THE APPLICATION OF RAPD MARKERS IN GENETIC VARIANCE DETECTION AMONG LOCAL DURIAN (*Durio zibethinus* Murr.) ON THE WEST HALMAHERA PROVINCE NORT MALUKU INDONESIA

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ABSTRACT

Durian is one of the most important tropical fruits with agricultural economic value in Indonesia. West Halmahera is one of centre of production of local durian at North Maluku. In this study, Random Amplification Polymorphisme DNA (RAPD) markers were applied to assess genetic diversity and genetic relationships of 37 variant of local durian (Durio zibethinus Murr.) from West Halmahera. Genomic DNA was extracted from fresh leaf samples of 37 variants of local durian collected from West Halmahera Island. Ten RAPD primers were initially screened for analysis and five primers (OPA 1,OPA 2,OPA16,OPA 18 and OPA 19) were chosen for further analysis. A total of 41 DNA fragments, varying from 100-2000 bp, were amplified, of which 41 (100%) were polymorphic. Banding pattern of DNA amplification show the existence of diversity among varieties of local durian on West Halmahera and also the diversity of local durian grown in different hotspot. A dendrogram showing genetic similarities among local durian was constructed based on polymorphic bands with UPGMA method using the MVSP program (version 3.22). The dendrogram showed eight clusters which could be separated with similarity coefficients ranging from 0.525-1.00. RAPD analysis promise an effective instrument in estimating genetic polymorphism in different accessions of local durian on West Halmahera province North Maluku Indonesia.

Keyword: Genetic Diversity, Local Durian West Halmahera, Rapd

Introduction

Durian is belong to family Malvaceae (APG II, 2003) and distinctive for its fruit shape, flesh color and texture, unique odour, and thorn-covered husk (Brown, 1997, Vanijajiva,2011). Durian (*Durio zibethinus* Murr.), "King of Fruits", is one of the most important agricultural economic tropical fruits in Indonesia. Indonesia is one of durian producer country , other than Malaysia and Thailand (Somsri, 2007). West Halmahera Island especially Jailolo district is one of the center of durian production in North Maluku (Anonymus, 2013). Local durian Jailolo is the name of the durian variant (*Durio zibethinus* Murr.) Jailolo durian grows naturally from the seeds in community garden and has been in local ownership from generation to generation (Sundari, *et al*, 2015). At this time information on genetic variation of local durian in West Halmahera island has not been assessed yet. Some research has been done based on morphological characters of trees, leaves, flowers and fruit of durian (Tolangara, *et al*, 2013; Sundari, *et al*, 2015). However, the difference between the cultivars is practically has not been studied. There is not much information available on the genetic relationship between cultivated local durian in west Halmahera island (Sundari, 2015), particularly in the North Maluku province, where it has been cultivated for hundreds of years. Earlier classification and evaluations of local durian were done primarily based on phenotypic expression of the plants such as shape of fruit, size of thorns on the skin and other morphological characters (Sundari, 2015).

Molecular identification method with RAPD was used to obtain the plant genetic identity because the data does not influenced by environmental factors. RAPD has some advantages including easy, quick and only requires very little DNA as template, and without the need for early information on the target genome (Vanijajiva *et al.*, 2005; Nandariyah,2011).The objective of this study was to use the RAPD technique to evaluate the genetic diversity and relatedness of 37 variant of local durian on the West Halmahera island province of Nonth Maluku using five RAPD primers.

Methodology

Plant materials

DNA isolation and RAPD analysis were carried out using fresh leaf samples from 37 variants collected from the Jailolo district, West Halmahera island North Maluku province.

DNA Isolation: Isolation of DNA from 37 samples of local durian Jailolo using modified CTAB method (Doyle & Doyle, 1987,). A total of 0.05 grams of fresh leaves crushed with a sterile pistil mortal with the addition of buffer extract {CTAB 2%, 1 M Tris-HCl (pH 8), 0.5M EDTA (pH 8), 5 M NaCl, 7.5 M Ammonium sulfate and 0.1 mg / mL RNase}, and then added with 2% β mercaptoetanol, and subsequent incubation of 60^0C for 180 minutes. The solution mixture then centrifuged at a temperature of 4^0C, 13,000 rpm for 10 minutes. The supernatant was collected and added with PCI (phenol: Chloroform: Isoamil alkohol) 25 : 24: 1 then centrifuged at a temperature of 4^0C, 13,000 rpm for 15 minutes (this step is repeated 2 times).The supernatant was coupled with CI (chloroform: Isoamil alkohol) 24: 1 and centrifuged at a temperature of 4^0C, 13,000 rpm for 5 minutes. The supernatant was transferred to new ependof tube and 0.1 mL 7,5M ammonium sulfate was added and then wasmixed gentle. The supernatant was collected and 2.5 volumes of absolute alcohol was added and shaken, and then incubated at a temperature of -20^0C for 16-18 hours, then centrifuged for 15 minutes at a temperature of 4^0C 13,000 rpm, next supernatant was discarded and the pellet plus 500µL as much as 70% alcohol and centrifuged for 15 minutes, discard the supernatant and the pellet dried for 1 hour later added TE buffer (pH 8) of 50 mL and ready durian DNA was stored at -20^0C for a long period. DNA qualitative tests was performed on agarose gel electrophoresis with 1.5% TBE 1X and visualization with GelDOC UV-transluminator, while the quantitative test was done by measuring the concentration of DNA using a spectronanodrop.

