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GENETIC RELATIONSHIP OF MANGO (*MANGIFERA INDICA* L.) ACCESSIONS BASED ON FRUIT TRAITS AND MOLECULAR MARKERS

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SUMMARY

Mango is a tropical fruit in Indonesia, rich in vitamins, minerals, and antioxidants. It plays a significant role in boosting farmers' income, supporting industrial development, and driving exports. Mangoes in Indonesia exhibit remarkable diversity in fruit shape, size, taste, and leaf characteristics. This study aimed to assemble the data on the diversity of 10 mango accessions using different fruit traits and molecular markers. This research took place at the Agricultural Technology Research and Assessment Installation (ATRAI)-Cukurgondang and the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD)-Bogor. A set of mango accessions, observed for their fruit traits, underwent molecular analysis using 20 RAPD and 15 SSR markers to generate their genetic relatedness. The results revealed that mango accessions varied considerably in fruit shape, scent, color, and taste. The RAPD primers produced more DNA bands than SSR markers. The PIC values were highly informative for three RAPD markers (15% of the total) and two SSR markers (13.33% of the total). Based on dissimilarity values, large-sized mangoes' separation from small-sized mangoes can be effective; however, one group emerged with a blend of both. Mango accessions Madu Segoro 127 and Delima 209 have a pure genetic composition, but Delima 209 is an essential crossing material as it belongs to a group distinct from the small mangoes. The two mango groups' development into smaller-sized mangoes can further succeed with a more attractive color and pronounced aroma to better attract consumers.

Keywords: Mango (*M. indica* L.), fruit traits, molecular markers, RAPD and SSR, genetic diversity, population structure

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Key findings: The fruits of 10 mango (*M. indica* L.) accessions in the Cukurgondang-ATRAI collection considerably vary in shape, scent, color, and taste. The RAPD and SSR markers succeeded in separating large-sized and small-sized mangoes. Mango accessions Madu Segoro 127 and Delima 209 revealed the pure genetic composition.

INTRODUCTION

Mango is a member of the family Anacardiaceae and the genus *Mangifera.* Historically, a belief prevailed that the genus *Mangifera* originated in Malaya, Indochina, Thailand, and Myanmar, and later dispersed throughout India, Pakistan, the Philippines, Malaysia, and Sri Lanka (Yadav and Singh, 2017). There are 69 species known to exist in the genus *Mangifera*. India has become a significant mango-producing nation (Sankaran *et al*., 2021). According to FAO (2024), the world's most considerable fruit production in 2023 was 262.53 million tons of tomatoes. Mango, guava, and mangosteen ranked eighth with 65.10 million tons of production. In 2023, Indonesia's mango production reached 3,302,620 tons, making it the second highest after banana production, which totaled 9,335,232 tons (BPS, 2024).

Among the fruits, the mangoes are highly nutritious (Govindan, 2019). It is an excellent source of vitamins A and C and minerals, such as copper, magnesium, potassium, and water, which are good sources of fiber. Mangoes, regarded as a significant fruit worldwide, had research spanning from morphological traits to genomic levels (Wang *et al*., 2020). Genetic diversity studies in mango germplasm can progress directly by examining their anatomy and morphology. By using the morphological method, the variations in the fruits, roots, stems, leaves, and other plant parts of each accession can be visible The examination and comparison with a predetermined manual are applicable for each section. In mango germplasm, the visual morphological variations can be help examine the genetic diversity and even combine it with SSR markers (Sridhar *et al*., 2022).

Numerous studies have materialized using molecular markers to investigate genetic diversity, and a growing number of molecular markers are available. The molecular markers employed range in complexity, from single-nucleotide polymorphism (SNP) markers to random amplified polymorphic DNA (RAPD) markers. Additionally, mango genome sequencing has been progressing to offer more comprehensive data, making it possible to develop more molecular markers as crucial tools in modern mango breeding programs, such as accurately dissecting the genetic variation of mango germplasm. Three mango cultivars from India have also undergone genomic sequencing of their core DNA (Bally *et al*., 2021; Singh *et al*., 2021). However, Song *et al*. (2023) also describe the latest developments in mango sequencing results. Himabindu and Rajasekhar (2021) and Jena and Chand (2021) have investigated the genetic diversity of mangoes using RAPD markers. Simple-sequence repeat (SSR) markers have also reached development (Srivastav *et al*., 2021), and efforts have succeeded to form a mango barcode from these markers (Kumar *et al*., 2023). These SSR markers have been beneficial to assemble the data on the genetic diversity of mangoes (Molla *et al*., 2019; Hidayat *et al*., 2021).

Using other molecular markers, such as SNP markers and the inter-simple sequence repeat (ISSR), has also appeared in limited research (Salsabila *et al*., 2021; Srivastav *et al*., 2023). In various studies, several DNA markers have succeeded in combining for use at once to obtain data on genetic diversity in mangoes. Among these are RAPD and ISSR (Khattab *et al*., 2022), RAPD and SSR (Hussein *et al*., 2023), start codon targeted (SCoT) and ISSR (Ghounim *et al*., 2022), and RAPD, ISSR, and directed amplification of minisatellites DNA (DAMD) (Jena and Chand, 2021).

