OPEN ACCESS
Journal of Multidisciplinary Applied Natural Science Vol. 6 No. 1 (2026) https://doi.org/10.47352/jmans.2774-3047.313
Check for updates
Research Article

Analysis of Tasikmalaya Mangosteen Diversity (*Garcinia mangostana* L.) Using Morphological and Genetic Studies Based on ISSR-PCR Markers

Adam Saepudin*, Rudi Priyadi, Tini Sudartini, Visi Tinta Manik, Muhamad Firmansyah Nugraha Adisty Erlangga, Ade Hilman Juhaeni, Gilang Vaza Benatar

Received : January 14, 2025	Revised : July 5, 2025	Accepted : July 17, 2025	Online : October 7, 2025

Abstract
Mangosteen (*Garcinia mangostana* L.) is a widely cultivated tropical fruit in Southeast Asia, including Indonesia, valued for its health benefits. This study assessed the morphological and genetic diversity using inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) markers of mangosteen from five locations in Tasikmalaya regency namely Sukaraja (M1), Tanjung Jaya (M2), Mangunreja (M3), Puspahiang (M4), and Leuwiliyang (M5). Molecular analysis was conducted on leaf DNA, with PCR products visualized via capillary electrophoresis. Data were analyzed using RStudio with vegan and poppr packages to construct dendrograms based on Jaccard and dissimilarity coefficients. Morphological observations complemented the genetic analysis. Due to amplification failure, no genetic data were obtained for M1, M2, and M3, and their similarity is inferred from morphological traits. M4 and M5 showed a Jaccard coefficient of 0.497 and a dissimilarity of 0.250, indicating moderate kinship and significant genetic variation, with 100% of amplified loci being polymorphic. The genetic diversity score (average variance 0.4966) suggests substantial variation, consistent with morphological differences influenced by factors like altitude. These findings provide insights for mangosteen breeding and conservation in Tasikmalaya, a region with unique agroclimatic conditions.

Keywords: mangosteen, genetic diversity, ISSR, dendrogram, Tasikmalaya, morphology

1. INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is one of the tropical fruits that is widely cultivated in Southeast Asia [1], including Indonesia [2]. Mangosteen in Indonesia also noted as one of the great exports [2]. This fruit reached its popularity because of its benefits in supporting health for people through biological and pharmalogical activities [3]. Previous studies have shown that mangosteen contains antibacterial, antifungal, anti-inflammatory, antihistamine, anticancer, antidiabetic, and many other bioactive compounds [4]. Pericarps, peel, pulp, and even mangosteen seed are widely used as nutritional supplements [5] [6].

Mangosteen, classified as an angiospermic plant, employs a unique reproductive mechanism that involves the development of adventitious pro-embryo tissue from ovular tissue. It results in the production of fruits with a consistent appearance and a singular variety. Despite this uniformity, variations in leaf and fruit shapes and sizes have been observed in mangosteen populations. Studies indicate that within naturally reproducing mangosteen populations, the presence of genetically distinct individuals is not uncommon, attributed to somatic mutations, genetic instability, and auto segregation, thereby contributing to individual variation within the species [7][8]. Although mangosteen seeds can form through apomixis and develop from adventitious asexual embryos, this mode of asexual regeneration leads to limited genetic variability in the plant [9].

Two primary methods are used to explore the genetic diversity in apomictic plants such as mangosteen: molecular analysis and progeny analysis [10]. Even though progeny analysis is insightful, it is time-consuming particularly during the juvenile stage of the plant [11]. Consequently, molecular analysis, specifically using DNA markers, is a more efficient alternative for studying the genetic diversity in mangosteen. Utilizing DNA markers can be done by several developed methods. One of them is ISSR-PCR. ISSR-PCR works based on former of the multi-locus markers through DNA sequence amplification using PCR methods, it can

Table 1. Primer used in PCR-ISSR.

No	Primer	Sequence	Annealing Temperature (°C)
1	PKBT 2	(AC)8 TT	53
2	PKBT 3	(AG)8 T	53
3	PKBT 4	(AG)8 AA	53
4	PKBT 8	(GA)9 C	54
5	PKBT 9	(GA)9 T	54
6	PKBT 11	(GT)9 C	54
7	ISSRED 20	(TCC)5 A	48
8	ISSRED 23	(CT)8 T	48
9	ISSRED 17	(GAC)5	48
10	ISSRED 12	(AGAC) 4	36
11	ISSRED 18	(GGAT) 4	48

also expand the usage by transform into co-dominant markers. The ISSR method is a prominent choice for DNA molecular markers. It is renowned for its precision, cost-effectiveness, ease of use, and higher polymorphism than random amplified polymorphic DNA (RAPD) methods [12]. The ISSR markers, being dominant, eliminate the need for primer design and typically have lengths ranging from 6 to 25 base pairs.

Research has extensively utilized, various analysis methods within the realm of DNA-based molecular techniques. The ISSR technique, consists of a microsatellite composed of non-coding regions generally consisting of mono-, di-, or trinucleotides, is particularly well-suited for plant genome research. The ISSR also does not require high knowledge of DNA information, still applicable even for a limited amount of DNA [12]. Compared to SSRs which is also common in use for diversity identification, ISSRs noted as more informative and sensitive. Several researches revealed ISSRs showed higher polymorphism than SSRs [13][14]. Furthermore, SSR markers just targeting the functional region of the open reading frame (ORF), while ISSR revealing genome's diversity through the founding in all over the genome [15].

