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Genetic diversity and phylogenetic analysis of matoa (*Pometia pinnata*) from South Sumatra, Indonesia based on ITS rDNA

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Abstract. *Hanum L, Usman SG, Oktariansyah Y. 2025. Genetic diversity and phylogenetic analysis of matoa* (Pometia pinnata) *from South Sumatra, Indonesia based on ITS rDNA. Biodiversitas 26: 5515-5524.* Matoa (*Pometia pinnata*) is an ecologically and economically significant tropical fruit tree, however, its genetic variation in South Sumatra, Indonesia remains poorly characterized. This study aimed to examine the genetic diversity and phylogenetic relationships of four matoa variants, i.e., red, yellow, green, and forest types using ITS rDNA sequences. PCR amplification yielded sequences of 681 bp for cultivated and 478 bp for forest matoa, revealing two InDel haplotypes, 141 InDel sites, and three InDel events with moderate haplotype diversity (Hd = 0.50) and low InDel diversity per site (πi = 0.001). These findings indicate limited but informative structural variation linked to insertion-deletion mutation events, supporting the ITS region's dual role as both a conserved barcode and a marker for intraspecific differentiation. Phylogenetic and AMOVA analyses showed clear separation between cultivated and forest variants, with almost all genetic variation (99.9997%) partitioned among populations (Fst = 0.999997), suggesting restricted gene flow and possible ecological divergence. Despite high sequence similarity of 99.85% to 100% confirming conspecificity, forest matoa formed a distinct clade and grouped separately, highlighting its potential as a unique genetic reservoir. These results provide foundational molecular insights for conservation, breeding, and germplasm authentication strategies for matoa in Indonesia.

Keywords: Genetic diversity, Internal Transcribed Spacer (ITS), matoa, phylogeny, *Pometia pinnata*

INTRODUCTION

Matoa (*Pometia pinnata* J.R.Forst. & G.Forst.), a member of the family Sapindaceae, is a tropical tree species widely distributed across Indonesia, Malaysia, the Philippines, and Papua New Guinea (POWO 2025). In Indonesia, it occurs in Papua, Kalimantan, and Sumatra (Suwardi et al. 2020; Sutomo et al. 2021; Indow et al. 2022). The species is valued for its edible fruits (Suwardi et al. 2020; Syamsuardi et al. 2022; Sagitarian et al. 2023), high-quality timber and medicinal properties (Suzuki et al. 2021; Putri et al. 2023).

Genetic diversity is central to conservation biology, ecological research, and sustainable agriculture (Ebert and Engel 2020). It underpins plant resilience to environmental stresses, pests, and diseases, while shaping traits, such as fruit yield, wood quality, and ecological adaptability (Dias et al. 2018; Massa et al. 2024; Rojas-Cortés et al. 2024). Matoa is expected to harbor high genetic variation, since it is a naturally cross-pollinated species (Hajar et al. 2021). In South Sumatra, four morphotypes are locally recognized, i.e., three cultivated forms (red, yellow, and green matoa) and a wild form known as forest matoa or *kungkil* (Novira and Hanum 2025; Usman and Hanum 2025). The cultivated types bear elongated fruits with bright-colored skins, smooth leaves, vivid petals, firm pulp, and wrinkled seeds, while forest matoa produces round dark fruits, coarse leaves, pale petals, sticky pulp, and smooth seeds (Marlini and Hanum 2025). These stable traits justify their recognition as morphological variants, but their genetic differentiation remains unknown.

Despite its ecological and economic importance, research on matoa genetic diversity across Indonesia remains uneven. While previous studies in Pekanbaru and Central Java have confirmed intraspecific genetic variation within matoa (Yuniastuti et al. 2023; Zulfahmi et al. 2023), little is known about the genetic structure of populations in South Sumatra. Since this region is experiencing severe deforestation, with an estimated 63% forest loss between 1990 and 2019, with the sharpest decline in the 1990s and only 0.8 million ha remaining by 2019 (Purnomo et al. 2023), this poses a direct threat to the survival of wild matoa populations. Therefore, assessing the genetic landscape of these variants is critical for developing effective conservation priorities and breeding programs.

Molecular markers are widely applied to assess genetic diversity and phylogenetic relationships in plants (Khal et al. 2023). To address this gap, the Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA was selected as a molecular marker. The ITS region has proven highly effective at resolving genetic variation within and between closely related species (Besse et al. 2021). The ITS region includes ITS1, 5.8S, and ITS2, is flanked by conserved rRNA genes, but contains high sequence variability, making it suitable for species-level studies (Nafisi et al. 2023; Berchtenbreiter et al. 2024). It has been successfully used in tropical plants with subtle morphological differences, such as *Menispermum dauricum* (Song et al.

2023) and has shown strong performance in detecting genetic diversity within Sapindaceae (Mahar et al. 2017). Thus, ITS might be a reliable tool to explore the diversity and evolutionary relationships of matoa variants.

