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Genetic Diversity of Durian (Durio Zibethinus Murr.) in Nias Island, North Sumatera Use Random Amplified Polymorphic Dna (Rapd) Markers

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Abstract. Durian (Durio zibethinus Murr.) is one of tropic fruits that has varian taste depending on the places of their production. Nias Island, well known as durian production place in North Sumatera. The information about it's varian was less studied and now day we need that information to analyze variant of durian especially in Nias Island. The aim of this research was to analyze genetic diversity of durian in Nias Island with Random Amplified Polymorphic DNA (RAPD). Ten shoots of durian was collected from each 5 regencies, which were: Nias Selatan, Nias Barat, Nias Utara, Nias Kota, dan Nias Induk. Durian's DNA has been isolated by using Cetyl Trimethyl Ammonium Bromide (CTAB) method and amplified with six RAPD primers namely: OPA 01, OPA 03, OPA 07, OPA 10, RAPD 05, and OPN 06. The amplified band were scored and translated to biner data, then analyzed using Numerical Taxonomy and Multivariate System (NTSys) and clustered by Unweighted Pair Group Method With Arithme Average (UPGMA) method. Isolated DNA showed a clear and unsmeared band. The size of amplified band was around 221-2855bp with 100% of polymorphism. The dendogram was constructed by 50 accession of Nias's durian with 0.51-0.59 of similarity value in each population. All of Nias's durian accession has similiar value of 67% and B2 (Nias Barat) as a farthest genetic distance from all accession.

Keyword: Durio zibethinus, Isolation DNA, Nias Island, NTSys, RAPD

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1 Introduction

Durian (*Durio zibethinus* Murr.) is a tropical fruit that originated in Southeast Asia. Durian is a fruit native to Indonesia, has been found 20 types of Durio. Eighteen species are found in Kalimantan (13 types of which are endemic to Kalimantan), 7 species are found in Sumatra, and only 1 type each in Java, Bali, Sulawesi, and Maluku [1]. Nine types are the types that can be consumed, but only Durio zibethinus Murr. Which is widely developed because it has a distinctive aroma and taste. Durio zibethinus spread throughout Indonesia and developed into region-specific genotypes [2].

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North Sumatra province is the second largest durian producer center in Indonesia after East Java province [3]. Durian in North Sumatra is spread in different regions of natural conditions with their respective peculiarities, so it can be estimated that there is a high genetic diversity. Nias Island is one of the regions of North Sumatra that produces a lot of durian. Nias Island is an excellent area for durian cultivation. Nias Island itself is surrounded by the ocean which makes it isolated from other sumatran regions that may have different natural conditions, so it is very unlikely that there is a gene interaction with species outside Nias Island. Durian variations on Nias Island have only been explored through their genetic diversity using the SSR mark [4], so further research is needed to support information about durian variations on Nias Island. One way to find out the diversity of durian nutfah plasma is to look at its genetic diversity.

Durian DNA diversity can be analyzed using several markers, including using RAPD (Random Amplified Polymorphic DNA) markers, RFLG (Restriction Fragment Length Polymorphism), and AFLP (Amplified Fragment Length Polimorphisms), SSR or microsatellite [5]The use of RAPD markings is easy to do, fast, requires only a small amount of DNA as a mold, and without the need for initial information on the target genome [6] . Rapd marks are an appropriate method to identify large amounts of DNA polymorphisms in the genome quickly and efficiently. This type of polymorphism makes RAPD appropriate for the study of genetic diversity, kinship relationships, genetic maps, and DNA fingerprints. The RAPD method uses short oligonucleotides (usually 10 bp) as a primer that will bind to the complement sites [7].

The use of RAPD markings for the analysis of the genetic diversity of durian plants has been widely done, including in durian breadfruit [5], durian from Jepara [8] durian crossed [9] and several durian clones from West Java [10]. In some regions of North Sumatra, research has been conducted on durian diversity using SSR markings [11] and on Nias Island using SSR markings [3] but rapd markings have never been done.

2. Research Method

2.1. Time and Place of research

This research was conducted from March 2018 to September 2019 in Nias Island, The Laboratory of Genetics and Molecular Biology of the Faculty of Mathematics and Natural Sciences, and the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra, Medan..

2.2. Sample Preparation

Durian plant samples in the form of leaf shoots were collected randomly selected (purposive random sampling) from 5 districts in Nias Island (North Nias, South Nias, West Nias, Nias Induk, and Nias City) as many as 10 trees from each district . The sample is stored in a plastic valve that has been given silica gel, then taken to the laboratory to be stored in the freezer..

2.3 Durian DNA Extraction

The DNA of the genome was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method [12]which has been modified at an increase in extraction buffer concentration, speed and centrifugation time. Durian leaf shoots weighed as much as 20 mg in small pieces, then added 1 ml of CTAB extraction buffer 4% and 2 mg Polivinilpirolidon (PVP), crushed with mortar and pestle until it becomes a fine slurry. The fine slurry is then transferred into a 1.5 mL microtube and incubated at 65°C for 1 hour while being flipped over regularly every 10 minutes. Chloroform solution: isoamil alcohol (24:1) is added as much as 500 µl, then turned back until homogeneous. The sample was dysenteryphized at a speed of 13,000 rpm for 15 minutes. Supernatan is moved to a new 1.5 mL microtube. Dna purification with chloroform solution: isoamil alcohol (24:1), repeated 2-3 times until supernatan clear and there is no boundary between the two phases. Supernatan is then deposited with cold isopropanol as much as 1.5× the volume of supernatan, then incubated overnight at a temperature of -20 °C. Supernatan disentrifius returns at a speed of 13,000 rpm for 15 minutes. The precipitating DNA pellets are suspended with a 200 µl TE buffer of 1× (Appendix 2), added 20 µl sodium acetate and 400 µl of absolute ethanol. The suspension is incubated at -20°C for 30 minutes, dysenteryphed at a speed of 13,000 rpm for 1 minute. Supernatants are discarded and the formed DNA pellets are washed with 70% ethanol, flipped for 10 seconds and then divortex. DNA pellets are applied and suspended in a TE 1× buffer of 30-50 µL. DNA suspension is stored at -20°C until the sample is used.

