Plant Breeding 116, 47—52 (1997)

ISSN 0179-9541

2260

Evaluation of random amplified polymorphic DNA (RAPD) markers in *Hevea brasiliensis*

Y. A. VARGHESE[1], C. KNAAK[2], M. R. SETHURAJ[1] and W. ECKE[2,3]

[1]Rubber Research Institute of India, Kottayam 686009, Kerala, India; [2]Institute of Agronomy and Plant Breeding, University of Göttingen, Von-Siebold-Strasse 8, D-37075 Göttingen, Germany; [3]Corresponding author

With 2 figures and 2 tables

Received August 15, 1996/Accepted October 11, 1996
Communicated by T. Börner

Abstract

The applicability of random amplified polymorphic DNA (RAPD) markers in the cultivated rubber tree, *Hevea*, was evaluated using 43 decamer oligonucleotide primers in a set of 24 clones selected in different South-East Asian countries. A total of 220 0.35–3.5 kb DNA fragments were amplified, of which 111 were polymorphic. Of these, 80 fragments (RAPD markers) which were repeatable and clearly scorable across all genotypes were used to estimate genetic distances among the clones tested. The estimated genetic distances ranged from 0.05 (RRII 308 and PB 5/51) to 0.75 (RRIC 100 and SCATC 88–13). A mean genetic distance of 0.5 indicates a rather high genetic variability among the tested clones. As expected, because of the breeding history of *Hevea*, UPGMA cluster analysis and Principal Coordinate Analysis (PCoA) indicated the absence of a distinct geographical grouping. The possible application of RAPD markers for clone identification and also for analysis of genetic relationships among *Hevea* clones is discussed.

Key words: *Hevea brasiliensis* — cultivar identification — genetic distance — genetic diversity — RAPD markers.

The genetic base of the cultivated rubber tree, *Hevea brasiliensis*, is assumed to be narrow; at least it is limited to a few surviving trees of the original collection from the Amazonian rain forests in Brazil by Sir Henry Wickham in 1876 . It is from this small foundation, referred to as the 'Wickham gene pool', that a spectacular yield improvement of about 10 times has been achieved. However, the genetic advance gained in the early years appears to have slowed down in some of the more recent phases, possibly due to the narrow genetic base, the wide use of clonal propagation systems, directional selection for yield and a cyclical generation-wise assortative mating (Varghese 1992). In addition, there has been frequent bilateral exchange of high-yielding clones between different countries, which were then used as parents in biparental crosses, thereby further reducing the genetic base in *Hevea* breeding. A pedigree of the present-day popular clones bred in Malaysia, India and other countries reveals that most of these can be traced back to a handful of parent clones. On the other hand, since *Hevea* is a predominantly open-pollinated tree species, a certain amount of genetic variability is expected among clones.

An understanding of the extent of genetic diversity among different breeding materials is essential for the selection of clones in recombination and polycross breeding programmes and also for the choice of component clones of clonal composites for multiclone planting recommendations. 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 progenies make conventional genetic analysis in *Hevea* difficult. Molecular markers such as restriction fragment length polymorphism (RFLP) (Botstein et al. 1980) or random amplified polymorphic DNA (RAPD) (Williams et al. 1990) offer attractive alternatives to conventional methods. The advantage of RAPD over RFLP includes its relative simplicity, rapidity and the requirement of only very small amounts of genomic DNA, making the method suitable for routine genetic studies that have to be carried out in breeders' laboratories. The use of RAPD to determine genetic relationships has been demonstrated in maize (Welsh et al. 1991), conifers (Carlson et al. 1991), Indian mustard (Jain et al. 1994), faba bean (Link et al. 1995), rapeseed (Förster and Knaak 1995) and in many other crop species.

Reports on application of molecular markers in *Hevea* are few. They include assessment of genetic variability in Wickham and wild material using isozymes (Chevellier 1988) and RFLPs (Besse et al. 1994), the estimation of phylogenetic relationships from mitochondrial DNA RFLPs (Luo et al. 1995) and the identification of mildew resistance genes by the RAPD technique (Shoucai et al. 1994). Reports on the analysis of genetic relationships in *Hevea* using RAPD markers are not known. The long juvenility period of around 7 years would make RAPD markers an extremely useful tool for identification of cultivars during propagation and planting. The objective of the present investigation was to examine whether the use of RAPD markers is feasible for clone identification in *Hevea*, and to evaluate the possibility of quantifying the extent of genetic variability between different clones.