Phylogenetic tree construction based on ITS sequences enables the identification of haplotypes, evolutionary relationships, and genetic differentiation among populations (Kapli et al. 2020; Vankan et al. 2021). Applying this approach to matoa will clarify whether cultivated and forest types represent genetically distinct groups, thereby providing insights into their evolutionary history and conservation value. Accordingly, this study aimed to analyze the genetic diversity and phylogenetic relationships of the four matoa variants from South Sumatra using ITS sequence data. The results are expected to provide foundational molecular information to support the conservation, breeding, and sustainable utilization of this valuable tropical species.

MATERIALS AND METHODS

Sampling locations

Leaf samples of matoa were collected from four distinct variants (red, yellow, green, and forest matoa) (Figure 1) across four different areas in South Sumatra, Indonesia, with one representative individual sampled per variant; thus, a total of four accessions were analyzed (Figure 2, Table 1). The four matoa variants were collected from different habitats. Forest matoa (*kungkil*) was obtained from secondary lowland forest, while the three cultivated variants, i.e., red, yellow, and green were collected from urban-edge gardens. Each matoa variant was represented by sample from different individuals, selected based on consistent and observable morphological traits, reflecting distinct morphotypes of matoa variants as reported by Marlini and Hanum (2025).

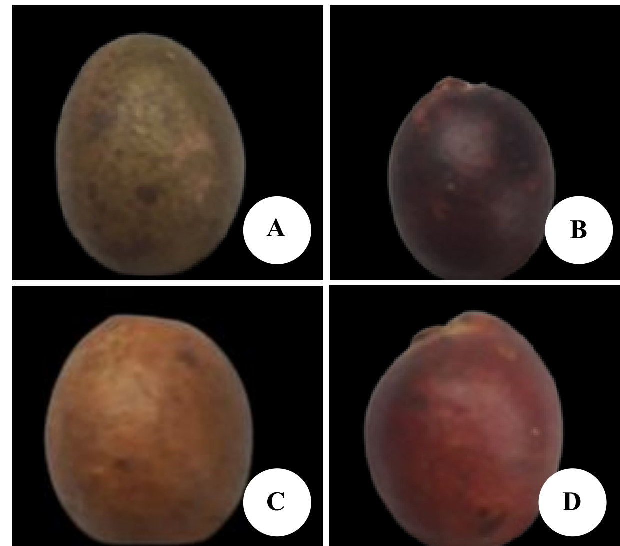

Figure 1. Representative fruit morphology of matoa variants in South Sumatra, Indonesia: A. Green matoa with green fruits (HIU), B. Forest matoa (*kungkil*) with round dark green to blackish fruits (KK), C. Yellow matoa with golden-yellow fruits (KN), D. Red matoa with reddish elongated fruits (MRH)

