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Genetic Relatedness among *Lansium domesticum* Accessions Using RAPD Markers

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Genetic relatedness among 85 *Lansium domesticum* Corr. accessions from Peninsular Malaysia were investigated using random amplified polymorphic DNA (RAPD) markers. Ten primers were used for amplification and yielded a total of 113 bands, of which 107 were polymorphic. Homology tests showed that the RAPD bands used in the study satisfy assumptions of homology and non-allelic behaviour. A dendrogram showing genetic similarities among accessions was constructed based on the 107 polymorphic bands using UPGMA cluster analysis. Jaccard similarity coefficients ranged from 0·25 to 1·00 among accessions indicating a diverse genepool in the species indicative of different species parentage. The dendrogram separated the 85 accessions into three main clusters, one comprising 56 accessions which possess thin-skinned fruit (mostly Dokong and Langsat), while the second has 28 accessions (mostly Duku-langsat, Duku Terengganu and Duku Johor) with thick fruit skin and the third comprising only one accession, namely Duku hutan. Thus, RAPD analysis was a useful tool for determining the genetic relatedness among accessions and identifying different types of *L. domesticum*.

Key words: *Lansium domesticum*, genetic relatedness, RAPD markers, cluster analysis.

INTRODUCTION

Lansium domesticum Corr. is a fruit tree species belonging to the family Meliaceae. This popular tropical fruit occurs mainly in South-East Asia, especially in the Philippines, where it is known as Lanzones, and South Sumatra in Indonesia (Othman and Bamroongrugsa, 1991). It also grows in Surinam, Puerto Rico and Australia (Othman and Suranant, 1995). Although many species have been ascribed to the genus *Lansium*, Mabberley (1985) recognized only three species, *L. membranaceum* Kosterm., *L. domesticum* Correa. *agg*. and *L. breviracemosum* Kosterm. In Peninsular Malaysia the genus is represented by only one species, *L. domesticum* (Mabberley and Pannell, 1989).

There are numerous forms of the species, and these belong to four main types: Dokong, Duku, Langsat and Duku-langsat. There is a fairly clear distinction between the two major types of *L. domesticum*: Langsat and Duku. Langsat fruits are oblong, thin-skinned and possess latex, while Duku fruits are round, thick-skinned and latex-free. The Duku-langsat (or Duku Terengganu) is the intermediate type, generally regarded as superior to both Duku and Langsat (Mabberley, 1985). Duku, Langsat and Duku-langsat are native to Peninsular Malaysia while Dokong originates from southern Thailand, and has been cultivated in Peninsular Malaysia for more than 10 years. Langsat can be found throughout the peninsula but predominates in the north, while Duku occurs in the southern region (Idris, 1987). Duku-langsat is a popular fruit tree on the east coast and is mainly cultivated in the states of Terengganu and Kelantan. Dokong is frequently cultivated in Kedah, Kelantan and Terengganu (Piper, 1990). These four types of *L. domesticum* are usually propagated by seeds. Embryological studies of Langsat and Duku by Prakash *et al.* (1977), and floral studies of Duku-langsat by Salma and Razali (1987), give evidence for apomictic reproduction in *L. domesticum*.

Other types of *L. domesticum* such as Duku Dewan, Duku Ayam, Langsat Pinang etc. are related types which differ morphologically especially in their fruit and tree characteristics. However, no convincing classification exists and the taxonomic status of the different types is still unknown. Complications arise as the vernacular names are inconsistent, with the same vernacular name being used for different types in different regions (Mabberley, 1985; Othman and Bamroongrugsa, 1991). Phenotypic characters (e.g. fruit character) can be used for the discrimination of plant types when trees are 5–10 years old and start bearing fruit.

RAPD (random amplified polymorphic DNA) analysis employs single short primers with arbitrary sequences to generate genome-specific fingerprints of multiple amplification products (Welsh and McClelland, 1990; Williams *et al.*, 1990). RAPD markers have proved useful in many genetic studies. These include the determination of DNA diversity between *Saccharum* varieties (Harvey and Botha, 1996); identification and classification of *Pisum sativum* genotypes (Samec and Našinec, 1996); phylogenetic studies of *Lepidium meyenii* and other Andean *Lepidium* species (Toledo *et al.*, 1998); evolutionary studies of finger millet (*Eleusine coracana* ssp. *coracana*) (Hilu, 1995); determination of the

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parentage of interspecific lilac hybrids (Marsolais *et al.*, 1993); and constructing genetic linkage maps of quantitative trait loci in *japonica* rice (Redoña and Mackill, 1996). RAPD markers have been used in laboratories throughout the world to identify different plant genotypes, e.g. apple (Koller *et al.*, 1993), cotton (Multani and Lyon, 1993), banana (Howell *et al.*, 1993), date palm (Corniquel and Mercier, 1994) and strawberry (Degani *et al.*, 1998).

We show here that RAPD analysis is a rapid and useful tool for distinguishing different types of *L. domesticum* Corr. as well as for estimating genetic relatedness among these types. The objectives of the present work were to identify polymorphic RAPD markers to distinguish the 85 accessions of *L. domesticum* Corr. and to estimate the genetic relatedness among these accessions.

