GENETIC DIVERSITY AMONG DURIAN (*DURIO ZIBETHINUS* MURR.) POPULATIONS FROM NIAS ISLAND, INDONESIA USING RAPD MARKERS

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Abstract. Indonesian durian (*Durio zibethinus* Murr.) germplasms cultivated on Nias Island were assessed for their genetic diversity and relationships using Random Amplified Polymorphic DNA (RAPD) markers. Fifty accessions were collected from five populations or zones in Nias Island, Indonesia. All RAPD fragments were polymorphic as revealed by the use of OPA-01, OPA-03, OPA-07, OPA-13, OPN-06, and RAPD-05 primers within and among the durian germplasms. Primer OPA-03 was evaluated as a highly informative marker. Similarity values (Jaccard) ranged from 0.216 to 0.423 between different populations and 0.00 to 0.89 within the populations. The genetic structure between populations ranged from 1.71 to 1.92 for mean number of alleles (n_a), and from 1.35 to 1.59 for effective number of alleles (ne). Shannon's diversity index (*I*) ranged from 0.34 to 0.51, and the Nei's gene diversity index (*h*) ranged from 0.21 to 0.34. Result of cluster analysis based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) grouped all germplasms into thirteen clusters, some were admixtures between different populations. Result of Principal Coordinate Analysis (PCoA) confirmed the UPGMA dendrogram with the results of five main clusters among durian accessions from Nias Island, Indonesia.
Keywords: *durian, germplasm, polymorphism, principal coordinate analysis, molecular diversity*

Introduction

Durian (*Durio zibethinus* Murr.) is one of the edible *Durio* species with an approximate number of 30 species dispersed throughout the world (Brown, 1997). This popular tropical fruit species has been cultivated mainly in Southeast Asia, including Malaysia, Thailand, and Indonesia as a potential export commodity. Indonesia's durian production is thought to increase following an average annual increment of 4.86% in 2013 which also coherently increased its selling price from year to year due to its popularity and high market demands (CDISA, 2014). The popularity basis of this fruit is due to its sweet and strong-pungent aroma with a fleshy texture of its edible pulps (Belgis et al., 2017). In addition, the fruits are rich in essential nutrients which may provide beneficial health properties to the consumers as well as supporting the local business of many durian farmers to improve their socio-economic life (Ho and Bhat, 2015).

There are currently two mostly known and favored durian species from Indonesia, namely durian (*D. zibethinus* Murr.) and lai (*D. kutejensis* Hassk. & Becc.), both were reported originate from and harvested mainly in Kalimantan (Borneo) (Reksodihardjo, 1962). Moreover, Borneo is described as one of the durian germplasm centers in the world with a total of 18 species, followed by Malaya (11 species), and Sumatra (7 species) while several Indonesian durian species has also been regarded as endemic germplasms (Kostermans, 1953; Nyffeler and Baum, 2000; Uji, 2005). Until 2015, the

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Government of Indonesia has listed 93 superior local durian cultivars compiled from three officially-commercialized durian species, the *D. zibethinus, D. kutejensis,* and *D. oxleyanus* with future possibilities on identifying other wild cultivars to be promoted and listed in Indonesian collection of indigenous durian cultivars (Santoso et al., 2016).

Information on the diverse local cultivars will also support the plant breeding sector, especially the crop breeder to produce progenies with adaptive and varying phenotypes or to select parental lines with improved productivity responding to future changes (Moreno and Trujillo, 2005). Among three durian species in Indonesia, *D. zibethinus* cultivars are mostly known and consumed especially in Sumatra and Java due to its abundance and availability regardless of harvesting period. Nias Island is one of the regions in North Sumatra, located in the eastern Indian Ocean, known for its intense trade of many local *D. zibethinus* cultivars compared to other provinces in Indonesia. The island is inhabited by local tribe or the Nias people who are practically cultivating *D. zibethinus* in many agricultural fields or harvesting the wild cultivar directly from the forest (Hannum et al., 2019). Moreover, Gunungsitoli as the central city of Nias Island, has been declared by the Government of Indonesia as an iconic city for durian tourism to increase the socio-economic life of the locals. However, there is no information and comprehensive study on their *D. zibethinus* germplasm originating from the island.

To date, studies on identifying or grouping the *D. zibethinus* populations (cultivars) based on their genetic characteristics using genetic markers have been reported. Genetic markers and polymerase chain reaction (PCR), produced a more informative result in characterizing the genetic diversity than morphological features, along with its advantages and limitations (Weising et al., 1995; Ibrahim et al., 2010). Genomic profiling using RAPD markers, has been reported as a preliminary investigation on the classification of durian cultivars in countries with abundant durian germplasms, including Indonesia (Ruwaida et al., 2009; Vanijajiva, 2011; Mursyidin and Daryono, 2016; Prihatini et al., 2016; Rosmaina et al., 2016). RAPD analysis was favored as the initial method in genetic fingerprinting due to its simplicity, low-cost, tiny amount of target DNA requirement, and non-requirement of target genetic sequences (Welsh and Mc-Clelland, 1990; Williams et al., 1990). Practicality of these markers increased their performance as attractive guidance in studying the overall genetic variation and population genetic structure (Lynch and Milligan, 1994). To our knowledge, RAPD-based profiling of local durian cultivars has been reported for some regions in Indonesia, but still no information for the cultivars originating from Nias Island in North Sumatra. In this study, six RAPD markers were utilized to evaluate the genetic variation among 50 durian accessions with an emphasize on the relationship between and within durian populations from Nias Island, Indonesia.

