Genetic Similarity of Three Durian (Durio zibethinus Murr.) Populations from Nias Island Sumatera Utara Based on Simple Sequence Repeat (SSR)

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Abstract. Genetic similarity of 3 durian populations [Nias Kota (NK), Nias Induk (NI), and Nias Barat (NB)] had been evaluated based on Simple Sequence Repeat (SSR) markers. The objectives of this study were to assess the genetic similarity of three durian population. There were 10 durian samples from each population. The genomic DNA was amplified using 4 primers (DzMtb021, Dz621, Dz535, and Dz504). All of locus had 100% polymorphism except Dz621 with 33.33% polymorphism. Dendrogram was constructed using Unweighted Group Method Arithmetic (UPGMA) based on Numerical Taxonomy and Multivariate System (NTSys) computer program. The result showed that the ten samples were not clustered within their populations. Genetic similarity of 30 durian from three population was 63%, and genetic similarity of durian within NK, NI, and NB population was 72, 74, and 59% respectively. The similarity of NK and NI population was the highest when compared to NB.

1. Introduction
Indonesia is an agraris country that have large area and various types of plant that can be used as economically valuable commodity. One of commodity supported Indonesia economy is durian (Durio zibethinus Murr.) [1]. Nias island is 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 Sumatera mainland. This causes gene flow or transference of species from this island or to this island is very small. On the other side, variation of durian in Nias Island has not even been explored and characterized. One way to find out similarity of durian is by looking the genetic diversity durian [2]. Diversity study are very important to find out the genetic diversity of some population. Therefore, it is necessary to conduct some conservation efforts to durian to maintain its sustainability and to improve durian plant breeding for the better [3].

Microsatellite is short tandem repeat where is the repetition consist of one until six nucleotide [4]. Utilization of SSR is very easy [5], informative, specific locus, and can read the codominant character, so it can applicated for analyze of genetic similarity [6]. DNA microsatellite marker has been used to identification genetic similarity of durian were isolation and characterized of microsatellite motive[7], 38 of microsatellite motive were successed isolated and designed 41 primer, then some of primer have been used to analyze genetic diversity of durian collection of Kebun Percobaan Cipaku [8] and analyze genetic diversity of durian of North Sumatera [9].
2. Method
Total of 10 samples of young leaves were taken randomly and purposively (Figure 1) 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 [10] and modified procedures by increasing the concentration of extraction buffer, speed and time of centrifugation.

| No. | Accession | Village       | Subdistrict   | Population   |
|-----|-----------|---------------|---------------|--------------|
| 1   | NK1       | Hilihao_1     | Gunungsitoli  | Nias Kota    |
| 2   | NK2       | Hilihao_2     | Gunungsitoli  | Nias Kota    |
| 3   | NK3       | Sisobahili_1  | Gamo          | Nias Kota    |
| 4   | NK4       | Sisobahili_2  | Gamo          | Nias Kota    |
| 5   | NK5       | Bawodesolo    | Gamo          | Nias Kota    |
| 6   | NK6       | Lasaratarakhaini_1 | Kec. Alo’oa | Nias Kota    |
| 7   | NK7       | Lasaratarakhaini_2 | Kec. Alo’oa | Nias Kota    |
| 8   | NK8       | Lasaratarakhaini_3 | Kec. Alo’oa | Nias Kota    |
| 9   | NK9       | Teluk Belukar | Gunungsitoli Utara | Nias Kota |
| 10  | NK10      | Afia          | Gunungsitoli Utara | Nias Kota |
| 11  | N1        | Lolowua       | Hiliserangkai | Nias Induk   |
| 12  | N2        | Botombawo_1   | Hiliserangkai | Nias Induk   |
| 13  | N3        | Botombawo_2   | Hiliserangkai | Nias Induk   |
| 14  | N4        | Hiliwaele     | Botomujoi     | Nias Induk   |
| 15  | N5        | Hilimbowo     | Botomujoi     | Nias Induk   |
| 16  | N6        | Lawe-lawe     | Botomujoi     | Nias Induk   |
| 17  | N7        | Lalai_1       | Hiliserangkai | Nias Induk   |
| 18  | N8        | Lalai_2       | Hiliserangkai | Nias Induk   |
| 19  | N9        | Lolofaoso Lalai | Hiliserangkai | Nias Induk   |
| 20  | N10       | Fadoro Lai’o  | Hiliserangkai | Nias Induk   |
| 21  | NB1       | Hili’uso_1    | Lolofitumoi   | Nias Barat   |
| 22  | NB2       | Hili’uso_2    | Lolofitumoi   | Nias Barat   |
| 23  | NB3       | Duria         | Lolofitumoi   | Nias Barat   |
| 24  | NB4       | Sisobawino    | Lolofitumoi   | Nias Barat   |
| 25  | NB5       | Ambukha_1     | Lolofitumoi   | Nias Barat   |
| 26  | NB6       | Ambukha_2     | Lolofitumoi   | Nias Barat   |
| 27  | NB7       | Hilimbowo Ma’u | Lolofitumoi   | Nias Barat   |
| 28  | NB8       | Hilimbuasi_1  | Lolofitumoi   | Nias Barat   |
| 29  | NB9       | Hilimbuasi_2  | Lolofitumoi   | Nias Barat   |
| 30  | NB10      | Tuwuna        | Mandrehe      | Nias Barat   |
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 supernatant 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 (OD\textsubscript{260/280}) 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 DNA with geldoc.

