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Primer Screening for *Dyera costulata* (Miq) Hook.f Random Amplified Polymorphic DNA Analyses

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ABSTRACT

Dyera costulata (Miq.) Hook.f (Apocynaceae) is a large tree of the lowland tropical rain forest of Southeast Asia, Thailand, Malay Peninsula and Indonesia, especially in Sumatra and Kalimantan (Borneo) islands. Its economic value was in its copious latex, used as gum in the manufacture of chewing gum. Today the timber of this species is much sought after for the manufacture of pencils and picture frames. Information on genetic diversity of the species is very limited. Hence studies were initiated to screen primers for RAPD analyses of *Dyera costulata* for use in genetic variation studies. Seventy one Operon primers (10 mer) were used to generate a total of 864 consistent and ambiguous amplification products ranging from 200 bp to 2.0 kb. Rare and genotype specific bands were identified which could be effectively used to distinguish the genotypes. 34 highly polymorphic primers (100%) are recorded from 71 primers used. Three primers (OPA-04, OPU-06, and OPU-07) produced highest variable RAPD profiles. The dendrogram separated the 8 genotypes into 2 groups. Genetic dissimilarity ranged from 0.07 to 0.71 %.

Key words: *Dyera costulata*, jelutong, primer screening, RAPD.

INTRODUCTION

Dyera costulata (Miq.) Hook.f (Apocynaceae), known as *jelutong* or "*jelutung*" in Indonesia, are spread in Sumatra and Kalimantan, and islands between them. Its timber is light and soft, easy to work and stable. The timber is used to manufacture of board, pencils, toys, packing board, photo frame, and furniture accessories. Although timber quality of *D. costulata* is moderate, it used for manufacturing of veneer and plywood. Its latex is used for manufacturing of chewing gum. Export of jelutong from Indonesia averaged 3600 t annually over the periods of 1988-1993. Kalimantan has always been the main area of the supply (Boer, 2001). Malaysia is also one of the exporters of the timber (Norwati, 2002).

The maintenance of genetic variation is considered essential for the long-term survival of a species since genetic diversity provides a species' evolutionary potential. Furthermore, a reduction in diversity through the loss of alleles reduces a population's ability to respond to biotic challenges (e.g. pathogens) and to changes in the abiotic environment (Pither et al., 2003). These make assessment of genetic diversity of a species is important. The species has not been studied genetically, even the total number of the chromosome has not been determined. The present study is conducted to obtain polymorphic primer suitable for distinguishing individual or variety of *D. costulata* for use in genetic diversity study and genetic identification (*fingerprinting)* based on Random Amplified Polymorphic DNA (RAPD). Study of genetic diversity of a species is becoming important for selection and domestication programs of *D. costulata* as well as for its conservation strategy. In this present research, individual/variety of *D. costulata* which represent genetic diversity from West Kalimantan, South Kalimantan, Jambi (DHL, Berbak) and from Palembang are used. Eight accessions of *D. costulata* collected from those areas are used for developing a protocol of DNA extraction and isolation, optimation of DNA amplification with Polymerase Chain Reaction (PCR), and primer using RAPD marker. RAPD analyses is used because of its simple and cost effective, and the analyses has been used with other species of tropical timber trees (Rath et al*.,* 1998; Shashidhara et al*.,* 2003; Pither et al*.,* 2003; Telles et al*.,* 2003; Mori et al*.,* 2004; Runo et al*.,* 2004; Singh et al*.,* 2005; Sarkhosh et al*.,* 2006) and used for other identification purposes (Poerba, 2003; Fernandez et al*.,* 2006; Parjanto et al., 2006,).

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MATERIALS AND METHODS

Materials

DNA materials consist of eight accessions of *D. costulata* collected from West and East Kalimantan, Jambi (DHL, Berbak) and Bogor Collection Garden of Dept of Forestry (from Palembang). The eight accessions were: 1 = DHL (Jambi), 2 = red jelutong (Jambi), 3 = green jelutong (Jambi), 4 = black jelutong (Jambi), 5 = West Kalimantan, 6 = South Kalimantan, 7 = Jambi, 8 = Palembang. DNA materials were leaf samples dried with silica gel collected from those areas using method for DNA sampling (Widjaja and Poerba, 2004).