Currently, the mango germplasm collection has its management by Cukurgondang-ATRAI at the Tropical Fruit Crop Research Institute, Indonesia. Established in 1938, this collection garden houses over 2000 mango trees, comprising various old varieties and crossbreeds. However, the material from these mango trees has yet to be widely utilized. Publications observing the morphology and molecular traits of Indonesian mangoes remain scarce. Most studies tend to concentrate solely on either morphological characteristics or molecular analyses. Therefore, the presented study aimed to assemble the data on the diversity of 10 mango accessions using various fruit traits and molecular markers.

MATERIALS AND METHODS

Plant material

This study began at two locations, in Bogor and Pasuruan, Indonesia. The observation of fruit traits in various mango accessions transpired at the Agricultural Technology Research and Assessment Installation (ATRAI) in Cukurgondang Village, Grati District, East Java Province, Indonesia, from February to December 2016. Meanwhile, molecular analysis continued from February to July 2017 at the Laboratory of Molecular Biology, the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor, Indonesia.

In this study, the 10 mango accessions used served as plant genetic materials, i.e., six from large-fruit mango clones—Madu Segoro 127, Gendewo 25, and Delima 209 from Indonesia; Keitt and Haden 217 from South Florida/USA; and Kensington Apple from Queensland/Australia—and four from small-fruit mango clones, viz., Gedong 261, Agung 365, and Wudel 425 from Indonesia and Z. Bombay 307 from India. The SSR markers totaled 15, while the RAPD had 20 markers, with both employed in the molecular analysis of mango germplasm.

Fruit traits

With the limited number of mango accessions in the collection garden, obtaining fruit samples came from a single healthy tree. Approximately 10 high-quality mango samples resulted from one accession. Various fruit traits' observation focused on the fruit's physical attributes and the total soluble solids (TSS) of each mango accession. The descriptors for mango (*M. indica*) became the reference for describing the physical attributes of the fruits (shape, weight, and thickness) (IPGRI, 2006). All the presentation of data was descriptive.

Molecular analysis

The collection of fresh leaves came from the trees of the mango accessions at ATRAI. The DNA extraction of the leaves used the cetyltrimethylammonium bromide (CTAB) extraction method, as described by Doyle and Doyle (1990).

Preparing PCR reactions used a 20–25 μL volume, 1× PCR buffer, 0.1 mM dNTP mix, 0.5 mM primer (F+R), 25–50 ng of mango DNA, and 1 unit of Taq DNA polymerase. The following profile for DNA replication comprised these steps: pre-denaturation/initial denaturation for two minutes at 94 °C continued for the RAPD—45 cycles of one minute at 94 °C, one minute at 37 °C, two minutes at 72 °C, and one cycle of seven minutes for final extension at 72 °C—and for SSR—35 cycles of one minute at 94 °C, one minute at 50 °C–55 °C (adjusted based on primer annealing), two minutes at 72 °C, and one cycle of seven minutes for final extension at 72 °C. Finally, the reaction reached cooling to 4 °C for indefinite storage.

RAPD and SSR fragments' separation employed electrophoresis in a 2% agarose gel, stained with ethidium bromide, and captured under UV light using the ChemiDoc XRS Gel Imaging System from Bio-Rad. The RAPD and SSR bands' scoring had a score of 1 for those on the same row having bands and a score of 0 for those without bands. Using the Excel program helped determine the number of bands, the number of band patterns (alleles), and the value of polymorphism information content (PIC) for each primer from both RAPD and SSR markers. The PIC values' calculation followed Hildebrand *et al*.'s (1992) formula. The PIC values classification consisted of PIC > 0.7 = very informative, 0.7 > PIC > 0.44 = moderate, and PIC < 0.44 = less informative.

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2$$

Where PIC = polymorphism information content, i = allele i in marker j, n = number of alleles in marker j, and p = allele frequency.

The development of a dendrogram utilized dissimilarity values in the Dissimilarity Analysis and Representation for Windows (DARwin 6.0.21) program (https://darwin.cirad.fr/). Forming the dissimilarity matrix employed the Dice coefficient, while the formation of the dendrogram used the neighbor-joining approach based on the weighted neighbor-joining method. Population structure analysis engaged the STRUCTURE 2.3.4 software, while predicting the best possible number of groups in a clustering, as performed, used a web-based, user-friendly software freely available at https://lmme.ac.cn/StructureSelector/. A graph display relied on the best K value by taking the graph from the Structure 2.3.4 analysis.

RESULTS AND DISCUSSION

Fruit traits

Presently, the most popular mangoes are Arumanis and Gedong Gincu in Indonesia. Arumanis mangoes have yellowish-green skin and have a sweet taste; however, the aroma is not too strong. Gedong Gincu mangoes have a bright color (yellow-red) with a strong aroma and sweet taste. In the latest study, all the material used was the accessions obtained from exploration in the field, locally and abroad (https://sdghorti.puslithorti.net/pn/crops/0305/03/daftar). Therefore, identifying fruit traits in detail would be very helpful in determining whether the mango accessions used were similar to the two mangoes.

From the results, the 10 mango accessions with an average weight of 751.066 g were specimens in this study (Table 1). The accession Wudel 425 was the smallest mango (75 g), and Madu Segoro 127 was the largest one (1066 g). Coral and Escobar-Garcia (2021) examined mangoes from Peru and observed the fruit weight to be less than 200 g. Likewise, Iranian mangoes' estimates secured the weight between 44.58 and 469.42 g (Khadivi *et al*., 2022).

