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Genetic Diversity of Papaya Using Molecular Markers Random Amplified Polymorphic DNA

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ABSTRACT: Papaya is a type of fruit that is widely consumed and cultivated by farmers. However, genetic analysis has not been carried out on various types of papaya available on the market. This aims to determine the genetic diversity of papaya plants that can become genetic resources to fulfill food needs and genetic resources for breeders. Genetic analysis was conducted by Random Amplified Polymorphic DNA (RAPD) method using 11 papaya varieties consisting of Calina, Bangkok, Red Pomegranate, Sunrise, Orange Lady, Red Lady, Taiwan, Arum, Miba, Golden, and Local and using 15 RAPD primers consisting of OPA-1, OPA-2, OPA-8, OPA-16, OPC-4, OPC-11, OPC-13, OPC-20, OPD-20, OPE-2, OPE-6, OPE-11, OPE-14, OPM-6, and OPY-15. PCR-RAPD results were translated into binary data, and then cluster analysis was conducted using the Unweighted Pair-Group Method Arithmetic (UPGMA) method and the Numerical Taxonomy and Multivariate System (NTSYS) program. The PCR-RAPD results of 11 papaya varieties successfully amplified 8 out of 15 primers, forming 112 DNA bands with 85 polymorphic bands. Genetic diversity analysis showed the results at 90% similarity coefficient formed 9 groups. Group 1 consists of Bangkok and Sunrise varieties. Group 2 consists of Red Pomegranate and Arum varieties. Groups 3 to 9 consisted of Calina, Miba, Local, Golden, Orange Lady, Red Lady, and Taiwan varieties.

Keywords: analysis, primers, varieties

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INTRODUCTION

Papaya (*Carica papaya* L.) originates from Mexico and is a tropical plant that is widely cultivated throughout the world (Chávez-Pesqueira & Núñez-Farfán, 2017). In Indonesia, papaya can be cultivated in lowland and highland areas. Suitable environmental conditions for papaya growth include 1500–2000 mm/year rainfall and a temperature of 15-43ºC. Papaya has a single tree trunk and single leaves with a five-finned shape that grow in threads on the upper tree trunk with hollow leaf stalks (Harsono, 2021).

Plant diversity can be determined by observing various markers, such as morphological, biochemical, and molecular markers. However, morphological and biochemical markers have a lower level of accuracy because environmental factors and plant growth phases can influence them. In contrast, these factors do not influence molecular markers (Hasnah, 2014). Molecular markers can be made using the Randomly Amplified Polymorphic DNA (RAPD) method. RAPD is a PCR-based DNA synthesis method using short primers with 8–10 base sequences to detect polymorphic differences in DNA between individuals (Zulfahmi, 2013). Using RAPD molecular markers can produce DNA band polymorphism as a study of genetic diversity,

kinship relationships, and genetic maps of various types of plants (Kumar et al., 2019).

The use of RAPD molecular markers has several advantages, such as a shorter processing time than the Restriction Fragment Length Polymorphism (RFLP) method; apart from that, it requires less sophisticated equipment and relatively cheaper costs, and the quantity of DNA required is small, and primers are required. What is needed has been widely commercialized (Zulfahmi, 2013).

The RAPD method can be used to determine the relationship between papaya plants. The RAPD method determined the genetic relationship between cultivated papaya plants. The genetic diversity of papaya plants has great potential based on RAPD markers, which can detect polymorphism in various papaya varieties (Kumar et al., 2019).

It is necessary to analyze the genetic diversity of papaya varieties available on the market because they can be used to meet food needs and genetic resources for breeders. Genetic diversity analysis was carried out using RAPD molecular markers.

MATERIALS AND METHODS

This research was conducted at the Plant Protection Laboratory, Faculty of Agriculture, Bengkulu University, from January to March 2023. The papaya varieties include Calina, Bangkok, Merah Delima, Sunrise, Orange Lady, Red Lady, Taiwan, Arum, Miba, Golden, and Local. RAPD methods consist of DNA extraction, DNA concentration and purity calculation, DNA amplification, and DNA visualization.

