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COMPARATIVE GENETIC DIVERSITY STUDIES OF *SHOREA LEPROSULA* (DIPTEROCARPACEAE) USING RAPD AND ALLOZYME MARKERS

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LEE, S. L., WICKNESWARI, R., MAHANI, M. C. & ZAKRI, A. H. 2001. Comparative genetic diversity studies of *Shorea leprosula* (Dipterocarpaceae) using RAPD and allozyme markers. Genetic diversity parameters of seven populations of *Shorea leprosula* from Peninsular Malaysia were estimated based on 50 RAPD fragments/putative RAPD loci using two approaches, i.e. phenotypic method using Shannon's index of diversity and genotypic method with null-allele frequency corrected for deviation from Hardy-Weinberg equilibrium (HWE). Comparison with previous allozyme results based on the same set of individuals and populations showed that the percentage of polymorphic loci and expected heterozygosity derived from RAPD genotypic method were consistently lower. This may suggest that RAPD allele frequencies corrected for HWE deviation may be underestimated. RAPD markers highlighted a larger amount of overall population differentiation (G_{ST}) than allozyme. However, G_{ST} derived from RAPD genotypic method was not significantly different from G_{ST} based on allozyme ($p = 0.372$). It is argued that genetic diversity parameters derived from RAPD genotypic method can be underestimated but might still able to provide a reliable estimate of G_{ST}, if the bias affects proportionally both the genetic diversity within population and total genetic diversity. No significant correlation between similarity matrices based on RAPD and allozyme may indicate that these marker types give different estimates of genetic relationship among the populations tested. However, the correlation found between dendograms constructed on RAPD genotypic and allozyme similarities was fairly high ($r = 0.839$, $p = 0.01$). In fact, RAPD genotypic method resulted in a more definitive separation of populations than allozyme. The greater discriminatory power of RAPD compared to allozyme may be due to its greater coverage of the genome.

Key words: RAPD - allozyme - *Shorea leprosula* - Dipterocarpaceae - genetic diversity

LEE, S. L., WICKNESWARI, R., MAHANI, M. C. & ZAKRI, A. H. 2001. Perbandingan kajian kepelbagaian genetik terhadap *Shorea leprosula* (Dipterocarpaceae) menggunakan penanda RAPD dan alozim. Parameter kepelbagaian genetik dianggar ke atas tujuh populasi *Shorea leprosula* dari Semenanjung Malaysia berdasarkan 50 serpihan RAPD/ lokus RAPD putatif dengan dua pendekatan iaitu kaedah fenotip menggunakan indeks kepelbagaian Shannon dan kaedah genotip menggunakan frekuensi alel nul yang dibetulkan untuk penyisihannya daripada keseimbangan Hardy-Weinberg (HWE). Perbandingan dengan keputusan kajian alozim terdahulu menggunakan set individu dan populasi yang sama menunjukkan RAPD genotip menghasilkan peratusan lokus

polimorfik dan keheterozigotan jangkaan yang lebih rendah. Ini mencadangkan frekuensi alel nul yang dibetulkan daripada penyisihan HWE mungkin kurang dijangka. Penanda RAPD memperlihatkan tahap perbezaan populasi (G_{ST}) yang lebih tinggi daripada alozim. Bagaimanapun, perbandingan antara G_{ST} terbitan kaedah RAPD genotip dan alozim adalah tidak bererti (p = 0.372). Dihujahkan parameter kepelbagaian genetik boleh dijangka kurang tetapi masih mampu memberikan pengiraan G_{ST} yang boleh percaya jikalau penyisihan terhadap kepelbagaian genetik dalam populasi dan keseluruhan adalah berkadaran. Tiada korelasi yang bererti antara matriks serupa RAPD dan alozim menunjukkan dua penanda ini memberikan anggaran yang berbeza terhadap pertalian genetik antara populasi yang dikaji. Bagaimanapun, korelasi antara dendogram RAPD genotip dan alozim adalah tinggi (r = 0.839, p = 0.01). Sememangnya, kaedah RAPD genotip mempunyai kuasa diskriminasi yang lebih baik daripada alozim. Kelebihan ini mungkin disebabkan oleh kebolehan RAPD merangkumi genom dengan lebih luas.

