**BIODIVERSITAS**
Volume 27, Number 3, March 2026
Pages: 10

ISSN: 1412-033X
E-ISSN: 2085-4722
https://doi.org/10.13057/biodiv/d270306

# Genetic diversity of *Dillenia indica* using iPBS markers and its implications for sustainability management in Koh Kret, Thailand

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Manuscript received: 28 October 2025. Revision accepted: 5 March 2026.

**Abstract.** *Rattanapittayapron A, Vanijajiva O, Scalone R, Guo X, Boonprajak D. 2026. Genetic diversity of* Dillenia indica *using iPBS markers and its implications for sustainability management in Koh Kret, Thailand. Biodiversitas 27 (3): d270306. https://doi.org/10.13057/biodiv/d270306*. Protecting genetic diversity in perennial fruit trees is essential for maintaining healthy ecosystems and supporting local communities under increasing urbanization. *Dillenia indica* is a culturally important fruit species on Koh Kret, Nonthaburi Province, Thailand, yet it has not previously been investigated using molecular markers. This study assessed the genetic diversity and population structure of *D. indica* using inter-Primer Binding Site (iPBS) markers together with information on local cultivation practices. A total of 82 individuals were sampled from seven villages across the island. Twenty iPBS primers generated 88 clear and reproducible bands, of which 39 (44.32%) were polymorphic, with a mean Polymorphism Information Content (PIC) of 0.209. Genetic diversity indices indicated moderate variation (H = 0.138±0.032, I = 0.270±0.307). Analysis of molecular variance showed that most genetic variation occurred within populations (78%), whereas 22% was distributed among populations (ΦST = 0.224, $p<0.001$). Neighbor-Joining clustering, principal coordinate analysis, and Bayesian inference consistently identified two genetic groups (K = 2) with high levels of admixture, suggesting considerable gene flow and weak spatial structuring. Interviews with local growers revealed that seed exchange, mixed home-garden cultivation, and natural regeneration contribute to genetic connectivity among communities. This research provides the first molecular evidence for *D. indica* on Koh Kret and demonstrates that traditional management practices help maintain intra-population genetic diversity. The findings support community-based conservation and sustainable utilization of this species within peri-urban agroecosystems.

**Keywords:** *Dillenia indica*, genetic diversity, iPBS markers, Koh Kret, Thailand

## INTRODUCTION

Plant genetic resources face growing threats from habitat fragmentation, land-use changes, and urban growth, all of which reduce population sizes and disrupt gene flow (Singh et al. 2021; Pansuwong et al. 2023). In Southeast Asia, many native perennial species that are important for cultural heritage and rural livelihoods are especially at risk (Ohtani et al. 2021; Phang et al. 2023). *Dillenia indica* L., known locally as "Matad," is an evergreen tree valued for its role in food production, traditional medicine, and as a habitat for wildlife (Rakarcha et al. 2018; Dasanayaka et al. 2022; Nahar et al. 2025). This species has features that help it spread its seeds in humid tropical areas (Figure 2), and it is found on Koh Kret in home gardens, mixed orchards, and temple grounds (Treetaruyanont et al. 2008; Asanok et al. 2021). While these community-managed areas help conserve the species, rapid urbanization threatens its natural regeneration and the long-term survival of its genetic diversity. For this reason, it is important to assess the current genetic diversity in these landscapes.

Genetic diversity is a key component of species persistence because it provides the basis for adaptation, resilience, and long-term survival under changing environmental conditions (Salgotra and Chauhan 2023; Chevin and Bridle 2025). In long-lived and outcrossing tree species, adequate genetic diversity is especially important because it supports reproductive success and population stability. When populations become small or isolated, they may lose allelic richness and become more vulnerable to inbreeding and genetic erosion. Such processes are often not detectable through morphology alone. For this reason, molecular markers are essential for revealing variation within and among populations and for identifying patterns of population structure and connectivity.

Among available marker systems, inter-Primer Binding Site (iPBS) markers are particularly suitable for studying non-model plant species. These markers target conserved retrotransposon regions, require no prior sequence information, and offer high reproducibility together with broad genome coverage (Vanijajiva and Pornpongrungrueng 2020; Bidyananda et al. 2024; Sameeullah et al. 2025). Compared with other dominant marker systems such as RAPD and ISSR, iPBS markers are often considered more reliable for detecting genetic variation in perennial species growing under traditional agroecosystem conditions (Arvas et al.

2023; Khapilina et al. 2025). Because *D. indica* has not previously been examined using this marker system in Thailand, the use of iPBS markers provides an opportunity to establish an initial molecular baseline for the species and to evaluate the extent of genetic differentiation across local populations on Koh Kret.

To obtain a more complete understanding of the genetic condition of *D. indica*, this study employed several population genetic parameters, including the percentage of polymorphic bands, Nei's Gene Diversity, Shannon's Information Index, Analysis of Molecular Variance (AMOVA), and Bayesian clustering. These complementary approaches allow the assessment of genetic variability within and among populations and help infer the degree of connectivity and differentiation across the study area (Nitiworakarn et al. 2023; Ragauskas et al. 2025). In addition, interpreting molecular results together with local cultivation practices is particularly relevant in Koh Kret, where traditional management, home-garden planting, seed exchange, and natural regeneration may all influence genetic patterns. Understanding the interaction between these socio-ecological factors and molecular diversity can help explain how community-managed agroecosystems contribute to the persistence of culturally important fruit tree species (Treetaruyanont et al. 2008; Qu 2025).

