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Genetic Diversity and Phylogenetic Relationships of *Pometia pinnata* Variants from South Sumatra Using RAPD Markers

Laila Hanum[1*], Sandra Novira[1], Yadi Oktariansyah[1]
[1]Jurusan Biologi, Universitas Sriwijaya, Indralaya, Indonesia;

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*Corresponding Author: **Laila Hanum**, Jurusan Biologi, Universitas Sriwijaya, Indonesia;
Email: lailahanum@unsri.ac.id

Abstract: Matoa (*Pometia pinnata* J.R. & G. Forst) is a tropical tree of the Sapindaceae family widely distributed in Southeast Asia, yet the genetic diversity and evolutionary relationships among its variants in South Sumatra remain poorly understood. This study aimed to clarify the genetic structure of four local variants forest, red, yellow, and green matoa using Random Amplified Polymorphic DNA (RAPD) markers. Leaf samples representing each variant were collected across different regions of South Sumatra and analyzed through standard molecular procedures, including DNA extraction and RAPD amplification using five primers. Cluster analysis based on Jaccard's coefficient and UPGMA revealed two major genetic groups, indicating substantial variation among the studied variants. Red and yellow matoa showed the closest genetic affinity, whereas forest matoa was the most distinct from the others. These findings demonstrate considerable intraspecific diversity within matoa populations in South Sumatra. The study provides essential baseline information that strengthens conservation strategies, supports potential breeding programs, and enhances understanding of the species' evolutionary patterns within tropical ecosystems.

Keywords: Biodiversity, Matoa, phylogenetic relationship, *Pometia pinnata*, RAPD.

Introduction

Genetic diversity is a fundamental component of plant resilience, influencing species adaptability, ecological stability, and long-term survival, especially in biodiverse tropical ecosystems (Ebert & Engel, 2020). In tropical tree species, high genetic variation is often associated with improved tolerance to environmental stress, enhanced resistance to pests and diseases, and better reproductive success (Dias et al., 2018; Massa et al., 2024; Rojas-Cortés et al., 2024). As environmental changes intensify in tropical regions, understanding the genetic structure of key forest species has become increasingly urgent for conservation, restoration, and sustainable utilization programs. One such species is matoa (*Pometia pinnata* J.R. & G. Forst.), a member of the Sapindaceae family widely distributed across Southeast Asia, including Indonesia, Malaysia, the Philippines, and Papua New Guinea (Jacobs, 1962). In Indonesia, *P. pinnata* occurs naturally in Papua, Kalimantan, and Sumatra (Sutomo et al., 2021; Suwardi et al., 2022; Indow et al., 2022), where it provides ecological and economic value as a source of food (Suwardi et al., 2020; Syamsuardi et al., 2022), timber (Maryanto & Ruzuqi, 2023), and traditional medicine (Suzuki et al., 2021; Putri et al., 2023).

In South Sumatra, recent reports have documented four distinct variants of matoa—commonly known as forest matoa (*kungkil*), yellow matoa, green matoa, and red matoa (Usman & Laila, 2025; Novira & Laila, 2025). Although P. pinnata exhibits natural cross-pollination and is therefore expected to display high genetic variability (Hajar et al., 2023), its genetic diversity in South Sumatra has not been comprehensively explored. Previous studies in

Indonesia have examined genetic variation in other regions, such as Pekanbaru City (Zulfahmi et al., 2023) and Central Java (Yuniastuti et al., 2023), yet comparable data for South Sumatra remain scarce. This lack of information is particularly concerning given the rapid environmental changes in the region, where approximately 63% of forest cover loss occurred between 1990 and 2019 (Purnomo et al., 2023). Understanding the genetic structure of local matoa populations is therefore crucial for species conservation, sustainable utilization, and anticipating future responses to ecological pressures.