Introduction

Randomly amplified polymorphic DNA (RAPD) markers (Williams *et al.* 1990) are based on amplification of DNA by the polymerase chain reaction (PCR) using single, arbitrary, decamer DNA oligonucleotide primers homologous to random target sites in the genome, a method also known as arbitrary-primed PCR (Welsh & McClelland 1990). The source of variation at the DNA level appears to be the result of a range of processes, including nucleotide substitution, insertion, deletion or inversion of a priming site or of segments between priming sites (Williams *et al.* 1993).

The technique enables rapid identification of a large number of markers at reasonably cost and has successfully been used to describe genetic diversity in many plant species, e.g. *Gliricidia sepium* (Chalmers *et al.* 1992, Dawson *et al.* 1995), *Gliricidia maculata* (Chalmers *et al.* 1992), *Theobroma cacao* (Russell *et al.* 1993), *Buchloë dactyloides* (Huff *et al.* 1993), *Camellia sinensis* (Wachira *et al.* 1995), *Eucalyptus globulus* (Nesbitt *et al.* 1995), *Medicago truncatula* (Bonnin *et al.* 1996), and more generally to study polymorphism at the species level (Kresovich *et al.* 1992, Mosseler *et al.* 1992, Boscherini *et al.* 1994, Link *et al.* 1995, Heinze *et al.* 1996, Kump & Javornik 1996). Several recent studies on RAPD variation have focused on the relative merit of RAPD marker as compared to other types of genetic markers such as allozymes (Liu & Furnier 1993, Heun *et al.* 1994, Isabel *et al.* 1995, Roesel *et al.* 1996, Szmidt *et al.* 1996, Bucci *et al.* 1997), RFLPs (Liu & Furnier 1993, Thormann *et al.* 1994), as well as morphological characteristics (Black-Samuelsson *et al.* 1997).

Shorea leprosula Miq., locally known as "meranti tembaga", belongs to the Dipterocarpaceae, the main timber family in the tropical forests of Southeast Asia (Ashton 1982). It is distributed from southern Thailand (Pattani), throughout Peninsular Malaysia (except for the seasonal areas in Perlis, northwest Kedah and Langkawi Island), Sumatra and to North Borneo (Symington 1943, Ashton 1982). The timber of *S. leprosula* is classified as light hardwood with a density range of 425–685 kg m^{-3} (Choo & Lim 1983). It is one of the main sources of light red meranti timber, which has already established a market both locally and

overseas. With continued exploitation for its timber and conversion of forests to other land uses, it is impossible for the species to remain abundant indefinitely and populations that are adapted to specific environments can easily be lost unless a proper conservation strategy is developed and practised. Moreover, current selective logging practices where trees with good form and straight bole are preferable can easily cause dysgenic effects, resulting in loss of the best genotypes.

Information on genetic diversity has several important applications for tree improvement and conservation. For genetic conservation programmes, there is general agreement that tropical forest reserves should be established and managed so as to preserve the maximum amount of genetic variation within species. But without data on the distribution of variation within or among populations, reasonable decisions cannot be made about the most effective ways to preserve this.

The aim of this study was to evaluate the genetic diversity and population genetic structure of *S. leprosula* on the basis of data provided by RAPD marker, as a complement to and comparison with the results reported by Lee *et al.* (2000) using allozyme marker on the same set of individuals and populations.