This study aimed to evaluate the level of genetic diversity of *D. indica* on Koh Kret using iPBS markers, determine the population structure and degree of differentiation among villages, and explore the relationship between genetic patterns and local cultivation practices. To our knowledge, this is one of the first applications of iPBS markers to *D. indica* in Thailand. The outcomes are expected to provide a molecular reference for future research and to support the sustainable use of this culturally significant species within peri-urban agroecosystems.

## MATERIALS AND METHODS

### Study area

The study was conducted in Koh Kret, Nonthaburi Province, Thailand, a riverine island within the Chao Phraya River basin. Geographically, Koh Kret is encircled by the river and administratively comprises seven villages (Figure 1, Table 1) under the authority of the Koh Kret sub-district. All seven villages were selected as research sites to capture the full extent of local variation. The landscape is characterized by traditional orchards, home gardens, and community-managed agroforestry systems, providing an appropriate context for in situ conservation of indigenous fruit tree species.

### Plant sampling

A total of 82 mature *D. indica* individuals were systematically sampled from seven villages on Koh Kret, Nonthaburi Province. A stratified sampling approach was employed, targeting 11-12 trees per village, with final sample numbers adjusted based on the availability of mature individuals. To minimize the likelihood of sampling genetically identical ramets or closely related individuals, a minimum spacing of 10-15 m was maintained between sampled trees. This spatial criterion was selected to reduce the probability of clonal sampling, particularly in community-managed landscapes where vegetative propagation may occasionally occur (Rajasekharan and Wani 2020). Young, fully expanded, symptom-free leaves were collected (Figure 2), immediately stored in sealed bags with silica gel, and desiccated on-site to preserve DNA quality. Voucher specimens from each population were deposited in the Phranakhon Rajabhat University Herbarium (PNRU) for long-term preservation and taxonomic verification. Specimen codes correspond to those listed in Table 1 and follow the official herbarium code PNRU.

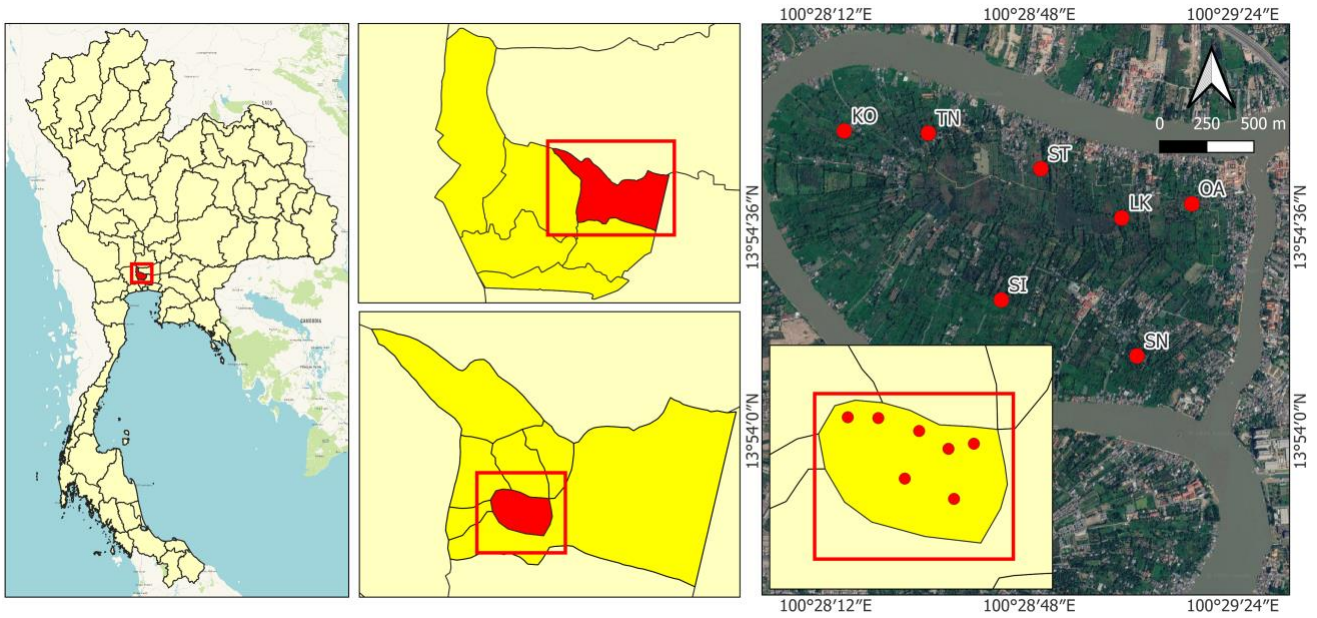


**Figure 1.** Map of study area and plant sampling in Koh Kret, Nonthaburi Province, Thailand

**Table 1.** Information on sample localities for all populations of *Dillenia indica* from Koh Kret, Thailand in this study

| Population code | Subdistrict (Village) | Sample site (n) | Latitude (°N) | Longitude (°E) | Voucher code |
|---|---|---|---|---|---|
| LK | Ban Lat Kret | 12 | 13°54'37.7" | 100°29'04.4" | KK001-012 |
| SN | Ban Salakul Nok | 11 | 13°54'13.3" | 100°29'07.1" | KK013-023 |
| SI | Ban Salakul Nai | 12 | 13°54'23.2" | 100°28'43.1" | KK024-035 |
| KO | Ban Khlong Sra Nam Oi | 12 | 13°54'53.1" | 100°28'15.3" | KK036-047 |
| TN | Ban Tha Nam | 12 | 13°54'52.7" | 100°28'30.2" | KK048-059 |
| ST | Ban Sao Thongthong | 11 | 13°54'46.4" | 100°28'50.1" | KK060-070 |
| OA | Ban Ong Ang | 12 | 13°54'40.2" | 100°29'16.8" | KK071-082 |