Methods

DNA extraction

Total genomic DNA was isolated from dried silica leaves according to Delaporta et al. (1983) with the addition of RNase treatment (100 mg/mL). Isolated DNA was visualized for its quantity and quality by running them in 1% Agarose gel electrophoresis. Amplification of RAPD was performed in Takara Thermocycler according to Williams et al*.* (1990) with total volume of PCR reaction of 11 µl consisting of 0.2 nM dNTPs; 1X reaction buffer; 2mM $MgCl_2$; 1-5 ng of DNA sample ; 0.5 pmole of single primer; and 1 unit of *Taq* DNA polymerase (Promega). Ten arbitrary RAPD primers from Operon Kit A, B, C, N, and U (Tabel 2) were screened for their polymorphic level. PCR reaction was conducted twice to ensure the reproducibility of RAPD. Only bands that appeared on both PCR reactions were scored. PCR products were visualized in 2% agarose gel electrophoresis for 50 min at 50 Volt. This was followed by EtBr staining (0.15 µl/mL) before photographed in UV transilluminator. 100 bp plus ladder (Promega) was used as DNA's size standard.

Total plant genomic DNA was isolated from 0.5 g dried silica leaves with the addition of RNase treatment of 1 µL RNase A (100 mg/mL). Leaves were collected in the field into plastic bags containing 5-10 g silica gel as desiccant, and then stored in a freezer at -20°C upon arrival to the laboratory. With silica gel, the plant material could be kept at the ambient temperature for up to 14 days without degradation of DNA quality. About 0.5 g of silica-dried leaf tissue was grinded to a fine powder using a mortar, pestle, and liquid nitrogen. The powder were then transferred to a 2.0 ml micro tube and were added 700 µL of hot (65°C) extraction buffer which contained 2% CTAB, 2% PVP, 20mM EDTA, 100mM Tris-HCl pH 7.5, and 1.4M HCl and 0.2% mercaptoethanol. The microtube was then vortexed until mixed well and incubated at 65°C for 60 min with occasional inversion. Centrifuge in a Beckman centrifuge at 12000 rpm for 10 min at 28^0C. The top aqueous layer was then transferred to a fresh 2-mL tube. Equal volume (~700 µL) of chloroform: isoamyl alcohol (41: 1) was then added, mixed by gentle inversion and centrifuged at 12000 rpm for 10 min at 28^0C to precipitate the DNA.

The top aqueous layer was then transferred to a fresh 2-mL tube. Equal volume (~700 µL) of chloroform: isoamyl alcohol (24: 1) and 5 M sodium acetate was then added, mixed and centrifuged at 12000 rpm for 10 min at 28^0C to precipitate DNA. 700µL of the top aqueous layer was transferred, and the DNA was precipitated by adding a ¾ volume of cold isopropanol and mix by gentle inversion, and incubated at -20^0C for 30 minute. Mix well by using gentle inversion and centrifuge for 3 min at 12000 rpm at 28^0C. The top aqueous layer was discarded, and removed the DNA pellet with a hook. The pellet was then washed with absolute alcohol and air-dried for a night or vacuumed for 30 min. At this step, DNA can be stored at -20^0C or add 100µL TE buffer to dissolve the pellet. Isolated DNA was visualized for its quantity and quality by running them in 1% Agarose gel electrophoresis, by comparing with Lambda DNA/ *Eco*R1+Hind III marker.

DNA amplification

Optimum PCR conditions for RAPD was standardized with various quantities of template DNA (5, 10, 15, 20, and 25 ng), primer concentration (5, 10, 25 pmole), and MgCl2 concentration (0, 1, 2 and 3 mM). DNA amplification was conducted based on the methods of Williams et al*.* (1990) using a selected arbitrary primer which consist of 10 oligo nucleotide (10-oligomer), OPN-14 (Operon Technology), a polymorphic primer previously tested for *Alstonia scholaris (*Apocynaceae) (Poerba, 2005). PCR reactions (15 µL) contained 5-25 ng of genomic DNA; 1X reaction buffer, 0.2 nM of each of dATP, dCTP, dGTP, and dTTP (Promega); 2.5 mM of $MgCl_2$; 5-25 pmole of 10-mer primer (Operon); and 1unit of *Taq* DNA polymerase (Promega). A thermocycler (Takara) was programmed for one preamplification step of 2 min at 94°C, followed by 45 cycles, with a denaturation step of 1 min at 94°C, annealing step of 1 min at 36^0C, and extension step of 2 min at 72°C. After 45 cycles, it was then followed by 5 min extension at 72°C. After amplification, 5 µL of the samples was loaded and fragments separated by Mupid Mini Cell electrophoresis in 2.0% agarose gels using a 1 × TEA running buffer for 50 min at 50 Volt. After electrophoresis, the gel was staining in ethidium bromide solution with final concentration of 1µg/ml for 10 min. The result of RAPD was documented using Gel document system (Biorad, USA).

