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Genetic Relatedness among Duku, Kokosan, and Pisitan in Indonesia Based on Random Amplified Polymorphic DNA Markers

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Abstract

Genetic relatedness among duku, kokosan, and pisitan from Indonesia were investigated using random amplified polymorphic DNA (RAPD) markers. Eleven primers (OPA-01, OPA-02, OPA-10, OPB-07, OPB-11, OPB-12, OPB-15, OPT-16, OPU-14, OPU-19, and OPU-20) were used for amplification and yielded a total of 174 DNA bands, of which 167 were polymorphic. Primer OPA-10, OPB-11, OPB-12, OPB-15, and OPU-19 produced all of the polymorphic DNA bands. The size of the amplified DNA fragments ranged from 41-1546 bp. The dendrogram separated into two clusters at a genetic similarity coefficient of 0.76. The cluster 1 consisted of subclusters duku and several pisitan (pisitan OKI, pisitan Sleman, pisitan Hatu, pisitan Punggur, and pisitan Tanjung), and cluster 2 consisted of subclusters kokosan and pisitan. In the kokosan subclusters, including duku Drendan. Dendrogram supported the determination of taxonomic status of duku, kokosan, and pisitan as one species, namely *Lansium domesticum* Corr. and its divided into two groups, namely *L. domesticum* 'duku group' and *L. domesticum* 'pisitan-kokosan group'. Thus, RAPD analysis was useful tool for determining the genetic variation and the genetics relatedness among duku, kokosan, and pisitan in Indonesia.

Key words: duku, kokosan, pisitan/langsat, genetic relatedness, RAPD

Introduction

Duku, kokosan, and pisitan/langsat (Meliaceae) are tropical tree plants originally come from Thailand, Malaysia, and Indonesia (East Kalimantan), and spreading to Vietnam, Myanmar, and India. The taxonomic status of duku, kokosan, and pisitan on the clasification system are unclear.

Previously study has been reported by Hasskarl in 1844 that duku, kokosan, and pisitan grouped as one species, namely *Lansium domesticum* Corr. and it is divided into *L. domesticum* Corr. var. duku Hasskl. (duku), *L. domesticum* Corr. var. kokosan Hasskl. (kokosan), and *L. domesticum* Corr. var. piedjietan Hasskl. (pisitan/langsat) (Sunarti, 1987).

According to Kostermans (1960), duku and kokosan stated as two different species, namely *L. domesticum* Corr. (duku) and *L. aqueum* (Jack) Miq. (kokosan), and pisitan is a hybrid between the duku and kokosan. Backer and Brink (1965) showed that based on specimens in the Java, classified duku, kokosan, and pisitan as one species, namely *L. domesticum* Corr.

Placement of duku, kokosan, and pisitan then revised again by Kostermans (1966) as three different species, namely *Aglaia dookkoo* Griff. (duku), *Aglaia aquea* (Jack) Kosterm. (kokosan), and *Aglaia domestica* (Corr. emend. Jack) Pellegrin (pisitan). Placement of duku, kokosan, and pisitan into the genus *Aglaia*

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as proposed Kostermans (1966) is difficult to acceptable, especially since Pennington and Styles (1975) showed that *Lansium* and *Aglaia* was distinguished by the structure of flower. This statement was supported by observations on the anatomy of the leaves in the duku, kokosan, and pisitan which collected from Bogor, Condet and Purworejo, and the results did not support the placement of duku, kokosan, and pisitan in the genus *Aglaia* and separate of them as different species (Sunarti, 1987).

Pudjoarinto and Hasanuddin (1996), based on the observations of pollen characteristics from the collections of Bogor Botanical Garden, Yogyakarta, Central Java and East Java revealed that duku, kokosan, and pisitan belongs to genus *Lansium*, with species name are *L. dookkoo*, *L. aqueum*, and *L. domesticum*, respectively. Retnoningsih *et al*. (2001) has conducted a genetics analysis of duku, kokosan, and pisitan collected from Yogyakarta and Central Java by the diversity of isozyme banding pattern. The data obtained support the findings of Pudjoarinto and Hasanuddin (1996).