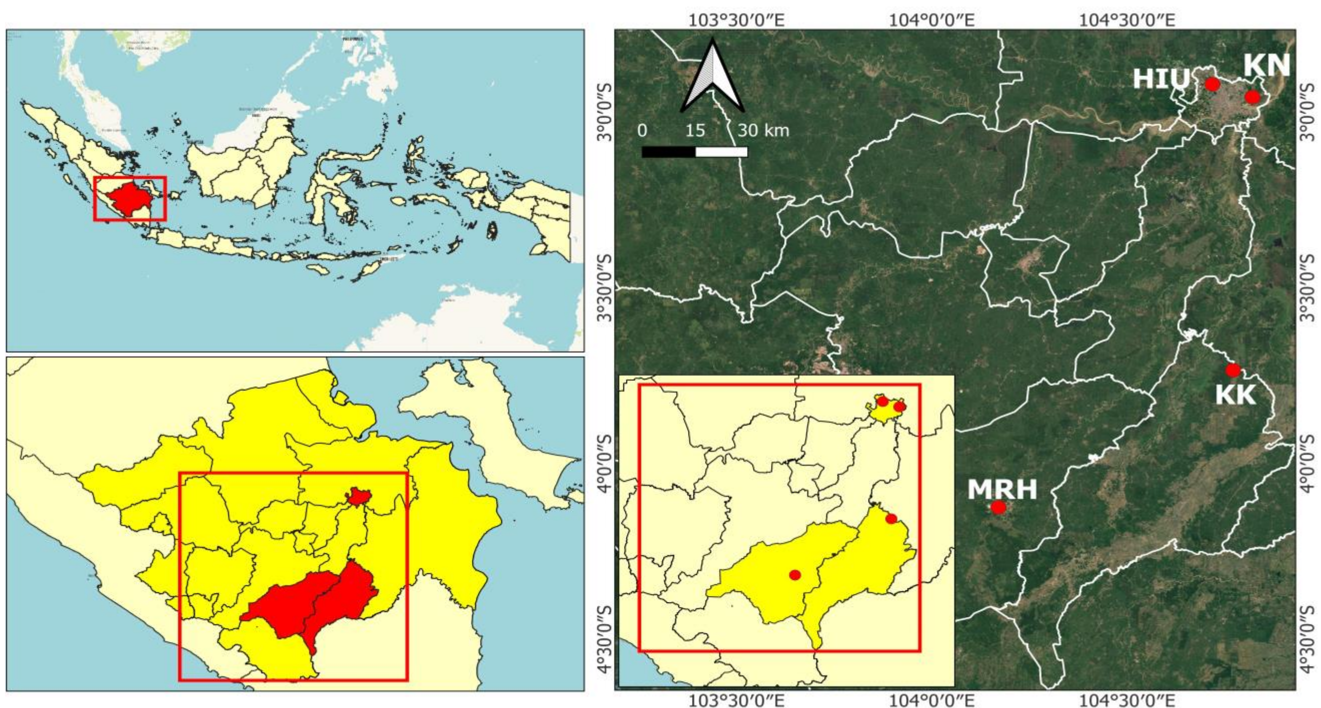

Figure 2. Sampling locations in South Sumatra, Indonesia. HIU: Sukodadi, Palembang, KK: Campang Tiga, Ogan Komering Ulu, KN: Kalidoni, Palembang, MRH: Baturaja, Ogan Komering Ulu

Table 1. Source of plant materials used in this study

Code	Sample identity	Status	Region	Habitat Type	Altitude (m asl.)	Coordinate	
						Latitude (S)	Longitude (E)
KK	Forest Matoa / *kungkil*	Wild	Campang Tiga, Ogan Komering Ulu	Secondary lowland forest	48.52	3° 44' 0.6"	104° 46' 19.6"
MRH	Red Matoa	Cultivated	Baturaja, Ogan Komering Ulu	Urban-edge garden	50.55	4° 7' 25.0"	104° 10' 16.3"
KN	Yellow Matoa	Cultivated	Kalidoni, Palembang	Urban-edge garden	4.64	2° 57' 23.0"	104° 49' 18.1"
HIU	Green Matoa	Cultivated	Sukodadi, Palembang	Urban-edge garden	6.06	2° 55' 10.9"	104° 43' 8.0"

Plant materials

The leaf samples of matoa variants were washed thoroughly to remove dirt stuck to the leaves. Then, the leaf samples were air-dried, wrapped using aluminum foil, labelled, and stored in the freezer (-20°C). These specimens are archived for future morphological verification and taxonomic reference. This research was conducted from September 2024 to January 2025 at the Genetics and Biotechnology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, and the Shared Facility Laboratory, Department of Biology, Universitas Gadjah Mada, Indonesia.

Voucher specimens representing each matoa variant were prepared and deposited at the UNSRI-Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indonesia. Each voucher was assigned unique code: MRH-01 (Red Matoa), KN-02 (Yellow Matoa), HIU-03 (Green Matoa), and KK-04 (Forest Matoa/*kungkil*).

Procedures

DNA isolation

DNA extraction from matoa leaves was conducted using the i-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology, Seongnam, South Korea).

Quantitative and qualitative tests

DNA concentration was assessed using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, n.d.) with the dsDNA setting. A260/A280 ratios were used to evaluate purity, where values of 1.7-2.0 and concentrations above 100 ng/µL indicate high-quality DNA (Dewanata and Mushlih 2021; Utaminingsih and Sophian 2022; Versmessen et al. 2024). All measurements were performed in technical duplicates, and only samples with consistent purity and concentration values were used for amplification and sequencing.

DNA quality was evaluated by agarose gel electrophoresis (1% agarose in 1× TBE buffer) stained with GelRed. Approximately 4 µL of DNA mixed with loading dye was loaded alongside a λ HindIII DNA ladder and run at 80 V for 60 min. Gels were visualized under a UV transilluminator (Accuris E3000, 115 VAC; Edison, NJ, USA), and only intact, high-molecular weight DNA without smearing was used for further analysis.

PCR amplification and electrophoresis

PCR amplification was performed in a 25 µL reaction mixture containing 12.5 µL MyTaq™ HS Red Mix (Bioline, Meridian Bioscience, London, UK), 1.5 µL each of forward and reverse primers (P4 and P5), 4.5 µL ddH_2O, and 5 µL genomic DNA template. The primers used were ITS P4 (forward: 5′-TCCTCCGCTTATTGATATGC-3′) and ITS P5 (reverse: 5′-GGAAGTAAAGTCGTAACAAGG-3′), as described by Mahar et al. (2017). PCR amplification was performed using a Bio-Rad T100™ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The PCR amplification process began with preheating the machine for 30 minutes before use. The PCR thermal cycling conditions, adapted from Mahar et al. (2017), were: initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute, and extension at 72°C for 1.5 minutes; followed by a final extension at 72°C for 7 minutes.

The PCR products were analyzed using agarose gel electrophoresis to verify the presence of amplified DNA fragments. A 1% agarose gel was prepared by dissolving 1 g of agarose powder in a solution of 90 mL of 1× TBE buffer and 10 mL of distilled water. The mixture was heated until fully dissolved, cooled to 50-60°C, stained with 1-2 µL GelRed, and poured into a casting tray with a comb to form wells before solidifying at room temperature. A 1 kb DNA ladder (A λ HindIII DNA ladder, Geneaid), mixed with 2 µL loading dye, was used as a molecular size marker. Electrophoresis was conducted at a constant voltage of 80 V and current of 400 mA for 60 minutes. Following electrophoresis, the gel was visualized under a UV transilluminator (Accuris E3000, 115 VAC; Edison, NJ, USA), and the resulting DNA band patterns were documented for further analysis.