Primer screening

Based on the results of PCR optimation, a total of eighty primers (Kits A, C, N and U from Operon Technologies, Alameda, California) were screened for usefulness in an initial survey. Amplification of DNA was performed in Takara Thermocycler according to Williams et al*.* (1990) with total volume of PCR reaction of 15 µl consisting of 0.2 nM dNTPs; 1X

reaction buffer; 2mM $MgCl_2$; 10 ng of DNA sample; 5 pmole of single primer; and 1 unit of *Taq* DNA polymerase (Promega). Eighty arbitrary RAPD primers from Operon Kit A, C, N, and U (Tabel 2) were screened for their polymorphic level. PCR for amplifying the DNA preparations was carried out in 15-µL vol of reaction mixture. Reaction tube contained 10 ng of DNA, 1 unit of *Taq* DNA polymerase enzyme, 0.2 nM of each dNTP, 1.5µl reaction buffer, 2mM $MgCl_2$, and 5 pmol decanucleotide primers. Amplifications were carried out by using DNA thermocycler (Takara) with the following parameters: preamplification step of 2 min at 94°C, followed by 45 cycles, with a denaturation step of 1 min at 94°C, annealing step of 1 min, and extension step of 2 min at 72°C. After 45 cycles, followed by 5 min extension at 72°C. PCR products were subjected to agarose gel (2.0 w/v) electrophoresis for 50 min at 50 volt, in 1x TAE buffer, along with 100-bp DNA ladders (Promega) as molecular weight markers. DNA was stained with ethidium bromide and photographed under UV light. PCR reaction was conducted twice to ensure the reproducibility of RAPD. Only bands that appeared on both PCR reactions were scored. PCR products were visualized in 2% agarose gel electrophoresis for 50 min at 50 Volt. This was followed by EtBr staining (0.15 µl/mL) before photographed in UV transilluminator. 100 bp plus ladder (Promega) was used as DNA's molecular weight standard.

Data scoring

Each band in the RAPD fingerprint pattern will be considered as a separate putative locus. Only the loci with clearly amplified bands will be manually selected and scored for presence (1) and absence (0) of a band. The binary matrices of RAPD phenotypes will then be assembled for different analyses. A similarity matrix was constructed and subjected to cluster analysis following the UPGMA method by computer program NTSYS-pc version 1.8 (Rohlf, 1993). Measurement of genetic similarity for pair-wise accessions was based on the simple matching coefficients (Dunn and Everitt, 1982; Rohlf, 1993), while measurement of genetic dissimilarity for pair-wise accessions was subtraction of genetic similarity values by 1 (Dunn and Everitt, 1982).

RESULTS AND DISCUSSION

DNA isolation

The DNA extraction of 8 accession of *Dyera costulata* was conducted using CTAB method of Delaporta et al. (1983). Mechanical grinding step was necessary to disrupt the cell wall for the release of DNA. The extraction process involves breaking or digesting away cell walls to release the cellular constituents. This is followed by disruption of the cell membranes to release DNA into the extraction buffer. This is normally achieved by using detergents such as sodium dodecyl sulphate (SDS) or cetyltrimethylammonium bromide (CTAB). In this research, we used CTAB. The released DNA should be protected from endogenous nuclease. For this purpose, hexadecyltrimethylammonium bromide (EDTA) is often included in the extraction buffer to chelate magnesium ions, necessary cofactor for nucleases. The initial DNA extracts often contain large amounts of RNA, proteins, polysaccharides, tannins, and pigments, which may interfere with the extracted DNA and were difficult to separate. Most proteins were removed by means of denaturation and precipitation from the extract with chloroform and/or phenol. RNAs, on the other hand, were normally removed by means of treatment of the extract with heat-treated RNase A. Polysaccharide like contaminants were particularly problematic, and more difficult to remove. Polysaccharides can cause anomalous reassociation kinetics. They can also co precipitate with DNA after alcohol addition during DNA isolation to form highly viscous solutions. The addition of NaCl at concentrations higher than 0.5 M, together with CTAB, is known to remove polysaccharides. Antioxidants were commonly used to address problems related to phenolics. Examples include the use of ß-mercaptoethanol, ascorbic acid, bovine serum albumin (BSA) sodium azide, and polyvinylpyrrolidone (PVP). In this research PVP was used for removing phenolic compounds. The concentration of ß-mercaptoethanol commonly used in DNA extraction protocols is 0.2%. However, our experimentation showed that as little as 0.05% ß-mercaptoethanol was sufficient to decrease polyphenol oxidation in jelutong young leaves.

Results of the experiment showed that method of Delaporta et al. (1983) produced DNA of the eight accession of *D. costulata* with good quality and quantity; so that DNA amplification can be proceed.

