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Genetic Diversity of Durian (Durio zibethinus Murr.) from North and South Nias using Simple Sequence Repeat (SSR)

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Abstract. Durian is a tropical fruit that has economic value for Nias Island especially in North and South Nias. Anthropogenic activity already occured in Nias and caused disturbance resulting in destruction and extinction of various durian. The aim of the research was to analyzed the genetic diversity of Durian from North and South Nias using SSR primers namely: Dz844, DzGCCG01, DzGCAG01, and DzMTb021. Total DNA was successfully isolated from 20 accessions. The DNA was successfully amplified resulting in DNA alleles sizing from 170-1100 bp. All primers showed 33 alleles with 28 polymorphic bands. The DNA profile were further analyzed with Ntsys which showed that 20 accession has coefficient of similarity 0.66-0.94 and clustered into nine groups at the similarity coefficient of 0.80. The research showed that the ninth accession of durian from North and South Nias has a high genetic diversity.

Keyword: DNA, Durian, Genetic Diversity, Nias, SSR

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1 Introduction

Durian is a tropical fruit that has unique flavor and aroma. Durian is also called the king of fruit which is very popular among other fruits because of its distinctive taste (Lestari et al., 2011). North and South Nias are Southern part of North Sumatera that cultivate durian with the highest numbers. The island is characterized by specific natural conditions that may be different from the other areas because it is surrounded by oceans that make it isolated from the Sumatra mainland. Recently, research of genetic diversity of durian in Nias Island has not ever been conducted yet reported. Durian is one of the diverse fruit germplasms that is important to be developed. Conversely, the anthropogenic activities that resulted in the destruction of the environment further causing potential extinction of various species of plants especially durian is unavoidable. Therefore, it is necessary to conduct some conservation efforts to durian to maintain its sustainability and to improve economical value of the people in Nias Island. One method to uncover the germplasm diversity of durian is by studying the genetic diversity (Yulita

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and Muna, 2010). The most widely used methods for obtaining plant genetic information is by using Simple Sequence Repeats (SSR) because it has the advantage of reading codominant, locus specific, and reliable.

2. Materials and Methods

Total of 10 samples of young leaves were taken randomly and purpossively from each subdistricts (Table 1). Samples were then stored in plastic bag filled in with silica gel at room temperature. Genomic DNA was extracted using CTAB method (Santoso et al., 2003) and modified procedures by increasing the concentration of extraction buffer, speed and time of centrifugation. The young leaves was cut into small segments weighing 20 mg, then added with 2 mg PVP, and homogenized in pre-heat (65°C) 4% CTAB solution using a pestle and mortar. The homogenized mixture was transferred into 1.5 mL eppendorf tubes and kept for incubation at 65°C for 1-1.5 hr, inverted for every 10 minutes, and then left a few minutes at room temperature. Five hundred µL solution of chloroform : isoamyl alcohol (24:1) was added into tube and vortexed, followed with centrifugation at 12,000 rpm for 10 minutes. Supernatant was removed to the new tube. The procedure is repeated 2-3 times. Supernatant was then precipitated with cold isopropanol 1:1.5 supernatan volume, then incubated overnight in the freezer (-20°C). The samples were centrifuged again at 12,000 rpm for 10 minutes. Pellets were diluted with 200 µl of TE 1X, then added with 20 µl of sodium acetate and 400 µl of absolute ethanol. Furthermore, the samples are incubated at a temperature of -20°C for 30 minutes, then centrifuged 12,000 rpm for 1 minute. The DNA pellets was washed with ethanol 70%, inverted for 10 sec and vortexed. Pellet was dried and suspended with TE 1X. Quality and quantity of RNA was checked with electrophoresis and nanophotometry. Optical density (OD260/280) was equivalent to standard ratio 1.8 for genomic RNA. Electrophoresis was done with 1,2% agarose gel and stained with ethidium bromide and visualization RNA with geldoc.

