RESEARCH ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

GENETIC DIVERSITY OF LOCAL SUGAR APPLE (*ANNONA SQUAMOSA* L.) POPULATIONS FROM CENTRAL JAVA, INDONESIA, REVEALED BY RAPD MARKERS

Parjanto*, Sri Hartati, Endang Setia Muliawati, Endang Yuniastuti, Ida Rumia Manurung and Nadia Azzahro

Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Indonesia

*Corresponding author: parjanto@staff.uns.ac.id/

ABSTRACT

Sugar apple (*Annona squamosa* L.) is an economically and nutritionally important tropical fruit, yet the genetic structure of its locally cultivated populations in Indonesia remains poorly documented. Such information is a prerequisite for evidence-based germplasm conservation and for selecting parental material in fruit breeding. This study assessed the intra- and inter-population genetic diversity of local sugar apple from two long-established cultivation centers in Central Java, namely Sukolilo-Pati (PT) and Gedangsari-Gunungkidul (GK). Genomic DNA was extracted from leaves of 24 trees (12 per population) and amplified by the Random Amplified Polymorphic DNA (RAPD) method using ten primers (A16, A18, B11, C11, C19, D8, D11, H20, W3, and W4). The ten primers generated 65 scorable loci, of which 20 (30.77%) were polymorphic, indicating a moderate level of variation. Nei's gene diversity within populations was higher in PT (0.4098) than in GK (0.3648), whereas diversity between the two populations was lower (0.2179). Analysis of Molecular Variance (AMOVA) confirmed this pattern, partitioning 95% of the total variation within populations and only 5% among populations. The predominance of within-population variation reflects the outcrossing reproductive biology of the species and suggests that in situ conservation at both sites, combined with parent selection across rather than between populations, would most effectively safeguard and exploit the available genetic resources for Indonesian sugar apple improvement.

***Keywords*:** Germplasm conservation, Molecular markers, Plant genetic resources, Population genetics, Tropical fruit crops.

Article History: ABR-26-206 || Received: 02-Jun-2026 || Revised: 30-Jun-2026 || Accepted: 04-Aug-2026 || Published Online: 13-Aug-2026

1. INTRODUCTION

Agricultural biodiversity is a fundamental pillar of food security, health, and sustainable development. In this context, the development and conservation of local fruit varieties, such as sugar apple (*Annona squamosa* L.), has strategic relevance that is aligned with the Sustainable Development Goals (SDGs). Sugar apple, as a tropical fruit commodity, is not only economically valuable but also contains nutrients and bioactive compounds that contribute to health (Gautam et al., 2024; Moussa et al., 2024; Shehata et al., 2021). Thus, its development directly supports the achievement of SDG 2 (Zero Hunger) and SDG 3 (Good Health and Well-being). Furthermore, sustainable sugar apple cultivation can drive the local economy (SDG 8) by creating livelihoods and adding value to agricultural products. On the other hand, efforts to conserve local sugar apple varieties are a tangible form of preserving agricultural biodiversity (agrobiodiversity), which is at the heart of SDG 15 (Terrestrial Ecosystems) (FAO, 2019).

Sugar apple is the most widely grown member of the genus *Annona* (Annonaceae) (Leal & Paull, 2022; Rodrigues et al., 2023). Although native to tropical America, the species has spread across the lowland tropics of Asia, Africa, and Oceania, and it is now cultivated commercially in India, China, Thailand, the Philippines, Egypt, Brazil, and much of Southeast Asia, including Indonesia (Hazra et al., 2025). Its broad geographic range reflects a marked tolerance of warm, seasonally dry climates and marginal soils, traits that make it attractive for smallholder orchards and home gardens in regions where few other fruit trees thrive. The fruit is valued for its sweet, aromatic pulp and is consumed fresh as well as processed into juices, ice cream, and other products, providing a dependable seasonal income for rural growers. Beyond its food value, the leaves, seeds, bark, and roots are rich in alkaloids, acetogenins, flavonoids, and other secondary metabolites that underpin a long history of ethnomedicinal use and a growing body of research into antioxidant, antidiabetic, antimicrobial, and anticancer properties (Paternina-Ricardo et al., 2025; Yadav et al., 2025). This combination of nutritional, economic, and pharmacological importance positions sugar apple

Citation: Parjanto, Hartati, S., Muliawati, E.S., Yuniastuti, E., Manurung, I.R., & Azzahro, N. (2026). Genetic diversity of local sugar apple (*Annona squamosa* L.) populations from central java, Indonesia, revealed by RAPD markers. Agrobiological Records, 25, 209-218. https://doi.org/10.47278/journal.abr/2026.058

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

as a strategic, climate-resilient crop whose genetic resources merit systematic documentation and protection.

In Central Java, Indonesia, sugar apple has long been cultivated traditionally, with two main production locations in Sukolilo Village (Pati Regency) and Gedangsari Village (Gunungkidul Regency). The sugar apple population in these locations has been planted for generations over a long period of time, thus potentially forming local varieties with specific and unique genetic characteristics. Such plant populations can evolve into local populations (landraces) that are adapted to local environmental conditions. These local varieties are invaluable genetic resources, but they are also vulnerable to genetic erosion due to land use change, climate change, and agricultural homogenization. Therefore, their conservation is crucial not only to preserve genetic identity but also as a source of resilience in the face of climate change (Demirboğa et al., 2024).

Effective conservation and utilization of genetic resources must be based on a comprehensive understanding of the level and pattern of genetic diversity. This knowledge forms the basis for breeding programs to develop new, more adaptive and productive varieties. Analysis of genetic diversity using molecular markers provides a more accurate and objective approach than phenotypic observation alone. The Random Amplified Polymorphic DNA (RAPD) technique has been proven to be effective, fast, and efficient for preliminary genetic diversity analysis in various plants, including fruit crops, in detecting polymorphism at the DNA level (Goutam et al., 2019; Sá et al., 2022). This technique is particularly suitable for exploring diversity in local sugar apple populations that do not yet have complete genomic information.

