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| RESEARCH ARTICLE

Genetic Diversity of the Mango Plant Based on Rapd Marks: Random Amplification of Polymorphic DNA

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| ABSTRACT
Mangosteen is a tropical fruit commodity that is Indonesia's leading export. The location and characterization of mangosteen germplasm and protective relatives are key in the conservation and use of genetic and mangosteen resources. Genetic improvement programs rely heavily on existing genetic resources. This research aims to analyze the genetic diversity of mangosteen plants based on RAPD markers. This research was carried out in Bulukumba Regency through genotypic observation and evaluation in the laboratory, as well as genetic observation through DNA band pattern analysis using the RAPD technique in the laboratory. The results of this research obtained information on phenotypic and genetic diversity, as well as obtaining superior candidate parents for mangosteen, obtaining information about the consistency of genetic variability between parent and progeny of Bulukumba mangosteen, recommending the most effective mangosteen characterization method in describing genetic variability and grouping mangosteen accessions and relatives involved.

| KEYWORDS

Mangosteen, Genetic Diversity, RADP.

| ARTICLE INFORMATION

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1. Introduction

Mangosteen (Garenia mangostanu L.), which is known as the Queen of Tropical Fruits (Popenoe 1974; Sanders, J. M., et al. 2019), is one of the tropical fruit commodities is Indonesia's leading export and ranks first among all fresh fruit exports. Indonesia's mangosteen exports in 2010 amounted to 84,538 tons; in 2011, it became 97,487 tons. This number increased in 2012; it was 107,409 tons, and in 2013, it was 113,096 tons (Directorate of Fruit Plant Cultivation). This increase in the economic value of mangosteen needs to be supported by increased production achieved with a more advanced technical culture and the use of superior seeds resulting from targeted and strategic breeding programs.

The genetic improvement program for mangosteen plants is very dependent on existing sources of genetic diversity. Considering that Indonesia is one of the distribution centers for mangosteen and Garcinia spp in Southeast Asia, it is predicted that there will be genetic diversity in this region. Development prospects can be known through botanical and agronomic studies. For this reason, the shrub was explored, identified, and characterized by mangosteen through morphological markers and RAPD. This information is important for obtaining new sources of genetic diversity for genetic improvement and increasing the mangosteen population.

Mangosteen has obligate apomixis; the seeds do not come from fertilization and are thought to have narrow genetic diversity, so it is estimated that mangosteen in nature is one clone, and its characteristics are the same as its parent (Cox 1996; Richards 1990;

Verherji 1992; and Salim, H. M. et al. 2010) The reality in the field shows that there is a diversity of mangosteen plants which is possibly caused by environmental factors or genetic factors due to natural mutations in line with history.

The mangosteen plant is thousands of years old (Ramage et al. 2004; Putro, P. W. N. 2008). In the Gesing River, Purwerjo Regency, Central Java, grafted seeds and productive plants that had brown stems were identified (Supriadi et al. 1994). Evaluation of mangosteen diversity in mangosteen production centers in Java and Lombok using isoenzyme analysis carried out by Supriyanto et al. shows that there are at least three mangosteen clones. Based on the morphology, mangosteen from West Sumatra and South Sumatra consists of seven clones (Mansyah et al. 1994; Mansyah, E et al. 2008b). West Sumatran mangosteen, based on analysis of the glucose phosphuietsomerase (GNP) isoenzyme with 14 samples tested, showed the same band pattern with varying phenotypes, in other words, narrow genetic diversity, but wide phenotypic diversity (Mansyah et al. 1999; Dewanata, P.A. & Mushlih, M., 2021). Several DNA and RNA analyses also show variations among mangosteen populations (Ramage et al. 2004). The DNA content of mangosteen and its close relatives (local Thai names, chamuang, mahput, pawa, and somkhang) by flow cytometry showed differences. Furthermore, gemon DNA sequencing and specific primers showed that mangosteen is more closely related to pawa, followed by somkhang and mahput (Te-Chato and Lim 2000; Salva-serra, F., L. 2018). It is not yet known whether diversity in mangosteen plants occurs at the level of parents with their progenies, between and within populations, or in a wider area. Genetic analysis using advanced techniques for a wider range of species allows the identification of male parents for hybridization with mangosteen as the female parent (Osman and Milan, 2006; Susilo, 2023)