This study is designed with the overarching goal of unraveling the intricate dynamics of genetic diversity within mangosteen populations in Tasikmalaya. An integral facet of this exploration involves a thorough characterization of observed variations in leaf and fruit shapes and sizes through detailed morphological analyses. Despite the uniform appearance and singular variety of mangosteen fruits resulting from its unique agamospermic reproductive mechanism, this study aims to shed light on the presence of genetic distinctions within naturally reproducing populations. The research compares the efficacy of molecular analysis, utilizing the ISSR method, with progeny analysis in studying genetic diversity. By focusing on the advantages of ISSR markers—precision, cost-effectiveness, and higher polymorphism [12]—the study seeks to discern correlations between morphological traits and genetic profiles, contributing to a comprehensive understanding of the intricate interplay between phenotype and genotype in mangosteen populations. In summary, this investigation is way to deepen our insights into the genetic intricacies of mangosteen through an integrated approach, combining morphological characterization with advanced molecular analysis techniques, specifically employing ISSR markers. By correlating morphological traits and genetic profiles, this study seeks to provide insight into the genetic structure of mangosteen populations in Tasikmalaya, supporting future breeding and conservation strategies.

2. MATERIALS AND METHODS

2.1. Materials

Mangosteen leaves were collected from

Table 2. Morphological characters from different accession and altitude.

No	Accession	Sample Code	Altitude (masl)	Morphological Characters			
				Flower	Stem	Leaf	Fruit
1	Sukaraja	M1	221	Round, pink petals, red corolla	Round, diameter ±135 cm, brown, 8 m high	Tapered, length 21.0–22.6 cm, width 11.7–11.8 cm	Round, diameter 7.5–8.0 cm, skin color blackish red, weight 83.3–100.0 g
2	Tanjung Jaya	M2	398	Round, red petals, pink crown	Round, diameter ±130 cm, brown, height ±8 m	Tapered, ±21 cm, width ±11 cm	Round, diameter ±7 cm, skin color blackish red, weight ±80 cm
3	Mangunreja	M3	549	Round, yellow petals, reddish yellow crown	Round, diameter ±54 cm, brown, tall ±20 m	Reniform shape, ±18 cm, width ±10 cm	Round, diameter 4.5 cm, weight ±70–90 cm, skin color purplish red, seed color brown
4	Puspahiang	M4	634	Round, greenish yellow, 4 petals, yellow crown with red edges	Round, smooth texture, horizontal branching, diameter 32 cm, height 10.9 m	Elliptical, length 20.0–29.5 cm, width 10.3–13.1 cm, leaf margins flat	Oval round, diameter 6 cm, skin color dark purple, weight 131.3 g, seed color dark brown
5	Leuwiliang	M5	534	Round, greenish yellow petals, 4 petals, reddish yellow crown	Round, diameter ±54 cm, brown, 20 m high	Reniform shape, length 17.8–22.6 cm, width 9.5–12.5 cm	Round, diameter 4.5 cm, weight 73.4–93.6 cm, skin color purplish red, seed color light brown

mangosteen trees in mangosteen orchards located in each research site. Sampling was done using a random sampling method, considering the different variations of mangosteen plants [15]. The number of samples taken was two trees per each research site. Mangosteen leaves were collected from mangosteen trees in mangosteen orchards located in each research site, they were Sukaraja (M1, 221 masl), Tanjung Jaya (M2, 398 masl), Mangunreja (M3, 549 masl), Leuwiliang (M5, 534 masl), and Puspahiang (M4, 634 masl).

2.2. Methods

2.2.1. Observation of Mangosteen Plant Morphology

Mangosteen plants from Sukaraja (M1), Tanjung Jaya (M2), Mangunreja (M3), Puspahiang (M4), and Leuwiliang (M5), were observed for their morphological characteristics. The characters observed are the stems, leaves, flowers, and fruits. Traits were standardized using the International Plant Genetic Resources Institute (IPGRI) descriptors for mangosteen [16]. The observation of morphological characters is carried out by conducting interviews or questionnaires with local farmers or mangosteen sellers. This information can provide an initial picture of the genetic diversity at each sample location.

2.2.2. Sample Preparation for ISSR Analysis

Sampling was carried out using a random sampling method, considering the variations in different mangosteen plants. The collected samples