Methodology

Plant materials

Durian (*Durio zibethinus* Murr.) accessions from five different populations comprising Nias City, Central Nias, West Nias, North Nias, and South Nias were collected randomly from Nias Island in 2019 (*Fig. 1*). The list of 50 durian accessions and their vernacular names is listed in *Table 1*. The foliar parts were sampled and preserved in a dried zip-lock bag filled with silica beads prior experimentation in the laboratory (*Fig. 2*).

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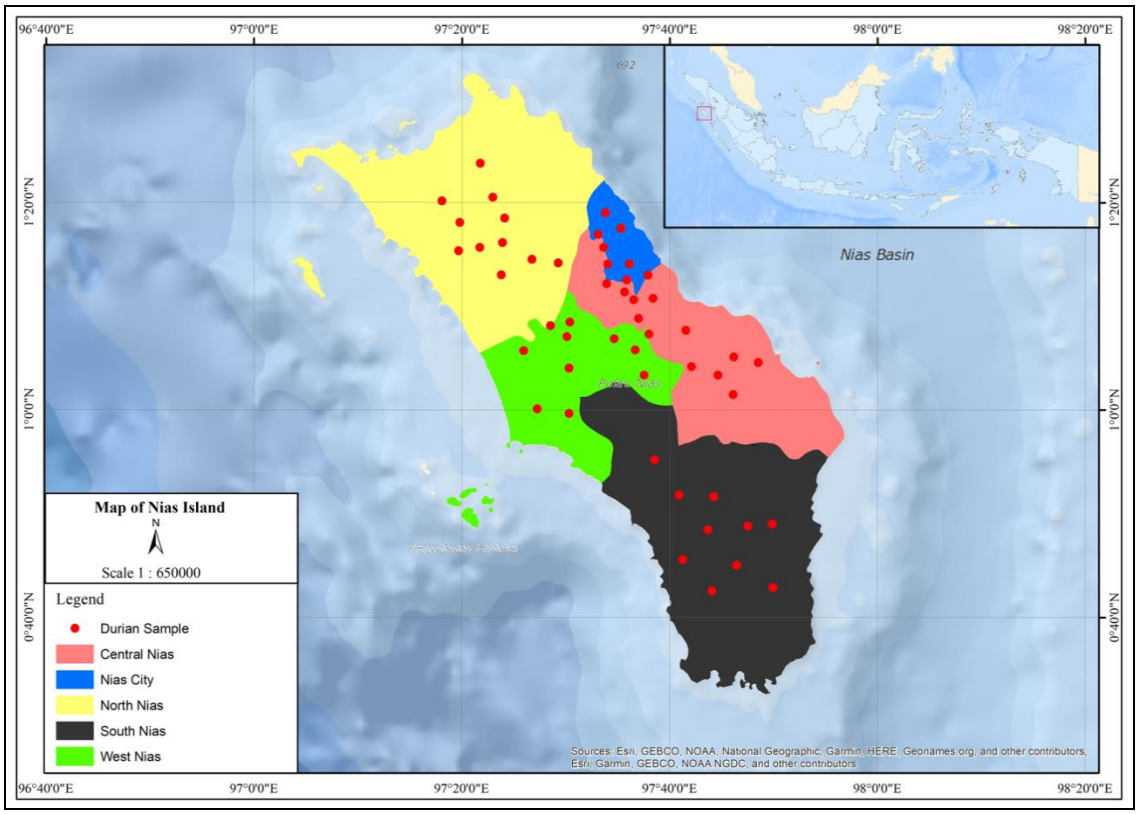

Figure 1. *Map showing sampling sites of Nias Island for study of durian cultivar diversity*

Figure 2. *A typical durian (D. zibethinus) tree and its fruit in Nias Island. Scale bar = 5 cm*

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Table 1. *List of 50 durian germplasms from Nias Island, Indonesia used in this study*