DNA sequencing

The PCR products were sent to PT Genetika Science Indonesia and the 1st BASE (Singapore) for sequencing using the Sanger method.

Data analysis

Genetic diversity and population structure were analyzed using DnaSP v6.12.03 (Rozas et al. 2017) and Arlequin v3.5 (Excoffier and Lischer 2010). For genetic diversity analysis, InDel polymorphism was examined using the diallelic and non-overlapping model in DnaSP, which estimated parameters, such as the total number of InDel sites and events, average InDel length, number of InDel haplotypes, InDel haplotype diversity (Hd), InDel diversity per site (πi), average number of InDel differences (ki), Watterson's theta for InDel polymorphism (θ(i)-W), and Tajima's D value. This approach is particularly suitable

for the ITS region of rDNA, which, although relatively conserved at the nucleotide level, often exhibits length variations caused by insertion-deletion events resulting from slipped-strand mispairing or unequal crossing over. Such InDels tend to reflect evolutionary changes more effectively than single-nucleotide substitutions, thereby providing a more accurate representation of genetic differentiation within and among ITS haplotypes. Consistent with this, a study on the yeast *Geotrichum candidum* revealed that although most rDNA polymorphisms were substitutions, a notable InDel polymorphism was detected in the ITS1 region, indicating intragenomic variability and emphasizing the importance of considering InDel events when assessing genetic diversity in ITS rDNA-based analyses (Alper et al. 2011). To assess genetic structure, sequences were grouped into two populations: (i) cultivated variants (red, yellow, and green matoa) and (ii) the wild variant (forest matoa). Analysis of Molecular Variance (AMOVA, $\alpha = 0.05$) and fixation index (F_{st}) values were calculated using the Pairwise Difference method, with significance tested through 1,000 permutations. Input files for Arlequin were prepared using PGDSpider v3.0.0.0 (Lischer and Excoffier 2012), and analyses were performed under the Tamura-Nei substitution model to maintain consistency with the phylogenetic framework.

Sequence data were processed using BioEdit (Hall 2021), MEGA 11 (Tamura et al. 2021), and BLAST via the NCBI platform (NCBI 2025). Forward and reverse chromatograms were assembled in BioEdit to generate consensus sequences in FASTA format, which were identified through BLAST by examining the top 100 hits for percent similarity. Three reference species with >85% similarity were selected for phylogenetic comparison. Multiple sequence alignment was performed in MEGA 11 using the ClustalW algorithm, and phylogenetic relationships were reconstructed with the Neighbour-Joining (NJ) method under the Tamura-Nei substitution model, supported by 1,000 bootstrap replicates. *Dimocarpus longan* (MW070202.1; EF532359.1), *Nephelium cuspidatum* var. *eriopetalum* (OK052923.2), and *Nephelium lappaceum* (MT704594.1) were used as outgroup taxa, because they belong to the family Sapindaceae, closely related to matoa, but are distinct species, making them appropriate references for rooting the phylogenetic tree.

RESULTS AND DISCUSSION

ITS rDNA region characteristics

Based on electrophoresis results, DNA band lengths of four matoa variants used in this study falls within the 500 bp to 750 bp range (Figure 3).

Using Bioedit software, the nucleotide sequences generated by P4 and P5 primers were combined to produce a nucleotide sequence process, yielding ITS rDNA regions ranging from 478 to 681 bp, with an average complete sequence length of 676 bp across all matoa variants. The ITS1 region ranges from 175 to 270 bp, the ITS2 region spans 141 to 227 bp, while the 5.8S region remains conserved at 162 bp (Table 2).

Molecular diversity

The molecular diversity analysis of the combined ITS rDNA sequence from four matoa variants is summarized in Table 3. Each variant (red, yellow, green, and forest matoa) was represented by a single sequence, preventing the calculation of intra-group diversity indices. Therefore, diversity parameters were evaluated based on the combined dataset. From the 337 bp region (excluding sites with gaps or missing data), a total of 141 InDel sites and three InDel events were identified, with an average InDel length of 141 bp. Two distinct InDel haplotypes were observed among the four sequences, yielding an InDel haplotype diversity (Hd) of 0.500 and an InDel diversity per site (πi) of 0.001. The average number of InDel differences (ki) was 0.500, and Watterson's theta (θ(i)-W) was estimated at 0.545. Tajima's D value was −0.612 and not significant (P>0.10), indicating no deviation from neutrality among the ITS rDNA sequences analyzed.

Analysis of Molecular Variance (AMOVA) is presented in Table 4. It revealed that nearly all genetic variation (99.99%) was distributed among matoa populations, whereas only a negligible proportion (0.0003%) was found within populations. The fixation index (F_{st} = 0.999997) indicated extremely high genetic differentiation between the cultivated variants (red, yellow, and green matoa) and the wild forest matoa. However, permutation testing based on 1,000 random replicates produced a p-value of 0.2617±0.0139, suggesting that the differentiation was not statistically significant at the α = 0.05 level, likely due to the small and unbalanced sample size, particularly the single sequence representing the wild population.

