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Genetic Diversity of Local Durian (*Durio zibethinus* Murr.) Cultivars of South Kalimantan's Province Based on RAPD Markers

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Abstract. South Kalimantan on the Borneo Island is one of the center origin and diversity of durian (*Durio* spp.) germplasm in Indonesia. This study was conducted to investigate the genetic diversity and relationships of local durian (*Durio zibethinus* Murr.) cultivars from South Kalimantan's Province by RAPD markers. Eleven plant samples of local durian cultivars trees and five selected RAPD primers, namely OPA-01, OPA-02, OPA-07, OPA-16, OPA-18, and OPA-19 were used. DNA samples were extracted from leaf tissues according to the protocol of Nucleon Phytopure (Amersham Bioscience). Amplifications were performed on a PCR Thermal Cycler (Applied Biosystem 9700). Variation of the PCR products of each primer was scored based on the presence (1) and absence (0) of bands on the images taken from the gels. The NTSys-PC software was used to compute Dice's coefficients of similarity, and the dendrogram was constructed using UPGMA method. Results showed that 11 local durian cultivars of South Kalimantan's Province have high diversity which indicated by polymorphism degree of 82.17% and clustering analysis where those cultivars were grouped into six distinct clusters. Based on the result, durian 'Likol' had closely related to the durian 'Sipisang' and 'Sihabuk' at the level 95% of similarity, while durian 'Enam Hapat' had separated at a distance from others. This information be used as a consideration in conservation and management improvement strategies (breeding program) of the durian in Indonesia.

INTRODUCTION

Studies on genetic diversity are very essential for the conservation and management strategies of the genetic resources [1, 2], included durian. In Indonesia, studies on genetic diversity of the durian (*Durio* spp.) has been conducted mostly through morphological assessment [3, 4, 5], and lack information on molecular data [6]. Moreover, most of these studies had been done in Java Island, Indonesia. Therefore, studies on genetic diversity of the durian in other part of this country, particularly in Kalimantan (Borneo) Island, is important to be carried out.

Kalimantan (Borneo) Island is one of the center origin and diversity of durian germplasm in the world [7]. There are 27 species of the durian in the world, and 19 of the 27 species have been found on the Island. Sixteen of the 19 species of the durian in Kalimantan are identified as endemic germplasm [4, 7]. *Durio zibethinus* is one of the most important durian species that interesting to be studied, because of their taste and commercial value in world market.

Random amplified polymorphic DNA or RAPD is one of familiar molecular markers that be used to investigate the genetic diversity of an organism [23]. This technique is suitable for many researcher because of its simplicity, inexpensive nature, and non-requirement of prior information of genetic sequences [23]. RAPD is also has several advantages than other markers, e.g RFLP, such as only entailed small amount of DNA samples in amplification process and generate a large number of DNA fragments, and do not exerted radioisotope for analysis [21, 24]. Over

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past ten years, RAPD have been employed for determine the genetic diversity of various plants, i.e. rice [8, 9], tomato [10], papaya [11], citrus [25], coconut [26], soybean [23], and durian [6, 27, 28].

The objectives of this study were to investigate the genetic diversity and relationships of local durian (*D. zibethinus* Murr.) cultivars from South Kalimantan's Province by using RAPD markers.

MATERIALS AND METHODS

Plant Samples

Plant samples were collected from the Botanical Garden of Indonesian Swampland Agriculture Research Institute (BALITTRA), South Kalimantan, Indonesia (Table 1). One durian sample was also collected from Central Java as comparison. The leaves samples were taken by random sampling method from each durian cultivars. Leave were restricted on healthy leaves, no infection and full developed for DNA extraction.

TABLE 1. *Cultivar names, code number and source of the plant materials used in the study*

Name of Cultivar	Code Number	Origin
Balade	D-01	South Kalimantan
Enam Hapat	D-02	South Kalimantan
Kamundai	D-03	South Kalimantan
Lakatan	D-04	South Kalimantan
Likol	D-05	South Kalimantan
Sahang	D-06	South Kalimantan
Sihabuk	D-07	South Kalimantan
Silanjung	D-08	South Kalimantan
Sipisang	D-09	South Kalimantan
Siitik	D-10	South Kalimantan
Petruk[a)]	D-11	Central Java

Note: [a)] as comparison

DNA Extraction

DNA was extracted and purified from leaf tissues according to the protocol of Nucleon Phytopure (Amersham Bioscience). The quantity of DNA was determined by UV spectrophotometer at a wavelength of 260-280 nm. DNA purity is determined by A260/A280 ratio of about 1.8 to 2.0 [12].

