GENETIC VARIABILITY OF LOCAL DURIAN (*Durio zibethinus* Murr.) IN TERNATE ISLAND BASED ON RAPD MARKERS

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ABSTRACT

Ternate Island is one of the centers of durian production in North Maluku, Indonesia. The genetic variability of local durian varieties in Ternate Island has not been thoroughly studied so far. Although morphological variation has been reported, further analysis of their genetic variability is still needed. In the present study, the local Ternate durian polymorphism based on RAPD molecular markers was assessed to confirm previous information about their morphological variation and simultaneously evaluated their genetic variability. Twenty-seven variants of local durian were obtained from 15 villages in Ternate Island. The genomic DNAs of those variants were isolated and amplified using six selected RAPD primers. The resulted RAPD bands were analyzed with NTSYSpc 2.2 Software to know the variability among the variants. T4 and T2 variants had the closest relation among the 27 variant samples with similarity index value of 0.938. T12- T15 variants had the similarity index value (0.854) while T7 variant had close relations with variant T2 and T4 with similarity index value of 0.896. Furthermore, the farthest distants were observed between T1 and T15, T15 and T16, and T26 and T19 with similarity index value of 0.417.

Keywords: Genetic variability; local durian Ternate; RAPD.

INTRODUCTION

Ternate Island is one of the centres of durian production in North Maluku, Indonesia. Based on their morphological characters, Ternate local durians are belonging to variants of *Durio zibethinus* Murr. Ternate durians grow naturally from the seeds in the garden of local ownership from generation to generation. In general, local durians have local names that given by local community based on the owner's names, addresses and the locations where durians are grown or based on the distinctive characters of fruit morphology. Explorations of Ternate durian have been obtained as many as 13 variants of local names given by the people of Ternate. Based on their morphological characters, they were categorized to three main groups namely: group I (Udi and Sina local durian); Group II (Air tege-tege and Luri kecil local durian); group III (Mentega and Gajah kuning local durian) (Sundari et al., 2015).

The genetic variation in local durians in Ternate and North Maluku has not been assessed yet. Mostly, many researches have intended to examine genetic variation of durian based on morphological characters of trees, leaves, flowers and fruits (Sundari et al., 2015; Tolangara and Sundari, 2014). Since morphological characters have limitations on distinguishing genetic variation within species that caused by environmental changes and the age of the plant, thus studies are needed using molecular markers to investigate the genetic variation of local durian. Molecular markers tend to be stable against environmental changes and age, thus molecular markers may relatively provide more accurate information (Weising et al., 1994; Sukartini, 2008; Vanijajiva et al., 2005).

DNA-based molecular markers are the best choice for assessing genetic variation. Random amplified polymorphic DNA (RAPD) is highly sensitive for detecting genomic polymorphism. RAPD-PCR method is based on PCR (Polymerase Chain Reaction) using random sequence primers for the purpose of random amplification of genomic loci (Williams et al., 1990; Rafalski et al., 1991). This method has advantages in simplicity of the technique and fast process (Hu and Quiros, 1991), thus it is suitable for analyzing large sample and useful for supporting plant breeding, population genetics and biodiversity study (Rafalski et al., 1991; Waugh and Powell, 1992; Yang and Quiros, 1993). Moreover, RAPD technique has many benefits to improve efficiency and reduce the costs for cultivar identification in seed industry (Horejsi and Staub, 1998).

Some research works using RAPD method have been done and revealed effectiveness in distinguishing genetic variation in durian (Ruwaida et al., 2009; Nandariyah, 2011; Vanijajiva, 2011). Information of genetic variation and genetic relationship within and between species are important for plant improvement. In breeding program, the estimation of genetic relationship is very useful to manage germplasms, to identify cultivars, and to help the selection of parents for crossing programs, as well as to reduce the number of individuals required for sampling from the wide range of diversity (Thorman et al., 1994). The present research examined polymorphism of local durian in Ternate using RAPD molecular markers to confirm previous information about local durian morphological variations and simultaneously evaluate their genetic realtionships of local durian in Ternate. It is expected that this research can be used as initial information for further research related to breeding and conservation of local durians in Ternate Island as genetic resources.

