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IDENTIFICATION OF DNA POLYMORPHISM AMONG CLONES OF *HEVEA BRASILIENSIS* MUELL. ARG. USING RAPD ANALYSIS

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RAPD (Random amplified polymorphic DNAs) were used to study the DNA polymorphism in 37 *Hevea* clones representing variability for several morphological, physiological and other characters. Eighty random sequences 10-mer primers were used of which nine produced clear and scorable bands while eight produced polymorphic amplification products between 300 to 4000 base pairs in size, sufficient to distinguish between the clones. Of bands from the 8 primers 51.5% were polymorphic in the *Hevea* clones studied. A dendrogram developed using Jaccard's coefficients indicated genetic relationships among these clones. Most of the primary clones were clustered together in the dendogram. UPGMA cluster analysis indicated that some of the clones are genetically close although they have been developed from different breeding programmes. The presence of polymorphic bands in the genomic DNA was further confirmed by Southern blot analysis. Detection of DNA polymorphism in the *Hevea* clones opens up the possibility of development of molecular map. This molecular approach will be useful for developing marker-assisted selection tools for genetic improvement of *Hevea*.

Key words: DNA polymorphism, *Hevea brasiliensis*, RAPD, Random oligonucleotide.

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INTRODUCTION

Crop improvement has been achieved in rubber tree (*Hevea brasiliensis*) by incorporating new sources of genetic variability for high rubber production and disease resistance (Costa *et al.*, 2000). If selection is only for certain desirable traits, it may lead to inbreeding depression in an advanced generation. Therefore, genetic variability in a breeding population needs to be preserved to sustain long-term breeding programmes. However, information on genetic relationships among clones is rather limited. The perennial nature, the long breeding and selection cycle and difficulties in raising F_2 population make conventional genetic analysis in *Hevea* difficult (Varghese *et al.*, 1997; Lespinasse *et al.*, 2000).

The DNA based marker procedures lead to a greater understanding of genetic relationships among clones or cultivars. These techniques are used by the breeders to identify genetic variability among the species and clones / cultivars by means other than morphological characteristics (Graham and McNicol, 1995). Morphological traits do not provide good estimates of genetic distance because they are influenced by the environment and are not variable enough to adequately characterize genetic differences among elite genotypes. Biochemical methods, such as isozyme analysis, have been used to determine the degree of variability within plant population. Isozyme analysis is limited by the small number of marker loci available, a general lack of polymorphism for these loci in elite breeding materials and variability in the banding patterns due to the stage of plant development (Bai *et al.*, 1998). Isozyme loci

are in limited number because they are restricted to genes encoding soluble proteins. In contrast DNA markers allow direct access to the coding and non-coding regions of the genome, making their number potentially unlimited (Roy *et al.*, 1992).

DNA markers are considered to be superior in the study of genetic relationships between clones/cultivars because of the availability of a large number of potential polymorphic sequences and the fact that they do not depend on environment and development. These markers include restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD). The techniques differ in their underlying principle and generate varying amounts of information (Das *et al.*, 1999). RFLP approach has been used successfully to identify genetic markers and to construct genetic linkage maps in *Hevea*. However, the RFLP technique requires specific gene probes for the target DNA sequences and is labour intensive, PCR based RAPD gained importance due to its simplicity, efficiency, relative ease of performance and non-requirement of prior information on DNA sequence.

Although molecular tools such as DNA markers are becoming increasingly important as effective tools in crop breeding programmes, their application in rubber tree improvement is lagging behind because of the limited knowledge of the genome. The genetics of rubber tree has been poorly investigated (Lespinasse *et al.*, 2000). The long juvenile period of around 7 years would make RAPD markers an extremely useful tool for early identification of potentially useful cultivars (Varghese *et al.*, 1997). A variety of molecular techniques have been used to study the extent of the genetic variation between different wild and cultivated *Hevea* clones. Among the different techniques, isozymes and RFLPs were used for the assessment of genetic variability between wild and cultivated populations (Chevellier, 1988; Basse *et al.*, 1994). RFLP was used to estimate phylogenetic-relationships from mitochondrial DNA (Luo *et al.*, 1995) and to assess the genetic variability from ribosomal DNA (Besse *et al.*, 1993b). Powdery mildew resistance genes were identified by the RAPD analysis (Shoucai *et al.* 1994). Varghese *et al.* (1997) also reported that DNA polymorphism could be detected within *Hevea* clones. However, they indicated that a greater number of *Hevea* clones need to be anslysed before drawing a definite conclusion. Recently, Lespinasse *et al.* (2000) established the first genetic map of *Hevea brasiliensis* using RFLP, AFLP, microsatellite and isozyme markers. In this report, the results of DNA polymorphism in 37 cultivated *Hevea* clones using RAPD analysis with 80 random oligonucleotide primers are discussed. The observed polymorphism may be useful for developing molecular markers for screening various traits in the crop improvement programmes of *Hevea*.