RAPD PCR Analysis

Reaction DNA amplification using PCR TAKARA brands. PCR cocktail used in this experiment was consisted of with 5 mL PCR mix (INTRON); 3 mL DdH2O; 1 mL primer OPA (1,2,16,18,19) and 1 mL DNA template. PCR program used was 45 cycles consisted of 94^0C Pradenaturasi phases for 5 minutes; Denaturation temperature of 94^0C for 30 seconds; Annealing temperature of 37^0C for 30 seconds; Extension temperature of 72^0C for 90 seconds; and Post Extension 72^0C temperature for 7 minutes. The amplicon then run on electrophoresis . To determine the size of DNA amplification product was used 1000 bp DNA marker (INTRON)

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 using the technique of UPGMA (*Unweight Pair Group Method with Aritmathic Mean*), with Multivariate Statistical Package program (MVSP) version 3:22 (Kovach: 2007) was employed.

Result And Discusion

RAPD Profil

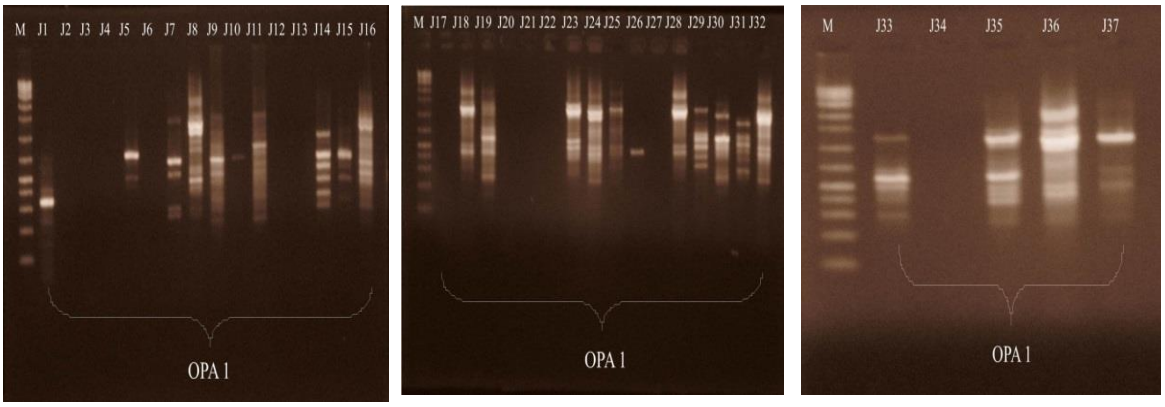
From 10 primers used in this study it was resulted 5 primer that has the capability of producing the DNA bands in DNA amplification (Table 2)

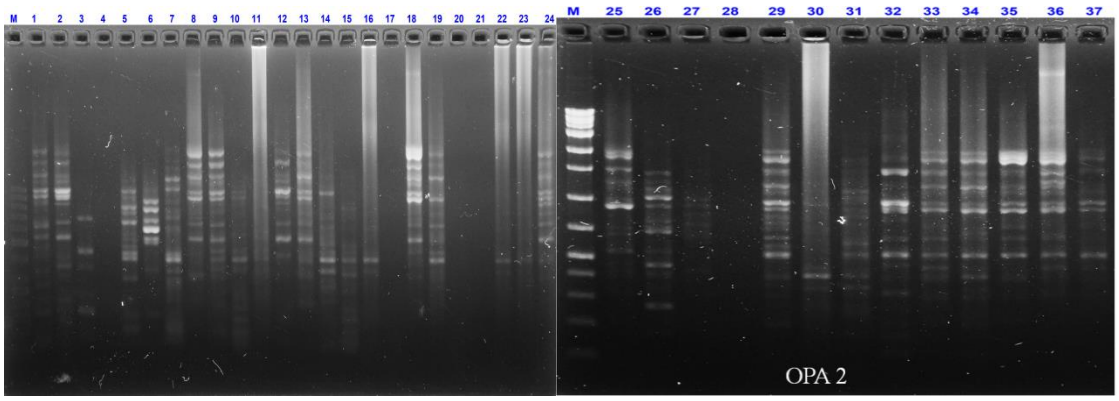
Table 1. Level 5 polymorphism Primer Based on the Pattern of DNA bands