MATERIALS AND METHODS

Sample Collection

Twenty-seven variants of durian (*Durio Zibethinus* Murr.) were collected from 15 villages at Ternate island (Table 1). Fresh leaves were collected from every variant of durian for DNA extraction.

Table 1. Samples of local durian used in the present experiment

Samples	Local name	Collection site in Ternate Island
T1	Cinta	Tobololo
T2	Urat	Tobololo
T3	Mentega	Tobololo,
T4	Tobokome	Tobokome
T5	Boso	Foramadiahi
T6	Coklat	Mariku
T7	Gosi	Foramadiahi
T8	Abu-abu	Foramadiahi
T9	Hijau	Ngade
T10	Gajah kuning	Sulamadaha
T11	Pare	Kulaba
T12	Rua 1	Rua
T13	Biji mati	Foramadiahi
T14	Rua 2	Rua
T15	Ratem	Sasa
T16	Luri	Sasa
T17	Pondak	Kulaba
T18	Biasa	Loto
T19	Sina	Tongole
T20	Udi	Tongole
T21	Air tege-tege	Tongole
T22	Afo	Moya, Mariku
T23	Moya	Moya, Mariku
T24	Bantal	Tabanga
T25	Poci	Tabanga
T26	Tusa	Kastela
T27	Balanga	Kastela

DNA Isolation

Isolation of DNA from twenty-seven samples was carried out using modified CTAB method. A total of 0.05 grams of fresh leaves were mashed with a sterile pistil mortal with addition of buffer extract {CTAB 2%, 1 M Tris-HCl (pH 8), 0.5 M EDTA (pH 8), 5 M NaCl, 7.5 M Ammonium sulfate and 0.1 mg/mL RNase}, and added with 2% β mercaptoetanol, and incubated at 60°C for 180 min. The homogenate was centrifuged at 13.000 rpm in 4°C, for 10 min. The protein were precipitated twice with PCI (phenol: Chloroform: Isoamil alcohol, 25:24:1), added to supernatant and centrifuged at 13.000 rpm in 4°C, for 15 min. The supernatant was mixed with CI (chloroform: Isoamil alcohol) 24:1 and centrifuged at 13.000 rpm in 4°C, for 5 min. The supernatant was transferred to new tube and 0.1 mL of 7.5 M ammonium sulfate was added and then mixed gentle. The supernatant was collected and 2.5 volumes of absolute alcohol was added and shaken, then incubated at -20°C for 16-18 hours. The supernatant was centrifuged at 13.000 rpm in 4°C, for 15 min. Pellet was washed with 500 µL 70% ethanol and centrifuged at 13.000 rpm in 4°C, for 15 min. The supernatant was discarded and pellet was dried for 1 hour then resuspended in 50 uL Tris-EDTA buffer (1 mM tris HCL and 0.1 mM EDTA, pH 8). DNA was stored at -20°C for a long period. DNA qualitative tests were performed using electrophoresis on 1.5% agarose gel with TBE 1X and photographed on GelDOC UV-transluminator, while the quantitative test was performed using a spectronanodrop.

RAPD- PCR Analysis

Amplification of genomic DNA was performed with Polymerase Chain Reaction (PCR). Among 20 OPA RAPD primers were screened and six primers (OPA 1, OPA 2, OPA 7, OPA 16, OPA 18, and OPA 19) were selected since it can reproduce more distinctive amplicons. PCR reaction mixture was 10 uL that containing 5 uL PCR mix (INTRON); 3 uL DdH2O; 1 uL primer OPA (1,2,7,16,18,19) and 1 uL DNA template. For RAPD amplification, PCR cycles consists of DNA denaturation at 94°C for 5 min, 45 cycles at 94°C for 30 sec, annealing at 37°C for 30 sec, extension at 72°C for 90 seconds and followed by final extension at 72°C for 7 min. RAPD fragment were separated electrophoretically on 1.5% agarose gels in TBE buffer1X, stained with ethidium bromide and documented with UV trans-illumilator. 1000 bp of DNA marker (INTRON) was used to determine the size of DNA amplification products.