2.4 DNA Quantity and Quality Test

Quantity tests are performed using a nanofotometer. Calibrate the nanofotometer first before measuring each DNA sample. A total of 2 µl of aquabidest is dripped on top of the nanofotometer cuvettes, place the lid on the cuvettes and press the blank button for calibration. A 1µl genome DNA sample is placed on an optical plate, the quantity of DNA is calculated based on the absorbance value at wavelengths of 260/280 nm.DNA quality testing is performed by electrophoresis method on agarosa gel concentration of 1%. To make a 1% agarosa gel, bring 1 gram of agarosa to a boil in 100 ml Tris Edta acetic acid (TAE) 1x. After the heat drops (± 37°C) the solution is molded into an electrophoresis mold and left to solidify. TAE Solution 1x is put in an electrophoresis tub until the agarosa gel is submerged. Marker 1 kb is inserted into the first well as a marker. Loading dye as much as 1 µl is picked up and placed on parafilm paper. Dna samples are pickpocketed as much as 5 µl, mixed with loading dye and then put in a agarosa gel well. Electrophoresis in running at 70 V for 45 minutes. The running agarosa gel is boiled in a 10 µl solution of Ethidium Bromide (EtBr) in 1 L of distilled acuades for 10 minutes, then soaked in aquades for 5 minutes. Agarosa gel is visualized under a UV transluminator and documented.

2.5 DNA amplification

DNA amplification is performed by PCR machine using 6 random primers (Table 1). Pcr reactions use 10 µl 2x Dream Taq Green PCR Master Mix (Thermo Scientific), 2 µl DNA template, 1 µl primer RAPD (Table 1), and 7 µl nuclease free water. The PCR program is set with a predenaturation temperature of 95 °C for 30 seconds, followed by 40 cycles consisting of 3 stages, namely denaturation of 95 °C for 1 minute, annealing (primary optimum temperature) of 34 °C for 30 seconds, and extension at 72 °C for 1 minute, followed by the final extension at 72 °C for 5 minutes, cooling after the cycle is completed at 4 °C.

Table 1 Primary sequence of RAPD and base array

NO.	Primer	Nucleotide Base Arrangement (5'-3'))	Refenence
1.	OPA-01	CAGGCCCTTC	[5]
2.	OPA-03	AGTCAGCCAC	[9]
3.	OPA-07	GAAACGGGTG	[5]
4.	OPA-13	CAGCACCCAC	[10]
5.	OPN-06	GAGACGCACA	[10]
6.	RAPD-05	AACGCGCAAC	[5]

$$\boldsymbol{Percent\ Polyformis} = \frac{\boldsymbol{Number\ of\ polyformist\ tapes}}{\boldsymbol{Total\ Ribbon}} \quad \textbf{(1)}$$

2.6 Data Analysis

2.6.1 Polymorphic Tape Scoring

Scoring the ribbon based on the presence or absence of the ribbon is done manually, with the provision of a value of 0 (zero) for no ribbon and a value of 1 (one) for the presence of a ribbon at the same position of each individual being compared. The data that has been obtained is processed using the Microsoft Excel 97-2003 application.

2.6.2 Genetic Kinship Analysis

Kinship estimates based on the number of similarities of the selected bands will be analyzed with the Numerical Taxonomy and Multivariate Analysis System (NTSys) program version 2.02. Polymorphic ribbon scoring that has been obtained in microsoft excel 97-2003 is changed to NTSys format in NTEdit. Data is clustered using the Unweighted Pair Group Method with Arithme Average (UPGMA) method based on Coefisient Dice (CD) to present data in the form of a dendrogram tree, in looking at the genetic distance between one species and another species.

3.Results And Discussion

3.1 DNA Total Durian Nias

The results of genomic DNA electrophoresis of fifty durian accessions showed the presence of one whole band and not smear on each well that had a band size above 10,000 bp. This shows

that the DNA isolation process using the CTAB buffer (Cetyl Trimethyl Ammonium Bromide) managed to get the INTACT DNA and can be used in the next analysis, namely the PCR stage. According to [5], if the DNA of the genome is well isolated it will show a intact band and a large (high) molecular weight. The same results were also obtained by [15], showing that the CTAB buffer used can isolate genomic DNA by producing intact bands. Genome DNA sizes above 10,000 bp are very suitable for pcr [16].

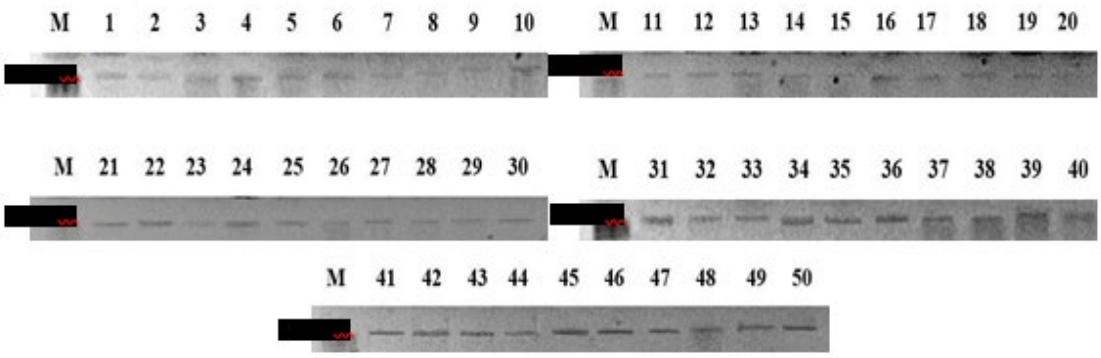

Figure 1 The results of dna isolation of durian genome from Nias Island. M: Marker (1 kb), 1- 1- 50: Durian genome DNA (1-10 from Nias City, 11-20 from West Nias, 21-30 from North Nias, 31-40 from Nias Induk, and 41-50 from South Nias).