DNA Amplification

PCR reactions were performed for genomic DNA in a total volume of 25 µl reaction volumes in 200 µl tubes. Each reaction mixture containing 20 µl PCR master mix (deionized water; PCR buffer; dNTPs; $MgCI_2$, Taq DNA polymerase), 2.5 ng/µL of each primer (100 picomoles) and 2.5 ng/µL of genomic/template DNA (2.5 ng). Amplification was performed on a DNA Thermal Cycler (Applic Biosystem 9700) with cycling conditions: initial denaturation process at 94°C for 5 minute, denaturation process at 94°C for 30 seconds, annealing process at 37°C for 30 seconds, extension process at 72°C for 90 seconds, and final extension at 72°C for 7 minutes [10]. Five selected RAPD primers [6] obtained from Research Instrument were used for PCR amplification (Table 2).

TABLE 2. Sequences of five RAPD primers used in PCR amplification

Primer	Sequences (5'→3')	GC content (%)
OPA-01	CAGGCCCTTC	70
OPA-07	AGCCAGCGAA	60
OPA-16	AGCCAGCGAA	60
OPA-18	AGGTCACCGT	60
OPA-19	CAAACGTCGG	60

Electrophoresis

PCR products of genomic DNA were separated by electrophoresis in a 2% agarose gel in 1x TBE along with DNA marker from Vivantis (100 bp). The gel was run in 1x TBE buffer at 100 volts for 40 minute. After that, the gel was stained in the ethidium bromide solution for 20 minutes and then washed in distilled water for 30 minutes [2]. The gels were observable on UV transilluminator, and the images of the stained gels were captured using digital camera.

RAPD Data Analysis

Variations of the PCR products will be scored based on the presence (1) and absence (0) of bands on the images taken from the gels. The NTSys-PC software [13] was used to compute Dice's coefficients of similarity [14], and the dendrogram will be constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) [15].

RESULTS AND DISCUSSION

DNA Amplification

Figure 1 shows the visualization of DNA fragment as a result of amplification by four RAPD primers (OPA-01, OPA-07, OPA-18, and OPA-19). Table 3 shows the number and size (lenght) of DNA fragment as a result of amplification by five RAPD primers used in this study.

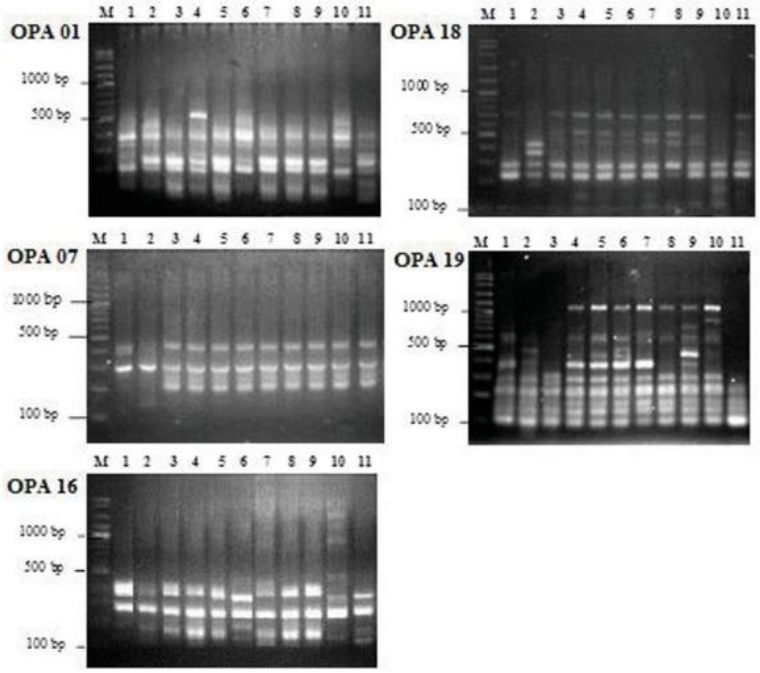

FIGURE 1. *Patterns of RAPD product by five primers. Lanes 1-10 are D. zibethinus cultivars from South Kalimantan's Province, Lane 11 is D. zibethinus cultivar from Central Java as comparison, M is 100 bp marker (see Table 1 = here include)*

