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GENETIC DIVERSITY IN JACKFRUIT (*Artocarpus heterophyllus* Lam.) BASED ON MOLECULAR CHARACTERS IN INDONESIA

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SUMMARY

Jackfruit (*Artocarpus heterophyllus* Lam.) is one of the most widely cultivated fruit in Indonesia. The Faculty of Forestry, Gadjah Mada Universtity, Indonesia has 11 provenances where jackfruitis widely grown in Indonesia. The aim of this study was to assessed variation injackfruit based on molecular marker RAPD (Random Amplified Polymorphic DNA). Seven RAPD primers namely A-13, A-27, B-4, H-15, L-1, OPB-1, and OPD-19 generated 70 DNA bands, of which 40 were polymorphic. In this study H-15 primers produced more polymorphic fragments than the other six primers and had the highest percentage of polymorphic fragments, namely 91.7%. Similarity matrix was calculated using coefficient of Jaccard. Unweighted Pair Group Method Using Arithmetic Mean (UPGMA) cluster analysis was performedto develop a dendogram. This data analysis was performed by NTSYS software ver. 2.1. In the present studies, jackfruit tress accessions from the eleven provenances were divided into two clusters with similarity coefficient ranging from 63.33 to 85.71%, provenance of East Java and West Java were the most closely similar (85.71%) based on molecular characters.

Key words: Jackfruit, molecular characters, genetic diversity, RAPD

Key findings: Detection of genetic differences and relationship between jackfruit from 11 provenances of Indonesia for sustainable cultivation and for conservation of plant genetic resources.

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INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.) is one of the oldest cultivated fruit tree belonging to Moraceae. Jackfruit originated from southern India and spread to the other tropical regions, including Indonesia. Jackfruit trees are wide spread in Indonesia as they are grown in almost every regions of the country (Widyastuti, 1993).

DNA molecules based markers enables rapid detection of genetic variations and are less influenced by the environmental factors. It enables the studies conducted a teach plant development phase, and most importantly, may be conducted in small number, yet representing the whole genome (de Vicente and Fulton, 2003).

Polymerase Chain Reaction (PCR) is a technique developed to produce millions of copies of certain DNA fragments for genetic analysis. PCR principle is to amplify a DNA sequence in a short-term cycle consisting of three stages of rapid temperature changes, covering; denaturation, primary annealing (attachment to template DNA) and the elongation or amplification (Weising *et al*., 1995).

Random Amplified Polymorphic DNA (RAPD) is a PCR researction based marker system developed by J. Williams in 1990 (Williams *et al*., 1990). This technique is based on Polymerase Chain Reaction (PCR) where DNA fragments are amplified using a single primer with a random nucleotide sequence. RAPD technique detects the DNA polymorphism by the presence and absence of the targeted DNA fragment amplification. The advantage of RAPD method when compared to the other PCR-based methods is that it simply uses a short single random primer sequence; the implementation is relatively easy and inexpensive and may produce numerous polymorphisms (Williams *et al*., 1990).

The research on jackfruit trees has been frequently conducted including genetic diversity analysis using RAPD method as reported earlier in China (Chunhai *et al*., 2005), India (Simon *et al*., 2007; Deivasigamani *et al*., 2009; Anburaj and Sudarmani, 2010), and Sri Lanka (Pusphakumara and Harris, 2007). However, the research on genetic diversity of the jackfruit trees grown mainly in Indonesia has never been conducted. Jackfruit research in Indonesia is limited to the analysis of flavonoid compounds resulted from the jackfruit bark (Musa, 2004). Globally, jackfruit research not only uses RAPD molecular markers but also other systems such as Amplified Fragment Length Polymorphism (AFLP) (Schnell *et al*., 2001; Shymalamma *et al*., 2008) and Restriction Fragment Length Polymorphism (RFLP) (Kanzaki *et al*., 1997).

This research aims at determining genetic diversity of Indonesian jackfruit from 11 provenances and a collection belonging to the Faculty of Forestry, Gadjah Mada University, Indonesia. Understanding of genetic variation would be highly important information in preserving and utilizing germplasm.