Table 1. List of durian germplasms used in the research

No.	Accession	Origin (Village, districts)	Population
1	U1	Hilimbosi, Sitolu Ori	North Nias
2	U2	Hilimbosi, Sitolu Ori	North Nias
3	U3	Umbu Balodano, Sitolu Ori	North Nias
4	U4	Hiligodu, Botombawo	North Nias
5	U5	Hilimaziaya, Lotu	North Nias
6	U6	Hilinduria, Lotu	North Nias
7	U7	Botolakha, Tuhemberua	North Nias
8	U8	Botolakha, Tuhemberua	North Nias
9	U9	Alo'oa, Tuhemberua	North Nias
10	U10	Hilimbosi, Sitolu Ori	North Nias

11	S1	Ambukha 1, Ulunoyo	South Nias
12	S2	Ambukha 2, Ulunoyo	South Nias
13	S3	Suka Maju, Ulunoyo	South Nias
14	S4	Amorosa, Ulunoyo	South Nias
15	S5	Caritas Sogawunasi, Lolomatua	South Nias
16	S6	Caritas Sogawunasi, Lolomatua	South Nias
17	S7	Koendrafo, Lolomatua	South Nias
18	S8	Lawa-lawa luo, Lolomatua	South Nias
19	S9	Hilisangowola, Lolomatua	South Nias
20	S10	Ehosakhozi, Huruna	South Nias

Table 2. List of loci used in the research

Locus	Primer Sequences	Annealing Temperature (°C)	References
Dz844	TGGTTGAATGCCCGCACGCT TCGGACCGATCCACCCCTGC	66	Kristianti (2005)
DzGCCG01	GGTGGGTTCAAGCACATCTT TCAAACCAGACCGAGGGTTA	56	Nafsi (2007)
DzGCAG01	GTTGAGCACCCGTACACTCA GAGAGGCAAAATACGCAAGC	59	Nafsi (2007)
DzMTb021	ATTGACCCATTCGAAATGTCCC CTTT TGCGCGGGAAATTGGTGTTTCA	55	Santoso (2016)

DNA amplification was performed using PCR. PCR program run by setting the temperature pre-denaturation 94°C for 4 min followed by 35 cycles, each cycle consists of three stages, denaturation 94°C for 30 seconds, annealing with adjustable temperature with each primer in the temperature range 55-66°C for 30 sec (Table 2.), then extension in 72°C for 45 sec, the final stages of elongation in 72°C for 7 min. Sequence analysis was peformed by using NTSys (Numerical Taxonomy and Multivariate Analysis System) Version 2.02.

3. Result and Discussion

There are 20 durian collections amplified with 4 SSR primers (Dz844, DzGCCG01, DzGCAG01, and DzMTb021). The DNA were visualized in agar electrophoresis with different sizes at the same locus (Table 3). Bennet (2000) stated that the differences in allelic size are due to differences in the number of repeated bases. Appearance of alleles indicate a character or trait on accession. Locus DzGCCG01 have the highest number of alleles with 13 alleles detected. The lowest number of alleles detected were from DzGCAG01 and DzMTb021 both with 6 alleles. The size of the amplified alleles is at length between 190 and 1100 bp (Figure 1). Polymorphic bands are bands that are always not present in all accessions. The percentage of polymorphic bands indicates the high variation in each of the studied durian accessions. The highest number of polymorphic bands were found in DzGCCG01 primers, consisted of 12 alleles. While the lowest number of polymorphic allels was found in DzGCAG01 primer, which was 3 alleles.