TABLE 3. The number and size (lenght) of DNA fragment as a results of amplification with five RAPD primers

Primer	Total Number of DNA fragment	Size (Lenght) of DNA Fragment (bp)
OPA-01	39	95 – 576
OPA-07	6	130 - 463
OPA-16	39	128 - 1754
OPA-18	41	124 - 678
OPA-19	43	104 – 1894
Total/Range	168	95-1894

Based on the figure and table above, five RAPD primers produced clear and distinct DNA banding patterns through all samples. These primers generate a total of 168 DNA fragments, comprise of 152 polymorphic bands and 16 as monomorphic, ranging from 95 to 1894 bp. The range of size of fragments obtained by RAPD markers was comparable to 150 to 1400 bp size as reported by Ruwaida et al. [6] in five durian cultivars from Java Island, amplified by six RAPD primers. The result of Ruwaida's was different from our study. According to Hartati [16], the differences of DNA fragment in number, size or lenght were generated per primer due to the differences in primer nucleotide base sequence or interaction between the primer and template DNA. Further, the differences of DNA fragment from the samples, were reflected the complexity of durian plant genome [16].

Based on the results, there are some specific bands that distinguished the genetic diversity among the durian cultivars from South Kalimantan (Fig. 1). RAPD primer of OPA-19 were generated the specific bands on the size of 800 bp (D-10) that differentiated those cultivars to other cultivars. In addition, the primer of OPA-16 were also produced the specific bands on the size of 1200 bp and 850 bp, owned by D-10. According to Jiang et al. [17], specific bands produced could provide references for the molecular identification on its authenticity and the construction of its fingerprints of germplasm. This result may provide as references for the rootstocks selection in the grafting of durian germplasm because identification of those germplasm were generally based on morphological characters required intensive observation of mature plants.

Molecular markers which used in the study, on the other hand, were able to distinguish the genotypes amongst individuals with relative high accuracy. It is relevant to the statement of Powell et al. [18], that molecular markers, for instance RFLP, RAPD, AFLP and SSR markers, could be used for differentiate the genotypes amongst individuals at the level of intra -species and distant relatives with high accuracy.

Polymorphism Degree

Table 4 shows the mean value of polymorphic fragment and polymorphism degree of DNA genome of durian samples. Base on this table, the mean value of polymorphic fragment was 50.67, higher than monomorphic one. The RAPD primers that were used in this study were produced the differences of DNA polymorphism in percentage. The primer of OPA-16 has a highest DNA polymorphism degree than others, with the percentage of 94.87%. Generally DNA genome of durian generated the polymorphism at the average of 82.17%. Hartati [16] said that the occurence of polymorphism in DNA fragment can be described the states of plant genome in population.

TABLE 4. Polymophism degree in DNA genome of local durian cultivars

Primer	Number of Polymorphic Fragment	Number of Monomorphic Fragment	Polymorphism (%)
OPA-01	35	4	89.74
OPA-07	3*	3	42.85*
OPA-16	37	2	94.87**
OPA-18	38	3	92.68
OPA-19	39**	4	90.70
Total	152	16	-
Mean	50.67	5.33	82.17

*Note: * the lowest value; ** the highest value*

The level of polymorphism and mean value of polymorphic fragments amplified for each primers tested in this study were higher as compared to that reported in durian cultivars by Ruwaida et al. [6], 81.89% and 12.33

fragment, respectively. This indicates that durian cultivars from South Kalimantan' Province has a high genetic diversity. According to McGregor et al. [19], polymorphism is an image obtained by amplification of DNA fragments where the differences were observed and scored as presence or absence of sequence differences, thus indicating a variation. In accordance with the statement of Cahyarini et al. [20], that the number, thickness, and mobility of DNA fragments were reflected the state of genetic diversity of those samples in population.

Genetic Diversity and Relationships

Genetic diversity amongst durian cultivars (used in the study) have been seen from the results of amplification by using RAPD primers (Fig. 1). Based on the figure, the number, thickness and mobility of DNA fragment that were produced were varied amongst durian cultivars. Cahyarini et al. [20] said that the state of genetic diversity of those cultivars in population can be seen actually on the number, thickness and mobility of DNA fragment that were produced. However, the cluster analysis with UPGMA methods can be used to see the clustering of those samples more comprehensive.