Over the past decade, a wide array of DNA markers has been applied to characterise diversity and population structure in fruit crops, including dominant markers such as RAPD and ISSR and codominant or sequence-based systems such as SSR and SNP (Bidyananda et al., 2024). Within the genus *Annona*, recent studies have used these tools to assess germplasm collections and wild populations, for example SSR-based characterisation of an *A. squamosa* collection (Sá et al., 2022) and combined molecular and morphological analysis of *A. senegalensis* in Africa (Donhouédé et al., 2023). While SSR and SNP markers offer higher reproducibility and locus specificity, they require prior sequence information, primer development, and greater cost, which are seldom available for orphan tropical fruits at the local scale. RAPD analysis, by contrast, needs no prior sequence data, surveys multiple anonymous loci across the genome with short arbitrary primers, and remains a practical first-pass approach for screening previously uncharacterised landraces (Goutam et al., 2019). For the Central Java sugar apple populations, which lack any published genomic or marker resources, RAPD therefore represents an appropriate and cost-effective entry point for an initial assessment of genetic structure that can later be refined with codominant markers.

However, to date, there has been insufficient scientific information regarding the structure and genetic diversity of local sugar apple populations in their production centers in Central Java. This knowledge gap hinders evidence-based conservation efforts and targeted variety development. This study aims to analyze the intra- and inter-population genetic diversity of local sugar apple from two main locations in Central Java using RAPD markers. This genetic diversity analysis will reveal the potential germplasm stored in natural populations. The results of the study are expected to identify specific genetic characteristics of each population, such as the level of heterozygosity and genetic distance between populations. This information is vital for developing effective breeding strategies, such as parent selection and the creation of adaptive superior varieties. Given the outcrossing reproductive biology of the species and the geographic proximity of the two cultivation centers, we hypothesised that the populations would retain a moderate level of polymorphism, that the larger share of genetic variation would be partitioned within rather than among populations, and that the two populations would show only weak genetic differentiation.

2. MATERIALS AND METHODS

2.1. Plant Material

The plant material for this study was in the form of samples of sugar apple leaves from two populations in sugar apple cultivation centers in Central Java, namely Sukolilo-Pati (PT) and Gedangsari-Gunungkidul (GK) (Fig. 1). Twelve plants were randomly selected from each population, resulting in a total of 24 samples analyzed.

The Sukolilo-Pati (PT) site is located in the northern lowland of Pati Regency (approximately 6°46′ S, 111°03′ E, ca. 50–120 m above sea level), whereas the Gedangsari-Gunungkidul (GK) site lies in the southern karst uplands of Gunungkidul Regency (approximately 7°53′ S, 110°38′ E, ca. 200–400 m above sea level). Both sites experience a tropical monsoon climate with a mean annual temperature of about 26–28°C, mean annual rainfall in the order of 1,900–2,300 mm concentrated in the November–April wet season, and relative humidity of roughly 75–85%. In each population, the sampled trees were mature, productive individuals estimated to be more than ten years old, grown in traditional smallholder orchards and home gardens with minimal external inputs and no formal pruning or fertilization program. To minimize the chance of sampling clonally related or closely related neighboring trees, sampled individuals were spaced at least 20–30 m apart. A sample of twelve trees per population was used to balance adequate representation of within-site variation against the practical availability of accessible, clearly identified mature trees at each location and the exploratory scope of this initial RAPD survey.

Citation: Parjanto, Hartati, S., Muliawati, E.S., Yuniastuti, E., Manurung, I.R., & Azzahro, N. (2026). Genetic diversity of local sugar apple (*Annona squamosa* L.) populations from central java, Indonesia, revealed by RAPD markers. Agrobiological Records, 25, 209-218. https://doi.org/10.47278/journal.abr/2026.058

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

Fig. 1: Location of sugar apple cultivation centers in Central Java, Indonesia.

2.2. DNA Extraction and Purification

DNA was extracted using a modified CTAB (hexadecyltrimethylammonium bromide) protocol supplemented with 1% PVP (Gill et al., 2025) and the extracts were purified with the GeneClean III Kit.

2.3. DNA Amplification

DNA amplification was performed on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). Each reaction (total volume 10 µL) contained 2.45 µL water, 1 µL 10× Stoffel buffer (10 mM Tris-HCl, pH 8.3, and 1.0 mM KCl), 0.05 units of Taq DNA polymerase Stoffel fragment (Applied Biosystems), 1.2 µL 25 mM $MgCl_2$, 0.8 µL 25 mM dNTP, 0.5 µL 10 µM primer, and 4 ng of template DNA. The cycling profile began with initial denaturation at 94°C for 60 seconds, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 37°C for 1 minute, and extension at 72°C for 2 minutes (Williams et al., 1990).

2.4. Electrophoresis

Electrophoresis was carried out on a 1.2% agarose gel in 1× TAE buffer. For loading, 10 µL of PCR product was combined with 2 µL of gel loading dye, and the gel was run at 120 volts for approximately 3 hours. Gels were stained with GelRed Nucleic Acid Stain (Biotium) (Sari et al., 2022) and imaged on a Fotodyne GelDoc Image Analyzer. A 100 bp ladder (Vivantis) served as the size standard for the amplified fragments.