At the level of infraspecies, there are two different taxonomic status of duku, kokosan and pisitan. Ridley (1931 *in* Lim 2012) suggested that duku, kokosan and pisitan belonging to two varietas which were *L. dommesticum* var. *typica* (duku) and *L. dosmesticum* var. *pubescens* (pisitan and kokosan), and Morton (1987) suggested that *L. domesticum* Corr. into two varieties, namely *L. domesticum* var. *domesticum* (duku) and *L. domesticum* var. *pubescent* (pisitan/langsat). Another study by Yee *et al*. (1993) based on the observation of the anatomy of leaves, flowers, and fruits of duku separated them as varieties within the same species, namely *L. domesticum*. Morton (1987) and Yee *et al*. (1993) did not mentioned kokosan taxonomic status at the level of varieties. Lim (2012) stated that duku, kokosan and pisitan were grouped into two groups namely *L. domesticum*'duku group' and *L. domesticum*'langsat-longkong group'.

Molecular approach is an effective technique in genetic analysis and its has been widely applied to determination of genetic relatedness, one of molecular approach is the Random Amplified Polymorphic DNA (RAPD). RAPD markers have proved useful in many genetic studies. Song *et al*. (2000) mentioned that RAPD is widely used to identify the genotype of plants due to its advantages are rapid, simple and reliable tool in estimating the genetic relatedness among accessions and identifying different type of *L. domesticum*. RAPD requires of DNA extraction, optimum amplification conditions, and data analysis which its can be done in a relatively fast. RAPD markers obtained by random amplification of DNA segments (random) from a single arbitrary primer, which is usually a 10 bp.

RAPD has been applied in studying the genetics diversity of longkong, langsat, and duku. Nualsri *et al*. (2000) conducted studies of genetics variation longkong (*L. domesticum* Corr.) based on specimens in Thailand by using primers OPB-07, 0PC-05, OPC-08, and Song *et al*. (2000) conducted studies of genetic relatedness among *L. domesticum* collected from several areas in Malaysia such as Kelantan, Terengganu, Selangor, Melaka and Johor with ten primers OPA-02, OPA-10, OPB-07, OPB-11 , OPB-12, OPB-15, OPT-16, OPU-14, OPU-19, and OPU-20. Another study by Konlasuk *et al*. (2001) identified longkong, langsat, and duku from Thailand using 100 primers. Te-Chato *et al*. (2005) identified that the longkong, langsat, duku (*Lansium* spp.) based on specimens in Thailand by using 36 primers. Yulita (2011) have analyzed genetics variation of *L. domesticum* 'duku', and *L. domesticum* 'kokosan' in Java, Sumatra, and Ceram using the five primers namely OPA-07, OPA-13, OPA-18, OPB-07, and OPN-12.

The present study was aimed to estimate the genetic relatedness among the accessions of duku, kokosan, and pisitan based on RAPD and to establish taxonomic status duku, kokosan, and pisitan with the wider sampling.

It is expected that samples from broader geographical distribution would provide a more comprehensive classification.

Materials and Methods

Plant materials

Samples used in this study were 29 sampel of plant include of duku, kokosan, and pisitan with all of their vernacular names summarized in Table 1.

DNA isolation

Total DNA was isolated from leaves using the Nucleon Phytopure Plant and Fungal DNA kit exraction RPN-8511/GE (Healthcare, U.K.) following the procedure described by Daryono and Natsuaki (2002).

Amplification condition and agarose gel electrophoresis

DNA amplification was conducted based on Williams *et al*. (1990) with slight modifications. DNA were amplified by polymerase chain reaction (PCR) using 10 arbitrary primers (OPA-01, OPA-02, OPA-10, OPB-07, OPB-11, OPB-12, OPB-15, OPT-16, OPU-14, OPU-19 and OPU-20). Each sample was prepared by PCR reaction mixture, PCR kit (Megamix-Blue PCR Master mix: the enzyme Taq polymerase, 2.75 mM MgCl2, 220 µM dNTPs, and blue agarose loading dye) 20 µl, 2.5 µl primer (100 pmol), DNA samples of 2.5 µl (10 ng/ml). PCR reaction was performed by 45 cycles consisting of 3 phases, namely (i) pre-denaturation for 5

