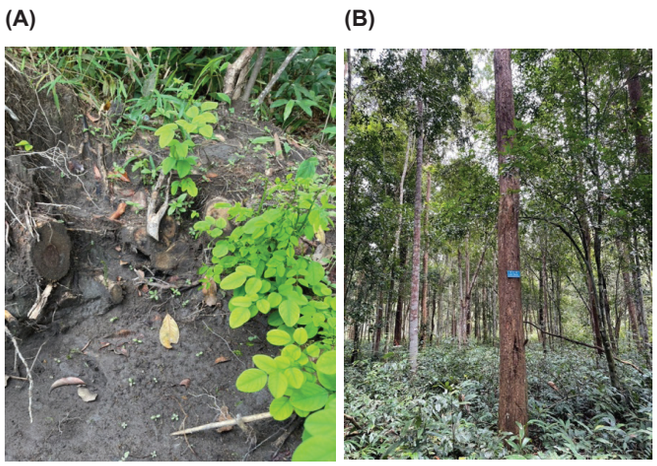

Fig. 1. *Dalbergia cochinchinensis* grows naturally alongside the road and in the Dak Uy Reserve Forest. (A) *D. cochinchinensis* saplings regenerate from the stump and roots of the felled mother tree; **(B)** Mature *D. cochinchinensis* tree individuals are preserved in Dak Uy Reserve Forest.

the Indochina area. In the remaining communes, *D. cochinchinensis* has only been documented in scattered occurrences. Globally recognised as a biodiversity hotspot, Southeast Asia faces substantial challenges stemming from the widespread destruction of its primary habitats and unprecedented levels of deforestation. Thus, protecting species is paramount to preventing widespread extinctions, as they significantly contribute to sustaining the delicate balance of ecosystems. *D. cochinchinensis* is classified as endangered (EN) in the Red Data Book of Vietnam (2007) [3] and critically endangered (CR) in the IUCN Red List of Threatened Species (2022) [1]. According to the IUCN Red List of Threatened Species, *D. cochinchinensis* is currently categorised as critically endangered under criteria A2cd+4cd (version 3.1) [1].

This endemic species, native to Southeast Asia, has been prioritised for conservation since 2020 due to its shrinking habitat in the region [4]. Wild populations of this rosewood species have declined rapidly due to uncontrolled exploitation, extensive trade, and habitat destruction. Evaluating genetic diversity requires the consideration of both qualitative and quantitative aspects. Characterisation based solely on morphological traits is less reliable, as these variations can be influenced by environmental factors.

Molecular markers provide a more accurate and reliable means of detecting variations within landraces compared with traditional approaches. The primary advantage of Inter-Simple Sequence Repeat (ISSR) markers lies in their ability to generate multiple bands at the same locus, demonstrating high reproducibility without requiring prior genomic information of the plant. Owing to their high level of polymorphism, ISSR markers are widely used. Furthermore, the findings of this study will contribute to enhancing the genetic diversity database for *D. cochinchinensis* and provide a foundation for conservation efforts aimed at improving this species in Southeast Asia.

Inter-Simple Sequence Repeat markers are widely recognised as effective molecular tools for assessing plant genetic diversity. Due to their simplicity, reproducibility, and high polymorphism, ISSR markers have been extensively applied in the genetic analysis of various plant taxa, including *Diospyros kaki* (persimmon) [5], barley genotypes [6], and species within the genus *Eplingiella* [7]. Furthermore, ISSR markers are often integrated with other marker systems to enhance the resolution of genetic diversity assessments. For instance, a combination of ISSR and RAPD markers has been employed in studies of *Bruguiera gymnorrhiza* [8], while ISSR and SCoT markers have been used to investigate the genetic variation of wheat [9].

Within the genus *Dalbergia*, ISSR markers continue to serve as valuable tools for evaluating genetic diversity among key species. Several studies have examined the genetic diversity of important *Dalbergia* species using molecular markers. For example, *Dalbergia sissoides* - a highly valued timber tree species in South Asia - has been studied using RAPD markers [10] and through a combination of ISSR and RAPD markers [11]. The genetic diversity of *Dalbergia odorifera* [12] and *Dalbergia latifolia* in Yogyakarta, Indonesia, has also been investigated using ISSR markers [13]. In addition, the genetic diversity of *D. cochinchinensis* has been explored using both RAPD and ISSR markers [14-16]. I. Hartvig, et al. (2018) [17] reported the population genetic structure of the endemic *D. cochinchinensis* and *Dalbergia oliveri*. Moreover, genetic variability within lowland forests in Cambodia and Thailand has been assessed in two tropical leguminous trees, *D. cochinchinensis* and *Dalbergia nigrescens*, using the MIG-seq method [18]. The genetic diversity of the endangered *D. oliveri* (Fabaceae) genotypes in Vietnam has also been evaluated using RAPD and ISSR markers [19].