2.5. Data Analysis

The RAPD profiles were manually scored as a binary matrix (1 for presence, 0 for absence), assuming two alleles per locus (Williams et al., 1990). Genetic diversity was analyzed using Nei (1972, 1973) genetic distance and Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992), both implemented via POPGENE (Yeh et al., 1997). Only clear, consistently reproducible bands between 250 and 3,000 bp were scored; faint, smeared, or non-reproducible bands were excluded. Each band position was treated as a separate locus and scored as a binary character (1 = presence, 0 = absence) for every individual, and a band was classified as polymorphic when it was present in some but not all individuals across the 24 samples. From this binary matrix, the percentage of polymorphic loci, Nei's gene diversity within and between populations, and the partitioning of variation by AMOVA were computed in POPGENE version 1.32, with the significance of among-population variation tested using 999 permutations.

Citation: Parjanto, Hartati, S., Muliawati, E.S., Yuniastuti, E., Manurung, I.R., & Azzahro, N. (2026). Genetic diversity of local sugar apple (*Annona squamosa* L.) populations from central java, Indonesia, revealed by RAPD markers. Agrobiological Records, 25, 209-218. https://doi.org/10.47278/journal.abr/2026.058

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

3. RESULTS & DISCUSSION

3.1. DNA Quantification

The concentration and purity of DNA extracted from 24 sugar apple plants, namely 12 plants from the Gunungkidul Regency population (GK) and 12 plants from the Pati Regency population (PT), are presented in Table 1. DNA concentration ranged from 107.3 to 265.7 ng/µL. A good value of DNA concentration and purity is if the isolated DNA concentration is greater than 20 ng/µL (Koetsier & Cantor, 2019; Utaminingsih & Sophian, 2022). The higher the DNA concentration value, the higher the DNA content in the solution. A concentration value that is too low will result in weak PCR. This DNA concentration can be influenced by the biological properties of the plant and the extraction method (Kaya & Şahin, 2025).

Table 1 also shows that the purity of DNA from Gunungkidul (GK) sugar apple ranged from 1.73 to 1.99 and Pati (PT) ranged from 1.49 to 2.00. Based on the results of measuring the level of DNA purity, it can be stated that the extracted DNA has good enough quality to be used in DNA amplification using the PCR-RAPD technique. DNA purity is measured based on the light absorption ratio or optical density (OD) at wavelengths $_{260}/_{280}$. DNA is said to be pure if the OD ratio $_{260}/_{280}$ ranges between 1.8 – 2.0 (Sambrook et al., 1989). Values below 1.8, such as those in PT-3 (1.60) and PT-9 (1.49), indicate protein residues that may be caused by the inhibition of phenolic compounds during deproteinization or precipitation imperfections (Bunu et al., 2020; Esfandani-Bozchaloyi et al., 2019). DNA of poor purity can be improved by washing the DNA using ethanol and then diluting the DNA before processing in the PCR stages (Yuniastuti et al., 2023).

Table 1: DNA concentration and purity

Sample plants	DNA concentration (ng/µL)	OD 260/280
GK-1	156.9	1.76
GK-2	198.7	1.77
GK-3	165.8	1.87
GK-4	251.6	1.99
GK-5	180.2	1.99
GK-6	190.2	1.79
GK-7	114.0	1.79
GK-8	182.4	1.73
GK-9	107.3	1.79
GK-10	116.5	1.90
GK-11	109.9	1.77
GK-12	213.9	1.95
PT-1	130.2	1.76
PT-2	192.8	2.00
PT-3	265.7	1.60
PT-4	158.0	1.62
PT-5	151.0	1.60
PT-6	143.6	1.68
PT-7	149.1	1.62
PT-8	139.9	1.80
PT-9	194.2	1.49
PT-10	149.5	1.96
PT-11	140.6	1.81
PT-12	144.7	1.62

GK: Gunungkidul; PT: Pati.

3.2. DNA Amplification

The amplification results using 10 selected primers on two populations of sugar apple (24 sample plants) can be seen in Fig. 2. The amplification resulted in 20 polymorphic loci. The number of loci produced by each primer ranged from 1 – 3 loci, with an average of 2 loci per primer (Table 2).

An initial set of eighteen RAPD primers was screened, and the ten primers that produced clear, reproducible, and polymorphic banding patterns (Table 2) were retained for the final analysis. Based on the results of RAPD analysis using ten primers, a genetic polymorphism level of 30.77% was detected in the sugar apple population studied. Polymorphism can be caused by the absence of amplification at a locus triggered by differences in the nucleotide base sequence at the primer binding site. This value indicates that of the total 65 amplified genomic loci, 20 loci (30.77%) were polymorphic, while 45 loci (69.23%) were monomorphic. This level of polymorphism indicates moderate genetic diversity in the analyzed population, which is consistent with the characteristics of perennial plants that generally have moderate genetic variability. Factors that can influence this polymorphism include a relatively narrow genetic sample population (Ellegren & Galtier, 2016), the dominant method of vegetative propagation (Wang et al., 2025), pollination system (Burgarella et al., 2024) and RAPD primar selection (Bidyananda et al., 2024).

Primer H20 is the most informative with a percentage of polymorphic bands of 50.00%, demonstrating its ability to detect genetic variation in half of the amplified loci. Primers C11 (42.86%) and W3 (37.50%) also have fairly high informative values. Primers C19 and D11 have the lowest (20.00% each), indicating that the target sequences of these primers are relatively conservative in the analyzed population. The percentage of polymorphic bands range of 20–50% reflects heterogeneity in the level of DNA sequence variation across different regions of the sugar apple plant genome.

Based on Fig. 2, it can be observed that samples PT-3 and PT-9 did not display DNA bands on all primers used in the RAPD analysis, indicating that both samples failed to amplify during the PCR process. This failure was most likely caused by poor sample DNA quality, which can occur due to genetic material degradation during storage or extraction (Schenk et al., 2023), or the presence of inhibitory contaminants such as polyphenolic compounds or polysaccharides commonly found in plant tissues (Kiss et al., 2024). In addition, technical factors such as suboptimal DNA concentration or unsuitable PCR reaction conditions may also contribute to the absence of bands. Because the absence of bands for PT-3 and PT-9 reflected amplification failure rather than true null genotypes, these positions

Citation: Parjanto, Hartati, S., Muliawati, E.S., Yuniastuti, E., Manurung, I.R., & Azzahro, N. (2026). Genetic diversity of local sugar apple (*Annona squamosa* L.) populations from central java, Indonesia, revealed by RAPD markers. Agrobiological Records, 25, 209-218. https://doi.org/10.47278/journal.abr/2026.058

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

were treated as missing data during scoring; the two individuals were nonetheless retained in the data set so that both populations remained represented by twelve trees in the molecular variance analysis (Table 4).