MATERIALS AND METHODS

Plant materials

Eighty-five accessions of *L. domesticum* from five states in Peninsular Malaysia, namely Kelantan, Terengganu, Selangor, Melaka and Johor were used for DNA isolation and RAPD analysis (Table 1). All vernacular names in Table 1 are given by locals or owners of the trees. Most of the accessions were obtained from field genebanks of the Malaysian Agricultural Research and Development Institute (MARDI) stations at Jerangau, Terengganu and Jeram Pasu, Kelantan. Voucher specimens of all accessions are kept in the Herbarium of Universiti Kebangsaan Malaysia.

DNA isolation

Total DNA was isolated from young leaves using the CTAB (hexadecyltrimethylammonium bromide) procedure described by Doyle and Doyle (1990). DNA concentration was estimated by subjecting samples to 0·85 % agarose gel electrophoresis and staining with ethidium bromide. Staining intensities of the total DNA were compared visually with a DNA molecular weight marker. DNA yields of 1–20 mg g^{-1} leaf tissues were obtained. Hence, total DNA was diluted with sterile distilled water to give a concentration of 10 ng μl^{-1}.

Amplification conditions and agarose gel electrophoresis

Ten decamer oligonucleotides (OPERON Technologies Inc.), OPA-02, OPA-10, OPB-07, OPB-11, OPB-12, OPB-15, OPT-16, OPU-14, OPU-19 and OPU-20, were used for PCR amplifications. PCR conditions, including the concentration of template DNA, primer, dNTP, magnesium and *Taq* polymerase were optimized to generate RAPD profiles of high intensity and sharp bands with a clear background. The 25-µl reaction mixture contained 10 ng template DNA, 200 µM each of dATP, dCTP, dGTP, dTTP, 7·5 pmoles of primer, 1 unit of *Taq* DNA polymerase (Boehringer Mannheim), 2·5 mM $MgCl_2$ and 1 × reaction buffer (10 mM Tris-HCl, 1·5 mM $MgCl_2$ and 50 mM KCl, pH 8·3). Amplifications were performed in a GeneAmp®PCR System 2400 (Perkin-Elmer) thermal cycler for an initial 3 min denaturation at 94°C followed by 40 cycles of 1 min at 93°C, 3 min at 35°C and 2 min at 72°C. All PCR products were separated by electrophoresis on 1·5 % w/v agarose gels in 1 × TAE buffer (Sambrook *et al.*, 1989), stained with ethidium bromide, viewed under ultraviolet light and photographed using Polaroid 665 film.

Homology test

Two assumptions are made in RAPD statistical analysis: (1) co-migrating bands are homologous; and (2) different band positions represent different loci. Hence, homology tests were carried out to test for homology and non-allelism of the amplified PCR products. One bright and well-resolved amplified band for each of the ten primers was used as a probe to determine homology with other bands. The bands were excised from agarose gels and the DNA was eluted from the agarose gel and purified using Geneclean III® kit (Bio 101 Inc.). Approximately 50–100 ng of DNA recovered from the band was then labelled with digoxygenin-dUTP by the random primer method (Feinberg and Vogelstein, 1984) using a commercially available kit (Boehringer Mannheim). For the remainder of the amplified products in the agarose gel, Southern blotting (Sambrook *et al.*, 1989) was performed overnight using Hybond N^+ membrane (Amersham). The blots were then hybridized with the labelled DNA. After hybridization, colorimetric detection was performed with the DIG DNA detection kit (Boehringer Mannheim). Procedures for Southern blotting, probe labelling, hybridization and colorimetric detection were performed according to the DIG System User's Guide for Hybridization (Boehringer Mannheim).

Data scoring and analysis

PCR reactions 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 ten primers, were used to compile a binary matrix for cluster analysis using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) version 1.80 package (Rohlf, 1993). Genetic similarity among accessions was calculated according to Jaccard's similarity coefficients (Jaccard, 1908) using the SIMQUAL (Similarity for Qualitative Data) routine. Jaccard's coefficients are defined as $a/(a + b + c)$, where a is the number of positive matches (i.e. bands common to two accessions), and b and c refer to the number of bands present only in accession 1 and 2, respectively. The similarity coefficients were then used to construct a dendrogram using the UPGMA (unweighted pair-group method with arithmetical averages) through the SHAN (sequential, hierarchical, agglomerative and nested clustering) routine of the NTSYS-pc package.