In the presented study, the largest fruit was of the mango accession Madu Segoro 127 (2.77 cm), with a flesh thickness of more than 2 cm, as demonstrated by the large fruit groups. Within the category of small fruits, the mango accession Wudel 425 has the smallest flesh, measuring less than 1.5 cm (Table 1). According to Khadivi *et al*. (2022), the flesh thickness of commercial mangoes was 0.595–2.992 cm in Iran. This demonstrates that Iranian mangoes were smaller than Indonesian mangoes and had thicker flesh than Indonesian mangoes.

Green mangoes were less visually appealing compared to red, orange, and purple ones (Figure 1). The accessions Madu Segoro 127, Gendewo 25, Agung 365, and Z. Bombay 307 have darker skin tones than the others. However, when the mango lacks sweetness, its appealing color will make consumers less interested in purchasing it. Indian mangoes also have this skin color variation, including green, yellow, and red (Sridhar *et al*., 2022).

Of the 10 mango accessions, the edible portion ranged from 52.99% (Z. Bombay 307) to 81.64% (Keitt). The flesh of the fruit is edible; the seeds and skin are not. A higher percentage of edible parts indicates thin skin and small seeds. Karsinah *et al*. (2022) reported that a mango's edible portion should ideally be at least 70%; thus, the mango genotypes that fall short of this threshold require improvement. The mango accessions used in this study were Haden 217 (67.48%), Delima 209 (69.65%), Kensington Apple (69.93%), Gedong 261 (64.15%), Agung 365 (68.75%), and Z. Bombay 307 (52.99%), and all of them had an edible portion of less than 70% (Table 1).

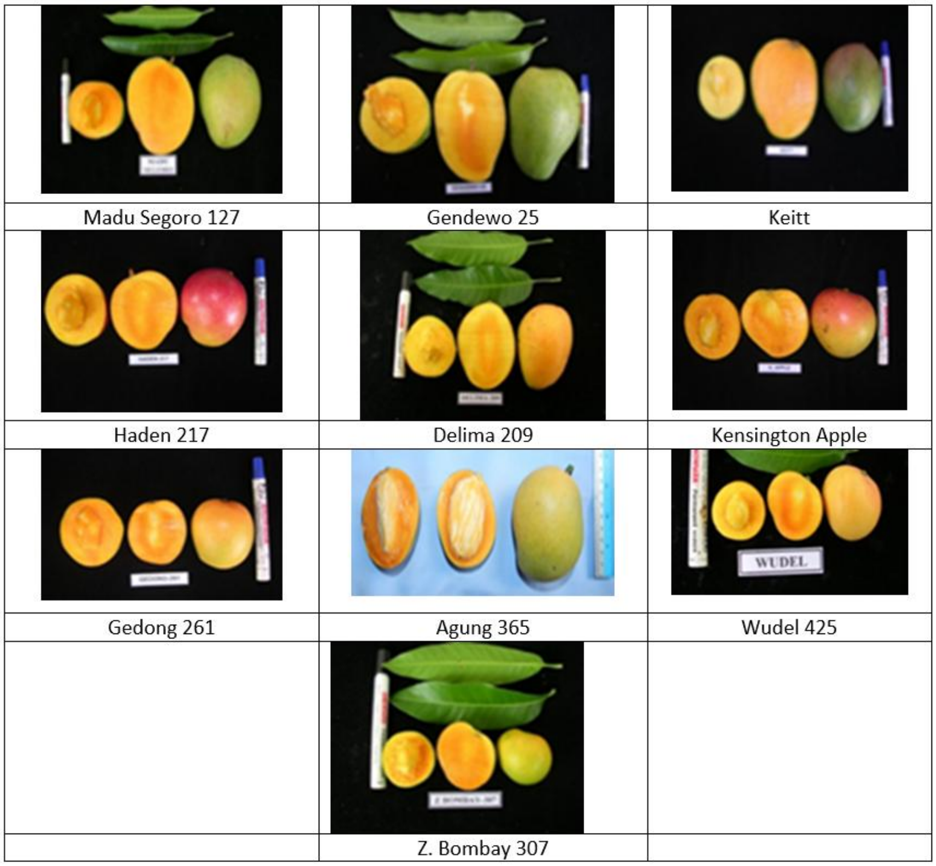
Table 1. The fruit traits of 10 mango accessions.

Characters	Big fruits						Small fruits			
	Madu Segoro 127	Gendewo 25	Keitt	Haden 217	Delima 209	Kensington Apple	Gedong 261	Agung 365	Wudel 425	Z. Bombay 307
Fruit shape	Round	Oval	Round	Round	Oval	Round	Round	Oval	Round	Round
Fruit weight (g)	1.066 ± 86.95	879 ± 19.48	651 ± 13.25	342 ± 20.23	450 ± 10.24	429 ± 6.38	175 ± 4.497	109 ± 3.23	75 ± 2.91	78 ± 1.76
Thickness of fruit flesh (cm)	2.77 ± 0.09	2.73 ± 0.01	2.39 ± 0.024	2.19 ± 0.02	2.07 ± 0.02	2.3 ± 0.15	1.40 ± 0.1	1.45 ± 0.01	1.13 ± 0.01	1.40 ± 0.03
Ripe fruit skin color	Yellowish green	Yellowish green	Greenish red	Yellowish red	Reddish yellow	Reddish yellow	Yellowish orange	Greenish yellow	Yellow	Yellowish green
Flesh color	Yellow-orange	Yellow-orange	Yellow	Yellow-orange	Yellow-orange	Yellow-orange	Orange	Yellow-orange	Yellow	Orange
Edible portion (%)	73.00 ± 0.82	80.61 ± 0.78	81.64 ± 0.88	67.28 ± 0.999	69.65 ± 0.83	68.93 ± 0.66	64.15 ± 0.8	68.75 ± 0.59	78.29 ± 0.66	52.99 ± 0.62
Fruit flesh texture	Hard	Medium	Hard	Medium	Medium	Medium	Medium	Medium	Medium	Soft
Fiber quantity in fruit flesh	Medium	Low	Medium	Medium	Medium	Medium	Low	High	Low	Medium
Scent/Aroma	Weak	Weak	Weak	Medium (Slightly fragrant)	Weak	Medium (Fragrant)	Strong	Weak	Strong	Strong
Flavor	Sweet and sour	Sweet and sour	Sweet and sour	Sweet and sour	Sweet and sour	Sweet	Sweet and sour	Sour and sweet	Sweet and sour	Sour
Sugar level (°Brix)	14.9 ± 0.74	12.98 ± 0.78	14.85 ± 0.56	16.04 ± 0.80	19.09 ± 0.78	15.01 ± 0.53	15.03 ± 0.48	12.06 ± 0.74	13.24 ± 0.80	12.15 ± 0.77