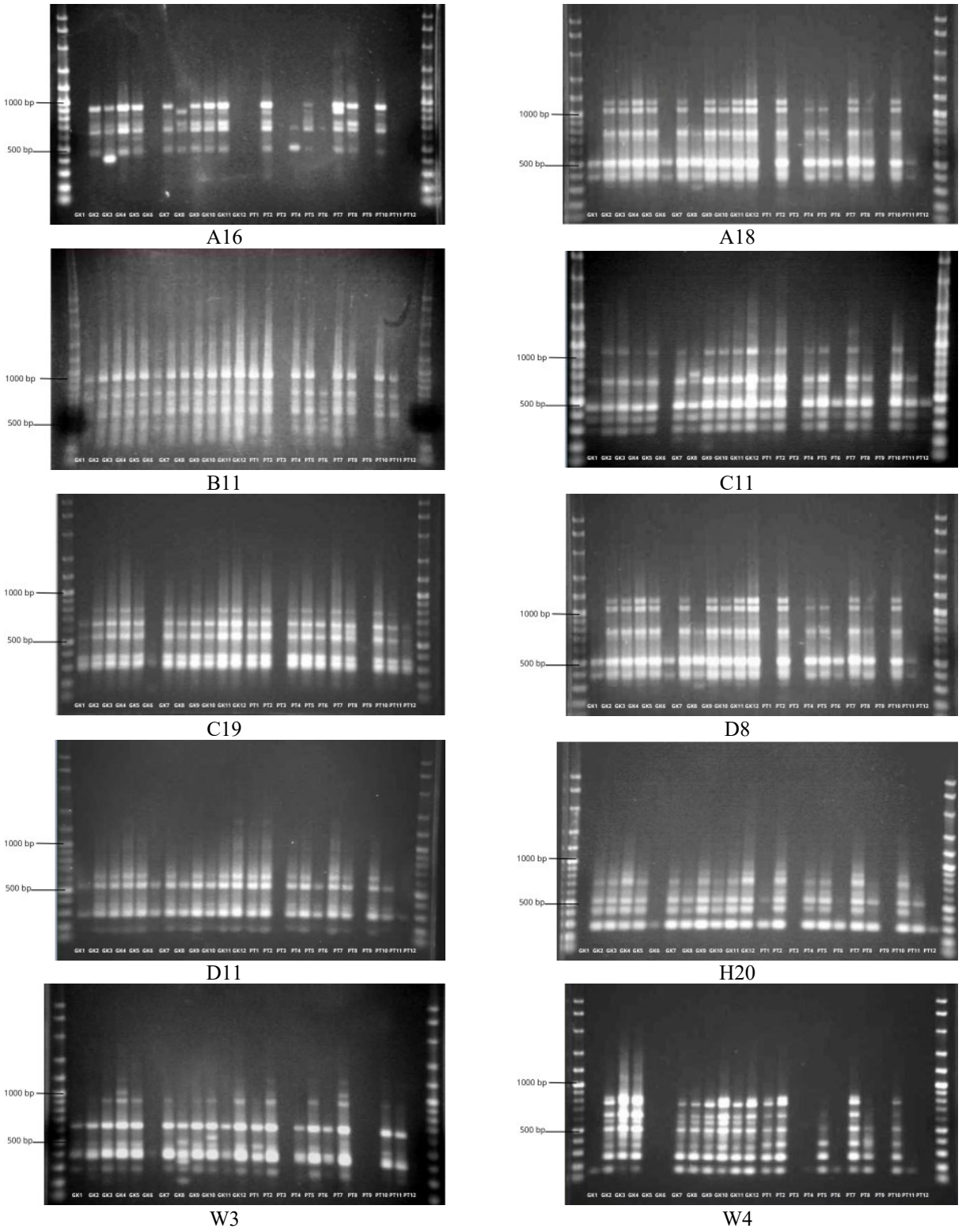

Fig. 2: RAPD amplification banding pattern in *Annona squamosa* using 10 different primers. (a) A16; (b) A18; (c) B11; (d) C11; (e) C19; (f) D8; (g) D11; (h) H20; (i) W3; (j) W4.

Citation: Parjanto, Hartati, S., Muliawati, E.S., Yuniastuti, E., Manurung, I.R., & Azzahro, N. (2026). Genetic diversity of local sugar apple (*Annona squamosa* L.) populations from central java, Indonesia, revealed by RAPD markers. Agrobiological Records, 25, 209-218. https://doi.org/10.47278/journal.abr/2026.058

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

Table 2: The primers used and the number of polymorphic banding patterns produced on sugar apple plants

Primer	Primer sequence (5′→3′)	Number of amplified bands	Number of polymorphic bands	Polymorphic bands (%)
A16	AGCCAGCGAA	8	2	25.00
A18	AGGTGACCGT	9	2	22.22
B11	GTAGACCCGT	4	1	25.00
C11	AAAGCTGCGG	7	3	42.86
C19	GTTGCCAGCC	5	1	20.00
D8	GTGTGCCCCA	6	2	33.33
D11	AGCGCCATTG	5	1	20.00
H20	GGGAGACATC	6	3	50.00
W3	GTCCGGAGTG	8	3	37.50
W4	CAGAAGCGGA	7	2	28.57
	Total	65	20	30.77

Across the ten primers, a total of 65 bands were amplified, of which 20 (30.77%) were polymorphic and 45 (69.23%) were monomorphic.