The use of molecular markers makes an important contribution to plant breeders in dealing with apomixis (Ramage et al., 2004; Acuna et al., 2005; Darrıgues et al., 2008; Tze et al., 2023). The genetic diversity of apomixis mangosteen can be revealed with molecular markers such as RAPD (Random Amplified Polymorphic DNA), E-RAPD (Enhanced-Random Amplified Polymorphic DNA), and AFLP (Amplified Fragment Length Polymorphism). In addition, a comparison of genetic and morphological data for each accession makes it possible to answer the important question of whether the reported superiority of a particular mangosteen accession is due to genetics or the environment.

2. Research Methods

Sample mangosteen plants were taken from the Mangosteen Garden location in Tanete District, Bulukumba Regency, at an altitude of 300 m above sea level. Molecular marker analysis will be carried out at the Bogor Genetic Resources and Biotechnology Laboratory, Balitbang, Ministry of Agriculture. The research was carried out through observation and genotypic evaluation in the laboratory. Genetic observation through DNA band pattern analysis using the RAPD technique in the laboratory.

2.1 Implementation of Research and Observations Genotypic Observations

Genotypic observations were carried out using DNA band pattern analysis based on the RAPD technique. Carrying out RAPD analysis includes two main activities, namely a) DNA isolation (template preparation) and b) RAPD analysis. DNA isolation was carried out by extracting DNA from young mangosteen leaves, which came from two parents and their derivatives from 30 F1 plants resulting from crosses. Isolation was carried out at the PKBT Molecular Laboratory, IPB Baranangsiang Bogor Campus, by amplifying the DNA template via a PCR machine. Electrophoresis, visualization, and documentation were carried out at the Molecular Biology Laboratory, PAU IPB Biotechnology Research Center, Darmaga Campus, Bogor.

2.2 Isolation and purification of mangosteen plant DNA

DNA isolation was carried out following the CTAB method (Doyle and Doyle, 1987; Deden et al., 2016; Muhammad et al., 2024). The material analyzed was 0.5 g of young leaves (base part) from the cross and cut into small pieces. The leaf pieces are put into a mortal, then liquid nitrogen PVPP is added, then finely crushed. The scoured results into a tube containing 600 µL of CTAB extract buffer solution (100 mH Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM ethylene diamine extraacetic acid (EDTA), pH 8.0, 2% (m/v) cetyltrimethylammonium bromide (CTBA), and 0.2% β-mercapto-ethanol, the mixture was shaken and heated in a water bath for 30 minutes at 650 C (every 10 minutes the mixture was turned over). The mixture was allowed to reach room temperature, and then 600 µL of chloroform-isoamyl alcohol solution was added (24:1) and shaken. Centrifuge the mixture at room temperature at 12000 rpm for 15 minutes so that two liquid phases are formed. The upper liquid phase (supernatant) is poured into a new tube, and then the same volume of cold isopropanol is added and stored in the freezer overnight. The mixture was thawed at room temperature and centrifuged at 12,000 rpm for 15 minutes. The liquid was carefully discarded, and 100 µL of cold 70% ethanol was added to the pellet, then centrifuged at 12,000 rpm for 5 minutes. The liquid was discarded, and the pellet was dried by inverting the tube. The dried pellets were added to 100 µL of ion-free water and shaken until dissolved.