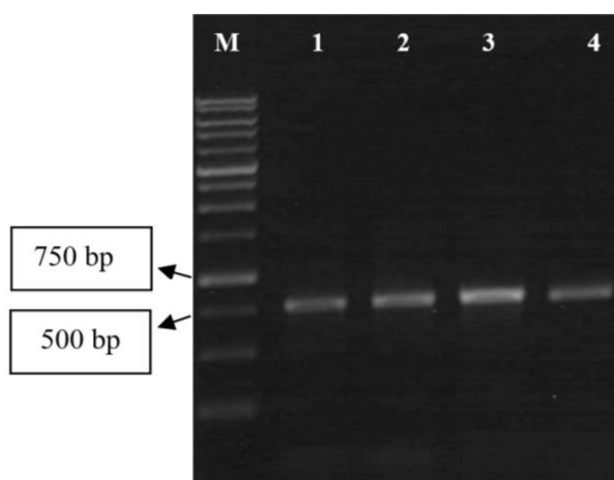

Figure 3. PCR-ITS rDNA electrophoregram of four matoa variants: M: Marker 1 kb DNA ladder, 1: Red matoa, 2: Yellow matoa, 3: Green matoa, 4: Forest matoa

Table 2. Size of the ITS rDNA regions for forest matoa/*kungkil*, red matoa, yellow matoa, and green matoa in South Sumatra, Indonesia

Code	18S (bp)	ITS 1 (bp)	5.8S (bp)	ITS 2 (bp)	28S (bp)	ITS region (ITS1; 5.8S; ITS2) (bp)	Total (bp)
KK	-	175	162	141	-	478	478
MRH	7	270	162	227	15	659	681
KN	7	270	162	227	15	659	681
HIU	7	270	162	227	15	659	681

Note: KK: Forest matoa, KN: Yellow matoa, MRH: Red matoa, HIU: Green matoa

Table 3. Molecular diversity indices of matoa variants (combined group)

Variant group	N	Sequence length (bp)	Total InDel Sites	InDel events	Average InDel length	InDel haplotypes (h)	InDel haplotype diversity (Hd)	InDel diversity per site (πi)	Average InDel differences (ki)	Watterson's Theta (θ(i)-W)	Tajima's D
KK	1	478	-	-	-	-	-	-	-	-	-
MRH	1	681	-	-	-	-	-	-	-	-	-
KN	1	681	-	-	-	-	-	-	-	-	-
HIU	1	681	-	-	-	-	-	-	-	-	-
All Combined	4	337*	141	3	141	2	0.500	0.001	0.500	0.545	-0.612 (ns)

Note: KK: Forest matoa, KN: Yellow matoa, MRH: Red matoa, and HIU: Green matoa. Sequence length: 337 bp (positions without gaps or missing data). Dashes (-) indicate that intra-group diversity could not be calculated due to a single sequence input per variant. Statistical significance for Tajima's D was not significant (P>0.10)

Table 4. AMOVA for genetic variation among and within matoa groups

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation (%)	F_{st}	p-value±SE
Among population	1	180,565.33	120,376.66 (Va)	99.9997	0.999997	0.261738±0.013901
Within population	2	0.66	0.33 (Vb)	0.0003	-	-
Total		180,565.99	120,376.99	100	-	-

Note: F_{st} and associated p-value calculated based on 1023 permutations using the pairwise difference method

Table 5. BLAST results of matoa variants based on ITS rDNA

Sample code	DNA fragments size (bp)	Highest similarity	Accession no.	E value	Max score	Query cover (%)	% Identity
MRH	681	*Pometia pinnata* voucher *Chase* 2135	EU720471.1	0.0	1258	100	99.85
KN	681	*Pometia pinnata* voucher *Chase* 2135	EU720471.1	0.0	1266	100	100
HIU	681	*Pometia pinnata* voucher *Chase* 2135	EU720471.1	0.0	1266	100	100
KK	478	*Pometia pinnata* voucher A4659	OK052934.1	0.0	883	100	100

Note: KK: Forest matoa, KN: Yellow matoa, MRH: Red matoa, and HIU: Green matoa

BLAST-based species confirmation

The ITS, sequences from forest, red, yellow, and green matoa were aligned in BioEdit, yielding from 478 to 681 bp and compared to reference accessions in the NCBI database using BLAST (Table 5). BLAST analysis of the ITS rDNA sequences revealed high similarity between all matoa variants and *P. pinnata* reference accessions. Red (MRH), yellow (KN), and green (HIU) matoa each produced sequences of 681 bp, showing near-complete alignment with *P. pinnata* voucher Chase 2135 (EU720471.1), with query cover values of 100% and identity ranging from 99.85% to 100%. Forest matoa (KK) generated a shorter sequence of 478 bp, which matched *P. pinnata* voucher A4659 (OK052934.1) with 100% identity and full query coverage. All samples returned maximum scores (883-1266) and e-values of 0.0, confirming highly significant alignments.