Measurement of quantity of durian genome DNA using nanophotometers with wavelengths of 260/280 nm is presented in Table 4.1. The resulting DNA concentration ranges from 210-5496 ng/µL. The highest concentration at B10 accession, while the lowest concentration at K3 accession. The concentration of DNA used in the Random Amplified Polymorphic DNA (RAPD) method ranges from 5-25 ng/µL [17], so the concentration obtained in this study is already qualified.

Table 2 Concentration and purity of durian DNA

No.	Accession	Purity (260/280)	Concentration (ng/µl)	No.	Accession	Purity (260/280)	Concentration (ng/µl)
1	I1	1,9	1140	26	B6	1,8	1340
2	I2	1,9	1130	27	B7	1,8	450
3	I3	1,8	2390	28	B8	1,7	488
4	I4	1,8	1400	29	B9	1,9	393
5	I5	1,6	2650	30	B10	1,7	5496
6	I6	1,9	1050	31	K1	1,8	4250
7	I7	1,8	852	32	K2	1,9	655
8	I8	1,6	560	33	K3	2,0	210
9	I9	1,5	820	34	K4	1,9	5323
10	I10	1,7	340	35	K5	1,8	940
11	U1	2,0	700	36	K6	2,0	660
12	U2	2,0	1080	37	K7	1,6	1320
13	U3	1,7	670	38	K8	1,5	670
14	U4	2,0	1800	39	K9	1,7	1620
15	U5	1,9	1420	40	K10	1,7	1650

16	U6	1,4	310	41	S1	2,0	2852
17	U7	2,0	2480	42	S2	1,8	5120
18	U8	1,8	980	43	S3	2,0	3136
19	U9	1,8	2080	44	S4	2,1	1790
20	U10	1,7	1120	45	S5	2,0	4752
21	B1	1,9	960	46	S6	2,0	4732
22	B2	1,7	620	47	S7	1,9	1911
23	B3	1,5	960	48	S8	1,5	1745
24	B4	1,7	2090	49	S9	2,0	968
25	B5	1,6	1775	50	S10	2,0	3410

Description: I = Nias Induk U = North Nias B = West Nias K = Nias Kota S = South Nias

The results of the genome DNA quantity test from 50 durian accessions from Nias Island using nanophotometers obtained DNA purity ranging from 1.4-2.1. According to [17], the ideal purity limit of DNA commonly used in molecular analysis for the next stage, PCR, is 1.8-2.0. According to [16], in the RAPD method the purity level of DNA below 1.8 can still be used in the PCR process. This is in accordance with the results of the [18] research, with a purity value of 1,069 can already be used to carry out the PCR-RAPD process. In addition, in research conducted by [19], a purity value of 1.37 can also be used in the PCR-RAPD process. [20], one of the advantages of using RAPD markings is that the DNA used does not need to be too pure.

3.2. Amplification of Total DNA of Durian Nias

Dna amplification results from 50 Nias durian accessions using 6 RAPD primers obtained the highest number of bands in the OPA 03 primary and in OPA 07 produced the least number of ribbons (Table 3).

Table 3 Band size and percentage of polymorphisms

No	Primer	Ribbon Size (bp)	Number of polyformist tape	Percent Polyformis (%)
1	OPA 01	431-2778	20	100
2	OPA 03	274-2240	22	100
3	OPA 07	259-2429	16	100
4	OPA 13	231-1738	16	100
5	RAPD 05	239-2855	20	100
6	OPN 06	221-2092	18	100

The total DNA amplification visualization results from the OPA 03 primer resulting in 22 bands measuring 274-2240 bp and the OPA 13 primer resulting in 16 bands measuring 231-1738 bp (Figure 2.2 A). In the primary OPA 13 (Figure 2.2B) there is one accession that has only two bands (S1) and one accession is not amplified (S3). According to [13], the difference in the number of bands produced by each primer is due to differences in the sequence of primary nucleotide bases or the interaction between the primer and the printed DNA.

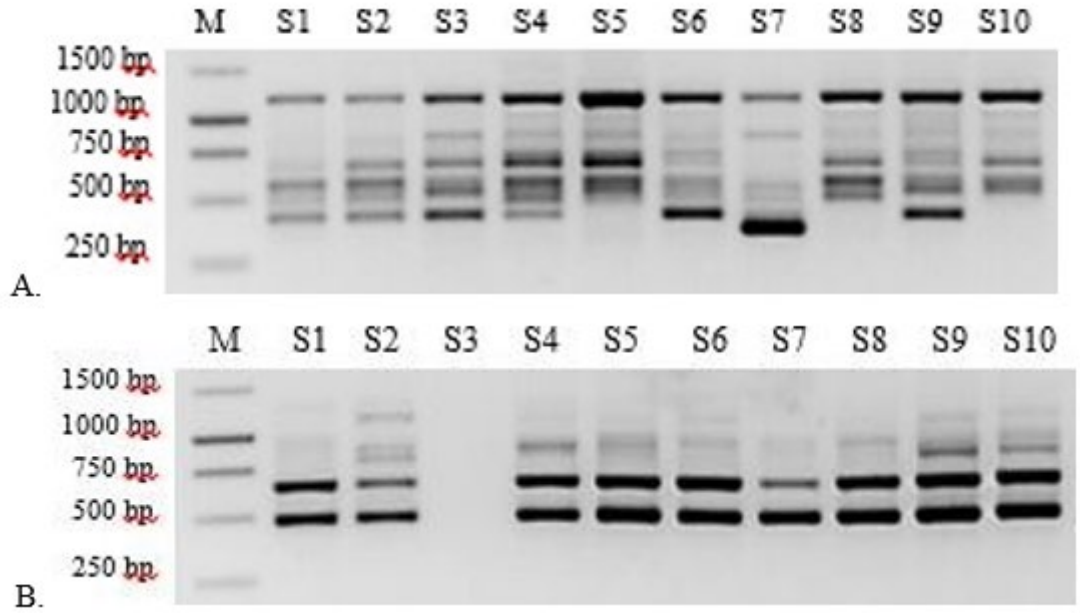

Figure 2 The result of amplification of 10 accession of South Nias durian. A. Primary OPA 03, B. Primary OPA 13. M: Maker, S1-S10: Accession of durian 1-10 from South Nias.