To the best of our knowledge, this is the first comprehensive study to evaluate the genetic diversity of *D. cochinchinensis* using ISSR markers across a large sample size and a broad range of geographic

locations in Vietnam. The findings provide critical insights into the genetic structure and diversity of this species, both within Vietnam and in a broader global context. Given its current status as Critically Endangered and inclusion on the IUCN Red List, this study offers a valuable scientific foundation for the development of targeted conservation and propagation strategies. Moreover, the genetic patterns revealed in this study generate important hypotheses regarding the historical formation, development, and dispersal of *D. cochinchinensis* populations in Vietnam's Central Highlands.

2. Materials and methods

2.1. Plant materials

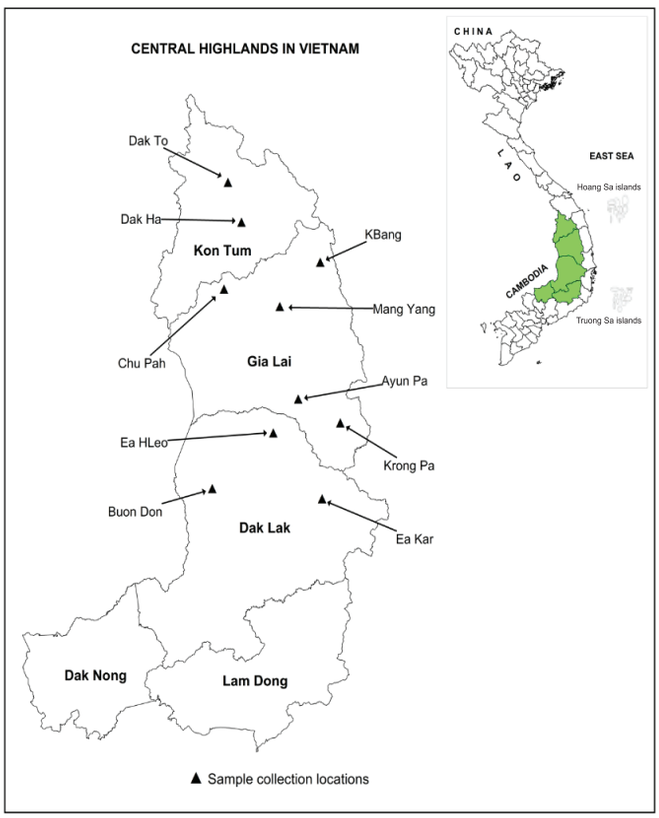

Fig. 2. The geographical location of three *D. cochinchinensis* populations in Northern Central Highlands (Vietnam). Source: Authors' compilation.

Individuals of *D. cochinchinensis* were gathered from various locations including the Ialy Protected Forest, Kon Ka Kinh National Park, Dak Uy Special Use Forest, and other natural resources (Fig. 2). A total of 115 individuals were collected from three distinct populations of *D. cochinchinensis* originating from Dak Lak, Kon Tum, and Gia Lai provinces in the Northern Central Highlands region of Vietnam. The Dak Lak population encompasses three areas, while the Kon Tum population covers two, and the Gia Lai population spans four (Table 1). The collection sites ranged in altitude from 162 to 996 meters and leaf tissue samples from these populations were preserved at -80°C for further experiments.

Table 1. Original sites and coordinates of the studied accessions.

Populations	Location	Latitude (N)	Longitude (E)
Gia Lai (GL)	Chu Pah	14° 09' 59.364" - 14° 09' 14.922"	107° 47' 39.617" - 107° 48' 26.442"
	Mang Yah	13° 48' 04.370" - 14° 15' 52.250"	108° 16' 23.030" - 108° 19' 51.647"
	Kbang	14° 15' 26.125" - 14° 16' 08.231"	108° 34' 28.834" - 108° 35' 09.694"
	Krong Pa	13° 13' 37.808" - 13° 21' 16.762"	108° 35' 09.694" - 108° 46' 19.171"
Kon Tum (KT)	Dak Ha	14° 32' 07.256" - 14° 33' 10.832"	107° 58' 11.435" - 107° 53' 42.662"
	Dak To	14° 37' 31.458" - 14° 40' 01.416"	107° 54' 20.653" - 107° 55' 53.555"
Dak Lak (DL)	Ekar	12° 55' 08.854" - 13° 00' 40.532"	108° 32' 05.071" - 108° 34' 49.724"
	Buon Don	13° 01' 51.564" - 12° 54' 29.401"	107° 37' 29.755" - 107° 48' 00.828"
	Eahleo	13° 14' 15.814" - 13° 23' 12.300"	107° 48' 00.828" - 108° 08' 25.148"

2.2. Genomic DNA extraction

Leaf tissue was ground into a fine powder in liquid nitrogen and subsequently washed with buffer (10 ml Tris HCl 1M, 10 ml EDTA, 12 g $Na_3PO_4.12H_2O$, 6.4 g Sorbitol for 100 ml) to remove secondary metabolites that could interfere with DNA extraction. Genomic DNA was extracted from the leaves of species *D. cochinchinensis* using the CTAB method [20]. DNA concentration and purity were determined using a JASCO V-730 spectrophotometer. The quality of the extracted DNA was assessed by electrophoresis on a 2% agarose gel, visualised alongside a 1 kb DNA ladder. All DNA samples were stored at -20°C until further use.

