BIODIVERSITAS
Volume 12, Number 3, July 2011
Pages: 125-130

ISSN: 1412-033X (printed edition)
ISSN: 2085-4722 (electronic)
DOI: 10.13057/biodiv/d120301

Genetic variations of *Lansium domesticum* Corr. accessions from Java, Sumatra and Ceram based on Random Amplified Polymorphic DNA fingerprints

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Manuscript received: 16 February 2011. Revision accepted: 8 June 2011.

ABSTRACT

Yulita KS (2011) Genetic variations of Lansium domesticum *Corr. accessions from Java, Bengkulu and Ceram based on Random Amplified Polymorphic DNA fingerprints. Biodiversitas 12: 125-130.* Duku (*Lansium domesticum* Corr.) is one of popular tropical fruits in SE Asia. The species has three varieties, known as duku, langsat and kokosan; and duku is the most popular one for being the sweeties fruit. Indonesia has several local varieties of duku, such as duku Condet, duku Sumber and duku Palembang. This present study aimed to assess genetic diversity of 47 accessions of duku from Java, Sumatra, and Ceram based on RAPD fingerprints. Ten RAPD's primers were initially screened and five were selected for the analysis. These five primers (OPA 7, 13, 18, OPB 7, and OPN 12) generated 53 scorable bands with an average of 10.6 polymorphic fragments per primer. Percentage of polymorphism ranged from 16.89% (OPA 7 and OPN 12) to 24.54% (OPB 7) with an average of 20.16% polymorphism. OPB 7 at 450 bp was exclusively possessed by accession 20 (Java), OPA 18 at 500 bp was by accession 6 (Java), 550 bp by 3 clones from Bengkulu. While OPN 12 at 300 bp and OPA 13 at 450 bp were shared among the accessions. Clustering analysis was performed based on RAPD profiles using the UPGMA method. The range of genetic similarity value among accessions was 0.02-0.65 suggesting high variation of gene pool existed among accessions.

Key words: *Lansium domesticum,* duku, RAPD, genetic variation.

INTRODUCTION

Duku (*Lansium domesticum* Corr.) belongs to the family of Meliaceae is one of the popular fruits in Indonesia. The main distribution of this species is in Southeast Asia, but the species has also found in Suriname, Puerto Rico and Australia (Othman and Suranant 1995). The species is closely related to *L. membranaceum* Kosterm. (Mabb.) and *L. breviracemosum* Kosterm. (Mabberley et al. 1995). Recent taxonomic treatment of Meliaceae (Mabberley et al. 1995) did not assign infra-specific rank for *L. domesticum*. Nevertheless, there are three varieties of *L. domesticum* that have been widely known namely duku, langsat and kokosan. Hence, for practical purpose, Mabberley et al (1995) suggested to write *L. domesticum* cv kokosan or *L. domesticum* 'kokosan' when referring to kokosan variety. These three varieties are widely known to the local fruit market and can be distinguished mainly on the basis of their fruit morphology. Among of these varieties, duku is the most preferable fruit since it has no latex, few seeds, sweet berry and pleasant aroma. Duku has also medicinal and cosmetic properties from its peel extract (Tilaar et al. 2008)

Some of widely known local cultivars of duku include duku Condet from Jakarta, duku Papongan from Tegal, duku Kalijajar from Purbalingga, duku Karangkajen and duku Klaten from Yogyakarta, duku Woro from Rembang, duku Sumber from Kudus, duku Palembang, duku Padang Batung from South Kalimantan. However, the most famous local cultivar is duku Palembang. This present study has collected 47 accessions of duku from Bengkulu (Sumatra), Kudus (Central Java), Germplasm Garden of Cibinong Science Centre (*Kebun Plasma Nutfah* Cibinong Science Centre; KPN-CSC) and Bogor Botanic Garden (*Kebun Raya Bogor*; KRB) with aimed to evaluate genetic variability among the accessions using Random Amplified Polymorphic DNA (RAPD).

RAPD is a molecular marker that has been widely used for genotyping plant species (Jimenez et al. 2002; Chakrabarti et al. 2006; Dnyaneshwar et al. 2006; Keller-Przyby³kowicz et al. 2006), evaluation of genetic relationship (Upadhyay et al. 2004; Goh et al. 2005) and variation (Martin et al. 2002; Ferriol et al. 2003; Fan et al. 2004; Adetula 2006; Guo et al. 2007; Jain et al. 2007). It is random fragment amplification technique, which based on amplification of DNA fragment randomly using single arbitrary primer. The main advantages of this marker include rapid and cost-efficient in terms of operational aspects.