DNA purification using the method of Sambrook et al. (1989). The DNA solution was added with 1 µL of RNAase and left at room temperature for 2 hours. Next, add 100 µL of cold isoamyl alcohol phenol chloroform and centrifuge at 12,000 rpm for 15 minutes. The supernatant was pipetted into a new tube, and an equal volume of isoamylalcohol chloroform was added, then centrifuged at 12,000 rpm for 15 minutes. The supernatant was pipetted into a new tube, and an equal volume of isoamylalcohol chloroform was

added. Then, it was centrifuged at 12,000 rpm for 15 minutes. The supernatant was pipetted into a new tube, and 1/10 volume of 3 M PH 5.2 sodium acetate and 2.5 volume of cold isopropanol were added. The solution was shaken until homogeneous and stored in the freezer overnight. The solution was centrifuged at 12,000 rpm for 15 minutes. The pellets obtained were added with 100 µL of 70% ethanol and centrifuged at 12,000 rpm for 5 minutes. The liquid was discarded, and the pellet was dried by inverting the tube. The dried pellets were added with 100 µL of ion-free water.

2.3 Determination of DNA quality

DNA quality is carried out after the DNA pellet has dissolved homogeneously. Estimation of DNA quantity was carried out through electrophoresis and compared with lambda DNA standards. A total of 5 µl of each DNA solution obtained was mixed with 1 µl of loading dye (10:2) and put into the well of a 1% agarose gel and run in an electrophoresis bath for 30 minutes with a voltage of 100 volts. The electrophoresed gel was soaked in 1% ethium bromuda solution for 15-20 minutes, rinsed with guides, and then the isolated DNA bands were visualized on a UV transilluminator and photographed with a digital camera. The DNA concentration is determined by comparing the thickness of the sample DNA with lambda DNA. The template DNA is then diluted to a concentration of 25 mg and ready to be used for the amplification reaction.

2.4 Data analysis

Data from field observations and RAPD analysis were analyzed using NTSYS-pc software version 2.02 and MINITAB Release 14. Before morphological data and RAPD data were analyzed, the data was first scored at zero (0) if not present and one (1) if they have the same morphological characters, while the RAPD DNA band profile is scored as zero (0) if there is no band, and one (1) if there is a band with the same genetic migration.

2.5 Similarity Analysis

The genetic similarity coefficient between mangosteen samples based on morphological markers and RAPD and the combined data of both were processed using the SIMQUAL (Similarity for Qualitative Data) procedure in the NTSYS-pc version 2.02 program and calculated based on the Nei and Li (1979) formula or the Dice (S) coefficient, namely S = 2ab/(na+nb); nab is the number of DNA bands in individuals a and b.

2.6 Clustering Analysis

Cluster analysis (clustering) of all data, both morphological data, RAPD, and combined data, were each analyzed using Sequential, Agglomerative, Hierarclucal, and Nested (SAHN)-UPGMA (Unweighted pair group method, arithmetic average) in the NTSYS-pe program version 2.02.

2.7 Principal Component Analysis

Three principal component analyses were carried out by extracting 3 eigenvectors from the 3 main eigenvalues that provide the highest level of diversity through the Ordination analysis procedure in the NTSYS-pc version 2.02 program.

3. Result and Discussion

3.1 Similarity Analysis Results

Genetic relationships between individual mangosteens can be found using a genetic similarity matrix based on the presence or absence of DNA bands amplified using 6 random primers. Based on cluster analysis of 6 random primers, the three progenies united at a genetic similarity of 89%, or 11%, in the diversity between offspring and parent accessions of Bulukumba mangosteen. Clusters between Bulukumba mangosteen progenies. Clusters between progeny are united at a similarity level of 93%, or there is diversity between children of 7%. The value of copenetic selection between Bulukumba mangosteen extracts was 0.44 or with goodness of fit according to the similarity matrix table. Picture :Primary:

