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Genetic diversity of local durian from Tidore Island based on morphological and molecular data for tropical fruit conservation in North Maluku

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Abstract. Durian is a tropical fruit that has substantial economic value. One of the durian production centers in North Maluku is Tidore Island. This study's objective was to identify genetic diversity, taxonomic status, and local durian conservation models in North Maluku. Data collection method with purposive sampling technique. Morphological data, including 142 characters (stems, leaves, flowers, and fruit), were analyzed descriptively and arranged by UPGMA dendrogram with the outgroup (*D. kutezensis*). Molecular data were obtained by using the PCR RAPD technic. Genetic diversity of local durian Tidore based on morphological characters produced 7 clusters with the closest genetic distance to local durian (R4-R10) with a similarity value of 0.887. Genetic diversity of local durian Tidore based on PCR RAPD technic produced 5 clusters with the closest genetic distance to local durian (R10-R12) with a similarity value of 0.977. The value of Tidore's local durian dissimilarity is close to 10%; it can be assumed that the genetic diversity of Tidore's local durian is at the level of intraspecies diversity. Gap analysis recommends that Tidore's local durian conservation model is appropriate with the durian breeding program through an in-situ selection of superior traits and conservation..

1. Introduction

Exploration of the genetic diversity of local durian resources relatives scattered in various regions is one of the efforts to build the image of the durian archipelago [1; 2; 3; 4]. Local durian is the name of a durian relative (*Durio* spp) which grows in various regions in Indonesia [5; 1; 6; 7]. *Durio zibethinus* var Tidore is the name of durian which grows on the Tidore island wildly, derived from seeds with hereditary ownership. Tidore island is one of the durian production centers in North Maluku province besides Ternate island and Jailolo sub-district on the western Halmahera island. Local durian production in Tidore island reaches 3.87 tons per year [8]. The diversity of local durian species in the Tidore island was not been reported yet. One parameter for the existence of local durian diversity in the Tidore island is the presence of local names of durian variants given by the local community. The local name was given based on morphological variations, the name of the durian plantation owner, and the location of the local durian grows. [9] reported that local durian diversity in Ternate based on taxonomic analysis there were divided into 7 groups with the highest similarity index is 90.32% for Udi and Sina durian, while taxonomic analysis of local durian in Jailolo were divided into 5 groups with the highest similarity index is 88, 66% for durian Boga and durian Malal.



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Exploration of the diversity of local durian in Tidore was showed that, there were nineteen local durian names spread over nine locations [10]. It is known that the local durian Tidore has a diversity of local names and morphological variations such as: habitus variation (canopy shape and branching type), leaf shape, fruit shape, fruit flesh color, seed shape, thorn shape and texture of different flesh of each variant [10]. This study aims to identify genetic diversity, taxonomic status and local durian conservation in North Maluku. Furthermore, this research is expected to contribute information on policies and strategies for managing local durian both in terms of utilization and conservation.

2. Material and Methode

This research was conducted in March-September 2015, sixteen samples of local durian plants were collected from the villages of Gurabunga, Gubukusuma, Afa-afa, Fobaharu, Foralaha, Jaya, Bobo, Dokiri and Rum Tidore Island. Morphological data including 142 characters (stems, leaves, flowers and fruit) refers to the International Biodiversity standard (2007). Tidore's local durian molecular data were obtained using PCR-RAPD technique. The laboratory work phase consists of: 1) DNA isolation, 2) DNA quantity and quality testing; 3) DNA amplification; 4) electrophoresis; 5) visualization and 6) analysis of DNA results. DNA isolation in sixteen local durian samples using the CTAB method [11] (modified by researchers). Qualitative DNA testing was carried out by electrophoresis with agarose Gel 1.5% TBE 1X and visualization with Geldoc Uvtransluminator, while quantitative tests were carried out by measuring concentrations with electronanodrop. DNA amplification reaction using six primers OPA 1, OPA2, OPA7, OPA16, OPA18, OPA19). Cluster analysis uses UPGMA (Unweight Pair Group Method with Arithmetic Mean) technique, the Multivariate Statistical Package (MVSP) version 3.22 [12]. Recommendations for local durian genetic conservation models in North Maluku were obtained through morphological and molecular data elaboration and Root Cause Analyze analysis [13].

3. Result and Discussion

3.1. Genetic diversity of local durian Tidore based on morphological data

Sixteen local durian names (*Durio zibethinus* var Tidore) were collected from 9 villages on the island of Tidore (Table 1). Several variations that can be distinctive morphological characters among the local names of durian Tidore include: crown shape, the type of branching, leaf shape, fruit shape, fruit color, and texture of the fruit [10]. Based on the analysis of 142 characters: stems, leaves, flowers and fruit on the local durian Tidore with outgroup *Durio kutezensis*. that the highest similarity of local durian Tidore is found in local durian with code R4 - R10 with similarity value of 0.887, while the lowest similarity there is a local durian with code R12-R15 with a similarity value of 0.620.