Data Analysis

Data were analyzed based on the presence or absence of DNA bands and scored as 0 for no band and 1 for the DNA bands. Cluster analysis was performed by UPGMA (Unweight Pair Group Method with Aritmathic Mean) technique, employed with NTSYSpc 2.2 [16]. The SIMQUAL (similarity for qualitative data) was used to calculate the Jaccard's coefficient similarity. A dendrogram was constructed based on UPGMA using Arithmetic average and SAHN (Sequential Agglomerative Hierarchical Non-overlapping) clustering. Polymorphic information content (PIC) was calculated by the formula PIC = $PIC = 1-\sum pi$ where pi is the frequency of the i^{th} allele (Smith et al., 1997).

RESULTS AND DISCUSSION

RAPD Profile

Based on the preliminary experiment on the 20 primers, six primers that had capability to produce DNA bands were selected (Table 2). All six primers were used for DNA amplification gave optimum results of RAPD profiles of all local durian samples. The result of amplification products ranged from 100 to 3000 bp (Table 3). A total of 123 bands were scored with eight-nine bands in every primer. One to five bands were monomorphic and 14-23 bands showed polymorphism (72%-94%). According to Table 2, the primer which has the highest polymorphic band was OPA-18 (94.1%). The highest Polymorphic Information Content (PIC) was observed in OPA-2 (Table 3) and the lowest was observed in OPA-18 (Fig. 1). The primer which produced the highest PIC was considered as the best primer in RAPD markers and reflects allele diversity and frequency among durians.

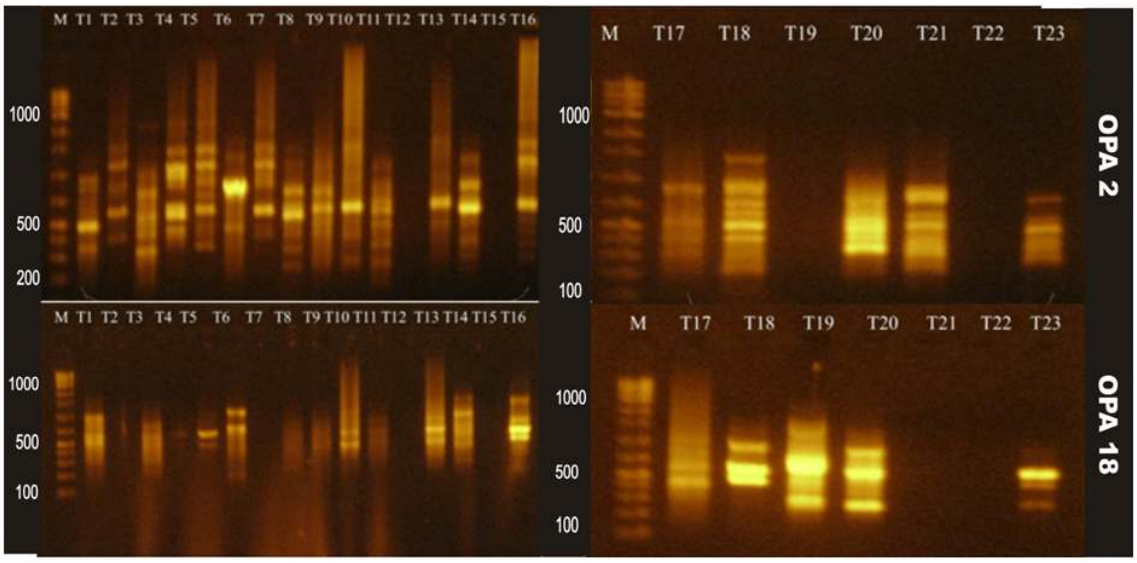

Fig. 1. RAPD profile showed the highest (OPA 2) and the lowest (OPA 18) polymorphism of durian in Ternate Island with 100 bp marker

Table 2. Data of RAPD primers used in this study

Primer	Sequence (5' to 3')	Annealing temperature (°C)
OPA-1	CAG GCC CTT C	38
OPA-2	TGC CGA GCT G	38
OPA-7	GAA ACG GGT G	38
OPA-16	AGC CAG CGA A	38
OPA-18	AGG TGA CCG T	38
OPA-19	CAA ACG TCG G	38