MATERIALS AND METHODS

Plant material

The jackfruit accessions used as the samples in this research are the collection belonging to the Faculty of

Forestry, Gadjah Mada University, Indonesia planted in the lands belonging to the Forestry and Plantation Service of Gunungkidul Regency of the Special Region of Yogyakarta Province, Indonesia. The samples were collected by selecting the jackfruit plants which have good quality and the same number of seedlot. The samples of DNA materials are also selected from the healthy, clean, and undamaged leaves.

Molecular character

RAPD analysis steps included: DNA isolation, Polymerase Chain Reaction (PCR), Electrophoresis, and visualization of amplified DNA fragments. DNA isolation methods were based on Daryono and Natsuaki (2002), using Nucleon-Phytopure Kit (Phytopure Reagent I, Phytopure Reagent II, and resin). Crush 0.1 gram of jackfruit plant leaves until smooth. Add 500 µL of phytopure reagent I and then 150 µL of phytopure reagent II. Incubate the samples at the temperature of 65 °C for 10 minutes. Let the samples stand for 20 minutes until cold. Add 400 µL of cold chloroform. Add 20 µL of phytopure resin carefully with the tube in perpendicular position. Centrifuge at 3000 rpm for 10 minutes. Carefully, move the supernatant to a new 1.5 mL tube. Add cold isopropyl alcohol to the same volume as the supernatant and shake slowly by hand. Centrifuge at 10,000 rpm for 10 minutes. Remove the white supernatant and further repeat the step to precipitate the DNA pellets. Wash the pellets by adding 50 µL of 70% alcohol and then centrifuge at 10,000 rpm for 5 minutes (repeat 3 times). Carefully remove the residual ethanol, dry the pellets, dissolve with 50 µL of TE and then store in a freezer at -20 °C.

PCR reaction was performed using seven RAPD primers. PCR conditions: 1) Initial denaturation at the temperature of 94 °C for 4 minutes; 2) denaturation at the temperature of 94 °C for 1 minute; 3) Annealing (primer attachment) at the temperature of 35 °C for 1 minute; 4) Extension at the temperature of 72 °C for 2 minutes and 45 cycles; 5) The final extension at the temperature of 72 °C for 10 minutes. The PCR amplification results were analyzed through electrophoresis by running the samples in 1.5% agarose gel at a voltage of 100 volts. The DNA fragments were visualized from the gel using UV transilluminator.

Data analysis

DNA fragments from the PCR amplification were scored as follows. A score of 1 was given for the presence of a fragment while a score of 0 was recorded for their absence. Data analysis was conducted using the software NTSYS ver. 2.1. The similarity Index values were obtained through Jaccard similarity coefficients and cluster analysis using Unweighted Pair-Group Methods Using Mean Arithmetic (UPGMA) method to determine the phenetic relationship between OTU's shown in the form of dendrogram.

RESULTS

Molecular characters of jackfruit accessions from 11 provenances

Random Amplified Polymorphic DNA (RAPD) is a PCR based technique for identifying genetic variation. It

involves the use of a single arbitrary primer in a PCR reaction, resulting in the amplification of many discrete DNA fragments. The principle of the RAPD technique is based on the ability of the primer to attach to the DNA template. The use of primers with a shorter base size is intended so that the primer can attach randomly to the genomic DNA of the organism to amplify more DNA fragments. In addition to the primer, the RAPD results were also determined by the quality and quantity of template DNA, the Taq polymerase enzyme, and the specifications of the PCR machine used. The use of appropriate extraction protocols and the purity of DNA from contaminants is important to produce sharp DNA ribbon quality to facilitate interpretation and accuracy of data. The best ratio of DNA and RNA with value between 1.8-2.2 is the ideal concentration. A ratio that is too low (<1.8) contains too much RNA, while a ratio that is too high (> 2.2) indicates the presence of many protein contaminants in the DNA solution. The jackfruit genome in our experiments had a purity ratio of 1.57 - 1.92. This value indicates that there were samples of jackfruit genome with a low purity of less than 1.8