PCR optimation

The RAPD technique, which uses single primers of arbitrary nucleotide sequence, allows random amplification of DNA sequences throughout the entire genome. Because RAPD polymorphisms result from either a nucleotide base change that alters the primer binding site, or from an insertion or deletion within the amplified region (Williams et al., 1993), polymorphisms usually result in the presence or absence of an amplification product from a single locus (Tingey et al., 1994). The products of these amplifications can be polymorphic and were useful as genetic markers. RAPD profiling is being increasingly used in genetic studies because of the easiness of methodology and the numerous polymorphic distinguishable. RAPD has proven to be quite efficient in detecting genetic variations (Williams et al. 1990). The technique is also very simple, fast, cost-effective,

highly discriminative, requiring very small amount of genomic DNA, without the need for blotting and radioactive detection, enabling assay to be performed at any stage of plant development (Fernandez et al*.,* 2006). RAPD fingerprinting techniques have been used for the identification of horticultural crop varieties, description of cultivar genotypes and for protecting breeder's rights. One of disadvantage of RAPD marker was its low reproducibility (Jones et al*.,* 1997). However, these can be minimized by optimizing PCR conditions. Different quality of amplification products can be observed based on number of bands and resolution of DNA fragments (Siregar et al*.,* 1998).

Results of the experiment showed that the best DNA amplification of *D. costulata* was performed with total volume of PCR reaction of 15 µl consisting of 0.2 nM dNTPs; 1X reaction buffer; 2mM $MgCl_2$; 10 ng of DNA sample; 5 pmole of single primer; and 1 unit of Taq DNA polymerase (Promega). Amplifications were carried out by using DNA thermocycler (Takara) with the following parameters: preamplification step of 2 min at 94°C, followed by 45 cycles, with a denaturation step of 1 min at 94°C, annealing step of 1 min at 36^0C, and extension step of 2 min at 72°C. After 45 cycles, followed by 5 min extension at 72°C, and cooling at 4^0C for 30 min. These PCR conditions produced more DNA amplicon and produced better DNA bands compared with other PCR conditions. Reproducibility of these selected primers was tested by repeating the PCR amplification for at least two times under the same amplification conditions. The results also showed that the optimized condition of PCR used in this experiment for *D. costulata* produced consistent DNA amplicon bands. Consistency of DNA bands produced indicated that RAPD markers can be used for distinguishing variation of *D. costulata* tested. Similar results was also observed in various plant for identifying individual plant, clone or cultivar (Shashidhara et al*.,* 2003; Mori et al*.,* 2004; Runo et al*.,* 2004; Fernandez et al*.,* 2006; Sarkhosh et al*.,* 2006).

Primer screening and RAPD profile analyses

For genetic identification purposes, primer used is important to be able to differentiate varieties or cultivars of the species. Primer screening, therefore, is an important step in genetic analyses. Optimum primer concentration for DNA amplification of different plant species depended on type of primer and plant species. Number of DNA amplification bands depended on how primer attached to its homolog at DNA template (Tingey et al., 1994).

Primers used for screening were arbitrary random primer consisted of 10-nucleotide and had a G+C content of 60-80%, and no palindromes. Results of DNA amplification showed that the eight accession of jelutong produced clear, scorable PCR products, so that they can be analyzed, except for primer OPC-7, OPC-9, OPC-10, OPC-13, OPC-17, OPU-04, OPU-09 and OPU-16. For the following experiment, only 71 primers were used i.e. 20 primers of Kit A, 14 primers of Kit C, 20 primers of Kit N and 17 primers of Kit U (Operon Technology) (Table 1). DNA amplification products of eight accession of jelutong showed different DNA profiles (Figure 1). The 71 RAPD primers resulted in 864 scorable band classes, ranging from 200 bp to 2 kb in size. The number of bands for each primer varied from 4 (OPC-16) to 19 (OPU-06) with an average of 12.1 bands per RAPD primer (Table 1).

The amplification products indicated that 9.26% were monomorphic, common to all the genotypes, and 90.74% were polymorphic bands. Among the selected primers OPU-06 produced maximum number of polymorphic 19 bands followed by OPU-01. Out of 71 primers used 34 (42.25%) were highly polymorphic (100% polymorphic). These results showed that the eight accessions of jelutong can be distinguished using these highly polymorphic primers. Similar results were observed in different plant species (Pither et al*.,* 2003; Poerba, 2003; Shashidhara et al*.,* 2003; Telles et al*.,* 2003; Mori et al*.,* 2004; Runo et al*.,* 2004; Singh et al*.,* 2005; Sarkhosh et al*.,* 2006; Fernandez et al*.,* 2006; Parjanto et al., 2006).

The profiles of the RAPD indicate that each primer could generate a major band (strong band) which could be used as RAPD markers for detecting the differences among the eight accessions. The differences in polymorphism may be due to the differences in amount of genetic variation that exist among the different accessions. The primers, and conditions for DNA amplification, chosen in this study produced reasonably consistent results.