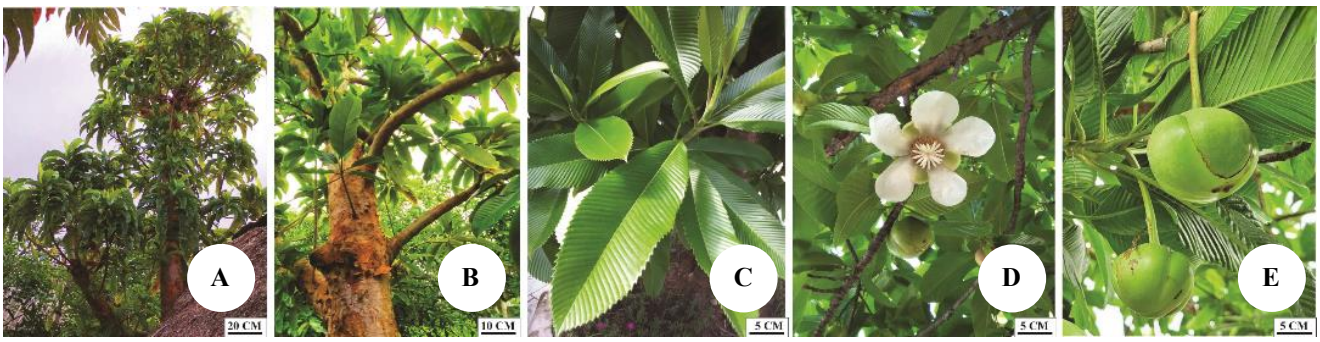


**Figure 2.** Morphological features of: A. *Dillenia indica* mature tree habit, B. Rough bark on trunk, C. Whorled serrated leaves, D. White flower with yellow stamens, E. Globose green fruits on branches

**DNA extraction and quantification**

Genomic DNA was isolated using a modified Cetyltrimethylammonium Bromide (CTAB) following Doyle and Doyle (1990) extraction protocol with 2% β-mercaptoethanol to prevent oxidative browning from phenolic compounds. The procedure included RNase A digestion to remove RNA and multiple chloroform:isoamyl alcohol (24:1) purification steps for improved DNA quality. Final DNA pellets were dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at -20°C. DNA concentration and purity were measured spectrophotometrically (A260/A280 and A260/A230 ratios), and DNA integrity was confirmed using 0.8% agarose gel electrophoresis (Nitiworakarn et al. 2023).

**iPBS marker amplification**

A total of twenty inter-Primer Binding Site (iPBS) primers were initially screened to evaluate amplification efficiency and polymorphism. All iPBS primers and the 100 bp DNA ladder were obtained from Ward Medic Co., Ltd. (Thailand) and utilized according to the manufacturer's specifications. Among these, primers that produced reproducible, well-resolved, and polymorphic amplification profiles were selected for detailed genetic analysis.

Polymerase Chain Reactions (PCR) were performed in 25 µL reaction mixtures containing 50 ng of genomic DNA, 1× PCR buffer supplemented with 2.0 mM $MgCl_2$, 200 µM of each dNTP, 0.4 µM primer, 1.0 U Taq DNA polymerase (Thermo Scientific, USA), and nuclease-free water. The thermal cycling program consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer-specific annealing (50-60°C; see Table 2), for 45 s, and extension at 72°C for 90 s, with a final elongation step at 72°C for 7 min (Siringam and Vanijajiva 2023).

To ensure data quality, each PCR batch included negative controls (no-template reactions) to detect potential contamination and duplicate amplifications of randomly selected samples to evaluate reproducibility. Additionally, replicate DNA extractions from approximately 10% of the samples were performed to estimate scoring error rates through comparison of banding patterns across runs. Amplified products were separated by electrophoresis on 1.5% agarose gels in 1× TBE buffer, stained with ethidium bromide, and visualized under UV illumination. Gel images were critically assessed for clarity, band sharpness, and reproducibility, and only distinct, non-smearing, and consistently scorable bands were retained for subsequent analyses (Siringam and Vanijajiva 2023). Approximately 10% of individuals were randomly selected for duplicate PCR and gel scoring to evaluate data consistency. The proportion of mismatched bands between replicates yielded an estimated scoring error rate of 1.2%, which is within the acceptable range for a dominant multilocus marker and supports the robustness of the dataset (Amiteye 2021).

**Data scoring and statistical analysis**

Statistical analyses of iPBS profiles were performed under the assumption that loci behave as diploid, dominant markers exhibiting presence/absence polymorphism, that co-migrating bands represent homologous loci, and that fragments originate from biparentally inherited nuclear DNA. Only clear and reproducible bands were included in the dataset. Polymorphism Information Content (PIC) was calculated as described by Roldán-Ruiz et al. (2000).