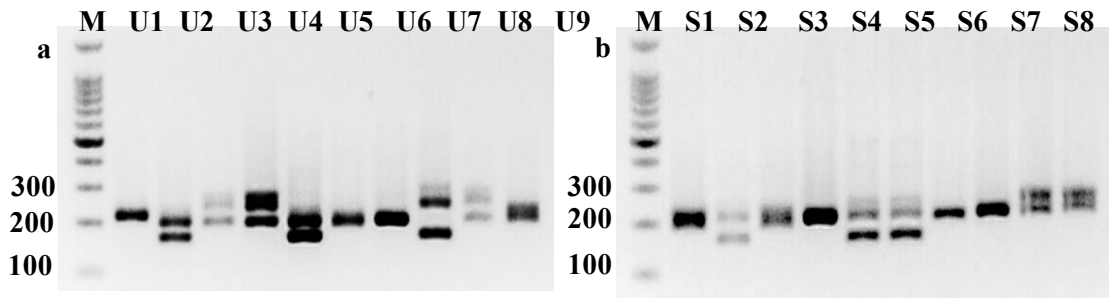

Figure 1. **a**. Visualization of amplified locus DzMTb021 durian North Nias **b**. Visualization of amplified locus DzMTb021 durian South Nias. M=Marker 100 bp.

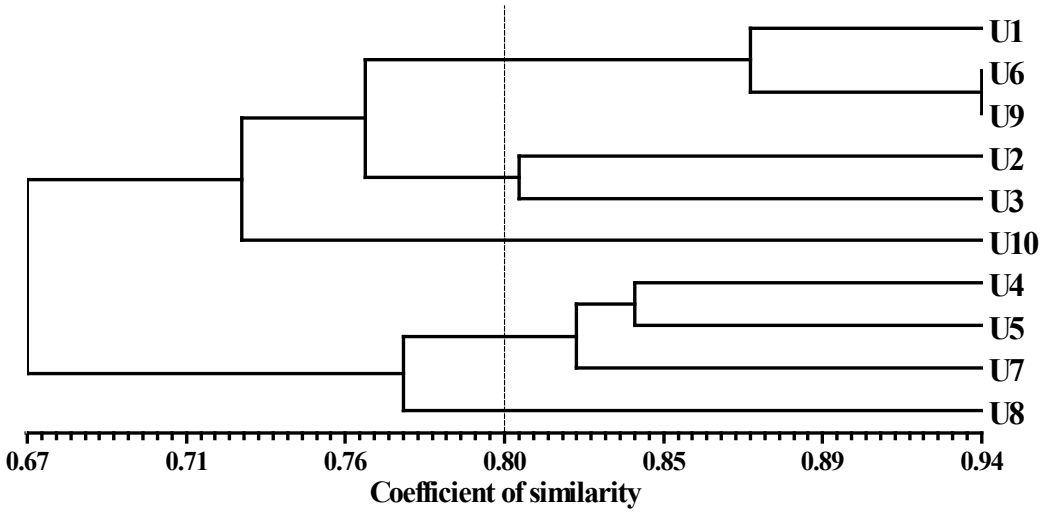

Figure 2. Dendogram of genetic diversity of accession North Nias with 4 locus SSR

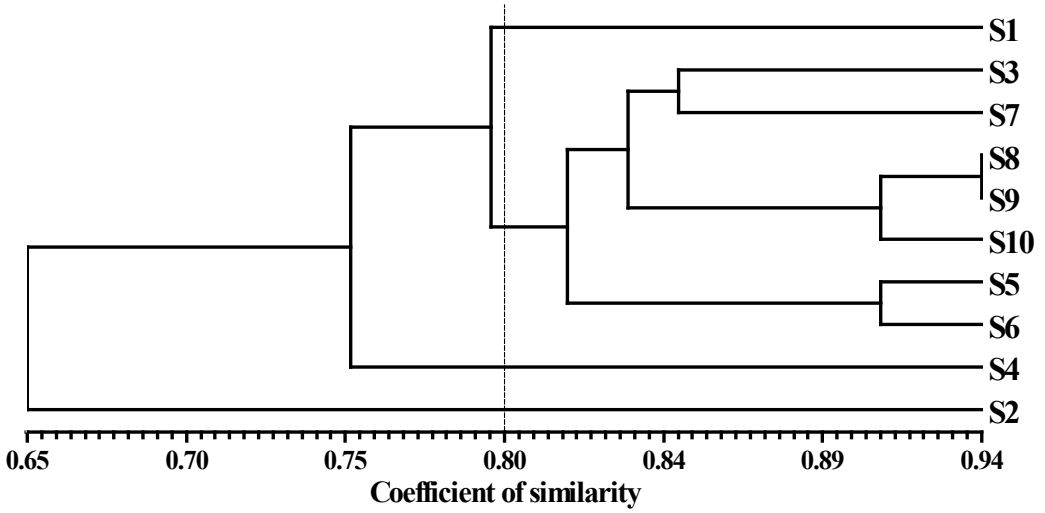

Figure 3. Dendogram of genetic diversity of accession South Nias with 4 locus SSR