MATERIALS AND METHODS

Sample

Forty-seven accessions consist of 43 *L. domesticum* 'duku', three *L. domesticum* 'kokosan' and one *Lansium*

sp. that were collected from KRB (10 accessions), KPN-CSC (9 accessions), Central Java (14 accessions) and Bengkulu (14 accessions) were being used in this study (Table 1). The biogeographical coverage of these collections include Java, namely Kudus in Central Java and Jakarta (26 accessions), Sumatra i.e. Bengkulu and Palembang (17 accessions), Ceram (1 accession) and unidentified locations within Malesian region (3 accessions). Samples were collected as dried leaves stored in silica gel.

Extraction of total DNA genome

Total DNA genome was extracted from the dried leaves using modified CTAB (Doyle and Doyle 1990) by addition of RNAse 200 µg/mL. The total DNA genome was electrophorezed on 0.7% agarose gel in 1X TAE buffer at 100 volt for 30 min, followed by ethidium bromide staining before photographed using gel documentation system (Atto Bioinstrument).

Tabel 1. List of samples used in the study.

Accession no	Vernacular/species name	Source of materials
KD 1	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 2	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 3	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 4	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 5	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 6	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 7	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 8	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 9	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 10	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 11	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 12	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 13	Duku Sumber	Golan Tepus Village, Mejobo Sub-district, Kudus District, Central Java
KD 14	Duku Sumber	Golan Tepus Village, Mejobo Sub-district, Kudus District, Central Java
CD 1	*L. domesticum*	XIX.F.124, KRB
CD 2	*L. domesticum*	III.C.35, KRB (origin: Malesia)
CD 3	*L. domesticum*	III.C.59, KRB
CD 4	*L. domesticum*	III.B.100A, KRB
CD 5	*L. domesticum*	III.B.100, KRB (origin: Malesia)
CD 6	*L. domesticum*	III.B.6, KRB (origin: Java)
CD 7	*Lansium* sp.	III.C.106, KRB (origin: Ceram, Maluku)
CD 8	*L. domesticum*	III.B.142, KRB
CD 9	*L. domesticum*	XI.B.VII.215, KRB (origin: Malesia)
CD 10	*L. domesticum*	XII.B.VIII.46, KRB
CD 11	Duku lokal	KPN-CSC, tag no. 1, I.B (origin: Jakarta)
CD 12	Duku kokosan	KPN-CSC, tag no. 2, I.B (origin: Jakarta)
CD 13	Duku kokosan	KPN-CSC, tag no. 3, I.B (origin: Jakarta)
CD 14	Duku lokal	KPN-CSC, tag no. 4, I.B (origin: Jakarta)
CD 15	Duku lokal	KPN-CSC, tag no. 5, I.B (origin: Jakarta)
CD 16	Duku kokosan	KPN-CSC, tag no. 8, I.B (origin: Jakarta)
CD 17	Duku Palembang	KPN-CSC, tag no. 35, I.B (origin: Palembang)
CD 18	Duku Palembang	KPN-CSC, tag no. 45, I.B (origin: Palembang)
CD 19	Duku Palembang	KPN-CSC, tag no. 65, I.B (origin: Palembang)
APH 22	Duku	Rena Panjang Village, Lubuk Sandi Sub-district, Seluma District, Bengkulu
APH 23	Duku	Rena Panjang Village, Lubuk Sandi Sub-district, Seluma District, Bengkulu
APH 24	Duku	Rena Panjang Village, Lubuk Sandi Sub-district, Seluma District, Bengkulu
APH 25	Duku	Gunung Agung Village, Arga Makmur Sub-district, North Bengkulu District, Bengkulu
APH 26	Duku	Gunung Agung Village, Arga Makmur Sub-district, North Bengkulu District, Bengkulu
APH 27	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 28	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 29	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 30	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 31	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 32	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 33	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 34	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 35	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu

PCR RAPD amplification

RAPD amplification was performed in Takara thermocycler following Williams et al. (1990) with random decamer primers (OPA 7, OPA 13, OPA 18, OPB 7, OPN 12) obtained from Operon Technologies (Almeda, USA). Amplifications were performed in 15 µl reaction volume containing 1x PCR Green Master Mix (Promega, USA), 2 µM primer (Operon Technology), and ~10 ng of DNA template. Amplified products were separated in 2% agarose gel in 1X TAE buffer at 50 volt for 120 min. The gels were stained with 0.5 µg/mL ethidium bromide solution and visualized and photographed using gel documentation system (Atto Bioinstrument). The PCR reactions were done twice to ensure the reproducibility and consistency of the PCR products.