Table 2. List of loci used in the research

| No | Locus | Primer forward dan reverse | TA (°C) | References |
|----|-------|---------------------------|--------|------------|
| 1  | DzMTb021 | ATTGACCATTCCAAATGTCCCTTTT TGCGGGGAATTTGGGTGTTTCA | 55     | Santoso (2016) |
| 2  | Dz621   | ACCGGACCAGGGTTGTGTT GCAAGCCGGGATCGACCCAG | 61     | Kristianti (2005) |
| 3  | Dz535   | GACTGAGCGCCCGATGCCC GTCCCCTCTGCGTGCTGTCG | 60     | Kristianti (2005) |
| 4  | Dz504   | CTGCGTGCGCTGGGGCTTA CCTTCTCGTGCTTGCTAGCG | 66     | Kristianti (2005) |

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 performed by using NTSys (Numerical Taxonomy and Multivariate Analysis System) Version 2.02.

3. Results and Discussions

Analyzed of genetic similarity of three durian population in Nias (Nias Kota, Nias Induk, and Nias Barat) used 4 locus SSR were DzMTb021, Dz621, Dz535, and Dz504 showed the result below.

3.1. Result of Isolated DNA Durian

Result of isolated DNA durian from 30 accession were visualized on 1% agarose (Figure 2). This test was done to find out the quality of DNA isolated so it could use to the next analyse were amplification of total DNA with PCR.

![Figure 2](image-url)

**Figure 2.** Visualization of isolated DNA from Nias’s durian (a) Nias Kota (b) Nias Induk (c) Nias Barat. M: Marker 1 Kb. 1-30 (Table 1)

**Table 3. Concentration and Purity of Durian DNA**

| No | Accession | Purity (260/280) | Concentration (ng/μl) | No | Accession | Purity (260/280) | Concentration (ng/μl) |
|----|-----------|----------------|------------------------|----|-----------|----------------|------------------------|
| 1  | NK1       | 1.8            | 4.250                  | 16 | NI6       | 1.9            | 1.050                  |
| 2  | NK2       | 1.9            | 0.655                  | 17 | NI7       | 1.8            | 0.852                  |
| 3  | NK3       | 2.0            | 0.210                  | 18 | NI8       | 1.6            | 0.560                  |
| 4  | NK4       | 1.9            | 5.323                  | 19 | NI9       | 1.5            | 0.820                  |
| 5  | NK5       | 1.8            | 0.940                  | 20 | NI10      | 1.7            | 0.340                  |
| 6  | NK6       | 2.0            | 0.660                  | 21 | NB1       | 1.9            | 0.960                  |
| 7  | NK7       | 1.6            | 1.320                  | 22 | NB2       | 1.7            | 0.620                  |
| 8  | NK8       | 1.5            | 0.670                  | 23 | NB3       | 1.5            | 0.960                  |
| 9  | NK9       | 1.7            | 1.620                  | 24 | NB4       | 1.7            | 2.090                  |
| 10 | NK10      | 1.7            | 1.650                  | 25 | NB5       | 1.6            | 1.775                  |
| 11 | NI1       | 1.9            | 1.140                  | 26 | NB6       | 1.8            | 1.340                  |
| 12 | NI2       | 1.9            | 1.130                  | 27 | NB7       | 1.8            | 0.450                  |
| 13 | NI3       | 1.8            | 2.390                  | 28 | NB8       | 1.7            | 0.488                  |
| 14 | NI4       | 1.8            | 1.400                  | 29 | NB9       | 1.9            | 0.393                  |
| 15 | NI5       | 1.6            | 2.650                  | 30 | NB10      | 1.7            | 5.496                  |