Cluster analyses

The eight jelutong accessions grouped into two clusters in Figure 2. 'Red jelutong' resolved separately from the remaining jelutong accessions. Cluster II grouped seven accessions, which was separated into two subgroups of five accessions and two accessions. The first subgroup consisted of five accessions from Jambi (DHL), West Kalimantan, South Kalimantan, Jambi and Palembang. The second subgroup consisted of green jelutong and black jelutong, both from Jambi. Clustering analysis indicates similarities between accession 7 (Jambi) and 8 (Palembang).

The genetic dissimilarity value ranges from 0.09 to 0.71 was observed. The highest dissimilarity 0.71 was detected between genotypes No 2 and No 6, and between No 2 and No 7, and the least 0.09 between genotypes N0 5 and No 6 (Table 2). The high genetic dissimilarity values indicated that these jelutong accessions were highly separated to each other and resulted in their clustering in the dendrogram (Figure 2).

Table 1. List of primers, their sequences, total number of amplified fragments and number of polymorphic bands generated by PCR using 71 RAPD primers.

No	Primer code	Primer nucleotide sequence (5'- 3')	Total bands	Mono-morphic bands	Polymorphic bands
1	OPA-01	CAGGCCCTTC	11	0	11 (100%)
2	OPA-02	TGCCGAGCTG	13	0	13 (100%)
3	OPA-03	AGTCAGCCAC	12	0	12 (100%)
4	OPA-04	AATCGGGCTG	14	2	12 (85.71%)
5	OPA-05	AGGGGTCTTG	9	0	9 (100%)
6	OPA-06	GGTCCCTGAC	10	0	10 (100%)
7	OPA-07	GAAACGGGTG	14	0	14 (100%)
8	OPA-08	GTGACGTAGG	13	0	13 (100%)
9	OPA-09	GGGTAACGCC	15	0	15 (100%)
10	OPA-10	GTGATCGCAG	15	0	15 (100%)
11	OPA-11	CAATCGCCGT	14	0	14 (100%)
12	OPA-12	TCGGCGATAG	12	0	12 (100%)
13	OPA-13	CAGCACCCAC	12	3	9 (75%)
14	OPA-14	TCTGTGCTGG	9	0	9 (100%)
15	OPA-15	TTCCGAACCC	8	1	7 (87.5%)
16	OPA-16	AGCCAGCGAA	12	0	12 (100%)
17	OPA-17	GACCGCTTGT	10	1	9 (90%)
18	OPA-18	AGGTGACCGT	16	1	15 (93.75%)
19	OPA-19	CAAACGTCGG	12	0	12 (100%)
20	OPA-20	GTTGCGATCC	17	0	17 (100%)
21	OPC-01	TTCGAGCCAG	7	1	6 (87.5%)
22	OPC-02	GTGAGGCGTC	9	3	6 (66.67)
23	OPC-03	CCGCATCTAC	7	0	7 (100%)
24	OPC-04	CCGCATCTAC	5	0	5 (100%)
25	OPC-05	GATGACCGCC	17	1	16 (94.12%)
26	OPC-06	GAACGGACTC	18	1	17 (94.44%)
27	OPC-08	TGGACCGTCC	14	3	11 (78.57%)
28	OPC-11	AAAGCTGCGG	14	4	10 (71.43%)
29	OPC-12	TGTCATCCCC	9	0	9 (100%)
30	OPC-14	TGCGTGCTTG	9	0	9 (100%)
31	OPC-16	CACACTCCAG	4	0	4 (100%)
32	OPC-18	TGAGTGGGTG	6	1	5 (83/33%)
33	OPC-19	GTTGCCAGCC	12	3	9 (75%)
34	OPC-20	ACTTCGCCAC	7	0	7 (100%)
35	OPN-01	CTCACGTTGG	13	1	12 (92.31%)
36	OPN-02	ACCAGGGGCA	15	1	14 (73.33%)
37	OPN-03	GGTACTCCCC	14	0	14 (100%)
38	OPN-04	GACCGACCCA	19	4	15 (78.95%)
39	OPN-05	ACTGAACGCC	19	3	16 (84.21%)
40	OPN-06	GAGACGCACA	14	3	11 (78.57%)
41	OPN-07	CAGCCCAGAG	18	1	17 (94.44%)
42	OPN-08	ACCTCAGCTC	16	2	14 (87.5%)
43	OPN-09	TGCCGGCTTG	13	1	12 (92.31%)
44	OPN-10	ACAACTGGGG	14	7	11 (73.33%)
45	OPN-11	TCGCCGCAAA	16	0	16 (100%)
46	OPN-12	CACAGACACC	16	3	13 (81.25%)
47	OPN-13	AGCGTCACTC	14	3	11 (92.86%)
48	OPN-14	TCGTGCGGGT	12	0	12 (100%)
49	OPN-15	CAGCGACTGT	12	0	12 (100%)
50	OPN-16	AAGCGACCTG	7	2	5 (71.43%)
51	OPN-17	CATTGGGGAG	12	1	11 91.67%)
52	OPN-18	GGTGAGGTCA	9	4	5 (55.56%)
53	OPN-19	GTCCGTACTG	12	2	10 (83.33%)
54	OPN-20	GGTGCTCCGT	13	0	13 (100%)
55	OPU-01	ACGGACGTCA	16	0	16 (100%)
56	OPU-02	CTGAGGTCT C	10	0	10 (100%)
57	OPU-03	CTATGCCGAC	12	1	11 (91.67)
58	OPU-05	TTG GCGGCCT	9	4	5 (55.56%)
59	OPU-06	ACCTTTGCGG	19	0	19 (100%)
60	OPU-07	CCTGCTCATC	14	0	14 (100%)
61	OPU-08	GGCGAAGGTT	13	0	13 (100%)
62	OPU-10	ACCTCGGCAC	8	0	8 (100%)
63	OPU-11	AGACCCAGAG	11	0	11 (100%)
64	OPU-12	TCACCAGCCA	4	0	4 (100%)
65	OPU-13	GGCTGGTTCC	17	3	14 (82.53%)
66	OPU-14	TGGGTCCCTC	15	0	15 (100%)
67	OPU-15	ACGGGCCAGT	9	1	8 (88.89%)
68	OPU-17	ACCTGGGGAG	13	1	13 (92.86%)
69	OPU-18	GAGGTCCACA	9	1	12 (92.31%)
70	OPU-19	GTCAGTGCGG	11	2	9 (81.82%)
71	OPU-20	ACAGCCCCCA	10	4	6 (60%)
		Total	864	80	784 (90.74%)