The DNA samples from the jackfruit of the 11 provenances were characterized using RAPD (Random Amplified Polymorphic DNA) method and seven primers such as A-13, A-27, B-4, H-15, L-1, OPB-1, and OPD-19, nucleotide sequences of seven primers (Table 1). All the primers amplified DNAs from the samples (Figures 1 to 7). The total number of DNA fragments produced by the seven primers consists of 70 DNA fragments at the size of 100 bp to 900 bp, of which 40 DNA fragments (57.1%) are considered as polymorphic fragments. On average, each primer produces 10 DNA fragments (Table 2). The primer that produced the least number of DNA fragments was A-27 with five DNA fragments of which only 1 was polymorphic (20%). Primer B-4 and L-1 evenly produce 9 DNA fragments. B-4 primer produces 5 polymorphic fragments (55.6%) while primer L-1 produces four polymorphic fragments (44.4%). Primer OPD-19 produces 11 DNA fragments with seven polymorphic fragments. Primer A-13, H-15, and OPB-1 evenly produce 12 DNA fragments with a different number of polymorphic fragments as primer A-13 produces 5 polymorphic fragments, primer H-15 produces 11 polymorphic DNA fragments, and primer OPB-1 produces seven polymorphic fragments. In this study, primer H-15 produces more polymorphic fragments than the other 6 primers and has the highest polymorphic fragment percentage by 91.7%.

Amplified DNA fragments using A-13 primers produced a total of 14 DNA fragments measuring 104 - 725 bp. DNA fragments produced from the jackfruit samples amounted to 12 fragments consisting of 5 polymorphic and seven monomorphic fragments. Amplified DNA fragments using A-27 primers produced seven DNA fragments measuring 150 - 602. DNA fragments of 302 bp and 602 bp were produced specifically from samples of C*empedak*, so that only 5 DNA fragments were amplified by A-27 primers and measured between 150 - 375 bp. Five fragments produced consisting of four monomorphic fragments and 1 polymorphic fragment, namely on a 352 bp DNA fragment that did not appear in the samples from provenances of South Kalimantan and Lombok.

Table 1. Primer used for RAPD PCR reaction in eleven jackfruit provenances.

Primer	Nucleotide Sequence (5′ – 3′)	Reference Source
A-13	GCGGCTGGAG	Chunhai *et al*. 2005
A-27	AGCACGGGCA	Chunhai *et al*. 2005
B-4	CTCTCGCCCC	Chunhai *et al*. 2005
H-15	AATGGCGCAG	Deivasigamani *et al*. 2005
L-1	TGCGCCTCAC	Chunhai *et al*. 2005
OPB-1	CTGGGGACTT	Phuspakumara dan Harris, 2007
OPD-19	GTTTCGCTCC	Anburaj dan Sudarmani, 2010

Table 2. Total number of 11 DNA fragments of jackfruit provenances produced by amplifying seven RAPD primers and polymorphic fragment percentage.

Primer	Sequence (5′- 3′)	Amplified Total Fragments	Monomorphic Fragments	Polymorphic Fragments	Polymorphic Percentage (%)
A-13	GCGGCTGGAG	12	7	5	41.7
A-27	AGCACGGGCA	5	4	1	20.0
B-4	CTCTCGCCCC	9	4	5	55.6
H-15	AATGGCGCAG	12	1	11	91.7
L-1	TGCGCCTCAC	9	5	4	44.4
OPB-1	CTGGGGACTT	12	5	7	58.3
OPD-19	GTTTCGCTCC	11	4	7	63.6
Total		70	30	40	
Average		10	4.3	5.7	57.1

Amplified DNA fragments using B-4 primers produced 11 DNA fragments measuring 148 - 575 bp. The DNA fragments produced from the jackfruit trees amounted to 9 fragments consisting of 5 polymorphic fragments and four monomorphic fragments.Amplified DNA fragments using H-15 primers produced a total of 15 DNA fragments measuring 102-900 bp. DNA fragments produced by provenance of jackfruit amounted to 12 fragments consisting of 11 polymorphic fragments and 1 monomorphic fragment.