2.3. ISSR markers used in the research and PCR conditions

In the present investigation, 17 ISSR primers were tested. The universal primers (UBC), designed at the Biotechnology Laboratory, University of British Columbia, Canada, are widely used by researchers (Table 2). For ISSR analysis, 17 different primers were tested, resulting in band formation with 9 of them. Initially, three individuals from each population were subjected to a preliminary screening. Then, primers yielding a minimal proportion of polymorphic markers were eliminated from further consideration. Finally, only primers generating consistent and clearly discernible bands were selected for the final assessment.

Table 2. Names of primers and their sequences used in the research from The University of British Columbia.

No	Primer	Sequence (5'-3')
1	UBC864	(ATG)6
2	UBC824	(TC)8G
3	UBC815	(CT)8G
4	UBC854	(TC)8RG
5	UBC853	(TC)8RT
6	UBC850	(GT)8YC
7	UBC895	AGAGTTGGTSGCTCTTGATC
8	UBC845	(CT)8RG
9	UBC866	(CTC)6
10	UBC874	(CCCT)4
11	UBC846	(CA)8RT
12	UBC852	(TC)8RA
13	UBC848	(CA)8RG
14	UBC900	ACTTCCCACAGGTTAACACA
15	UBC860	(TG)6RA
16	UBC857	(AC)8YG
17	UBC834	(AG)8YT

The PCR reaction selecting the optimal annealing temperature was performed on a Proflex PCR machine (Applied Biosystem, USA) according to the temperature range design. The PCR reaction was performed on the PCR System 9700 (Applied Biosystem, USA) with a total volume of 25 µl/sample which contained the following ingredients and concentrations of reactants: DreamTaq Green Master Mix 2X 12.5 µl; Primer 1 µl (10 µM); DNA template (50 ng/µl) 2 µl; nuclease-free water 9.5 µl. The PCR amplification program was carried out using the following conditions of an initial denaturation at 95°C for 4 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at the primer specific temperature for 70 seconds, extension at 72°C for 2 min and final extension was adjusted at 72°C for 10 min, followed by saturation at 4°C. Each PCR batch included a negative control containing nuclease-free water to verify the absence of contamination and ensure the accuracy and purity of the reagents.

2.4. Statistical analysis

All *D. cochinchinensis* samples were compared based on the banding pattern of specific primers, and ISSR primers were analysed by scoring the presence and absence of bands as "1" and "0", respectively. The binary data generated were used to compute primer banding characteristics, including the number of scored bands and the percentage of polymorphic bands. Standard marker analysis was conducted to enhance the discriminatory power, informativeness, and polymorphism level of ISSR markers, along with PCoA using GenAIEx 6.5 [21]. The genetic differentiation coefficient (G_{ST}), total genetic diversity (H_T), genetic diversity within populations (H_S), Shannon index (I), and gene flow (Nm), as well as Na (observed number of alleles), Ne (effective number of alleles), H_E (expected heterozygosity), I (Shannon's information index), PPB% (percentage of polymorphic loci), and uH_E (unbiased expected heterozygosity), were calculated using Popgene 1.32 software [22]. Bayesian clustering algorithms were employed to detect plant population structure using STRUCTURE software, with a burn-in period of 1,000 iterations and 10,000 MCMC repetitions after burn-in [23]. The resulting output files from STRUCTURE were visualised using the CLUMPAK website [24, 25].

3. Results

3.1. ISSR markers informativeness

A total of 9 ISSR primers were selected from a pool of 17 ISSR primers employed for evaluating the genetic diversity within 115 samples of *D. cochinchinensis*. The size of amplification products ranged from 150 to 3500 bp (Fig. 3). The range of polymorphism percentages (%PPB) extended from 75% to 100% (Table 3).

Table 3. Summary of ISSR primers used in this study and their extent of polymorphism.

No	Primer	Ta	Size range (bp)	TB	PB	MB	PPB
1	UBC815	50	600-3000	7	6	1	85.71
2	UBC866	57	690-3500	6	6	0	100
3	UBC846	50	300-1500	9	8	1	88.88
4	UBC848	55	260-3500	18	15	3	83.33
5	UBC857	55	190-3500	16	16	0	100
6	UBC834	52	150-3000	14	14	0	100
7	UBC850	56	250-3000	16	16	0	100
8	UBC900	41.5	250-1000	7	6	1	85.71
9	UBC853	52	1400-3000	8	6	2	75

Ta: annealing temperature; TB: total number of amplified bands; PB: polymorphic bands; MB: monomorphic bands; %PPB: the percentage of polymorphism.