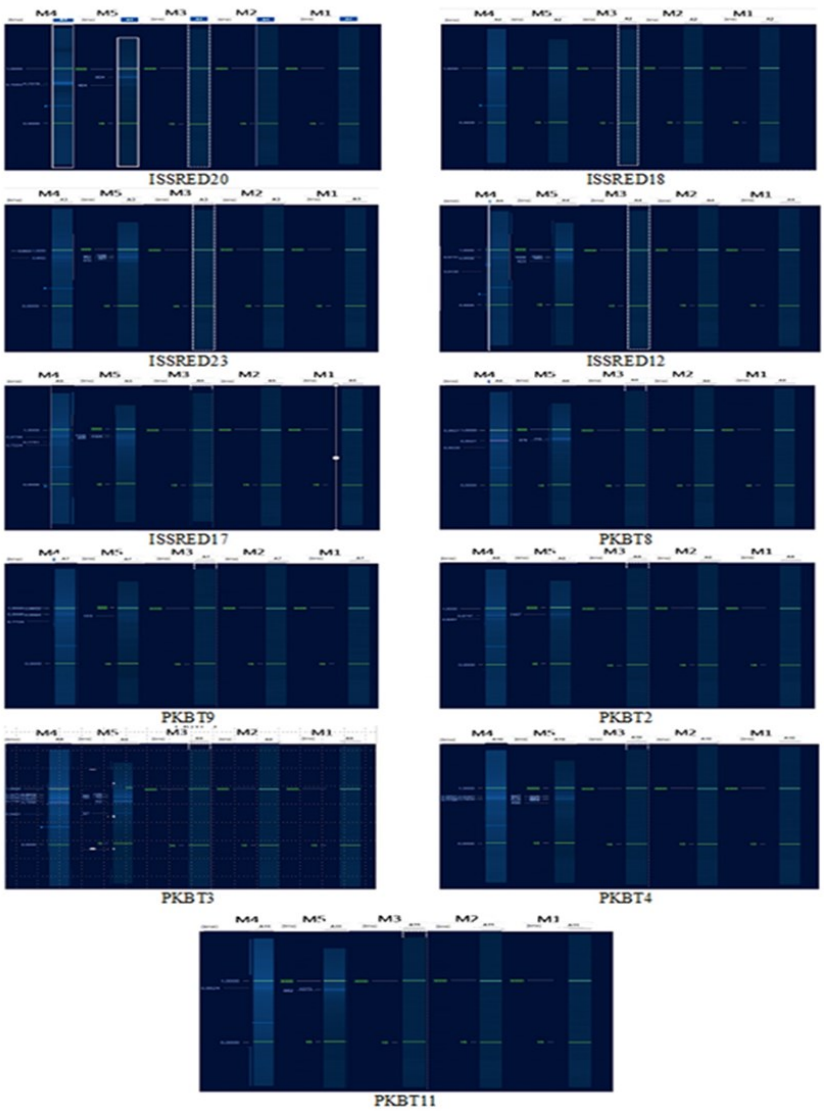

Figure 1. ISSR profile from Tasikmalaya mangosteen in five populations.

were then prepared by washing them with running water, followed by spraying with 70% alcohol for sterilization [15]. Subsequently, the samples were air-dried, placed in plastic, and supplied with silica gel.

2.2.3. DNA Extraction

DNA extraction was performed following the method outlined in the DNeasy Plant Mini Kit (Qiagen, Germany) according to the extraction protocol. The quality and quantity of the extracted DNA were assessed using the nanophotometer (Implen, Germany) to measure the concentration and purity of the DNA. The A260/A280 ratio ranging from 1.8 to 2.0, indicating pure DNA, and a concentration between 50–100 ng/µL [17].

2.2.4. PCR-ISSR

The reaction mixture should be prepared with an appropriate composition [18]. The reaction mixture consisted of 10 µL mastermix MyTaq™HS Red Mix (Bioline, UK) which is containing with deoxynucleoside triphosphates (dNTPs), $MgCl_2$, and Taq Polymerase, sample DNA, and filled with distilled water to increase the volume upto 25 µL. The detail information of primers presented in Table 1.

2.2.5. Visualization through Electrophoresis and Interpretation

The PCR results using ISSR were visualized using the capillary electrophoresis method using the QiaxCel Advanced digital electrophoresis device (Qiagen, Germany) using the DNA HIGH-RESOLUTION KIT. The procedure used follows the QiaxCel Advanced manual from Qiagen, Germany. Before operation, the cartridge is placed at room temperature for ±20 min. Then, the separation and wash buffer is poured into the buffer tray and placed in the buffer tray holder. Alignment markers (15–3,000 bp) to match the migration time of DNA were homogenized and placed on the wells in the buffer tray in an open tube position. Visualization and determination of the size of DNA fragments were carried out using QIAxcel ScreenGel software. Data were analyzed using the RStudio program includes calculating genetic polymorphism, diversity values, kinship coefficients, and dendrogram construction. The dendrogram constructed using binary data), where 1 is for visualized band and 0 is for no band visualized [19].

2.2.6. Genetic Diversity

Binary data matrices were created and analyzed using RStudio (version 1.4.1717) with the vegan package for cluster analysis based on Jaccard similarity coefficient and the poppr package to calculate genetic diversity indices, including mean gene diversity (MH), variance of diversity (VH), and others. Dendrograms were created using the unweighted pair group method with arithmetic mean (UPGMA) to visualize genetic relationships. The Mantel test was performed to assess the correlation between genetic distance and geographic distance (altitude), showing a significant positive correlation ($r = 0.6$, $p = 0.04$) [20]-[22].

3. RESULTS AND DISCUSSIONS

3.1. The Morphological Characters

The results of observations of the morphological characters of each research location show unique differences (Table 2). Through this study, it was found that samples from Sukaraja (M1) and Puspahiang (M4) had larger fruit, weighing 83.3–100.0 g and 131.3 g respectively, compared to Mangunreja (M3) and Leuwiliyang (M5) which had smaller fruit weighing 70.0–90.0 g and 73.4–93.6 g. The striking differences between these accessions are the color of the petals and crown, leaf shape, fruit skin color, and fruit size.

The differences in morphological characters that appear in each of these accessions can be influenced by external factors such as altitude or internal factors such as genetic composition, and both can even influence each other. Differences in altitude can produce visible morphological differences in plants, although of course this phenomenon is not exactly the same for every type of plant [23]. The higher altitude shows smaller fruit size than the lower one [24][25]. These morphological differences are likely influenced by genetic and environmental factors, such as altitude, which ranges from 221 masl (M1) to 634 masl (M4). Genetic factors such as genetic dynamics greatly influence the variation in morphological

Table 2. Primer amplification and polymorphism levels.

Primer	M1	M2	M3	M4	M5	Number of polymorphic loci	Number of amplified loci	% polymorphic
ISSRED20	0	0	0	0	1	2	3	66.7
	0	0	0	1	1			
ISSRED23	0	0	0	0	1	6	8	75.0
	0	0	0	0	1			
	0	0	0	1	1			
	0	0	0	0	1			
	0	0	0	1	1			
	0	0	0	1	1			
	0	0	0	1	1			
ISSRED12	0	0	0	0	1	5	7	71.4
	0	0	0	0	1			
	0	0	0	1	0			
	0	0	0	0	1			
	0	0	0	1	0			
ISSRED17	0	0	0	0	1	5	7	71.4
	0	0	0	1	1			
	0	0	0	1	1			
	0	0	0	0	1			
PKBT8	0	0	0	1	1	3	4	75.0
	0	0	0	1	0			
	0	0	0	0	1			
PKBT9	0	0	0	1	1	3	4	75.0
	0	0	0	1	0			
PKBT2	0	0	0	1	1	2	3	66.7
	0	0	0	1	0			
	0	0	0	1	0			
	0	0	0	1	1			
	0	0	0	1	1			
PKBT3	0	0	0	1	1	7	12	58.3
	0	0	0	1	1			
	0	0	0	1	0			
	0	0	0	1	1			
	0	0	0	1	1			
PKBT4	0	0	0	1	1	6	8	75.0
	0	0	0	1	1			
	0	0	0	1	1			
	0	0	0	0	1			
PKBT11	0	0	0	0	1	2	5	40.0
	0	0	0	1	1			
	0	0	0	0	1			

characters. Furthermore, these two factors are then interconnected, altitude influences genetic diversity through differences in living environments and high gene flow [26]. Even though it does not happen immediately.