Table 1. List of samples used in the study

No	Vernacular Name	Source of Materials	Province
1	Duku Tembung	Deli Serdang	North Sumatera
2	Duku Komering	Ogan Komering Ilir; Ogan Komering Ulu	South Sumatera
3	Duku Moncong	Ogan Komering Ilir	South Sumatera
4	Duku Rasuan	Ogan Komering Ilir	South Sumatera
5	Duku Bulat	Ogan Komering Ilir	South Sumatera
6	Duku Karangkajen	Bantul	Yogyakarta
7	Duku Sleman	Sleman	Sleman Yogyakarta
8	Duku Singosari	Malang	East Java
9	Duku Matesih	Karanganyar	Central Java
10	Duku Klaten	Klaten	Central Java
11	Duku Dendan	Bengkalis	Riau
12	Duku Papongan	Tegal	East Java
13	Duku Sabu	Pesawaran	Lampung
14	Duku Condet	Jakarta	Jakarta
15	Duku Sumber	Kudus	Central Java
16	Duku Woro	Rembang	Central Java
17	Duku Hatu	Ambon	Ambon
18	Duku Kumpe	Muaro Bungo	Jambi
19	Duku Kalikajar	Purbalingga	Central Java
20	Langsat OKI	OKI	South Sumatera
21	Langsat Sleman	Sleman	Yogyakarta
22	Langsat Singosari	Malang	East Java
23	Langsat Matesih	Karanganyar	Central Java
24	Langsat Klaten	Klaten	Central Java
25	Langsat Tanjung	Tabalong	South Kalimantan
26	Langsat Punggur	Kubu Raya	West Kalimantan
27	Langsat Hatu	Ambon	Ambon
28	Kokosan Kaliurang	Kaliurang	Yogyakarta
29	Kokosan Klaten	Klaten	Central Java

min denaturation at 94°C, (ii) denaturation for 5 min at 94°C, (iii) annealing for 1 min at 36°C, (iv) elongation for 2 min at 72°C, and (v) post-elongation for 10 min at 72°C. All PCR products were separated by electrophoresis on 1.5 %w/v agarose gel in 1xTBE, stained with 2.5 µl GoodView (Fermentas), viewed under ultraviolet light and photographed using digital camera (Cannon).

Data scoring and analysis

PCR reaction and electrophoresis were repeated at least twice to ascertain the reproducibility of the bands. Only reproducible bands were scored as present (1) or absent (0) in this study. These RAPD data, generated with eleven primers, were used to compile a binary matrix for cluster analysis using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) version 2.1 software. Genetic similarity among accessions was calculated according to Jaccard's similarity coefficients and the similarity coefficients were then used to construct a dendrogram using the UPGMA (Unweighted Pair-Group Methode with Arithmetical Averages) through the SHAN (Sequential, Hierarchical, Agglomerative and Nested Clustering) routine of the NTSYS-pc software (Rohlf, 2000).

Result and Discussion

DNA isolation

DNA isolation from accessions of duku, kokosan, and pisitan showed a fairly good DNA quality with quantity varying from 108.4 to 1162.2 ng/µl and the purity of DNA between 1.806 -1.897. Sambrook *et al.* (1989) stated that pure DNA has a A260/A280 rate ranged from 1.8 to 2.0. According to Poerba and Martanti (2008) the purity of the template DNA strongly influence the intensity of the amplified DNA bands. Template DNA contain with polysaccharides and phenolic compounds will produce a faint band of DNA amplification or unclear. Ferriol *et al.* (2003) demonstrated that RAPD was very sensitive to reaction conditions and purity of template DNA.

Genetic polymorphism and RAPD patterns

The results of total genomic DNA amplification using eleven primer at 29 accesions of duku, kokosan, and pisitan, resulted in 174 DNA bands that can be read and scored (Table 2).

The amplification of eleven primers produced an amount of various DNA sequences (Table 2). The amount of DNA band was entirely from 12 (OPB-07 and OPB-15) to 21 (OPU-20) per primer. The size of amplified DNA fragments ranged from 41 bp (OPU-20) to 1546 bp (OPA-02). Primers producing the least amount of DNA band were OPB-07 (12 DNA bands), while those producing the largest amount of DNA band were OPU-20 (21 DNA bands). Hartati *et al.* (2007) reported that one of requirements for the amplification of DNA is that primers have nucleotide basa sequence, which is the complement of two DNA template sequences with contrary positions. The same statement was also expressed by Tingey *et al.* (1994) that the amount of DNA bands produced was dependent on how primers recognized the sequence of complementary DNA at DNA template.

The selection of primers in RAPD analysis determined level of resultant DNA band polymorphism. In this study, the selection of primers referred to Song *et al.* (2000) on the accessions of genetic relatedness between *L. domesticum* using RAPD marker. The obtained data indicates that all ten primers produced high level of polymorphism.