MATERIALS AND METHODS

Plant material

The *Hevea* clones used in this study and their origin are given in Table 1. The study involved a selection of 37 clones, originating from 6 countries. Fully expanded and disease free leaves were collected from plants growing in the nursery as well as experimental farm of Rubber Research Institute of India (RRII), Kottayam.

Preparation of genomic DNA

Genomic DNA from young leaves of selected clones were isolated and purified following the modified CTAB extraction procedure (Doyle and Doyle, 1990). One gram of fresh leaf tissue was ground to a find powder in liquid nitrogen with a mortar and pestle and homogenized in DNA

Table 1. **Pedigree details of *Hevea* clones**

Clone	Parentage
India	
RRII 105	Tjir 1 x Gl 1
RRII 201	Tjir 1 x PB 25
RRII 202	PB 86 x Mil 3/2
RRII 203	PB 86 x Mil 3/2
RRII 204	PB 86 x Mil 3/2
RRII 205	PB 86 x BD 10
RRII 206	Mil 3/2 x AVROS 255
RRII 207	Mil 3/2 x AVROS 255
RRII 208	Mil 3/2 x AVROS 255
RRII 209	Mil 3/2 x BD 10
Malaysia	
RRIM 600	Tjir 1 x PB 86
PB 217	PB 5/51 x PB 6/9
PB 235	PB 5/51 x PB S/78
PB 255	PB 5/51 x PB 32/36
PB 260	PB 5/51 x PB 49
PB 280	PBIG seedlings
PB 31	RRIM 600 x PB 5/51
PB 311	RRIM 600 x PB 235
PB 312	RRIM 600 x PB 235
PB 314	RRIM 600 x PB 235
PB 6/9	PB 24 x PB 28
Gl 1	Primary clone
PB 86	Primary clone
RRIM 501	PilA 44 x Lun N
PB 5/51	PB 86 x PB 24
PB 25	Primary clone
PB 5/63	PB 56 x PB 24
Indonesia	
PR 107	Primary clone
BD 10	Primary clone
AVROS 255	Primary clone
Tjir 1	Primary clone
Thailand	
KRS 25	Primary clone
KRS 128	RRIM 501 x PB 5/63
KRS 163	RRIM 501 x PB 5/65
China	
SCATC 93-114	TR 31-45 x Haiken 3-11
Haiken 1	Primary clone
Sri Lanka	
Mil 3/2	Primary clone

isolation buffer [2% CTAB (hexadecyltriethylammonium bromide), 1.4 M NaCl, 20 m EDTA (pH 8.0), 100 mM Tris-HCl (pH 8.0), 1% polyvinyl polypyrolidone (PVPP), 1% 2-mercaptoethanol]. The homogenate was incubated in a water bath at 65°C for 30 min with gentle mixing. The extracts were centrifuged for 15 min (12000xg) and the supernatant was treated with an equal volume of phenol : chloroform : isoamylalcohol (25:24:1) and spun at 10,000 rpm for 10 min. The aqueous phase was carefully removed to new tubes and incubated at 37°C for 1 h after the addition of 10 ml of RNase (10 mg/ml). The samples were extracted with chloroform and spun at 10,000 rpm for 5 min and re-extracted until a clear aqueous phase was obtained. The DNA was precipitated with an equal volume of isopropanol. After 15 min of centrifugation at 10,000 rpm, the DNA pellet was washed with 70% ethanol, air-dried and dissolved in about 300 ml of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0). DNA quality was analysed by 0.8% agarose gel electrophoresis and stored at –20°C until use for PCR amplification.