**Table 2.** Summary of iPBS primer polymorphism in this study

| Primer | Sequence (5'-3') | Optimal annealing, Ta (°C) | Total band number | Scored band sizes (bp) | Polymorphic band number | Polymorphism percentage | Polymorphism Information Content (PIC) value |
|---|---|---|---|---|---|---|---|
| 2076 | GCTCCGATGCCA | 59.2 | 5 | 150-750 | 2 | 40.00 | 0.198 |
| 2077 | CTCACGATGCCA | 55.1 | 4 | 300-1,000 | 2 | 50.00 | 0.243 |
| 2079 | AGGTGGGCGGCCA | 65.2 | 5 | 250-1,000 | 3 | 60.00 | 0.197 |
| 2080 | CAGACGGGCCCA | 63.3 | 4 | 400-1000 | 1 | 25.00 | 0.121 |
| 2081 | GCAACGGGCGCCA | 65.0 | 5 | 250-1,500 | 2 | 40.00 | 0.196 |
| 2083 | CTTCTAGGGCCA | 54.6 | 5 | 250-1,000 | 2 | 40.00 | 0.194 |
| 2085 | ATGCCGGATACCA | 52.8 | 4 | 500-1,200 | 1 | 25.00 | 0.125 |
| 2272 | GGCTCAGATGCCA | 55.0 | 3 | 250-750 | 1 | 33.33 | 0.162 |
| 2273 | GCTCATGCCA | 56.5 | 4 | 200-1,000 | 2 | 50.00 | 0.245 |
| 2277 | GGGCATGCCA | 52.0 | 5 | 150-1,200 | 2 | 40.00 | 0.198 |
| 2279 | AATGAAAGGCCA | 52.0 | 4 | 200-1,000 | 2 | 50.00 | 0.233 |
| 2374 | CCCAGGAACCGA | 53.5 | 5 | 500-2,500 | 3 | 60.00 | 0.265 |
| 2378 | GGTCCCTATCA | 53.0 | 4 | 250-1,000 | 1 | 25.00 | 0.122 |
| 2380 | CAACCTAGGCCA | 50.5 | 5 | 200-1,500 | 3 | 60.00 | 0.293 |
| 2382 | TGTTGGGTTCCA | 50.5 | 3 | 400-750 | 1 | 33.33 | 0.166 |
| 2389 | ACATCTCCAGCCA | 50.0 | 5 | 250-1000 | 3 | 60.00 | 0.282 |
| 2391 | ATCTGTCAGCCA | 52.6 | 4 | 250-800 | 1 | 25.00 | 0.124 |
| 2392 | TAGAGTGGCCA | 52.2 | 4 | 200-1,000 | 2 | 50.00 | 0.242 |
| 2393 | GAGCCTAGGCCA | 54.0 | 5 | 150-1,000 | 3 | 60.00 | 0.279 |
| 2394 | TAGGGTAGGCCA | 51.0 | 5 | 250-1,500 | 2 | 40.00 | 0.285 |
| Total/Means | | | 88 | 150-2,500 | 39 | 44.32 | 0.209 |

Cluster analyses were conducted in PAST v3.14 (Hammer et al. 2001) using Neighbor-Joining (NJ) and Principal Coordinate Analysis (PCoA) based on Jaccard's genetic distance matrix (Cardoso et al. 2024). The robustness of the NJ topology was evaluated using 1,000 bootstrap replications. Genetic diversity parameters, including Percentage of Polymorphic Bands (PPB), observed and effective allele numbers (Na, Ne), Nei's Gene Diversity (H), and Shannon's Information Index (I), were calculated in POPGENE v1.32 under Hardy-Weinberg equilibrium assumptions appropriate for dominant markers. Population diversity indices (Hs, Ht, Gst) and estimates of gene flow (Nm) were obtained, and molecular variance was partitioned using AMOVA, with ΦST calculated in ARLEQUIN v3.5.

Population structure was analyzed using STRUCTURE v2.3.4 under an admixture model with correlated allele frequencies. Ten independent runs were performed for each K value (K = 1-10), with a burn-in of 10,000 iterations followed by 100,000 MCMC iterations to ensure convergence. The "recessive alleles" option was enabled to accommodate the dominant nature of iPBS markers, as described by Falush et al. (2007). The most likely number of genetic clusters was determined using the ΔK method of Evanno et al. (2005) as implemented in STRUCTURE HARVESTER (Earl and von Holdt 2012). Replicate runs were aligned, and consensus membership coefficients were visualized using CLUMPAK to account for label switching and multimodality (Kopelman et al. 2015).

**Integration with socio-ecological context**

To complement molecular analyses, semi-structured interviews were conducted with local cultivators and community representatives. These interviews documented ethnobotanical knowledge, cultivation practices, and perceived values of *D. indica.* The integration of molecular data with local knowledge provided a framework for interpreting genetic diversity in relation to sustainability management and community-based conservation practices.

**Ethics and consent statement**

The authors state that the questionnaire-based interviews were conducted with volunteer participants who were fully informed of the study's objectives, procedures, and confidentiality safeguards. All participants provided informed consent prior to participation. The survey focused exclusively on general knowledge, cultivation practices, and perceptions regarding *D. indica* management within the Koh Kret community. It did not involve the collection of sensitive personal, medical, or identifying information. Participation was entirely voluntary, with the option to withdraw at any time without consequence. All responses were anonymized and used solely for academic and research purposes. Phranakhon Rajabhat University granted ethical approval for this study under research number 216376, and all procedures adhered to institutional and national guidelines for ethical research involving human participants.

## RESULTS AND DISCUSSION

**iPBS marker polymorphism**

A total of 20 iPBS primers were used to evaluate the genetic diversity of *D. indica* populations collected from seven villages on Koh Kret, Nonthaburi Province. All primers produced clear, reproducible, and scorable amplification patterns, generating 88 bands ranging from

150 to 2,500 bp, of which 39 were polymorphic (Table 1). The percentage of polymorphism ranged from 25.00% (iPBS 2080, 2085, 2378, and 2391) to 60.00% (iPBS 2079, 2374, 2380, 2389, and 2393), with an average of 44.32%, indicating a moderate level of genetic variation among the studied populations. The number of amplified fragments per primer varied from three (iPBS 2272 and 2382) to five (e.g., 2076, 2079, 2081, 2374, 2380, 2389, 2393, and 2394), suggesting that the selected primers were effective in detecting polymorphisms across loci.