Table 1. Collection of local durian Tidore

Collection Number	Local name	Location
R4	Nona	Rum, Gurabunga, Gubukusuma
R6	Baba	Gurabunga
R7	Boso	Gurabunga
R9	Tusa	Dokiri, Rum
R10	Batu/ gulinga	Gubukusuma
R12	Mafu	Fobaharu
R13	Malal	Jaya
R15	Gajah	Rum, Gurabunga, Foralaha, jaya
R16	Mentega	Rum
R19	blek	Bobo, Rum
R20	Malal	Jaya
R23	Nangka	Rum
R24	Kusi	Rum
R25	Kohori	Rum
R28	Takate	Rum
R32	Sambiki	Dokiri

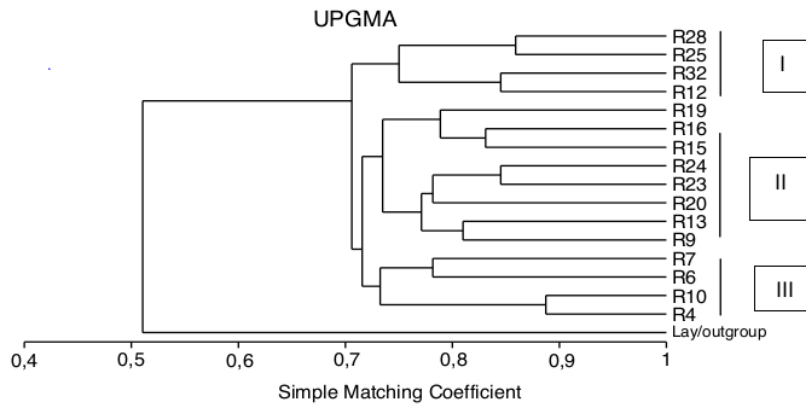


Figure 1. Genic diversity of local durian Tidore using UPGMA dendrogram based on morphological data.

The dendrogram analysis using the UPGMA method is shown in Figure 1. The genetic diversity of local Tidore durian based on morphological data shows that the UPGMA analysis results in 3 clusters with the closest genetic distance to the local durian (R4-R10) with a similarity value of 0.887.

3.2. Genetic diversity of local durian Tidore based on molecular data

The RAPD profile of DNA amplification using 6 (six) OPA primers can provide optimum results for all durian samples. Amplification products produced between 100 to 3000 bp. Polymorphism between local durian varieties Tidore based on RAPD with 6 primers was shown on the Figure 2:

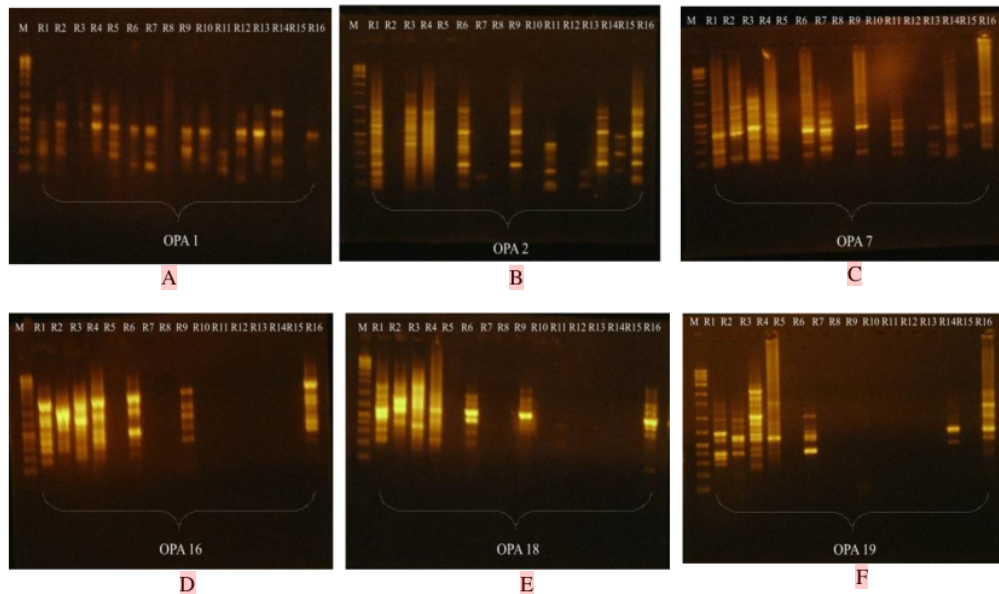
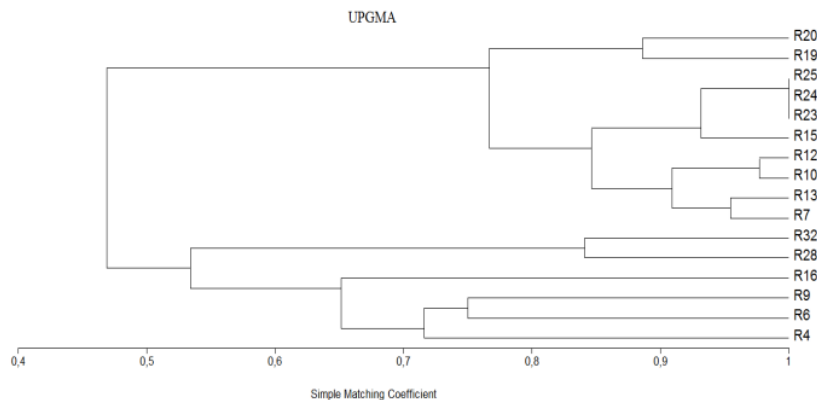


