



GENETIC DIVERSITY OF MATOA (*POMETIA PINNATA*) SPECIES BASED ON RAPD MARKERS

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SUMMARY

Matoa (*Pometia pinnata*), comprising three cultivars, i.e., green matoa (*Emme Anokhong*), yellow matoa (*Emme Khabelaw*), and red matoa (*Emme Bhanggahe*), belongs to the family Sapindaceae. The three cultivars have similar morphological characteristics. However, the analysis of DNA banding patterns can provide information on the genetic diversity of matoa. The presented research sought to examine the genetic diversity of matoa based on the DNA banding pattern of green, yellow, and red matoa using the RAPD technique. DNA isolated from green, yellow, and red matoa leaves' amplification used the RAPD method with primers OPA 19, OPB 15, OPD 08, OPD 11, and OPD 13. The results showed that green and yellow matoa had a closer genetic similarity, with a coefficient of 0.698. The most distant genetic similarity came from yellow and red matoa, with a coefficient of 0.566. The number and pattern of DNA bands produced from each primer occurred at different loci among the one and other cultivars.

Keywords: DNA amplification, DNA isolation, genetic similarity, matoa (*Pometia pinnata*), polymorphism, RAPD primers

Key findings: The study described the genetic diversity in matoa based on the DNA banding pattern using the RAPD method. The results can serve as an indicator of genetic diversity from DNA bands and support diversity at the morphological level. The research result can help support agricultural production.

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INTRODUCTION

Matoa (*P. pinnata*) is an annual fruit plant native to Papua, further spreading to several

other islands in Indonesia, such as, Sumatra, Sulawesi, and Java. Matoa fruit has a distinctive flavor similar to rambutan, with a mixture of longan and durian (Nuryadi *et al.*,

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2020). Matoa cultivars are distinguishable by their skin color, i.e., green matoa (*Emme Anokhong*), yellow matoa (*Emme Khabelaw*), and red matoa (*Emme Bhanggahe*). Matoa can grow at an altitude of 500 masl and is, therefore, a lowland plant. Matoa mainly grows in groups in swamps and valleys that have an average rainfall of 1500–5000 mm/year, with average temperatures of 5 °C–16 °C, 18 °C – 24 °C, and 22 °C–28 °C (Furay, 2019).

Before ripening, the red matoa fruit is green, but after ripening, it becomes red or yellowish red. This type of matoa is known as matoa papeda, which has soft, slightly juicy flesh and no loose seeds. A yellow matoa fruit is also green before ripening and turns yellow when ripe, with their cultivars known as matoa coconut. A green matoa fruit before ripening is green; however, after ripening, it becomes dark green, and these cultivars are typically coconut matoa, which have sweet, chewy, and loose fruit flesh (Furay, 2019).

Matoa stem can be usable in the wood industry, while its fruits, seeds, and leaves consumption can be helpful as traditional medicine. Matoa plant parts like leaves and bark are also popular as effective indigenous medicines. A decoction of matoa leaves has the Papuan people believe to relieve hypertension (Sutomo *et al.*, 2021). Matoa fruits contain flavonoids, which are antioxidants and perform as free-radical antidotes (Pamangin *et al.*, 2020). Matoa also contains active compounds and secondary metabolites, namely, flavonoids, phenols, tannins, and alkaloids, that can benefit as medicines (Rahmawati *et al.*, 2021). Previous research on phytochemical screening for 96% ethanol extract from matoa leaves showed that these components were alkaloids, flavonoids, tannins, steroids, and triterpenoids in matoa leaves (Rahmawati *et al.*, 2021).

The three matoa cultivars have similar morphological characteristics in the shape of their leaves and fruits. Morphological traits widely function as a basis for identification and diversity analysis. However, morphological features cannot show the true nature of genetic diversity because the plant cultivars incur more environmental influences (Yuniastuti *et al.*, 2021). Morphological

characteristics also require a relatively long observation time to determine the genetic diversity of matoa. Therefore, a molecular genetic diversity analysis is crucially necessary. Molecular analysis for genetic diversity can serve as an indicator to support genetic diversity based on said traits.