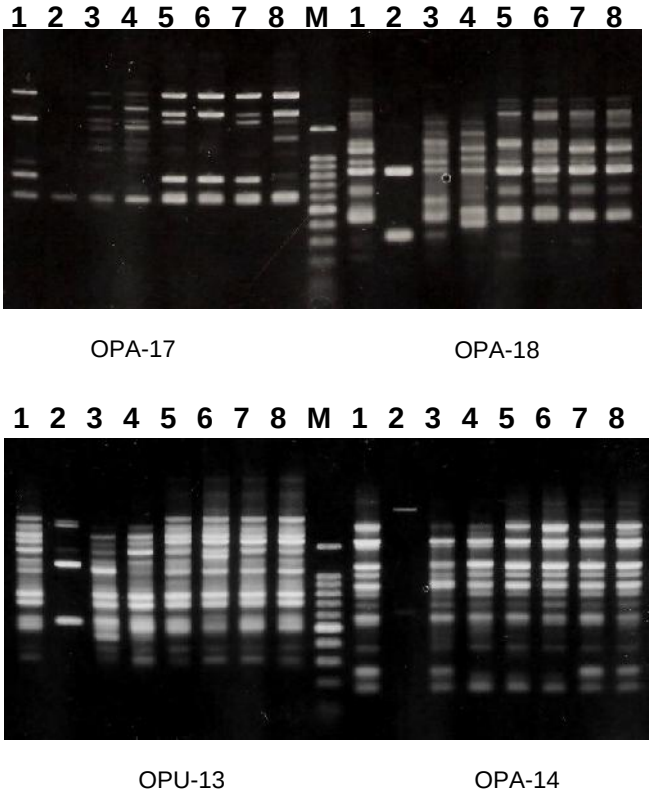

Figure 1. RAPD profile of eight jelutong accessions using four different primers. Note: 1-4 = Jambi, 1 = DHL, 2 = red jelutong, 3 = green jelutong, 4 = black jelutong, 5 = West Kalimantan, 6 = South Kalimantan, 7 = Jambi, 8 = Palembang

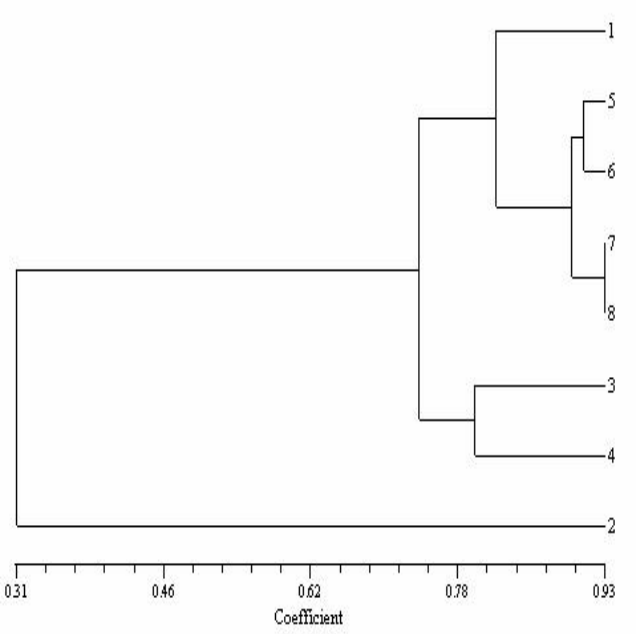

Figure 2. Dendrogram of eight accessions of jelutong. Note: 1-4 = Jambi, 1 = DHL, 2 = red jelutong, 3 = green jelutong, 4 = black jelutong, 5 = West Kalimantan, 6 = South Kalimantan, 7 = Jambi, 8 = Palembang.