Clustering ability test

The clustering abilities of ten different primers were tested in this study. The primary objective of the test was to

TABLE 1. *List of* Lansium domesticum *accessions used in the study*

Accession number	Vernacular name	Source of materials	Voucher number
DKK01	Dokong bulat	MARDI Jeram Pasu, Kelantan	MWNS 1001
DKK02	Dokong bulat	MARDI Jeram Pasu, Kelantan	MWNS 1002
DKK03	Dokong bulat	MARDI Jeram Pasu, Kelantan	MWNS 1003
DKK04	Dokong bulat	MARDI Jeram Pasu, Kelantan	MWNS 1004
DKK05	Dokong bujur	MARDI Jeram Pasu, Kelantan	MWNS 1005
DKK06	Dokong bujur	MARDI Jeram Pasu, Kelantan	MWNS 1006
DKK07	Dokong bujur	MARDI Jeram Pasu, Kelantan	MWNS 1007
DKK08	Dokong bujur	MARDI Jeram Pasu, Kelantan	MWNS 1008
DKK09	Dokong bujur	MARDI Jeram Pasu, Kelantan	MWNS 1009
DKK10	Dokong Bawang	MARDI Jeram Pasu, Kelantan	MWNS 1010
DKK11	Dokong butir	MARDI Jeram Pasu, Kelantan	MWNS 1011
DKK15	Dokong butir	MARDI Jeram Pasu, Kelantan	MWNS 1012
DKK16	Dokong butir	MARDI Jeram Pasu, Kelantan	MWNS 1013
DKK24	Dokong	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1014
DKK26	Dokong	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1015
DKK27	Dokong	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1016
DKT27	Dokong air	MARDI Jerangau, Terengganu	MWNS 1017
DKT28	Dokong air	MARDI Jerangau, Terengganu	MWNS 1018
DKT29	Dokong air	MARDI Jerangau, Terengganu	MWNS 1019
DKR07	Dokong	Experimental Plot UKM, Bangi, Selangor	MWNS 1020
DKR08	Dokong	Experimental Plot UKM, Bangi, Selangor	MWNS 1021
DKJ07	Dokong Naratiwat	Agriculture Department Serdang, Selangor	MWNS 1022
DKJ09	Dokong kering	Agriculture Department Serdang, Selangor	MWNS 1023
DKJ10	Dokong Tokki	Agriculture Department Serdang, Selangor	MWNS 1024
DUT19	Duku Johor	MARDI Jerangau, Terengganu	MWNS 1025
DUR01	Duku	Experimental Plot UKM Bangi, Selangor	MWNS 1026
DUR02	Duku	Experimental Plot UKM Bangi, Selangor	MWNS 1027
DUP01	Duku	Taman Pantun UKM Bangi, Selangor	MWNS 1028
DUJ01	Duku Johor	Agriculture Department Serdang, Selangor	MWNS 1029
DUJ02	Duku Johor	Agriculture Department Serdang, Selangor	MWNS 1030
DUH09	Duku	Sg. Mati, Muar, Johor	MWNS 1031
DUH10	Duku	Sg. Mati, Muar, Johor	MWNS 1032
DUH11	Duku	Sg. Mati, Muar, Johor	MWNS 1033
LSK29	Langsat	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1034
LSK31	Langsat	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1035
LSK35	Langsat	Kg. Padang Lati, Wakaf Bharu, Kelantan	MWNS 1036
LSR03	Langsat	Experimental Plot UKM Bangi, Selangor	MWNS 1037
LSR04	Langsat	Experimental Plot UKM Bangi, Selangor	MWNS 1038
LSP04	Langsat	Taman Pantun UKM Bangi, Selangor	MWNS 1039
LSP05	Langsat	Taman Pantun UKM Bangi, Selangor	MWNS 1040
LSP06	Langsat	Taman Pantun UKM Bangi, Selangor	MWNS 1041
LSJ03	Langsat	Agriculture Department Serdang, Selangor	MWNS 1042
LSJ04	Langsat	Agriculture Department Serdang, Selangor	MWNS 1043
LSM01	Langsat Mengkabau	Kg. Londang, Masjid Tanah, Melaka	MWNS 1044
LSM05	Langsat Mengkabau	Kg. Londang, Masjid Tanah, Melaka	MWNS 1045
LSM06	Langsat Jerendang	Kg. Durian Daun, Masjid Tanah, Melaka	MWNS 1046
LSM07	Langsat Jerendang	Kg. Durian Daun, Masjid Tanah, Melaka	MWNS 1047
LSM08	Langsat Jerendang	Kg. Durian Daun, Masjid Tanah, Melaka	MWNS 1048
LSH15	Langsat	Sg. Mati, Muar, Johor	MWNS 1049
UGK19	Duku Dewan	MARDI Jeram Pasu, Kelantan	MWNS 1050
UGK20	Duku Dewan	MARDI Jeram Pasu, Kelantan	MWNS 1051
UGK22	Duku Dewan	MARDI Jeram Pasu, Kelantan	MWNS 1052
UGK23	Duku Dewan	MARDI Jeram Pasu, Kelantan	MWNS 1053
UGK28	Duku Pramare	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1054
UGK30	Duku Pramare	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1055
UGK32	Duku Dewan	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1056
UGK34	Duku Dewan	Kg. Kulim, Wakaf Bharu, Kelantan	MWNS 1057
UGT01	Duku Terengganu J1	MARDI Jerangau, Terengganu	MWNS 1058
UGT02	Duku Terengganu J35	MARDI Jerangau, Terengganu	MWNS 1059
UGT03	Duku Terengganu J40	MARDI Jerangau, Terengganu	MWNS 1060
UGT04	Duku Terengganu J8	MARDI Jerangau, Terengganu	MWNS 1061
UGT05	Duku Terengganu J24	MARDI Jerangau, Terengganu	MWNS 1062
UGT06	Duku Terengganu J27	MARDI Jerangau, Terengganu	MWNS 1063