Polymorphism Information Content (PIC) ranged from 0.121 (iPBS 2080) to 0.293 (iPBS 2380), with an average of 0.209, reflecting moderate informativeness of the primers. Primers 2380, 2374, 2389, and 2393 exhibited relatively high PIC values (>0.26), suggesting their utility for fine-scale discrimination of genetic variation within *D. indica* populations. The overall moderate polymorphism percentage and PIC levels indicate that iPBS markers are reliable tools for detecting genetic variability in perennial tropical trees. These results highlight the effectiveness of iPBS markers for assessing intra- and inter-population diversity in *D. indica*, providing a valuable molecular foundation for future conservation, propagation, and sustainable management of genetic resources on Koh Kret.

**Genetic diversity within populations**

Genetic diversity parameters estimated from iPBS markers revealed moderate variation among the seven *D. indica* populations sampled from Koh Kret, Nonthaburi Province. The Percentage of Polymorphic Bands (PPB) ranged from 34.09% in Ban Khlong Sra Nam Oi (KO) and Ban Tha Nam (TN) to 38.64% in Ban Salakul Nok (SN), with an overall mean of 44.32%. The observed Number of alleles (Na) varied narrowly from 1.340 (KO) to 1.386 (SN). In contrast, the effective Number of alleles (Ne) ranged from 1.208 in Ban Salakul Nai (SI) to 1.255 in KO, indicating a relatively uniform allelic distribution across populations.

Nei's Gene Diversity (h) values ranged from 0.178 (SI) to 0.205 (KO). The population total was 0.212±0.186. Shannon's Information Index (I) showed a similar trend, ranging from 0.262 (SI) to 0.293 (KO), with a total of 0.307±0.270. The population means gene diversity within populations (Hs) was 0.138±0.032. The total gene diversity (Ht) was 0.185±0.045. The coefficient of gene differentiation (Gst) was 0.258. The estimated gene flow (Nm) was 1.436. These figures indicate moderate genetic differentiation and limited but ongoing gene exchange among Koh Kret populations. Collectively, the results suggest that *D. indica* populations on Koh Kret maintain measurable but modest genetic polymorphism and allelic richness. This pattern reflects localized gene flow and a moderate degree of population connectivity within the island landscape.

The Analysis of Molecular Variance (AMOVA) based on iPBS marker data revealed significant genetic differentiation among the seven *D. indica* populations collected from Koh Kret (Table 4). The total molecular variance was partitioned into two hierarchical levels: among populations and within populations. A substantial proportion of genetic variation (78%) was found within populations, indicating high intra-population diversity and suggesting that most genetic variation is maintained among individuals rather than being strongly structured by population boundaries. Conversely, 22% of the total variance was attributed to differences among populations, demonstrating a moderate level of genetic differentiation across the sampled sites. The fixation index ($\Phi_{ST}$ = 0.224; $p$<0.001) confirmed that the populations are moderately genetically structured, implying limited but significant gene flow among them. This pattern suggests that while populations of *D. indica* across Koh Kret remain genetically connected, spatial or ecological factors may still contribute to some degree of genetic divergence. The proportion of molecular variance assigned to within-population differences demonstrates that individual populations harbor substantial allelic diversity, consistent with the patterns typically observed in cross-pollinated tropical tree species (Uddin et al. 2024).

**Table 3.** Summary of iPBS variation for seven populations of *Dillenia indica* collected from Koh Kret

| Population code | Sample size (n) | Percentage of Polymorphic Bands (PPB, %) | Observed Number of alleles (Na) | Effective Number of alleles (Ne) | Nei's (1973) gene diversity (h) | Shannon's Information Index (I) |
|---|---|---|---|---|---|---|
| LK | 12 | 37.50 | 1.375±0.487 | 1.224±0.331 | 0.201±0.134 | 0.271±0.202 |
| SN | 11 | 38.64 | 1.386±0.490 | 1.227±0.337 | 0.185±0.136 | 0.271±0.205 |
| SI | 12 | 37.50 | 1.375±0.487 | 1.208±0.318 | 0.178±0.126 | 0.262±0.193 |
| KO | 12 | 34.09 | 1.340±0.477 | 1.255±0.383 | 0.205±0.142 | 0.293±0.205 |
| TN | 12 | 34.09 | 1.341±0.477 | 1.251±0.388 | 0.204±0.139 | 0.291±0.202 |
| ST | 11 | 37.50 | 1.375±0.487 | 1.241±0.343 | 0.192±0.142 | 0.281±0.216 |
| OA | 12 | 37.50 | 1.375±0.487 | 1.243±0.340 | 0.192±0.144 | 0.286±0.213 |
| Total | 82 | 44.32 | 1.443±0.450 | 1.325±0.379 | 0.212±0.186 | 0.307±0.270 |

| Average gene diversity within populations (Hs) | Total gene diversity (Ht) | Coefficient of gene differentiation (Gst) | Estimate of gene flow (Nm) |
|---|---|---|---|
| 0.138±0.032 | 0.185±0.045 | 0.258 | 1.436 |

**Table 4.** Analysis Molecular Variance (AMOVA) for iPBS variation based on seven populations of *Dillenia indica* collected from Koh Kret

| Source of variation | Degree of Freedom (df) | Sum of Squares (SS) | Mean Squares (MS) | Variance Components | Percentage of Total Variance (%) | Fixation Index ($\Phi_{ST}$) | P value* |
|---|---|---|---|---|---|---|---|
| Among populations | 6 | 199.223 | 33.204 | 2.187 | 22 | 0.224 | $p$<0.001 |
| Within populations | 75 | 569.386 | 7.592 | 7.592 | 78 | | $p$<0.001 |
| Total | 81 | 768.610 | | 9.779 | 100.00 | | |