Code	Local name	Zone of collection	GPS Coord.	Code	Local name	Zone of collection	GPS Coord.
N1-1	Afia	Nias City	1°22’35”N 97°32’51”E	N3-6	Hilimbowo Ma’u	West Nias	1°3’47”N 97°37’47”E
N1-2	Bawodesolo	Nias City	1°20’4”N 97°31’47”E	N3-7	Hilimbuasi_1	West Nias	1°2’43”N 97°35’25”E
N1-3	Hilihao_1	Nias City	1°15’3”N 97°35’23”E	N3-8	Hilimbuasi_2	West Nias	1°3’12”N 97°35’3”E
N1-4	Hilihao_2	Nias City	1°15’3”N 97°36’21”E	N3-9	Sisobawino	West Nias	1°2’12”N 97°36’43”E
N1-5	Lasaratarakhaini_1	Nias City	1°18’56”N 97°30’30”E	N3-10	Tuwuna	West Nias	1°5’14”N 97°34’18”E
N1-6	Lasaratarakhaini_2	Nias City	1°20’18”N 97°31’48”E	N4-1	Alo’oa	North Nias	1°27’31”N 97°29’16”E
N1-7	Lasaratarakhaini_3	Nias City	1°19’49”N 97°32’25”E	N4-2	Botolakha_1	North Nias	1°26’3”N 97°29’56”E
N1-8	Sisobahili_1	Nias City	1°19’59”N 97°35’17”E	N4-3	Botolakha_2	North Nias	1°26’16”N 97°30’24”E
N1-9	Sisobahili_2	Nias City	1°19’26”N 97°35’0”E	N4-4	Hiligodu	North Nias	1°23’53”N 97°23’18”E
N1-10	Teluk Belukar	Nias City	1°23’54”N 97°31’26”E	N4-5	Hilimaziaya	North Nias	1°23’31”N 97°25’39”E
N2-1	Botombawo_1	Central Nias	1°12’31”N 97°34’21”E	N4-6	Hilimbosi_1	North Nias	1°24’2”N 97°29’30”E
N2-2	Botombawo_2	Central Nias	1°12’14”N 97°34’10”E	N4-7	Hilimbosi_2	North Nias	1°23’57”N 97°29’39”E
N2-3	Fadoro Lai’o	Central Nias	1°11’46”N 97°34’7”E	N4-8	Hilinduria	North Nias	1°23’19”N 97°23’51”E
N2-4	Hilimbowo	Central Nias	1°10’49”N 97°32’48”E	N4-9	Umbu Balodano	North Nias	1°24’6”N 97°29’27”E
N2-5	Hiliwaele	Central Nias	1°10’6”N 97°35’27”E	N4-10	Ambukha 1	North Nias	0°54’5”N 97°39’56”E
N2-6	Lalai_1	Central Nias	1°11’22”N 97°36’42”E	N5-1	Ambukha 2	South Nias	0°53’31”N 97°40’34”E
N2-7	Lalai_2	Central Nias	1°9’46”N 97°35’36”E	N5-2	Amorosa	South Nias	0°54’53”N 97°40’1”E
N2-8	Lawe-lawe	Central Nias	1°12’29”N 97°33’21”E	N5-3	Caritas Sogawunasi_1	South Nias	0°56’55”N 97°38’14”E
N2-9	Lolofaoso Lalai	Central Nias	1°9’51”N 97°37’2”E	N5-4	Caritas Sogawunasi_2	South Nias	0°57’10”N 97°38’21”E
N2-10	Lolowua	Central Nias	1°11’16”N 97°34’38”E	N5-5	Ehosakhozi	South Nias	1°1’16”N 97°36’25”E
N3-1	Ambukha_1	West Nias	1°2’8”N 97°35’28”E	N5-6	Hilimbosi_3	South Nias	1°24’11”N 97°29’54”E
N3-2	Ambukha_2	West Nias	1°2’24”N 97°35’11”E	N5-7	Hilisangowola	South Nias	0°57’15”N 97°36’52”E
N3-3	Duria	West Nias	1°2’14”N 97°36’56”E	N5-8	Koendrafo	South Nias	0°52’51”N 97°40’3”E
N3-4	Hili’uso_1	West Nias	1°5’12”N 97°34’58”E	N5-9	Lawa-lawa luo	South Nias	0°57’0”N 97°37’40”E
N3-5	Hili’uso_2	West Nias	1°4’9”N 97°35’11”E	N5-10	Suka Maju	South Nias	0°54’39”N 97°39’36”E

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DNA isolation and PCR amplification

Extraction of DNA genomes was carried out from foliar parts of selected accessions following the principle of hexadecyltrimethylammonium bromide (CTAB) with modification on using 4% CTAB solution and additional 2 mg of polyvinylpyrrolidone (PVP) during extraction (Murray et al., 1980). The isolated DNA was quantified at $A_{260/280}$ using NanoPhotometer P-Class® (Implen, US) and was visually checked on 1% agarose. Six decamer RAPD primers namely OPA-01, OPA-03, OPA-07, OPA-13, OPN-06, RAPD-05 were screened for amplification of scorable and reproducible DNA fragments. PCR reaction of a 20 µL reaction mixture contains: 10 µL (0.5 unit) of DreamTaq Green PCR Master Mix (2×), 2 µL (100 ng) of DNA template, 1 µL (0.6 mM) of primer, and 7 µL nuclease-free water. Thermal cycler (SensoQuest GmbH, Germany) was used for PCR-RAPD reactions with specification as follows: 95 °C (30 s), 34 °C (30 s), 72 °C (1 min), and 72 °C (5 min). The reaction products (5 µL) were mixed thoroughly with 2 µL loading dye prior loading. Both PCR products and DNA ladder (1 kb) were separated in 1.5% agarose with 1× TAE buffer. The PCR products were resolved by running gel at 70 V for 45 min. The gels were stained in 1 L of distilled water added with 10 µL of ethidium bromide for 10 min. The gels were observed under UV light and documented inside of a gel imaging device (G:Box Syngene, India). PCR-RAPD was repeated twice to ensure the reproducibility of reactions.