Table 1 continues on next page

TABLE 1. *continued*

Accession number	Vernacular name	Source of materials	Voucher number
UGT07	Duku Terengganu J47	MARDI Jerangau, Terengganu	MWNS 1064
UGT08	Duku Terengganu J48	MARDI Jerangau, Terengganu	MWNS 1065
UGT10	Duku Terengganu J21	MARDI Jerangau, Terengganu	MWNS 1066
UGT11	Duku Ayam	MARDI Jerangau, Terengganu	MWNS 1067
UGT12	Duku Pulau	MARDI Jerangau, Terengganu	MWNS 1068
UGT14	Duku Serongkoh	MARDI Jerangau, Terengganu	MWNS 1069
UGT16	Duku hutan	MARDI Jerangau, Terengganu	MWNS 1070
UGT18	Duku Palembang	MARDI Jerangau, Terengganu	MWNS 1071
UGT20	Duku Dewan Beta	MARDI Jerangau, Terengganu	MWNS 1072
UGT26	Duku Terengganu J60	MARDI Jerangau, Terengganu	MWNS 1073
UGT30	Duku hutan	MARDI Jerangau, Terengganu	MWNS 1074
UGR05	Duku-langsat	Experimental Plot UKM Bangi, Selangor	MWNS 1075
UGR06	Duku-langsat	Experimental Plot UKM Bangi, Selangor	MWNS 1076
UGP02	Duku-langsat	Taman Pantun UKM Bangi, Selangor	MWNS 1077
UGJ05	Duku-langsat	Agriculture Department Serdang, Selangor	MWNS 1078
UGJ06	Duku-langsat	Agriculture Department Serdang, Selangor	MWNS 1079
UGJ08	Duku Dewan Beta	Agriculture Department Serdang, Selangor	MWNS 1080
UGM02	Langsat Embun/hutan	Kg. Londang, Masjid Tanah, Melaka	MWNS 1081
UGM04	Langsat Embun/hutan	Kg. Londang, Masjid Tanah, Melaka	MWNS 1082
UGH12	Duku-langsat	Sg. Mati, Muar, Johor	MWNS 1083
UGH13	Duku-langsat	Sg. Mati, Muar, Johor	MWNS 1084
UGH14	Duku-langsat	Sg. Mati, Muar, Johor	MWNS 1085

determine the primer with the highest clustering ability and the optimal number of primers needed to discriminate the 85 accessions of *L. domesticum* used in the study reliably. The primer with the highest ability to cluster the 85 accessions, i.e. OPB-12 was used alone as the first primer combination, PC1.J (primer combination 1, based on Jaccard's similarity coefficients). The following nine combinations of primers comprised primers in ascending order. The last primer combination, PC10.J comprised all ten primers used in the study. The ten combinations tested are given in Table 4.

RESULTS

Genetic polymorphism and RAPD patterns

The ten primers used produced an average of 11·3 scorable bands per primer with six to 15 bands per primer. The total number of scorable bands amplified in this study was 113, ranging in size from 311 to 1352 bp (Table 2). Of the 113 bands, 107 (94·7 %) were polymorphic and six were monomorphic among the *L. domesticum* accessions. Most of the accessions studied possessed unique combinations of bands thereby enabling their identification. The optimized PCR protocol resulted in highly reproducible banding patterns. Examples of the RAPD profiles resulting from OPB-12 and OPT-16 are shown in Fig. 1.

Homology test of RAPD markers

Homology tests performed using a DIG-labelled probe for one of the amplification products from each primer showed that the labelled DNA probe hybridized only with other co-migrating bands in the Southern blot. No hybridization with bands of different mobilities was observed. Thus, we can assume that the ten RAPD bands examined are homologous and non-allelic. We can then infer that the 113 RAPD bands generated by the ten primers used in the study are most likely homologous and non-allelic too. Figure 2 shows results of the homology test for primer OPA-10.

TABLE 2. *Sequences of the ten primers, with the number of scorable amplified and polymorphic bands*

Primer	Sequence	No. of amplified bands	No. of polymorphic bands
OPA-02	TGCCGAGCTG	11	10
OPA-10	GTGATCGCAG	7	7
OPB-07	GGTGACGCAG	15	14
OPB-11	GTAGACCCGT	12	12
OPB-12	CCTTGACGCA	15	15
OPB-15	GGAGGGTGTT	15	15
OPT-16	GGTGAACGCT	9	7
OPU-14	TGGGTCCCTC	6	5
OPU-19	GTCAGTGCGG	11	11
OPU-20	ACAGCCCCCA	12	11
Total		113	107

Genetic relatedness

The use of Jaccard's (1908) similarity coefficient to estimate genetic relatedness among accessions gave similarity values ranging from 0·25 for UGT30 and DUH10 to 1·00 for the pairs which could not be distinguished from each other. There are 14 groups of accessions which yielded similarity values of 1·00 among group members. The similarity values among all accessions are available on request from the authors.