RAPD amplification in all six primers shows that the number of bands produced in each primer is different. The resulting band size varies from 221-2855 bp with a polymorphism percentage of 100% (Table 2). The lowest band size is owned by the PRIMARY OPN 06 as much as 221 bp and the highest is owned by the primer RAPD 05 as much as 2855 bp (Table 2). Primary selection on rapd analysis affects the polymorphism of the resulting band, since each primary has its own sticking site. The polymorphic DNA bands produced by each primer are different, both in the size of the number of base pairs and the number of DNA bands produced [5].

3.3 Analysis of Genetic Diversity of Nias Durian in One Popolation

3.3.1 Analysis of the genetic diversity of South Nias durian

The results of the diversity analysis of 10 South Nias durian accessions based on RAPD markers with 6 primers obtained a matrix of genetic similarities (Table 3) and dendogram construction results presented in Figure 4.3. Based on the matrix of genetic similarities formed (Table 3), the highest likeness values found at one point are caritas S_1 (S5) with Advanced Likes (S3) with a similarity value of 0.73 or a genetic distance value of 0.27. The lowest genetic similarity value was found at one point, namely the accession of Lawa-lawa luo (S8) to Ambukha_2 (S2) with a similarity value of 0.48 or a genetic distance value of 0.52.

In the dendrogram construction (Figure 3) it can be seen that the entire accession of The South Nias durian clustered at a similarity of 0.59. This proves that durian accession in the South Nias population has the lowest genetic diversity compared to durian accession in other Nias populations. Cluster analysis based on dendrogram construction separates the accession of South Nias into 5 clusters at a similarity coefficient of 0.64, where the S6 accession from Caritas Sogawunasi_2 has the furthest genetic distance. According to [21], the kinship between

individuals indicated by dendograms correlates with the genetic distance of the individual. Close kinship indicates low genetic distance and distant kinship indicates a high genetic distance.

Table 4 Matrix of genetic similarities of South Nias durian

	Ambukha_1 (S1)	Ambukha_2 (S2)	Suka Maju (S3)	Amorosa (S4)	Caritas S_1 (S5)	Caritas S_2 (S6)	Koendrafo (S7)	Lawa-lawa luo (S8)	Hilisangowola (S9)	Ehosakhozi (S10)
Ambukha_1 (S1)	1.00									
Ambukha_2 (S2)	0.66	1.00								
Suka Maju (S3)	0.69	0.69	1.00							
Amorosa (S4)	0.61	0.67	0.67	1.00						
Caritas S_1 (S5)	0.55	0.58	**0.73**	0.69	1.00					
Caritas S_2 (S6)	0.59	0.59	0.66	0.61	0.61	1.00				
Koendrafo (S7)	0.64	0.58	0.64	0.72	0.59	0.61	1.00			
Lawa-lawa luo (S8)	0.55	**0.48**	0.61	0.63	0.63	0.67	0.63	1.00		
Hilisangowola (S9)	0.52	0.55	0.61	0.72	0.66	0.58	0.59	0.69	1.00	
Ehosakhozi (S10)	0.56	0.53	0.63	0.64	0.67	0.59	0.55	0.70	0.70	1.00

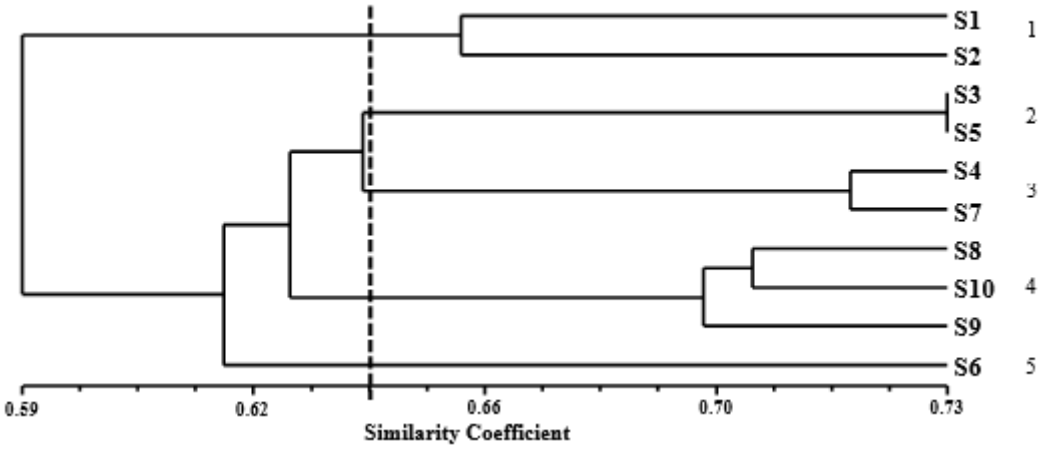

Figure 3 Dendogram 10 accession of South Nias durian based on amplification results using 6 primer RAPD. 1-5: cluster (group).

3.3.2 Analysis of the genetic diversity of North Nias durian

The results of the diversity analysis of 10 north Nias durian accessions based on RAPD markers with 6 primers obtained a genetic similarity matrix (Table 4.4) and dendogram construction results presented in Figure 4.4. Based on the matrix of genetic similarities formed (Table 4.4), the highest similarity values are found at one point also namely Hilinduria (U6) with Hilimbosi_3 (U10) with a similarity value of 0.75 or a genetic distance value of 0.29. The lowest genetic similarity values were found at two points, namely the accession of Umbu Balodano (U3) with Hilimbosi_2 (U2) and hilimaziya accession (U4) with Hilimbosi_2 (U2) with a similarity value of 0.44 or a genetic distance value of 0.56.