Fruit flesh's fiber content and texture showed a correlation. Some customers dislike the fruit's tough flesh, particularly when it has an excessive amount of fiber. Customers like their fruit flesh to be medium-textured and low in fiber. The mango 'kuweni' is a type of mango with an extremely potent aroma, much like the fruit itself. Three out of the 10 mango accessions studied—Gedong 261, Wudel 425, and Z. Bombay 307—revealed a considerably strong aroma (Table 1).

Except for the accession Z. Bombay 307, all other mango accessions have a predominantly sweet and sour taste. Consumers prefer mangoes with a sweet flavor. Only the Kensington Apple mango, out of the 10 mango accessions, had a pure sweet flavor without any sour undertones. The sweet taste of mango acquires a great influence from the sugar content (°Brix). Based on this research, sugar content data obtained ranged between 12.15° Brix (Z. Bombay 307) and 19.09° Brix (Delima 209). This means the mango accession Delima 209 was the sweetest among all the studied accessions. Duyen *et al*. (2023) reported the sugar brix of several mangoes taken from Vietnam had a sugar brix ranging from 13.82% to 23.43%. This figure means that every 100 g of mango in Vietnam contains 13.82 to 23.43 g of sugar.

The mango accession Gedong 261 has appeared to be similar in properties to the accession Gedong Gincu, and its immediate introduction to consumers can proceed. The accessions Haden 217 and Kensington Apple have the potential for high consumer choices because of their bright color (yellow-orange), which is similar to Gedong 261. Unfortunately, the aroma was not as strong as with Gedong 261. In terms of taste, both mangoes have a sweet taste. Perhaps by crossing it with Gedong 261, the mango will have a strong aroma. The accession Gedong 261 has similar characters to the Gedong Gincu. The mango accessions Wudel 425 and Z. Bombay 307 have a strong aroma and sweet taste. The shape of the fruit also resembles Gedong 261. However, the size was tiny, and at the ripening stage, the fruit skin color becomes yellowish green. The mango accessions Wudel 425 and Z. Bombay 307 require improving their ripe fruit color with large size.



Madu Segoro 127
Gendewo 25
Keitt
Haden 217
Delima 209
Kensington Apple
Gedong 261
Agung 365
Wudel 425
Z. Bombay 307

Figure 1. The 10 mangoes utilized in the study.

Molecular analysis

Based on the results, the bands produced by the RAPD markers had an average of 7.7 alleles with a range of 3–13 and 1–9 bands (Figure 2, Table 2). Using RAPD markers, Hussein *et al*. (2023) observed six to 26 bands, with an average of 14.5, measuring approximately 200 to 1100 bp. Khattab *et al*. (2022) demonstrated several more bands ranging from three to 16 than this study's results. Himabindu and Rajasekhar (2021) reported the band results at 100–5000 bp by using RAPD markers to amplify the mangoes, and the bands obtained range from about 100 to 1000 bp.

In the relevant study, the SSR markers produced several bands with an average of 3.53 alleles and a range of 2–9. The SSR markers used in this study generated bands measuring 80–1000 bp, with a band gap of 1–6 (Figure 2, Table 2). Typically, the SSR bands generated by the mango genotype SSR analysis have a size of less than 500 bp (Molla *et al*., 2019). Hidayat *et al*. (2021) discovered SSR markers resulting in 600–700 bp bands. In the presented study, the maximum band size of the other SSR markers was 500 bp; however, the SSR marker AY942819 has a band size of 1000 bp, and AY942817 has a size of 750 bp. The largest band size found in the RAPD marker was between 625 and 1000 bp, suggesting that the band generated by this marker was larger than the one produced by the SSR marker.

The RAPD markers (OPO01, OPR06, and OPZ14, around 15% of the total) seem to have values >0.7 (very informative) based on Hildebrand *et al*. (1992). Two SSR markers (AY942823 and M312, around 13.33% of the total) also have the values >0.7 (very informative) (Table 2). According to Botstein *et*

Table 2. Tabulation of the characteristics of the RAPD and SSR markers used.