There was the different of the thickness of the bands on each well on the gel. The clear bands found on almost the entire gel. However, the isolated band not too thick. This is because of technique was still not optimal when grinded or when separated the pellet and supernatant. Isolated DNA had a size...
above 10,000 bp. [11] The thickness of a band proportional with the concentration of DNA, when the thicker DNA obtained then the higher concentration obtained.

**Table 3.** qualitative DNA test results showed the total value of the durian purity ranging from 1.5 – 2.0. The value of the purity of 1.8 – 2.0 indicates a good quality DNA purity, that is free from contaminants protein or RNA [12]. The results showed that the CTAB method was a good method for isolation of durian DNA. Concentration or purity of DNA produced were heavily influenced by technical factors during DNA isolation, one of which was at the time the move supernatan containing the DNA to a new tube. Pellet drying process on the final stage is also very influential. If drying is not good, then the pellets may still contain aqueous-solution such as alcohol or ethanol.

3.2. Amplification of DNA Total Durian Nias with SSR Markers
Electrophoresis results indicated that locus DzMTb021 and Dz535 successed amplified. Bands on the locus DzMTb021 based on the DNA ladder-sized 171-300 bp and 125-566 sized Dz535 Amplification of SSR motifs in the two pairs of the other primer also successfully carried out. [13] the DNA with the purity of 1.12 – 1.64 also capable amplified and yielding a clear amplification band. Visualization of two amplified locus SSR can be seen in **Figure 3.**

![Figure 3. Visualization of amplified locus on Nias Kota accession used primer: a. DzMTb021 b. Dz535. M: Marker 100 bp. 1-10 (Table 1)](image)

| No | Locus   | Allele Size (bp) | Percentage of Polymorphism (%) |
|----|---------|------------------|--------------------------------|
| 1  | DzMTb021 | 171-300          | 100%                           |
| 2  | Dz621   | 58-379           | 33.33%                         |
| 3  | Dz535   | 125-566          | 100%                           |
| 4  | Dz504   | 94-388           | 100%                           |

From **Table 4.** it can be seen that the lowest allele sizes owned by locus Dz504 and the highest by locus Dz535. The highest percentage of polymorphism present on all loci except locus Dz621 33.33%. There are marked with the polymorphism and the absence of the band as well as the size of the resulting band each accession.

3.3. *The Analysis of the Genetic Similarity of Durian Nias*
From the results of the clustering with the UPGMA method based on Coefisient Dice using NTSys Software can be seen politically different on each population.
Dendrogram of genetic similarity of accession Nias Kota with 4 locus SSR.

Dendrogram of genetic similarity of accession Nias Induk with 4 locus SSR.

Dendrogram of genetic diversity of accession Nias Kota

Figure 4. showed the results of the construction tree dendrogram 10 accession durian Nias Kota. All of accession durian Nias Kota clustered on the value of the coefficient of similarity was 0.72. On the value of the coefficient of 0.72 accession clustered into 2 big cluster. Cluster analysis based on the construction of the dendrogram tree separate accession into 5 clusters on the value of the coefficient of 0.84. The cluster is formed of 5 there is 1 cluster with the highest number of accessions, namely cluster 1. This cluster is composed of 3 accessions, namely accession NK1, NK10, and NK9. These accessions located in the different sub district. The other cluster is composed of 1 or 2 accessions originating from different sub district. There are 6 accession which has the closest genetic similarity, namely accession by NK1 with NK10, NK2 with NK7, NK3 with NK8 with the value of the coefficient of similarity 0.93. This means that there are 93% genetic similarity among the six accession with the difference of just 7%. Based on the 5 clusters that formed it can be concluded that
the genetic distance to accession Nias Kota high. This is because the cluster should be clustered on the original cluster sub district each cluster on the cluster that is different or separate.