Table 3. Genetic variability of each RAPD primer used in this study

Primer	Range of marker size (bp)	No. of polymorphic bands/total bands	No. of monomorphic bands	Rate of polymorphism (%)	PIC
OPA-1	100-1500	20/23	3	87,0	0.25
OPA-2	100-1500	17/22	5	72,3	0,35
OPA-7	100-1500	17/19	2	89,8	0,19
OPA-16	100-1500	14/16	2	87,5	0,22
OPA-18	100-1500	16/17	1	94,1	0,11
OPA-19	100-3000	23/26	3	88,5	0,21

Genetic Relationship among the Local Durians in Ternate Island

Genetic relationship among the local durians in Ternate Island was shown as the similarity index (Fig. 2). The highest similarity index value, 0.938, was between local durian T2 (Urat) and T4 (Tobokome). Both durians were from similar location, namely Tobololo. These durians had similar characteristics such as strong smell and yellow color in fruit. Actually, durians with these characteristics are mentioned as durian Mentega. But due to diversity of local ethnic language, they have been mentioned as different names. Sometimes, these facts make durians in Ternate Island have similar morphology and genetic characteristic, but different names.

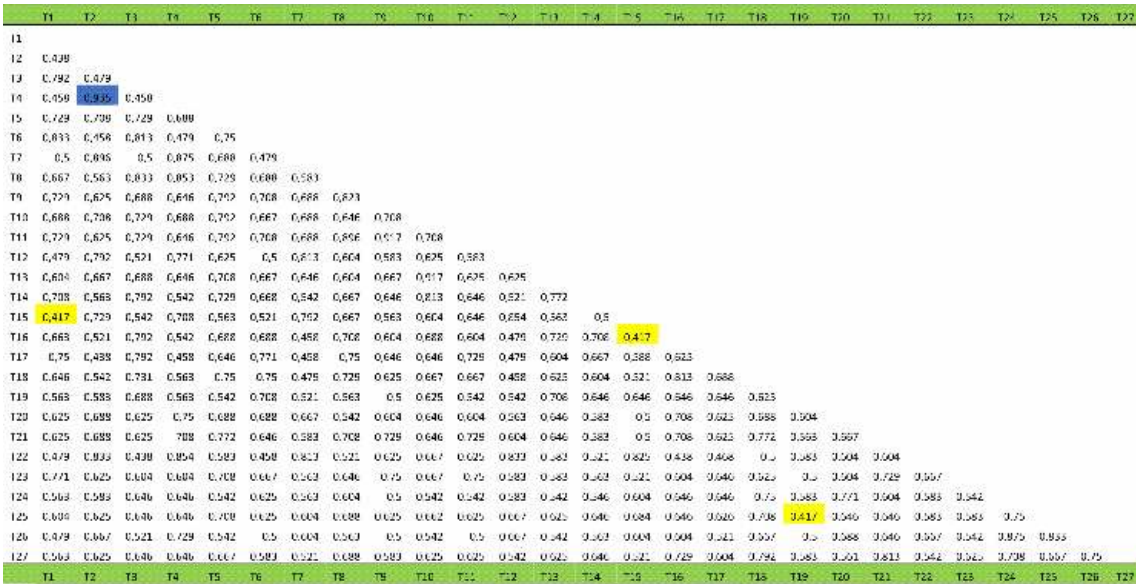

Fig. 2. Similarity Index in local durians in Ternate Island. Blue: The highest similarty index (T2-T4), Yellow: The lowest similarty index (T1-T15), (T15-T16) and (T19-T25)

The lowest similarity index (0.417) was obtained between T1 (Cinta) and T15 (Ratem), T15 (Ratem) and T16 (Luri), and T19 (Sina) and T25 (Poci). Durian Cinta and Ratem were located in different area. According to Fig. 3, Durian Cinta was located in Tobololo, in the North of Gamalama Mountain and Durian Ratem was located in Sasa, in the South of Gamalama Mountain. The existence of Gamalama Mountain created a barrier that caused durian has genetic and morphological diversity. Durian Ratem and Durian Luri were located in the same area, namely Sasa. Although both durians were located in the same area, they had genetic and morphological diversity. The highly of genetic and morphological diversity mostly caused by its location in the west of Gamalama Mountain that was not exposed by lava of Gamalama Mountain. Durian Luri and Durian Poci also had the lowest similarity index. Both durians were located in different area, namely, Sina in Tongole and Poci in Tabanga. Both areas were located in adjacent location in the southeast of Gamalama Mountain. Although both durians were adjacent, they showed diversity in RAPD markers.