3.2. PCR-ISSR Analysis

Observation of ISSR electrophoresis results revealed a fascinating picture of genetic variation in the studied population. Data from visualization through electrophoresis provide deep insight into the DNA fragment patterns produced by the ISSR PCR reaction. By analyzing these patterns, we can identify genetic polymorphisms between individuals in the population. The results of ISSR electrophoresis also provide important information on the level of genetic diversity and evolutionary relationships between these individuals. Therefore, a deep understanding of ISSR pattern visualization electrophoresis results is the key to uncovering the information of genetic diversity in the context of this research. The results of ISSR electrophoresis readings showed several DNA band patterns appearing in the genetic samples studied. Various DNA bands produced by the ISSR PCR reaction using specific primers have been identified in this analysis showed in Figure 1 and Table 3. Electrophoresis results showed DNA banding patterns for M4 and M5. No bands were seen for M1, M2, and M3 due to amplification failure, indicating that genetic analysis was only performed for M4 and M5. A value of 0 indicates no DNA band at that position, while a value of 1 indicates a DNA band at that position. Then, that binary data used for constructing the dendrogram. It should be noted that primer ISSRED18 did not show any DNA bands at the identified positions. The ISSRED20 primer produces 2 DNA bands, ISSRED23 produces six bands, ISSRED12 and ISSRED17 each produce five bands, PKBT8 and PKBT9 show 3 bands each, PKBT2 shows 2 bands, PKBT3 shows 7 bands, PKBT4 shows 6 bands, and PKBT11 shows 2 bands at the identified positions.

Those data from Table 2 above were used for constructing dendrogram for similarity and dissimilarity among all accessions using R-studio. The % polymorphic value is percentage of number of polymorphic loci compared with number of amplified loci [27]. The ISSR-PCR analysis using 11 primers (Table 1) resulted in the amplification of 41 unique loci among the five accessions, with all amplifications occurring in M4 and M5 due to amplification failure in M1, M2, and M3. This failure was likely due to DNA degradation, PCR inhibitors, or primer mismatches, with potential solutions including re-extraction or use of alternative primers. For M4 and M5, all 41 amplified loci were polymorphic, indicating high genetic diversity.

Dendrogram visualized that M4 and M5 have different clusters with a Jaccard coefficient value of 0.497. The dissimilarity dendrogram (Figure 2) showed a genetic distance of 0.25 between M4 and M5, confirming their genetic divergence. Since there were no genetic data for M1, M2, and M3, their relationships were inferred from morphological traits alone. This kinship coefficient describes the level of kinship relationship between the samples analyzed [28]. A higher kinship coefficient value indicates a closer kinship

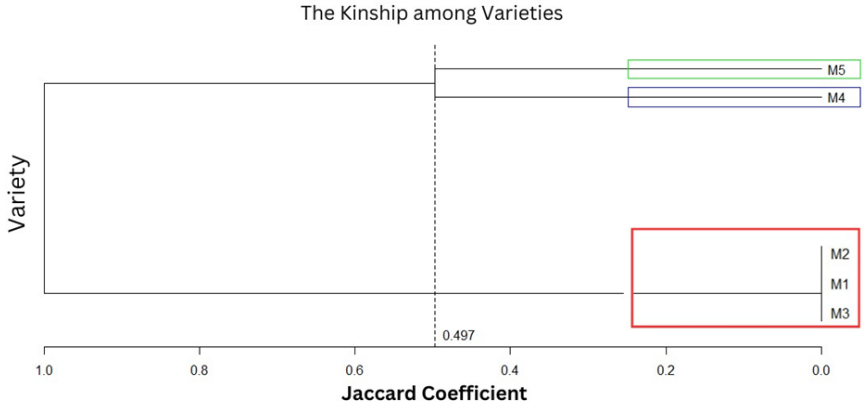

Figure 2. Dendrogram visualized Kinship through Jaccard Coefficient.

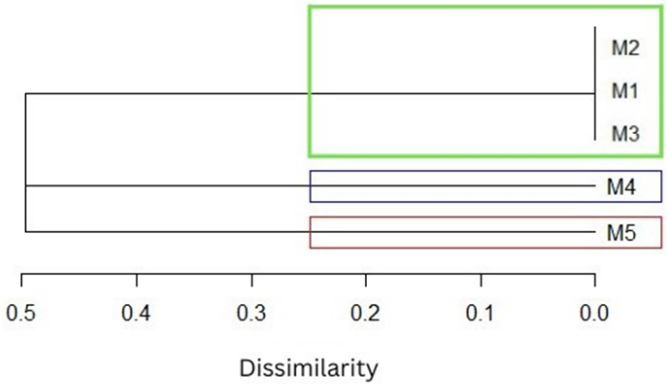

Figure 3. Dissimilarity Dendrogram among varities.

relationship between samples [29]. Therefore, the resulting kinship value is lower than expected. A higher kinship coefficient is expected between individuals of angiospermic plants due to asexual reproduction, which tends to maintain minimal genetic diversity. Studies have shown that asexual reproduction, such as apomixis (angiospermic), can lead to reduced genetic diversity and increased kinship coefficients among individuals. For example, a study comparing genetic variability of the dioecious plant of asexually and sexually reproducing *Marchantia inflexa* (liverwort) found differences in genetic structure and fecundity between the two reproductive modes [30]. Additionally, research on apomictic plants has revealed the deregulation of reproductive pathways and gene expression patterns compared to sexual plants [31].

