



## Identification and Classification of Menoreh Durians Based on RAPD Molecular Markers

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### Abstract

Menoreh durian is an accessions durian fruit originally from Kulonprogo district, Special Region of Yogyakarta, Indonesia. In the context of proposing Menoreh durian to be a new superior variety, it is necessary to have an identity and position in the molecular genetic group. The aim of the study was to obtain the identity and position of the of Menoreh Durian in the molecular genetic classification. The wet laboratory experiment was using PCR-based RAPD method. Eleven accessions of durian were identified and characterized then grouped based on molecular analysis which were pink Menoreh and 3 Yellow Menoreh accessions, Petruk, Sunan, Sukun, Sitokong, Montong, Kani and Aspar. The RAPD method was carried out in stages: DNA extraction, DNA quantity and quality test, amplification, electroforesis, visualization and analysis of results. The RAPD classification of 11 durian accessions, resulted in three groups; namely group I which consisted of Menoreh Kuning, Petruk, Aspar Sunan and Menoreh Jambon; Group II consisted of Montong, Sitokong, and Kani from Thailand; and group III consisted of only Sukun accession. Based on this grouping, Menoreh Kuning dan Menoreh Jambon which were separated from other groups at a genetic distance of more than 0.25 indicated that the accession of Menoreh Kuning and Menoreh Jambon only as variety.

**Keywords:** durian; molecular; RAPD.

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### Introduction

Menoreh durian is a leading commodity in the Kulonprogo Regency, the Special Region of Yogyakarta, because of the superiority of the delicious fruit taste, and the thick flesh of the fruit. There are two types of durian, namely Menoreh Kuning with buttery yellow flesh and Menoreh Jambon with the fruit flesh is pink in color. In order to release the Menoreh durian variety, identification and molecular genetic characterization are required. The aim of the study was to identify and classify Menoreh durians through molecular genetic tools. The release of superior Menoreh durian varieties will enrich the variety of nationally superior durian varieties.

The aim of the study was to obtain the identity and position of Menoreh Durian in the molecular genetic classification. This research was using PCR-based RAPD method. Eleven accessions of durian were identified and characterized then grouped based on molecular analysis which were pink Menoreh and 3 Yellow Menoreh accessions, Petruk, Sunan, Sukun, Sitokong, Montong, Kani and Aspar. The RAPD method was carried out in stages: DNA extraction, DNA quantity and quality test, amplification, electroforesis, visualization and analysis of results. DNA extraction using the CTAB method according to the protocol from (1) modified by (2).

## Material and Methods

Molecular identification and classification of durian Menoreh was carried out in the laboratory of the center for forest Plant Research and Breeding using the PCR-based RAPD method (3). The materials used were 11 accessions of durian; which consisted of 3 accessions of Menoreh (Menoreh Kuning 1, Menoreh Kuning 2, and Menoreh Kuning 3); Menoreh Jambon, Petruk, Sunan, Sukun, Montong, Sitokong, Kani and Aspar durians. The research stages were: (1) DNA extraction, (2) DNA Quantity and Quality test, (3) DNA amplification (PCR-RAPD process), (4) Electrophoresis, (5) Visualization of DNA amplification results and (6) Result Analysis. Twelve primers were used; they were selected from 18 primers of OPA1-OPA18. The extraction method followed the method for Salak DNA extraction (2, 3).

DNA amplification (PCR-RAPD) followed these stages: Stage I was pre amplification for 3 minutes at a temperature of 950C. Stage II was the amplification process for 40 cycles, started with denaturation at 940C for 1 minute, annealing at a temperature of 37<sup>0</sup>C for 45 second, extension at a temperature of 72<sup>0</sup>C for 1.5 minutes. Stage III (final extention), at a temperature of 72<sup>0</sup>C for 5 minutes.