Note: df: Degree of freedom, P-value: Probability of null hypothesis. *Significance tests after 1000 permutations

**Table 5.** Assignment of *Dillenia indica* individuals from Koh Kret to two genetic clusters (CL1 and CL2) inferred by STRUCTURE (K = 2) based on iPBS markers. The mean membership coefficient (Q±SD) of each cluster was used to characterize genetic ancestry patterns in each population

| Population code | n | CL1 (Green) | CL2 (Red) |
|---|---|---|---|
| LK | 12 | 0.869±0.044 | 0.132±0.044 |
| OA | 12 | 0.882±0.040 | 0.118±0.040 |
| SI | 12 | 0.860±0.048 | 0.140±0.048 |
| SN | 11 | 0.861±0.051 | 0.139±0.051 |
| ST | 11 | 0.872±0.031 | 0.128±0.031 |
| KO | 12 | 0.210±0.064 | 0.790±0.064 |
| TN | 12 | 0.139±0.039 | 0.861±0.039 |
| Mean | 82 | 0.668±0.269 | 0.333±0.269 |

## Cluster and ordination analyses

Cluster and ordination analyses based on iPBS marker data revealed clear genetic groupings among the 82 *D. indica* individuals collected from seven subpopulations on Koh Kret. The Neighbor-Joining (NJ) dendrogram (Figure 3.A) grouped the individuals into two primary clusters. The first cluster comprised individuals predominantly from LK, OA, SI, SN, and ST, while the second cluster contained most individuals from TN and KO. Principal Coordinate Analysis (PCoA) (Figure 3.B) further supported the dendrogram results, showing two groups corresponding to the same general cluster distribution observed in the NJ tree. The first two coordinates explained a substantial proportion of the total molecular variation, effectively separating individuals into two major but not entirely distinct clusters. Individuals from LK, OA, SI, SN, and ST were positioned mainly in the green shade of the plot, whereas those from KO and TN were distributed toward the red shade. The grouping pattern corresponds broadly to geographic proximity among villages, suggesting that spatial adjacency may influence local gene flow and the maintenance of shared genetic pools across the island landscape.

Bayesian clustering analysis (STRUCTURE; Figure 4) identified two major genetic clusters among the 82 *D. indica* individuals. The Evanno ΔK method indicated that K = 2 is the most likely partition (mean lnP(D) = -3743.600; ΔK = 42.70). The STRUCTURE barplot revealed high admixture across all seven populations, indicating weak genetic subdivision. Ancestry coefficients (Table 5) showed that five populations were mainly associated with Cluster 1 (Green): LK (0.869±0.044), OA (0.882±0.040), SI (0.860±0.048), SN (0.861±0.051), and ST (0.872±0.031). KO (0.210±0.064) and TN (0.139±0.039) were primarily assigned to Cluster 2 (Red). All populations exhibited mixed membership in both clusters, and standard deviations were moderate across replicates. These results indicate two partially differentiated but interconnected genetic groups with substantial admixture.

## Integration with socio-ecological context

Field observations indicated that the sampled *D. indica* trees were predominantly seed-derived, lacked visible clonal structures, and were sufficiently spaced to minimize ramet inclusion. Community interviews confirmed that propagation is mainly through seed, while vegetative propagation is practiced only occasionally. These management patterns are relevant to the genetic results, as seed-based regeneration is expected to maintain higher allelic diversity within populations.

Socio-ecological information from seven villages showed that *D. indica* is maintained in home gardens, temple grounds, and mixed orchards for food, medicinal, and ornamental uses. Regular exchange of seedlings among households and natural recruitment along waterways create opportunities for both human-mediated and natural dispersal. Such practices provide a plausible mechanism for the high within-population variation detected by AMOVA (78%) and the extensive admixture observed in STRUCTURE analyses.

The correspondence between cultivation behavior and molecular patterns suggests that local management contributes to genetic connectivity across Koh Kret. The absence of strict genetic boundaries among villages, together with moderate population differentiation, supports the view that both traditional propagation and natural dispersal shape the current population structure.

## Discussion

*Genetic polymorphism and marker informativeness*

The iPBS marker system proved effective for detecting polymorphisms and assessing genetic diversity in *D. indica* populations from Koh Kret, Nonthaburi Province. All 20 primers produced clear and reproducible bands, generating 88 scorable loci, 39 of which were polymorphic. The overall polymorphism rate of 44.32% and mean PIC value of 0.209 indicate moderate marker informativeness. These findings align with previous studies on tropical woody plants analyzed using iPBS markers, such as *Aniba rosaeodora* Ducke (Baloch et al. 2022, *Melientha suavis* Pierre (Siringam and Vanijajiva 2023), and *Zanthoxylum* species (Zhang et al. 2024), all of which exhibit similar levels of polymorphism and heterogeneity.