DNA amplification by PCR

PCR was carried out in a 20 ml reaction mixture containing 15 ng of template DNA, 250 nM of primer, 1.5 mM $MgCl_2$, 100 mM each of dATP, dGTAP and dTTP (Amersham-Pharmacia, UK), 0.5 unit of Taq DNA Polymerase enzyme and 1x reaction buffer. In order to avoid evaporation, the reaction mixture was overlaid with 25ml of mineral oil (Sigma, USA). Amplification was performed in 0.5 ml tubes placed in a 48-well thermal cycler (Perkin-Elmer, USA). Tubes containing all the reaction components except DNA template were included as control for each primer used. The PCR programme included a 4 min initial denaturation step at 94°C followed by 35 cycles each comprising 1 min denaturing at 94°C, 1.30 min at 38°C for annealing and 2.0 min at 72°C for extension. The last cycle was followed by 7 min at 72°C to ensure that primer extension reactions proceeded to completion. Eighty random 10 mer oligonucleotide primers *viz.*, OPA, OPB, OPC, OPD and OPE (Operon Technologies Inc.,

Alameda, CA, USA) were used as single primers for the amplification of genomic DNA. The primers which produced clear banding pattern after PCR amplification were selected for further RAPD analysis of 37 clones. In order to confirm that the amplified products are reproducible amplification with each primer was repeated at least thrice.

Gel electrophoresis

After PCR amplification, 6x loading buffer was added to the amplified products. The RAPD products were separated by electrophoresis in 1.5% agarose gels containing 0.5 mg/ml ethidium bromide in 0.5 X TBE buffer (Sambrook *et al.*, 1989). Electorphoresis was performed at50V for about 4 h until the bromophenol blue dye front had migrated to the bottom of the gel. The molecular standard used was the lambda DNA double digested with EcoRI/ Hind III. The gels were visualized under UV-light and photographed.

DNA blotting

Amplified RAPD products were separated by electrophoresis on 1.5% agarose gel in TBE buffer (0.045 M Tris-borate and 0.001 M EDTA) at 25 V for 8 h. Genomic DNA from *Hevea* clones were digested with Hind III restriction enzyme and separated by 1.2% agarose gel electrophoresis. After depurination in 0.25 M HCl for 10 min, denaturation of the DNA in the gels was carried out in a solution containing 1.5 M NaCl and 0.5 M NaOH for 30 min and then neutralized for 30 min in a solution of 1.5 M NaCl and 1.0 M Tris-HCl (pH 7.4). The DNA was then transferred onto a nylon membrane (Hybond N^+, Amersham-Pharmacia, UK) in 10X SSC buffer (1X SSC is 0.15 M NaCl, 0.015 M trisodium citrate) for 18 h (Sambrook *et al.*, 1989). After DNA transfer, the nylon membranes were rinsed in 2X SSC buffer, UV-crosslinked and stored at 4°C until use.

DNA probe preparation, labelling and hybridization

The selected polymorphic band was cut out from the low-melting agarose gel and DNA was eluted (Sambrook *et al.*, 1989). This was reextracted once with phenol : chloroform and the DNA pellet was dissolved in sterile double distilled water and used for labelling. Radioctive probes were synthesized with a-^{32}PdATP (BARC, Trombay, Mumbai, India, 4000 Ci/mmol) using the random primer labelling kit (Amersham-Pharmacia, UK). The nylon membranes with DNA were placed in hybridization bottles and prehybridized for 4 h (Hybridization buffer is 6X SSC, 5X Denhardt's, 0.5% SDS) at 65°C. After 4 h, the radio labelled DNA probe was added into the prehybridization buffer and hybridization was performed at 65°C for 20 h in a rotary hybridization oven (Amersham-Pharmacia, UK). After completion of hybridization, membranes were washed at low stringency at room temperature twice in 2X SSC + 0.1% SDS for 5 min and 1X SSC + 0.1% SDS for 15 min and at high stringency at 65°C, twice in 0.5X SSC + 0.1% SDS for 30 min and 0.1X SSC + 0.1% SDS for 30 min, followed by radio active signal generation. The labelled blots were then exposed to X-ray film (X-Omat, Kodak) with intensifying screens at –80°C.

RAPD data analysis

A conservative approach to score the amplified fragments was adopted and only consensus bands were included for the analysis. Individual amplified bands were indicated by the primer used and its size in bp. Data were scored on the basis of presence or absence of the amplified bands of a given length. If a band was present in a genotype it was designated as "1" and if absent, it was designated as "0". Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared

polymorphic bands were used to generate complementation of Jaccard's similarity coefficients. The matrix of dissimilarities was used to construct a dendrogram according to the UPGMA (unweighed pair-group method with arithmetical average) using the TREECON programme (Van de Peer and De Wachter, 1994).