## Results and Discussion

Molecular analysis of RAPD using 10 selected primers resulted in a clear DNA polymorphism bands so that it can be used in DNA analysis of Menoreh Durian. Results of DNA amplification with primer OPA-1 from 11 accessions shows three polymorphism band patterns (Fig.1). Menoreh (Rows 1-4), and other accessions, resulted in clear bands. Difference in the number and pattern of bands shown by the DNA of durian Sukun (Row.7) on genetic distance 380 bp and Aspar on 1000 bp. Amplification of DNA with OPA-02 shows similarity in patterns in Menoreh 1, 2 and 4 (Menoreh Jambon). Montong only showed 1 band, Sukun showed specific pattern. (4) studied the genetic diversity of coconut cultivars using RAPD produced bands measuring between 250-3080 bp. The use of short primers usually will amplify DNA sequences smaller than 4000 bp. (2) identified and classified 12 salak accessions in Java based on molecular RAPD using 5 primers; the results showed that the salak pondoh group from Sleman Special Region of Yogyakarta was different from the salak group from East Java and West Java.

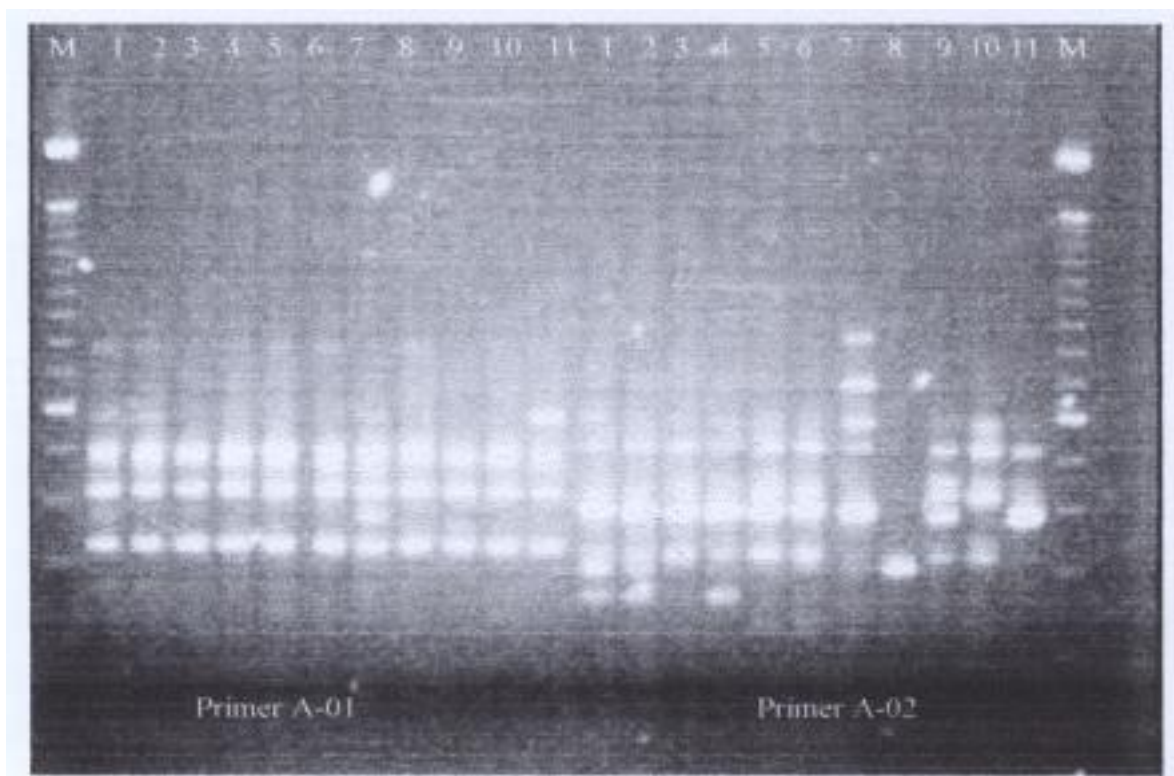


Figure 1. Results of DNA amplification by OPA-1 and OPA-2 primers.  
Description: No. 1-3 Menoreh Kuning, 4. Menoreh Jambon, 5. Petruk, 6. Sunan, 7. Sukun, 8. Montong, 9. Sitokong, 10. Kani, 11. Aspar