Materials and Methods

Plant materials: A total of 24 vegetatively propagated clones bred in India, Malaysia, Indonesia, Sri Lanka, Thailand and China were used in the present investigation (Table 1). These include both primary clones resulting from natural hybridizations selected from seedling progenies by means of plus tree or mother tree selection and also hybrid clones from biparental crosses between heterozygous parents.

Extraction of DNA: Total genomic DNA was extracted from detached mature fresh leaves and from lyophilized samples according to a slightly modified procedure described by Rogers and Bendich (1988): 50–100 mg of leaf tissue frozen in liquid nitrogen was ground to a fine powder and mixed with five volumes of warm (65°C) 2 × CTAB extraction buffer (2% CTAB, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl) and 7 µl of proteinase K (10 mg/ml). After incubation at 65°C for 30 min the solution was extracted once with 1 volume of chloroform–isoamyl

Table 1: Plant materials used in the present analysis

No.	Clone	Parentage	Country of origin
1	RRII 5	Primary clone	India
2	RRII 105	Tjir 1 × Gl 1	India
3	RRII 208	Mil 3 2 × Av 255	India
4	RRII 308	PB 5 60 × Gl 1	India
5	RRIM 600	Tjir 1 × PB 86	Malaysia
6	PB 5/51	PB 86 × PB 24	Malaysia
7	PB 28/59	Primary clone	Malaysia
8	PB 86	Primary clone	Malaysia
9	PB 217	PB 5 51 × PB 6 9	Malaysia
10	PB 235	PB 5 51 × PB S.78	Malaysia
11	PB 260	PB 5 51 × PB 49	Malaysia
12	PB 280	Primary clone	Malaysia
13	PB 312	RRIM 600 × PB 235	Malaysia
14	PCK 1	Tjir 1 × PR 107	India
15	Tjir 1	Primary clone	Indonesia
16	Gl 1	Primary clone	Malaysia
17	GT 1	Primary clone	Indonesia
18	SC ATC 88-13	RRIM 600 × Pil B 84	China
19	KRS 25	Primary clone	Thailand
20	HP 82/14	RRII 105 × RRII 100	India
21	HP 86/120	RRII 105 × RRII 118	India
22	RRIC 100	RRIC 52 × PB 83	Sri Lanka
23	HP 86/602	RRII 203 × PB5/51	India
24	HP 86/68	PB 5/51 × RRII 208	India

alcohol (24:1). Phases were separated by centrifugation at 11 000 × *g* for 10 min at 4° C and the aqueous upper phase was recovered. The DNA was precipitated by addition of 0.8 volume of cold isopropyl alcohol, followed by centrifugation at 11 000 × *g* for 8 min at 20° C. The DNA pellet was washed with 1 ml 70% alcohol, again centrifuged at 13 500 × *g* for 8 min at 20° C, and the final pellet dissolved in 50–100 µl of TE buffer (1 mM Tris HCl and 0.1 mM EDTA, pH 8). DNA concentrations were determined using a DNA fluorometer (Hoefer, Scientific Instruments, San Francisco, California) and the DNA was diluted to a final concentration of 10 ng/µl.

RAPD assay: Decamer oligonucleotide primers from Kits A, AL, AK, AM, AN, Q, R, S and T (Operon Technology, Alameda, California) were used for PCR amplification of DNA following a protocol from Quiros et al. (1991) with minor modifications. Each reaction mixture of 25 µl comprised 0.1 mM dNTPs, 1.9 mM $MgCl_2$, 1 × buffer, 0.2 µM primer, 1.4 U *Taq* DNA polymerase from AGS (Angewandte Gentechnologie Systeme GmbH, Heidelberg, Germany) and 50 ng of genomic DNA. Amplifications were performed in a Perkin Elmer Cetus Thermal Cycler TC480 with an initial cycle of 30 s at 94° C, 45 cycles of 1 min at 92° C, 1 min at 35° C and 2 min at 72° C and a final cycle of 5 min at 72° C. After amplification, the reaction products were separated by electrophoresis at 90 V for 5 h in 1.8% agarose gels in 1x TAE buffer with 0.1 µg/ml ethidium bromide. A 1 kb DNA ladder was used as molecular weight standard. The gels were photographed under ultraviolet light on a transilluminator using a video camera.