Data analysis

The RAPD bands were scored manually based on the profiles obtained from gel electrophoresis photos, as present (1) or absent (0), each of which was treated as a putative locus. Data analyses were performed using NTSys-PC (Numerical Taxonomy System, version 2.02i, Rohlf 1998). The SIMQUAL (Similarity for Qualitative Data) program was used to calculate the Jaccard's similarity coefficient, a common estimator of genetic identity. Similarity matrices were utilized to construct the UPGMA (unweighted pair group method with arithmetical average) dendrograms. Finally, a principal coordinate analysis was performed in order to highlight the resolving power of the ordination.

RESULTS AND DISCUSSION

Pattern of RAPD fingerprints

Amplifications of genomic DNA of 47 accessions using five primers yielded 53 fragments that could be scored. The number of amplified fragments ranging from 9 (OPA 7 and OPN 12) to 13 (OPB 7), with an average of 10.6 polymorphic fragment per primer and which varied in size from 300 (OPA 18 and OPN 12) to 1700 pb (OPA 18). Percentage of polymorphism ranged from 16.89% (OPA 7 and OPN 12) to 24.54% (OPB 7) with an average of 20.16% polymorphism (Table 2). The selection of primers and the number of primers used to amplify the DNA template would be crucial to produce polymorphic bands because these selections will determine attachment of primers to their complementary sequences of the DNA templates (Tingey et al. 1994). This present study used only bands that were existed between 300-1700 bp. Generally, bands below 300 bp were inconsistent, while bands above 1700 bp could not be well separated during electrophoresis.

Genetic variations observed were mainly based on differences on RAPD profiles found in all accessions. Generally all the 53 RAPD bands were found in all accessions (Figure 1-3). Common bands that were existed in all accessions was OPN 12 at 300 bp (Figure 3). However, few bands were only existed within certain accessions, i.e. OPB 7 at 450 bp and OPA 18 at 500 bp were recorded at two accessions from Java (no 20 and 6, respectively). Thus, these unique bands may potentially be served as provenance diagnostic character. Nevertheless, in order to enable RAPD be used as diagnostic marker for provenance identification, may need more sampling at populational level because when more samples were included there might be more unique bands found or vice versa, the specific bands observed in certain provenance may not be any longer unique bands.

Estimation of genetic diversity

A dendrogram based on UPGMA analysis grouped the 47 accessions into two clusters (A and B) and five minor clusters (C) located outside the main cluster, with Jaccard's similarity coefficient ranging from 0.02 to 0.65 (Figure 4). This may be implied considerable wide range of genetic variations among accessions. A rather different result was recorded from a study on *Lansium domesticum* in Peninsular Malaysia (Song et al. 2000) that reported high genetic similarity (0.43-0.98). The main cluster (A and B, united by coef. 0.15) mainly comprises duku collected from Java in addition to kokosan, duku Palembang dan six of duku Bengkulu (Figure 4). Meanwhile, eight accessions of duku Bengkulu and Ceram were placed outside the main cluster. Dendrogram did not indicate any clear pattern of clustering to the location in which they were collected because the putative similar bands originating from RAPDs in different individuals are not necessarily homologous although they may share the same size. Similar results were observed in blackgram (Souframanien and Gopalakrishna 2004) and *Xanthomonas axonopodis* pv *dieffenbachiae* strain (Khoodoo and Jaufeerally-Fakim 2004).

Table 2. Percentage of polymorphism in five RAPD primers and their distribution in each bioregion, namely Java, Sumatra, and Ceram.