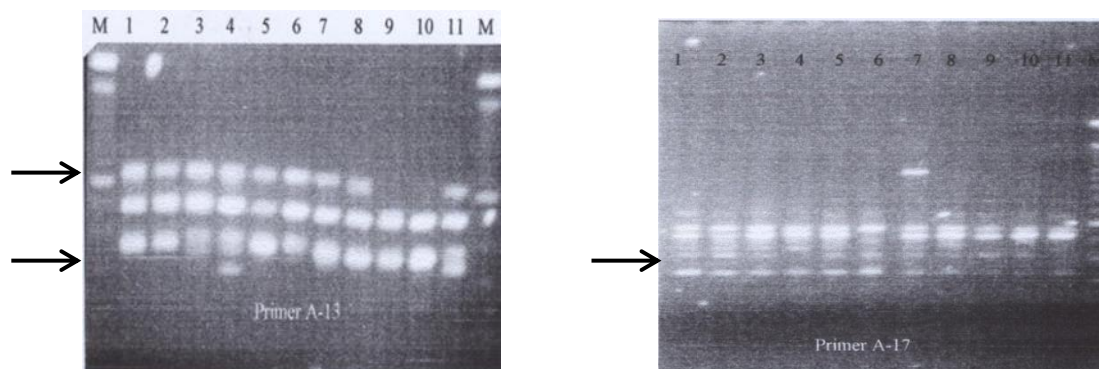


Figure 3 and Figure 4. Profiles of RAPD amplified by OPA-13 and OPA-17 primers.  
Description: No. 1-3 Menoreh Kuning,.4. Menoreh Jambon, 5. Petruk, 6. Sunan 7.Sukun, 8. Montong, 9. Sitokong, 10. Kani, 11. Aspar

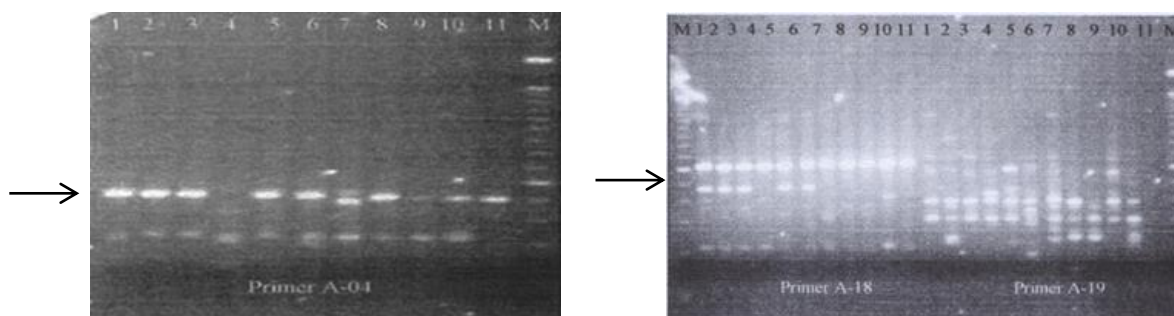


Figure 5 and Figure 6. RAPD profiles amplified by OPA-4; OPA-18 and OPA-19 primers  
Description: No. 1-3 Menoreh Kuning,.4. Menoreh Jambon, 5. Petruk, 6. Sunan 7. Sukun, 8. Montong, 9. Sitokong, 10. Kani, 11. Aspar

Amplification with primer OPA-02 shows the number and pattern of bands with more variety; there were 1-6 bands with 7 patterns. Menoreh durian 3 different with Menoreh 1 and Menoreh 2, with genetic distance of 300 and 380 bp. Amplification with primer OPA-13 resulted in very clear bands. There were 4 patterns: patterns of 2 bands (Sitokong and Kani); 2) patterns of 3 bands (Durian Menoreh Kuning1, 2, 3, Petruk, Sunan, Sukun, Montong and Aspar on number

5, 6, 7, 8 and 11); (3) patterns of 4 bands consisted of one accession; the Menoreh Jambon which was different with other accessions with genetic distance of 250 bp. Amplification DNA with primer OPA-17 shows differences in patterns of durian Sukun in genetic distance of 500 dan 1050 pb. Primer OPA-19 resulted in bands patterns of durian Sukun (No 7) which was different with other accessions.