In this study, primers producing the total amount of polymorphic DNA bands were primers of OPA-02 (17 DNA bands), OPA-10 (14 DNA bands), OPB-07 (12 DNA bands), OPT-16 (16 DNA bands), and OPU-20 (21 DNA bands). In general, result of the amplification of eleven primers produced level of DNA band polymorphism for 95.98% (167 polymorphic DNA bands). The polymorphic DNA bands were mostly produced in primers of OPU-20 (21 DNA bands with the polymorphism level of 100%) and the least was produced in primers of OPU-14 (11 DNA bands with the polymorphism level of 84.62%).

Table 2. Number of RAPD fragment, fragment size, and number of polymorphic fragment from primers to be used

Primers name	DNA sequence (5'-3')	Number of amplified bands	Number of polymorphic bands	% Polymorphic bands	Fragment RAPD sized (bp)
OPA-01	CAGGCCCTCC	15	14	93,33	119-795
OPA-02	TGCCGAGCTG	17	17	100	99-1546
OPA-10	GTGATCGCAG	14	14	100	70-897
OPB-07	GGTGACGCAG	12	12	100	65-666
OPB-11	GTAGACCCGT	19	18	94,74	78-623
OPB-12	CCTTGACGCA	16	15	93,95	85-1092
OPB-15	GGAGGGTGTT	13	12	92,31	97-430
OPT-16	GGTGAACGCT	16	16	100	68-617
OPU-14	TGGGTCCCTC	13	11	84,62	122-578
OPU-19	GTCAGTGCGG	18	17	94,44	74-973
OPU-20	ACAGCCCCCA	21	21	100	65-579
	Total	174	167	95,76	41-1546

Hartati *et al*. (2007) showed that the high percentage of polymorphic DNA bands compared with monomorphic DNA bands indicates great out-crossing and gene flow frequency in the plants as in *Alstonia scholaris*. In our study, although generally all primers have the high level of polymorphism, there were primers that produced only one DNA band. It occurred for Kaliurang kokosan with the primer of OPU-19 (Figure 1). Susantidiana *et al*. (2009) reported that polymorphism as a result of the RAPD analysis was occured from insertion, deletion, and mutation. It caused less or loss of landing site, causing the primers fail to attach, so amplification did not occur and eventually did not generate DNA band.

Yulita (2011) studied that genetics variation at several accessions of *L. domesticum* 'duku' and *L. domesticum* 'kokosan' with RAPD marker using five primers (OPA-07, OPA-13, OPA-18, OPB-07, and OPN-12) producing 53 DNA bands. The amount of polymorphic DNA bands ranged from 9 (OPA-07 and OPN-12) to 13 (OPB-07) with variation in size of DNA fragments from 300 bp (OPA-18 and OPN-12) to 1700 bp (OPA-18). This study used the same primers, i.e. OPB-07, producing 12 DNA bands that were entirely polymorphic DNA bands with size of DNA fragments ranging from 65 to 666 bp.

According to Poerba and Martanti (2008), the selection of primers in RAPD analysis affected the resultant band polymorphism. It was because each primer has its own attaching site, so the DNA band resulted by each primer was different, both in size of multiple basa pairs and in amount of DNA bands. Hartati *et al*. (2007) stated that polymorphism is a form of genetic variation that occurs in DNA sequence. Moreover, Hartati (2006) and Ruwaida *et al*. (2009) explained that the amount of resultant polymorphic DNA bands determined the diversity of a population because they described the condition of plant genome.

The amplification of PCR-RAPD with eleven primers produced 89 patterns of DNA bands is shown in Table 3.

Table 3 shows that highest variability in DNA band pattern was resulted from primers of OPB-07 and OPB-11 for 16 and 15 patterns, respectively, while lowest variability in DNA band pattern was resulted from primer of OPB-15 (4 patterns). RAPD used differences in the pattern of PCR amplification generated form differences in its attaching position of primers at genome DNA from the different individuals. The difference in DNA band pattern was because of the amplification of DNA sequence at certain