On the construction of the formed dendrogram (Figure 4) It can be seen that the entire accession of North Nias durian has a similarity coefficient of 0.53. Cluster analysis based on dendrogram

construction separates North Nias accession into 5 clusters at a similarity coefficient value of 0.64, where U2, U3, U4 accession does not cluster and U2 accession from Hilimbosi_2 has the furthest genetic distance.

Table 5 Matrix of genetic similarities of North Nias durian

	Hilimbosi_1 (U1)	Hilimbosi_2 (U2)	Umbu Balodano (U3)	Hiligodu (U4)	Hilimaziaya (U5)	Hilinduria (U6)	Botolakha_1 (U7)	Botolakha_2 (U8)	Alo'oa (U9)	Hilimbosi_3 (U10)
Hilimbosi_1 (U1)	1.00									
Hilimbosi_2 (U2)	0.54	1.00								
Umbu Balodano (U3)	0.68	**0.44**	1.00							
Hiligodu (U4)	0.52	**0.44**	0.52	1.00						
Hilimaziaya (U5)	0.56	0.60	0.46	0.56	1.00					
Hilinduria (U6)	0.71	0.60	0.62	0.62	0.59	1.00				
Botolakha_1 (U7)	0.65	0.54	0.59	0.59	0.68	0.65	1.00			
Botolakha_2 (U8)	0.68	0.51	0.56	0.65	0.59	0.65	0.59	1.00		
Alo'oa (U9)	0.70	0.59	0.51	0.51	0.67	0.67	0.70	0.70	1.00	
Hilimbosi_3 (U10)	0.66	0.51	0.62	0.68	0.52	**0.75**	0.68	0.65	0.60	1.00

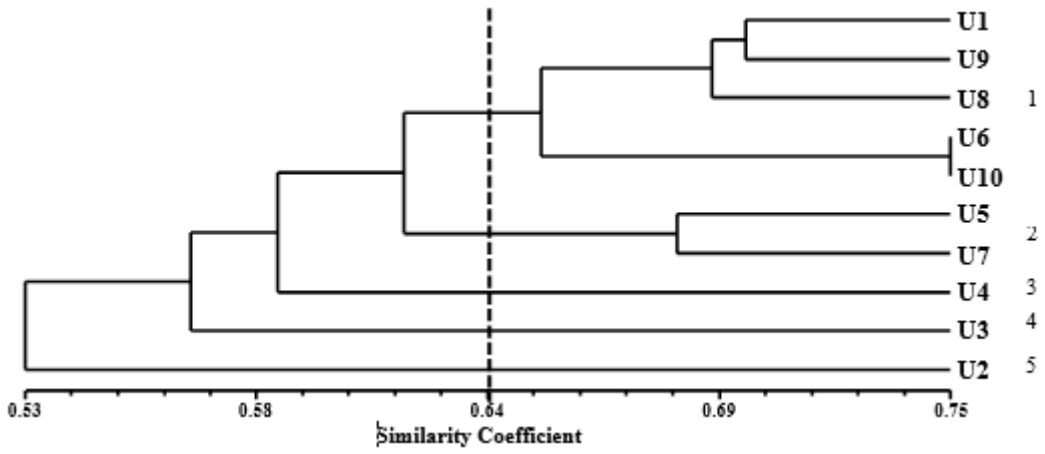

Figure 4 Dendogram 10 accession of North Nias durian based on amplification results using 6 primers rapd. 1-3: cluster (group).

3.3.3 Analysis of genetic diversity of West Nias durian

The results of the diversity analysis of 10 west Nias durian accessions are based on RAPD markers with 6 primers. Based on the matrix of genetic similarities formed (Table 6), the highest likeness values are found at one point, namely the accession of Hilimbuasi_2 (B9) with Hilimbuasi_1 (B8) with a similarity value of 0.89 or a genetic distance value of 0.11. The lowest similarity value was found at one point, namely Hili'uso_2 (B2) with Hili'uso_1 (B1) with a similarity value of 0.47 or a genetic distance value of 0.53.

Table 6 Matrix of genetic similarity of West Nias durian

	Hili'uso_1 (B1)	Hili'uso_2 (B2)	Duria (B3)	Sisobawino (B4)	Ambukha_1 (B5)	Ambukha_2 (B6)	Hilimbowo Ma'u (B7)	Hilimbuasi_1 (B8)	Hilimbuasi_2 (B9)	Tuwuna (B10)
Hili'uso_1 (B1)	1.00									
Hili'uso_2 (B2)	**0.47**	1.00								
Duria (B3)	0.68	0.55	1.00							
Sisobawino (B4)	0.70	0.62	0.68	1.00						
Ambukha_1 (B5)	0.79	0.50	0.71	0.79	1.00					
Ambukha_2 (B6)	0.80	0.52	0.70	0.77	0.83	1.00				
Hilimbowo Ma'u (B7)	0.71	0.58	0.76	0.77	0.77	0.76	1.00			
Hilimbuasi_1 (B8)	0.56	0.55	0.48	0.74	0.62	0.64	0.61	1.00		
Hilimbuasi_2 (B9)	0.55	0.62	0.53	0.70	0.61	0.59	0.65	**0.89**	1.00	
Tuwuna (B10)	0.53	0.58	0.58	0.68	0.56	0.64	0.58	0.82	0.80	1.00