A dendrogram based on the similarity values was generated using UPGMA clustering analysis (Fig. 3). In

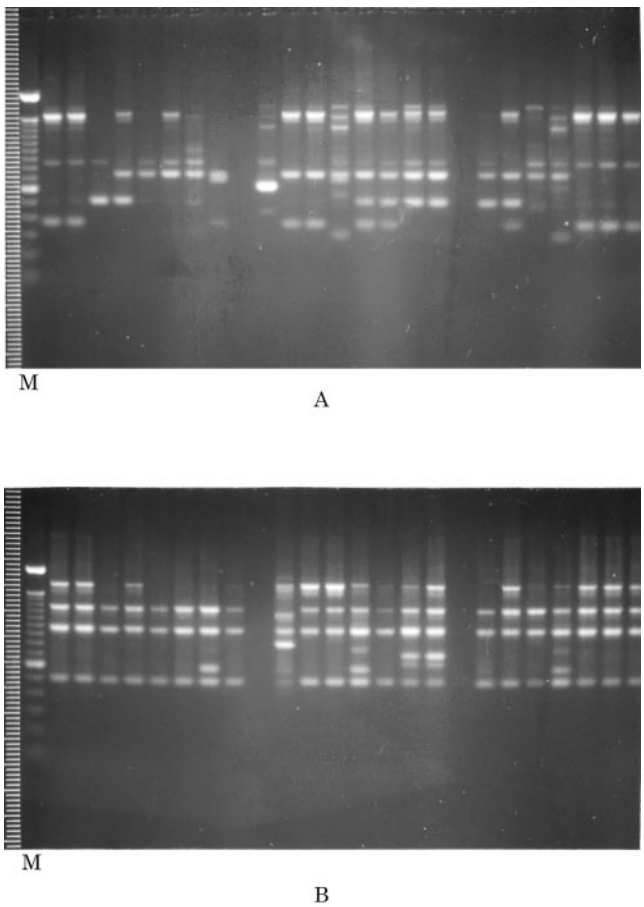

FIG. 1. RAPD profiles of representative accessions using primer OPB-12 (A) and OPT-16 (B). Lane M is a 100 bp ladder marker, the first band (2072 bp) and the 600 bp band are brighter than other bands.

general, the dendrogram separated the accessions into three main branches (X, Y and Z) with branch X being further sub-divided into six major clusters (X1, Dokong kering; X2, Dokong butir; X3, Langsat; X4, Duku Dewan; X5, Dokong air; and X6, a miscellaneous cluster) and Y into three major clusters (Y1, Duku Johor; Y2, Duku Terengganu; and Y3, Langsat hutan). Branch Z comprises only one accession, namely Duku hutan. A total of 56 clusters could be discerned for the 85 accessions of *L. domesticum* examined in this study.

The mean genetic similarities between the ten major clusters of *L. domesticum* discerned from this study are presented in Table 3. The mean genetic similarity between these major clusters of *L. domesticum* ranged from 0·287 between Duku Johor (Y1) and Duku hutan (Z) to 0·794 between Langsat (X3) and Duku Dewan (X4). The genetic similarity between Duku Johor and Duku Terengganu (or Duku-langsat which was reported to be an intermediate type) was about 51 %, whereas the genetic similarity between Langsat and Duku Terengganu was only about 33 %.

Test for clustering ability of primer combinations

The minimum number of primers needed for optimal accuracy is a consideration in a phenetic study (Nei, 1978; Marillia and Scoles, 1996). Table 4 shows the number of individual subsets resulting from the successive clustering analyses with the ten primer combinations. The number of subsets clustered by using different combination of primers was found to increase significantly from 35 in PC1.J to 56 in PC7.J. The clustering power reached a maximum point at 56 subsets after the RAPD data of the seventh primer were added. The addition of the eighth, ninth and tenth primers resulted in minor modifications to the dendrogram (PC8.J, PC9.J and PC10.J; data not shown).

DISCUSSION

Our study has shown that the method of Doyle and Doyle (1990) is very efficient for extraction of DNA from *L. domesticum*. The method used in this study gave DNA yields of 1–20 mg g^{-1} leaf tissue. This is considered a satisfactory result when compared to figures reported by Rogers and Bendich (1988), where the same DNA isolation method was used for species such as *Zea mays* and *Triticum aestivum* yielding 0·4–52 mg g^{-1} leaf of total DNA.