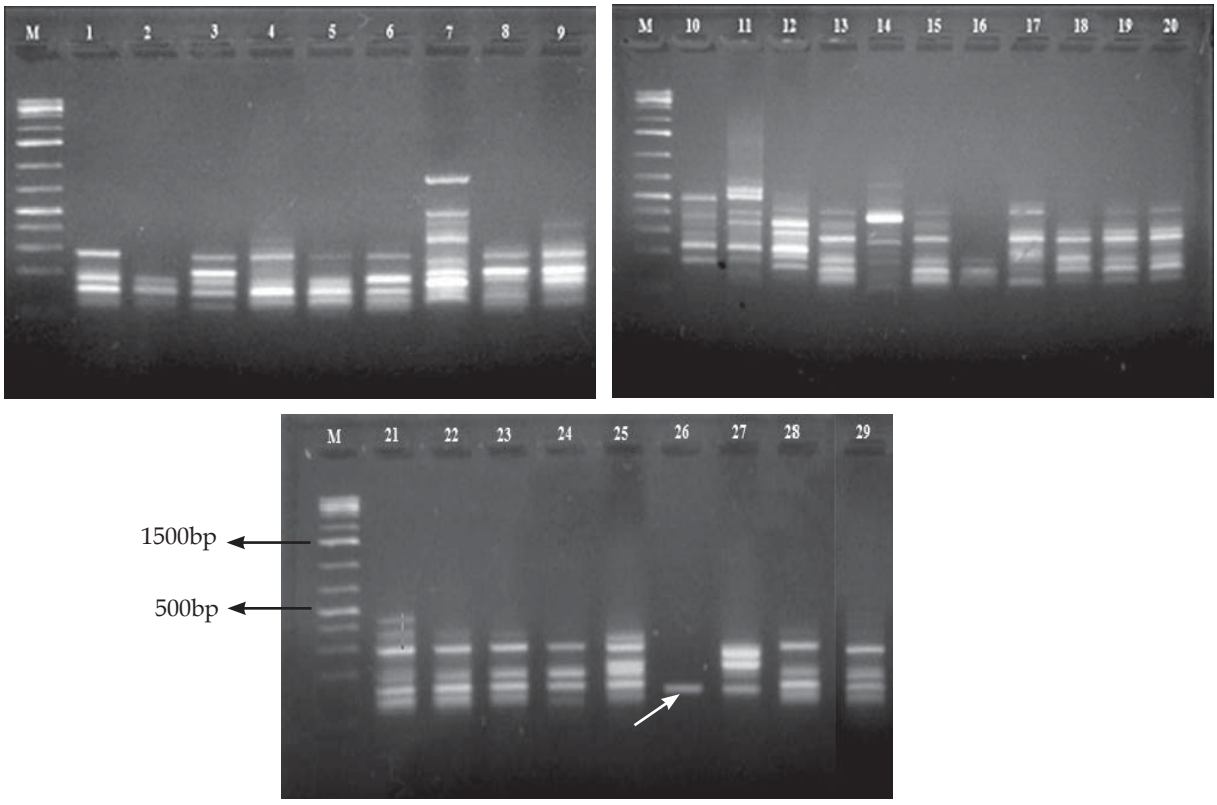

Figure 1. DNA bands patterns duku, kokosan, and pisitan in Indonesia using OPU 19 primer. M: DNA marker (1 kb ladder Fermentas); 1. Duku Tembung; 2. Duku Komering; 3. Pisitan OKI; 4. Duku Moncong; 5. Duku Rasuan; 6. Duku Bulat; 7. Duku Karang Kajen; 8. Pisitan Hatu; 9. Pisitan Sleman; 10. Duku Sleman; 11. Duku Singosari; 12. Pisitan Singosari; 13. Duku Matesih; 14. Pisitan Matesih; 15. Duku Klaten; 16. Pisitan Klaten; 17. Pisitan Punggur; 18. Pisitan Tanjung; 19. Duku Papongan; 20. Duku Sabu; 21. Duku Condet; 22. Duku Sumber; 23. Duku Woro; 24. Duku Drendan; 25. Kokosan Klaten; 26. Kokosan Kaliurang; 27. Duku Hatu; 28. Duku Kumpe; 29. Duku Kalikajar.