The extraction of total plant DNA using the CTAB (Cetyl Trimethyl Ammonium Bromide) method refers to the method used by Doyle & Doyle (1987) which has been modified. Plant samples in leaf tissue were weighed at 0.1 grams and then crushed using a mortar and pistil by adding liquid nitrogen. After being powdered, transfer it to 2 mL Eppendorf tube, add 500 µl CTAB buffer, and then vortex to make it homogeneous. Incubate at 65°C for 60 minutes (turning back and forth every 10 minutes), then leave for ± 2 minutes at room temperature. Add 500 µl of chloroform and isoamyl alcohol (24:1), centrifuge at 12000 rpm for 15 minutes, then transfer 300 µl of the supernatant to a new 2 mL Eppendorf tube. Add sodium acetate as much as 1/10 of the supernatant volume, then add isopropanol as much as 2/3 of the supernatant volume and incubate for 24 hours at -20°C. After that, it was incubated at room temperature for 1 minute, then centrifuged at 12000 rpm for 10 minutes, then carefully discarded the supernatant (you will see DNA pellets attached to the edge of the tube), then washed the DNA pellets with 500 µl of 70% ethanol, then centrifuged at 8000 rpm for 5 minutes, then discarded the ethanol and air-dry the tube containing the DNA pellets, then added 50 µl of nuclease-free water.

Calculation of DNA concentration and purity using the formula:

The sample used was extracted DNA diluted 250×. 4 µL of extracted DNA was added with 996 µL of dH_2O and homogenized. Measurements were carried out at wavelengths of 260 nm and 280 nm. The measurement result value is calculated using the following formula:

DNA concentration µg/mL = A260 × 50 × dilution of DNA in a cuvette

DNA purity = A260: A280

DNA amplification refers to the article by Kumar et al. (2019) modified in the reagent composition section. The composition of the reagents used in the amplification reaction is 0.5 µl DNA template, 2 µl RAPD primer, 3.75 µl nuclease-free water, and 6.25 µl Green Taq DNA Polymerase. The PCR program used is predenaturation at 94 °C for 5 minutes, 35 cycles (denaturation at 94 °C for 45 seconds, annealing at a different temperature for each primer according to Table 1 for 25 seconds, extension at 72 °C for 1 minute), and final extension at 72°C for 5 minutes.

DNA visualization using 1% agarose gel at 50 volts for 50 minutes After that, the agarose gel was soaked in diamond nucleic acid dye from promega, USA (1:10,000 v/v) for 15 minutes and visualized with an ultra-slim LED illuminator.

Table 1. RAPD primer sequence and annealing temperature

No	Primary name	Sequence	Ta (°C)
1	OPA 1	CAAT CGCC GT	38
2	OPA 2	TGCC GAGC TG	44
3	OPA 8	GTGA CGTA GG	38
4	OPA 16	AGCC AGCG AA	44
5	OPC 4	CCGC ATCT AC	38
6	OPC 11	AAAG CTGC GG	39
7	OPC 13	AAGC CTCG TC	38
8	OPC 20	ACTT CGCC AC	38
9	OPD 20	ACCC GGTC AC	36
10	OPE 2	GGTG CGGG AA	35
11	OPE 6	AAGA CCCC TC	35
12	OPE 11	GAGT CTCA GG	35
13	OPE 14	TGCG GCTG AG	35
14	OPM 6	CTGG GCAA CT	35
15	OPY 15	AGTC GCCC TT	38

DNA visualization results were translated into binary data with a score of 1 and 0. The scoring results were processed using Microsoft Excel software. The scoring data is used to calculate genetic diversity values. Grouping matrix data (cluster analysis) and creating dendrograms were carried out using the Unweighted Pair-Group Method Arithmetic (UPGMA) method using the Numerical Taxonomy and Multivariate System (NTSYS) program.