3.3. Genetic Diversity

The results of the genetic diversity analysis across two sugar apple (*A. squamosa*) populations, specifically the Gunungkidul (GK) and Pati (PT) populations, reveal the presence of both intra-population and inter-population variation. The genetic diversity within the GK population was recorded at 0.3648, while the PT population showed a value of 0.4098. In contrast, the genetic diversity between the populations (inter-population) was 0.2179 (Table 3). These findings indicate that genetic diversity within populations is greater than the diversity between them.

Table 3: Value of genetic diversity within the population (diagonal) and between populations (bottom diagonal) based on Nei's gene diversity (1972; 1973)

Population	Gunungkidul	Pati
Gunungkidul	0.3648	
Pati	0.2179	0.4098

The genetic diversity within each sugar apple (*A. squamosa*) population (PT and GK) is classified as relatively high. The genetic diversity values of 0.4098 for the PT population and 0.3648 for the GK population fall within the moderate-to-high category according to Botstein et al. (1980). The application of the Polymorphism Information Content (PIC) index, introduced by Botstein et al. (1980), remains highly relevant and is extensively utilized in contemporary plant genetic studies to evaluate the discriminatory power of molecular markers (Chesnokov & Artemyeva, 2015).

The sugar apple (*A. squamosa*) exhibits a protogynous dichogamy flowering system, in which the female and male reproductive organs of the hermaphroditic flowers mature at different times. This mechanism naturally prevents self-pollination and promotes a dominance of cross-pollination, leading to high genetic diversity within sugar apple populations (Kishore et al., 2012; Bisht et al., 2025).

With a high level of genetic diversity, both populations possess significant potential for adaptation to environmental changes and support the success of breeding programs (Hoban et al., 2021; O'Brien et al., 2025). This variation is essential because genetic diversity provides the raw material for natural selection and long-term fitness in shifting environments (Abdul-Rahman et al., 2021). Furthermore, maintaining such diversity is a critical prerequisite for genetic gain and resilience in modern breeding strategies (Li et al., 2022).

The genetic diversity between *A. squamosa* populations (PT and GK) was 0.2179, which is classified as low (Botstein et al., 1980). This inter-population diversity was notably lower than the diversity found within each population (Table 3). These findings are corroborated by the Analysis of Molecular Variance (AMOVA), which revealed that the vast majority of genetic variation (95%) occurs within populations, while only a minor portion (5%) is due to differences between populations (Table 4). Low inter-population diversity is a frequent phenomenon in plant species and has been documented in various studies, including research on *Annona senegalensis*, where the bulk of genetic variation was identified at the intra-population level (Donhouédé et al., 2023). The observed inter-population diversity suggests the existence of certain geographical barriers or environmental factors that may restrict full gene flow between the Gunungkidul and Pati populations, thereby allowing for some degree of genetic differentiation. Nonetheless, the low overall diversity values imply that these two populations have not undergone significant genetic differentiation.

Table 4: Molecular variance analysis (AMOVA) in two populations of *A. squamosa*

Source of Variation	df	Sum of Squares	Mean Square	Variance Component Estimate	Percentage of Variation	P Value
Between Populations	1	8.833	8.833	0.280	5	P = 0.412
Within Populations	22	120.417	5.473	5.473	95	–
Total	23	129.250	–	5.753	100	–

The moderate polymorphism recorded here (30.77%) sits within the range reported for other sugar apple germplasm assessed with dominant and codominant markers. In India, RAPD-based studies of custard apple landraces

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

in Madhya Pradesh similarly detected intermediate levels of variation among accessions (Goutam et al., 2019), while SSR analysis of an active *A. squamosa* collection in Brazil revealed most variation residing among individuals within the collection rather than between defined groups (Sá et al., 2022). A comparable structure was reported for *A. senegalensis* populations sampled across Benin and Mozambique, where the majority of genetic variation was partitioned at the within-population level (Donhouédé et al., 2023). The agreement between these tropical and subtropical studies and the present data points to a shared biological explanation (Abuelmaali et al., 2024). The dominance of cross-pollination in *Annona*, enforced by protogynous dichogamy, continually reshuffles alleles among individuals and inflates heterozygosity within each stand. At the same time, the exchange of seedlings and scion material by smallholders across the two regencies, together with their geographic proximity within Central Java, would maintain enough gene flow to keep the populations from diverging (Depecker et al., 2023; Liu et al., 2023; Phang et al., 2024). These two forces, abundant outcrossing within sites and ongoing germplasm movement between sites, jointly account for the simultaneous observation of high within-population diversity, moderate overall polymorphism, and low among-population differentiation.

These patterns carry direct practical implications for Indonesian fruit improvement. Because variation is concentrated within rather than between populations, parent selection for breeding should focus on capturing diverse individuals from across each site, and crosses that deliberately combine Gunungkidul and Pati genotypes offer a means of broadening the genetic base of new varieties while exploiting the modest differentiation detected between the two stands. The substantial within-population diversity is also a favorable starting point for selecting climate-resilient material, since it supplies the standing variation on which selection for drought tolerance, heat tolerance, and adaptation to marginal karst soils can act (Hoban et al., 2021; Li et al., 2022). At the same time, the findings should be read in light of the limitations of the marker system. RAPD profiles are dominant, so heterozygotes cannot be distinguished from dominant homozygotes, and the technique is known to be sensitive to reaction conditions, which can compromise reproducibility relative to codominant SSR or sequence-based SNP markers (Bidyananda et al., 2024). The amplification failure of two Pati samples illustrates this sensitivity. The estimates reported here should therefore be regarded as a robust first approximation of genetic structure rather than a definitive account; future studies employing SSR or SNP genotyping, larger sample sizes, and additional populations across Java would allow heterozygosity, gene flow, and fine-scale structure to be quantified more precisely, in line with the shift toward sequence-based breeding in other crops (Varshney et al., 2019).