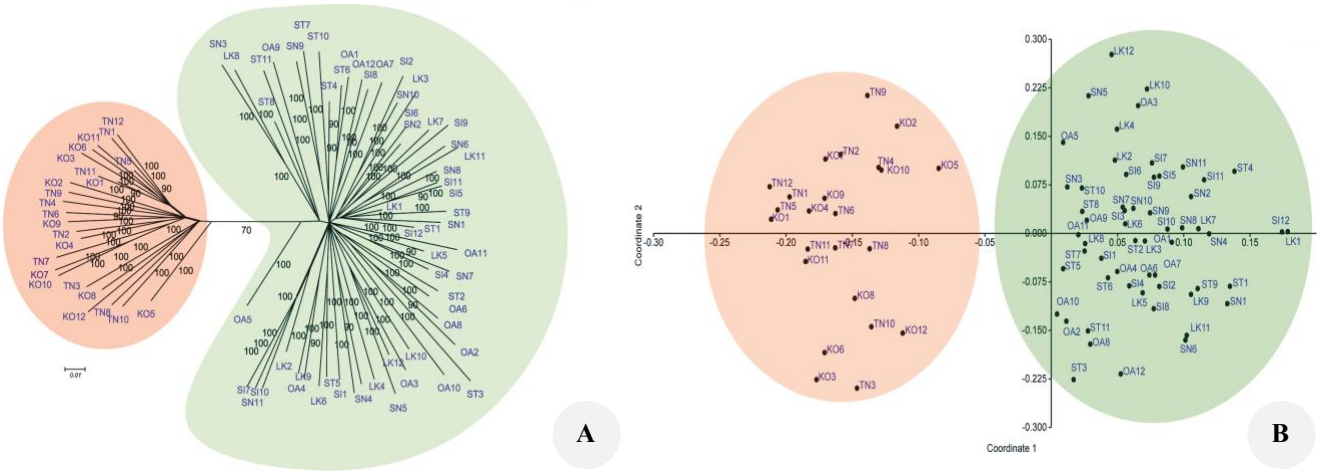


**Figure 3.** Genetic relationships among 82 *Dillenia indica* individuals from seven populations on Koh Kret, Nonthaburi Province, Thailand, based on Jaccard's genetic distance matrix. A. Neighbor-Joining (NJ) dendrogram, B. Principal Coordinate Analysis (PCoA) plot. Axis 1: 32.41%, Axis 2: 18.27%

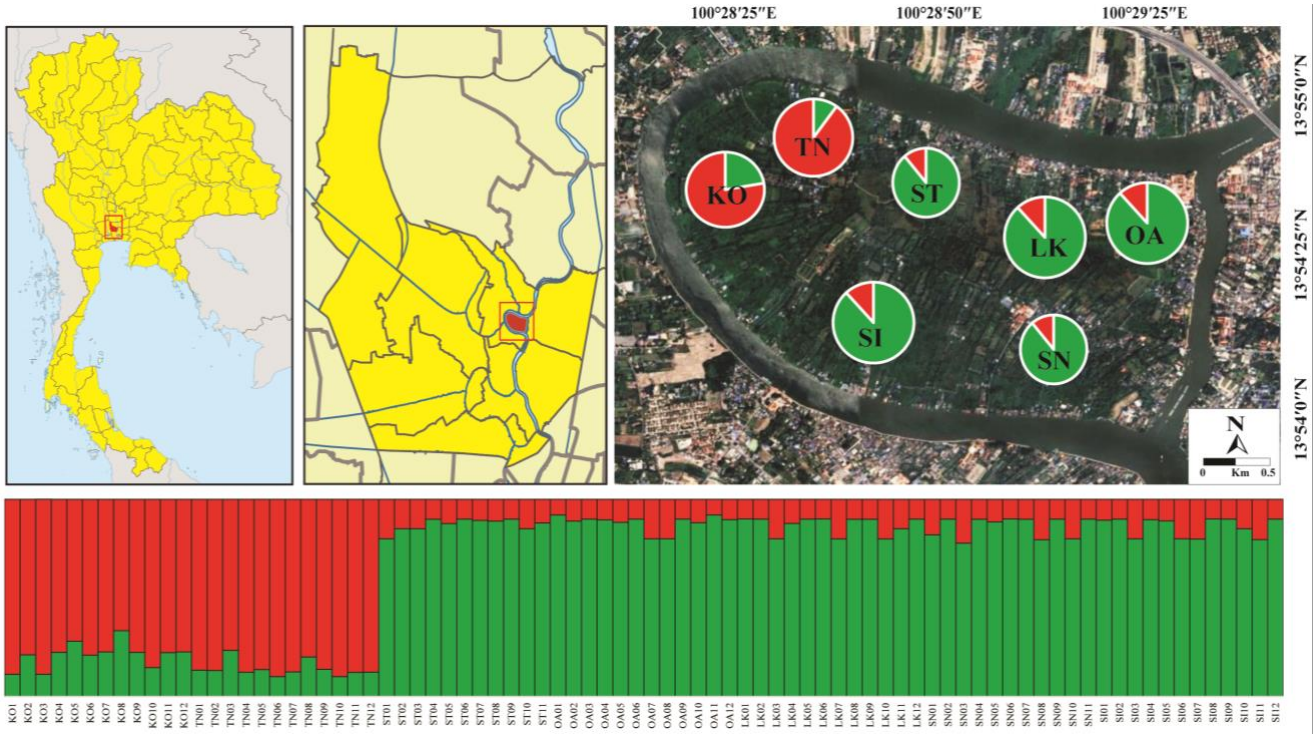


**Figure 4.** Geographic distribution and genetic structure of 82 *Dillenia indica* individuals from seven populations on Koh Kret, Nonthaburi Province, Thailand, based on Bayesian clustering analysis (K = 2). Upper panels: Sampling locations and population membership proportions are represented by pie charts, Lower panel: STRUCTURE bar plot showing individual membership coefficients to the two inferred clusters (green and red)

High PIC values for primers 2380 (0.293), 2374 (0.265), 2389 (0.282), and 2393 (0.279) suggest these loci are particularly effective for distinguishing closely related individuals and detecting fine-scale genetic structure. The variation in polymorphism percentage among primers (25-60%) reflects heterogeneity in retrotransposon insertion sites within the *D. indica* genome, consistent with the dynamic nature of iPBS loci as molecular markers that target conserved PBS (Primer Binding Site) regions of retrotransposons. Retrotransposon-based markers such as iPBS are well-suited for non-model tropical tree species because they require no prior sequence information and capture genome-wide diversity shaped by both evolutionary and ecological processes (Sameeullah et al. 2025). Thus, the current results confirm that iPBS markers are a reliable and informative tool

for evaluating genetic variability in *D. indica*, particularly in semi-managed agroecosystems such as Koh Kret.