Table 3. Patterns of DNA bands and specific DNA band of duku, kokosan, and pisitan

No	Primers	Number of patterns of DNA bands	DNA bands that were present in all accessions (bp)	Specific DNA band (bp)	Accessions of duku, kokosan, and pisitan
1	OPA-01	5	119; 146; 188; 270	527	Duku Hatu
2	OPA-02	5	122, 212, 264	-	-
3	OPA-10	6	-	-	-
4	OPB-07	16	-	65	Duku Moncong
				145	Duku Singosari
5	OPB-11	15	167	120	Pisitan Klaten
				449	Pisitan Matesih
				559	Pisitan Matesih
				623	Pisitan Singosari
6	OPB-12	8	-	-	-
7	OPB-15	4	97; 134	430	Duku Tembung
8	OPT-16	5	193	-	-
9	OPU-14	6	167; 205; 252; 280	-	-
10	OPU-19	14	-	-	-
11	OPU-20	5	-	-	-
	Total	89	-	-	-

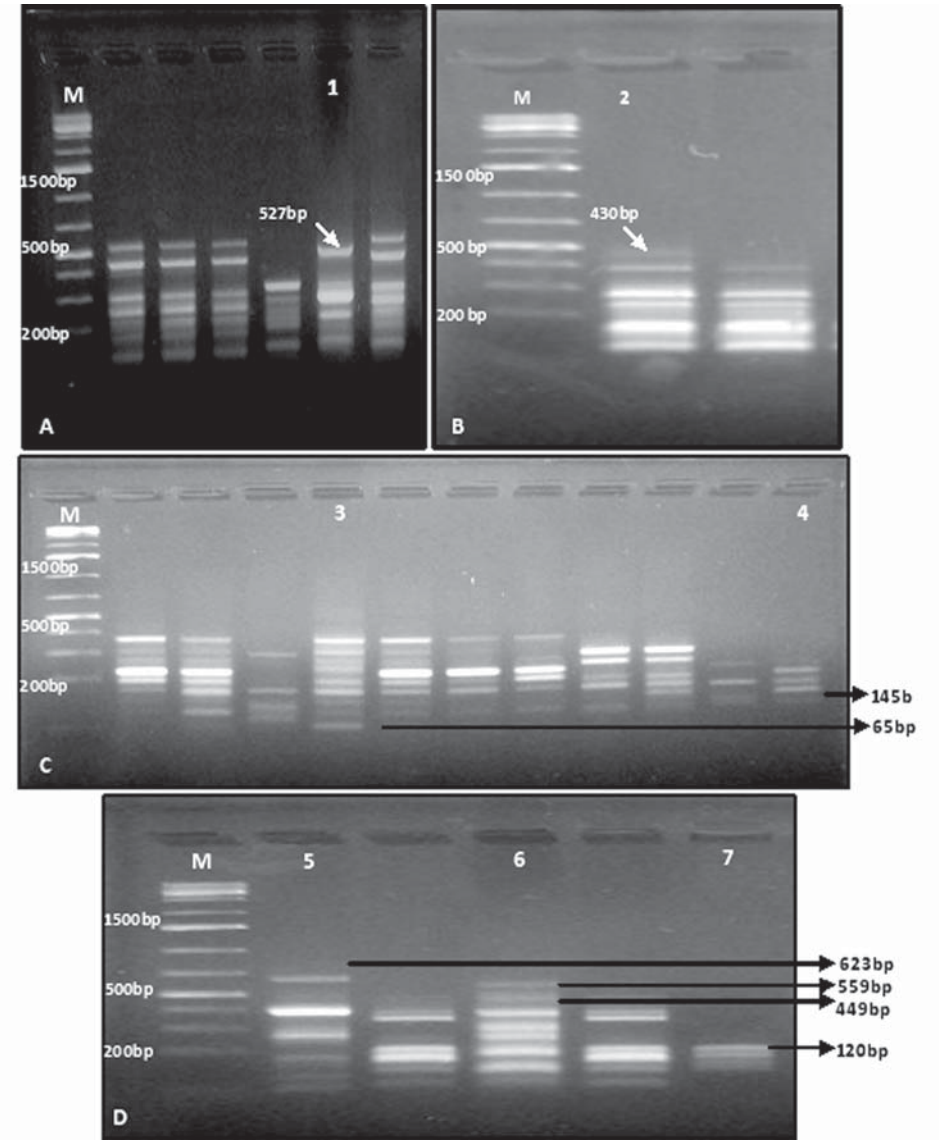

Figure 2. Specific DNA bands formed in the primers OPA-01, OPB-07, OPB-11, OPB-15. A). PCR product of OPA-01; B). PCR product of OPB-15; C). PCR product of OPB-07; D). PCR product of OPB-11; (M) DNA Marker (1 kb Ladder fermentas); (1). Duku Hatu; (2). Duku Tembung; (3). Duku Moncong; (4). Duku Singosari; (5). Pisitan Singosari; (6). Pisitan Matesih; (7). Pisitan Klaten.

positions. Difference in basa pairs in DNA sequence caused amplification not taking place because of unsuitability between primary DNA sequence and its complementary one. Hadipoentyanti *et al*. (2001) stated that the RAPD analysis can be used to determine and recognize the characteristics of genetic variability between plant genotypes, and the genetic variability can be seen through DNA polymorphism.