Primer's name	DNA sequence	Number and percentage of polymorphic loci (PPL)	Size range (bp)	Common bands (bp)	Unique bands observed in each bioregion (bp)		
					Java	Sumatra	Ceram
OPA 7	5′ GAA ACG GGT G 3′	9 (16.89%)	450-1200	-	450	-	-
OPA 13	5′ CAG CAC CCA C 3′	11 (20.75%)	400-1400	450	-	-	-
OPA 18	5′ AGG TGA CCG T 3′	11 (20.75%)	300-1700	-	500	550	-
OPB 7	5′ GGT GAC GCA G 3′	13 (24.54%)	400-1500	-	-	-	-
OPN 12	5′ CAC AGA CAC C 3′	9 (16.89%)	300-1500	300	-	-	-
Average		10.6 (20.16%)					

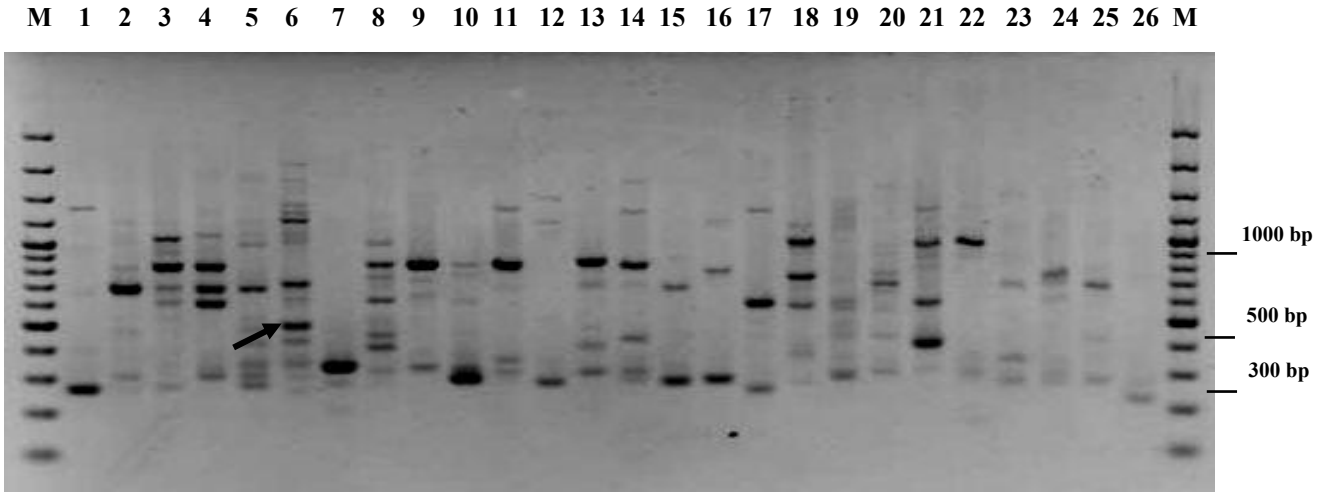

Figure 1. RAPD profiles of 26 accessions of *Lansium domesticum* using OPA 18. M: 100 bp plus DNA marker (Fermentas), Lane 1-14: Duku Sumber, Lane 15-20: *L. domesticum*, Lane 21: *Lansium* sp., Lane 22-24: *L. domesticum*, Lane 25: Duku lokal, Lane 26: Kokosan. Arrow: specific band found in accession 6.