Statistical analysis: Data were scored as discrete variables using 1 to indicate the presence and 0 the absence of a band. Only bands reproducible in repeated amplifications were considered for estimation of genetic distances. Genetic distance was estimated as a complement of Jaccard's (1908) coefficient of similarity according to the following equation:

$$GD_{ij} = 1 - (N_{ij})/(N_{ij} + N_i + N_j)$$

where:

N_i = Number of fragments found in clone i but not in clone j
N_j = Number of fragments found in clone j but not in clone i
N_{ij} = Number of fragments found in clones i and j

Thus, GD_{ij} reflects the proportion of DNA fragments (RAPD markers) distinguishing the two clones compared relative to the total number of fragments occurring in this pair. GD values may range from 0 to 1.

A pairwise distance matrix between genotypes was the basis for cluster analysis by the Unweighted Pair-Group Method using Arithmetic average (UPGMA) and for Principal Coordinate Analysis (PCoA). All computations were performed using the computer program SYNTAX IV (Podani 1990).

Results

Out of a total of 126 random primers screened for DNA amplification using six test clones of apparently different pedigree, 97 primers produced clearly distinguishable amplification products. The remaining primers did not produce any amplification products, or only faint bands that could not be reliably scored. From the 97 positive primers 57 (59%) showed polymorphisms between the six test clones, 40 primers (41%) produced only monomorphic fragments. Out of the 57 informative primers, 42 were tested further with 24 clones. These primers amplified a total of 220 fragments ranging from 0.35 kb to 3.5 kb, of which 111 were polymorphic. Examples of the amplification of RAPD markers in *Hevea* are shown in Fig. 1. The number of polymorphic DNA fragments per primer ranged from 1 to 5 with

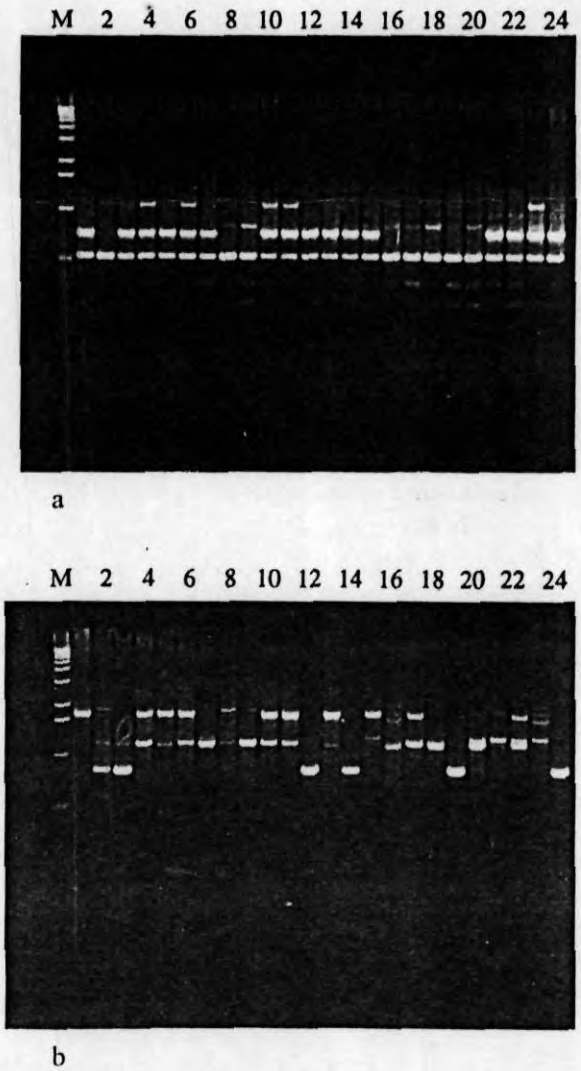

Fig. 1: RAPD patterns of 24 *Hevea* clones generated by primers OPAN12 (A) and OPAN16 (B). Numbers 1–24 refer to clone numbers as listed in Table 1. M indicates molecular size marker

an average of 2.6. Only 80 polymorphic bands which were clearly scorable across all 24 clones in repeated amplifications were considered for estimation of genetic distances. All *Hevea* clones tested could be distinguished by these 80 DNA polymorphisms.

Based on the 80 RAPD markers scored, genetic distances were estimated between the different clones. The results revealed a range of 0.05 (RRII 308 and PB 5/51) to 0.75 (RRIC 100 and SCATC 88–13) with an average genetic distance of 0.5 (Table 2).