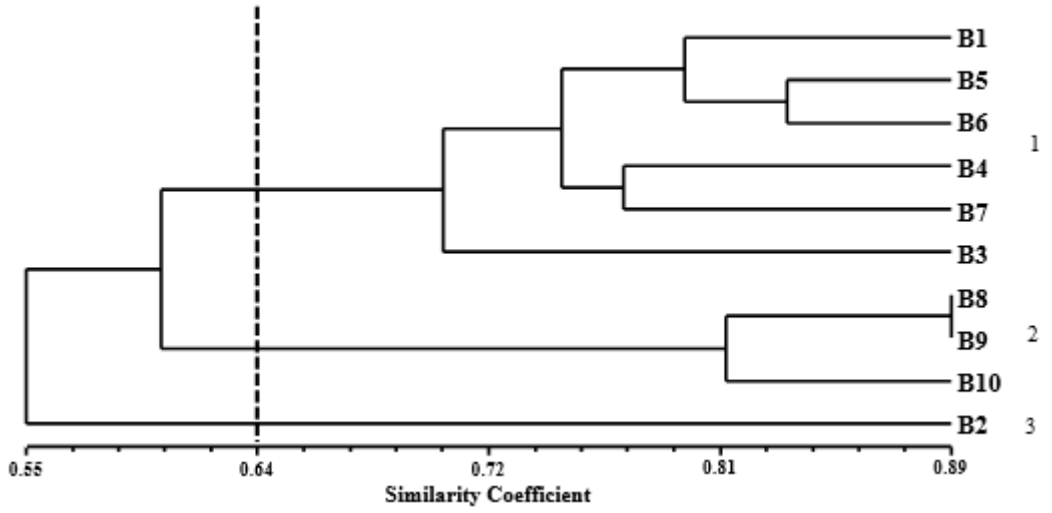

Figure 5 Dendogram 10 accession of West Nias durian based on amplification results using 6 primers rapd. 1-3: cluster (group)

In the construction of the dendrogram formed (Figure 5) it can be seen that the entire accession of West Nias durian has a similarity coefficient of 0.55. Cluster analysis based on dendrogram construction separates west Nias accession into 3 clusters at a similarity coefficient value of 0.64, where B2 accession from Hili'uso_2 has the furthest genetic distance. These results show that B2 accession has the furthest genetic distance from other accessions and does not cluster. This grouping is based on the primary polymorphic band 6 and does not correlate with the distance of the West Nias durian collection geographically.

3.3.4 Analysis of the genetic diversity of Nias City durian

The results of the diversity analysis of 10 Nias Kota durian accessions are based on RAPD markers with 6 primers. Based on the matrix of genetic similarities formed (Table 7), the highest similarity values are found at one point also namely Lasara T_2 (K7) with Bawodesolo (K5) with

a similarity of 0.84 or a genetic distance value of 0.16. The lowest similarity values are found in the value of two points, namely Afia accession (K10) with Hilihao_1 (K1) and Afia accession (K10) with Sisobahili_2 (K4) with a similarity value of 0.43 or a genetic distance value of 0.57.

Table 7 Matrix of genetic similarity of Nias City durian

	Hilihao_1 (K1)	Hilihao_2 (K2)	Sisobahili_1 (K3)	Sisobahili_2 (K4)	Bawodesolo (K5)	Lasara T_1 (K6)	Lasara T_2 (K7)	Lasara T_3 (K8)	Teluk Belukar (K9)	Afia (K10)
Hilihao_1 (K1)	1.00									
Hilihao_2 (K2)	0.65	1.00								
Sisobahili_1 (K3)	0.80	0.69	1.00							
Sisobahili_2 (K4)	0.76	0.65	0.73	1.00						
Bawodesolo (K5)	0.82	0.78	0.82	0.71	1.00					
Lasara T_1 (K6)	0.73	0.73	0.69	0.65	0.67	1.00				
Lasara T_2 (K7)	0.82	0.75	0.78	0.75	**0.84**	0.75	1.00			
Lasara T_3 (K8)	0.76	0.76	0.69	0.73	0.78	0.76	0.78	1.00		
Teluk Belukar (K9)	0.65	0.76	0.65	0.61	0.67	0.65	0.75	0.61	1.00	
Afia (K10)	**0.43**	0.63	0.47	**0.43**	0.45	0.63	0.49	0.51	0.59	1.00

In the construction of the dendrogram formed (Figure 6) it can be seen that the entire accession of Nias Kota durian has a 51% resemblance. Cluster analysis based on dendrogram construction separates nias kota accession into 2 clusters at a similarity coefficient of 0.64, where K10 accession from Afia has the furthest genetic distance. Result Dendograms showed that durian accession in nias city populations has the highest genetic diversity compared to durian accession in other populations. According to [22], the higher the genetic distance, the higher the genetic diversity of individual populations. Conversely, the lower the genetic distance, the lower the genetic diversity. High genetic diversity is one of the high factors for assembling new superior varieties.

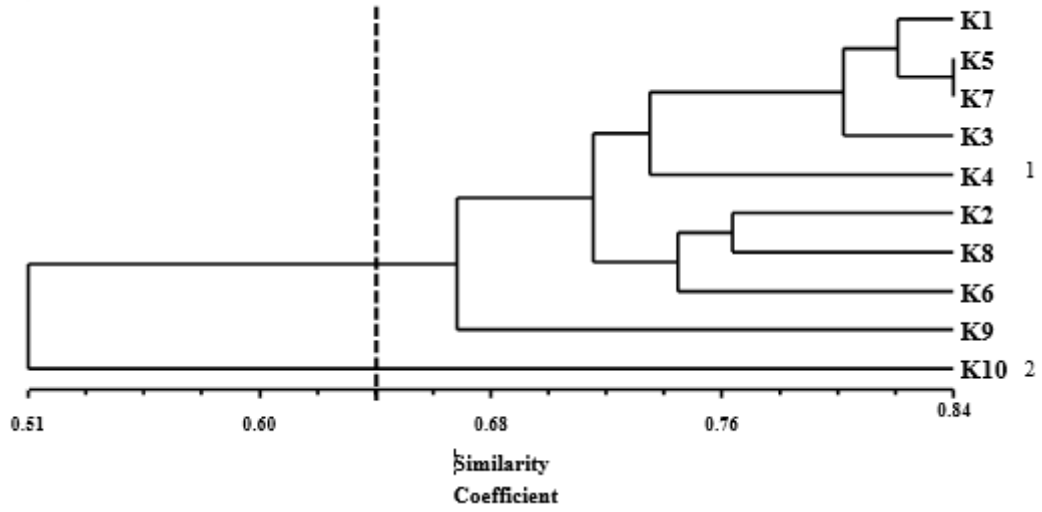

Figure 6 Dendogram 10 accession of City Nias durian based on amplification results using 6 primers rapd. 1-3: cluster (group)