No.	Primers	Sequence (5′-3′)	Band range (bp)	Number of bands	Number of alleles	PIC value
RAPD markers						
1	OPD-20	ACTTCGCCAC	200–750	3–5	8	0.616**
2	OPJ-06	TCGTTCCGCA	250–750	1–6	6	0.682**
3	OPK-06	CACCTTTCCC	250–750	2–5	5	0.548**
4	OPM-10	TCTGGCGCAC	150–1000	2–8	13	0.68**
5	OPM-13	GGTGGTCAAG	150–875	2–8	10	0.524**
6	OPM-17	TCAGTCCGGG	250–750	1–6	7	0.571**
7	OPM-18	CACCATCCGT	300–750	2–6	7	0.614**
8	OPN-03	GGTACTCCCC	150–750	6–8	9	0.376
9	OPN-04	GACCGACCCA	250–875	4–9	11	0.521**
10	OPN-17	CATTGGGGAG	250–750	1–3	4	0.565**
11	OPO-01	GGCACGTAAG	250–1000	1–5	10	0.811*,**
12	OPP-02	TCGGCACGCA	250–700	4–7	8	0.55**
13	OPR-06	GTCTACGGCA	200–750	1–3	7	0.871*,**
14	OPR-08	CCCGTTGCCT	100–625	2–5	7	0.544**
15	OPR-11	GTAGCCGTCT	250–1000	2–3	3	0.28
16	OPR-20	ACGGCAAGGA	300–875	3–6	8	0.605**
17	OPV-10	GGACCTGCTG	200–750	1–4	4	0.265
18	OPV-14	AGATCCCGCC	200–875	5–6	7	0.243
19	OPZ-13	GACTAAGCCC	250–875	3–6	10	0.736*,**
20	OPZ-19	GTGCGAGCAA	250–750	4–9	10	0.442
	Average		-	-	7.7	0.552**
	Range		100–1000	1–9	3–13	0.243–0.871
SSR markers						
1	AJ635165	F-GATGAAACCAAAGAAGTCA R-CCAATAAGAACTCCAACC	300–500	1–2	2	0.435
2	AY942823	F-AGAATAAAGGGGACACCAGAC R-CCATCATCGCCCACTCAG	100–250	1–6	7	0.761*,**
3	AJ938179	F-TCGGTCATTTACACCTCT R-TTATTGAGCTTCTTTGTGTT	100–250	1–2	2	0.375
4	AJ635178	F-AGCTGTTTTGGCCTT R-ATGTGGTTTGTTGCTTC	90–250	1–2	2	0.375
5	AJ635187	F-ATCCCCAGTAGCTTTGT R-TGAGAGTTGGCAGTGTT	90–250	1–2	2	0.095
6	AY942819	F-AAACGAGGAAACAGAGCAC R-CAAGTACCTGCTGCAACTAG	90–1000	2–4	4	0.338
7	AJ635168	F-TTCTAAGGAGTTCTAAAATGC R-CTCAAGTCCAACATACAATAC	90–200	1–2	2	0.18
8	AJ635171	F-TAAAGATAAGATTGGGAAGAG R-CGTAAGAAGAGCAAAGGT	90–200	1–2	2	0.095
9	AJ635175	F-TGCGTAAAGCTGTTGACTA R-TCATCTCCCTCAGAACA	80–200	2–3	3	0.333
10	AY942825	F-CGAGGAAGAGGAAGATTATGAC R-CGAATACCATCCAGCAAAATAC	90–250	1–2	2	0.35
11	AY942817	F-TAACAGCTTTGCTTGCCTCC R-TCCGCCGATAAACATCAGAC	90–700	1–4	4	0.255
12	AY942822	F-CAACTTGGCAACATAGAC R-ATACAGGAATCCAGCTTC	90–200	1–2	2	0.575**
13	T302	F-CAGCACCTGGAAGTTTTCC R-TCATTTTCCAGGGCTGTTGC	80–300	1–4	4	0.665**
14	AJ635166	F-CTTGAAAGAGATTGAGATTG R-AGAAGGCAGAAGGTTTAG	80–350	5–6	6	0.165
15	M312	F-TTGCGTAAAGAGAACGAGCA R-GTGTGGAAAATTCACCTGAGC	80–200	1–6	9	0.954*,**
	Average		-	-	3.533	0.397
	Range		80–1000	1–6	2–9	0.435–0.954

*Very informative according to Hildebrand *et al*. (1992), ** very informative according to Botstein *et al*. (1980).