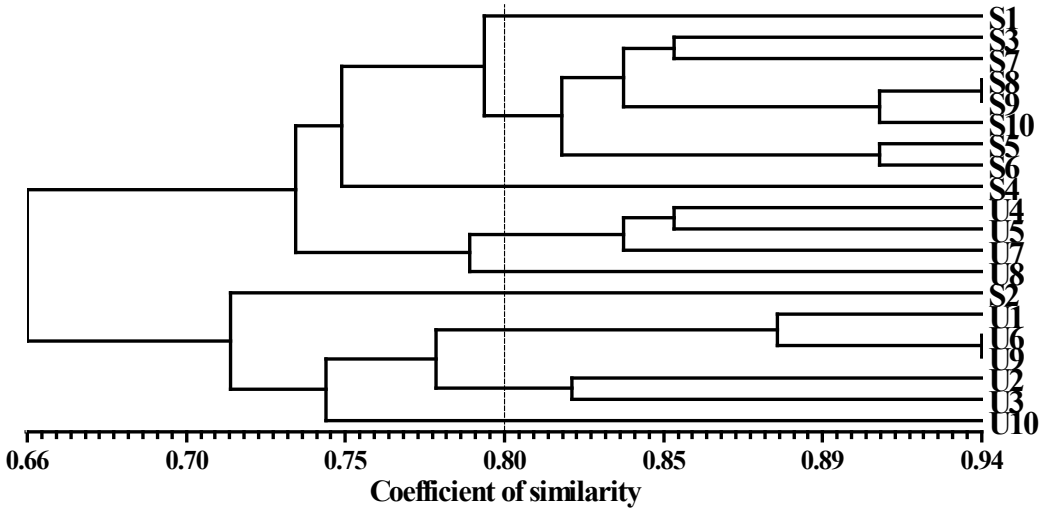

Figure 4. Dendogram of genetic diversity of accession North and South Nias with 4 locus SSR

Proper selection of SSR primers selection is necessary to support the succession of the amplification process that will produce desirable amplicons. The good selection of SSR primers are based on (1) the length of primer, (2) base compositiion, (3) annealing temperature, (4) selection of base at 3', and (5) avoid the similar primer annealing (Millah et al., 2012).

Dendogram of genetic diversity of accession North Nias

Based on the dendogram, it can be seen that the accession durian North Nias were clustered at similarity coefficient value of 0.67 (Figure 2) and the genetic diversity measuring 6-33%. The North Nias cluster analysis based on dendogram tree construction separates the accession into 5 clusters at a value of 0.80. The first clusters are U1, U6, and U9, both U2 and U3, third U10, fourth U4, U5, and U7, fifth U8. The cluster with the greatest accession is the first and the fourth cluster. This cluster is dominated by several villages located in different sub-districts, namely Hilimbosi, Hilinduria, Alo'oa, Hiligodu, Hilimaziaya, and Botolakha Village. Nurmiyati et al (2009) stated that the more coefficient reaching to 1.0 then members within a group were perfectly similar, while coefficient closer to zero means the more dissimilar members within a group.

Dendogram of genetic diversity of accession South Nias

Based on the construction of dendogram trees, it can be seen that accession of durian South Nias is grouped in the coefficient of similarity 0.65 (Figure 3) and the genetic diversity measuring 6-35%. The South Nias cluster analysis based on dendogram tree construction separates the accession into 4 clusters at a value of 0.80. The first cluster is S1, second S3, S5, S6, S7, S8, S9, S10, third S4, fourth S2. The cluster with the greatest accession is the second cluster. This cluster is dominated by several villages located in different sub-districts, namely Suka Maju, Caritas Sogawunasi, Koendrafo, Lawa-lawaluo, Hilisangowola, and Ehosakhozi Village. Kristamtini et al (2014) stated that the implications of dendogram are cultivars that are in one group showed a great genetic similarity or have a small genetic distance.