Table 2. Genetic dissimilarity of eight jelutong accessions as Obtained from RAPD markers.

	1	2	3	4	5	6	7	8
1	0							
2	0.67	0.00						
3	0.25	0.67	0.00					
4	0.22	0.68	0.21	0.00				
5	0.16	0.70	0.24	0.27	0.00			
6	0.19	0.71	0.28	0.30	0.09	0.00		
7	0.19	0.71	0.26	0.30	0.10	0.10	0.00	
8	019	0.70	0.25	0.28	0.09	0.11	0.07	0.00

Note: 1-4 = Jambi; 1= DHL, 2 = red jelutong, 3 = green jelutong, 4 = black jelutong, 5 = West Kalimantan, 6 = South Kalimantan, 7 = Jambi, 8 = Palembang.

CONCLUSION

Optimized PCR condition for RAPD analyses of *D. costulata* was obtained to produce clear and consistent DNA amplification bands. The amplification of DNA was performed in Takara Thermocycler according to Williams et al*.* (1990) with total volume of PCR reaction of 15 µl consisting of 0.2 nM dNTPs; 1X reaction buffer; 2mM $MgCl_2$; 10 ng of DNA template ; 5 pmole of single primer; and 1 unit of *Taq* DNA polymerase (Promega). The DNA amplifications were carried out with the following parameters: preamplification step of 2 min at 94°C, followed by 45 cycles, with a denaturation step of 1 min at 94°C, annealing step of 1 min at 36^0C, and extension step of 2 min at 72°C. After 45 cycles, followed by 5 min extension at 72°C, and cooling at 4^0C for 30 min.

The 71 RAPD primers resulted in 864 scorable band classes, ranging from 200 bp to 2 kb in size. The number of bands for each primer varied from 4 (OPC-16) to 19 (OPU-06) with an average of 12.1 bands per RAPD primer. Out of the amplification products recorded, 90.74% were polymorphic bands with OPU-06 produced maximum number of polymorphic 19 bands Operon primers of OPA-04, OPU-06, and OPU-07 were highly polymorphic primers for distinguishing jelutong accessions tested.

The eight jelutong accessions grouped into two clusters. 'Red jelutong' resolved separately from the remaining jelutong accessions. Cluster II grouped seven accessions, which was separated into two subgroups of five accessions and two accessions. The first subgroup consisted of five accessions from Jambi (DHL), West Kalimantan, South Kalimantan, Jambi and Palembang. The second subgroup consisted of green jelutong and black jelutong, both from Jambi. Clustering analysis indicates similarities between accession 7 (Jambi) and 8 (Palembang).

Result of the experiment showed the accessions of jelutong showed a broad genetic variation and can be detected using highly polymorphic RAPD markers, such as OPA-04, OPU-06, and OPU-07. From the above study, we concluded that RAPD is reliable, rapid and inexpensive screening method to discriminate the jelutong genotype. RAPD analysis also revealed genetic diversity among these investigated species, which may be beneficial to crop improvement and the detection of genetic variation of the species. Furthermore, this technique is less restricting than other techniques like RFLPs (no hybridization and no use of radioisotopes), and therefore is more convenient for use in research. Knowledge on genetic diversity will help in the efficient management of jelutong germplasm by breeders and conservationist.

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REFERENCES

Boer, E. 2001. **Dyera** Hook.f. *In:* Boer, E. and A.B. Ella (eds.). *Plant Resources of South-East Asia. PROSEA 18. Plant Producing Exudates.* Bogor: PROSEA.

Delaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation. Version II. *Plant Molecular Biology Reporter* 4: 19-21.

Dunn, G. and B.S. Everitt. 1982. An *Introduction to Mathematical Taxonomy*. Cambridge: Cambridge University Press.