Amplified DNA fragments using L-1 primers reduced 10 DNA fragments measuring 125-723 bp. DNA fragments produced by provenance of jackfruit amounted to 9 fragments consisting of four polymorphic fragments and five monomorphic fragments. The DNA fragments from amplification using primary OPB-1 produced 14 DNA fragments measuring 100-598 bp. DNA fragments amounted to 12 fragments specifically seven fragments were polymorphic and 5 monomorphic fragments. The DNA fragments from amplification using OPD-19 primer produced 15 DNA fragments measuring 123-800 bp. DNA fragments in this study amounted to 11 fragments consisting of seven polymorphic fragments and four monomorphic fragments.

DISCUSSION

DNA fragments amplified from jackfruit samples of the 11 provenances using A-13 primers

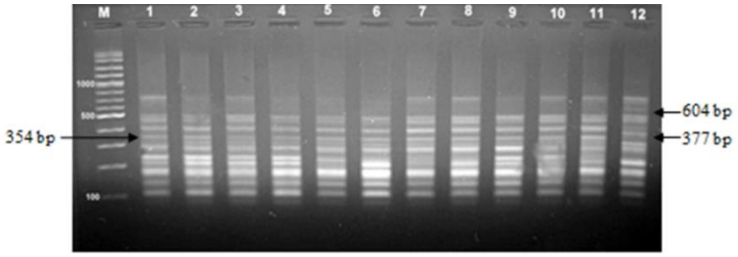

Figure 1. Jackfruit DNA Fragments amplified using primer H-15.*

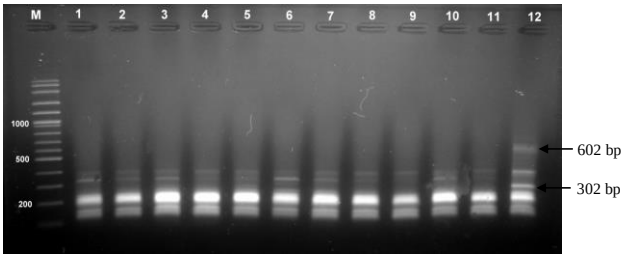

Figure 2. Jackfruit DNA Fragments amplified using primer A-27.*

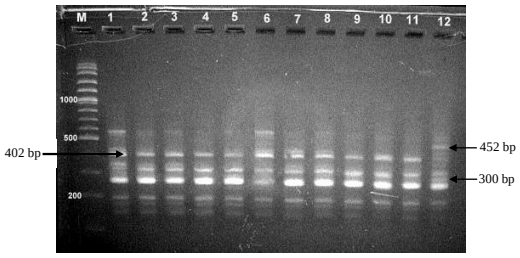

Figure 3. Jackfruit DNA Fragments amplified using primer B-4.*

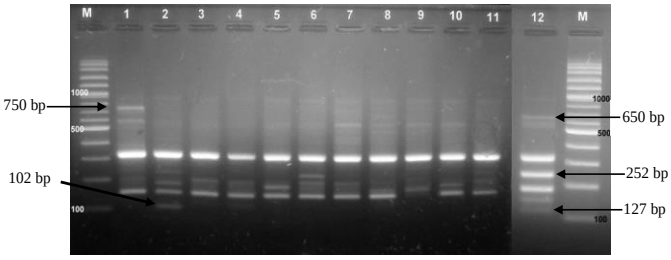

Figure 4. Jackfruit DNA Fragments amplified using primer H-15.*

*Description: M = Vivantis marker 100 bp; 1 = East Java provenances; 2 = Central Java provenances; 3 = West Java provenances; 4 = Bali provenances: 5 = Medan provenances; 6 = Pekanbaru provenances; 7 = Lampung provenances; 8 = East Kalimantan provenances; 9 = South Kalimantan provenances; 10 = Kendari provenances; 11 = Lombok provenances; 12 = Out group (*cempedak*/*Artocarpus integer*)