From the results obtained, we can conclude that Ternate Durians have high genetic diversity. Since RAPD method has generated less statistical information per marker in F2 population (Williams et al., 1990), RAPD method is rarely used to evaluate genetic variation. Nevertheless RAPD is simple and low cost technique (Muthusamy et al., 2008), thus it is still used in wide range in biology, such as genetic mapping, developing genetic markers linked to trait, population and evolutionary genetics, plant and animal breeding (Bardakci, 2001).

Cluster analysis has been employed using UPGMA to know the grouping of durian in Ternate Island. The dendogram consists of five main clusters (based on minimal similarity index of 0.7) (Fig. 3). The first cluster with similarity index, 0.8, consist of six local durians in Ternate (T15: Ratem, T12: Rua, T22: Afo, T7: Gosi, T4: Tobokome and T2: Urat). This durian was located in the south of Gamalama Mountain. The second cluster with similarity index of 0.7 consisted of 4 durians (Fig. 4) located in the south to east of Gamalama Mountain (Fig. 3). It was composed of durians Poci from Kastela, Tusa from Kastela, Bantal from Tabanga and Udi from Tongole.

The third clusters with similarity index of 0.75 consisted of durians Rua from Rua, Biji Mati (infertile seed) from Foramadiahi and Gajah Kuning from Sulamadaha. The fourth cluster

consisted of durians Pare from Kulaba, Gajah Hijau from Ngade, Gajah Abu from Foramadiahi, Boso from Foramadiahi, Pondak from Kulaba, Mentega from Tobololo, Coklat from Mariku and Cinta from Tobololo. According to Fig. 3, it is showed that Durian Sina from Tongole and durian Moya from Moya were located in adjacent location in the East of Gamalama Mountain. From this cluster, it can be concluded that part of Ternate Island which has high diversity were ranged from the south to east of Gamalama Mountain.

Genetic variation is influenced by parental origin. The variants that have a close genetic relationship were predicted to be derived from closely related parents, whereas variants with high relative genetic distances probably derived from distant parents (Williams et al., 1990). The results presented here can be used as a basic reference of parent determination for development of local durian seedlings in the North Maluku in the future. Breeding between individuals with close genetic relationships have an effect in increasing homozygosity, otherwise the breeding between individuals with a large genetic variation or far relationship have an effect in increasing heterozygosity (Yang and Quiros, 1993). This information is useful for developing process of breeding local durian seedlings in the future. Study of genetic diversity in local durians in Ternate using RAPD molecular markers provides information about polymorphism among them. Thus it is possible to conduct breeding programs in durian for the future. RAPD method is relatively simple yet accurate to examine polymorphisms in order to develop policy on breeding program and conservation of genetic resources of Ternate local durian. Since RAPD had many disadvantages, further research with different technique to obtain more specific result will be needed.