3.3.5 Analysis of genetic diversity of Nias Induk durian

The results of the diversity analysis of 10 accessions of Nias Induk durian are based on RAPD markers with 6 primers. Based on the matrix of genetic similarities formed (Table 4.7), the highest similarity value is found at one point also namely Lolofaoso Negligent (I9) with a Botombawo_2 (I3) with a similarity value of 0.92 or a genetic distance value of 0.08. The lowest genetic similarity value was found at one point, namely lawe-lawe accession (I6) with Botombawo_2 (I3) with a similarity value of 0.34 or a genetic distance value of 0.66.

In the construction of the dendrogram formed (Figure 7) it can be seen that the entire accession of nias induk durian has a similarity coefficient of 0.52. Cluster analysis based on dendrogram construction separates the parent Nias accession into 4 clusters at a similarity coefficient value of 0.64, where cluster 4 has the most accession and I10 accession does not cluster.

Table 8 Matrix of genetic similarity of Induk Nias durian

	Lolowua (I1)	Botombawo_1 (I2)	Botombawo_2 (I3)	Hiliwaele (I4)	Hilimbowo (I5)	Lawe-lawe (I6)	Lalai_1 (I7)	Lalai_2 (I8)	Lolofaoso Lalai (I9)	Fadoro Lai'o (I10)
Lolowua (I1)	1.00									
Botombawo_1 (I2)	0.64	1.00								
Botombawo_2 (I3)	0.43	0.64	1.00							
Hiliwaele (I4)	0.64	0.74	0.49	1.00						
Hilimbowo (I5)	0.62	0.64	0.43	0.60	1.00					
Lawe-lawe (I6)	0.64	0.58	**0.34**	0.55	0.68	1.00				
Lalai_1 (I7)	0.57	0.58	0.68	0.40	0.49	0.59	1.00			
Lalai_2 (I8)	0.43	0.72	0.85	0.49	0.55	0.49	0.68	1.00		
Lolofaoso Lalai (I9)	0.40	0.64	**0.92**	0.49	0.47	0.38	0.68	0.89	1.00	
Fadoro Lai'o (I10)	0.64	0.62	0.57	0.43	0.60	0.62	0.66	0.60	0.60	1.00

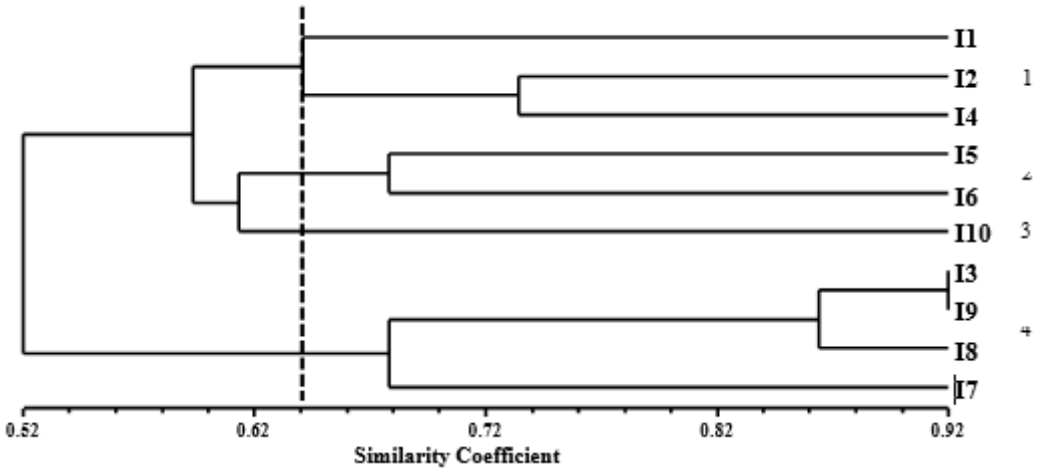

Figure 7 Dendogram 10 accession of Nias Induk durian based on amplification results using 6 primers rapd. 1-3: cluster (group)

3.4 Analysis of Genetic Diversity of Nias Durian Between Populations

The results of the diversity analysis of 50 Nias durian accessions based on RAPD markers with 6 primers obtained a matrix of genetic similarities and dendogram construction results presented in Figure 4.8. Based on the matrix of genetic similarity of 50 nias durian accessions can be known accession with the highest similarity value found at one point, namely the accession of Lolofaso Negligent (I9) with Botombawo_2 (I3) with a similarity value of 0.98 or a genetic distance value of 0.02. Accession I9 and I3 is an accession originating from the same sub-district, namely Hiliserangkai District, Nias Induk regency.

The lowest similarity values were found at five points, namely Hilisangowola accession (S9) with Hili'uso_2 (B2), Ehosakhozi accession (S10) with Hili'uso_2 (B2), Koendrafo accession (S7) with Ambukha_1 (B5), Koendrafo accession (S7) with Ambukha_2 (B6), and Hilimbosi_2 accession (U2) with Lawe-lawe accession (I6) with a similarity value of 0.54 or genetic distance

value of 0.46. Geographically, the location of this inter-accession collection is quite far away because it is in a different district from each other.