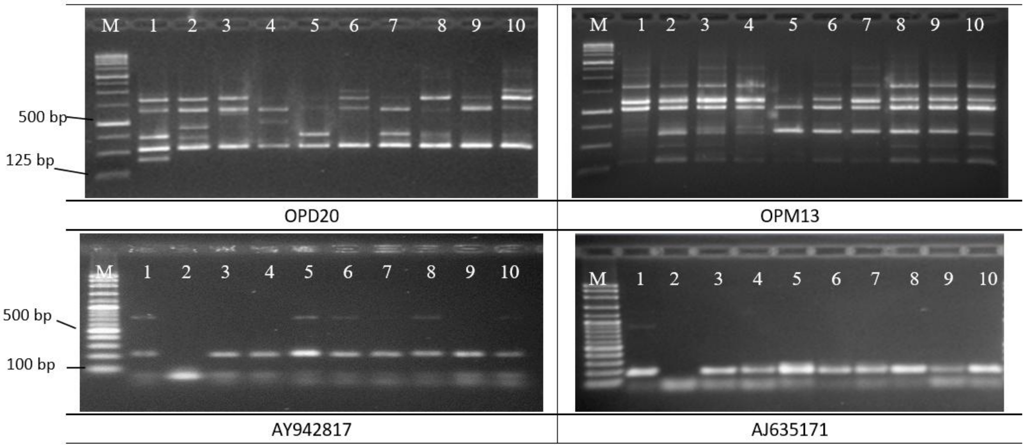


Figure 2. The outcomes of using RAPD (top row) and SSR (bottom row) and to separate the amplified DNA bands. M = 100 bp DNA marker. 1 = Madu Segoro 127, 2 = Gendewo 25, 3 = Keitt, 4 = Haden 217, 5 = Delima 209, 6 = Kensington Apple, 7 = Gedong 261, 8 = Agung 365, 9 = Wudel 425, and 10 = Z. Bombay 307.

al. (1980), the PIC criteria were as follows: very informative (PIC > 0.5); moderate information (0.5 > PIC > 0.25); and less informative (PIC < 0.25). By applying these criteria, four markers (26.67%) for the SSR markers and up to 15 RAPD markers (75%) were highly informative (PIC > 0.5). Ajayi *et al*. (2019) mentioned that most PIC values were below 0.5, and the highest PIC value was 0.58 by using SSR markers. Soliman *et al*. (2020) observed different results, and five out of seven SSR markers produced PIC values above 0.5, and the parent and 30 derivatives from Egyptian mango, namely Zebda and Ewais, produced these markers. Using 60 genotypes of Bangladesh mangoes, Rahman *et al*. (2022) discovered that the six SSR markers used had PIC values ranging from 0.65 to 0.94.

The dissimilarity value matrix of 10 mango accessions analyzed using SSR and RAPD markers appears in Table 3. The mango accession Mango Wudel 425 vs. Gendewo 25 has the highest dissimilarity value (0.329 = similarity value: 0.671), while the lowest one occurred for the accession Madu Segoro 127 vs. Agung 365, at around 0.157 (similarity value = 0.843). Generally, the development of the genetic diversity depended on similarity values. Fewer studies applied the dissimilarity values to perform genetic diversity than those that used similarity values, and some of those past findings include the investigations of Agre *et al*. (2019) and Kumar *et al*. (2022).

The dendrogram based on the dissimilarity matrix value is available in Figure 3. One can see that the 10 mango accessions used further attained division into three groups. Group A comprises the large mangoes, such as accessions Gendewo 25, Keitt, Haden 217, Delima 209, and Kensington Apple. Group B consists of a mixture of large mangoes (Madu Segoro 127) and small mango accessions (Gedong 261 and Agung 365), while Group C contains small mango accessions (Wudel 425 and Z. Bombay 307). This dissimilarity value (marked by a line measuring 0.1) located below the dendrogram showed the first separation occurred in the accession Agung 365, and the last was in the accession Gendewo 25. The accession Agung 365 has the smallest dissimilarity value (0.157), while Gendewo 25 has the highest dissimilarity value (0.329). However, in this case, the high dissimilarity value for the accession Wudel 425 vs. Gendewo 25 does not reflect the origin, as both were from Indonesia but differ in their fruit size.

Population structure analysis employment determined the genomic makeup for each mango accession. The optimal K value that captured the likely genetic composition at

Table 3. The dissimilarity matrix of 10 mango accessions using RAPD and SSR markers.

Accessions	1	2	3	4	5	6	7	8	9	10
1	0									
2	0.302	0								
3	0.251	0.239	0							
4	0.239	0.247	0.196	0						
5	0.265	0.306	0.256	0.243	0					
6	0.252	0.293	0.243	0.230	0.229	0				
7	0.222	0.294	0.225	0.213	0.257	0.244	0			
8	0.157	0.275	0.279	0.197	0.238	0.239	0.195	0		
9	0.269	0.329	0.251	0.266	0.293	0.279	0.261	0.242	0	
10	0.252	0.312	0.262	0.250	0.275	0.269	0.244	0.225	0.241	0

1 = Madu Segoro 127, 2 = Gendewo 25, 3 = Keitt, 4 = Haden 217, 5 = Delima 209, 6 = Kensington Apple, 7 = Gedong 261, 8 = Agung 365, 9 = Wudel 425, and 10 = Z. Bombay 307.