A dendrogram constructed by UPGMA cluster analysis showed a grouping of the 24 clones into seven clusters, the number of clones in a cluster varying from one to 10 (Fig. 2). The clones are grouped irrespective of their country of origin, indicating the absence of any relationship between geographic diversity and genetic diversity. A principal coordinate (PCoA) analysis based on genetic distance values also gave similar results (data not shown).

Discussion

In *Hevea*, a perennial plantation crop species with an economic life span of around 30 years and an immaturity period of 7 years, identification of elite cultivars prior to field planting assumes much significance. Since morphological variations among *Hevea* clones are not very distinct, phenotypic identification based on morphological traits is difficult. RAPD markers have been used for cultivar identification in celery (Yang and Quiros 1993), broccoli and cauliflower (Hu and Quiros 1991), apple (Koller et al. 1993), grape vine (Moreno et al. 1995) and many other species. Considering the degree of polymorphism between the *Hevea* clones studied and the consistency of the RAPD markers in repeated amplifications, it can be concluded that the RAPD marker technique is feasible in *Hevea* also and represents an effective method for clone identification and for the analysis of genetic relationships among clones.

The observation that more than half of the RAPD primers that give discernible amplification products in *Hevea* show polymorphisms between the clones analysed and the high average genetic distance between these clones shows that a rather high degree of genetic variability does still exist within the cultivated rubber tree, despite the small foundation and the breeding history of this material. This variability should be useful for further genetic improvement in *Hevea*. Similar results have been reported by Markose (1984) and Mydin et al. (1992) using conventional genetic analysis, and by Chevallier (1988) using isozymes.

In clone breeding, parent clones should have a high degree of heterozygosity as well as optimum genetic distances. Measurement of genetic distances facilitates identification of diverse parents in order to maximize the expression of heterosis (Smith et al. 1990). Among the different clones tested, RRIC 100 displayed the maximum and highest average genetic distance from the others (Table 2), suggesting the use of this clone as a potential parent in biparental crosses. In HP 82/14, a progeny of RRIC 100 and RRII 105, a very high heterotic increase for latex yield over the first three years of tapping has been observed (Licy, pers. comm.). A large genetic distance of 0.61 between RRIC 100 and RRII 105, as revealed in the present study, could be one of the factors contributing to the high performance of the hybrid HP 82/14. Similarly, Tjir 1 and Gl1, the parents of a very high-yielding clone, RRII 105, are genetically distinct with a large genetic distance of 0.49. On the other hand, a desirable and high magnitude of heterosis is not always directly related to pronounced parental divergence (Arunachalam et al. 1984) and intermediate divergent classes might also have a high probability of producing heterotic hybrids (Thakur and Zarger 1989). Hence, for the improvement of a complex quantitative trait like rubber yield it is suggested that parents with desirable yield components be selected from clusters separated by medium to large genetic distances. Similar criteria should be applied for the selection of component clones of polyclonal seed gardens aimed at production of polycross progeny. This procedure would result in a better exploitation of heterosis in F_1 hybrids. Further investigations between parents and hybrids at the molecular level in *Hevea* should reveal useful information on the degree of heterozygosity and genetic distance between parents and its correlation with the performance of the progeny.

With a view to restrict a tendency towards monoculture, the Rubber Research Institute of India has recently introduced a multiclone concept for planting of potential clones in order to maintain genetic heterogeneity in the plantations (Varghese et al. 1996). The clones for commercial planting as clonal composites should be the first for which genetic distances are studied.

The geographic origin of the clones investigated in the present analysis did not reveal any correlation with their genetic diversity (Fig. 2), as was also observed by Markose (1984) and Mydin et al. (1992) after conventional analysis. This is in agreement with the breeding history of *Hevea*, since all clones bred in different South-East Asian countries originate from the same source — the Wickham base collected from a minute part of the genetic range of *Hevea* in Brazil, resulting in a sparse representation of the geographic diversity. Added to this, there were frequent exchanges of materials between different countries which facilitated the distribution of genes in various clones irrespective of the country in which they were developed. Most of the clones developed in India can be traced back to Malaysian and Indonesian clones, as is also evident from the dendrogram shown in Fig. 2.