High internal genetic health (95%) indicates that these populations are currently genetically robust and are unlikely to suffer from inbreeding depression (Charlesworth & Wilis, 2009; Hedrick & Garcia-Dorado, 2016). Since most genetic variation resides within individual populations, the primary conservation focus must prioritize the protection of natural habitats (in situ conservation) at both GK and PT sites. This approach is essential for maintaining natural ecological processes and preserving the full spectrum of existing internal genetic diversity (Frankham, 2019; O'Brien et al., 2025). Furthermore, the availability of recent high-quality genomic resources for *A. squamosa* could facilitate more effective plant breeding and crop improvement programs in the future (Talavera et al., 2023; Bisht et al., 2025).

4. CONCLUSION

RAPD analysis of local sugar apple (*Annona squamosa* L.) from the Gunungkidul and Pati cultivation centers revealed a moderate level of genetic polymorphism and showed that most of the variation is held within rather than between populations, with only weak differentiation separating the two sites. This pattern is consistent with the outcrossing reproductive biology of the species and indicates that both populations remain genetically healthy reservoirs of diversity. For conservation, the predominance of within-population variation argues for prioritizing in situ protection of mature trees at both locations, supported where possible by ex situ collections that capture individuals from across each site. For breeding, the appreciable within-population diversity provides a useful pool of parental material, and the genetic distance detected between the two populations suggests that crosses combining Gunungkidul and Pati accessions could be exploited to broaden the genetic base of improved varieties. The results provide the first molecular baseline for these Central Java landraces and a foundation for future work using codominant SSR or SNP markers to refine estimates of structure, gene flow, and adaptive potential, thereby strengthening germplasm utilization within Indonesian fruit improvement programs.

Declarations

Funding: This research was funded by Universitas Sebelas Maret and the Ministry of Higher Education of the Republic of Indonesia.
Acknowledgement: The authors gratefully acknowledge Universitas Sebelas Maret and the Ministry of Higher Education of the Republic of Indonesia for funding this research.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

Conflicts of Interest: The authors declare no potential conflict of interest.

Data Availability: All the data are available in the article.

Ethics Statement: This study involved only plant materials. No human subjects, animal experiments, or genetically modified organisms were used; therefore, formal ethics committee approval was not required.

Author's Contributions: Parjanto: Conceptualization, Supervision, Formal analysis, Writing – original draft, Writing – review & editing. S. Hartati, E.S. Muliawati, and E. Yuniastuti: Plant material collection, DNA extraction, Writing – review & editing. I.R. Manurung and N. Azzahro: Molecular analyses, Data curation, Writing – review & editing. E. Yuniastuti: Statistical analyses, Writing – review & editing.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Abdul-Rahman, F., Tranchina, D., & Gresham, D. (2021). Fluctuating Environments Maintain Genetic Diversity through Neutral Fitness Effects and Balancing Selection. Molecular Biology and Evolution, 38(10), 4362–4375. https://doi.org/10.1093/molbev/msab173

Abuelmaali, S. A., Mashlawi, A. M., Ishak, I. H., Wajidi, M. F. F., Jaal, Z., Avicor, S. W., & Kassim, N. F. A. (2024). Population genetic structure of Aedes aegypti subspecies in selected geographical locations in Sudan. Scientific Reports, 14(1), 2978–2978. https://doi.org/10.1038/s41598-024-52591-6

Bidyananda, N., Jamir, I., Nowakowska, K., Varte, V., Vendrame, W. A., Devi, R. S., & Nongdam, P. (2024). Plant Genetic Diversity Studies: Insights from DNA Marker Analyses. International Journal of Plant Biology, 15(3), 607–640. https://doi.org/10.3390/ijpb15030046

Bisht, M. S., Mahajan, S., Chakraborty, A., & Sharma, V. K. (2025). A high-quality genome assembly of Annona squamosa (custard apple) provides functional insights into an emerging fruit crop. DNA Research, 32(3), dsaf007. https://doi.org/10.1093/dnares/dsaf007

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. American Journal of Human Genetics, 32, 314–331.

Bunu, S., Otele, D., Alade, T., & Dodoru, R. (2020). Determination of serum DNA purity among patients undergoing antiretroviral therapy using NanoDrop-1000 spectrophotometer and polymerase chain reaction. Biomedical and Biotechnology Research Journal (BBRJ), 4(3), 214. https://doi.org/10.4103/bbrj.bbrj_68_20

Burgarella, C., Brémaud, M.-F., Von Hirschheydt, G., Viader, V., Ardisson, M., Santoni, S., Ranwez, V., De Navascués, M., David, J., & Glémin, S. (2024). Mating systems and recombination landscape strongly shape genetic diversity and selection in wheat relatives. Evolution Letters, 8(6), 866–880. https://doi.org/10.1093/evlett/qrae039

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. Nature Reviews Genetics, 10, 783–792. https://doi.org/https://doi.org/10.1038/nrg2664

Chesnokov, Y. V., & Artemyeva, A. M. (2015). Evaluation of the measure of polymorphism information of genetic diversity. Agricultural Biology, 50(5), 571–578. https://doi.org/10.15389/agrobiology.2015.5.571eng

Demirboğa, G., Demirboğa, Y., & Özbay, N. (2024). Local varieties and their importance. Biodiversity Studies, 3(1), 15–18.