Molecular markers have emerged as indispensable tools for examining genetic diversity, evolutionary processes, and phylogenetic relationships in plants (Khal et al., 2023). Among them, Random Amplified Polymorphic DNA (RAPD) markers are widely used due to their simplicity, cost-effectiveness, and ability to detect genome-wide polymorphisms without prior sequence information (Babu et al., 2020; Alsaffar, 2023; Aydin et al., 2022; Yusvita & Idami, 2024; Katad et al., 2024). RAPD is particularly suitable for non-model species such as matoa, whose genetic resources and genomic information are still limited. Coupling molecular markers with bioinformatic approaches further strengthens evolutionary inference.

Phylogenetic tree construction enables the identification of genetic clusters, evolutionary affiliations, and divergence patterns within and between species (Kapli et al., 2020; Vankan et al., 2021; Hibbins et al., 2022; Zhang et al., 2023). Tools such as NTSYS 2.1 facilitate the conversion of RAPD banding patterns into binary matrices, computation of similarity coefficients, and development of dendrograms to visualize genetic relationships (Hanum et al., 2020; Tamura et al., 2021; Emelianova et al., 2023). Together, these methods allow for a robust assessment of genetic variability while contributing essential data to biodiversity studies (Kardos et al., 2021).

Despite its ecological, cultural, and economic significance (Suwardi et al., 2020; Syamsuardi et al., 2022), the genetic diversity and evolutionary relationships of P. pinnata variants in South Sumatra remain underexplored, representing a clear research gap. No molecular-based study has yet evaluated the genetic structure of forest, yellow, green, and red matoa in this region, even though such information is critical for identifying genetically rich populations, guiding breeding programs, and informing conservation planning (Kesari & Rangan, 2011; Shelke et al., 2020; Salgotra & Chauhan, 2023; Riaz et al., 2025; Viana et al., 2022).

Therefore, this study aims to (1) analyze the genetic diversity among four P. pinnata variants in South Sumatra using RAPD markers, (2) assess their evolutionary relationships through phylogenetic analysis based on Jaccard's similarity coefficient and UPGMA clustering, and (3) provide baseline genetic information to support conservation, breeding, and sustainable management efforts for this tropical species. Addressing this research gap will enhance our understanding of matoa's genetic architecture and contribute to broader strategies for safeguarding biodiversity in rapidly changing tropical environments.

Materials and Methods

Research Design

This study employed an exploratory molecular approach to characterize the genetic diversity of four matoa variants (forest, red, yellow, and green matoa) collected from different regions in South Sumatra. A Random Amplified Polymorphic DNA (RAPD) marker system was used to assess genetic variation, while cluster analysis and similarity coefficients were applied to evaluate phylogenetic relationships among variants.

Sample Collection

The research population consisted of matoa individuals occurring naturally in the South Sumatra region. Four representative variants were sampled: forest matoa (*kungkil*), red matoa, yellow matoa, and green matoa. One healthy individual per variant was selected purposively based on field identification, accessibility, and verified local information (Usman & Laila, 2025; Novira & Laila, 2025). Fresh leaves were collected from each variant,

cleaned, air-dried, labeled, and stored at –20°C. Sampling coordinates are presented in Table 1. 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, Sriwijaya University, Indonesia and the Shared Facility Laboratory, Department of Biology, Gadjah Mada University, Indonesia.

Table 1. Sample data used for research

Code	Sample identity	Region	Coordinate	
			X	Y
KK	Kungkil	Campang Tiga, Ogan Komering Ulu	104.7721	–3.7335
MRH	Matoa Merah	Baturaja, Ogan Komering Ulu	104.1712	–4.1236
KN	Matoa Kuning	Kalidoni, Palembang	104.8217	–2.9564
HIU	Matoa Hijau	Sukodadi, Palembang	104.7189	–2.9197

Procedures

DNA Isolation

Genomic DNA was extracted using the i-genomic Plant DNA Extraction Mini Kit following the manufacturer's protocol. Approximately 0.10 g of leaf tissue was ground in liquid-cooled conditions, lysed, and purified through spin-column procedures. DNA was eluted in 100 µl elution buffer. The protocol followed standard plant DNA extraction workflows as described in Dewanata & Mushlih (2021).