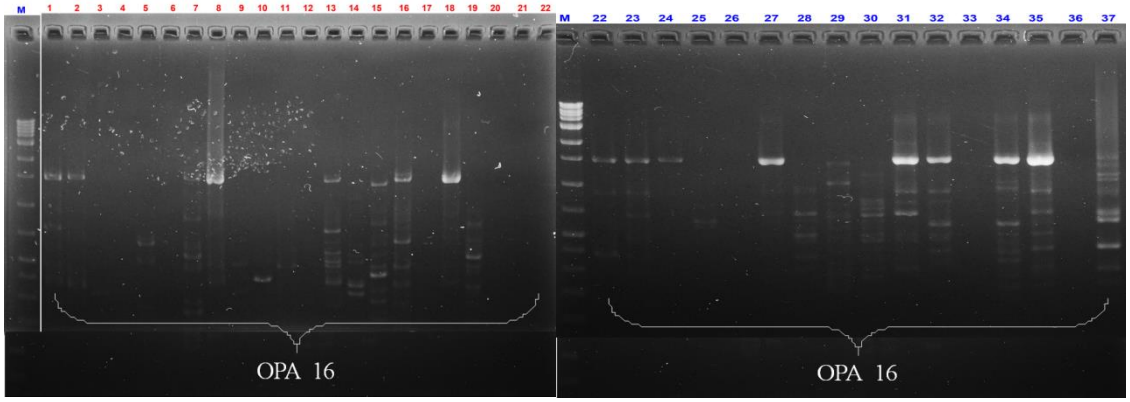
Primer	Seq 5 to 3	Seq 5 to 3 bands	Polymorphic bands	Monomorphic bands	Polymorphism (%)
OPA-1	CAG GCC CTT C	07	07	00	100
OPA-2	TGC CGA GCT G	14	14	00	100
OPA-16	AGC CAG CGA A	06	06	00	100
OPA-18	AGG TGA CCG T	09	09	00	100
OPA-19	CAA ACG TCG G	05	05	00	100
Total		41	41	00	100

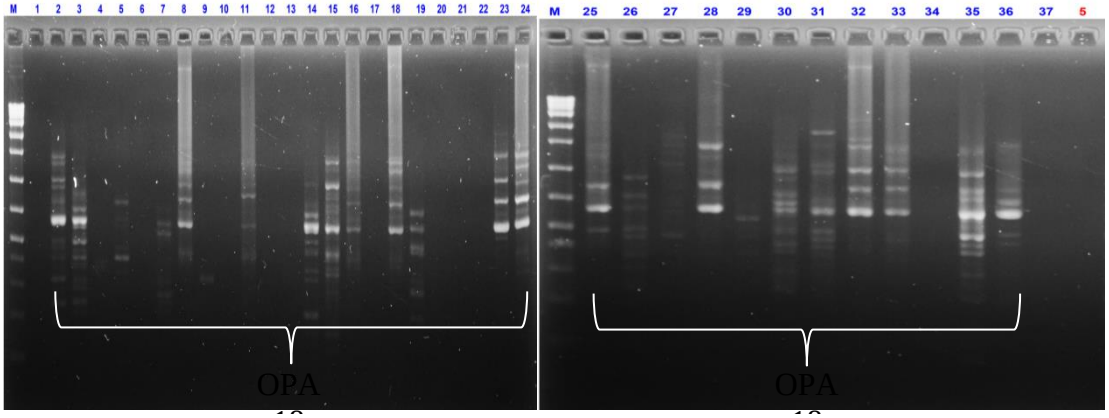
The five primers used in the amplification of DNA can give optimum results of RAPD profiles of all samples. There are 5 to 14 band generated by five primers used. The resulted amplification products ranging from 100 to 2000 bp. Polymorphisms was indicated between all Jailolo durian varieties (Fig 1).

Figure 1: Profile RAPD Polymorphism of 37 Local Durian Jailolo District West Halmahera.