RESULTS AND DISCUSSION

The 37 cultivated *Hevea* clones selected for the present study represent a wide spectrum of variation for their origin and for several phenotypic traits (Table 1). In the RAPD assay, 9 primers produced clear and readable bands, 43 primers showed poor amplification and the remaining failed to amplify or generated only smears. Primer OPC 11 produced monomorphic bands among the different clones and it was excluded since it was not informative. The remaining 8 primers were selected as informative primers which produced RAPD profiles in all the clones used. The nucleotide sequences of these primers are illustrated in Table 2. Easily detectable, well-resolved bands were those which were reproducible over repeated runs with sufficient intensities to determine their presence or absence.

Molecular markers using RAPD assay have been used in constructing genetic linkage maps and identification of markers associated with agronomically important traits, which is very essential for marker-assisted selection in crop improvement programmes. The most commonly used molecular tools for germplasm identification and genetic relationship studies are isozymes, seed storage proteins and RFLPs. The use of isozyme and protein markers have been limited by the number of loci that can easily be detected (Skroch *et al.*, 1992). Both isozymes and RFLPs are limited by the low level of polymorphism among cultivars in various crop species (Zhang *et al.*, 1996). On the other hand, the RAPD technique is more efficient and less expensive. The application of RAPD does not need any prior knowledge of genomic nucleotide sequences (Williams *et al.*, 1990).

Table 2. **Nucleotide sequence of RAPD primers and number of application products in *Hevea* clones**

Primer code	Primer sequence (5′ – 3′)	Number of bands	
		Total	Polymorphic
OPA-01	CAGGCCCTTC	16	7
OPA-04	AATCGGGCTG	21	7
OPA-07	GAAACGGGTG	19	9
OPA-16	AGCCAGCGAA	13	8
OPA-A7	GACCGCTTGT	15	11
OPA-18	AGGTGACCGT	16	7
OPA-12	CCTTGACGCA	14	9
OPC-05	GATGACCGCC	18	10
	Total	132	68

The agarose gels showing polymorphism with three primers OPC-05, OPA-04 and OPA-17 are illustrated in Figures 1, 2 and 3, respectively. Primers varied greatly in their ability to resolve variability among the clones. Some primers (*eg.*, OPA-17 and OPC-05) generated several markers and were able to show high genetic diversity while others (*eg.*, OPA-04) generated fewer markers and showed less variability. Out of the total 132 bands generated in 37 clones using 8 primers, 68 bands were polymorphic. The total number of bands produced per primer varied from 5 to 21. The size of bands ranged from 300 to 4000 base pairs. Dissimilarity between clones varied from 15 to 69% indicating a very high degree of genetic diversity (Fig. 3). This molecular information concurs with the reported high morphological variability in *Hevea*. The clones are classified into seven major groups based on DNA markers. The phenogram showed that the popular clones RRII 105 (India) and RRIM 600 (Malaysia) were clustered together, since one of the parents is common for both the clones. They are also closely related to the PB clones (Malaysia) and Chinese clones. It is interesting to note

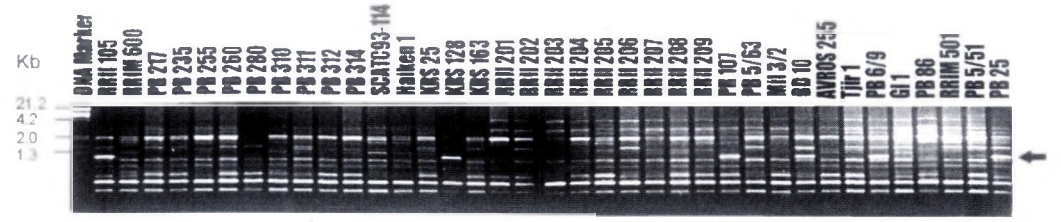

Fig. 1. RAPD profiles generated by OPC-05 primer for the 37 cultivated *Hevea* clones showing DNA polymorphism (indicated by arrow)