These findings support the notion that asexual reproduction can lead to higher kinship coefficients and reduced genetic diversity in angiospermic plants. Several factors can influence a lower kinship coefficient value. Environmental factors such as selection pressure, environmental change, or climate change can also influence genetic diversity in plant populations [32]. In addition, seed dispersal or human plant propagation can also introduce new genetic variations to populations [33][8]. Based on the results, the kinship coefficient obtained is lower than expected. These factors may be necessary in shaping genetic diversity in the studied mangosteen populations. These findings highlight the complexity of the mangosteen reproductive system that still requires further genetic research to understand the factors that influence genetic diversity in angiospermic plants.

Despite the informations above, M1, M2, and M3 samples show a Jaccard coefficient of 0.0 due to the amplification results having a value of n/a or not applicable because there are no visualized bands from all the primer pairs used so that the samples it is not detected. Data that is "not applicable" in R-Studio displays by value of 0.0 [34]. This value indicates that no alleles or bands can be compared between samples, because of failure in amplification. R-Studio assigns a value of 0.0 as the default result when data are not available, reflecting that no genetic relationship can be calculated between the samples.

Even though the M1, M2, and M3 show a 0,0 Jaccard coefficient which is shows by dendrogram in Figure 2, but it also gives information that they are closely related than with M4 and M5. This information also confirmed by morphological character from Table 2, where M1, M2, and M3 tend to more similar than with M4 and M5. In purpose to convincing these information, so we also constructed the dendrogram for dissimilarity that shows below in Figure 3. The dissimilarity dendrogram shows a genetic distance of 0.25 between M4 and M5, confirming their genetic divergence. Since there is no genetic data for M1, M2, and M3, their relationships are inferred from morphological traits alone. In line with kinship dendrogram from Jaccard coefficient, the dissimilarity dendrogram shows that M1, M2, M3 more closely related by the 0.0 coefficient while the M4 and M5 show ± 0.25 dissimilarity coefficient.

3.3. Genetic Diversity Analysis

Based on the visualization in Figure 1, samples M4 and M5 are able to be analyzed further to

determine their diversity. Genetic diversity analysis has 2 components, the single locus effect and the two loci effect that shows in Table 4. First, the *Single Locus Effect* with parameters: mean gene diversity (MH), variance of diversity (VH), Wahlund's effect (WH). Second, two locus effect with parameters: mean gene disequilibrium (MD), Wahlund effect (WC), interaction between MD and WC (AI), variance of interaction (VD), covariance of intersection (CI), the total variance (MH+VH+WH+MD+WC+AI) and average variance (MH+MD+AI+VD+CI). Genetic diversity parameters were calculated only for M4 and M5 due to the lack of amplified bands in M1, M2, and M3. The MH was 1.112668, indicating high allelic variation, comparable to the previous findings, who reported 86% polymorphism in 43 bands [7][35]. Although M1 and M4 had large fruits, M1 showed morphological similarities with M2 and M3, possibly due to environmental influences such as low altitude (221 masl) rather than genetic factors [36]. The Mantel test showed a significant positive correlation ($r = 0.6$, $p = 0.04$) between genetic distance and altitude, indicating that geographic isolation contributed to genetic differentiation among mangosteen populations in Tasikmalaya.

The "Single Locus Effect" parameter describes the genetic diversity at one particular gene locus in the sample [37]. The high MH by 1.112668 indicates that there is significant variation in the alleles at this gene locus. MH value is number of different alleles present in the population and the even distribution of these allele frequencies, where an MH value ≥ 1.0 indicates significant allelic variation and an MH value < 1.0 indicates insignificant allele variation [38]. According to previous work [39], H score means higher diversity or variance and lower dominancy, vice versa. The Variance of Diversity (VH) of 0.011068 indicates that this variation is well distributed in the sample. Meanwhile, the "Two-Locus Effect" reveals the sample's interaction between two gene loci [40]. A negative MD (-0.637263) indicates a negative correlation between these gene loci, which means that alleles at a particular gene locus tend to be unrelated to alleles at other gene loci in the sample. A positive Wahlund's Effect (WC) (0.004877) indicates a population structure effect that influences variation in allele frequencies between subpopulations in the sample.

The interaction between MD and WC (AI), which is close to zero (-0.000079), indicates that the effects of population structure and correlation between gene loci do not strengthen or weaken each other significantly. The VD of 0.001992 reflects the variability in genetic correlation between gene loci. At the same time, the CI of 0.019317 describes the covariation between gene locus interactions and the

Table 4. Genetic diversity analysis.

Parameter	Score	Description
Single Locus Effect:		
Mean Gene Diversity (MH)	1.112668	High allele variation
Variance of Diversity (VH)	0.011068	Evenly distributed variation
Wahlund's Effect (WH)	0.005560	Increased variance of allele frequencies due to subpopulation structure
Two Locus Effect:		
Mean Disequilibrium (MD)	-0.637263	Negative correlation between loci
Wahlund Effect (WC)	0.004877	Effect of population structure on allele frequencies
Interaction between MD and WC (AI)	-0.000079	Minimal interaction between MD and WC
Variance of Intersaction (VD)	0.001992	Variability in locus correlation
Covariance of Intersection (CI)	0.019317	Covariation of locus interaction and population structure
Total Variance		
(MH+VH+WH+MD+WC+AI)	**0.49683**	Holistic genetic variation
Average Variance		
(MH+MD+AI+VD+CI)	**0.49663**	Average genetic variability

effects of population structure. A total variance of 0.496830 (rounded to 0.5) and an average of 0.496635 (rounded to 0.5) provide a holistic picture of genetic variation in the sample.