Data analysis

DNA polymorphism was evaluated and scored manually based on the presence (1) and absence (0) for each scoreable DNA fragment to generate a binary data set. The number of alleles and polymorphic bands were calculated for each primer as the percentage of polymorphisms (%). Determination of the marker suitability was evaluated through three parameters:

The polymorphic information content (PIC) value of each primer was the average of all loci in a primer (Powell et al., 1996):

$$Polymorphic\ Information\ Content\ (PIC) = 2fi(1 - fi) \quad \text{(Eq.1)}$$

The marker index (MI) value which explains the usefulness of a marker to discriminate the polymorphism. The effective multiplex ration (EMR) was obtained from a multiplication of the number of loci polymorphic in the germplasm set of interest (Varshney et al., 2007):

$$Marker\ Index\ (MI) = EMR\ x\ PIC \quad \text{(Eq.2)}$$

The resolving power (RP) value shows the ability of the most informative primers to discriminate genotypes of interest, where i_b is the band informativeness (Prevost and Wilkinson, 1999):

$$Resolving\ Power\ (RP) = \sum i_b \quad \text{(Eq.3)}$$

Genetic diversity among populations was estimated by following parameters: the average number of alleles (n_a), effective number of alleles (n_e), heterozygosity (h) (Nei,

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1973), Shannon's diversity index (*I*) (Shannon, 1949), and percentage of polymorphic loci (P%) using POPGENE ver. 1.32 (Yeh and Boyle, 1997). The binary data of each population and accession were calculated for the similarity values (Jaccard, 1908) using NTSYS-pc ver. 2.1 by applying Similarity for Qualitative Data, Simple Matching (SimQUAL-SM) feature (Rohlf, 2000). The genetic relatedness or cluster analysis among populations and within 50 durian accessions was visualized in a UPGMA dendrogram by applying the Sequential Agglomerative Hierarchical and Nested (SAHN) module in NTSYS-pc. In addition, a Principal Coordinate Analysis (PCoA) was used to plot the ordination matrices using EIGEN and PROJ features (NTSYS-pc).

Results

The assessment of genetic diversity among 50 durian germplasms used six RAPD primers to characterize their genetic information based on the presence/absence of polymorphic bands (*Fig. 3*). We observed that all primers produced legible and reproducible polymorphic patterns in two repetitions. Two factors in determining the genetic diversity using molecular markers are marker informativeness and marker performance with the results listed in *Table 2*. Each RAPD primer was evaluated for its informativeness based on the following parameters (*Table 2*). Sixty-six clear and reproducible RAPD fragments (200–2800 bp) was generated in this study. Interestingly, all RAPD bands showed a high level of polymorphism (100%) which also meant that no conserved pattern or monomorphic bands were observed. The average of polymorphic bands was eleven with the range from nine (OPA-01, RAPD-05) to fourteen (OPN-06) with an average of eleven. The polymorphic RAPD fragments were carefully scored and utilized for clustering and ordination analysis herein.

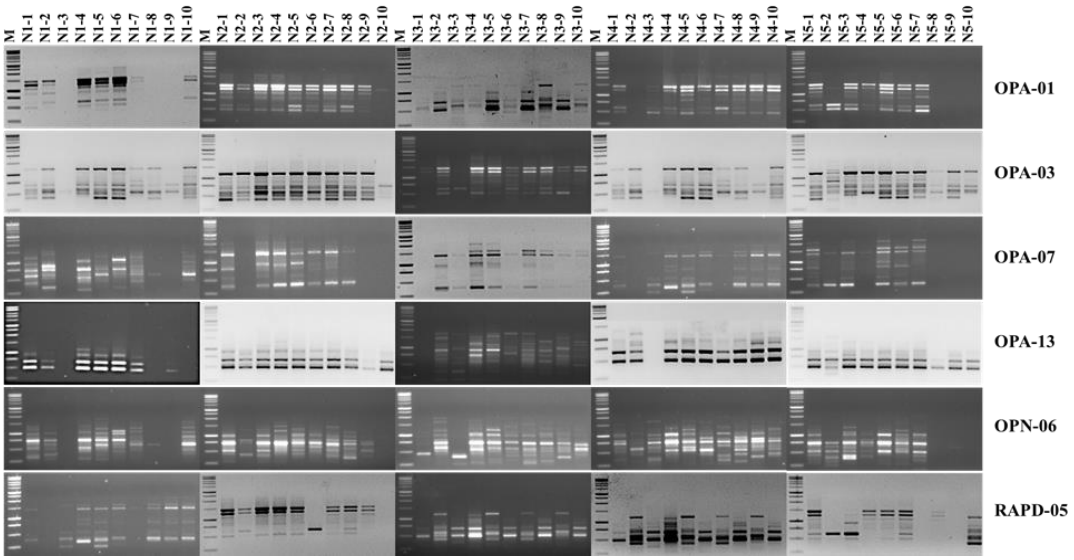

Figure 3. *Genetic profile of the 50 Durio zibethinus Murr. accessions (N1-1–N5-10) generated by five RAPD primers. Marker used was 1 kb (250-10,000 bp)*

Each accession was analyzed for the evaluation of its marker performance based on the following parameters (*Table 2*). The average PIC value was 0.42 for 66 polymorphic bands with the range between 0.29 to 0.48. Forty-three RAPD fragments were regarded

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as greatly informative (PIC ≥ 0.4), four fragments were low (0.0–0.2), while the remaining nineteen fragments were medium (0.2–0.4) (*Fig. 4*). OPA-01 was observed as with the highest PIC value (0.48) while the lowest (0.29) was obtained from RAPD-05. The highest EMR (7.36) was observed for primer OPA-03 with the mean per primer was 4.46 (*Table 2*). The overall performance from each RAPD marker was evaluated from its marker index (MI). The MI for the six primers ranged from 0.50 (RAPD-05) to 3.46 (OPA-03) with an average of 1.94. The resolving power (RP) of RAPD primers is a parameter which indicates a discriminatory properties, ranged from 3.48 (RAPD-05) to 10.24 (RAPD-03) with an average of 7.47. There were significant correlations for PIC–MI ($r = 0.85$, $P = 0.032$), EMR–MI ($r = 0.99$, $P = 0.000$), EMR–RP ($r = 0.94$, $P = 0.006$), and MI–RP ($r = 0.90$, $P = 0.015$) which showed that the parameters of marker performance resulted in our study were considerably related each other.