Depecker, J., Verleysen, L., Asimonyio, J. A., Hatangi, Y., Kambale, J., Mwanga, I. M., Ebele, T., Dhed'a, B., Bawin, Y., Staelens, A., Stoffelen, P., Ruttink, T., Vandelook, F., & Honnay, O. (2023). Genetic diversity and structure in wild Robusta coffee (Coffea canephora A. Froehner) populations in Yangambi (DR Congo) and their relation to forest disturbance. Heredity, 130(3), 145–153. https://doi.org/10.1038/s41437-022-00588-0

Donhouédé, J. C. F., Marques, I., Salako, K. V., Assogbadjo, A. E., Ribeiro, N., & Ribeiro-Barros, A. I. (2023). Genetic and morphological diversity in populations of Annona senegalensis Pers. Occurring in Western (Benin) and Southern (Mozambique) Africa. PeerJ, 11, e15767. https://doi.org/10.7717/peerj.15767

Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity. Nature Reviews Genetics, 17(7), 422–433. https://doi.org/10.1038/nrg.2016.58

Esfandani-Bozchaloyi, S., Sheidai, M., & Kalalegh, M. H. (2019). Comparison of DNA extraction methods from Geranium (Geraniaceae). Acta Botanica Hungarica, 61(3–4), 251–266. https://doi.org/10.1556/034.61.2019.3-4.3

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics, 131(2), 479–491. https://doi.org/https://doi.org/10.1093/genetics/131.2.479

FAO (2019). Voluntary guidelines for the conservation and sustainable use of farmers' varieties/landraces.

Frankham, R. (2019). Conservation Genetics. In Encyclopedia of Ecology (2nd ed., Vol. 1, pp. 382–390).

Gautam, D. K., Singh, V., Singh, S. K., Tiwari, P., & Prabakaran, S. (2024). Custard Apple (Annona squamosa L.): Exploring its Health Benefits and Medicinal Properties. European Journal of Nutrition and Food Safety, 16(10), 129–140. https://doi.org/10.9734/ejnfs/2024/v16i101563

Gill, K., Negi, S., Kumar, P., & Irfan, M. (2025). Improved genomic DNA extraction from citrus species using a modified CTAB method. Molecular Biology Reports, 52(1), 638. https://doi.org/10.1007/s11033-025-10763-1

Goutam, K., Sharma, T. R., Verma, B. K., Chanderia, U. K., & Pandey, S. K. (2019). Genetic Variability in Custard Apple Landraces of Madhya Pradesh, India. International Journal of Current Microbiology and Applied Sciences, 8(10), 2201–2209. https://doi.org/10.20546/ijcmas.2019.810.256

Hazra, S., Nahar, N., Sarkar, T., & Chakraborty, R. (2025). Custard Apple and Its By-products (pp. 229–270). https://doi.org/10.1201/9781003352341-7

Hedrick, P. W., & Garcia-Dorado, A. (2016). Understanding Inbreeding Depression, Purging, and Genetic Rescue. Trends in Ecology & Evolution, 31(12), 940–952. https://doi.org/10.1016/j.tree.2016.09.005

Hoban, S., Campbell, C. D., Da Silva, J. M., Ekblom, R., Funk, W. C., Garner, B. A., Godoy, J. A., Kershaw, F., MacDonald, A. J., Mergeay, J., Minter, M., O'Brien, D., Vinas, I. P., Pearson, S. K., Pérez-Espona, S., Potter, K. M., Russo, I.-R. M., Segelbacher, G., Vernesi, C., & Hunter, M. E. (2021). Genetic diversity is considered important but interpreted narrowly in country reports to the Convention on Biological Diversity: Current actions and indicators are insufficient. Biological Conservation, 261, 109233. https://doi.org/10.1016/j.biocon.2021.109233

Kaya, Ç., & Şahin, E. (2025). Determination of the Effects of Different DNA Isolation Methods on Quantity and Quality. Akademik Ziraat Dergisi, 14(1), 65–73. https://doi.org/10.29278/azd.1689058

Kishore, K., Shukla, A. K., Babu, N., Sarangi, D. N., & Patanayak, S. (2012). Pollination biology of Annona squamosa L. (Annonaceae): Evidence for pollination syndrome. Scientia Horticulturae, 144, 212–217. https://doi.org/10.1016/j.scienta.2012.07.004

Kiss, T., Karácsony, Z., Gomba-Tóth, A., Szabadi, K. L., Spitzmüller, Z., Hegyi-Kaló, J., Cels, T., Otto, M., Golen, R., Hegyi, Á. I., Geml, J., & Váczy, K. Z. (2024). A modified CTAB method for the extraction of high-quality RNA from mono-and dicotyledonous plants rich in secondary metabolites. Plant Methods, 20(1), 62. https://doi.org/10.1186/s13007-024-01198-z

Koetsier, G., & Cantor, E. (2019). A Practical Guide to Analyzing Nucleic Acid Concentration and Purity with Microvolume Spectrophotometers. New England Biolabs Inc, 1(8), 1–8.

Leal, F., & Paull, R. E. (2022). The genus Annona: Botanical characteristics, horticultural requirements and uses. Crop Science, 63(3), 1030–1049. https://doi.org/10.1002/csc2.20833

Li, Y., Kaur, S., Pembleton, L. W., Valipour-Kahrood, H., Rosewarne, G. M., & Daetwyler, H. D. (2022). Strategies of preserving genetic diversity while maximizing genetic response from implementing genomic selection in pulse breeding programs. Theoretical and Applied Genetics, 135(6), 1813–1828. https://doi.org/10.1007/s00122-022-04071-6

Liu, J., Magige, E. A., Fan, P., Wambulwa, M. C., Luo, Y., Qi, H., Gao, L., & Milne, R. I. (2023). Genetic imprints of grafting in wild iron walnut populations in southwestern China. BMC Plant Biology, 23(1), 423–423. https://doi.org/10.1186/s12870-023-04428-z

Moussa, A. Y., Siddiqui, S. A., Elhawary, E. A., Guo, K., Anwar, S., & Xu, B. (2024). Phytochemical constituents, bioactivities, and applications of custard apple (Annona squamosa L.): A narrative review. Food Chemistry, 459, 140363. https://doi.org/10.1016/j.foodchem.2024.140363

Nei, M. (1972). Genetic Distance between Populations. The American Naturalist, 106(949), 282–292. https://doi.org/https://doi.org/10.1086/282771

Nei, M. (1973). Analysis of Gene Diversity in Subdivided Populations. Proceedings of the National Academy of Sciences, 70(12), 3321–3323.