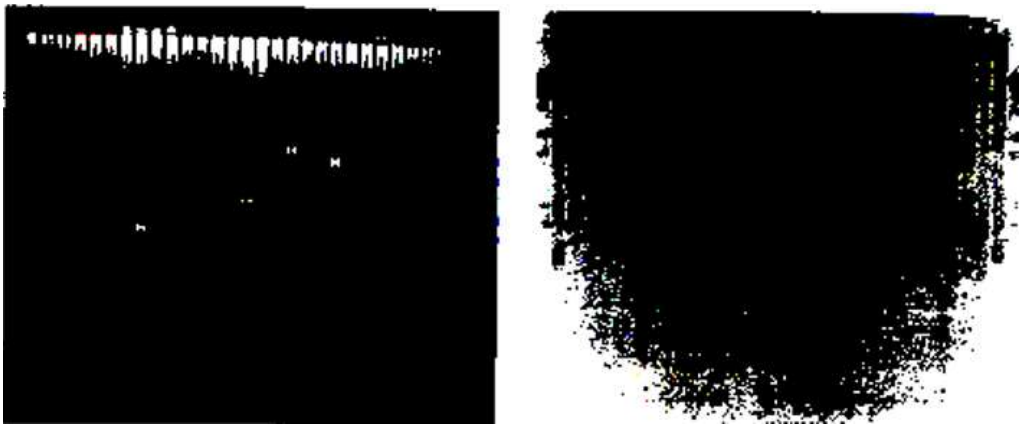

Figure 1. DNA band propyl 30 exec RAPD marker with exec primer OPE 03 M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M178, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, M2930

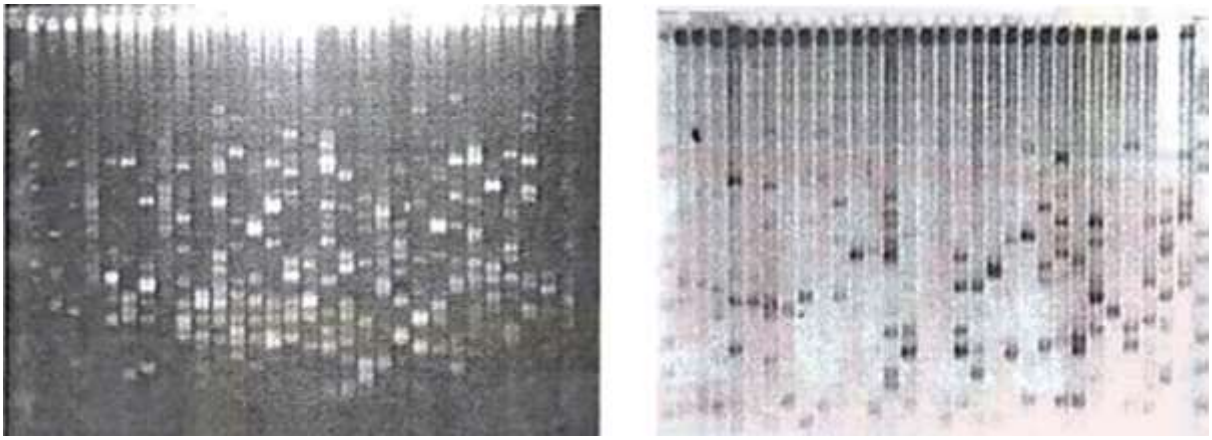

Figure 2. DNA band propyl 30 exec RAPD marker with excretion OPD 02 primer M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M178, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, M2930