In certain cases, clones with a common pedigree, such as PB 5/51, PB 235, PB 217, PB 312, HP 86/602, were observed to cluster together (Fig. 2). RRII 308 and PB 5/51 appear genetically very close although they are not apparently related. It is quite likely that PB 5/60, one of the parents of RRII 308, is genetically very close to PB 5/51, both clones originating from the same series. However, clones RRII 105 and PB 86 which are very close to each other in the dendrogram do not have any apparent relationship according to pedigree information. In *Hevea*, being a predominantly cross-pollinated tree species where F_1 hybrids are fixed vegetatively, the clones are highly heterozygous. As a result of segregation and independent assortment in these clones, the proportion of visible marker alleles in the hybrid from each parent can vary considerably, with the result that parents and hybrids may or may not cluster together. Thus, in highly heterozygous species with a common ancestry, pedigree information may not always reveal the exact nature of genetic relationships. Tinker et al. (1993), in a study on RAPDs and pedigree relationships among spring barley lines with varying amounts of common ancestry concluded that RAPD markers can be used to gain information about genetic similarities or differences that are not evident from pedigree information.

The present study shows clearly that it is possible to identify RAPD markers that can be scored reliably in an extensive *Hevea* material and can be used for clone identification and

Table 2: Genetic distances between the 24 *Hevea* clones tested

	RR II 5	RR II 105	RR II 208	RR II 308	RR IM 600	PB 5/51	PB 28/59	PB 86	PB 217	PB 235	PB 260	PB 280	PB 312	PCK 1	TJIR 1	GL 1	GT 1	SC ATC 88-13	KRS 25	HP 82-14	HP 86-120	RR IC 100	HP 86-602	HP 86-68
RRII 5		0.603	0.600	0.491	0.554	0.462	0.589	0.589	0.547	0.407	0.483	0.636	0.491	0.557	0.559	0.414	0.483	0.527	0.500	0.661	0.536	0.644	0.404	0.569
RRII 105	0.603		0.593	0.537	0.462	0.537	0.607	0.143	0.566	0.509	0.525	0.609	0.509	0.525	0.500	0.458	0.474	0.571	0.571	0.392	0.500	0.614	0.500	0.509
RRII 208	0.600	0.593		0.586	0.617	0.586	0.554	0.579	0.564	0.533	0.525	0.327	0.533	0.548	0.500	0.531	0.525	0.593	0.544	0.455	0.600	0.453	0.547	0.365
RRII 308	0.491	0.537	0.586		0.451	0.050	0.547	0.574	0.333	0.250	0.407	0.557	0.385	0.464	0.491	0.448	0.407	0.589	0.564	0.500	0.519	0.655	0.321	0.412
RRIM 600	0.554	0.462	0.617	0.451		0.420	0.528	0.471	0.417	0.426	0.525	0.587	0.265	0.446	0.526	0.458	0.525	0.519	0.621	0.481	0.527	0.683	0.364	0.536
PB 5/51	0.462	0.537	0.586	0.050	0.420		0.519	0.574	0.333	0.250	0.407	0.557	0.353	0.464	0.491	0.448	0.407	0.564	0.589	0.527	0.519	0.655	0.321	0.412
PB 28/59	0.589	0.607	0.554	0.547	0.528	0.519		0.566	0.604	0.518	0.509	0.525	0.491	0.534	0.536	0.492	0.509	0.500	0.582	0.621	0.564	0.672	0.508	0.519