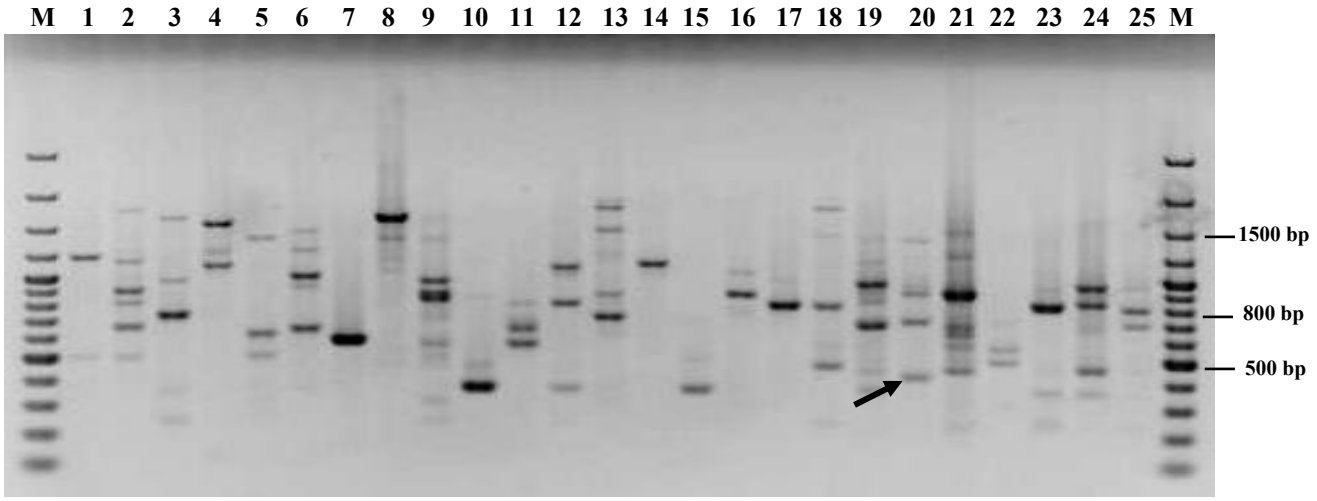

Figure 2. RAPD profiles of 25 accessions of *Lansium domesticum* using OPB 7. M: 100 bp plus DNA marker (Fermentas), Lane 1-14: Duku Sumber, Lane 15-20: *L. domesticum*, Lane 21: *Lansium* sp., Lane 22-24: *L. domesticum*, Lane 25: Duku lokal. Arrow: specific band found in accession 20.

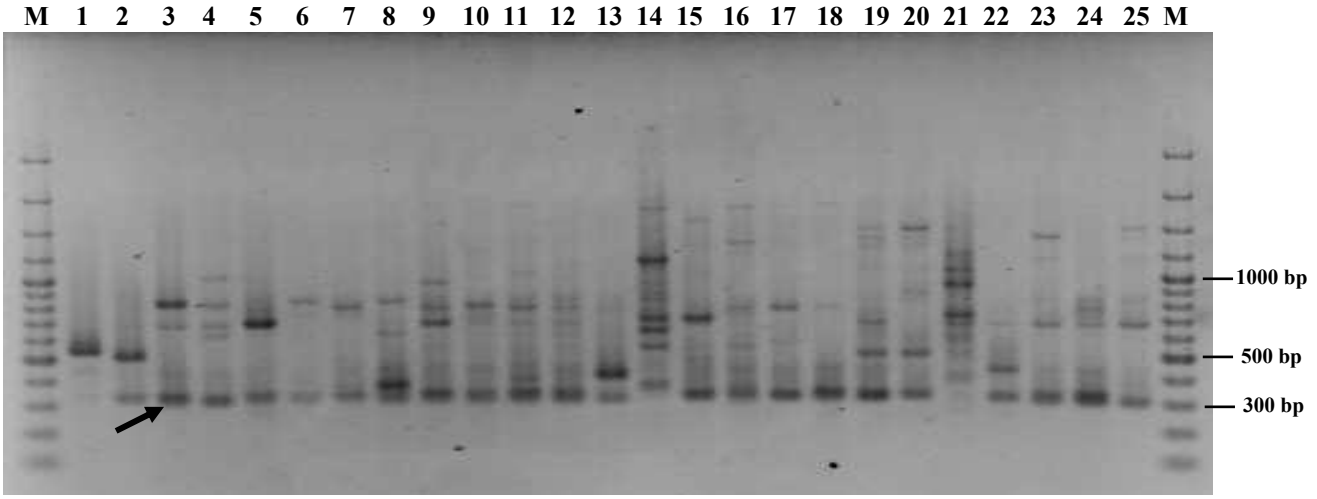

Figure 3. RAPD profiles of 25 accessions of *Lansium domesticum* using OPN 12. M: 100 bp plus DNA marker (Fermentas), Lane 1-14: Duku Sumber, Lane 15-20: *L. domesticum*, Lane 21: *Lansium* sp., Lane 22-24: *L. domesticum*, Lane 25: Duku Lokal. Bold arrow: common band.