Generally, the use of molecular markers and morphological traits in practice are not contradictory but rather complementary (Enggarini, 2022). Molecular analysis is a favorable selection tool compared with phenotypic selection. Selection using molecular markers depends solely on plant genetic characteristics, with no influence from environmental factors, making plant breeding activities more precise and faster. Molecular techniques are strategic tools that can also shorten the selection time. DNA molecular markers have a high level of polymorphism with an unlimited number, not influenced by the environment, and the level of heritability is almost 100% (Swandari *et al.*, 2019).

The RAPD method uses multiple markers having a dominant nature with a length of 10 base pairs, which can attach to random DNA strands. The RAPD method is delicate and has weaknesses in reproducibility. The slightest change in the reaction can alter the amount and intensity of amplification products. The RAPD method cannot distinguish between homozygotes and heterozygotes due to amplification by primers in unknown genome parts; hence, codomain loci often have DNA bands of different sizes (Ifah *et al.*, 2018). Codominant markers are according to differences in the size of DNA bands from one individual to another, compared with dominant ones having the presence or absence of bands of the same size. In simple terms, differences in the size of DNA bands visible on a gel have reference as alleles. Codominant markers can consist of many different alleles, while dominant pointers only have two alleles. According to Majid (2022), RAPD cannot distinguish among the individuals of homozygotes and heterozygotes because it is a dominant marker.

Generating the RAPD method results from PCR (Polymerase Chain Reaction). The RAPD method operates on almost all crop

plants; the analysis is cheaper and quick and provides fast results. Said method does not require large quantities of DNA because it does not require radioactive processes, blotting, and hybridization (Anggraheni and Mulyaningsih, 2018). Previous studies used the RAPD method in identifying genetic diversity in breadfruits (Ifah *et al.*, 2018), pigeon peas (Khoiriyah *et al.*, 2018), polymorphism of cayenne pepper and gendot chili (Purnomo and Ferniah, 2018), persimmon genetic identification (Wardani and Yuniastuti, 2019), and genetic diversity and kinship in mulberry plants (Majid, 2022). The promising study aimed to determine the genetic diversity of matoa displayed through DNA banding patterns analysis and detect differences in DNA bands of green, yellow, and red matoa based on the RAPD method.

MATERIALS AND METHODS

The presented research ran from June to November 2022 at the Plant Breeding Laboratory, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia. The materials used were the leaf samples of green matoa (*Emme Anokhong*), yellow matoa (*Emme Khabelaw*), and red matoa (*Emme Bhanggahe*), with each cultivar represented by a single plant. The green and red matoa came from the Karanganyar Regency, Central Java, while the yellow matoa was from Pati Regency, Central Java, Indonesia. The research consisted of various stages, i.e., DNA isolation, DNA purity and concentration test, DNA amplification, electrophoresis, visualization of electrophoresis results, and statistical analysis of all the recorded data.

DNA isolation

The DNA isolation of three matoa cultivars ensued by using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1990), modified by the addition of 2% PVP (polyvinylpyrrolidone) and removal of liquid nitrogen.

DNA quantification

DNA quantification uses a Gene Quant Spectrophotometer to determine the concentration and purity of the obtained DNA. Absorbance measurement was at wavelengths of 260 nm and 280 nm, while the DNA purity obtained had the comparison value of A260/A280.

DNA amplification

DNA amplification using PCR (Bio-Rad T100 Thermal Cycler) continued with the following steps: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 37 °C for 1 min, and elongation at 72 °C for 1 min and 30 s, followed by a final elongation at 72 °C for 7 min, and inactivation at 12 °C. The primers used were OPA 19 (CAAACGTCGG), OPB 15 (GGAGGGTGTT), OPD 08 (GTGTGCCCCA), OPD 11 (AGCGCCATTG), and OPD 13 (GGGGTGACGA). Each PCR tube contained a total volume of 12.5 µL to perform the PCR reaction. The PCR reaction used consisted of 6.25 µL Gotaq green 2× PCR Mix (Promega), 0.5 µL 100 µM primers (IDT-Integrated DNA Technologies), 3 µL template DNA, and 2.75 µL Nuclease Free Water.