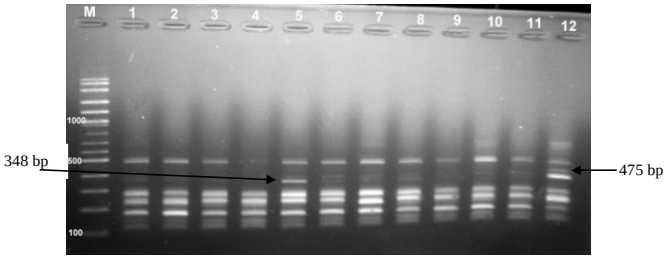

Figure 5. Jackfruit DNA Fragments amplified using primer L-1.*

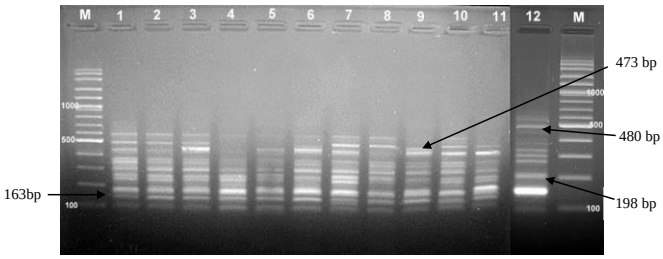

Figure 6. Jackfruit DNA Fragments amplified using primer OPB-1.*

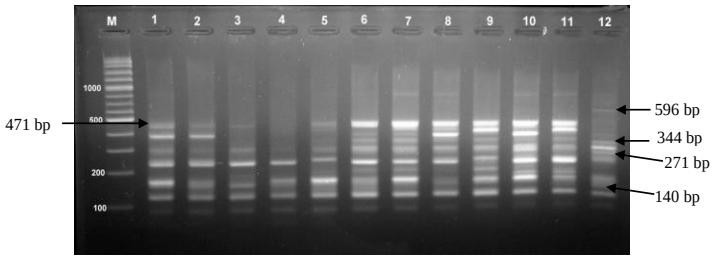

Figure 7. Jackfruit DNA Fragments amplified using primer OPD-19.*

*Description: M = Vivantis marker 100 bp; 1 = East Java provenances; 2 = Central Java provenances; 3 = West Java provenances; 4 = Bali provenances: 5 = Medan provenances; 6 = Pekanbaru provenances; 7 = Lampung provenances; 8 = East Kalimantan provenances; 9 = South Kalimantan provenances; 10 = Kendari provenances; 11 = Lombok provenances; 12 = Out group (*cempedak*/*Artocarpus integer*)

amounted to 9-12 DNA fragments, while the number of fragments produced by the samples of the *Cempedak* was 12 DNA fragments. DNA fragments of 377 bp and 604 bp appeared in *Cempedak* indicating their specificityto the region while 354 bp DNA fragment appeared in other provenance while results from Chunhai *et al*. (2005) showed that DNA fragments amplified using the same primer in jackfruit plant samples of China produced a total of 11 DNA fragments which were all polymorphic.

DNA fragments from amplification using A-27 primers produced only 5 DNA fragments jackfruit measuring between 150-375

bp, namely four monomorphic fragments and 1 polymorphic fragment. DNA fragments measuring 352 bp did not appear in the provenances of South Kalimantan and Lombok. Past research showed that DNA fragments of jackfruit plants from amplification using A-27 primers in China produced 6 DNA fragments which were all polymorphic (Chunhai *et al*., 2005). More polymorphic DNA fragments were produced because the samples used were more than 65 jackfruit accessions.

The results of amplification using B-4 primers produced between 4-9 DNA fragments; while from *Cempedak* has 5 fragments. The DNA fragment measuring 402 bp only appears in the provenance of jackfruit. Research results from Chunhai *et al*. (2005) showed that DNA fragments from amplification using the same primer in jackfruit plant samples in China produced a total of 8 DNA fragments which were all polymorphic.