These results indicate significant genetic diversity in the samples studied, with population structure effects influencing some of this variation. The formation of the ancestral mangosteen did not come from a single hybridization event, it would cause variance among different mangosteen populations [7]. Genetic diversity was observed between mangosteen maternal plants and their progeny, and many types of genetic diversity may have emerged following the interbreeding of sexual ancestors with distinct reproductive characteristics. However, the lower relationship values and significant genetic diversity that differ from the authors' hypothesis are precisely in accordance with the differences in morphological characters that appear between the two varieties. Low or distant relationships tend to show differences in morphological characters, where kinship is divided into 2 subgroups showing differences in the unique morphology of each subgroup member [41].

Overall, the high genetic diversity in M4 and M5 reflects the adaptation of mangosteen to different environmental conditions in Tasikmalaya, such as variations in altitude and soil types. The correlation between genetic distance and altitude ($r = 0.6$, $p = 0.04$) is consistent with previous studies showing that environmental factors can drive genetic divergence in angiospermic plants such as mangosteen [35]. Morphological differences, such as larger fruit size in M4 (131.32 g) compared to M5 (73.4–93.6 g), likely reflect a combination of genetic variation and environmental influences, such as nutrient availability and rainfall. Amplification failure in M1, M2, and M3 was likely caused by DNA degradation, the presence of PCR inhibitors (such as polysaccharides or phenolics from mangosteen leaves), or primer mismatch with template DNA [42]. This is similar to challenges reported in other studies on tropical plants [14]. To address this, future studies could use alternative DNA extraction methods, such as modified CTAB, or additional ISSR primers with more diverse sequences. The implications for breeding and conservation are significant. The high level of polymorphism (100% for amplified loci) suggests that Tasikmalaya mangosteen has sufficient genetic diversity to support breeding programs, especially for traits such as fruit size and environmental tolerance. However, the failure of amplification for M1–M3 highlights the need for further studies to ensure all accessions are represented in genetic analyses, which could provide a more complete picture of genetic diversity in the region.

4. CONCLUSIONS

This study revealed significant genetic and morphological diversity among mangosteen accessions from Tasikmalaya, with M4 (Puspahiang) and M5 (Leuwiliyang) showing polymorphic loci out of 41 amplified loci, with a Jaccard coefficient of 0.497 and dissimilarity of 0.25. Failure of amplification for M1 (Sukaraja), M2 (Tanjung Jaya), and M3 (Mangunreja) limited genetic analysis, but morphological data showed clear variation, likely influenced by genetic and environmental factors such as altitude. The Mantel test ($r = 0.6$, $p = 0.04$) confirmed the correlation between genetic distance and altitude. These findings provide a basis for mangosteen breeding and conservation strategies in Tasikmalaya, a region with unique agroclimatic conditions, with recommendations for optimization of PCR protocols to include all accessions in future genetic analyses.

AUTHOR INFORMATION

Corresponding Author

Adam Saepudin — Graduate Program in Agricultural Science, Universitas Siliwangi, Tasikmalaya-46112 (Indonesia);
orcid.org/0009-0006-9346-9941
Email: adamsaepudin@unsil.ac.id

Authors

Rudi Priyadi — Graduate Program in Agricultural Science, Universitas Siliwangi, Tasikmalaya-46112 (Indonesia);
orcid.org/0000-0002-3325-8177

Tini Sudartini — Agrotechnology Study Program, Universitas Siliwangi, Tasikmalaya-46191 (Indonesia);
orcid.org/0009-0009-1090-6280

Visi Tinta Manik — Agrotechnology Study Program, Universitas Siliwangi, Tasikmalaya-46191 (Indonesia);
orcid.org/0000-0001-6761-796X
Muhamad Firmansyah Nugraha Adisty Erlangga — Agrotechnology Study Program, Universitas Siliwangi, Tasikmalaya-46191 (Indonesia);
orcid.org/0000-0003-3974-7745
Ade Hilman Juhaeni — Agrotechnology Study Program, Universitas Siliwangi, Tasikmalaya-46191 (Indonesia);
orcid.org/0000-0002-5833-800X
Gilang Vaza Benatar — Agrotechnology Study Program, Universitas Siliwangi, Tasikmalaya-46191 (Indonesia);
orcid.org/0000-0002-3174-2416

Author Contributions

Conceptualization, Methodology, Writing – Review & Editing, A. S. and V. T. M.; Software, Formal Analysis, Investigation, and Resources, M. F. N. A. E.; Validation, A. S., V. T. M. and M. F. N. A. E.; Data Curation, M. F. N. A. E. and G. V. B.; Writing – Original Draft Preparation, A. S.; Visualization, A. H. J.; Supervision, and Funding Acquisition, R. P.; Project Administration, T. S.

Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This research was supported by the Ministry of Education, Culture, Research, and Technology, Siliwangi University, Institute for Research and Community Service (LPPM) via PGB Research Grant, contract number: 250/UN58.21/PP/2023 fiscal year 2023.

DECLARATION OF GENERATIVE AI

Not applicable.

REFERENCES

[1] Z. Xie, M. Sintara, T. Chang, and B. Ou. (2015). "Daily consumption of a mangosteen-based drink improves in vivo antioxidant and anti-inflammatory biomarkers in healthy adults: a randomized, double-blind, placebo-controlled clinical trial". *Food Science & Nutrition.* **3** (4): 342-8. 10.1002/fsn3.225.

[2] M. R. Syahputra, H. Setiado, L. A. M. Siregar, and R. I. Damanik. (2021). "Morphological characteristics of Mangosteen plants (Garcinia mangostana L.) in Langkat District, North Sumatera, Indonesia". *IOP Conference Series: Earth and Environmental Science.* **782** (4). 10.1088/1755-1315/782/4/042056.

[3] L. S. Kalick, H. A. Khan, E. Maung, Y. Baez, A. N. Atkinson, C. E. Wallace, F. Day, B. E. Delgadillo, A. Mondal, R. Watanapokasin, S. M. Barbalho, and A. Bishayee. (2023). "Mangosteen for malignancy prevention and intervention: Current evidence, molecular mechanisms, and future perspectives". *Pharmacological Research.* **188** : 106630. 10.1016/j.phrs.2022.106630.