Phylogenetic tree and sequence variability

The phylogenetic tree was constructed using the Neighbour-Joining (NJ) method based on the Tamura-Nei model with 1,000 bootstrap replicates. The tree, rooted using ITS nrDNA sequences *D. longan*, *Nephelium cuspidatum*, and *N. lappaceum* revealed that the four matoa variants were grouped into two distinct clusters (Figure 4). Red matoa (Cult_MRH; PX452870), yellow matoa (Cult_KN; PX452868), and green matoa (Cult_HIU; PX452869) clustered together with *P. pinnata* voucher Chase 2135 (EU720471.1), forming Cluster I with strong bootstrap support (100). Forest matoa (Wild_KK; PX452867) clustered

separately with *P. pinnata* voucher A4659 (OK052934.1), forming Cluster II, also supported by a bootstrap value of 100. The two clusters were clearly differentiated from the outgroup taxa, which included *D. longan* (MW070202.1; EF532359.1), *N. cuspidatum* var. *eriopetalum* (OK052923.2), and *N. lappaceum* (MT704594.1). All major branches in the tree were strongly supported (bootstrap = 100), indicating robust and reliable clustering patterns.

DNA sequences from red, yellow, green, and forest matoa were aligned alongside reference sequences of *P. pinnata*-voucher A4659 (OK052934.1) and voucher Chase 2135 (EU720471.1) is presented in Table 6. The sequence alignment highlights both conserved and variable sites. At positions 27-37, several substitutions were observed: red matoa differed from the other three variants, which shared the same nucleotide composition as the reference sequences at most sites. At positions 506-517, differences were also detected; red matoa showed a deletion at position 507, whereas yellow, green, and forest matoa shared identical bases. In contrast, the reference sequences displayed additional substitutions at multiple positions, such as 508, 510, and 512. Several sites, including positions 33, 510, and 512, were conserved across all variants, indicating regions of stability within the ITS rDNA.

Discussion

The finding of two InDel haplotypes within the four matoa sequences indicates that the ITS rDNA region exhibits significant length variability despite its largely consistent nucleotide composition. The InDel haplotype diversity (Hd = 0.50) and InDel diversity per site (πi = 0.001) signify restricted yet noticeable structural variation linked to insertion-deletion events. These mutations are probably the consequence of slipped-strand mispairing or uneven crossing over during concerted evolution inside rDNA repeats. Despite the overall diversity being relatively low, these findings affirm that the ITS region is effective in differentiating closely related matoa variants, in accordance with the species' cross-pollinating characteristics that foster allelic heterogeneity among individuals (Suwardi et al. 2020; Hajar et al. 2021). A study on the yeast *G. candidum* demonstrated that, although most rDNA polymorphisms were substitutions, a significant InDel polymorphism was identified in the ITS1 region, highlighting intragenomic variability and emphasizing the necessity of incorporating InDel events in evaluations of genetic diversity in ITS rDNA-based analyses (Alper et al. 2011).

The variation in genetic diversity among matoa variants illustrates trends typically seen in tropical outcrossing tree species, influenced by reproductive biology and environmental variability that determine population structure. Similar diversity indices have been documented for various tropical tree species, indicating that outcrossing behavior and environmental variability play significant roles in genetic differentiation (Gonias et al. 2019; Katad et al. 2024). The presence of 141 InDel sites and 3 InDel events, despite each matoa variant being represented by a single sequence, indicates that the ITS region exhibits observable structural variation, even when analyzed within a constrained dataset. The sequence lengths observed (478-681 bp) align with earlier studies in Sapindaceae, indicating that ITS1 and ITS2 show more length variability compared to the highly conserved 5.8S rDNA (Mahar et al. 2017; Hariri et al. 2021). The observed moderate InDel haplotype diversity (Hd = 0.50) and low InDel diversity per site (πi = 0.001) reinforce the idea that insertion-deletion variation, despite its constraints, can serve as a reliable indicator of intraspecific differentiation within matoa populations.