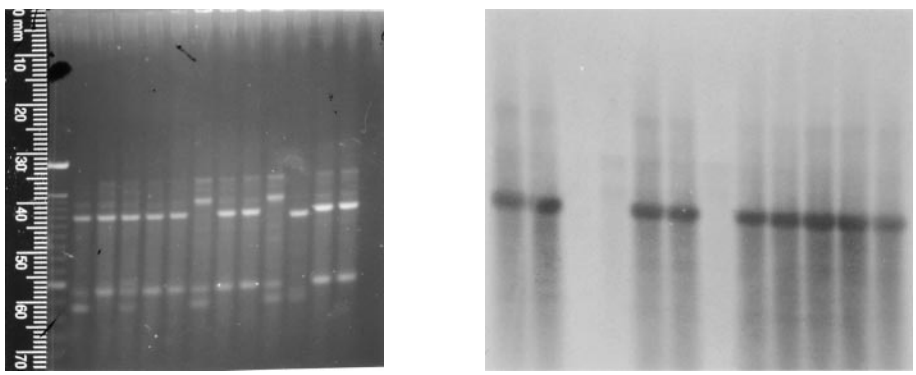

FIG. 2. Southern hybridization of some accessions for primer OPA-10. A, Agarose gel of RAPD products produced by primer OPA-10, Lane M is a 100 bp ladder marker. B, Southern blot probed with DIG-labelled amplified band OPA-10_{1169}. Only co-migrating bands hybridized in the Southern blot.

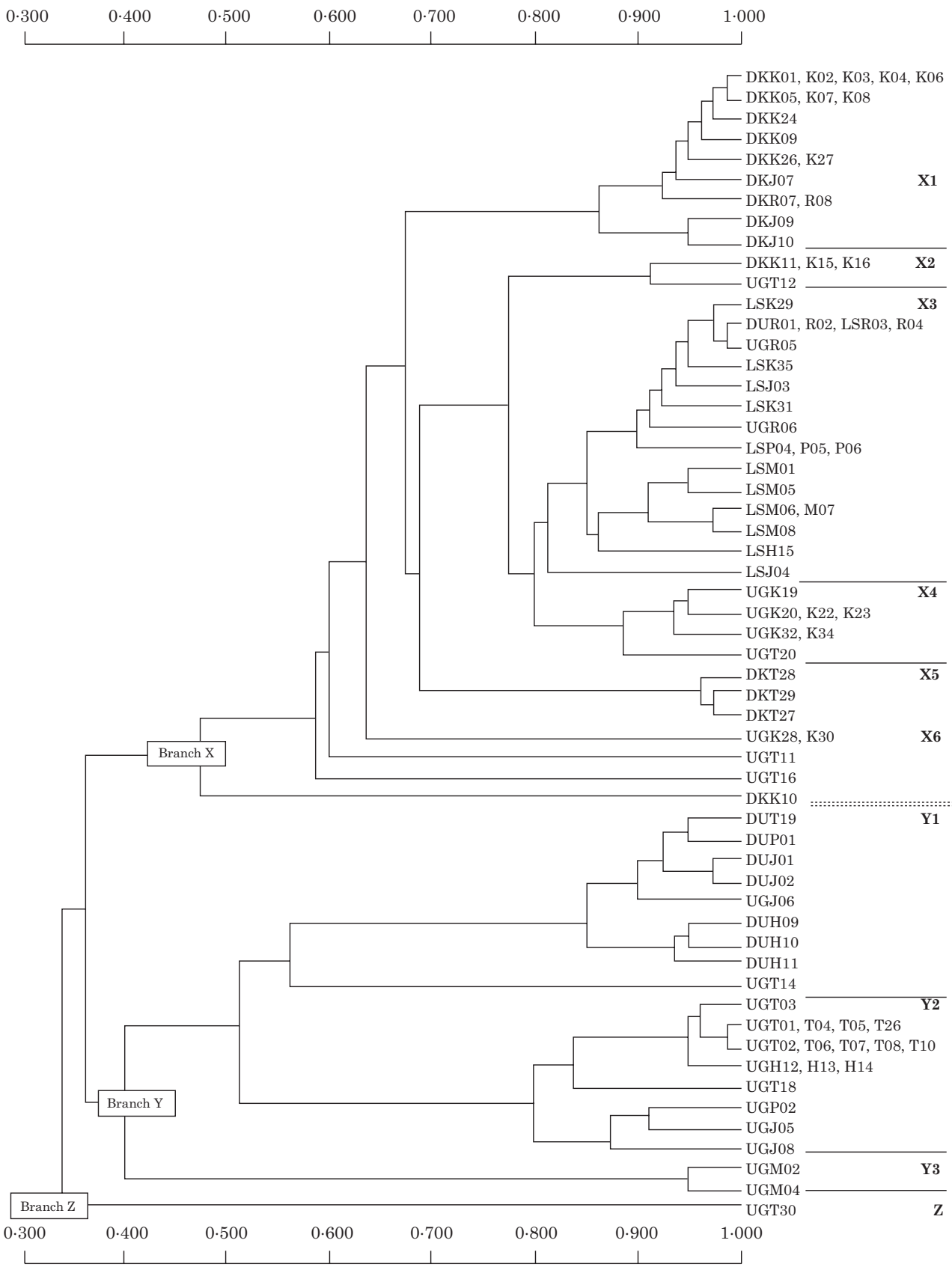

FIG. 3. Dendrogram illustrating genetic relatedness among 85 accessions of *L. domesticum* generated by the UPGMA cluster analysis based on 113 RAPD bands. Scale shown is Jaccard's coefficient of similarity.