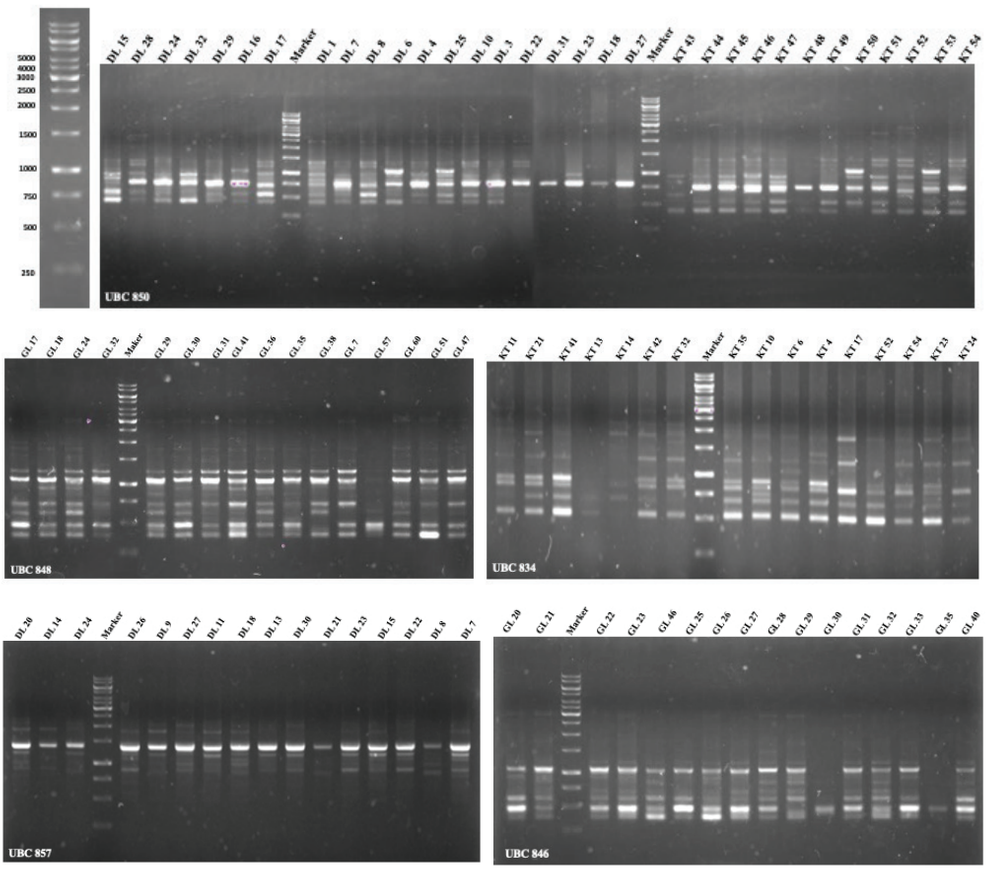

Fig. 3. Genetic profiling of different individuals of *D. cochinchinensis* using ISSR primers (DNA marker 1 kb).

A total of 101 ISSR loci were amplified across 115 individuals of *D. cochinchinensis* selected in our study. Out of 101 loci, 93 (92.08%) were found to be polymorphic and 8 (7.92%) loci were monomorphic. Most of the primers were found to include from 6 to 16 polymorphic loci.

3.2. Genetic diversity in population

The highest value of the Shannon index (I) was 0.296 in the DL population. The KT population exhibited the lowest observed number of alleles (N_a=1.108), effective number of alleles (N_e=1.174), expected heterozygosity (H_E=0.117), and unbiased expected heterozygosity (uH_E=0.118). The percentage of polymorphic loci for each population ranged from 58.06% (GL) to 73.12% (DL), with an average of 61.65% (Table 4). Genetic variability among the populations, as indicated by the expected heterozygosity (H_E), showed that the DL population possessed a greater level of variability, with a value of 0.188, compared with the other populations (Table 4).

Table 4. Mean over loci for each population.

Populations	N	N_a	N_e	I	H_E	uH_E	PPB%	PrB	BF
Kon Tum	43	1.108	1.174	0.191	0.117	0.118	53.76	9	44
Dak Lak	28	1.538	1.305	0.296	0.188	0.192	73.12	17	68
Gia Lai	44	1.183	1.231	0.229	0.145	0.146	58.06	3	50
Mean	38.33	1.276	1.237	0.239	0.150	0.152	61.65	9.66	54

N (sample size), N_a (observed number of alleles), N_e (effective number of alleles), I (Shannon's information index), H_E (expected heterozygosity), uH_E (unbiased expected heterozygosity), PPB% (percentage of polymorphic bands), PrB (number of private bands), BF (number of different bands with a frequency ≥5%).

3.3. Genetic distance

The result of Nei's genetic identity was shown in Table 5. ISSR analysis showed that the genetic identity between the three populations ranged from 0.7498 (DL and KT) to 0.8960 (GL and KT) and also indicated that GL population exhibited the highest similarity with the KT population, while DL and KT showed the lowest similarity. The genetic distance ranged from 0.1098 (GL and KT) to 0.2879 (DL and KT) (Table 5).

Table 5. Pairwise population matrix Nei (1978): genetic identity (above diagonal), genetic distance (below diagonal).