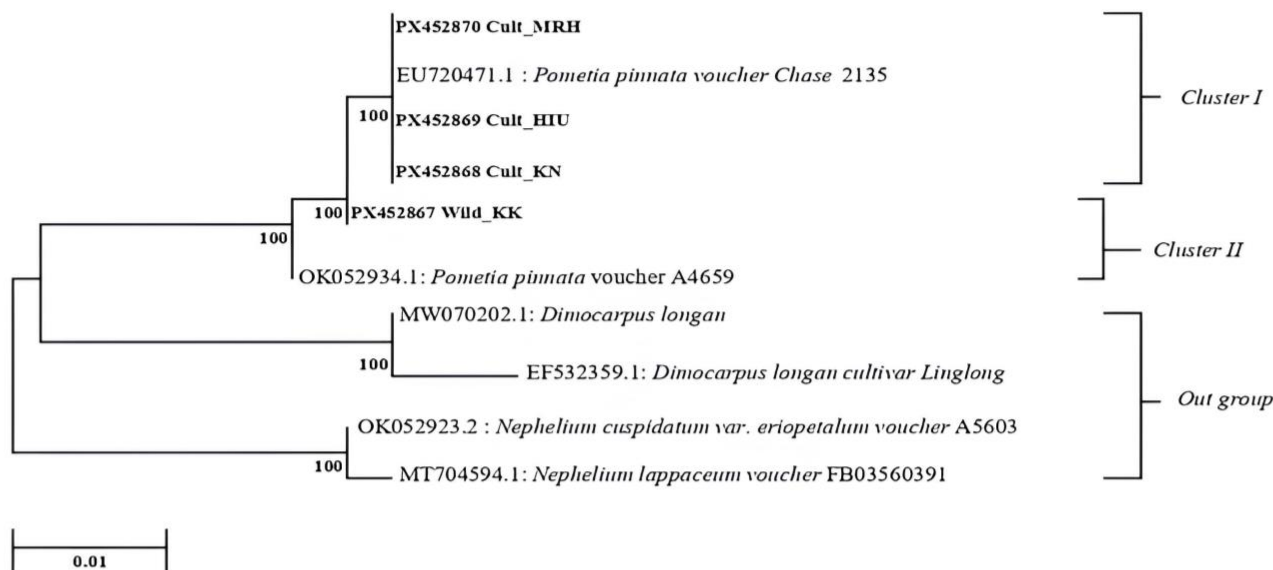

Figure 4. Phylogenetic tree based on ITS rDNA sequences of matoa and related taxa. The tree was constructed using the Neighbor-Joining method with 1,000 bootstrap replicates (values shown at nodes). The sequences labeled PX452867-PX452870 represent matoa variants (Wild_KK, Cult_KN, Cult_HIU, and Cult_MRH) obtained in this study. Other sequences were retrieved from GenBank and their corresponding accession numbers are shown

Table 6. Alignment segments of the ITS rDNA sequences from forest, red, yellow, and green matoa, along with reference sequences of *Pometia pinnata* (OK052934.1 and EU720471.1)

Species/Abbrv	27*	28	29	30	31	32	33*	34	35	36	37
Red matoa/ MRH	G	C	C	G	C	C	A	C	A	A	T
Yellow matoa/ KN	G	T	T	C	T	C	A	T	G	C	G
Green matoa/ HIU	G	T	T	C	T	C	A	T	G	C	G
Forest matoa/ KK	G	T	T	C	T	C	A	T	G	C	G
Pometia pinnata voucher A4659/ OK052934.1	G	T	C	T	T	G	A	A	A	G	T
Pometia pinnata voucher Chase 2135/ EU720471.1	G	T	C	T	T	G	A	A	A	G	T
		2 (28–29)			2			1		2	

Species/Abbrv	506*	507	508	509	510*	511	512*	513	514	516	517
Red matoa/ MRH	C	-	C	T	G	G	C	A	A	T	C
Yellow matoa/ KN	C	G	T	T	G	G	C	C	G	T	C
Green matoa/ HIU	C	G	T	T	G	G	C	C	G	T	C
Forest matoa/ KK	C	G	T	T	G	G	C	C	G	T	C
Pometia pinnata voucher A4659/ OK052934.1	C	G	C	G	G	C	C	C	G	A	A
Pometia pinnata voucher Chase 2135/ EU720471.1	C	G	C	G	G	C	C	C	G	A	A
		3		1		1		1	1 (514–516)		1

Note: 1: Transversion, 2: Transition, 3: Deletion, *: Conserved

Beyond length variation, nucleotide changes were also evident. Substitution mutations (both transitions and transversions) and deletion events, indicated by alignment gaps, were detected among the variants. Such mutations are commonly reported in nuclear ribosomal spacers and contribute to intraspecific variation. Deletions have been shown to drive genetic differentiation among closely related taxa (Abdullah et al. 2021), while transitions and transversions reflect typical mutation mechanisms in plant genomes (Morihoto et al. 2017). These mutational events provide important signals of divergence, even when overall sequence conservation is high. Hermansyah et al. (2018) emphasized that the mutation rate of the ITS region, compared to coding regions, enhances its utility in distinguishing closely related species.

Taken together, the balance between conserved and variable sites underscores the dual function of the ITS region. It serves as a reliable barcode for species-level identification while also providing phylogenetically informative variation at the intraspecific level. The mutational patterns observed here may reflect adaptive responses to environmental pressures, as nucleotide changes are key drivers of evolutionary processes (Triandiza et al. 2020; Mursyidin et al. 2021). The strong similarity among cultivated variants, in contrast to the subtle divergence observed in forest matoa, may reflect ecological separation, with wild populations maintaining higher heterogeneity due to cross-pollination and environmental gradients (Suwardi et al. 2020).