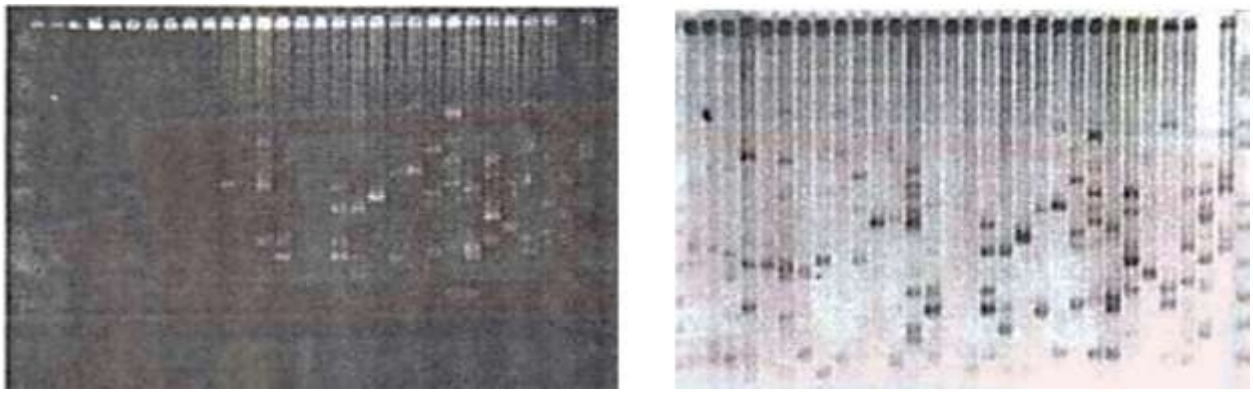

Figure 3. DNA band propyl 30 exec RAPD marker with exec OPM 03 primer M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M178, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, sM2930

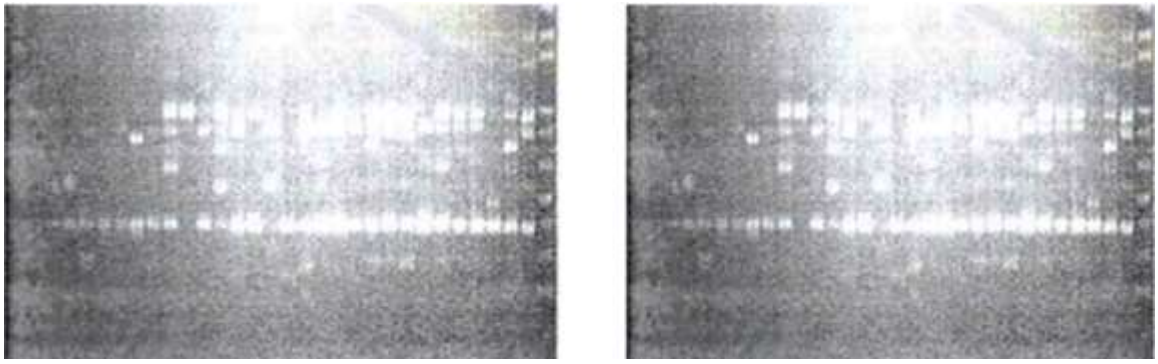

Figure 4. DNA band propyl 30 exec RAPD marker with exec OPR 10 primer M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M178, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, M2930

Figure 5. DNA band propyl 30 exec RAPD marker with exec OPR 15 primer M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M178, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, M2930

Figure 6. DNA band propyl 30 exec RAPD marker with exec OPR 01 primer M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M178, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, M2930

The picture above is the total DNA of several mangosteen plants and their close relatives. DNA application using 6 random primers on 30 excretions of mangosteen and its close relatives produced the number of DNA bands for each primer varying between 6-13 bands with an average of 10 sample DNA bands. The primer produced the fewest bands (6 bands), while the most bands were produced by the SBH primer. 13 (13 bands), the size of the amplified bands ranges from 200. 2000 bp. The number of bands produced by each primer depends on the distribution of sites that are homologous to the primer sequence in the genome. The RAPD band is formed from the extension of the primer attached to the template DNA, which occurs repeatedly. Each primer can usually attach simultaneously to several homologous sites spread across the template DNA, and the resulting primer extension varies greatly in size. Therefore, the electrophoresis results observed in agarose showed several bands of different sizes. The

number of DNA bands detected in each primer and whether there are variations in certain genotypes (Chauhan S et al., 2018; Te-chato, S & Lim, M 2000).

All primers produced a high level of polymorphism, namely 80 bands (100%). The polymorphism produced by the RAPD technique comes from variations in the DNA sequence at the primer attachment site and from differences in length between the primer attachment site, which experiences misplacement of the target homolog and results in band loss (Wijaya I.M.A.S., I.W. Tika, I G. P. Mangku 2004; Ma'arif B; Susilo, 2023).