The DNA fragments amplified using primer H-15 on jackfruit from the 11 provenances and *Cempedak* produce a total of 15 DNA fragments with the size of 102-900 bp, 12 DNA fragments are produced by the jackfruit from the 11 provenances consisting of 11 polymorphic fragments and 1 monomorphic fragment. Jackfruit from each provenance has at least 3 to 6 DNA fragments, while *Cempedak* has 8 DNA fragments. The DNA fragment pattern showed similarity between West Java and Bali provenances, while the other provenances exhibit considerable variations. The DNA fragments with the size of 127 bp, 252 bp and 650 bp appeared only in *Cempedak*. There were 3 specific fragments, covering those with the size of 102 appearing only in the samples of Central Java provenances, those with the size of 400 bp appearing only in samples from Lampung provenances and those with the size of 750 bp appearing only from jackfruits of East Java provenances.

The DNA fragments with the size of 146 bp from the analysis using primer H-15 do not appear in samples from South Kalimantan provenances that can be used as a reference or markers for the region. The research conducted by Deivasigamani *et al*. (2009) show that the amplification using primer H-15 in 5 Indian jackfruit varieties produce a total of 31 DNA fragments with3 polymorphic fragments. The average DNA fragment produced by each Indian jackfruit variety using primer H-15 is 6.2, while the average DNA fragments produced by each Indonesian jackfruit provenance using the same primer is 10.1.

A total of DNA fragments produced in the research conducted by Deivasigamani *et al*. (2009) is more than that of this research since the total of fragments calculated is the number of DNA fragments appearing in each variety. The fragments calculated in this study are DNA fragments with different sizes, although some appears simultaneously or partially on provenances, yet calculated only once that the calculated fragments are fewer in number. The produced DNA fragments are then analyzed by the presence or absence of DNA fragments on samples of each provenance. The similarity coefficient used is Jaccard coefficient (Table 3).

DNA fragments with L-1 primers number between 5-8, while from *Cempedak* samples produces 9 DNA fragments. DNA fragments measuring 475 bp were specific DNA fragments

Table 3. Association coefficient (%) of 11 Indonesian jackfruit provenances considered as the collection belonging to the Faculty of Forestry, Gadjah Mada University, Indonesia based on molecular character.

OTU	1	2	3	4	5	6	7	8	9	10	11	12
1	100											
2	83.05	100										
3	85.71	85.45	100									
4	71.43	74.07	83.67	100								
5	69.49	78.18	77.36	75.51	100							
6	76.66	79.31	75.44	70.37	71.43	100						
7	79.03	70.31	72.13	64.41	65,57	75.41	100					
8	83.61	77.42	76.67	63.33	70.00	77.05	79.37	100				
9	64.52	66.67	71.43	69.23	73.58	68.97	63.49	73.33	100			
10	71.43	73.77	75.86	67.86	68.97	73.33	78.69	74.60	72.41	100		
11	63.49	68.33	73.21	67.92	69.09	73.68	70.49	72.13	75.93	80.36	100	
12	50.67	50.00	53.86	47.76	47.14	49.32	52.00	55.41	52.17	56.34	51.43	100

for *cempedak* region. The 348 bp DNA fragment is a specific DNA fragment that appears on the provenance of Medan so that DNA fragments can be used as a marker for the provenance of Medan. The 498 size DNA fragment does not appear in the provenance of Bali so that it can be used as a reference for identification or markers of Bali provenance if amplified with L-1 primers. Chunhai *et al*. (2005) showed that DNA fragments from amplification using the same primer on 65 accessions of jackfruit in China produced seven DNA fragments with 6 polymorphic fragments. The number of DNA fragments produced was less than this study but more polymorphic fragments were produced.

The DNA fragments produced using OPB-1 primers amounted to 12 fragments consisting of seven polymorphic and 5 monomorphic fragments. DNA fragments measuring 198 bp and 480 bp were specific to *Cempedak* region, while 163 bp DNA fragments only appeared in proven jackfruit DNA fragments. The 473 bp DNA fragment is specific to the samples from provenance of South Kalimantan. The 373 bp DNA fragment did not appear in Bali's provenances so that it could be used as a reference for identification or markers for Bali provenance samples if amplified with OPB-1 primers. The results of Pushpakumara and Harris (2007) showed that DNA fragments from amplification using the same primer in jackfruit plant samples in Sri Lanka produced 9 DNA fragments consisting of seven polymorphic and 1 monomorphic fragment.