DNA Quality and Quantity Assesment

DNA purity and concentration were measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific, n.d.) under the dsDNA setting. Samples with A260/A280 ratios between 1.8–2.0 and concentrations above 100 ng/µl were considered suitable for downstream PCR analysis (Hikmatyar et al., 2015). DNA integrity was verified through 1% agarose gel electrophoresis in 1× TBE buffer and visualized under UV illumination.

DNA Amplification and Electrophoresis

DNA samples with good purity and concentration were then subjected to the amplification stage. The RAPD primers employed for matoa in this study were based on the research of Yuniastuti *et al.* (2023). The sequences of the primers used in the PCR–RAPD reactions included OPA 19 (5′–CAAACGTCGG–3′), OPB 15 (5′–GGAGGGTGTT–3′), OPD 08 (5′–GTGTGCCCCA–3′), OPD 11 (5′–AGCGCCATTG–3′), and OPD 13 (5′–GGGGTGACGA–3′).

Amplification was performed in 12.5 µl reaction volumes containing MegaMix Blue mastermix (Thistle Scientific, Cat. No. 2MMB-05; Glasgow, UK), primer, nuclease-free water, and DNA template. PCR conditions consisted of initial denaturation at 94°C (5 min), followed by 35 cycles of denaturation (95°C, 1 min), annealing (37°C, 1 min), extension (72°C, 1 min), and final extension at 72°C for 7 minutes. PCR products were resolved on 2% agarose gels stained with GelRed and documented using an UV transilluminator (Accuris E3000, 115 VAC; Edison, NJ, USA).

Operational Definitions and Research Variables

This study defines genetic diversity as the variation in RAPD banding patterns among various matoa variants, assessed by the presence or absence of certain amplified DNA fragments. A polymorphism band refers to a DNA fragment that exhibits variability among individuals, signifying genetic differentiation, while a monomorphic band is a segment that is uniformly present throughout all analysed samples. The similarity coefficient quantifies genetic relatedness among variations and is computed using Jaccard's coefficient derived from binary scoring of RAPD data. Cluster grouping refers to the categorisation of variations based on their genetic similarity patterns, executed by the Unweighted Pair–Group Method with Arithmetic Mean (UPGMA) to produce a dendrogram that depicts the genetic links among the sampled variants.

Data analysis

Genetic variation analysis through RAPD produces polymorphic DNA banding patterns. Genetic polymorphism refers to the occurrence of variations in genetic structure within a population. To ensure objectivity in identifying polymorphic bands, the electropherogram results from RAPD PCR were printed in 10 copies per primer and distributed to 10 different individuals for independent interpretation. The data collected were used to determine the molecular weight of the DNA bands by measuring the migration distance manually, from the well to the point of band migration on the gel. The migration distances were recorded in Microsoft Excel. The molecular sizes of the standard DNA marker bands were converted into logarithmic values to serve as Y-axis data, while the corresponding migration distances were used as X-axis data. A linear regression analysis was performed to generate a standard curve represented by the equation Y = ax + b. Using this equation, the migration distances of unknown DNA bands were used to calculate their corresponding logarithmic values (Y), which were then converted back through antilogarithmic transformation to determine the base pair sizes of the amplified fragments.