3.2 Genetic similarity matrix of thirty mangosteen plant samples based on 6 RAPD primers.

The results of the grouping analysis derived from the DNA similarity matrix of the mangosteen plant did not provide a grouping that followed the area of origin; however, the two parents of the mangosteen (G. hombroniana and G. maccensis) did not group separately from the mangosteen but were spread among the mangosteen accessions. This phenomenon supports the opinion that the mangosteen plant does not originate from genetic hybridization of its two parents but is the result of repeated hybridization of its parents, but the result of repeated hybridization of both parents in various places. Ninety mangosteen accessions and their close relatives were divided into three groups based on the main group at a similarity coefficient level of 26%. Cluster analysis is an effective genetic method for analyzing genetic similarity using RAPD data.

3.3 Genetic Variability of Bulukumba Mangosteen

In the geographical area of Indonesia, mangosteen plants are genetically diverse, both with isoenzyme markers and AFLP, as well as RAPD. Based on the isoemzyme and AFLP peramides with 89% and 77% similarity, which were closely related to G. malaccensis, it is suspected that the clone still carries G. malaccensis characters as the parent of the mangosteen accession. Furthermore, Indonesian mangosteen with the RAPD marker at 44.4% similarity produced 31 clones. Clones formed based on genetic variability tend to follow the location where the mangosteen grows. Similarity analysis of genetic variability using isoenzyme markers and AFLP was carried out to obtain a more comprehensive picture of the genetic variability of the mangosteen plant and its close relatives. These two markers can be used to group mangosteen plants and distinguish close relatives.

Analysis of genetic variability using isoenzyme markers, AFLP, and their combinations gave diversity in mangosteen accessions ranging from 40-42%, while mangosteen and its close relatives ranged from 62-79%. In line with the two markers above, analysis of the variability of Indonesian mangosteen and its close relatives using the RAPD marker on 30 accessihs can reveal genetic diversity in the mangosteen group and its close relatives in the range of 8-75%. This wide range of genetic variability could be due to the greater number of accessions used, both mangosteen accessions and their close relatives, and originating from a wider distribution area. However, this range is still within the scope of the previous two markers. Furthermore, using the E-RAPD marker, the diversity within the population was 40%, while the diversity between populations was 53%.

Based on the isoenzyme marker, AFLP, and its combination on 30 accessions of mangosteen and its close relatives, along with RAPD on 30 accessions of Bulukumba mangosteen, it shows that the accessions of close relatives of mangosteen (G. hombromuiana and G. malaccensis), which are thought to be parents of mangosteen, are within the range of variability of source mangosteen accessions from repeated crosses of the allotettrapolid G. homroniana and G. mulaccensis thousands of years ago. This phenomenon is shown by the morphological characteristics of the mangosteen, which is intermediate to its two parents. Overall, the mangosteen accession G. hombroniana has morphological characteristics that are more similar to G. mangostana compared to G. celebica, which is only similar in flower and fruit type to G. mangostana (Jones 1980). Morphological characters are controlled by many genes so they are stronger in describing kinship relationships. Based on AFLP and RAPD markers, G. hombroniana is more closely related to G. mangostana than G. celebica (Qonytah. 2004; Vikas et al., 2021).

3.4 Cluster Analysis

The results of grouping based on RAPD markers show that the 4 mangosteen populations from Bulukumba have a fairly close genetic relationship, as shown by grouping the mangosteen populations at a similarity level of 80-97%. This technique can group mangosteen accessions based on their region of origin. Based on cluster analysis, it can be seen that the mangosteen population in Bulukumba is not separated into each population group. The presence of RAPD bands that are only present in these two populations indicates a close genetic relationship between the populations, so it is suspected that the seeds used originate from the same place.

4. Conclusion

The genetic diversity of mangosteen based on similarity analysis ranges from 0.19 – 0.44%, while based on RAPD, mangosteen selection at 80% similarity can be made into two selection steps, namely M28 and M29 as step one and selection as step two.

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