Electrophoresis

DNA obtained from PCR results sustained electrophoresis using the horizontal electrophoresis set, with a voltage of 100 V for 50 min. Agarose gel electrophoresis results used a UV transilluminator documentation.

Band scoring and data analysis

Data analysis considered the presence and absence of DNA bands. DNA band profile translation into binary data employed the provisions of the value 0 for blank bands and 1 for bands that have DNA. Genetic similarity analysis ran the NTSYSpc program version 2.02 SimQual (Similarity for Qualitative Data) function with the genetic similarity matrix calculated based on the SM coefficient.

RESULTS AND DISCUSSION

DNA quantification

The quality and quantity of isolated DNA need processing before pursuing further DNA analyses. The DNA quality and quantity are the results of the purity values and the concentration of isolated DNA. The RAPD method is a technique for testing DNA polymorphism based on the amplification of random DNA fragments using a single primer whose nucleotide sequence has irregular determination with a size of 10 base pairs. The RAPD method is a marker tolerant of the level of DNA purity. The quality of the genomic DNA produced from the DNA isolation process can affect the storability of the DNA and the appearance of enzymes and inhibitors during the DNA amplification stage (Sundari, 2018; Juliantari *et al.*, 2021).

DNA purity with A260/A280 absorbance ranged from 1.4 to 2.0, with yellow matoa having the lowest and green matoa as the highest (Table 1). Past studies also authenticated that DNA is classified as good quality if the range of purity values is between 1.8–2.0 and has a concentration above 100 ng/μL (Sambrook and Russel, 2001; Rizko *et al.*, 2020). Rizko *et al.* (2020) findings further revealed that the comparison value (A260/A280), if less than 1.8 nm, indicates that the DNA has contaminants of phenol or protein compounds during the extraction process. DNA with poor purity attains purification by washing the DNA using ethanol and then diluting the DNA before processing in the PCR stages. The DNA concentration ranged between 800–4300 ng/μL, with the highest concentration (4300 ng/μL) in yellow matoa.

However, past studies recognized that two factors can affect the concentration of extracted DNA, i.e., the speed of extraction and the composition of the lysis buffer (Naharia and Nanlohy, 2020; Elshahawy *et al.*, 2022; Sudirga *et al.*, 2022). The extraction speed will affect the DNA concentration, as the supernatant collection as the cell lysis and precipitation step needs a quick application to avoid DNA precipitation.

DNA amplification

The five primers, viz., OPA 19, OPB 15, OPD 08, OPD 11, and OPD 13 used in this study, have been successfully applied to several plants, such as, the study of plant genetic diversity in breadfruit (Ifah *et al.*, 2018). In this study, amplification produced polymorphic bands, indicating that the RAPD method can help evaluate genetic diversity in matoa (*P. pinnata*) plants.

Molecular analysis with the RAPD method using five primers produced significant polymorphic DNA bands that can benefit matoa DNA analysis (Figure 1). The amplification results totaled 53 DNA bands with sizes of 150 to 2100 bp (Table 2). Previous research conducted by Khoiriyah *et al.* (2018) on pigeon peas using the RAPD method produced band sizes from 300 to 1300 bp. According to Purnomo and Ferniah (2018), most of the informative DNA bands on RAPD usually are within 300–3000 bp. The DNA band sizes' differences were due to length variations of DNA threads that the primers can further extend. However, RAPD primers ably amplified the DNA with a scope of 100 to 1300 bp, depending on the genomics and primers used (Wahyudi *et al.*, 2020).

Table 1. Results of DNA purity and concentration values.