The results of Analysis of Molecular Variance (AMOVA) indicated that almost the entirety of genetic variation (99.9997%) was distributed among populations, with a mere 0.0003% present within populations. The fixation index (F_{st} = 0.999997) significantly surpasses Wright's threshold for strong genetic structuring (F_{st}>0.25), suggesting nearly complete genetic differentiation between cultivated variants (red, yellow, and green) and forest matoa (Pojskić 2017). This pattern indicates that gene flow between cultivated and wild habitats is highly restricted, probably because of ecological isolation and human-driven propagation methods. Despite permutation testing (p = 0.2617±0.0139) showing that the differentiation was not statistically significant at α = 0.05, the notably high Fst value indicates a clear genetic subdivision, suggesting limited connectivity between populations with forest matoa possibly representing a genetically distinct lineage. Expanded datasets incorporating multilocus markers, such as SNPs (Fasanella et al. 2024), will be needed to confirm whether the observed differentiation reflects true population structure or sampling artifacts.

BLAST analysis confirmed that all four matoa variants share strong homology with matoa, with e-values of 0.0 and percent identity values ranging from 99.85% to 100%. Red, yellow, and green matoa aligned with voucher Chase 2135 (EU720471.1), while forest matoa matched voucher A4659 (OK052934.1). These results verify that the samples belong to the same species, consistent with the interpretation that e-values of 0 indicate highly significant alignments and that percent identity above 99% reflects conspecificity (VanderWeele and Ding 2017; Gunnels et al. 2020; Andariyusti and Roslim 2021). However, the different voucher matches point to subtle genetic differences between cultivated and wild populations.

Phylogenetic reconstruction further supported this pattern, producing two well-supported clusters with bootstrap values of 100. The cultivated variants (red, yellow, and green) formed a single group, while forest matoa clustered separately. Short branches within the cultivated cluster indicate strong genetic homogeneity, likely reflecting shared ancestry and common agroforestry practices, such as vegetative propagation (Suwardi et al. 2020; Hajar et al. 2021). In contrast, the distinct placement of forest matoa suggests reduced gene flow and possible ecological divergence in its native lowland habitat.

Sequence alignment revealed conserved sites across all samples (e.g., positions 27, 33, 506, 510, 512), which highlight the genetic stability of ITS regions. At the same time, mutational events including substitutions (transitions and transversions) and deletions were also detected. Such mutations are typical of ribosomal spacers and provide useful phylogenetic signals at lower taxonomic levels (Morihoto et al. 2017; Sofiyanti and Isda 2019; Abdullah et al. 2021). The coexistence of conserved and variable regions illustrates the dual role of ITS markers are reliable for species identification yet informative for detecting fine-scale variation. From a taxonomic perspective, all variants are confirmed as matoa. Nevertheless, the distinct grouping of forest matoa suggests it retains unique alleles that may be important for conservation and breeding. Future research should integrate broader geographic sampling and multilocus markers, such as SNPs to resolve cryptic diversity (Fasanella et al. 2024).

The genetic uniformity observed among cultivated matoa variants suggests possible homogenization driven by domestication and agroforestry practices. In contrast, the distinct clustering of forest matoa emphasizes its role as a reservoir of unique alleles, which could be crucial for traits such as pest resistance, environmental tolerance, and broader adaptability. Preserving in situ populations of forest matoa is therefore essential to safeguard these alleles, while ex situ conservation of cultivated forms should prioritize broad ecological representation to maximize genetic variability (Suzuki et al. 2021; Syamsuardi et al. 2022; Putri et al. 2023). Such strategies are vital not only for maintaining biodiversity but also for ensuring the sustainable use of matoa in local communities.

Conserved ITS regions may serve as reliable molecular markers to track stable genetic traits, while variant-specific mutations could provide targets for developing improved cultivars. However, the ITS marker has certain limitations, as it exists in multiple copies and may include pseudogenes that introduce intragenomic variation (Xie et al. 2019; Stadler et al. 2020; Cedeño-Sanchez et al. 2024). Although no pseudogene signals were detected in this study, integrating additional nuclear and plastid loci, along with high-resolution SNP markers will improve molecular resolution (Fasanella et al. 2024). Future work that combines broader sampling across Sumatra, Kalimantan, and Papua with ecological and morphological data will be essential to capture the full extent of matoa diversity and to harness its potential in conservation and breeding programs.

In conclusion, this study illustrated that ITS rDNA markers effectively captured genetic diversity and phylogenetic divergence among four matoa variants from South Sumatra. Two InDel haplotypes, 141 InDel sites, and three InDel events exhibited measurable structural variation, even in the context of the sequence's overall conservation. Phylogenetic and AMOVA analyses clearly indicated a distinct separation between cultivated (red, yellow, and green-matoa) and forest matoa. Almost all genetic variation (99.9997%) was detected among populations, paired with an exceptionally high fixation index (F_{st} = 0.999997), suggesting limited gene flow and possible ecological divergence. Despite the limited sample size, the strong population structure indicates that forest matoa serves as a distinct reservoir of alleles. The findings highlight the importance of maintaining wild populations in their natural ecosystems while utilizing cultivated varieties in managed settings for effective breeding and genetic resource stewardship.

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