Materials and methods

Plant materials and DNA isolation

Seven natural populations of *S. leprosula* distributed throughout Peninsular Malaysia were selected for this study (Table 1). A transects line-sampling method, as described by Lee *et al.* (2000), was utilised for the sampling activities. A preliminary survey was carried out to locate an area (approximately 50 ha) in each of the sampling sites, where adult *S. leprosula* were abundantly found and well distributed. A transact line, approximately 2 km long, was marked across the sampling site. As fruits of this species are rarely dispersed more than 50 m from the mother tree, 1–2 seedlings (0.7–2.5 m in height with 2–7 small branches) were collected from the area around each individual mother tree. Selected mother trees were separated by approximately 50 m along the transact line. The total genomic DNA was extracted from leaf tissues using the method described by Murray and Thompson (1980) with modifications.

Table 1. Site description of the populations surveyed

Population	Pop. code	Location	Latitude	Longitude	Sample size
Bukit Perak	BP	Kedah	6° 00'N	100° 38'E	41
Segari Melintang	SM	Perak	4° 21'N	100° 34'E	40
Bangi	BA	Selangor	2° 54'N	101° 48'E	40
Sungai Menyala	SG	N. Sembilan	2° 27'N	101° 54'E	42
Pasoh	PA	N. Sembilan	2° 59'N	102° 19'E	35
Beserah	BE	Pahang	3° 50'N	103° 17'E	42
Panti	PT	Johor	1° 52'N	103° 53'E	40

RAPD analysis

Reactions were performed in 25 µl consisting of 5–100 ng of template DNA, 1.8 mM Mg^{2+}, 0.4 µM primer, 1 unit of *Taq* polymerase (Boehringer Mannheim), 1X *Taq* polymerase buffer, and 100 µM each of dNTP. All reactions were performed strictly according to the following scheme: (i) a master mix (buffer and dNTPs) was prepared using distilled water, (ii) the master mix was divided up and the primer was mixed in thoroughly, (iii) *Taq* polymerase was added and mixed gently, (iv) 24 µl reaction mixture was added into each tube, and (v) 1 µl of DNA sample (5 ng/µl) was added into the tubes and covered with two drops of mineral oil. The reaction mixture was subjected to amplification in a Techne PH2 thermocycler for 40 cycles consisting of 1 min at 94 °C, 1 min at 34 °C and 2 min at 72 °C. A final cycle of 10 min at 72 °C was used to complete the extension of any remaining products before holding the samples at 4 °C until analysed. Two kits (OPB and OPD from OPERON Technologies Inc., USA), containing 20 decamer primers of random sequence each were screened. Genomic DNA of three individuals from each of the BP, PT, BE and PA populations were used as templates for primer selection. Primers were evaluated based on quality of amplification fragments and consistency across a 20-fold span of template concentrations. Amplification fragments were separated on 2% agarose gels in 1X SEB (0.04 M Tris-base pH7.85, 0.02 M sodium acetate, 1 mM EDTA), stained with ethidium bromide, visualized by illumination with ultraviolet light and recorded by photography, using Polaroid 667 film. To test whether the comigrated fragments were homologous and different fragment positions represented different loci, one of the amplified fragments from each of the primer was eluted, labelled and hybridised to Southern blots of some RAPD gels (Smith *et al.* 1994).

Data analysis

Amplification fragments were scored as discrete character states, (1) if present or (0) if absent, based on the following criteria: (i) only fragments with molecular weight less than 2 kb were considered, (ii) a constant fragment, which appeared in most of the samples, was served as the cutting point for the lowest molecular weight fragment, and (iii) there was no bias in scoring monomorphic fragmented versus polymorphic fragments. The lengths of the amplification fragments were estimated by comparison to a molecular standard (123bp marker, BRL, USA). Each scorable fragment was designated with number (starting from 1 with primer type as initial, e.g. OPB06-01) in decreasing order of relative base pair lengths and entered into a binary data matrix. Genetic diversity parameters were calculated using two approaches: (i) phenotypic method using Shannon's index of diversity (King & Schaal 1989), and (ii) genotypic method with null-allele frequency corrected for deviation from Hardy-Weinberg equilibrium (HWE) with allozyme markers (Chong *et al.* 1994).