Table 2. *Polymorphism and marker characteristics of RAPD primers*

Primer name	Sequence (5′- 3′)	Number of fragments			% Polymorphism	PIC	EMR	Marker index	Resolving power
		Total	Mono-	Poly-					
OPA-01	CAGGCCCTTC	9	0	9	100	0.48	4.80	2.30	7.28
OPA-03	AGTCAGCCAC	13	0	13	100	0.47	7.36	3.46	10.24
OPA-07	GAAACGGGTG	11	0	11	100	0.44	4.54	2.00	7.96
OPA-13	CAGCACCCAC	10	0	10	100	0.44	3.86	1.70	7.32
OPN-06	GAGACGCACA	14	0	14	100	0.38	4.44	1.69	8.56
RAPD-05	AACGCGCAAC	9	0	9	100	0.29	1.74	0.50	3.48
Total	-	66	-	66	-	-	-	-	-
Average	-	11	-	11	100	0.42	4.46	1.94	7.47

Genetic diversity of five durian populations from Nias Island was estimated based on the RAPD amplification results under following range of values: effective number of alleles (n_e) from 1.35 to 1.59, mean number of alleles (n_a) from 1.71 to 1.92, Nei's gene diversity index (h) from 0.21 to 0.34, and Shannon's diversity index (I) from 0.34 to 0.51 (*Table 3*). Based on the polymorphic loci (P%), West Nias and North Nias displayed the highest variation of durian cultivars with the percentage of 92.42 for both zones. According to the overall variation resulted from P%, the ranking among Nias durian population was West Nias/North Nias > South Nias > Central Nias > Nias City. Our findings then strongly suggested that the durian germplasms cultivated in five different regions were highly varying as evidenced effectively through RAPD genetic analysis.

Table 3. *Estimates of genetic diversity within five populations (zones) using RAPD markers*

Index	Nias City	Central Nias	West Nias	North Nias	South Nias	All
n_a	1.71	1.88	1.92	1.92	1.89	2.00
n_e	1.35	1.55	1.52	1.59	1.56	1.77
h	0.21	0.32	0.31	0.34	0.33	0.42
I	0.34	0.47	0.47	0.51	0.49	0.60
P%	71.21	87.88	92.42	92.42	89.39	100.0

n_a = Average number of alleles; n_e = Effective number of alleles; h = Heterozygosity; I = Shannon's diversity index; P% = Percentage of polymorphic loci

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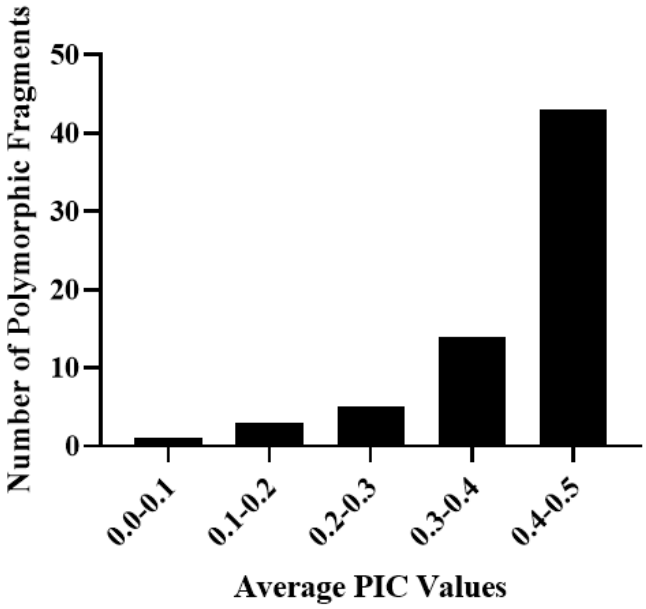

Figure 4. *Average PIC values for polymorphic bands generated by RAPD primers in 50 durian accessions*

The similarity values among populations were estimated using Jaccard's similarity matrices retrieved from RAPD binary data. The similarity values ranged from 0.216 to 0.423 with an average of 0.31 (*Table 4*). A UPGMA dendrogram was constructed to depict the populations grouping (*Fig. 5*). The result showed the ingrouping of Nias City, West Nias, and North/South Nias populations with Central Nias being an outlier. In addition, results showed that populations of North Nias and South Nias were more similar (42%) than others while the population of North Nias was separated indicating its high genetic dissimilarity.

Table 4. *Similarity matrix based on Jaccard's coefficient from five populations (zones)*

Index	Nias City	Central Nias	West Nias	North Nias	South Nias
Nias City	1.000	-	-	-	-
Central Nias	0.263	1.000	-	-	-
West Nias	0.333	0.294	1.000	-	-
North Nias	0.216	0.233	0.367	1.000	-
South Nias	0.410	0.175	0.342	0.423	1.000

Genetic diversity among 50 durian individuals or accessions were based on the Jaccard's similarity values. The similarity value of overall accessions ranged from 0.00 to 0.89 with an average of 0.44 (data not shown) indicating a close genetic relationship within the accessions. A UPGMA dendrogram revealed the eight main clusters and four outliers (cluster 6,7,8,10), with cluster (1) comprises the largest members with fourteen germplasms (*Fig. 6*). In order to visualize a better degree of differentiation among accessions, the spatial analysis using PCoA was utilized to represent the relative genetic similarities among individuals. The result was plotted into a two-dimensional plot generated from PCoA based on the calculated eigenvalues and eigenvectors.