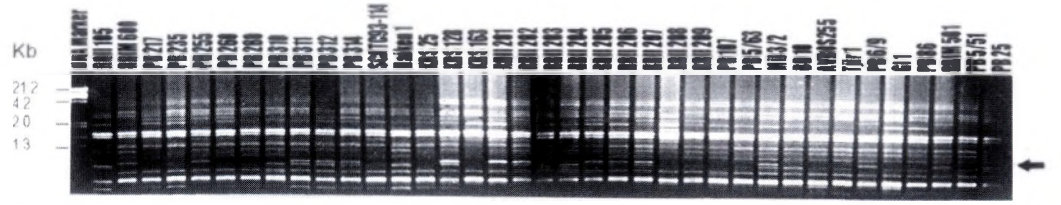

Fig. 2. PCR products from 37 cultivated *Hevea* clones generated by RAPD analysis using OPA-04 primer showing DNA polymorphism (indicated by arrow)

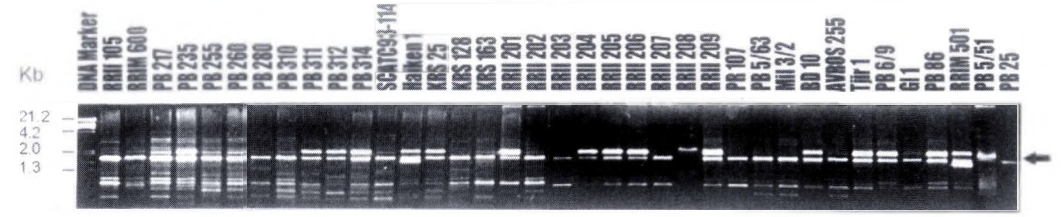

Fig. 3. Genomic DNA from 37 cultivated *Hevea* clones amplified with RAPD primer OPA-17 showing DNA polymorphism (indicated by arrow)

that most of the Malaysian clones have been grouped into two clusters. Three clones from Thailand have fallen into the same cluster. The two clusters formed by the Indian clones were distinct with one group (RRII 202, RRII 204, RRII 205, RRII 207 and RRII 209) clustering with one Indonesian clone, PR 107 and the other three clones *viz.*, RRII 201, RRII 203 and RRII 206 forming a distinct group.

RAPD analysis clearly distinguished all the 37 *Hevea* clones from one another. The clones RRIM 501 and PB 25 were grouped together and were separated from the remaining clones with 0.425% dissimilarity. As expected of clones belonging to same series, the clones PB 312 and PB 314 were grouped together with a maximum dissimilarity of 0.152%. In the present study, 69% of the RAPD were polymorphic. This seems to be relatively high when compared to earlier reports of RAPD studies in *Hevea* (Varghese *et al.*, 1997). Table 3 shows the distance matrix developed on the basis of RAPD data. The clone RRII 203 was separated from the other clones of its cluster. The Indian clone RRII 203 is very dissimilar to the Malaysian clone PB 255 (0.692). The next most dissimilar clones are PB 25 and RRII 203 with a distance coefficient of 0.672. From the dendogram (Fig. 4), it is interesting to note that several primary clones developed in different countries such as Tjir 1, Gl 1, PB 86, Mil 3/2, AVROS 255 and BD 10 were

Table 3. **Genetic distance matrix of thirty seven *Hevea* clones based on RAPD**