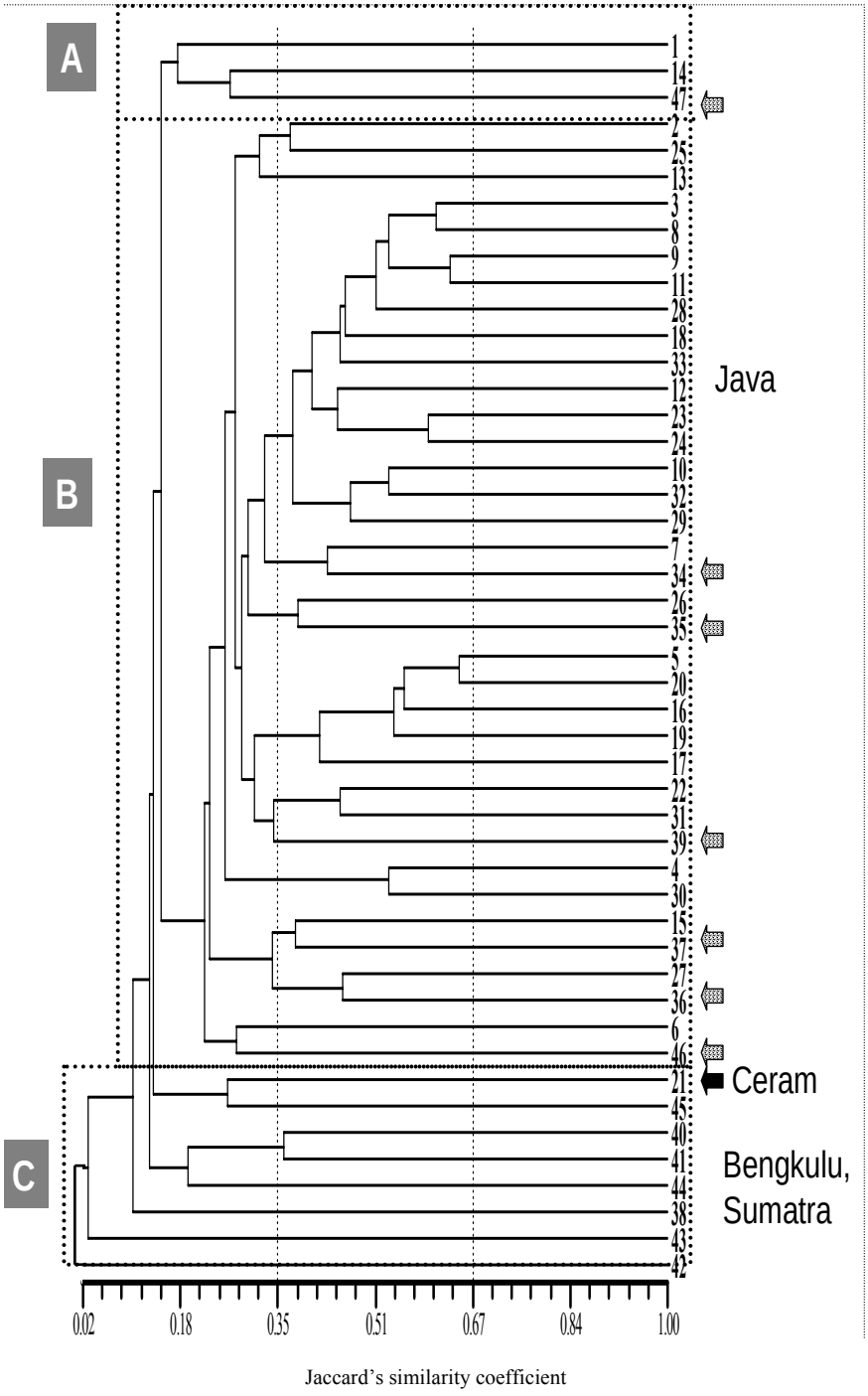

Figure 4. Dendrogram indicating genetic relatedness among 47 accessions of *L. domesticum* 'duku' and *L. domesticum* 'kokosan' based on Jaccard coefficient of similarity and UPGMA algorithm. Reference line indicated by vertical dotted lines. Solid arrow: accession from Ceram. Dotted arrow: accessions from Sumatra. Numbers correspond to sample used in Table 2.

Cluster A (coef. 0.18) and B (coef. 0.22) forming a big cluster, with most of the accessions were grouped in cluster B. Unidentified species (*Lansium* sp., no. 21) collected from KRB that was originated from Ceram has genetic similarity of 0.25 with Duku Bengkulu. The grouping of duku Palembang collected from KPN-CSC as well as kokosan into the main cluster may be implied genetic similarity between them and duku from Java. This may due to domestication process that has taken a very long period of time raising the possibility of outcrossing among local varieties. Kokosan (26, 27 and 30) were not forming a group but were grouped with duku from Central Java (coef. 0.55-0.69). Marsolais et al. (1993) suggested that the range of 0.50 using RAPD may be implied the occurrence of interspecific hybrid, while range between 0.61-0.99 could suggest genetic similarity at the species level in Lilac. While interspecific hybrid in *Mentha spicata* and *M. arvensis* shared 56 and 49% similarity to the parents (Shasany et al. 2005). In addition, Kiew et al. (2003) have observed the existence of hybrid in Duku-langsat (*L. domesticum* var. *domesticum*).