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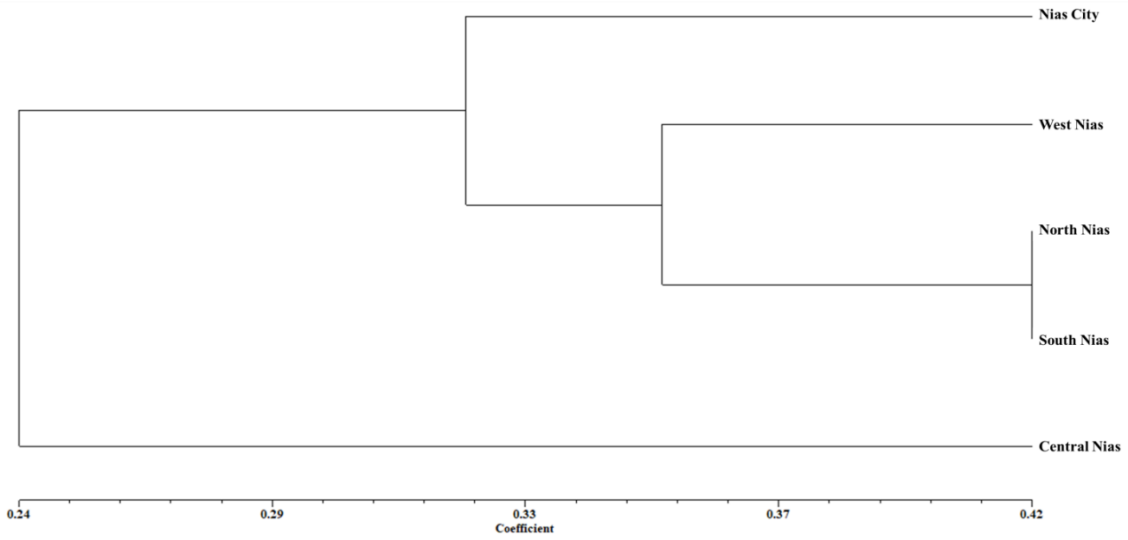

Figure 5. *A UPGMA dendrogram among five populations (zones) based on Jaccard´s similarity coefficient calculated from RAPD data set*

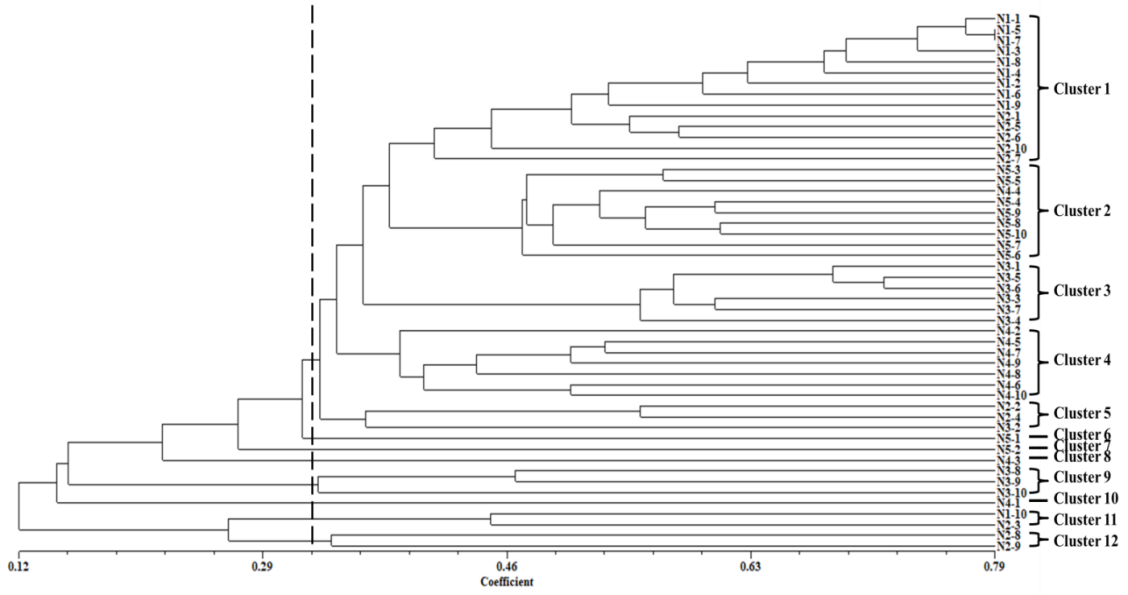

Figure 6. *A UPGMA dendrogram among 50 durian cultivars (N1-1–N5-10) based on Jaccard´s similarity coefficient calculated from RAPD data set*

Results showed that the first two principal coordinates contributed for 53.9% of the total variation, with lesser contribution of other coordinates (<4%) each (*Fig. 7*). The accessions were later grouped into five clusters based on their coordinates; cluster (1) comprises four germplasms, cluster (2) with one germplasm, N4-1, cluster (3) with three germplasms, cluster (4) with sixteen germplasms, and cluster (5) with twenty-six germplasms. Moreover, the three-dimensional plot was generated and confirmed the coordinative trend with the 2D plot indicating a clear distinction among the 50 accessions (*Fig. 8*).