	RRII 105	PB 312	PB 314	SCATC 93-114	Haiken 1	KRS 25	KRS 128	KRS 163	RRII 201	RRII 202	RRII 203	RRIM 600	RRII 204	RRII 205	RRII 206	RRII 207	RRII 208	RRII 209	PR 107	PB 5/63	Mil 3/2	BD 10	PB 217	AVROS 255	TJIR 1	PB 6/9	GI 1	PB 86	RRIM 501	PB 5/51	PB 25	PB 235	PB 255	PB 260	PB 280	PB 310	PB 311
RRII 105	0																																			
PB 312	0.490	0.000																																		
PB 314	0.458	0.152	0.000																																	
SCATC 93-114	0.571	0.345	0.275	0.000																																
Haiken 1	0.489	0.333	0.292	0.275	0.000																															
KRS 25	0.477	0.408	0.437	0.500	0.469	0.000																														
KRS 128	0.375	0.416	0.413	0.509	0.377	0.307	0.000																													
KRS 163	0.596	0.444	0.527	0.525	0.471	0.355	0.468	0.000																												
RRII 201	0.511	0.500	0.437	0.578	0.557	0.488	0.465	0.576	0.000																											
RRII 202	0.460	0.456	0.454	0.532	0.534	0.555	0.416	0.551	0.375	0.000																										
RRII 203	0.595	0.649	0.629	0.666	0.654	0.632	0.617	0.648	0.404	0.490	0.000																									
RRIM 600	0.243	0.416	0.377	0.509	0.413	0.428	0.400	0.530	0.500	0.509	0.586	0.000																								
RRII 204	0.547	0.456	0.425	0.508	0.534	0.500	0.448	0.500	0.375	0.339	0.547	0.538	0.000																							
RRII 205	0.607	0.465	0.436	0.491	0.491	0.537	0.519	0.508	0.450	0.351	0.555	0.547	0.288	0.000																						
RRII 206	0.442	0.555	0.500	0.578	0.529	0.522	0.500	0.603	0.341	0.440	0.441	0.533	0.408	0.450	0.000																					
RRII 207	0.627	0.516	0465	0.492	0.540	0.561	0.545	0.603	0.481	0.413	0.442	0.545	0.440	0.339	0.481	0.000																				
RRII 208	0.586	0.523	0.500	0.523	0.524	0.593	0.527	0.587	0.517	0.368	0.535	0.553	0.368	0.290	0.462	0.355	0.000																			
RRII 209	0.535	0.423	0.392	0.476	0.474	0.491	0.442	0.540	0.462	0.368	0.586	0.500	0.277	0.290	0.462	0.383	0.310	0.000																		
PR 107	0.578	0.584	0.587	0.582	0.564	0.610	0.518	0.580	0.535	0.440	0.527	0.571	0.357	0.368	0.481	0.426	0.327	0.298	0.000																	
PB 5/63	0.533	0.476	0.451	0.500	0.500	0.564	0.550	0.538	0.609	0.428	0.603	0.500	0.500	0.387	0.540	0.462	0.295	0.375	0.462	0.000																
Mil 3/2	0.500	0.466	0.410	0.416	0.410	0.535	0.431	0.533	0.561	0.413	0.578	0.461	0.440	0.396	0.509	0.372	0.355	0.355	0.372	0.311	0.000															
BD 10	0.509	0.423	0.363	0.451	0.474	0.491	0.472	0.540	0.490	0.423	0.677	0.500	0.450	0.433	0.543	0.507	0.419	0.366	0.507	0.375	0.355	0.000														
PB 217	0.363	0.403	0.431	0.491	0.461	0.479	0.489	0.509	0.568	0.517	0.641	0.309	0.543	0.551	0.568	0.550	0.580	0.508	0.596	0.531	0.473	0.456	0.000													
AVROS 255	0.543	0.433	0.431	0.460	0.457	0.444	0.423	0.524	0.500	0.406	0.639	0.509	0.433	0.362	0.500	0.468	0.293	0.263	0.419	0.333	0.310	0.232	0.491	0.000												
Tjir 1	0.462	0.433	0.431	0.484	0.483	0.576	0.481	0.548	0.500	0.321	0.599	0.481	0.406	0.416	0.500	0.515	0.350	0.350	0.444	0.333	0.338	0.350	0.465	0.275	0.000											
PB 6/9	0.545	0.483	0.508	0.531	0.533	0.603	0.537	0.525	0.553	0.431	0.620	0.537	0.457	0.466	0.527	0.538	0.426	0.426	0.467	0.327	0.389	0.426	0.517	0.383	0.267	0.000										
GI 1	0.545	0.457	0.456	0.531	0.508	0.553	0.509	0.500	0.500	0.403	0.571	0.509	0.457	0.440	0.471	0.442	0.344	0.400	0.442	0.380	0.362	0.372	0.517	0.355	0.327	0.321	0.000									
PB 86	0.596	0.431	0.482	0.553	0.508	0.527	0.509	0.446	0.527	0.431	0.620	0.509	0.375	0.357	0.578	0.492	0.400	0.372	0.416	0.454	0.389	0.400	0.491	0.383	0.327	0.379	0.226	0.000								
RRIM 501	0.549	0.508	0.535	0.580	0.535	0.557	0.540	0.527	0.584	0.534	0.654	0.568	0.534	0.436	0.500	0.516	0.474	0.448	0.540	0.523	0.491	0.524	0.596	0.457	0.431	0.508	0.400	0.339	0.000							
PB 5/51	0.509	0.450	0.500	0.567	0.500	0.543	0.471	0.465	0.568	0.450	0.633	0.527	0.423	0.433	0.517	0.507	0.419	0.419	0.507	0.400	0.383	0.444	0.533	0.428	0.377	0.315	0.344	0.372	0.363	0.000						
PB 25	0.600	0.482	0.508	0.532	0.508	0.528	0.509	0.500	0.631	0.533	0.672	0.566	0.533	0.540	0.607	0.539	0.523	0.500	0.606	0.476	0.440	0.475	0.593	0.507	0.483	0.457	0.375	0.375	0.425	0.396	0.000					
PB 235	0.400	0.403	0.431	0.542	0.431	0.446	0.456	0.509	0.596	0.543	0.641	0.386	0.568	0.600	0.596	0.573	0.580	0.508	0.640	0.507	0.473	0.508	0.304	0.491	0.491	0.542	0.542	0.542	0.596	0.508	0.568	0.000				
PB 255	0.454	0.450	0.448	0.535	0.416	0.532	0.511	0.557	0.647	0.563	0.692	0.365	0.614	0.571	0.647	0.593	0.622	0.551	0.661	0.524	0.490	0.526	0.355	0.508	0.508	0.586	0.586	0.561	0.642	0.576	0.614	0.318	0.000			
PB 260	0.479	0.415	0.442	0.473	0.380	0.520	0.500	0.461	0.603	0.551	0.622	0.400	0.576	0.559	0.603	0.557	0.587	0.540	0.603	0.515	0.456	0.465	0.204	0.500	0.474	0.473	0.525	0.525	0.627	0.516	0.551	0.319	0.404	0.000		
PB 280	0.456	0.423	0.420	0.508	0.420	0.468	0.478	0.470	0.560	0.535	0.660	0.333	0.561	0.568	0.588	0.542	0.596	0.525	0.612	0.546	0.491	0.473	0.209	0.508	0.508	0.508	0.508	0.508	0.637	0.525	0.561	0.326	0.302	0.186	0.000	
PB 310	0.395	0.333	0.326	0.340	0.288	0.478	0.418	0.509	0.541	0.490	0.531	0.300	0.518	0.527	0.478	0.444	0.508	0.508	0.576	0.508	0.384	0.508	0.333	0.491	0.491	0.543	0.462	0.490	0.547	0.508	0.518	0.404	0.422	0.311	0.355	0.000
PB 311	0.434	0.306	0.297	0.436	0.333	0.446	0.456	0.450	0.540	0.490	0.641	0.309	0.490	0.472	0.568	0.525	0.508	0.400	0.573	0.459	0.446	0.456	0.340	0.465	0.381	0.464	0.464	0.464	0.545	0.456	0.517	0.340	0.318	0.387	0.326	0.404