Population	KT	DL	GL
KT	0.0000	0.7498	0.8960
DL	0.2879	0.0000	0.8513
GL	0.1098	0.1610	0.0000

3.4. Genetic structure

POPGENE 1.32 software was used to calculate the genetic differentiation levels among different populations of *D. cochinchinensis*. The total genetic diversity for the species (H_T) was 0.261, while the mean heterozygosity within populations (H_S) was 0.166. The total genetic variation in the three populations of *D. cochinchinensis*

occurred among populations (G_{ST}=0.361), indicating the most genetic variation existed within populations. Gene flow levels (N_m) of the populations based on ISSR analyses was 0.882, suggesting significant genetic differentiation among populations (Table 6). The gene flow (N_m) based on G_{ST} was measured at 0.882. The results of this analysis suggest that the gene flow among populations throughout the entire distribution area is restricted with a high genetic differentiation.

Table 6. Genetic diversity of *D. cochinchinensis* populations based on ISSR markers.

	H_T	H_s	N_m	G_{ST}	F_{ST}	H	I
Mean	0.261	0.166	0.882	0.361	0.432	0.251	0.395

H_T: total genetic diversity, H_S: genetic diversity within populations, G_{ST}: genetic differentiation coefficient, N_m: geneflow, H: Nei's genetic diversity.

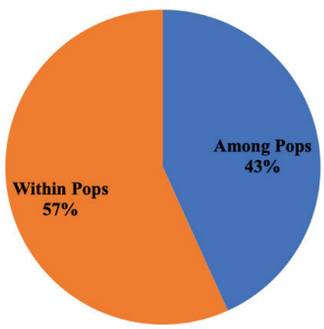

Fig. 4. Percentages of molecular variance (AMOVA analysis).

Overall, the results show that three populations of *D. cochinchinensis* exhibit a high level of genetic variation and high level of genetic differentiation between them (with G_{ST}=0.361 and F_{ST}=0.432).

AMOVA analysis uncovered significant genetic diversity within populations, accounting for 57% of the total variation, while indicating comparatively lesser genetic variance among populations at 43% (Fig. 4).

Principal Coordinates Analysis (PCoA) revealed that groups geographically close to one another exhibited the lowest genetic distances, whereas populations originating from distinct locations displayed the greatest genetic distances. Among the three studied populations, samples from the KT population of *D. cochinchinensis* demonstrated the highest level of genetic similarity. The genetic distances among the three populations were distributed relatively independently, indicating a substantial degree of differentiation among them.

Using ISSR markers, the first principal coordinate accounted for 38.6% of the total variation, followed by the second coordinate with 12.21% and the third coordinate with 6.79%. Minimal introgression between the gene pools was observed in the comparison between dimensions 1 and 2 (Fig. 5).

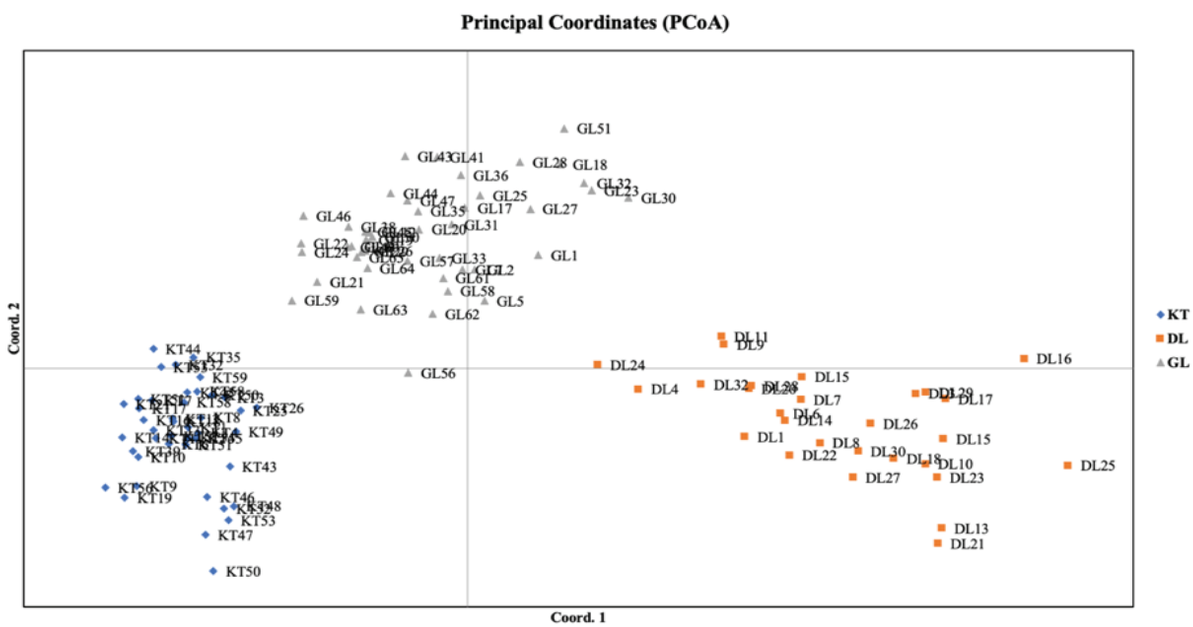

Fig. 5. Principal Coordinates (PCoA) illustrating genetic relationship among different samples of *D. cochinchinensis* using 9 ISSR markers.