The genetic closeness among accessions can be explained by the high degree of commonness in their pedigree. Accessions 5 and 20 have the highest genetic similarity (coef. 0.65, Figure 4). Accession 5 was a parent tree of duku Sumber, while accession 20 was identified as *Lansium domesticum* from Java collected from KRB. Thus, accession 20 may have been collected from an area of where duku Sumber was mainly distributed. Accession 38, 43 and 42 (from Bengkulu) were placed at the basal dendrogram and not forming a group with any of the accessions. These accessions were seemed to be the most distinct and their DNA profiles are likely to contain the greatest number of novel alleles.

The result of PCA was to some extent not comparable to the cluster analysis (Figure 3) except for duku Bengkulu (Figure 5). Four accessions (39, 30, 4 and 28) appear to be distinct from other accessions in the PCA while these accessions were having considerably high similarity with other accessions from Java in the dendrogram. Otherwise, the remaining components were grouped in PCA may contributed the total variation correspond to the polymorphic locii.

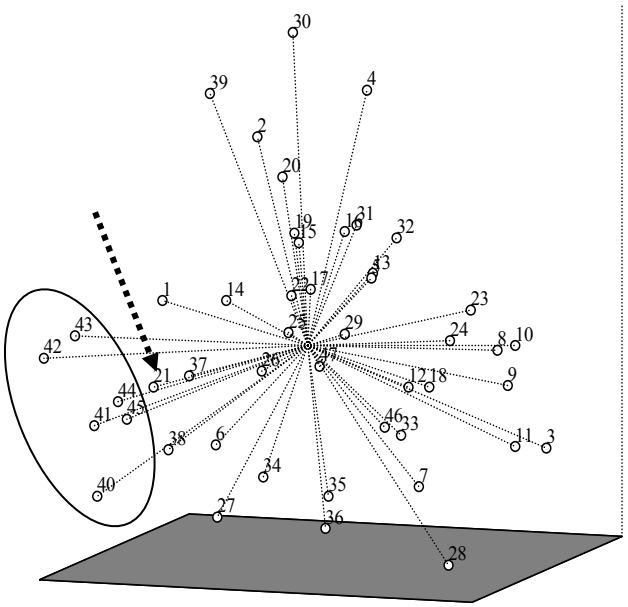

Figure 5. Three-dimensional plot of principal coordinate analysis using 47 accessions of *Lansium domesticum* 'duku' and *L. domesticum* 'kokosan'. The numbers plotted represent individual sample and corresponds to notes in Figure 2. Dotted arrow: *Lansium* sp. from Ceram. Circle correspond to the grouping at Figure 4 as Duku Bengkulu. Vertical line axis Z.

CONCLUSION

Five RAPD primers produced 53 polymorphic bands ranging in size from 300-1700 bp. These bands were used to assess genetic variation among 47 accessions of duku and kokosan. The genetic similarity range between 0.02 and 0.65 indicating wide range of genetic variations among the accessions. Of the 5 selected primers, OPB 7 produced the highest number of bands (13) while OPA 7 and OPN 12 yielded the least number of bands (9) with the average of 10.6 polymorphic bands per primer. Common bands that were existed in all accessions were OPN12 at 300 bp and OPA 13 at 450 bp. While unique bands were recorded from OPB 7 at 450 bp and OPA 18 at 500 bp belong to two accessions from Java (no 20 and 6 respectively); OPA 18 at 550 bp was found in Bengkulu's provenances (39, 42, 44). Results from cluster analysis suggested that all accessions were grouped randomly into some clusters not in accordance to the locations where they were collected. This study indicated the presence of genetic variability among accessions of duku and kokosan that could be detected by RAPD marker.

ACKNOWLEDGEMENTS

The study was financially supported by DIPA project 2010 entitled "Genetic assessment of Indonesian fruits" by Research Centre for Biology of the Indonesia Institute of Sciences, Cibinong-Bogor, West Java. I am thanking to Herlina for her assistance during the experiments.

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