TABLE 3. *Mean values of genetic similarity between major clusters of* Lansium domesticum

Group	X1	X2	X3	X4	X5	X6	Y1	Y2	Y3	Z
X1, Dokong kering	–									
X2, Dokong butir	0·635	–								
X3, Langsat	0·664	0·774	–							
X4, Duku Dewan	0·699	0·779	0·794	–						
X5, Dokong air	0·657	0·698	0·704	0·666	–					
X6, Unnamed group	0·563	0·649	0·590	0·615	0·598	–				
Y1, Duku Johor	0·424	0·345	0·359	0·409	0·344	0·368	–			
Y2, Duku Terengganu	0·393	0·282	0·333	0·361	0·338	0·335	0·514	–		
Y3, Langsat hutan	0·357	0·338	0·312	0·321	0·334	0·354	0·456	0·367	–	
Z, Duku hutan	0·407	0·356	0·342	0·326	0·337	0·336	0·287	0·328	0·318	–

TABLE 4. *Primer combinations and the number of subsets they distinguish*

Dendrogram no.	Primer combinations	No. of RAPD bands	No. of subsets distinguished
PC1.J	OPB-12	15	35
PC2.J	OPB-12, OPB-15	30	41
PC3.J	OPB-12, OPB-15, OPU-19	41	44
PC4.J	OPB-12, OPB-15, OPU-19, OPB-07	56	51
PC5.J	OPB-12, OPB-15, OPU-19, OPB-07, OPU-20	68	52
PC6.J	OPB-12, OPB-15, OPU-19, OPB-07, OPU-20, OPB-11	80	54
PC7.J	OPB-12, OPB-15, OPU-19, OPB-07, OPU-20, OPB-11, OPA-10	87	56
PC8.J	OPB-12, OPB-15, OPU-19, OPB-07, OPU-20, OPB-11, OPA-10, OPU-14	93	56
PC9.J	OPB-12, OPB-15, OPU-19, OPB-07, OPU-20, OPB-11, OPA-10, OPU-14, OPA-02	104	56
PC10.J	OPB-12, OPB-15, OPU-19, OPB-07, OPU-20, OPB-11, OPA-10, OPU-14, OPA-02, OPT-16	113	56

The results presented here demonstrate that 1·5 % agarose gel gives a satisfactory resolution of PCR amplification products, which range in size from 200 to 1400 bp. PCR products with molecular weights of less than 200 bp were inconsistently amplified. Products more than 1400 bp could not be separated clearly. Harris (1995) and He *et al.* (1995) also used this range of RAPD bands in their systematic studies of the genus *Leucaena* and of sweetpotato (*Ipomoea batatas*), respectively. Hence, only products found in both replicates of a PCR amplification, sharp and clear enough to be scored, and in a molecular size range of 200 to 1400 bp were scored in this study.

Our studies show an average number of 10·7 polymorphic bands per primer in *L. domesticum*. Comparative values in other crops are 3·8 in rapeseed (Mailer *et al.*, 1994), 5·2 in common bean (Briand *et al.*, 1998), 16·7 in sweetpotato (He *et al.*, 1995) and 18·6 in pea (Samec and Našinec, 1996).

Our results proved that reproducibility of RAPD experiments can be very high if care is taken to avoid any changes in experimental parameters. The conditions that must be strictly controlled include cycling temperature profiles and the brand or sources and concentrations of PCR reagents, especially *Taq* DNA polymerase and primers. No differences were observed among the reproducible RAPD profiles of PCR replicates of the same accessions. This is in agreement with reports of similar studies, e.g. Castiglione *et al.* (1993), Hilu and Stalker (1995), Multani and Lyon (1995) and Virk *et al.* (1995).

Hilu and Stalker (1995) reported that a labelled probe can hybridize with co-migrating bands and fragments of different molecular weights in a Southern blot. Lee *et al.* (1996) demonstrated that one of the primers used in RAPD analysis of *Shorea leprosula* showed cross-hybridization to almost all the bands present in the blot, further suggesting that the amplification products could be allelic or contain repetitive sequences. Some workers (e.g. Warbuton *et al.*, 1996) have used Southern hybridization to show allelic segregation and co-dominant behaviour of amplified fragments. These reports strongly suggest the need for homology tests of amplified products in RAPD analysis especially in studies of genetic relatedness. However, results of the homology tests in this study eliminated doubts that co-migrating bands were homologous and showed that individual bands represented independent characters. Thus, the data from all ten primers in the study can be used for the similarity analysis.

In general, the higher the number of amplified products, the better the discrimination of accessions. Nei (1978) suggested that a minimum number of 50 different loci should be used for estimating genetic distances. Hence the number of primers used is critical in a phenetic analysis. The present study, which used 107 polymorphic RAPD bands (loci) to determine the genetic relatedness among 85 accessions of *L. domesticum*, is adequate.