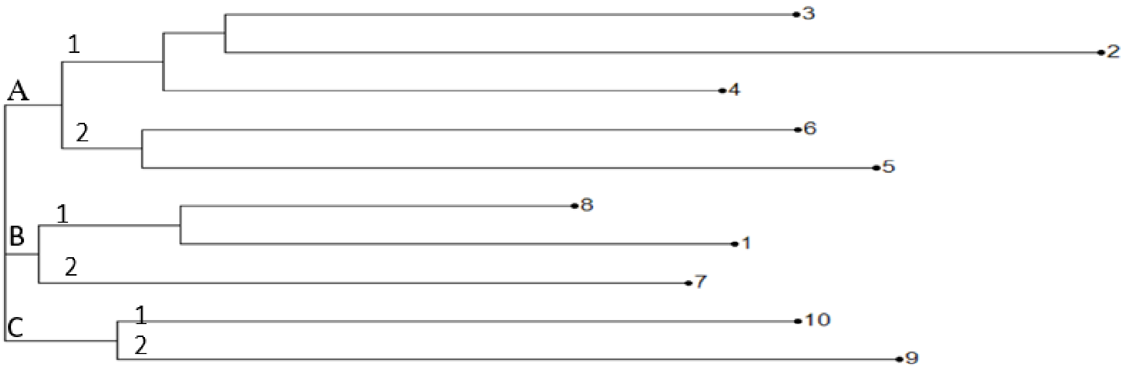
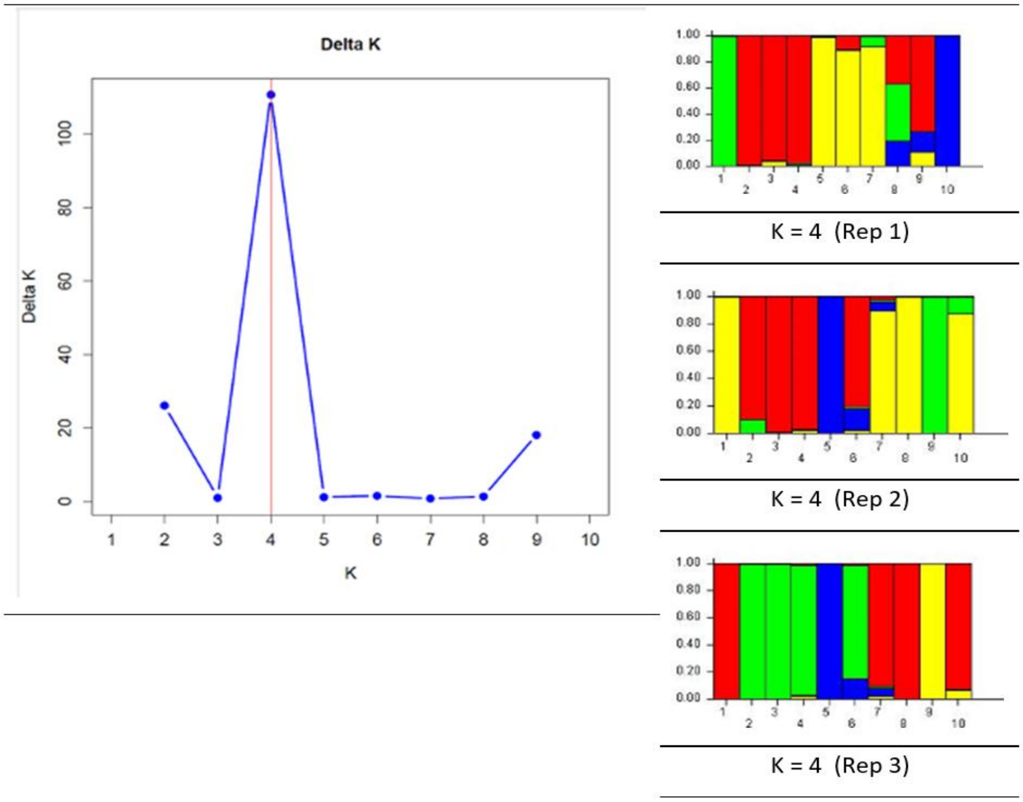


Figure 3. Dendrogram of 10 mango accessions using RAPD and SSR markers based on dissimilarity value. 1 = Madu Segoro 127, 2 = Gendewo 25, 3 = Keitt, 4 = Haden 217, 5 = Delima 209, 6 = Kensington Apple, 7 = Gedong 261, 8 = Agung 365, 9 = Wudel 425, and 10 = Z. Bombay 307.

a given point sustained selection through analysis. Each K value (K = 1-10) incurred three iterations of 10,000 repetitions. The resulting K graph indicated that K = 4 was optimal, displaying the highest K value (Figure 4). The colorful display of each genotype's histogram in the structural harvester's best simulation results demonstrated how the same genomes could be assumed based on color similarity.

In this study, based on the K value formulation, a colored histogram depicting the genetic composition of each mango accession existed. Following the K value (K = 4), one can see the production of four different colors. From the three replications, it can be evident that the mango accessions with full color and no variation in the three replications were the accessions Madu Segoro 127 and Delima 209. The genetic composition of the two mango accessions has not reached mixing with the genomes of the other eight mango accessions. Razak *et al*. (2019) reported SSR markers could describe the genetic condition of the mango accessions, whether they were polyembryonic or monoembryonic. Liang *et al*. (2024) succeeded in explaining the genetic composition of 284 mango accessions in three sections based on sample origin using SNP markers.

Figure 4 shows mango accessions with various color mixtures at varying levels. In Gendewo 25, Keitt, Haden 217, Kensington Apple, Gedong 261, and Z. Bombay 307 accessions, a dominant color has blended with one other color. Conversely, the Agung 365 and Wudel 425 accessions displayed mixtures of three colors. These occurrences are common in nature, where genetic variations arise from natural crossing or mutations that may have



K = 4 (Rep 1)

K = 4 (Rep 2)

K = 4 (Rep 3)

Figure 4. Population structure analysis with K = 4 using STRUCTURE 2.3.4.
1 = Madu Segoro 127, 2 = Gendewo 25, 3 = Keitt, 4 = Haden 217, 5 = Delima 209, 6 = Kensington Apple, 7 = Gedong 261, 8 = Agung 365, 9 = Wudel 425, and 10 = Z. Bombay 307.

occurred centuries ago. The mango accessions grown in the Cukurgondang collection garden originate from various regions, where such genetic diversity is likely to occur. Mangoes exhibit self-crosspollination, self-incompatibility, and self-sterility systems, though some cultivars are semi-compatible or fully compatible (Ramírez and Davenport, 2016).