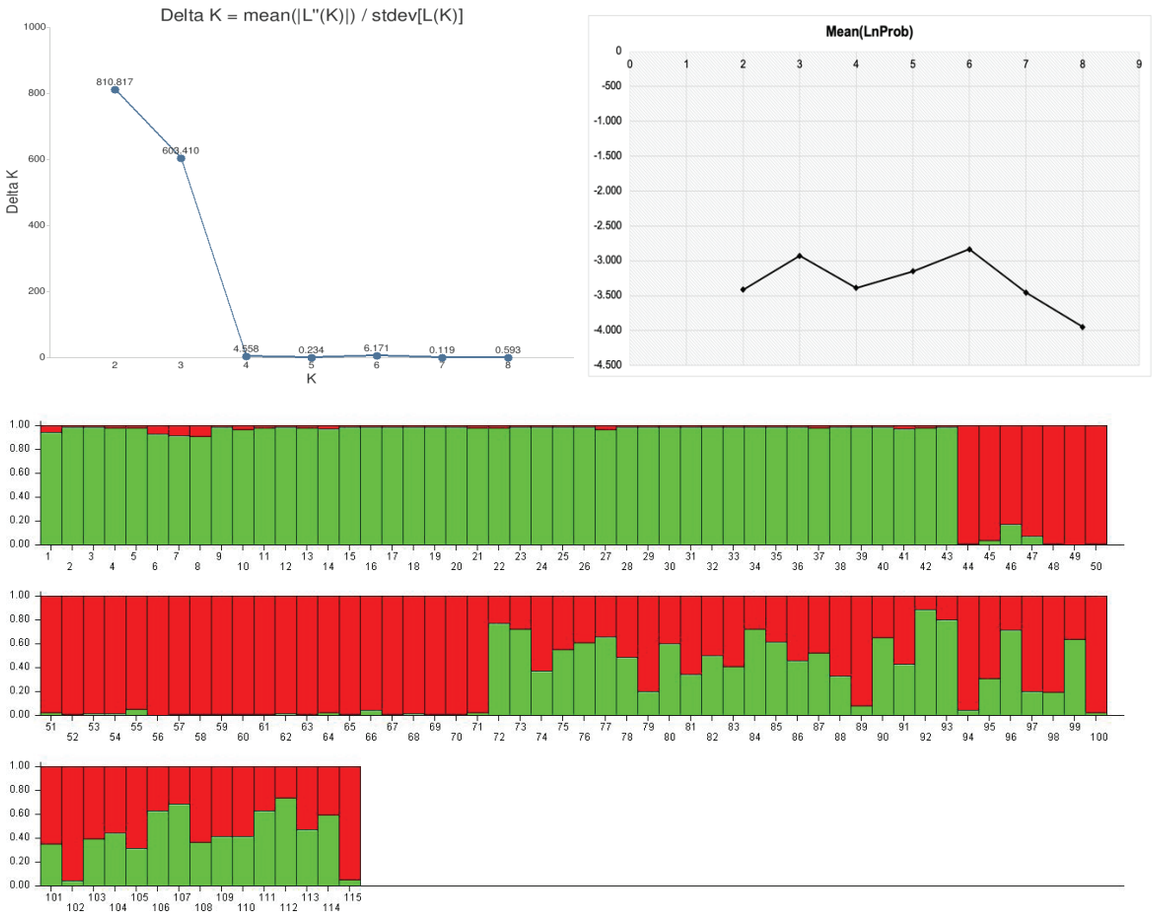

Fig. 6. Genetic structure plot showing similarities and differences in the *D. cochinchinensis* populations K=2, similarity score =0.995 (From 1 to 43: KT, from 44 to 71: DL, from 72 to 115: GL).

The STRUCTURE software utilises a Bayesian clustering algorithm to infer the most probable number of genetic clusters within a population. The results were visualised using the CLUMPAK platform (Fig. 6). Based on the ΔK, which identifies the uppermost hierarchical level of population structure, the optimal number of clusters (K) was determined to be two with similarity score=0.995. Accordingly, population *D. cochinchinensis* was partitioned into three distinct genetic groups. The first group (KT) and the second group (DL) comprised individuals with high genetic purity, whereas the third group (GL) exhibited evidence of admixture, sharing genetic components with both the DL and KT groups.

STRUCTURE analysis revealed that the DL and KT populations were the most genetically distinct, with clear clustering into three groups, indicating significant genotypic differentiation. Notably, individuals from the GL population exhibited high levels of introgression and possessed the highest proportion of heterozygous loci. These findings support the hypothesis of historical gene flow - either natural or anthropogenic - among populations.