PB 86	0.589	0.143	0.579	0.574	0.471	0.574	0.566		0.549	0.518	0.583	0.597	0.518	0.534	0.561	0.466	0.482	0.607	0.582	0.431	0.509	0.625	0.533	0.57
PB 217	0.547	0.566	0.564	0.333	0.417	0.333	0.604	0.549		0.412	0.463	0.559	0.380	0.463	0.571	0.474	0.434	0.538	0.593	0.500	0.574	0.729	0.436	0.500
PB 235	0.407	0.509	0.533	0.250	0.426	0.250	0.518	0.518	0.412		0.357	0.531	0.302	0.467	0.492	0.255	0.414	0.482	0.509	0.474	0.436	0.552	0.390	0.446
PB 260	0.483	0.525	0.525	0.407	0.525	0.407	0.509	0.583	0.463	0.357		0.523	0.441	0.459	0.345	0.339	0.484	0.500	0.500	0.466	0.483	0.613	0.410	0.411
PB 280	0.636	0.609	0.327	0.557	0.587	0.557	0.525	0.597	0.559	0.531	0.523		0.484	0.476	0.524	0.551	0.545	0.631	0.587	0.483	0.594	0.533	0.453	0.431
PB 312	0.491	0.509	0.533	0.385	0.265	0.353	0.491	0.518	0.380	0.302	0.441	0.484		0.467	0.517	0.426	0.441	0.396	0.583	0.474	0.491	0.623	0.304	0.474
PCK 1	0.557	0.525	0.548	0.464	0.446	0.464	0.534	0.534	0.463	0.467	0.459	0.476	0.467		0.404	0.419	0.459	0.550	0.550	0.466	0.400	0.656	0.435	0.439
Tjir 1	0.559	0.500	0.500	0.491	0.526	0.491	0.536	0.561	0.571	0.492	0.345	0.524	0.517	0.404		0.492	0.483	0.526	0.500	0.464	0.396	0.593	0.379	0.436
Gl 1	0.414	0.458	0.531	0.448	0.458	0.448	0.492	0.466	0.474	0.255	0.339	0.551	0.426	0.419	0.492		0.339	0.375	0.458	0.475	0.414	0.571	0.446	0.500
GT 1	0.483	0.474	0.525	0.407	0.525	0.407	0.509	0.482	0.434	0.414	0.484	0.545	0.441	0.459	0.483	0.339		0.500	0.474	0.517	0.533	0.613	0.410	0.492
SC ATC 88-13	0.527	0.571	0.593	0.589	0.519	0.564	0.500	0.607	0.538	0.482	0.500	0.631	0.396	0.550	0.526	0.375	0.500		0.545	0.561	0.472	0.746	0.500	0.61
KRS 25	0.500	0.571	0.544	0.564	0.621	0.589	0.582	0.582	0.593	0.509	0.500	0.587	0.583	0.550	0.500	0.458	0.474	0.545		0.536	0.554	0.614	0.525	0.561
HP 82/14	0.661	0.392	0.455	0.500	0.481	0.527	0.621	0.431	0.500	0.474	0.466	0.483	0.474	0.466	0.464	0.475	0.517	0.561	0.536		0.569	0.472	0.492	0.444
HP 86/120	0.536	0.500	0.600	0.519	0.527	0.519	0.564	0.509	0.574	0.436	0.483	0.594	0.491	0.400	0.396	0.414	0.533	0.472	0.554	0.569		0.689	0.458	0.518
RRIC 100	0.644	0.614	0.453	0.655	0.683	0.655	0.672	0.625	0.729	0.552	0.613	0.533	0.623	0.656	0.593	0.571	0.613	0.746	0.614	0.472	0.689		0.609	0.554
HP 86/602	0.404	0.500	0.547	0.321	0.364	0.321	0.508	0.533	0.436	0.390	0.410	0.453	0.304	0.435	0.379	0.446	0.410	0.500	0.525	0.492	0.458	0.609		0.467
HP 86/68	0.569	0.509	0.365	0.412	0.536	0.412	0.519	0.571	0.500	0.446	0.411	0.431	0.474	0.439	0.436	0.500	0.492	0.610	0.561	0.444	0.518	0.554	0.467	
Mean	0.535	0.514	0.533	0.458	0.496	0.454	0.548	0.529	0.502	0.432	0.468	0.535	0.450	0.486	0.491	0.446	0.476	0.539	0.550	0.498	0.515	0.616	0.444	0.486
Minimum	0.404	0.143	0.327	0.050	0.265	0.050	0.491	0.143	0.333	0.250	0.339	0.327	0.265	0.400	0.345	0.255	0.339	0.375	0.458	0.392	0.396	0.453	0.304	0.365
Maximium	0.661	0.614	0.617	0.655	0.683	0.655	0.672	0.625	0.729	0.552	0.613	0.636	0.623	0.656	0.593	0.571	0.613	0.746	0.621	0.661	0.689	0.746	0.609	0.610