The RAPD data were encoded as binary values, where the presence of a DNA band was scored as "1" and the absence as "0." These binary matrices were input into Microsoft Excel to assess the genetic distance and relatedness among individuals. The data were subsequently exported to NTedit and analyzed using NTSYS-pc version 2.1 software. According to Lukmanasari et al. (2024), each RAPD primer used produced 100% polymorphic DNA bands, indicating a high level of genetic variation among the tested samples. This high polymorphism percentage is likely due to the taxonomic diversity among the plant specimens analyzed. The percentage of polymorphic bands was calculated using the following formula:

$$PP(\%) = \frac{\Sigma PB}{\Sigma PB + \Sigma MB} \times 100\% \quad \text{.....(1)}$$

Abbreviation:
PP = Polymorphic percentage
PB = Polymorphic bands
MP = Monomorphic bands

Molecular sizes of DNA bands were estimated by comparing migration distances with a DNA size marker, followed by logarithmic transformation and linear regression to generate a standard curve. Binary matrices were used to compute genetic similarity using the JCoA (Jaccard Coefficient of Similarity). Cluster analysis was conducted using NTSYS-pc version 2.1, employing the UPGMA algorithm to produce a dendrogram illustrating genetic relationships among variants (Yuniastuti et al., 2023). Analysis of the percentage of polymorphic bands to calculate the percentage of polymorphic bands that appear in DNA samples.

Results and Discussion

Genetic Diversity Revealed by RAPD Profiling

PCR–RAPD amplification using five primers generated clear and reproducible banding patterns that varied across the four P. pinnata variants (Figure 1). The presence of both polymorphic and monomorphic bands indicates genetic differentiation among individuals, which is expected in a predominantly outcrossing tropical tree species. The overall polymorphism percentage reached 96%, demonstrating a high level of genomic variation within the analyzed population (Table 2). In population genetics, polymorphism levels above 50% typically reflect wide allelic richness and substantial divergence among individuals (Radwan et al., 2021), consistent with the reproductive biology of P. pinnata, which is insect-pollinated and dispersed across heterogeneous habitats. According to Lukmanasari et al. (2024), the elevated polymorphism levels are attributed to cross–pollination, which leads to genetically diverse offspring.

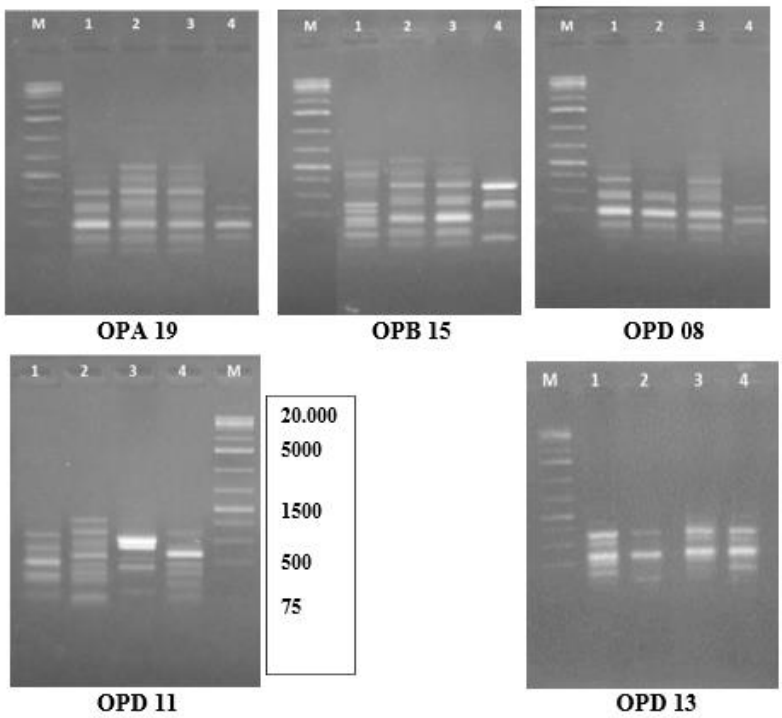

Figure 1. PCR–RAPD electrophoregram (Abbreviations: (M) 1kb Markers (Fermentas): 75bp, 500bp, 700bp, 1000bp, 1500bp, 2000bp, 3000bp, 5000bp, 7000bp, 8000bp, 10,000bp, 20,000bp. (1) Green matoa (2) Red matoa (3) Yellow matoa, (4) Forest matoa)

The predominance of polymorphic loci across all primers (67 of 69 bands) suggests that the RAPD markers effectively targeted highly variable genomic regions. Similar high-polymorphism patterns have been reported in other tropical Sapindaceae species where RAPD markers successfully captured inter-varietal diversity (Mahar et al., 2017; Yuniastuti et al., 2023). The broad fragment size range observed (158–2299 bp) also indicates amplification of multiple genomic loci, which strengthens the ability of RAPD to detect inter-variant variability.