O'Brien, D., Bader, E., Hall, J., Hoban, S., Segelbacher, G., Vilaça, S., & Leigh, D. (2025). Genetic diversity is key to a nature-positive future. Biodiversity. https://doi.org/10.32942/X2Z91S

Paternina-Ricardo, S., Castillo, M. D. del, Sánchez-Martín, V., Espiñeira, M., Ceballos, L., Arroyo-Salgado, B., & Benitez, L. B. (2025). Exploring the efficacy of the biological properties of Persea americana Mill seed: Useful studies and perspectives in functional foods. Journal of Agriculture and Food Research, 24, 102428–102428. https://doi.org/10.1016/j.jafr.2025.102428

Phang, A., Niissalo, M. A., Ruhsam, M., Pezzini, F. F., Neo, W. L., Burslem, D. F. R. P., Middleton, D. J., Wilkie, P., & Khew, G. (2024). Genetic erosion in a tropical tree species demonstrates the need to conserve wide-ranging germplasm amid extreme habitat fragmentation. Biodiversity and Conservation, 33, 2527–2548. https://doi.org/10.1007/s10531-024-02870-5

Rodrigues, B. R. A., Gasser, C. S., Pimenta, S., Pereira, M. C. T., & Nietsche, S. (2023). Seedless fruit in Annona squamosa L. is monogenic and conferred by INO locus deletion in multiple accessions. Plant Reproduction, 37(2), 71–84. https://doi.org/10.1007/s00497-023-00464-9

Sá, G. H., Lima, F. C. D., Viana, J. P. G., Lopes, Â. C. A., Carvalho, L. C. B., Valente, S. E. S., & Lima, P. S. C. (2022). Genetic diversity and structure of an active germplasm collection of Annona squamosa L. Ecological Genetics and Genomics, 25, 100143. https://doi.org/10.1016/j.egg.2022.100143

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular cloning: A laboratory manual (2nd ed.). Cold Spring Harbor Laboratory Press.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

Sari, R. M., Achyar, A. A., Ahda, Y., & Hilda Putri, D. (2022). Genotyping of Sumatera local variety of citrus using random amplified polymorphism DNA (RAPD) technique. Tropical Genetics, 2(2), 56–65. https://doi.org/10.24036/tg.v2i2.29

Schenk, J. J., Becklund, L. E., Carey, S. J., & Fabre, P. P. (2023). What is the "modified" CTAB protocol? Characterizing modifications to the CTAB DNA extraction protocol. Applications in Plant Sciences, 11(3), e11517. https://doi.org/10.1002/aps3.11517

Shehata, M. G., Abu-Serie, M. M., Abd El-Aziz, N. M., & El-Sohaimy, S. A. (2021). Nutritional, phytochemical, and in vitro anticancer potential of sugar apple (Annona squamosa) fruits. Scientific Reports, 11(1), 6224. https://doi.org/10.1038/s41598-021-85772-8

Talavera, A., Fernandez-Pozo, N., Matas, A. J., Hormaza, J. I., & Bombarely, A. (2023). Genomics in neglected and underutilized fruit crops: A chromosome-scale genome sequence of cherimoya (Annona cherimola). Plants, People, Planet, 5(3), 408–423. https://doi.org/10.1002/ppp3.10366

Utaminingsih, S., & Sophian, A. (2022). Analysis of Purity and Concentration of DNA Isolation Results on Chondroitin Samples. BiosciED: Journal of Biological Science and Education, 3(2), 56–61. https://doi.org/10.37304/bed.v3i2.5425

Varshney, R. K., Pandey, M. K., Bohra, A., Singh, V. K., Thudi, M., & Saxena, R. K. (2019). Toward the sequence-based breeding in legumes in the post-genome sequencing era. Theoretical and Applied Genetics, 132(3), 797–816. https://doi.org/10.1007/s00122-018-3252-x

Wang, X., Feng, Q.-H., Zeng, Z.-H., Zhang, Z.-Q., Cai, J., Chen, G., Li, D.-Z., Wang, H., & Zhou, W. (2025). Effects of mode of reproduction on genetic polymorphism and divergence in wild yams (Dioscoreaceae: Dioscorea). Plant Diversity, 47(1), 136–147. https://doi.org/10.1016/j.pld.2024.09.009

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18(22), 6531–6535. https://doi.org/10.1093/nar/18.22.6531

Yadav, A., Jha, S., Choudhary, G., Ram, A., Kumar, R., Kumar, N., Anuragi, H., Garg, S., Kumar, A., Singh, P. K., Dwivedi, R. P., Arunachalam, A., & Jinger, D. (2025). Nutritional valorization of Manila tamarind accessions through antioxidant analysis and UHPLC-Q-TOF-MS-based metabolomic profiling. Frontiers in Nutrition, 12, 1646522–1646522. https://doi.org/10.3389/fnut.2025.1646522

Yeh, F. C., Yang, R. C., Boyle, T. B., Ye, Z. H., & Mao, J. X. (1997). POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada, 10.

Yuniastuti, E., Yuliana, A., Sukaya, & Nandariyah. (2023). Genetik diversity of matoa (Pometia pinnata) species based on RAPD markers. SABRAO Journal of Breeding and Genetics, 55(6), 1941–1949. https://doi.org/10.54910/sabrao2023.55.6.8