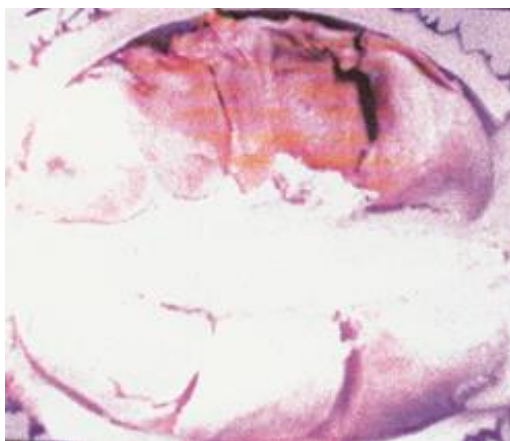


Figure 7. Durian Menoreh Jambon



Figure 8. Durian Menoreh Kuning

A research by (2) on salak cultivars with six primers (OPA-11, OPA-16, OPA-17, OPA-18, OPX-15 dan OPX-17) obtained 3-6 polymorphic bands sized between 250-3000 bp. The results of DNA amplification using primer OPA-4 showed that the DNA bands of Durian Jambon was different with other accessions with genetic distance of 490 bp.

Whereas the results of amplification with OPA-18 showed that durian Jambon different from the other accessions with genetic distance of 400 dan 480 bp. Based on the number and patterns of DNA bands from the 11 accessions, a genetic similarity matrix can be arranged using the Jaccard formula (5).

Table 1. Matrix of genetic similarity among 11 Durian accessions

OUT	1	2	3	4	5	6	7	8	9	10	11
1	0										
2	0.200	0									
3	0.467	0.429	0								
4	0.529	0.588	0.571	0							
5	0.500	0.467	0.273	0.600	0						
6	0.706	0.687	0.455	0.643	0.500	0					
7	0.632	0.684	0.687	0.722	0.625	0.824	0				
8	0.600	0.667	0.667	0.800	0.583	0.750	0.733	0			
9	0.600	0.667	0.667	0.714	0.583	0.750	0.733	0.444	0		
10	0.571	0.733	0.636	0.692	0.545	0.833	0.714	0.556	0.556	0	
11	0.571	0.643	0.500	0.692	0.545	0.600	0.615	0.556	0.556	0.667	0

Description: 1=Menoreh kuning, 2=Menoreh kuning 2, 3=Menoreh kuning 3, 4=Menoreh jambon, 5=Petruk, 6=Sunan, 7=Sukun, 8=Montong, 9= Sitokong, 10=Kani, 11=Aspar.