Figure 2. RAPD profile of local durian Tidore using 6 OPA primers

Table 2. RAPD Markers, DNA Sequence, and Polymorphism (%) of sixteen local durian Tidore Analyzed Using the UPGMA Technique

Primer	Seq 5 to 3	Seq 5 to 3 bands	Polymorphic bands	Monomorphic bands	Polymorphism (%)
OPA 1	CAG GCC CTT C	21	19	04	90,4
OPA 2	TGC CGA GCT G	17	14	03	82,3
OPA 7	A ACG GGT G	23	19	09	82,6
OPA 16	AGC CAG CGA A	16	10	06	62,5
OPA 18	AGG TGA CCG T	15	09	06	60,0
OPA 19	CAA ACG TCG G	17	11	06	64,7
Total		109	82	34	75.22

The total number of DNA bands found was 109 bands (82 polymorphic bands and 34 monomorphic bands). The average percentage of polymorphism was 75,22%. Based on UPGMA analysis show the highest similarity of local durian Tidore is found in local durian with code R10 - R12 with a similarity value of 0.977, while the lowest similarity is found in local durian with code R7-R16 with a similarity value of 0.409. Furthermore, the dendrogram analysis using the UPGMA method is shown in Figure 3 below:

**Figure 3.** Genetic diversity of local durian Tidore using UPGMA dendrogram based on PCR-RAPD

Genetic diversity of local durian Tidore based on PCR-RAPD analysis showed that the results of UPGMA analysis based on PCR-RAPD data produced 5 clusters with the closest genetic distance to local durian (R10-R12) with a similarity value of 0.977. Based on morphological and molecular data, it can be seen that there is genetic diversity local durian in Tidore with a value of dissimilarity approaching 10%, it can be assumed that Tidore local durian genetic diversity is at the level of intraspecies diversity. Variations that occur at the level of species (intraspecies diversity) are influenced by the origin of the parents [14; 15].

3.3. Gap Analysis

Based on morphological and molecular data, gap analysis can be carried out to determine a conservation strategy for local durian Tidore. The results of Gap Analysis of the local durian conservation in Tidore island North Maluku based on morphological and molecular data elaboration as in table 4 below:

Table 3. Gap analysis of the of local durian from Tidore island

Variables and Criteria	Object	Standart	Gap analysis	
			+	-
Genetic diversity of local durian	Variation of morphology	Similarity index	√	
	Variation of molecular	Polimorfisme	√	
	Local names	Nomenclature		√
quality	Use value	Usefulness	√	
	Economic value	Sale value	√	
	Aesthetic value	Preference value		√
konservation	Ex situ	Cultivation and breeding (collection)		√
	In situ	Local cultivation		√

Information on genetic diversity possessed by local durians is needed to determine the kinship of these variants. Local durians that have genetic proximity, are thought to originate from close relatives, and vice versa [16; 17]. Germplasm that is closely related is needed in determining the crossing elders to assemble hybrid varieties [19; 19]. Furthermore, the Gap analysis results recommend that the Tidore local durian conservation model that is appropriate is the durian breeding program through selection of superior traits and in-situ conservation. One method of analyzing biodiversity conservation models both wild or effective cultivation is Gap analysis [13; 20].

4. Conclusion

The genetic diversity of local durian from Tidore islands based on morphological characters produces 3 clusters with the closest genetic distance to local durian (R4-R10) with a similarity value of 0.887. The Tidore local durian variant's genetic diversity based on RAPD analysis produced 5 clusters with the closest genetic distance to the local durian (R10-R12) with a similarity value of 0.977. The value of Tidore's local durian dissimilarity close to 10%, can be assumed that the genetic diversity of Tidore's local durian is at the level of intraspecies diversity. Gap analysis recommends that Tidore's local durian conservation model is appropriate with the durian breeding program through an in-situ selection of superior traits and conservation.

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