Table 2. Polymorphism percentage

Primer	Primer Sequences (5' – 3')	Total of Bands		Total	%Bands	Fragments size (bp)	
		Polymorphic	Monomorphic			Lowest size	Highest size
OPA 19	CAAACGTCGG	8	2	10	80	203	1685
0PB 15	GGAGGGTGTT	18	0	18	100	158	1314
OPD 08	GTGTGCCCCA	13	0	13	100	168	1685
OPD 11	AGCGCCATTG	15	0	15	100	216	1160
OPD 13	GGGGTGACGA	13	0	13	100	705	2299
Total/Average/Highest/Lowest		67	2	69	96	158	2299

Specific bands identified in several primers (Table 3) likely represent genotype-unique loci that may serve as potential molecular identifiers. The presence of primer-specific amplicons, particularly in OPB 15, OPD 08, OPD 11, and OPD 13, confirms the existence of variant-exclusive genomic regions. Such diagnostic bands have been used in other genetic identification studies to distinguish morphologically similar accessions (Lukmanasari et al., 2024). These findings reinforce the potential of RAPD markers as preliminary tools for varietal authentication in matoa.

Table 3. Length of PCR–RAPD Amplified Bands

Primer	Amplified Locus Length (bp)	Specific DNA Bands	
		Bands size	Accession
OPA 19	203, 216, 277, 295, 314, 429, 623, 705, 963, 1314, 1685	–	–
0PB 15	158, 179, 191, 203, 295, 314, 379, 429, 486, 517, 663, 705, 850, 963, 1234, 1314	179	KK
OPD 08	168, 191, 216, 277, 295, 379, 403, 550, 663, 799, 905, 1234, 1685	168 1685	KK KK
OPD 11	216, 230, 277, 334, 356, 429, 486, 550, 585, 623, 663, 799, 905, 1160	230 1160	KN MRH
OPD 13	705, 905, 963, 1090, 1234, 1314, 1488, 1583, 1908, 2030,2299	2299	HIU

Description: Forest matoa (KK), Yellow Matoa (KN), Red Matoa (MRH), Green Matoa (HIU)

Genetic Relationships Among Matoa Variant

Jaccard similarity coefficients (Table 4) and UPGMA clustering (Figure 2) consistently separated the four matoa variants into two major genetic groups. Red and yellow matoa exhibited the highest similarity (65.22%), indicating strong genetic affinity. This relationship aligns with previous work on Sapindaceae trees, where phenotypically similar fruit color morphs often share close genotypic backgrounds. Conversely, forest matoa formed a distinct cluster, reflecting its unique banding profile and lower similarity values (39.13–44.93%). These findings support the hypothesis that forest matoa may represent an ecotype adapted to different environmental conditions, which typically drives divergence in tropical perennials (Asmara et al., 2023).

Table 4. Similarity matrix

	HIU	MRH	KN	KK
HIU				
MRH	0.4928			
KN	0.4348	0.6522		
KK	0.4058	0.4493	0.3913	

Description: Forest matoa (KK), Yellow matoa (KN), Red matoa (MRH), Green matoa (HIU)

Based on Table 4, the similarity index between green matoa and red matoa is 0.4928 (49.28%), while green matoa and yellow matoa share a similarity of 0.4348 (43.48%). The similarity between red matoa and yellow matoa is higher, at 0.6522 (65.22%). When compared with forest matoa, green matoa has a similarity index of 0.4058 (40.58%), red matoa 0.4493 (44.93%), and yellow matoa 0.3913 (39.13%). Based on these results, red and yellow matoa have a similarity index greater than 50%, indicating a close genetic relationship, while yellow matoa and forest matoa have a similarity index below 50%, suggesting a more distant kinship. According to Kundariati et al. (2021), a similarity index closer to 1 indicates a closer kinship, while a value closer to 0 reflects a more distant kinship. The similarity index ranges from 0 to 1.