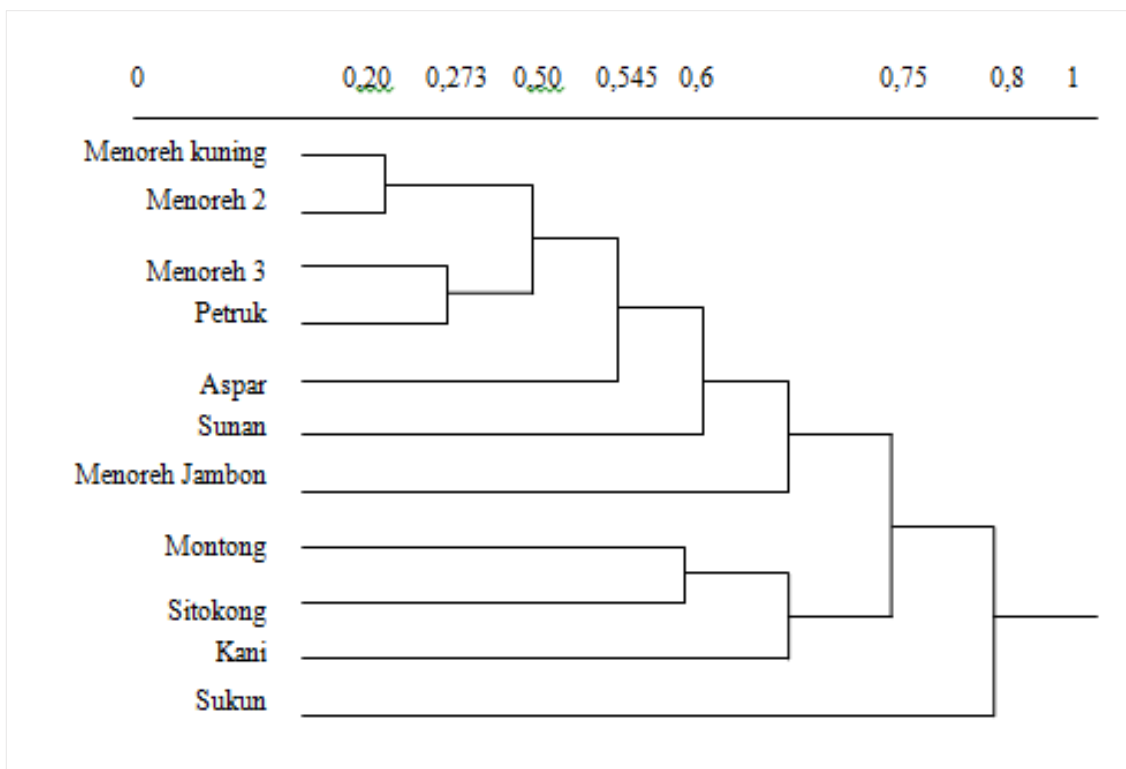


Figure 9. Dendrogram clusters of 11 Durians accessions

The smallest genetic distance in the durian Menoreh Kuning 1 and Menoreh Kuning 2 was 0.2. Both plants came from the same parent so it is natural to have a small genetic distance. Durian Menoreh Kuning and Menoreh Jambon are superior local durian from Kulonprogo. To determine differences in genetic characteristics between these varieties, an analysis is needed and in this case the Jaccard formula was used (5) The genetic distance between Menoreh Jambon dan Menoreh Kuning was 0.529-0.588. According to (6) the genetic distance above 20 % determines the position of an accession as a different variety from the other. Based on the results of the analysis, a dendrogram of 11 durian accessions was compiled using the UPGMA method (5).

Molecular clustering of 11 durian accessions using RAPD method showed that there were three groups, namely: (1) Menoreh, Petruk, Aspar, Sunan and Menoreh Jambon, (2) Montong, Sitokong and Kani (this durian came from Thailand) and (3) Sukun accession. This clustering placed Menoreh Kuning 1, 2, 3 durians in separate sub cluster at a genetic distance from other durians which were between 0.25-0.273 so that Menoreh Kuning durians met the requirements to be placed as a separate variety different from other varieties. While Menoreh Jambon durian which located in sub-cluster one at a genetic distance of 0.70, with the light pink flesh colour is different from other durians accessions. Therefore, Menoreh Jambon durian according to (7) guidelines can be proposed as a separate variety.

The decree of Minister of Agriculture of the Republik of Indonesia: No. 38/Permentan/OT.140/7/2011 (8), plant varieties hereinafter referred to as a group of plants of a type or species characterized combinations of candidate by plant, form, plant growth, leaves, flowers, fruit, seeds, and variable expressions of candidate varieties or varieties that can distinguish them from the same type or species by at least one characteristic that determine and when reproduced there is no change. Based on molecular genetic grouping using the Jaccard formula with genetic distance above 0.20 according to the above definition, Menoreh Jambon and Menoreh Kuning durians can be positioned as a separate variety, different from other varieties.

## Conclusion

The results of the identification and clustering of 11 durians accessions by RAPD method within which Menoreh Kuning and Menoreh Jambon durians were in them, placed the two durian accessions at different genetic distances from other durian accessions. Based on the results above, Menoreh Kuning and Menoreh Jambon durians can be proposed as different varieties from other varieties durians.

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## Conflict of Interest

All authors declare no conflicts of interest

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