[4] B. Retnoaji, P. Paramita, and L. U. Khasanah. (2023). "Mangosteen Garcinia mangostana L. simplicia effect on bone structure and behaviour of Wader Fish Rasbora lateristriata (Bleeker, 1854) Embryo". *Indonesian Journal of Pharmacy.* 10.22146/ijp.6348.

[5] B. Ovalle-Magallanes, D. Eugenio-Perez, and J. Pedraza-Chaverri. (2017). "Medicinal properties of mangosteen (Garcinia mangostana L.): A comprehensive update". *Food and Chemical Toxicology.* **109** (Pt 1): 102-122. 10.1016/j.fct.2017.08.021.

[6] W. M. Aizat, F. H. Ahmad-Hashim, and S. N. Syed Jaafar. (2019). "Valorization of mangosteen, "The Queen of Fruits," and new advances in postharvest and in food and engineering applications: A review". *Journal of Advanced Research.* **20** : 61-70. 10.1016/j.jare.2019.05.005.

[7] S. Sobir, R. Poerwanto, E. D. Y. Santosa, S. Sinaga, and E. Mansyah. (2011). "Genetic variability in apomictic mangosteen (Garcinia mangostana) and its close relatives (Garcinia spp.) based on ISSR markers". *Biodiversitas Journal of Biological Diversity.* **12** (2). 10.13057/biodiv/d120201.

[8] T. L. Yao, M. Nazre, D. McKey, R. Jalonen, and J. Duminil. (2023). "The origin of cultivated mangosteen (Garcinia mangostana L. var. mangostana): Critical assessments and an evolutionary-ecological perspective". *Ecology and Evolution.* **13** (3): e9792. 10.1002/ece3.9792.

[9] S. Vranken, A. Scheben, J. Batley, T. Wernberg, and M. A. Coleman. (2022). "Genomic consequences and selection efficacy in sympatric sexual versus asexual kelps". *Frontiers in Marine Science.* **9**. 10.3389/fmars.2022.921912.

[10] F. A. de Oliveira, B. B. Z. Vigna, C. C. da Silva, A. P. Favero, F. P. de Matta, A. L. S. Azevedo, and A. P. de Souza. (2020). "Coexpression and Transcriptome analyses identify active Apomixis-related genes in Paspalum notatum leaves". *BMC Genomics.* **21** (1): 78. 10.1186/s12864-020-6518-z.

[11] F. Liu, J. Cheng, X. Liu, and X. Q. Wang. (2021). "High-Throughput and Accurate Determination of Transgene Copy Number and Zygosity in Transgenic Maize: From DNA Extraction to Data Analysis". *International Journal of Molecular Sciences.* **22** (22). 10.3390/ijms222212487.

[12] S. Amiteye. (2021). "Basic concepts and methodologies of DNA marker systems in plant molecular breeding". *Heliyon.* **7** (10): e08093. 10.1016/j.heliyon.2021.e08093.

[13] M. Ramzan, S. Sarwar, N. Kauser, R. Saba, I. Hussain, A. A. Shah, M. N. Aslam, J. Alkahtani, and M. S. Alwahibi. (2020). "Assessment of Inter simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers to reveal genetic diversity among Tamarix ecotypes". *Journal of King Saud University - Science.* **32** (8): 3437-3446. 10.1016/j.jksus.2020.10.003.

[14] Sabreena, M. Nazir, R. Mahajan, M. J. Hashim, J. Iqbal, M. N. Alyemeni, B. A. Ganai, and S. M. Zargar. (2021). "Deciphering allelic variability and population structure in buckwheat: An analogy between the efficiency of ISSR and SSR markers". *Saudi Journal of Biological Sciences.* **28** (11): 6050-6056. 10.1016/j.sjbs.2021.07.061.

[15] K. Setiawan, P. B. Timotiwu, A. Agustiansyah, M. S. Hadi, M. Kamal, A. Ardian, and W. A. Setiawan. (2023). "Root Yield and Starch Synthase Type IV Gene Activity under Different Micro Nutrient Fertilizer and Harvest Ages on Cassava (Manihot esculenta Crantz) ". *Natural and Life Sciences Communications.* **22** (1): e2023002.

[16] IPGRI. (2023). "Descriptors for Mangosteen (Garcinia mangostana)". International Plant Genetic Resources Institute. Rome.

[17] A. Abdel-Latif and G. Osman. (2017). "Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize". *Plant Methods.* **13** : 1. 10.1186/s13007-016-0152-4.

[18] J. Kumar and V. Agrawal. (2019). "Assessment of genetic diversity, population structure and sex identification in dioecious crop, Trichosanthes dioica employing ISSR, SCoT and SRAP markers". *Heliyon.* **5** (3): e01346. 10.1016/j.heliyon.2019.e01346.

[19] M. C. Mehta, K. Srivastava, and M. Raghuraman. (2023). "Student friendly approach of molecular data analysis for the evaluation of genetic diversity". *The Pharma Innovation Journal.* **10** : 1622-1629.

[20] *Vegan: Community Ecology Package*. (2015). R Package Version 2.2-1.

[21] Z. N. Kamvar, J. F. Tabima, and N. J. Grunwald. (2014). "Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction". *PeerJ.* **2** : e281. 10.7717/peerj.281.

[22] R. Temizgul, M. Akbulut, and D. Lafiandra. (2016). "Genetic diversity of high-molecular-weight glutenin subunit compositions in bread wheat landraces originated from Turkey". *Plant Genetic Resources: Characterization and Utilization.* **16** (1): 28-38. 10.1017/s1479262116000356.

[23] S. Ö. Keleş. (2020). "The Effect Of Altitude On The Growth And Development Of Trojan Fir (Abies nordmanniana subsp. equi-trojani [Asch. & Sint. ex Boiss] Coode & Cullen) Saplings". *Cerne.* **26** (3): 381-392. 10.1590/01047760202026032734.