Based on the construction of the dendrogram tree (Figure 4.8) it can be known that the entire accession of Nias durian clustered at a similarity coefficient of 0.67. Cluster analysis based on dendrogram tree construction separates accessions into 8 groups at a similarity coefficient value of 0.73. Based on the 8 clusters formed, there are 3 clusters with a high number of accessions, namely cluster 3 consisting of 13 accessions (B4, I2, B8, B9, I3, I9, K10, I8, B10,U1, I7, U3, and I10), cluster 5 it consists of 10 accessions (I1, K1, K3, K5, K7, K4, K8, K2, K9, and K6) and cluster 7 consists of 10 accessions (S1, S3, S2, S4, S7, S5, S6, S8, S9, and 10). Cluster 3 is filled by accessions originating from several districts, cluster 5 comes from two districts while cluster 7 comes from the same district.

There is one accession that groups itself, namely B2 accession originating from West Nias Regency in cluster 6. There are several clusters consisting of accessions originating from the same district, namely clusters 1 (B1, B5, B6, and B7) from West Nias Regency, clusters 4 (I5 and I6) from The Parent Nias regency, and clusters 8 (U2, U4, U6, U10, U8 U9, U5, and U7) from North Nias regency.

Accession that has the farthest genetic distance from other accessions is B2 accession originating from the West Nias population precisely in Hili'uso_2 Village, Lolofitumoi District. Based on this difference in genetic distance, B2 accession can be used as a source of germ plasma for the plant breeding process. According to [23], high genetic diversity in a population can be caused by natural populations that have not experienced disorders and the occurrence of random marriages that result in the stability of genetic diversity is maintained.

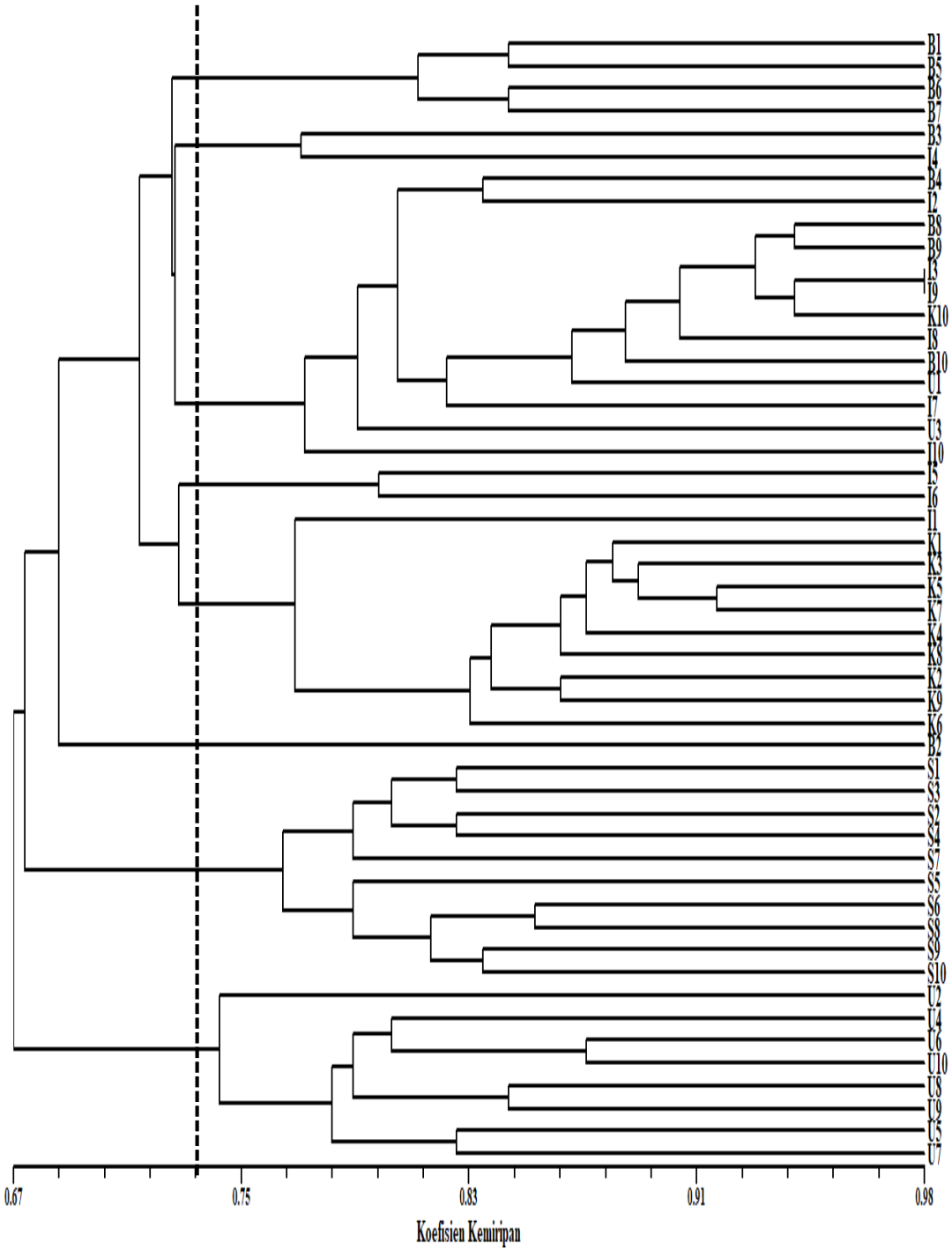

Figure 8 Dendrogram 50 accession of Nias durian based on amplification results using 6 primers rapd. 1-8: cluster (group).

4. Conclusion

The conclusions of this study are as follows:

a. The results of genomic DNA amplification from 50 Nias durian accessions using 6 primers of RAPD, namely OPA 01, OPA 03, OPA 07, OPA 13, RAPD 05, and OPN 06 produce polymorphic bands of 100% with ribbon sizes ranging between 221-2855 bp.

b. The similarity coefficient value of each durian accession population from Nias Island ranges from 0.51-0.59 and the overall accession clustered on similarities. 67%. B2 accession from West Nias is an accession that has the furthest genetic distance from other durian accessions.

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