Table 3 shows that the formation of specific DNA band. The specific DNA band was resulted by the primers of OPB-11 (4 bands), OPB-07 (2 bands), OPB-15 (1 band), and OPA-01 (1 band) (Figure 2). In a taxonomic study, the specific DNA bands can be categorized as analytical and diagnostic characters. Davis and Heywood (1973), reported that the analytical characters is an attribute used to identify, recognize, and limit taxonomy with

limited and typical availability to distinguish taxonomy and it is near kinship. A study carried out by Yulita (2011) founded that specific DNA bands in the accessions of *L. domesticum* 'duku' and *L. domesticum* 'kokosan', i.e. those formed at sizes of 450 bp (OPB-07) and 500 bp (OPA-18). Further, they have potentials as diagnostic characters that can be used to identify or determine a cultivar.

Genetic relatedness based on RAPD

The use of Jaccard's similarity coefficient to estimate genetic relatedness among accessions gave similarity in value ranging from 78.17% to 97.33%. Mean genetic similarity between the major clusters of duku, kokosan, and pisitan ranged from 0.76 to 0.97, as shown in Figure 3. The high level of similarity among the accessions of duku, kokosan, and pisitan was because the plants of duku, kokosan and pisitan were apomixis plants and able to be proliferated in a vegetative manner. Other possibility was the event of cross-pollination because the plants of duku, kokosan, and pisitan were annual plants whose existence was still in forest. Song *et al*. (2000) showed that the similarity index values of 28.7%-79.4% with a distance of genetics similarity coefficients of 0.34 (34%)-0.98 (98%) in several accessions of *L. domesticum*. According to Song *et al*. (2000), it was an indication of the apomixis, vegetative proliferation, or a wide range of hybridation. Yulita (2011) founded that the similarity index values of 0.02-0.65, indicating a large genetics variation among the accessions of *L. domesticum* 'duku' and *L. domesticum* 'kokosan'.