The kinship relationship among matoa variants samples is depicted in Figure 2. The kinship relationship between matoa variants shows that the dendrogram divides the four samples into two main clusters at a similarity coefficient of 0.43, namely Cluster I and Cluster II. Cluster I, with a similarity coefficient of 0.47, is further divided into two sub–clusters: Sub–cluster A, which consists of HIU, and Sub–cluster B, which includes MRH and KN, forming at a similarity coefficient of 0.65. Meanwhile, Cluster II contains only one sample, KK. This indicates genetic differences between the variants. According to Azizah et al. (2019), the differences in kinship relationships observed based on RAPD markers are due to variations in DNA fragment amplification across the samples.

The dendrogram structure shows that the divergence threshold among matoa variants is moderate (0.43 similarity coefficient), implying that while they share a common species background, substantial genomic differentiation has accumulated. This may result from geographic isolation, pollination dynamics, or domestication-related selection pressures. The clustering pattern also parallels RAPD-based genetic structure reported for other Indonesian fruit trees, where wild accessions consistently group separately from cultivated types due to reduced gene flow (Azizah et al., 2019).

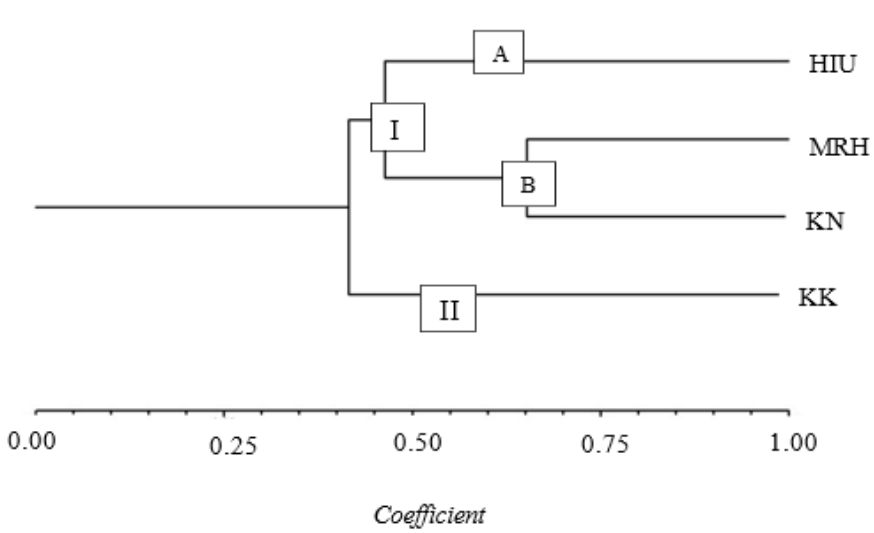

Figure 2. The dendrogram of matoa variants based on PCR–RAPD analysis (Description: Forest matoa (KK), Yellow matoa (KN), Red matoa (MRH), Green matoa (HIU)).

The RAPD molecular markers revealed that the forest matoa sample falls into a different cluster, as evidenced by the DNA bands detected from each primer (Figure 2). The forest matoa sample showed 25 DNA bands, while the red and yellow Matoa samples each displayed 34 bands, and the green matoa sample showed 35 bands. This indicates differences in the gene arrangement among matoa variants. Each detected DNA band is considered a single character representing a DNA locus. According to Saleky & Dailami (2021), factors contributing to differences in genetic profiles include habitat conditions.