Dendogram of genetic diversity of accession North and South Nias

Based on the construction of dendogram trees, it can be seen that the durian accession of North and South Nias are grouped in the coefficient of similarity 0.66 (Figure 4) and the genetic diversity measuring 6-34%. North and South Nias cluster analysis based on dendogram tree construction separates accession to 9 clusters at a value of 0.80. The first cluster is S1, second S3, S5, S6, S7, S8, S9, S10, third S4, fourth U4, U5, U7, Fifth U8, Sixth S2, Seventh U1, U6, U9, Eight U2, U3, U10 ninth. The cluster with the greatest accession is the same cluster as the dendogram of genetic diversity of accession of South Nias is the first cluster. A unique feature

of accession from South Nias (S2) that is assumed to be clustered on other South Nias accessions is separated in different clusters. There are several factors that may cause the results such as the unique of the character possessed by accession, the amount of accession and the locus used.

Based on the above explanation, it can be seen that 20 accessions of North and South Nias durians with 4 SSR locus indicate high genetic variation. Accessions of durian North and South Nias are obtained from forestry and smallholder plantations. Genetic differentiation of durian plants may occur due to pollination that may occur in the same tree or in different trees, intentional crosses by humans, or the introduction of external plants. Of these three most likely, variations in North and South Nias durians are caused by natural pollination of a tree species or by a different tree. This is because the durian plantation is not yet available in Nias. Durian plants obtained is a durian plant that mostly grows from the seeds of durian are accidentally planted by people of Nias.

4. Conclusions

a. Twenty accessions of North and South Nias durian were analyzed using 4 SSR loci Dz844, DzGCCG01, DzGCAG01, and DzMTb021, had different values. The highest number of polymorphic allels was found in DzGCCG01 primer, which are 12 alleles. While the lowest number of polymorphic allels was found in DzGCAG01 primer, which is 3 alleles.

b. Based on the use of 4 SSR locus it is known that 20 accessions of durian North and South Nias has high genetic diversity with a coefficient of similarity from 0.66-0.94 and was divided into 9 clusters at the similarity coefficient of 0.80.

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REFERENCES

[1] Lestari, S., Fitmawati, Ninik, N.-W., 2011. Keanekaragaman Durian (Durio zibethinus Murr.) Di Pulau Bengkalis Berdasarkan Karakter Morfologi. Bultein Kebun Raya 14(2),29-44.

[2] Yulita, K.-S., Muna, M., 2010. Keragaman Genetik Beberapaklon Durian (Durio zibethinus Murray) Asal Jawa Barat Berdasarkan Sidik Random Amplified Polimorphic DNA. Berita Biologi 10(3), 269-275.

[3] Santoso, P.-J., Saleh, G.-B., Saleh, N.-M., Napis, S., 2003, Double Extraction Procerdure for Higher Quality DNA of Mucilaginous Plants. Exhibition of Invention & Research. Universitas Putra Malaysia.

[4] Bennet, P., 2000. Microsatellites. J. Clin. Phatol: Mol. Phatol 53, 177-183.

[5] Millah, M.-I., Habibah, N.-A., Suwarni, E., Retorningsih, A., 2012. Analysis of Genetic Diversity and Differentiation of Java and Madura Teak Based on Microsatellite Markers to Support Teak Fingerprint. Biosaintifika 4(2), 113-120.

[6] Nurmiyati, Sigiyarto, Sajida., 2009. Kimpul (Xanthosomo spp.) Characterization Based on Morphological Characteristic and Isozymic Analysis. BioScience 1(3),138-145.

[7] Kristamtini, Taryono, Basunanda, P., Murti, R.-H., 2014. Keragaman Genetik Kultivar Padi Beras Hitam Lokal Berdasarkan Penanda Mikrosatelit. Jurnal AgroBiogen 10(2), 67-76.