Furthermore, Bayesian clustering analysis conducted using STRUCTURE indicated that *D. cochinchinensis* individuals were divided into two genetically distinct groups with minimal overlap. Most individuals within these groups exhibited high genetic purity and low admixture levels. These results are consistent with previous observations of limited gene flow and rare interpopulation contact, as well as low heterozygosity (H_E). Collectively, these indicators raise conservation concerns, suggesting that without effective propagation and management strategies, the species may face a decline in genetic diversity. This could lead to the loss of valuable alleles within *D. cochinchinensis* populations in Vietnam's Central Highlands and, more broadly, throughout Southeast Asia.

4. Discussion

Genetic diversity is crucial for the long-term survival of species and plays a significant role in improving them through genetic breeding programmes. Despite its importance, limited information is available on the genetic diversity of *D. cochinchinensis*. Several evolutionary factors, such as mating system, gene flow, seed dispersal, reproductive mode, and natural selection, influence the genetic structure of a species [26]. The genetic differentiation coefficient (G_{ST}) of the investigated *D. cochinchinensis* populations is 0.361, exceeding that of most dicotyledonous plants, which typically have a G_{ST} value of approximately 0.32 [27]. The G_{ST} value ranges from 0 to 1, where values below 0.05 indicate minimal genetic differentiation, 0.05-0.15 represent moderate differentiation, and 0.151-0.25 denote substantial differentiation. G_{ST} values greater than 0.25 suggest significant genetic divergence. Importantly, the estimation of G_{ST} is heavily dependent on the H_T value [28].

The gene flow (N_m) for *Dalbergia sissoo* is calculated to be 3.3125 and G_{ST}=0.1311 [10]. *D. sissoo*, a durable woody plant, relies on insect pollination, and its seeds are equipped with wings for dispersal by wind, facilitating gene flow between populations. The extensive planting of *D. sissoo* on plantations reduces the distance between natural populations, facilitating an uninterrupted genetic interchange among them [29]. The gene flow (N_m) for *D. odorifera* calculated 2.58 [30], whereas for *D. cochinchinensis*, it stands at 0.882. In this research, the gene flow for *D. cochinchinensis* is 0.882 and G_{ST}=0.361. Restricted gene flow between *D. cochinchinensis* populations leads to increased genetic differentiation, with a G_{ST} value of 0.361. Low gene flow enhances the effect of genetic drift, which greatly influences the genetic structure of *D. cochinchinensis*. Research data indicates that the genetic structure of *D. cochinchinensis* is characterised by limited gene flow between populations. This is due to overexploitation, which has reduced the species' distribution area, and geographical separation, which restricts pollination. Consequently, low gene flow results in significant genetic differences between populations. Genetic drift randomly alters allele frequencies from generation to generation. This phenomenon has its most significant impact when gene flow is at a low level. The random changes in frequency can lead to the extinction of successful mutants and decrease the genetic variation in these smaller populations, compared to those not experiencing genetic drift [31]. Genetic drift significantly affects populations with small sizes and low gene flow. It typically reduces genetic variation in these populations, unlike in large populations, where genetic drift tends to increase the variation of gene flow [32]. In small effective populations size, random genetic drift plays a more significant role than selection in determining the fate of new alleles. Consequently, small populations tend to accumulate harmful mutations. If these fixed alleles are not counteracted, they can diminish the reproductive capacity of the species, potentially leading to extinction. However, new beneficial mutations, if fixed by selection, can help recover some of the lost fitness [33]. Subpopulation size is the most influential factor in determining genetic load. Additionally, connecting subpopulations through migration becomes crucial for population fitness when subpopulations are small and gene flow is limited [34]. Gene flow functions by reducing genetic differences between populations. In *D. cochinchinensis* populations with low Nm values, the transfer of genetic information between populations is severely restricted. This limitation can be attributed to the dwindling distribution of *D. cochinchinensis* in the wild, largely due to exploitation. As an obligate pollination species, the considerable geographical distances and restricted distribution range contribute to the constrained gene flow (N_m) between populations, with the N_m of *D. cochinchinensis* measured at only 0.882.

The *D. cochinchinensis* populations analysed in this study exhibited a high level of genetic diversity, as indicated by an F_{ST} value of 0.432. This value is considerably higher than that reported for *Dalbergia melanoxylon* (F_{ST}=0.133) [35], whose low F_{ST} suggests limited genetic differentiation and, consequently, lower genetic diversity. Similarly, *Dalbergia stevensonii* exhibited an F_{ST} of only 0.063 [36], also indicating minimal genetic differentiation. In *Dalbergia nigra*, the F_{ST} value was 0.118 [37], while in another study, *D. odorifera* showed an even lower F_{ST} of 0.034 [38]. A comparison of F_{ST} and G_{ST} statistics reveals marked genetic differentiation among the clusters of *D. cochinchinensis* identified in this study, indicating a high level of genetic isolation. This finding contrasts sharply with previous studies on other species within the genus *Dalbergia*, which generally exhibit lower interpopulation divergence.