[24] P. R. Rokaya, D. R. Baral, D. M. Gautam, A. K. Shrestha, and K. P. Paudyal. (2016). "Effect of Altitude and Maturity Stages on Quality Attributes of Mandarin (<i>Citrus reticulata</i> Blanco)". *American Journal of Plant Sciences.* **07** (06): 958-966. 10.4236/ajps.2016.76091.

[25] S. Sharma and V. S. Rana. (2023). "Altitude Variation on Fruit Development and Quality Attributes of Kiwifruit (Actinidia Deliciosa Chev.) In North-Western Himalaya". *Bangladesh Journal of Botany.* **52** (2): 369-379. 10.3329/bjb.v52i2.67038.

[26] F. Wang, X. Cheng, S. Cheng, W. Li, and X. Huang. (2023). "Genetic diversity of the wild ancient tea tree (Camellia taliensis) populations at different altitudes in Qianjiazhai". *PLoS One.* **18** (4): e0283189. 10.1371/journal.pone.0283189.

[27] M. Yılmaz, A. Işık Özgüven, and Y. Aka Kaçar. (2024). "Molecular characterization of Turkish hazelnut cultivars and genotypes using SSR markers". *International Journal of Agriculture Environment and Food Sciences.* **8** (1): 88-93. 10.31015/jaefs.2024.1.10.

[28] B. Kirkpatrick, S. Ge, and L. Wang. (2019). "Efficient computation of the kinship coefficients". *Bioinformatics.* **35** (6): 1002-1008. 10.1093/bioinformatics/bty725.

[29] R. K. Waples, A. Albrechtsen, and I. Moltke. (2019). "Allele frequency-free inference of close familial relationships from genotypes or low-depth sequencing data". *Molecular Ecology.* **28** (1): 35-48. 10.1111/mec.14954.

[30] J. R. Brzyski, C. R. Stieha, and D. Nicholas McLetchie. (2018). "The impact of asexual and sexual reproduction in spatial genetic structure within and between populations of the dioecious plant Marchantia inflexa (Marchantiaceae)". *Annals of Botany.* **122** (6): 993-1003. 10.1093/aob/mcy106.

[31] M. Podio, C. Colono, L. Siena, J. P. A. Ortiz, and S. C. Pessino. (2021). "A study of the heterochronic sense/antisense RNA representation in florets of sexual and apomictic Paspalum notatum". *BMC Genomics.* **22** (1): 185. 10.1186/s12864-021-07450-3.

[32] Y. Pacheco-Hernandez, N. Villa-Ruano, E. Lozoya-Gloria, C. A. Barrales-Cortes, F. E. Jimenez-Montejo, and M. D. C. Cruz-Lopez. (2021). "Influence of Environmental Factors on the Genetic and Chemical Diversity of Brickellia veronicifolia Populations Growing in Fragmented Shrublands from Mexico". *Plants (Basel).* **10** (2). 10.3390/plants10020325.

[33] S. Susilo. (2023). "Deciphering the Analysis of the Genetic Diversity of Mangosteen (Garcinia mangostana L.) from Various Regions of Origin in Java, Indonesia". *Biosciences Biotechnology Research Asia.* **20** (1): 163-171. 10.13005/bbra/3078.

[34] E. De Jonge and M. Van Der Loo. (2013). "An introduction to data cleaning with R".

[35] S. Noor, A. Muhammad, A. Shahzad, I. Hussain, M. Zeshan, K. Ali, S. Begum, M. Aqeel, M. Numan, R. M. Muazzam Naz, S. Shoukat, H. Hafeez, I. U. Zaid, and G. M. Ali. (2022). "Inter Simple Sequence Repeat-Based Genetic Divergence and Varietal Identification of Banana in Pakistan". *Agronomy.* **12** (12). 10.3390/agronomy12122932.

[36] E. Mansyah, P. J. Santoso, I. Muas, and Sobir. (2013). "Evaluation of Genetic Diversity among and within Mangosteen (Garcinia Mangostana L.) Trees". *Acta Horticulturae.* (975): 73-79. 10.17660/ActaHortic.2013.975.6.

[37] O. P. Rajora, A. J. Eckert, and J. W. Zinck. (2016). "Single-Locus versus Multilocus Patterns of Local Adaptation to Climate in Eastern White Pine (Pinus strobus, Pinaceae)". *PLoS One.* **11** (7): e0158691. 10.1371/journal.pone.0158691.

[38] M. Rodriguez, D. Rau, D. O'Sullivan, A. H. Brown, R. Papa, and G. Attene. (2012). "Genetic structure and linkage disequilibrium in landrace populations of barley in Sardinia". *Theoretical and Applied Genetics.* **125** (1): 171-84. 10.1007/s00122-012-1824-8.

[39] M. Ulfah, S. N. Fajri, M. Nasir, K. Hamsah, and S. Purnawan. (2019). "Diversity, evenness and dominance index reef fish in Krueng Raya Water, Aceh Besar". *IOP*

Conference Series: Earth and Environmental Science. **348** (1). 10.1088/1755-1315/348/1/012074.

[40] M. Pontz, J. Hofbauer, and R. Burger. (2018). "Evolutionary dynamics in the two-locus two-allele model with weak selection". *Journal of Mathematical Biology.* **76** (1-2): 151-203. 10.1007/s00285-017-1140-7.

[41] R. Rubiyo, S. Sudarsono, M. F. Anshori, N. Nurdebyandaru, Y. A. Dewi, and M. R. Akbar. (2022). "Determining kinship pattern of robusta and arabica coffee clones using multivariate analysis". *Chilean Journal of Agricultural Research.* **82** (2): 276-284. 10.4067/s0718-58392022000200276.

[42] W. A. Qosim, S. Patarapuwadol, and K. N. Watanabe. (2011). "Development of SSR markers of mangosteen (Garcinia mangostana L.)". *International Research Journal of Biotechnology.* **2** (1): 1-8.