The closest kinship is observed in samples like red matoa and yellow matoa, which have a high similarity index, while the forest matoa samples exhibit the most distant kinship with the green, red, and yellow matoa samples. This suggests a high level of genetic diversity, as genetic factors influence the traits expressed. According to Fitriyanti et al. (2023), samples with high genetic variation have significant potential to serve as sources of genetic diversity. Such variation is crucial for enhancing species adaptation to environmental changes and boosting productivity. The greater the genetic distance between samples, the lower the likelihood of successful crossbreeding, but the chances of obtaining superior genotypes are higher if the crossbreeding is successful.

Biological Interpretation and Implications

The high level of genetic variation detected in matoa suggests that the South Sumatra population maintains rich allelic diversity, which is biologically important for long-term adaptability and resilience to environmental changes. The distinct clustering of forest matoa implies unique genetic resources that may hold adaptive traits, such as tolerance to specific microhabitats. Meanwhile, the close kinship between red and yellow matoa reflects a shared lineage and suggests limited differentiation despite phenotypic variation in fruit characteristics.

From a conservation perspective, maintaining genetically distinct groups—particularly the divergent forest variant—is essential to avoid loss of rare alleles. For breeding programs, cross-compatibility potential is indicated by moderate similarity levels; however, crosses involving highly divergent types may yield greater heterosis, as seen in other cross-pollinated fruit trees (Hajar et al., 2021).

Study Limitations and Future Prospects

This study provides an initial assessment of genetic diversity among matoa variants using RAPD markers; however, several limitations restrict the depth of inference. First, the sampling design included only one representative per variant, which prevents estimation of intra-variant variability and may overemphasize individual-specific loci. Broader sampling across

populations and ecological gradients is necessary to accurately characterize within- and among-variant genetic structure. Second, RAPD markers are dominant, anonymous, and sensitive to amplification conditions. They cannot distinguish heterozygous from homozygous states, and therefore yield limited resolution compared to co-dominant markers. Consequently, the observed patterns should be interpreted as preliminary indicators of genomic differentiation rather than definitive measures of genetic distance. Third, the dendrogram lacks statistical support such as bootstrapping, and no complementary multivariate analyses (e.g., PCoA) or variance partitioning methods (e.g., AMOVA) were applied, which limits confidence in the inferred clustering patterns. Finally, environmental variables were not incorporated, preventing evaluation of whether ecological factors contribute to the observed genetic divergence—particularly relevant for the distinct forest matoa group.

Future studies should integrate larger and spatially diverse sampling to capture both within-variant and regional genetic variability. Employing high-resolution marker systems such as SSRs or SNP genotyping would enable robust estimation of allele frequencies, heterozygosity, gene flow, and population structure (Choudhury et al., 2023; Adjebeng–Danquah et al., 2020). Incorporating bootstrapped clustering, ordination analyses, and AMOVA will strengthen statistical validity and clarify the hierarchical distribution of genetic variation. In addition, coupling molecular data with ecological parameters (e.g., habitat type, microclimate, soil characteristics) would allow tests of local adaptation, which may explain the genetic distinctiveness of the forest matoa ecotype. Sequencing of accession-specific bands identified in this study could support the development of diagnostic markers for varietal authentication. Ultimately, improved genomic resolution and ecological integration will enhance conservation strategies, identify priority germplasm for breeding programs, and support sustainable utilization of matoa as an important tropical genetic resource.

Conclusion

This study sought to evaluate the genetic diversity and evolutionary relationships among Pometia pinnata variants from South Sumatra utilizing RAPD markers and bioinformatic analysis. The RAPD results demonstrated a significant degree of polymorphism (96%), signifying considerable genetic variety within the population. Cluster analysis with the similarity index categorized the varieties into two primary categories, differentiating forest matoa from farmed kinds. The most proximate genetic link was noted between red and yellow matoa, whereas forest matoa displayed the most genetic divergence. These data illustrate considerable genetic difference among P. pinnata variants, underscoring the efficacy of RAPD markers for evaluating genetic diversity and their importance in informing future conservation and breeding initiatives.

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