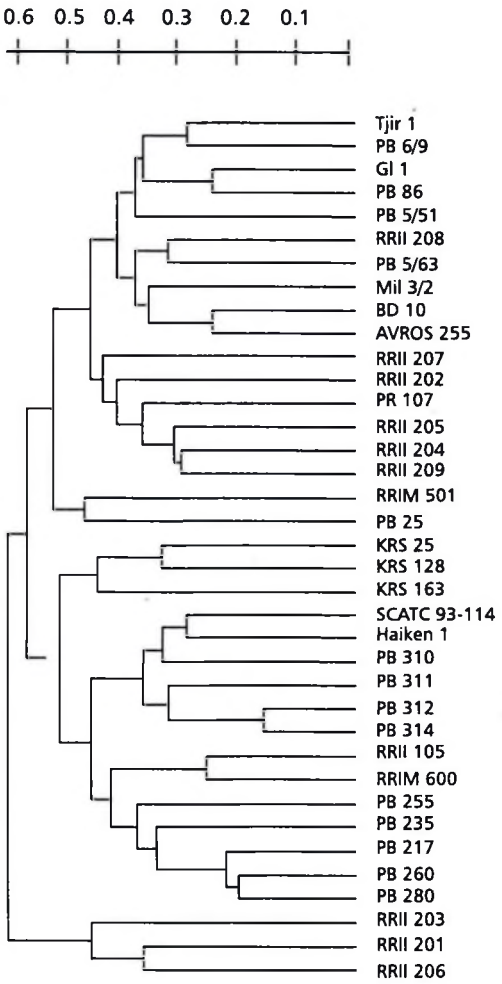

Fig. 4. The dendrogram generated by UPGMA analysis. The bar on the top represents dissimilarity index based on Jaccard's coefficients.