No.	Cultivars code	Purity value (A260/A280)	Concentration value (ng/μL)
1	H	2,000	800
2	K	1,433	4300
3	M	1,583	1900

Description: 1: Green Matoa (*Emme Anokhong*), 2: Yellow Matoa (*Emme Khabelaw*), 3: Red Matoa (*Emme Bhanggahe*).

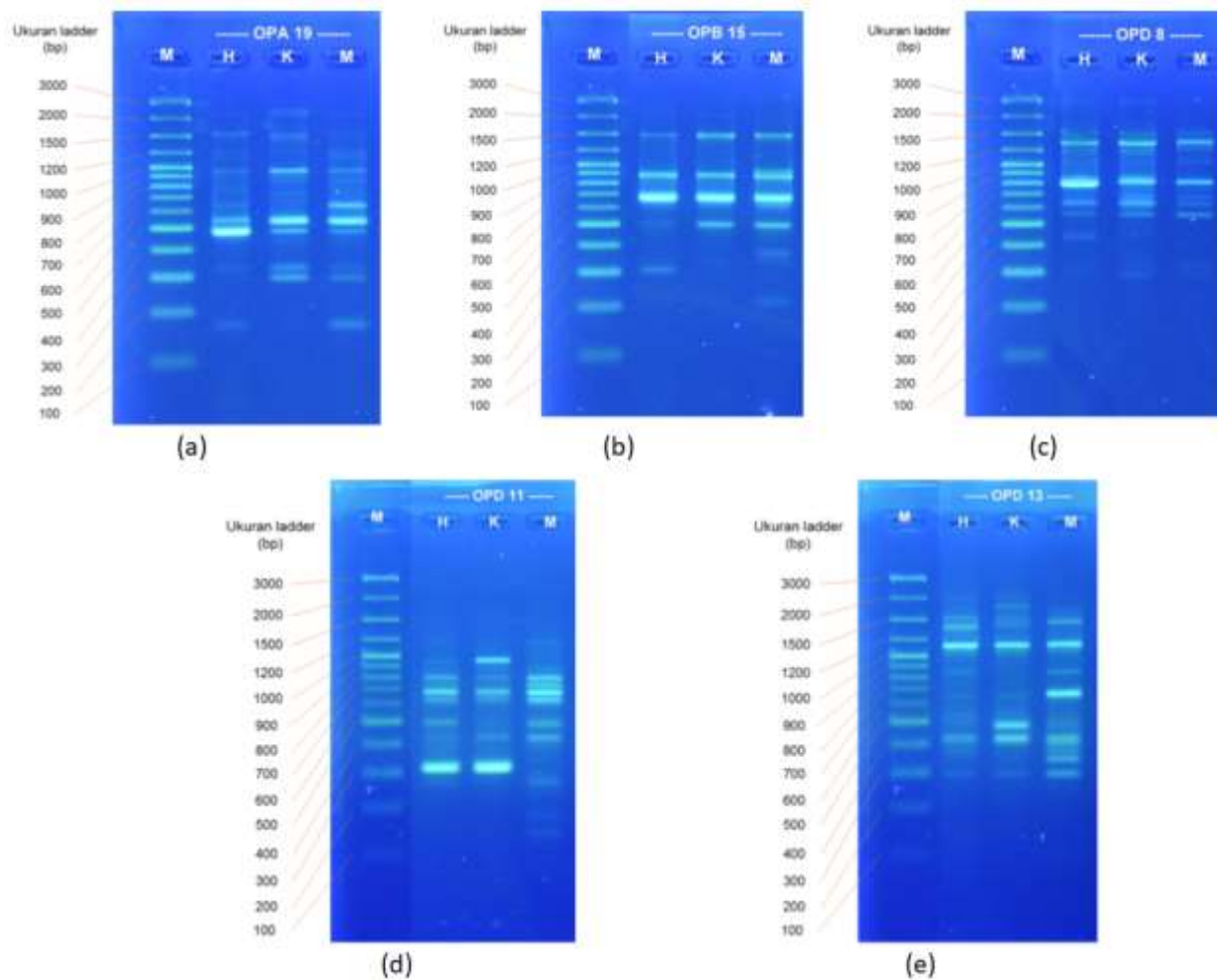


Figure 1. DNA amplification results with primers: a) OPA 19, b) OPB 15, c) OPD 08, d) OPD 11, and e) OPD 13. Description: H: Green Matoa, K: Yellow Matoa, M: Red Matoa.