**Figure 6.** Dendrogram of genetic similarity of accession Nias Barat with 4 locus SSR.

**Dendrogram of genetic diversity of accession Nias Induk**

**Figure 5.** Showed the results of the construction tree dendrogram 10 accession durian Nias Induk. All of accession durian Nias Induk clustered on the value of the coefficient of similarity was 0.74. Separate accession into 2 large cluster on the value of the coefficient of 0.74. Cluster analysis based on the construction of the dendrogram trees separates the accessions into 4 clusters on the value of the coefficient of 0.92. Cluster 1 is a cluster with the highest number of accessions that is made up of 6 accessions (NI1, NI7, NI8, NI9, NI10, and NI3). The cluster is derived from the same subdistrict but from the different village. On cluster 1 contained 5 accessions that has closest of genetic similarity namely NI1, NI7 NI8 NI9, and NI10 with a coefficient of similarity of 1.00 or 100% similarity. From the resemblance of the fifth accession can be inferred that the layout of the geography of the genetic similarities determined the accession of each accession. There are 1 the accession should be presumed clustered in clusters 1 because it comes from the same subdistrict, namely accession NI2. But these accessions were clustered in cluster 4 with the value of the coefficient of 0.74. This is likely caused by the activities of humans or animals that cause seed accessions NI2 brought to this area.

**Dendrogram of genetic diversity of accession Nias Barat**

**Figure 6.** showed the results of the construction tree dendrogram 10 accession durian Nias Barat. All of accession durian Nias Barat clustered on the coefficient if similarity of 0.59. Accession separated into 2 large cluster on the value of the coefficient 0.59. Cluster analysis based on the construction of the dendrogram trees separates the accessions into 4 clusters on the value of the coefficient of 0.76. Cluster 1 is a cluster with the highest number of accessions that is made up of 5 accessions (NB1, NB4, NB6, NB7, and NB8). Accessions of genetic similarity value is nearby is NB3 with coefficient of similarity NB5 1.00 or 100%. This population is the population that has a value of genetic similarity is low compared to other populations.
Figure 7. Dendrogram of genetic similarity of accession Nias Kota, Nias Induk, and Nias Barat with 4 locus SSR.

Dendrogram of genetic diversity of accession Nias Kota, Nias Induk, and Nias Barat

Figure 7. showed the results of the construction tree dendrogram 30 accessions of durian. All of accession durian Nias clustered on the value of the coefficient of similarity was 0.63. Accession separated into 3 large cluster on the value of the coefficient of 0.63. Cluster analysis based on the construction of the dendrogram tree separate into 8 clusters of accessions in the value of the coefficient of 0.81. Cluster 4 is the highest number of accessions with a cluster that is composed of 12
accessions. These accessions were from a third of the population. In this cluster, there are 5 accessions from Nias Induk and 1 accession from Nias Kota which has a very close genetic similarities namely NK3, NI1, NI7 NI8, N9, and NI10 with the value of the genetic similarity of 1.00 or 100%. This showed that the SSR locus were used on the distinguishes research *Durio zibethinus* is not accession by region of origin, but based on the phylogenetic relationship. Cluster 1 is composed of the same accession that is derived from the Nias Kota. Cluster 6 and 7 comprised of accessions originating from Nias Barat but with a fewer number of accessions. Cluster 3 and 4 is a cluster consisting of accessions originating from populations of Nias Kota, Nias Induk and Nias Barat. There is also a cluster of its own accession, namely the accession of NK6 in cluster 2, NB1 in cluster 5, and NB10 in cluster 8. Accessions with the lowest genetic similarity was NB10 with the coefficient of similarity of 0.63 or 63%. If seen from its geographical location, it is located at Kecamatan accession Mandrehe which accessions originating from Sub Mandrehe there is only one i.e. accession NB10. [14] The number one on the dendrogram analysis indicates the accession has the perfect similarity between groups, while getting closer to zero means the farther distance similarity. So, it can be inferred that if further genetic distance between individuals owned the higher its genetic diversity.

4. Conclusions
a. Thirty accessions of durian Nias Kota, Nias Induk and Nias Barat were analyzed using 4 SSR locus DzMTb021, Dz621, Dz535, and Dz504 had different values. All of locus had the highest percentage of polymorphic except Dz621 locus.
b. Genetic similarity of 30 durian from three population was 63%, and genetic similarity of durian within NK, NI, and NB population was 72, 74, and 59% respectively. The similarity of NK and NI population ws the highest when compared to NB.

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