Various primer combinations were used in cluster analysis of *L. domesticum* accessions to estimate the minimum number of primers necessary to estimate the genetic relatedness among the 85 accessions. The maximum resolution of 56 subsets clustered by UPGMA analysis could be obtained using only 87 RAPD bands. The general

topology of the dendrogram was conserved when the data of the eighth and ninth primers were added to generate dendrograms PC8.J and PC9.J, respectively. These dendrograms (from PC1.J to PC9.J) retained their structure (two-branch tree) until the dataset of RAPD bands of the tenth primer (OPT-16) was added when accession UGT30 (Duku hutan) formed a third branch (branch Z) which showed the greatest dissimilarity from all other accessions.

The similarities among the accessions ranged from 34·3 % to 98·7 % indicating that the accessions have a diverse genepool. The dendrogram shows accession UGT30 to be the most divergent—a wild type with only a 34·3 % similarity to the remaining 84 accessions which are cultivated types. Marsolais *et al.* (1993) showed that a similarity value of about 50 % using RAPD markers is indicative of interspecific hybrid origin in lilac. The mean genetic similarity between the major clusters of *L. domesticum* shown in Fig. 3 ranged from as little as 0·287 to a maximum of 0·794; this is indicative of different species parentage or the occurrence of extensive hybridization and introgression in this species. Embryological studies which have demonstrated apomixis in Duku and Langsat varieties of *L. domesticum* (Prakash *et al.*, 1977) support this inference as most hybrids between distant species show reduced fertility (Grant and Grant, 1960). Duku Terengganu (or Duku-langsat) was reported by Mabberley (1985) to be an indeterminate type, being superior to Duku and Langsat. Duku Johor which exhibited a 51 % genetic similarity with Duku Terengganu may be a possible parent of this intermediate type. However, Langsat which was 33 % similar to Duku Terengganu may not be the only other parent of Duku Terengganu.

Using RAPD markers, Marsolais *et al.* (1993) showed that within species genetic similarity usually ranged from 61 to 99 % in lilac. Hence, it is tempting to postulate that the four main types of *L. domesticum* investigated in this study could be different species of *Lansium* or sub-species of *L. domesticum*. Further studies, including reexamination of morphological traits, chloroplast DNA polymorphisms and internally transcribed spacer (ITS) region polymorphisms may be needed to understand the systematic relationship of *Lansium*.

Based on the dendrogram, two main clusters could be discerned within the 85 accessions in this study. Fifty-six accessions, ranging from DKK01 to DKK10, formed branch X, while branch Y comprised 29 accessions, which covered accessions DUJ01 to UGM04. Most of the accessions in branch X (Dokong and Langsat) are types that possess thin fruit skins while those in branch Y (mostly Duku Terengganu, Duku Johor and Langsat hutan) have thick fruit skins.

Several accessions that were found to have identical RAPD profiles are shown in Fig. 3. Two possible explanations for this are: (1) the seedlings arose apomictically from the same mother plant; or (2) the accessions may have been propagated by grafting. Vernacular names appear to have been used rather inconsistently, with the same name being applied to different types in different regions. Without any biological characterization of the samples, identity of accessions may be misleading.

Dokong, which originates from Thailand, is increasingly cultivated throughout Malaysia. Dokong possesses good market potential both locally and abroad. There are three main types of Dokong, namely Dokong kering, Dokong butir and Dokong air, each of which forms a different group in branch X. The dendrogram indicates that Dokong butir (group X2), which was previously expected to group next to, or at least near, the other two types of Dokong (group X1 and X5), is surprisingly more closely related to Duku Dewan (group X4) ($I = 0{\cdot}779$) accessions than to other accessions of Dokong. One possible reason for this is the fruit characters of Dokong butir which are comparatively closer to those of Langsat (group X3) and Duku Dewan (group X4). However, the fruits of both Duku Dewan and Dokong air are thin-skinned, possess latex and have a sweeter flavour compared to Langsat, whilst Dokong kering and Dokong butir differ from Dokong air in terms of latex content in the fruit skin.

Generally accessions of the same type clustered together. However, a few accessions (UGJ06, UGJ08, DUR01, DUR02, UGR05 and UGR06) did not conform to the expected clustering. Since all the accessions obtained were named by local people or the tree owner, it is possible that these accessions were mislabelled. Without any biological characterization of the samples, the names may be misleading. Re-checking of the herbarium voucher specimens and morphological characters is needed to confirm the real identity of these accessions. Mislabelling within germplasm collections and detection using RAPD markers have been reported by Marmey *et al.* (1994) in the evaluation of a cassava germplasm collection and Van de Ven and McNicol (1995) in the fingerprinting of Sitka spruce clones.

The present study is a first step in elucidating the genetic relatedness in *L. domesticum* using RAPD markers. Further studies using accessions representing more types from a broader geographic distribution may provide a better understanding of genetic relatedness in this species. We conclude that used correctly, RAPD analysis is a rapid, simple and reliable tool in estimating the genetic relatedness among accessions and identifying different types of *L. domesticum*.

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