Fernandez, R.M., A.S. Valenzuela, and L.C. Balocchi. 2006. RAPD and freezing resistance in **Eucalyptus globulus**. *Electronic Journal of Biotechnology* 9 (3). http://www.scielo.cl/scielo.php?script-sci_arttext&pid

Jones, C.J., K.J. Edwards, S. Castagiole, M.O. Winfield, F. Sala, C. Van del Wiel, G. Bredemeijer, B. Vosman, M. Matthes, A. Daly, R. Brettsshneider, P. Bettini, M. Buiatti, E. Maestri, A. Malcevschi, N. Marmiroli, R. Aert, G. Volckaert, J. Rueda, R. Linacero, A. Vasquez, and A. Karp. 1997. A Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding* 3 (5): 382-390.

Mori, E.S., P.Y. Kageyama, R.F. de A Veiga, L. Zimback, and J.R.S. Mello Junior. 2004. Genetic structure of **Trichilia pallida** Swartz (Meliaceae) populations by RAPD markers. *Scientia Forestalis* 65: 114-119.

Norwati, M. 2002. Jelutong. *In*: Krishnapillaly, B. (ed). *A Manual for Forest Plantation Establishment in Malaysia. Malayan Forest Record* No. 45: 165-171.

Parjanto, S. Moeljopawiro, W.T. Artama dan A Purwantoro. 2006. Indentifikasi penanda RAPD untuk penentuan jenis kelamin salak (**Salacca zalacca** Gart. Voss). *Berkala Ilmiah Biologi* 5 (1): 57-63.

Pither R, J.S. Shore and M. Kellman. 2003. Genetic diversity of the tropical tree **Terminalia amazonia** (Combretaceae) in naturally fragmented populations. *Heredity* 91 (3): 3017-313.

Poerba, Y.S. 2003. Penampilan karakter agronomi dan analisis Random Amplified Polymorphic DNA (RAPD) genotipe mutan **Sonchus arvensis** L. *Buku Kumpulan Abstrak Seminar Nasional X Persada.* Persada Cabang Bogor dan Badan Pengurus Pusat Persada. Jakarta 4 Juli 2003.

Poerba, Y.S. 2005. *Studi Keragaman Genetik* **Alstonia scholaris** *(L.) berdasarkan Marka Random Amplified Polymorphic DNA.* [Laporan Teknik]. Bogor: Pusat Penelitian Biologi LIPI.

Rath, P., G. Rajaseger, C.J. Goh, and P. Kumar. 1998. Phylogenetic analysis of Dipterocarps using Random Amplified Polymorphic DNA markers. *Annals of Botany* 82: 61-65.

Rohlf, F.J. 1993. *NTSYS-pc Numerical Taxonomy and Multivariate Analysis.* Version 1.80. Applied Biostatistics Inc.

Runo MS, G.M. Muluvi, and D.W. Odee. 2004. Analysis of genetic structure in **Melia volkensii** (Gurke.) populations using random amplified polymorphic DNA. *African Journal of Biotechnology* 3 (8): 421-425.

Sarkhosh A., Z. Zamani, R. Fatahi and A. Ebadi. 2006. RAPD markers reveal polymorphism some Iranian pomegranate (**Punica granatum** L.). *Scientia Horticulturae* 111 (1): 24-29.

Shashidhara, G., M.V. Hema, B. Koshy, and A.A. Farooqi. 2003. Assessment of genetic diversity and identification of core collection in sandalwood germplasm using RAPDs. *Journal of Horticultural Science & Biotechnology* 78 (4): 528-536.

Singh, D.R.P., R. Singh, K. Malik, and G.J. Randhawa. 2005. Assessment of genetic diversity and genetic relationship among 29 populations of **Azadirachta indica** A. Juss. using RAPD marker. *Genetic Resources and Crop Evolution* 52 (3): 285-292.

Siregar, U.J., E. Sudarmonowati, and N.S. Hartati. 1998. Development of RAPD protocol for **Shorea laevis***. Annales Bogorienses* 5 (2): 85-92.

Telles, M.P.C., A.S.G. Coelho, L.J. Chaves, J.A.F. Diniz-Filho, and F. D'Ayala Valva. 2003. Genetic diversity and population structure of **Eugenia dysenterica** DC. (cagaiteira - Myrtaceae) in Central Brazil: spatial analysis and implications for conservation and management. *Conservation Genetics* 4: 685-695.

Tingey, S.V, J.A. Rafalski, and M.K. Hanafey. 1994. Genetic analysis with RAPD markers. *In:* Coruzzi, C. and P. Puidormenech (eds.). *Plant*. *Molecular Biology*. Belin: Springer-Verlag

Widjaja, E.A. dan Y.S. Poerba. 2004. Pengumpulan data plasma nutfah dan genetika. *Dalam*: Rugayah, E.A. Widjaya dan Praptiwi (ed.). *Pedoman Pengumpulan Data Keanekaragaman Flora.* Bogor: Pusat Penelitian Biologi LIPI.

Williams, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalsky, and S.V. Tingev. 1990. DNA plolymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* 18 (22): 6531-6535.