M J1 J2 J3 J4 J5 J6 J7 J8 J9 J10 J11 J12 J13 J14 J15 J16
OPA 1
M J17 J18 J19 J20 J21 J22 J23 J24 J25 J26 J27 J28 J29 J30 J31 J32
OPA 1
M J33 J34 J35 J36 J37
OPA 1


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
M 25 26 27 28 29 30 31 32 33 34 35 36 37
OPA 2


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
OPA 16
M 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
OPA 16

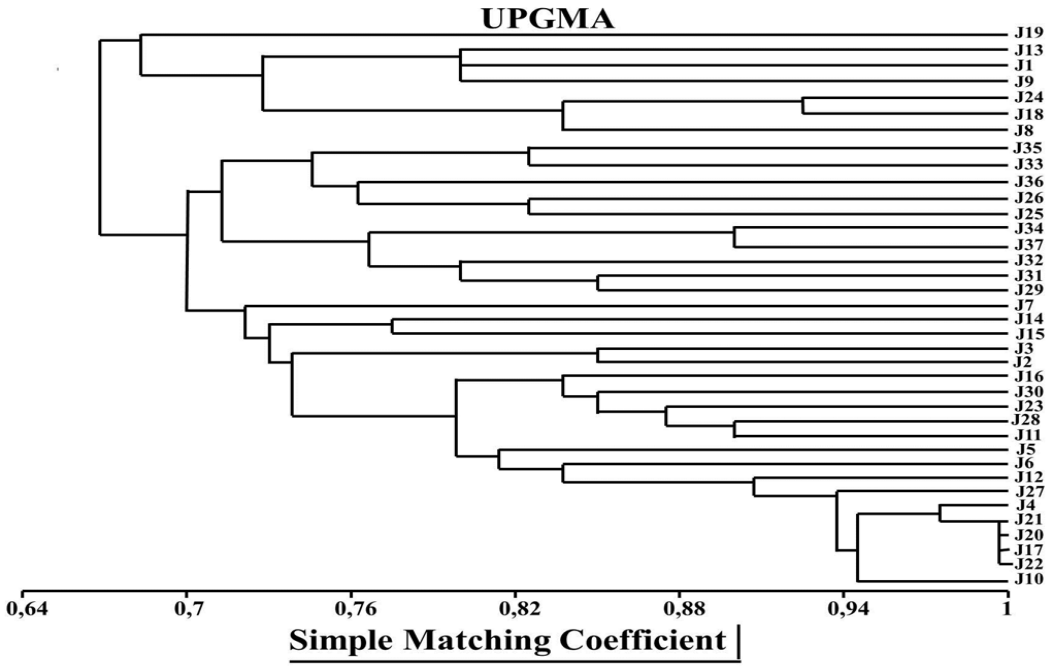

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
OPA
M 25 26 27 28 29 30 31 32 33 34 35 36 37 5
OPA

The amplification result of the primer OPA-01,02,16 and 18 showed the diversity of patterns of DNA bands of 37 Jailolo durian. This diversity can be seen on the 100-2000 bp band showed by the varieties studied. Primer OPA-02 show the most divers DNA banding pattern of the 37 Jailolo durian. This diversity can be seen on the profil DNA at 100-2000 bp.

Relationships between variant lokal durian Jailolo

In order to estimate the genetic variability among local durian Jailolo, genetic similarity coefficients were calculated. Similarity coefficients ranged from 0.525-1.00 in 37 local durian in the present experiment, with the lowest value obtained showed by J2-J37, whereas J17-J20, J17-J21, J17-J22 showed the highest similarity values. Based on the RAPD bands genetic distances among the 37 local durian were calculated and a dendrogram was constructed by UPGMA method (Fig. 2). The dendrogram consisted of eight clusters.

Fig. 2. Dendrogram based on UPGMA analysis of genetic similarity of 37 local durian Jailolo obtained from RAPD, showing relationships among individual plants.



Cluster1I consists of one variants, J19, cluster 2 consists of six variants, J13, J1, J9, J24, J18 and J8; cluster 3 consists of five variant: J35, J33,J36,J26,J25; cluster 4 consists of five variant: J34,J37,J32,J31,J29; cluster 5 consist of one variant, J7; cluster 6 consist of four variant: J14,J15,J3 and J2; cluster 7 consist of five variant: J16,J30,J23,J28,J11, and cluster 8 consist of ten variant: J5,J6,J12,J27,J4,J21,J20,J17,J22,J10.

The results of the genetic diversity analysis can be used as a basic reference in the determination of the parent for the manufacture of local durian seedlings featured in North Halmahera. Study of genetic diversity and phylogenetic relationship of local durian Jailolo (West Halmahera) with RAPD analysis promises an effective instrument in estimating genetic polymorphism in different accessions of local durian Jailolo polymorphism is high, so it is possible to do breeding durian for the future, the method RAPD is a method that is relatively simple yet accurate to examine polymorphisms in order policy of breeding and conservation of genetic resources of local durian on Jailolo (West Halmahera).

Conclusion

The banding pattern of DNA amplification show the existence of diversity among varieties of local durian on West Halmahera which in turn show the diversity of local durian grown in different hotspot. The dendrogram constructed based on the RAPD bands showed eight clusters which could be separated with similarity coefficients ranging from 0.525-1.00. RAPD analysis promises an effective instrument in estimating genetic polymorphism in different accessions of local durian in Indonesia.

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