Table 2. Percentage polymorphism of five RAPD primers in three matoa (*P. pinnata*) cultivars.

No.	Primer	Sequence (5'-3')	Size (bp)	Total Bands Amplified	Polymorphic Bands	Monomorphic Bands	Polymorphic (%)	Monomorphic (%)
1	OPA 19	CAAACGTCGG	190-2100	11	5	6	45	55
2	OPB 15	GGAGGGTGTT	205-1500	7	3	4	43	57
3	OPD 08	GTGTGCCCA	300-1300	8	3	5	37.5	62.5
4	OPD 11	AGCGCCATTG	150-1200	12	7	5	58	42
5	OPD 13	GGGGTGACGA	300-1900	15	10	5	67	33
Total				53	28	25	52.8	47.2

Description: Polymorphic (%) = (Polymorphic bands / Total DNA bands) × 100, Monomorphic (%) = (Monomorphic bands / Total DNA bands) × 100.

The research data produced 28 polymorphic bands with an average percentage of 52.8%. The number of monomorphic bands was 25, with an average share of 47.2%. In the sample, the percent polymorphic bands are a key to success in finding genetic diversity. Arsyad *et al.* (2022) findings revealed that the success of primers in amplifying template DNA can be distinguishable by the presence and absence of homologous primer nucleotide sequences with template DNA nucleotide sequences. According to Ifah *et al.* (2018), DNA fragments generated from each RAPD primer were different and are suitable to determine the similarity characteristics of each cultivar, with the presence of increasingly alike snippets can enhance the level of similarity.

DNA amplification outcomes obtained with OPA 19 primers from three matoa cultivars resulted in differences in the number and pattern of the DNA band shown by yellow matoa at the 750 bp and 850 bp loci. DNA amplification with primer OPB 15 in three matoa cultivars resulted in variations in the number and pattern of DNA bands shown by green matoa at the 300 bp locus and red matoa DNA at the 205 bp and 350 bp loci. Khoiriyah *et al.* (2018) findings expressed the number of bands produced after DNA amplification by PCR was highly dependent on how the primer recognizes the complementary DNA sequence in the used DNA template.

Primer OPD 08 also amplified DNA bands of the matoa cultivars, resulting in differences in the number and pattern of DNA bands as shown by varying numbers and patterns of DNA bands in green matoa at the 450 bp locus, yellow matoa DNA at the 300 bp locus, and red matoa DNA at the 1200 bp locus. These results were in analogy with past findings of Ifah *et al.* (2018), who mentioned that the lowest polymorphic percentage occurred in primer OPD 08 at 68%. Based on the presented research, primer OPD 08 has the lowest ratio of polymorphic bands (37.5%). In contrast to persimmon genetic analysis, the study by Wardani and Yuniastuti (2019) showed that primer OPD 08 has a percentage of polymorphic bands above 50%. However, the five RAPD primers used can still be helpful in genetic diversity analysis, as they have

confirmed an average polymorphic banding pattern (52.8%).

Primer OPD 11 ably amplified 12 bands of DNA in the three cultivars of matoa, which resulted in differences in the number and pattern of DNA bands shown by variances in the number and pattern of bands by red matoa DNA at loci 150, 175, and 1200 bp. Ifah *et al.* (2018) results revealed that the more polymorphic DNA fragments formed indicate diverse genetic variations, and the genotypes of each cultivar can also be distinct from one another. The resulting monomorphic fragments were the loci of a specific size shared by all the genotypes in one cultivar. Past research reported that in a genetic diversity analysis, the number of polymorphic DNA bands determines the level of diversity of a population and also describes the plant genome state (Nienhuis *et al.*, 1994; Yuniastuti, 2007).