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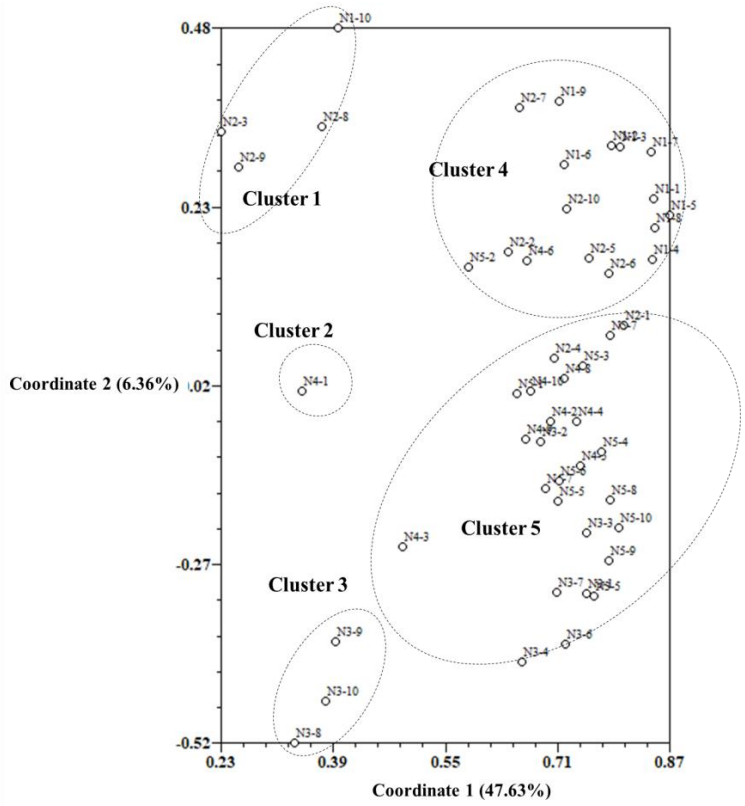

Figure 7. *Two-dimensional plot generated from Principal Coordinate Analysis (PCoA) of 50 durian cultivars using RAPD markers*

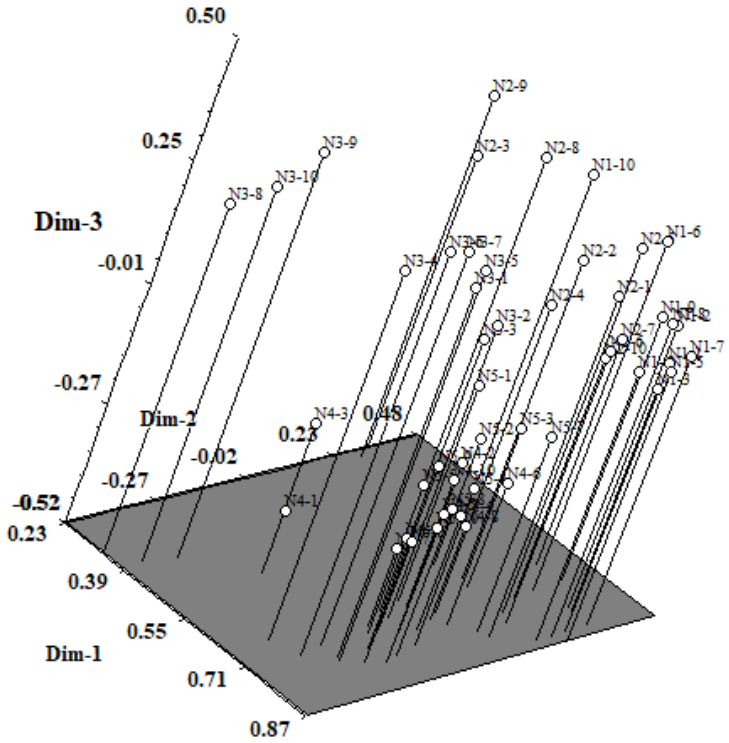

Figure 8. *Three-dimensional plot generated from Principal Coordinate Analysis (PCoA) of 50 durian cultivars using RAPD markers*

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Discussion

Identification of new crop germplasms by using DNA fingerprint is important prior the release and introduction of new varieties/cultivars to the agricultural fields and the differentiation of wild accessions bearing beneficial phenotypic traits. Genetic diversity and structure among 50 durian (*D. zibethinus* Murr.) accessions cultivated in different zones (regions) of Nias Island, Indonesia were analyzed using RAPD markers in this study. The PCR-RAPD has been utilized to employ the genetic characterization and profiling among germplasms for the major reason of its simplicity and ease of application. However, RAPD technique had some limitations, such as dominant type of inheritance, poor reproducibility, and co-migration issue (Munthali et al., 1992; Lowe et al., 1996). In this study, we optimized the reaction conditions and found out that during two repetitions, good and scorable DNA fragments were generated and they were consistent hereafter. Six RAPD primers (OPA-01, OPA-3, OPA-07, OPA-13, OPN-06, RAPD-05) produced an absolute polymorphism percentage (100%) among 50 accessions based on the absence/presence or binary data of DNA fragments. Therefore, the six RAPD markers used in this study were regarded as equally effective in determining polymorphisms as revealed from its PIC value in the level from moderate to high informative (Hildebrand et al., 1992).