*Genetic diversity and structure analyses*

Genetic diversity estimates from iPBS markers indicated moderate and relatively uniform variation among the seven *Dillenia indica* populations on Koh Kret. Polymorphic bands ranged from 34.09-38.64%, with Na = 1.443±0.450 and Ne = 1.325±0.379, while Nei's diversity (H = 0.212±0.186) and Shannon Index (I = 0.307±0.270) confirmed consistent allelic richness across sites. The comparison of Hs (0.138±0.032) and Ht (0.185±0.045) produced Gst = 0.258 and Nm = 1.436, indicating moderate connectivity typical of cross-pollinated tropical tree species (Hamrick and Godt 1996; Chung et al. 2023).

AMOVA showed that 78% of variation occurred within populations and 22% among populations (ΦST = 0.224, $p<0.001$), a pattern consistent with outcrossing perennials with effective pollen and seed dispersal (Garnier and Lafontaine 2022). STRUCTURE and clustering analyses identified two partially differentiated groups with extensive admixture, suggesting continuous rather than discrete differentiation. Comparable dominance of intra-population variation has been reported in other tropical fruit trees such as *Artocarpus heterophyllus* Lam. (Gwokyalya et al. 2024), *Psidium guajava* L. (Lima et al. 2024), and *Mangifera indica* L. (Kumar et al. 2022), where overlapping generations and mixed dispersal maintain local heterogeneity.

Both biological and human factors likely support the maintenance of genetic connectivity on Koh Kret. *D. indica* is primarily entomophilous, enabling cross-pollination among neighboring trees (Omi 2022; Gwokyalya et al. 2024), while seed movement by frugivores and hydrochory along the Chao Phraya River further enhances gene flow (Dasanayaka et al. 2022). Local exchange of planting material and home-garden cultivation also reduces population isolation (Pathirana and Carimi 2022). These combined processes explain the weak spatial structuring observed and indicate that current management practices contribute to the persistence of intra-population diversity.

*Integration with socio-ecological context and broader implications*

The integration of molecular evidence with socio-ecological observations provides a broader perspective on the dynamics of *D. indica* on Koh Kret. Local practices such as seed exchange, limited vegetative propagation, and tolerance of natural recruitment are consistent with the genetic patterns of high within-population diversity and weak spatial structure observed in this study. Similar roles of traditional agroforestry as informal germplasm exchange networks have been described in other community-managed systems (Treetaruyanont et al. 2008).

The level of polymorphism (44.32%) and PIC (0.209) detected here is comparable to values reported for traditionally cultivated tropical trees such as *Syzygium cumini* (L.) Skeels (Uddin et al. 2024) and *M. suavis* (Siringam et al. 2024), where substantial gene flow maintains intra-population variation. The predominance of within-population variance (78%) and moderate differentiation (ΦST = 0.224) align with patterns documented for outcrossing perennials in heterogeneous landscapes (Urquía et al. 2019; Nazareno et al. 2021).

General conservation literature suggests that maintaining diverse seed sources and avoiding excessive clonal propagation can help preserve locally adapted germplasm (Schulze et al. 2016). At the same time, seed movement among communities may introduce non-local genotypes, which highlights the importance of balancing connectivity with the retention of local genetic identity. These considerations, derived from previous studies, provide useful context for interpreting the present results without implying that specific sites are prescribed.

From a conservation genetics perspective, the predominance of within-population variation and extensive admixture observed in this study indicate that existing propagation and dispersal processes are currently sufficient to maintain genetic connectivity of *D. indica* on Koh Kret. The molecular results support general principles emphasized in previous research, such as prioritizing seed-derived propagation, retaining diverse maternal lineages, and avoiding excessive clonal planting (Clarke and Merlin 2016; Kim et al. 2023). These implications are drawn directly from the genetic patterns detected here and provide a scientific context for interpreting the sustainability of community-managed populations without proposing site-specific prescriptions.

In conclusion, this study presents the first molecular evaluation of *D. indica* populations on Koh Kret, Thailand, integrating iPBS marker analysis with socio-ecological context. The results revealed a moderate level of genetic diversity (44.32% polymorphism; mean PIC = 0.209), with most genetic variation occurring within populations (78%) and moderate differentiation among populations (ΦST = 0.224, $p<0.001$). Multivariate and Bayesian analyses, including Neighbor-Joining, PCoA, and STRUCTURE clustering, consistently identified two weakly differentiated genetic groups (K = 2) with extensive admixture, suggesting substantial gene flow and limited spatial genetic structuring across the island. However, the study is constrained by geographically restricted sampling within a single island system and the use of dominant molecular markers, which may underestimate allelic variation relative to codominant marker systems. Despite these limitations, the concordance between molecular patterns and local cultivation practices suggests that seed exchange among villages and natural regeneration likely help maintain genetic connectivity within the island landscape. Collectively, these findings provide an initial genetic baseline for *D. indica* in a peri-urban socio-ecological system and establish a foundation for future population genetic monitoring and broader comparative studies.

## ACKNOWLEDGEMENTS

This study was supported by the Fundamental Fund of Phranakhon Rajabhat University, Thailand. The authors sincerely thank the Koh Kret community in Nonthaburi Province for their cooperation and for providing plant

materials. Grateful acknowledgment is extended to Shinawatra University and Phranakhon Rajabhat University for laboratory facilities and technical support, and to Saruda Nitiworakarn for invaluable field assistance.

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