The authors received advice to briefly describe how population structure obtained across 10 mango genotypes used in this study can help the maintenance of mango germplasm collection, thereby accelerating the mango breeding programs in the future. For readers' consideration is the following suggested statement: The information on population structure obtained in this study could provide basic information on the genetic background of the mango germplasm collection that could help mango breeders identify accessions as potential parents for crossbreeding in the future.

This study utilized 10 mango accessions. However, population structure analysis identified two accessions that did not exhibit genomic mixtures from the other eight accessions. Based on these findings, a comprehensive population structure analysis should continue for all mango accessions in the Cukurgondang collection garden. The selected markers should be abundant and easy to work with. SNP markers are the most suitable for this purpose, as their development and commercial application have reached wide release (Sherman *et al*., 2015; Kuhn *et al*., 2019; Liang *et al*., 2024).

This work does not necessarily complete in a single phase but can happen progressively, depending on available funding. Results obtained at different times can be collective, provided the consistent use of the same SNP markers. The resulting data will be useful for kinship and population structure analysis. Mango accessions with minimal genomic mixture from other mango varieties should be a priority as crossbreeding parents, concerning the dendrogram.

Implications for breeding

Mango breeding varies from one country to another, as the choice of preferred mango varieties differs across regions (Bura *et al*., 2023). Using fruit morphology data and molecular analysis, a cross-breeding design can be an outcome to produce mangoes that align closely with consumer preferences. In Indonesia, some of the most popular mango varieties include Arumanis/Harumanis, Manalagi, Golek, Lalijiwo, Gedong, and Indramayu. Beyond these, there are other varieties available, even though in smaller quantities. Arumanis and Gedong are two prominent mango varieties in Indonesia, both widely recognized as export commodities (Hardiyanto *et al*., 2020). The crossbreeding of mangoes in this study, if aimed at resembling these popular varieties, is likely to have more ready acceptance from the public. According to Figure 3, a crossbreeding program involving group A (Gendewo 25, Keitt, Haden 217, Delima 209, and Kensington Apple) and group C (Wudel 425 and Z. Bombay) would yield diverse mango characteristics. This is because group A consists of large-fruited mangoes, while group C represents small-fruited mangoes. The resulting variations would include fruit weight, color, aroma, and taste differences.

Gendewo 25 and Keitt mangoes are large, making them less popular among consumers. Their flavor is sweet and sour, with a mild aroma. When crossing Gendewo 25 or Keitt with Wudel 425 and Z. Bombay 307 (Gendewo 25 × Wudel 425, Gendewo 25 × Z. Bombay 307, Keitt × Wudel 425, and Keitt × Z. Bombay 307), the expected progeny will exhibit desirable traits, such as a mango weight of approximately 300 grams, a bright yellow shade, a strong aroma, and a sweet taste without sourness. In the Cukurgondang collection garden, conducting crossings typically occurs once to obtain F_1 seeds. These seeds then sustain planting, and after fruiting, they undergo a two-year evaluation process to determine their suitability based on the desired traits. The Arumanis mango, known for its purely sweet flavor without any sourness, represents the standard taste consumers prefer. The genetic combination of these two distinct types of mangoes (big and small mangoes) will result in diverse offspring, which will need careful selection based on the intended breeding objectives.

The mango accessions Haden 217, Delima 209, and Kensington Apple displayed near alignment with the ideal mango weight preferred by consumers. They can be crossed with the two other accessions, Wudel 425 and Z. Bombay 307, to produce mangoes similar to Gedong 261. These two groups of mangoes have a long genetic distance, proving that they are in different groups. The crossing will produce various genetic variations, which will be similar to the mango Gedong 261. The new mango will have a bright color (reddish yellow), which comes from the accessions Haden 217, Delima 209, and Kensington Apple with a strong aroma, coming from the accessions Wudel 425 and Z. Bombay 307. However, the size is similar to Gedong 261 because it comes from a combination of large mangoes (Haden 217 and Kensington Apple) with small mangoes (Wudel 425 and Z. Bombay 307). The new mango variety produced from this cross, which exhibits several favorable traits, has the potential to be a preference for consumers.

The results of the study showing Madu Segoro 127 and Delima 209 mangoes still have a pure genetic composition (not mixed with other mangoes) are usually evident in the population structure analysis. These results are only the results of statistical analysis by comparing them with eight other mango samples analyzed in this study. If the mango sample is added to another mango, these results could have potential changes.

If this data sustains linkage to a current mango breeding—specifically Madu Segoro 127 and Delima 209—the resulting offspring will exhibit a contrasting blend of the genomes from these two mango varieties with

Wudel 425 and Z. Bombay 307. However, according to the dendrogram (Figure 3), Delima 209 is a better choice than Madu Segoro 127 for use as crossbreeding material with Wudel 425 or Z. Bombay 307, as Madu Segoro 127 belongs to the same group as small-fruited mangoes.

CONCLUSIONS

Both RAPD and SSR markers can generally differentiate between the large- and small-fruit mango groups and identify genetic diversity among the 10 mango germplasm collections. These findings can be applicable in a mango breeding strategy involving a crossing scenario to generate superior offspring. The combination of fruit morphological traits could produce offspring with diverse variations of the mango fruit. The Delima 209 accession shows strong potential as the primary material for the mango-crossing scenario in this study.

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