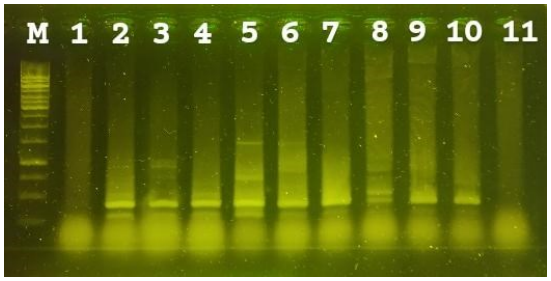
RESULTS AND DISCUSSION

Based on the extraction results, the papaya DNA concentration obtained ranged from 862.5 to 2,687.5 µg/mL with a purity of 1.6 to 2.4 (Table 2). DNA contains purine and pyrimidine bases, which can absorb UV light. The DNA band absorbs UV light at 260 nm, while the contaminant parts of DNA, such as proteins and phenols, absorb light at 280 nm. DNA is classified as pure if the purity index is >1.75 (Puspitaningrum et al., 2018). The papaya DNA extraction results with a purity of <1.75 were only Miba papaya variety DNA. According to (Murtiyaningsih, 2017) the DNA concentration required for RAPD is 10 ng. Therefore, the papaya DNA extraction method is optimal and can be used for genetic diversity analysis.

PCR results from the selection of 15 primers obtained 8 primers that successfully amplified papaya DNA sequences (Figure 1). The number of marker variations obtained ranged from 4 markers (OPC-4 and OPY-15) to 22 markers (OPD-20), with marker sizes ranging from 250 to 3000 base pairs (bp). The total number of markers obtained was 112, which formed 85 polymorphic bands. The dominant markers appeared in the primers OPA-2 with a 250-bp DNA band, OPD-20 with a 400-bp DNA band, and OPE-2 with a 1500-bp DNA band (Table 3).

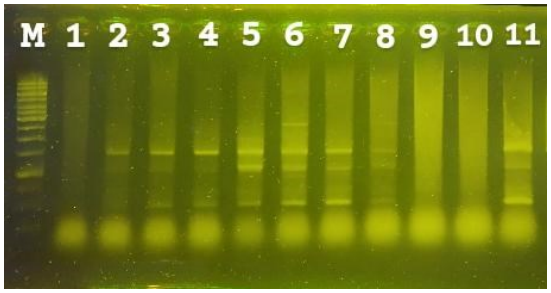
Table 2. Results of papaya plant DNA extraction

No	Papaya varieties	DNA concentration (µg/mL)	Ratio A260/A280
1	Calina	1.525	2,4
2	Bangkok	2.687,50	2,1
3	Merah Delima	1.312,50	1,9
4	Sunrise	862,5	1,9
5	Orange Lady	1.025	2
6	Red Lady	862,5	2
7	Taiwan	912,5	2
8	Arum	1.487,50	2,1
9	Miba	1.900	1,7
10	Golden	1.325	1,6
11	Lokal	1.712,50	2,2



Note: M: Marker 1 kb; 1:Calina; 2:Bangkok; 3: Pomegranate Red; 4: Sunrise; 5: Orange Lady; 6: Red Lady; 7: Taiwan; 8: Arum; 9: Miba; 10: Golden; 11: Local.

Figure 1. Papaya DNA visualization results using OPD 20 primer



Note: M: Marker 1 kb; 1:Calina; 2:Bangkok; 3: Pomegranate Red; 4: Sunrise; 5: Orange Lady; 6: Red Lady; 7: Taiwan; 8: Arum; 9: Miba; 10: Golden; 11: Local.

Figure 2. Papaya DNA visualization results using OPE 2 primers

The marker patterns that were successfully amplified showed polymorphism in 11 papaya varieties. According to Sitepu et al. (2019), the success of polymorphic DNA is caused by the presence of the DNA polymerase enzyme, while differences in the firmness of the DNA bands are influenced by the primer attachment site on the template DNA, purity, and concentration of the template DNA in the reaction.

Contaminants can cause DNA amplification failure during DNA extraction. To reduce contaminants, you can use polyvinylpolypyrrolidone (PVP) and β-mercaptoethanol, which are capable of degrading phenolic compounds and polysaccharides (Sari & Restanto, 2022). Apart from that, amplification failure can also be caused by the absence of a complementary sequence between the primer and the template DNA, so no amplification occurs (Herman et al., 2018).