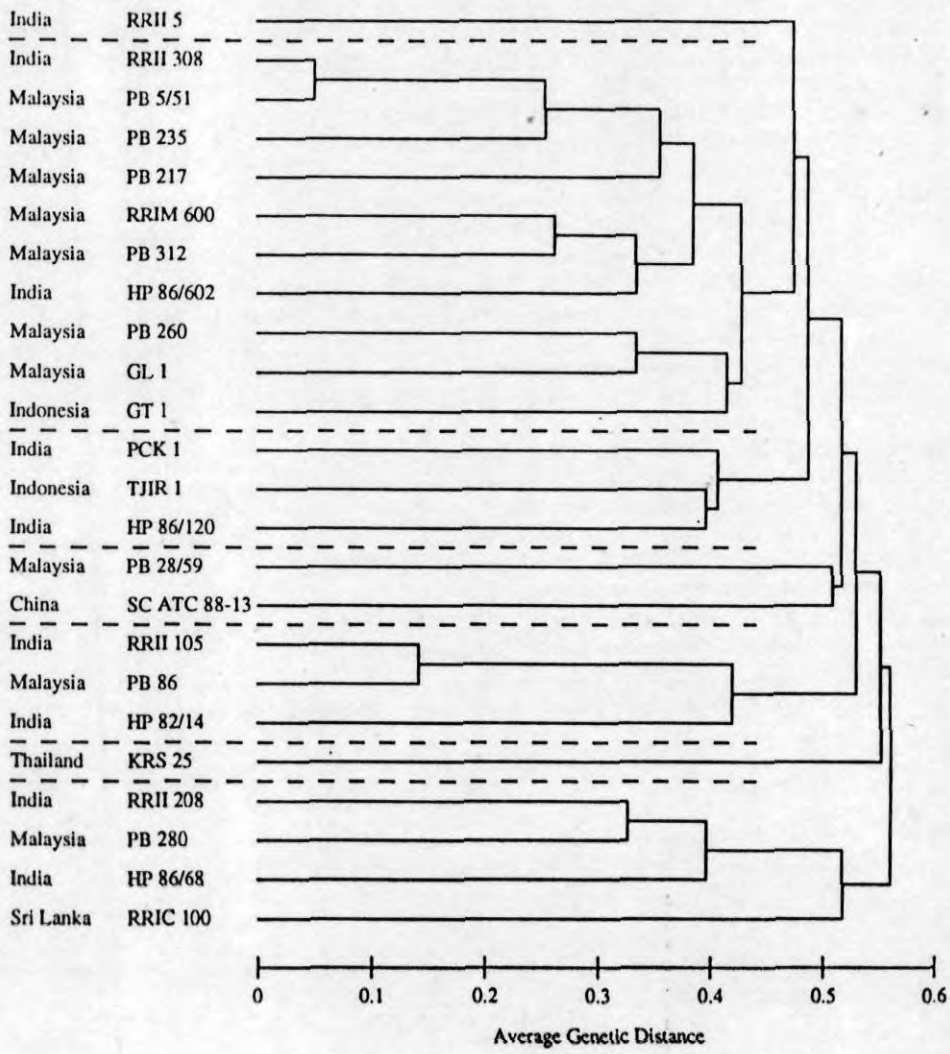

Fig. 2: Dendrogram of 24 *Hevea* clones based on 80 RAPD polymorphisms. Genetic distances were estimated according to Jaccard (1908) and clustering was carried out using the 'average linkage' method

estimation of genetic distances. Genetic distance estimates should be useful in *Hevea* breeding but before drawing definitive conclusions, further studies with a larger number of clones and more DNA polymorphisms are required.

Acknowledgements

The authors are grateful to Professor G. Röbbelen and Professor H. Becker, Institute of Agronomy and Plant Breeding, Georg-August University Göttingen, Germany, for their support in carrying out this investigation. Valuable suggestions and technical help given by Dr M. Uzunova, Dr J. Plieske and other colleagues in the institute are gratefully acknowledged. This research was supported by a World Bank-aided training programme. The senior author is grateful to the Chairman, Rubber Board, Ministry of Commerce, Government of India for granting the work, and to Dr C. K. Saraswathyamma, Dy. Director, Botany Division for her encouragement and support.

References

Arunachalam, V., A. Bandyopadhyay, S. N. Nigam, and R. W. Gibbons, 1984: Heterosis in relation to genetic divergence and specific combining ability in groundnut (*Arachis hypogea* L.). Euphytica **33**, 33—39.

Besse, P., M. Seguin, P. Lebrun, M. H. Chevallier, D. Nicolas, and C. Lanaud, 1994: Genetic diversity among wild and cultivated populations of *Hevea brasiliensis* assessed by nuclear RFLP analysis. Theor. Appl. Genet. **88**, 199—207.

Botstein, D., R. L. White, M. Skolnick, and R. W. Davis, 1980: Construction of a genetic map in man using restriction fragment length polymorphisms. Amer. J. Human Genet. **32**, 314—331.

Carlson, J. E., L. K. Tulsieram, J. C. Glaubitz, V. W. K. Luk, C. Kauffeldt, and R. Rutledge, 1991: Segregation of random amplified DNA markers in F_1 progeny of conifers. Theor. Appl. Genet. **83**, 194—200.