The GL population, located geographically between the KT and DL populations, was shown - based on Bayesian clustering analysis - to have a low proportion of genetic pure individuals and signs of genetic admixture, likely resulting from gene flow between neighbouring populations. In contrast, both the KT and DL populations exhibit strong genetic differentiation and a high

proportion of purebred individuals, underscoring their conservation value.

Consequently, KT and DL should be prioritised for conservation efforts through targeted breeding programs and facilitate gene flow, such as the introduction of individuals from nearby populations. Given that species *D. cochinchinensis* is dominantly insect-pollinated and its seeds are eliminated by wind via flattened seedpods, natural gene dispersal is limited, especially considering that large trees flower and fruit infrequently [17, 39]. Therefore, the strategic translocation of individuals and the implementation of assisted genetic breeding are recommended to enhance connectivity and ensure the long-term sustainability of *D. cochinchinensis* populations in the wild.

The variation in F_{ST} values among these studies can largely be attributed to differences in sampling scope and the types of molecular markers used to assess genetic diversity. Despite these factors, F_{ST} remains a robust and widely used metric for quantifying genetic differentiation among populations. Generally, a low F_{ST} value implies little genetic differentiation and homogeneity within the population, whereas a high F_{ST} reflects greater genetic divergence and diversity. A higher F_{ST} may also suggest more pronounced reproductive isolation among subpopulations, contributing to increased heterozygosity.

Genetic diversity is a critical reservoir of evolutionary potential and raw material for natural selection, playing an essential role in maintaining population stability. Both G_{ST} and F_{ST} results in this study support the conclusion that the *D. cochinchinensis* population possesses substantial genetic diversity and significant genetic differentiation.

In *D. odorifera*, the PPB is 40.9%, the Shannon diversity index is 0.2048, and Nei's genetic diversity is 0.1353 at the population level. For *D. sissoo*, the PPB is 68.7%, the Shannon diversity index is 0.358, and Nei's genetic diversity is 0.239 [40]. In *D. monticola*, the PPB is 51%, the Shannon diversity index is 0.223, and Nei's genetic diversity is 0.15 [14]. In *D. latifolia*, Nei's genetic diversity is 0.23, Shannon diversity index is 0.34, PPB is 64.71% [13]. PPB, Shannon diversity index, and Nei's genetic diversity are key metrics for evaluating variation within a population. While PPB measures the proportion of polymorphic bands, Nei's genetic diversity takes into account both polymorphism and gene heterozygosity. Finally, the Shannon diversity index is calculated for each locus and averaged across loci to indicate the level of variation within the population. In this study, *D. cochinchinensis* had values of the PPB, Shannon diversity index and Nei's genetic diversity of 61.65%, 0.3955, and 0.2518, respectively, at the population level. Based on these values calculated by polymorphic and monomorphic loci, genetic diversity in *D. cochinchinensis* was found to be moderate compared to other plants in the *Dalbergia* genus.

In this study, the *D. cochinchinensis* populations in the Northern Central Highlands of Vietnam had an expected heterozygosity (H_E) of 0.15, which is lower than in some endemic tree species like *D. odorifera* (H_E=0.37), a species restricted to small areas on Hainan Island. *D. nigra*, found in the Brazilian Atlantic Forest, had an H_E of 0.73. Notably, the expected heterozygosity (H_E) is much lower than that reported in previous publications on *D. cochinchinensis* in Southeast Asia like *D. cochinchinensis* (H_E=0.55) and *D. oliveri* (H_E=0.75) [4]. The variations observed could stem from the varying quantities and kinds of molecular markers utilised in the research, or conversely, from the distinct population sizes examined in the two studies. A significant factor directly impacting diversity is illegal mining activities, which have reduced the distribution region of *D. cochinchinensis* in Northern Central Highlands, Vietnam.

5. Conclusions and implications for gene conservation

This study offers an initial evaluation of genetic diversity and structure of *D. cochinchinensis,* utilising 9 ISSR markers. Significant genetic diversity at the species level, as well as notable differentiations among populations, was revealed for this endangered species of endemic tree.

This pattern of genetic variation may primarily be caused by *D. cochinchinensis* as an obligate pollination species. The considerable geographical distances and restricted distribution range contribute to the constrained gene flow between populations, creating large genetic differentiation between populations. To strengthen the sustainable development of the *D. cochinchinensis* population, it is necessary to establish a plan for breeding *D. cochinchinensis* individuals at an appropriate density, facilitating effective pollen dispersal and pollination, as the *Dalbergia* family primarily relies on wind and insect pollinators. This approach helps ensure genetic diversity and the long-term sustainability of the *D. cochinchinensis* population.

The *D. cochinchinensis* population exhibits low heterozygosity (H_E), restricted gene flow, and a shrinking distribution area. To prevent the adverse effects of genetic drift, it is crucial to boost the breeding of this species to restore population size. Hence, efforts

should focus on preserving the genetic diversity of the DL subpopulation to prevent the loss of valuable alleles. Further, enhancing gene flow (N_m) and heterozygosity (H_E) by introducing individuals from neighbouring populations is essential to restore the population diversity of *D. cochinchinensis*.

CRediT author statement

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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