RAPD primer with high PIC value also indicated the more usefulness of its utilization among primers, following other parameters such as EMR, MI, and RP in determining the genetic distance within a population (Kalinowski, 2002). Variety of RAPD primers have been reported to determine the genetic diversity of other local durian cultivars. Ruwaida et al. (2009) also employed six RAPD primers (OPA-01, OPA-02, OPA-07, OPA-16, OPA-18, OPA-19) in assessing the diversity of five durian cultivars from Java, Indonesia and reported them to be sufficient in separating between cultivars. Mursyidin and Daryono (2016) utilized five RAPD primers (OPA-01, OPA-07, OPA-16, OPA-18, OPA-19) to differentiate 11 durian accessions from South Kalimantan, Indonesia. Li and Midmore (1999) suggested that the use of few primers will be satisfactory in differentiating genotypes with high variations.

The high level of diversity in durian cultivars may due to the existence of wild hybrids within populations since durian is known as open pollinating plants in which pollination being facilitated by mostly fruit or nectarivorous bats (Bumrungsri et al., 2009). In addition, utilization of different DNA markers and other profilling techniques may produce different results in estimating the durian genetic diversity. Rosmaina et al. (2016) reported the high variation among five durian cultivars from Indonesian germplasm with a low genetic relationship or Nei and Li's genetic distance (0.27 to 0.47) based on RAPD analysis. Prihatini et al. (2016) reported a high variation among 32 durian F_1 hybrids from Indonesia based on RAPD analysis (0.14 to 0.77). Vanijajiva (2012) applied the inter simple sequence repeat (ISSR) markers to detect genetic variation among 14 durian accessions from Thailand and reported the less genetic relationship (0.63 to 1.00). Sundari et al. (2017) reported a medium to high level of genetic diversity (0.41 to 0.93) 15 durian cultivars from Ternate Island, Indonesia based on RAPD analysis. Nurlaila et al. (2019) reported the medium level of variation among 18 local durian varieties from South Sulawesi, Indonesia by using the morphological traits (0.08 to 0.50).

Based on our results, the fifty durian accessions collected from five different zones or populations in Nias Island were highly diverse (0.21 to 0.42) among populations. Hence, the result again supported the fact that the genetic diversity of Nias cultivars

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were slightly higher than previous reports and worth investigated. Based on the cluster analysis, the dendrogram showed a close relationship between North and South durian populations, while Central Nias and Nias City were different despites its adjacent location. Furthermore, the dendrogram among 50 accessions showed that some accessions with different geographical origins or zones were clustered together in the same group. We assumed that the phenomenon may due to the mobility of local farmers from the Central Nias/Nias city or any initial geographical points to other regions cultivating the durian seeds in the new agricultural locations. The similar finding was also reported from sesame (*Sesamum indicum* L.) populations in West Bengal, India as revealed from the RAPD analysis (Saha et al., 2019). Human factor may then be responsible for the geographical detachment of associated cultivars in some cases (Stankiewicz et al., 2001).

Deeper analysis among accessions was done by plotting the converted binary data into eigenvalues and projected using principal coordinate analysis (PCoA). Based on the 2D plot, we assigned five clusters out of the thirteen durian clusters constructed previously from UPGMA dendrogram analysis, with some admixtures among populations. Confirmatory analysis using the 3D plot projection using three coordinates supported our grouping although some accessions between cluster (4) and (5) were indiscriminative to some coordinates. N4-1 was the only cultivar discriminated and far distantly from other accessions hence grouped into own cluster (2). The existence of N4-1 cultivar in Nias Island revealed its unique genetic identity which may be explored thoroughly for any beneficial and tolerant traits to environmental changes.

In practical term, N4-1 may divert even more further than its members in the long term which can later be categorized as a new species or sub-species. Further investigations are needed to uncover this phenomenon since no comprehensive study on the botanical backgrounds or information of durian cultivars from Nias Island so far. The PCoA method has been used to discriminate many crop cultivars based on the RAPD database, such as Brazilian coffee plants (Sera et al., 2003), Indian coconut accessions (Upadhypay et al., 2004), Turkish marijuana accessions (Pinarkara et al., 2009), Thailand durian populations (Vanijajiva, 2011), durian populations from Indonesia (Santoso et al., 2016), and our study. Interestingly, Santoso et al. (2016) found that the Sumatra and Kalimantan durian cultivars were closely related based on the PCoA projection through the use of microsatellites as molecular markers. However, the study only used the accessions from the mainland of North Sumatra and still not incorporated the accessions from Nias Island. We assumed that more distinctive genotypes may be observed by studying the durian cultivars from Nias Island as evidenced from RAPD analysis in this study, but this will need a more comparative studies in the future.

Conclusion

Based on the genetic similarity and population genetic structure analysis, the study concluded that the local durian cultivars originating from Nias Island were highly diverse. The result of clustering analysis produced thirteen clusters with some admixtures between different geographical populations. Under population level, the North Nias and South Nias were closely related revealing a possible human factor in durian diversification in the level of varieties or cultivars. Based on PCoA projection, we further grouped the durian populations into five clusters with major accessions

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grouped into cluster (4) and (5). Moreover, N4-1 is the only cultivar placed in its own cluster (2) indicated a distinct genetic identity among cultivars. As a recommendation related to this study, we suggest that N4-1 may be possibly harnessed and studied for its beneficial genotypic and phenotypic agricultural traits such as its fruit quality and productivity with other characteristics in agronomical aspect. Therefore, a correlation study between agro-morphological and genetical traits from each cultivar must be assessed for a rapid identification of cultivars under practical terms in the future.

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