closely clustered. In most cases, clones with a common pedigree, such as RRII 105, RRIM 600, PB 311, PB 312, PB 314, KRS 128, KRS 163, PB 217, PB 255, PB 260, RRII 204, RRII 209 were observed to cluster together. Similar results were reported earlier in *Hevea* (Varghese *et al.*, 1997). Low *et al.* (1996) observed that *Hevea* cultivars which share two common parents such as, PR 255 and PR 261, RRIM 901 and RRIM 905 and RRIM 937 and RRIM 938, were distinguished by their DNA polymorphism.

The DNA polymorphism observed in the study could be attributed to the selection of clones with diverse characteristics including geographic origin as well as the specificity of primers used in the RAPD analysis. These genotypes will be useful for developing new hybrid lines as well as mapping populations for future breeding programmes. However, associating polymorphic DNA fragments with different traits needs further study using the selected trait-specific clones and their progenies. This study indicated the use of the RAPD technique to detect genetic variation at the level of DNA among cultivated rubber clones.

Figure 5 demonstrates that the labelled product from PCR amplification using the primer OPA-17 was observed to hybridize to the corresponding amplified bands from the clones namely PB 235, PB 311, PB 312, PB 314, Haiken 1, KRS 25, RRII 201, RRII

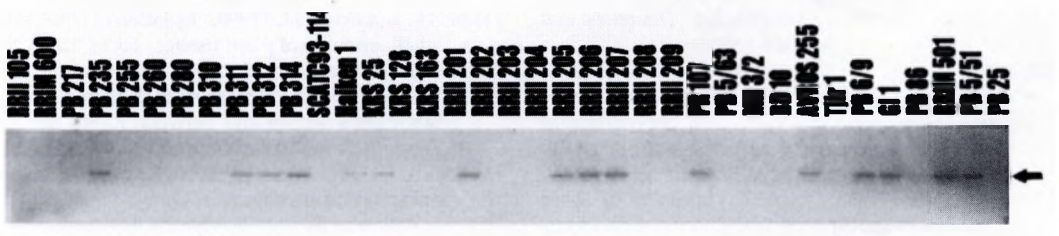

Fig. 5. Hybridization observed when the polymorphic band (Fig. 3) was used to probe a Southern blot of amplified DNA fragments.

205, RRII 205, RRII 206, RRII 209, BD 10, Tjir 1, PB 6/9, PB 86 and RRIM 501 but not with other clones. In addition, genomic DNA was digested with Hind III restriction enzyme and the selected polymorphic band was used as probe for hybridization. The Southern blot results confirmed that the amplified polymorphic band is a part of genomic DNA (Fig. 6).

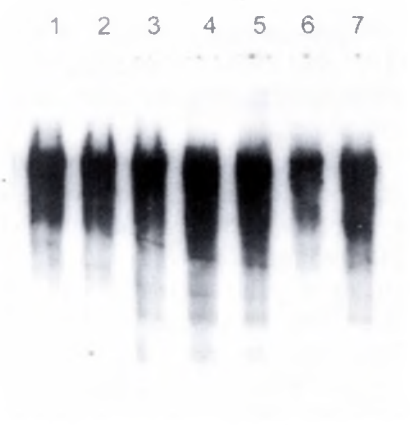

Fig. 6. Genomic Southern blot hybridization analysis of *Hevea* clones. The radiolabelled probe used was a selected polymorphic RAPD band (2.0 kb) identified with OPA-17 primer. Lanes 1-7 genomic DNA samples from different *Hevea* clones digested with Hind III restriction enzyme.

To conclude, this study indicated the presence of DNA polymorphism within the cultivated *Hevea* clones using RAPD analysis. Among the different clones tested, RRII 203 displayed the maximum and highest average distance from others followed by PB 255, PB 25, RRIM 501, PB 280 (Table 3). This result suggests that these clones could be used as potential parents in future breeding programmes. This opens up the possibility for developing a molecular genetic map that will lead to the application of marker-assisted selection tools for genetic improvement of *Hevea*.

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