A dendrogram based on the similarity index values was generated using UPGMA

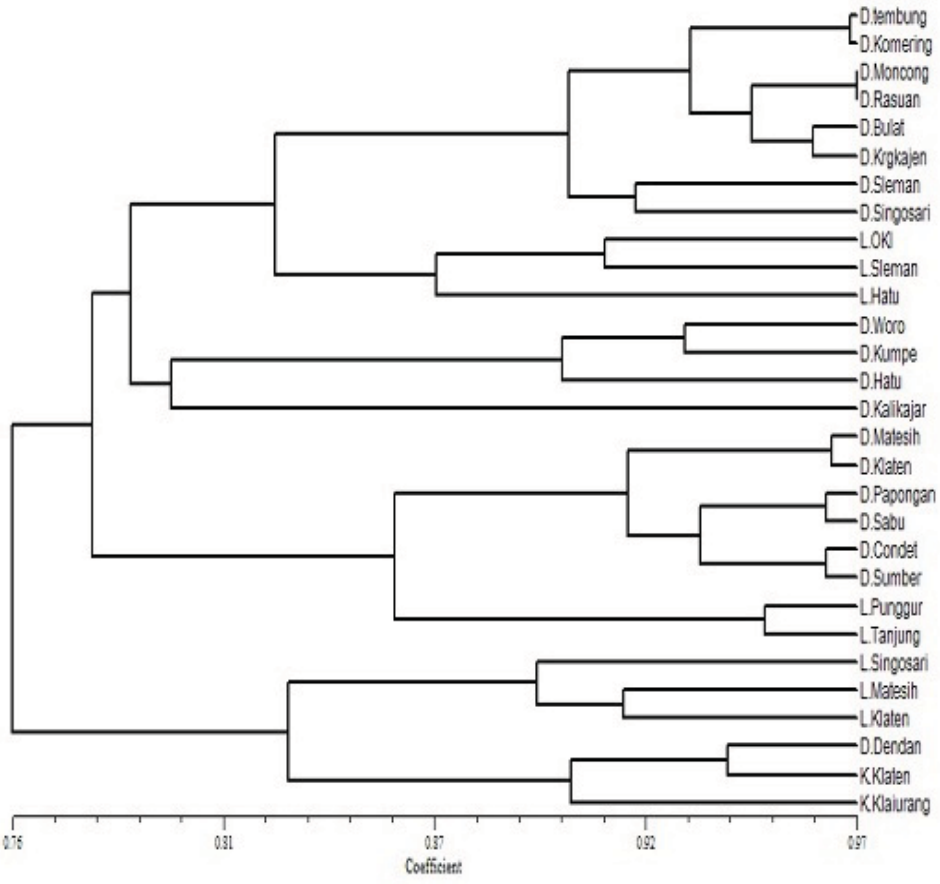

Figure 3. Dendrogram indicating genetics relatedness among accessions of duku, kokosan, and pisitan based on Jaccard coefficient of similarity and UPGMA algorithm.

clustering analysis. In Figure 2, the resultant dendrogaram was separated into two main cluster at genetic similarity coefficient of 0,76.

Cluster 1 was separated into two subcluster at genetic similarity coefficient of 0,79. Cluster 1 consisted of the accession of duku (duku Tembung, Komering, Moncong, Rasuan, Bulat, Karangkajen, Sleman, Singosari, Wono, Kumpe, Hatu, Kalijajar, Matesih, Klaten, Papongan, Sabu, Condet, and Sumber), but found also the accessions of pisitan (pisitan OKI, Sleman, Hatu, Punggur, and Tanjung). The inclusion of pisitan into the groups of duku was possible because cross-pollination occurred naturally. In this study, most sampling of duku, kokosan, and pisitan are the plants inherited from ancestors with $\pm$ 50 - 150 years old growing in wild areas. Even in some regions such as Sumatera, population's gardens were in forests. Thus, the possibility for cross-pollination was very great. Other possibility was that the inclusion of pisitan into the groups of duku taken place as a result of the cultivation efforts through transplantation or inoculation.

Cluster 2 consisted of two subclusters are pisitan and kokosan with genetic similarity coefficient of 0,82. In the subclusters of kokosan, duku Drendan was included into the subclusters of kokosan with the similarity index value of 92.2%. The inclusion of duku Drendan in the subcluster of kokosan was possibly occured by mistake in the naming of region by local comunities. Duku Drendan was collected from Bengkalis Island, Pekan Baru. Recently, it is known that kokosan has only been recognized and found in Java Island. The same result was also found from study conducted by Song *et al.* (2000) in the accessions of *L. domesticum* collected from Malaysia Peninsular. The same taxonomy was grouped into one, except in duku (Selangor), duku-langsat (Selangor), and duku Dewan Beta (Selangor). Song *et al*. (2000) reported that it was possibly caused by misnaming or cross-pollination with local varieties. The same problem was also founded in another study by Yulita (2011), indicated that duku Palembang, duku Java and kokosan grouped into same clusters, its possibly because there was the domestication efforts and cross-pollination in a long period of time.

Based on the dendrogram (Figure. 3) showed that the grouping was not in accordance with geographical positions. The grouping did not indicates that the nearer the geographical distance, but the nearer the genetic similarity. It shows genetics diversity in the accessions of duku, kokosan, and pisitan, being possibly caused by the recombination of genes. According to Hartati *et al*. (2007), the grouping based on the geographic positions taken place because the RAPD marker indicates DNA variation, both in coding and non-coding regions. Moreover, RAPD property was unrepreducable, so to get an accurate grouping; DNA analysis was required using more primers.

The same result was shown from study by Yulita (2011), on some accessions of duku and kokosan, the formed pattern of grouping did not show the grouping based on the region of origin (sampling location). In this study, cultivars that came from the same location were not grouped into one cluster. Its was different with data reported by Song *et al*. (2000), indicating that the grouping was generally appropriate with its geographic region of origin.

Result of the analysis on the genetic relatedness of duku, kokosan, and pisitan and the RAPD approach showed that duku, kokosan, and pisitan were grouped as one species, namely *Lansium domesticum* Corr. and each of divided into two groups, namely *L. domesticum* 'duku group' and *L. domesticum* 'pisitan-kokosan group'. The determination of duku, kokosan, and pisatan species supported the placement of duku, kokosan, and pisitan grouped into two groups as proposed by Lim (2012).

Further investigation are necessary to be conducted to phylogenetic relationship duku, kokosan, and pisitan using squencing of DNA Internal Transcribed Spacer (ITS) of

nucelar ribosomal DNA because can be used to assessing relationship infrageneric level.

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