Table 5 shows the Dice's coeficient value amongst durian cultivars, and Fig. 2 represented the intra-species clustering of those samples as a dendogram. Local durian cultivar of South Kalimantan were clustered into six groups, with highest similarity were shown by durian 'Likol' and 'Sipisang', and 'Likol' and 'Sihabuk' (95.5%), while the lowest similarity were shown by durian 'Enam Hapat' and 'Siitik' (64.5%). Frankham et al. [21], state that the divergence of germplasm based on clustering analysis reflected the evolutionary potential of those cultivars for the future adaptation with environmental changes.

TABLE 5. Dice's coefficient (%) amongst 11 local durian cultivars, comprices ten samples from South Kalimantan's Province and one sample from Java Island (Petruk cultivar as comparison)

OTU	D-01	D-02	D-03	D-04	D-05	D-06	D-07	D-08	D-09	D-10	D-11
D-01	100										
D-02	76.0	100									
D-03	80.8	66.7	100								
D-04	81.3	66.7	74.2	100							
D-05	86.7	71.4	82.8	88.6	100						
D-06	73.0	67.8	65.6	79.5	84.1	100					
D-07	83.3	67.9	82.8	85.7	83.9	78.2	100				
D-08	82.8	66.7	85.7	85.3	93.8	77.6	93.8	100			
D-09	85.2	66.7	81.4	87.3	95.5[a)]	80.0	95.5[a)]	95.4	100		
D-10	68.9	59.6[b)]	61.0	67.6	68.7	74.3	68.7	70.8	70.6	100	
D-11	83.6	70.6	90.6	83.1	91.8	75.0	91.8	94.9	90.3	64.5	100

Note : D-01 = Balade; D-02 = Enam Hapat; D-03 = Kamundai; D-04 = Lakatan; D-05 = Likol; D-06 = Sahang; D-07 = Sihabuk; D-08 = Silanjung; D-09 = Sipisang; D-10 = Siitik; D-11 = Petruk. [a)] the far relationships; [b)] The close relationships

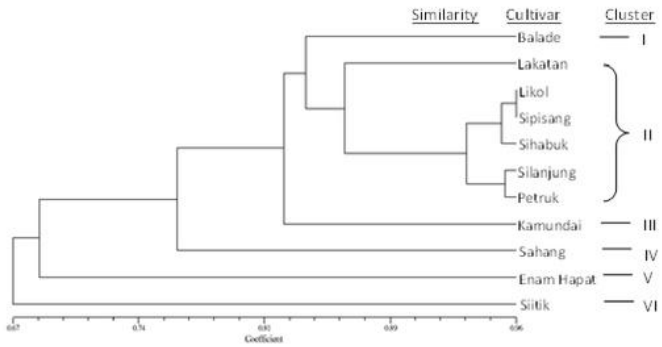

FIGURE 2. *Dendogram of 11 local durian cultivars, comprices ten from South Kalimantan's Province and one sample from Java island (Petruk cultivar as comparison)*

A dendogram shows that durian 'Petruk' cultivar from Central Java was closely related to 'Silanjung' cultivar from South Kalimantan, at the level of 0.949 similarity coefficient (Fig.2). In comparison to the repoted of Ruwaida et al. [6], that durian 'Petruk' has closely related to the durian 'Sunan' (also the local durian cultivar from Central Java) at the level of 0.830 similarity coeficient. Therefore, it was likely that durian 'Silanjung' would be close related to the durian 'Sunan' cultivar.

Despitefully, result of cluster analysis indicating a higher degree of genetic divergence amongst durian samples. The durian cultivar of 'Siitik' (D-10) was far distantly from the other durian cultivars at the level of 0.645 similarity coefficients. This cultivar was possible to become a new species (speciation) in the long-term. Chaveerach et al., [22], state that the samples at a level of 0.650 similarity coeficient, can be categorized as a new species. According to Frankham et al. [21], the divergence of germplasm based on clustering analysis were reflected the evolutionary potential of those germplasms for the future or adaptation to environmental changes.

CONCLUSION

Based on the polymorphism and clustering analysis, this results were concluded that local durian cultivars of South Kalimantan's province has a high diversity. The result of clustering analysis shows that those cultivars were clustered into six groups, with the highest similarity were shown both by durian 'Likol' and 'Sipisang', and 'Likol' and 'Sihabuk' (95.5%), while the lowest were shown by durian 'Enam Hapat' and 'Siitik' (59.6%). The result of RAPD analysis was also showed that OPA-16 can be applied for determine the polymorphism or genetic diversity of local durian cultivars.

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