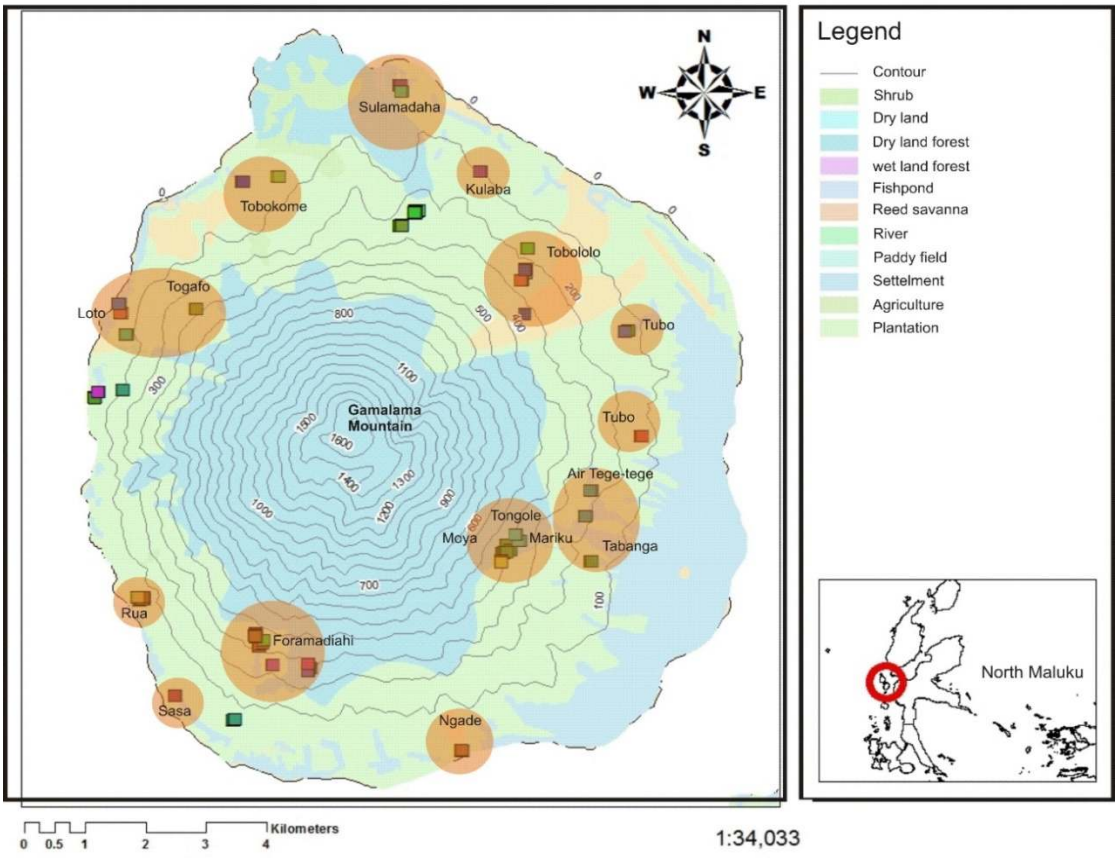

Fig. 3. Distribution of durian varieties in Ternate Island, North Maluku, Indonesia

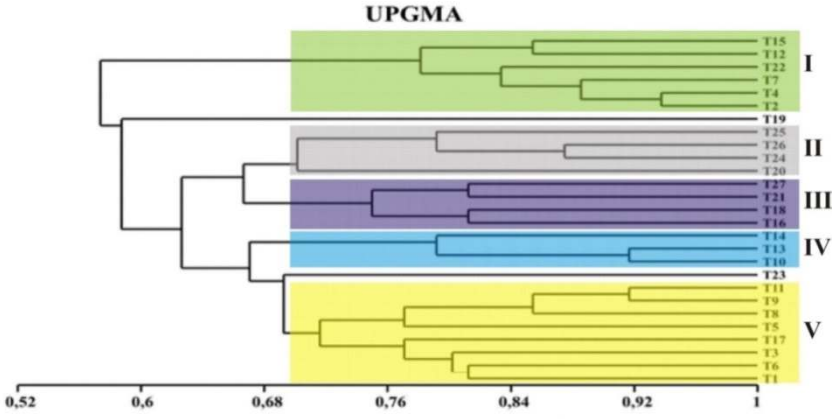

Fig. 4. UPGMA dendogram among 27 variants of local durians in Ternate Island

CONCLUSION

Analysis of genetic variability using RAPD markers showed that the genetic variability of local durian Ternate can be categorized as high polymorphism. Primer that useful to determine allele diversisty and polymorphism in durian as well as have the highest PIC value is OPA 2. Furthermore, the groups that showed farthest distances, between T1 and T15, T15 and T16, and T26 and T19 with similarity index values of 0.417, can be used as parent determination for breeding program to conserve genetic diversity of durian in Ternate Island.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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