Chevallier, M. H., 1988: Genetic variability of *Hevea brasiliensis* germplasm using isozyme markers. J. Nat. Rubb. Res. **3**, 42—53.

Förster, J., and C. Knaak, 1995: Estimation of the genetic distance of 21 winter rapeseed varieties by RAPD analysis in comparison to RFLP results. Proc. 9th Int. Rapeseed Congr. Cambride, UK, 1184—1186.

Hu, J., and C. F. Quiros, 1991: Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep. **10**, 505—511.

Jaccard, P., 1908: Nouvelles recherches sur la distribution florale. Bull. Soc. Vand. Sci. Nat. **44**, 223—270.

Jain, A., S. Bhatia, S. S. Banga, S. Prakash, and M. Lakshmikumaran, 1994: Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. Theor. Appl. Genet. **88**, 116—122.

Koller, B., A. Lehmann, J. M. McDermott, and C. Gessler, 1993: Identification of apple cultivars using RAPD markers. Theor. Appl. Genet. **85**, 901—904.

Link, W., C. Dixkens, M. Singh, M. Schwall, and A. E. Melchinger, 1995: Genetic diversity in European and Mediterranean faba bean germplasm revealed by RAPD markers. Theor. Appl. Genet. **90**, 27—32.

Luo, H., B. V. van Coppenolle, M. Seguin, and M. Boutry, 1995: Mitochondrial DNA polymorphism and phylogenetic relationships in *Hevea brasiliensis*. Mol. Breeding **1**, 51—63.

Markose, V. C., 1984: Biometric analysis of yield and certain yield attributes in the para rubber tree. PhD thesis, Kerala Agricultural Univ., Kerala, India.

Moreno, S., Y. Gogorcena, and J. M. Ortiz, 1995: The use of RAPD markers for identification of cultivated grapewine (*Vitis vinifera* L). Scientia Horticulturae **62**, 237—243.

Mydin, K. K., V. G. Nair, M. R. Sethuraj, P. Saraswathy, and A. O. N. Panickar, 1992: Genetic divergence in *Hevea brasiliensis*. Indian J. Nat. Rubb. Res. **5**, 120—126.

Podani, J., 1990: SYN-TAX IV. Computer Programs for Data Analysis in Ecology and Systematics on Computers. Exeter Software, Setauket, NY.

Quiros, C. F., J. Hu, P. This, A. M. Chevre, and M. Delseny, 1991: Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. Theor. Appl. Genet. **82**, 627—632.

Rogers, S. O., and A. J. Bendich, 1988: Extraction of DNA from plant tissues. Plant Mol. Biol. Man. **A6**, 1—10.

Shoucai, C., S. Hansuang, H. Dong Qiong, L. Sheng, and Z. Xueqin, 1994: Identification of mildew resistance gene from *Hevea* tree by RAPD technique. Chin. J. Trop. Crops **15**, 26.

Smith, O. S., J. S. C. Smith, S. L. Bowen, R. A. Tenborg, and S. J. Wall, 1990: Similarities among a group of elite maize inbreds as measured by pedigree, F_1 grain yield, grain yield, heterosis, and RFLPs. Theor. Appl. Genet. **80**, 833—840.

Thakur, H. L., and M. A. Zarger, 1989: Heterosis in relation to genetic divergence and specific combining ability in Indian mustard *Brassica juncea* L. Czern. Indian J. Genet. Plant Breed. **49**, 223—226.

Tinker, N. A., M. G. Fortin, and D. E. Mather, 1993: Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet. **85**, 976—984.

Varghese, Y. A., 1992: Germplasm resources and genetic improvement. In: M. R. Sethuraj, N. M. Mathew (eds), Development in Crop Science 23. Natural Rubber: Biology, Cultivation and Technology, 89—115. Elsevier, Cambridge.

Varghese, Y. A., C. K. Saraswathyamma, and M. R. Sethuraj, 1996: Strategies of rubber breeding in Kerala. In: Semin. Breeding of Plantation Crops in Kerala. University of Kerala, January 1996, p. 19.

Welsh, J., R. J. Honeycutt, M. McClelland, and B. W. S. Sobral, 1991: Parentage determination in maize hybrids using arbitrarily primed polymerase chain reaction (AP-PCR). Theor. Appl. Genet. **82**, 473—476.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey, 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. **18**, 6531—6535.

Yang, X., and C. Quiros, 1993: Identification and classification of celery cultivars with RAPD markers. Theor. Appl. Genet. **86**, 205—212.