DNA amplification with primer OPD 13 in three matoa cultivars provided variations in the number and pattern of DNA bands shown by yellow matoa at loci 495, 1400, and 1700 bp and red matoa at loci 350 and 375 bp. According to Yuniastuti (2007), differences in the number and polymorphism of bands produced by each primer illustrate the complexity of the plant genome. Polymorphic bands are DNA strips that appear in a sample but do not appear in other specimens at the same observation time. Gusmiaty *et al.* (2021) findings revealed the need for polymorphic bands to analyze the genetic diversity in crop plants by showing the variety of banding patterns. Monomorphic bands appear on the amplified DNA stripes because the sample genome has the same base arrangement at the exact observation time.

Genetic similarity

Binary data analysis proceeded based on the number and pattern of DNA bands amplified from each primer to obtain a measure of similarity in the form of genetic resemblance. Among the three cultivars of matoa, attaining the genetic similarity matrix used the SM coefficient (Sokal and Sneath, 1963). The results showed the similarity coefficient values

ranged from 0.566 to 0.698 (Table 3). The highest similarity coefficient appeared for green and yellow matoa cultivars, with a value of 0.698, which indicates that green matoa (*Emme Anokhong*) and yellow matoa (*Emme Khabelaw*) tend to have the highest genotypic similarity. Green and yellow matoa have sweet, chewy, and loose flesh. Red matoa has a soft and slightly juicy pulp and does not release seeds. The skin color of green and yellow matoa is almost the same when ripe; thus, they have a higher degree of similarity (Furay, 2019). Matoa cultivars green and red have a similarity coefficient of 0.679. Matoa cultivars yellow and red have the lowest similarity coefficient value of 0.566, indicating these matoa cultivars have the maximum genetic differences. According to Faizah (2021), the greater the similarity coefficient value until it approaches unity implies that, genetically, the plant cultivars are more alike. Meanwhile, the smaller the similarity coefficient value, the closer to zero, the more genetically different the plant cultivars are.

The higher the genetic similarity among the cultivars, the lower the genetic diversity. Conversely, the lower the genetic similarity among the cultivars, the higher the genetic diversity. According to past studies, the genetic diversity that arises can be due to environmental factors, such as, soil type and condition, rainfall, and climate, and internal factors, such as, mutations, besides the influence of human intervention in the distribution process, which is also vital and affects the course of genetic variation (Ifah *et al.*, 2018). Sawitri *et al.* (2019) revealed that the higher the genetic variation, the greater the possibility of obtaining superior genotypes in various crop plants. The low genetic diversity of matoa can refer to influences of environmental factors, such as, variety, soil conditions, rainfall, and the same climate during research. The occurrence of mutations can also result in low matoa genetic diversity because the samples used came from seeds. Further research on matoa genetic diversity needs implementation to provide more information on matoa genetic diversity.

Table 3. Genetic similarity matrix of three matoa (*P. pinnata*) cultivars based on SM coefficient.

Matoa cultivars	H	K	M
H	1.000	-	-
K	0.698	1.000	-
M	0.679	0.566	1.000

Description: H: Green Matoa, K: Yellow Matoa, M: Red Matoa.

CONCLUSIONS

DNA amplification of three matoa (*Pometia pinnata*) cultivars, i.e., green matoa (*Emme Anokhong*), yellow matoa (*Emme Khabelaw*), and red matoa (*Emme Bhanggahe*), using five RAPD primers, showed 28 total polymorphic bands, with an average percentage of 52.8%. The monomorphic bands were 25, with an average share of 47.2%. Green and yellow matoa cultivars have a closer level of genetic similarity, with a coefficient of 0.698. The most distant genetic similarity was evident in matoa

cultivars yellow and red, with a coefficient of 0.566. The number and pattern of DNA bands produced by each primer emerged at different loci among matoa cultivars.

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