Genetic diversity analysis using the RAPD method was carried out based on the results of DNA visualization using random primers (Zulfahmi, 2013). The results of the diversity of 11 papaya varieties based on genetics produced 21 characters and obtained a similarity coefficient value of 58–90%.

Table 3. Number of DNA bands from amplification of 11 papaya varieties using 8 primers

Varieties	Primary								Total
	OPA 2	OPA 8	OPA 16	OPC 4	OPC 20	OPD 20	OPE 2	OPY 15	
Calina	1	1	0	0	0	0	0	0	2
Bangkok	1	2	2	0	0	2	1	0	8
Merah Delima	2	1	0	0	1	3	2	0	9
Sunrise	2	3	1	0	1	2	2	0	11
Orange Lady	3	3	3	1	0	4	3	3	20
Red Lady	2	2	3	1	1	4	4	0	17
Taiwan	3	2	2	2	2	2	3	1	17
Arum	2	1	0	0	0	3	3	0	9
Miba	2	1	2	0	1	1	0	0	7
Golden	1	0	2	0	2	1	0	0	6
Lokal	1	1	1	0	0	0	3	0	6
Total	20	17	16	4	8	22	21	4	112

Sabara & Vakharia (2018) reported that RAPD of 13 papaya cultivars using 16 RAPD primers produced 58 polymorphic bands from 126 DNA bands with similarity coefficients ranging from 67-99%. According to Prihatini & Budiyanti (2019), RAPD of 5 papaya varieties using 14 primers produced 90 polymorphic bands from 119 DNA bands with similarity coefficients ranging from 70-100%. The difference in similarity coefficient values is caused by differences in the number of samples and primers used, so the resulting polymorphic bands are also different.

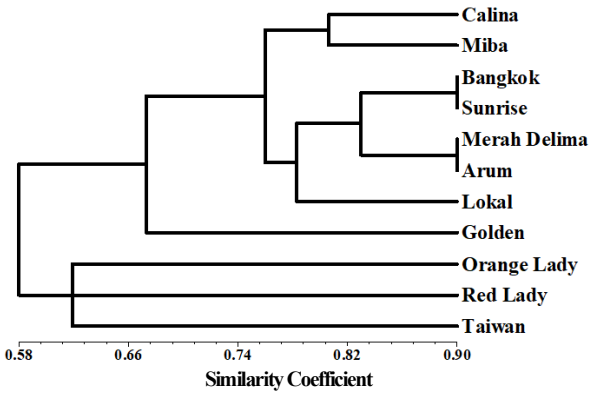


Figure 3. Dendogram of genetic and morphological analysis results of 11 papaya varieties

At a similarity coefficient of 58%, all papaya varieties are in the same group; however, at a similarity coefficient of 90%, the papaya varieties are divided into 9 groups, including Bangkok and Sunrise, Merah Delima and Arum, as well as Calina, Miba, Lokal, Golden, Orange Lady, Red Lady, and Taiwan (Figure 3).

With a similarity coefficient of 90%, the Merah Delima and Arum varieties are in the same group because the number of polymorphic bands formed is the same, namely 9 bands. Papaya varieties Calina, Miba, Local, Golden, Orange Lady, Red Lady, and Taiwan form each group at a similarity coefficient of 90%. Differences in polymorphic bands cause this difference in kinship.

The RAPD technique can be used to analyze an individual's genetic diversity; however, several things need to be considered in the process, such as the quality of the DNA and the number of primers used, as well as the accuracy of the process.

CONCLUSION

The genetic diversity of 11 papaya varieties using 8 RAPD primers produced the highest similarity coefficient value of 90%, which formed 9 groups, including group 1 consisting of the Bangkok and Sunrise varieties. Group 2 consists of the Merah Delima and Arum varieties. Groups 3 to 9 each consist of Calina, Miba, Local, Golden, Orange Lady, Red Lady, and Taiwan varieties.

SUGGESTION

Morphological observation variables can be added, such as observing leaf shape, stem shape, leaf color, and other morphological observations.

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