DNA fragments produced by provenance of jackfruit using OPD-19 primers in this study amounted to 11 fragments consisting of seven polymorphic and four monomorphic fragments. DNA fragments of each provenance of jackfruit are between 4-10, while *Cempedak* region has 8 DNA fragments. The 471-size DNA fragment did not appear in Bali's provenances. The results of Anburaj and Sudarmani (2010) study showed that DNA fragments from amplification using OPD-19 primers in 5 individual jackfruit plants in India produced a total of 67 DNA fragments with 18 polymorphic fragments.

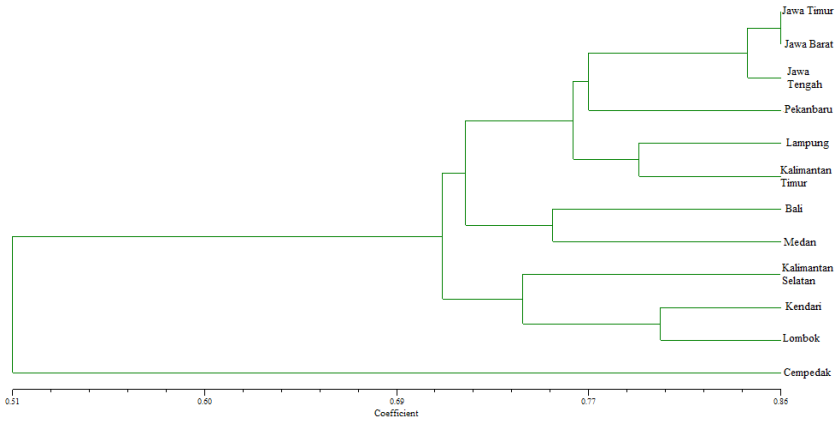

Figure 8. The dendogram of 11 Indonesian jackfruit provenances considered as the collection belonging to the Faculty of Forestry, Gadjah Mada University, Indonesia and 1 out group (*cempedak*) based on molecular character.

Jackfruit provenances are divided into two major groups at the association coefficient value of 1%, while from C*empedak* used as the separating comparison of 11 jackfruit provenances is at the association coefficient value of 51.4% (Figure 8). Group I consists of East Java, West Java, Central Java, Pekanbaru, Lampung, East Kalimantan, Bali, and Medan provenances, while group II consists of South Kalimantan, Kendari, and Lombok provenances. The highest similarity is the East Java and West Java provenances with the association coefficient of 85.7%, which means having a close kinship, probably because both provenances come from adjacent regions.

The research results show that the molecular character of RAPD method using seven primers on 11 Indonesian jackfruit provenances produced 70 DNA fragments measuring 100 bp to 900 bp, of which 40 DNA fragments (57, 1%) were polymorphic fragments. The research results show that the further research is required to obtain more complete data related to each jackfruit provenance characteristics using more number of RAPD primers or the other molecular markers. The research results on genetic diversity of 11 Indonesian jackfruit provenances considered as the collection of the Faculty Forestry of Gadjah Mada University, Indonesia based on the molecular character may be used for the conservation and cultivation purposes.

In Indonesia, young jackfruit or known in Indonesia as *gori* is very popular as a vegetable material. Young jackfruit in Yogyakarta generally cooked into *gudeg* and becomes a typical food of Yogyakarta. A sustainable and exploitative utilization of young jackfruit may reduce the production of the ripe jackfruit. A direct understanding should be given to the society due to their role in conserving the genetic diversity of jackfruit plants by not harvesting and consuming the all relatively young jackfruit that the seeds as a genetic diversity source may be obtained from the ripe jackfruit.

Eleven Indonesian jackfruit provenances have high genetic diversity based on molecular characters using RAPD marker. Detection of genetic differences and discrimination of genetic relationship between jackfruit provenances are for sustainable utilization and conservation of plant genetic resources.

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