For the phenotypic method, the presence (1) and absence (0) of fragments were treated as phenotypic characteristics. The degree of polymorphism for a particular population was quantified with Shannon's index of diversity, $H'_o = -(\Sigma\pi_i \ln\pi_i)$, where π_i is the frequency of phenotype *i*. The average diversity over *n* different populations was calculated as $H_{pop} = (1 / n) \Sigma H'_o$ and the total diversity was calculated from the phenotypic frequencies π_i in all population considered together as $H_{sp} = \Sigma (-\pi_i \ln\pi_i)$. The proportion of diversity present within population was calculated as H_{pop}/H_{sp} and among populations (G'_{ST}) as $(H_{sp} - H_{pop})/H_{sp}$. Gower's (1985) asymmetrical quantitative similarity was used to generate a population based similarity matrix. This was done by using the R Package-CMS (IBM) computer program (Legendre & Vaudor 1991) and the similarity matrix obtained was then transformed to NTSYS-PC computer program format (Rohlf 1990) and used for cluster analysis via the unweighted pair group method (UPGMA) to construct a dendogram.

For the genotypic approach, the following properties of RAPD markers in a population of diploid individuals were assumed: (i) each polymorphic fragment will be considered as a locus with two alleles, identified by the presence or absence of the fragment, (ii) the null-allele may fail to amplify, either due to loss of a primer site or an insertion has caused the distance between primer sites to exceed the capacity of the PCR, (iii) RAPD profile was interpreted without ambiguity that is alleles from different loci do not comigrate to the same position on a gel, (iv) fragments that comigrate in two individuals represent homologous regions, and (v) loci are independent. With the assumption that deviation from HWE affects RAPD and allozyme markers in a similar manner, the RAPD null-allele frequency was estimated using the previous estimate of the departure from HWE (F_{is}) with allozyme data (Lee *et al.* 2000). Suppose that *A* and *a* are the dominant and recessive (null) allele respectively at a RAPD locus in a diploid population, then the expected frequencies of presence of fragment (*A*-) and absence of fragment (*aa*) are given as Freq(*A*-) = $1 - [q^2 + (1 - q) qF_{is}]$ and Freq(*aa*) = $q^2 + (1-q) qF_{is}$ respectively, where *q* is the frequency of recessive allele. For a given value of F_{is}, *q* was estimated using the iteration approach with the initial trial value of [Freq (*aa*)]$^{1/2}$. The frequency of dominant allele (*p*) was calculated as (1 - *q*). These corrected allele frequencies were then used for the estimations of the following genetic diversity parameters: percentage of polymorphic loci (*P*, 95% criterion), expected heterozygosity (H_e; Nei 1978), coefficient of population differentiation (G_{ST}; Nei 1973, 1977) and unbiased genetic identity (*I*; Nei 1978). Corrected allele frequencies and genetic identity were calculated using POPGENE program release 1.2 (Yeh & Boyle 1997). The corrected allele frequency dataset was then converted into BIOSYS-1 (Swofford & Selander 1981) type 3 format for the estimations of *P*, H_e and G_{ST}. As for the phenotypic method, similarity matrix obtained was transformed to NTSYS-PC computer program format and used for cluster analysis via the UPGMA method to construct a dendogram.

To make the comparisons between RAPD and allozyme analyses, allozyme data reported by Lee *et al.* (2000) were reanalysed based on the seven populations from Peninsular Malaysia (BP, SM, BA, SM, PA, BE and PT) for *P*, H_e, G_{ST}, *I* and

construction of dendogram via UPGMA method. The significant difference between G_{ST} values derived from RAPD data and allozyme data was tested using Student *t*-test (unequal variance of completely randomised two-group design; Sokal & Rohlf 1981). Similarity matrices based on different marker types and approaches (allozyme, RAPD phenotypic and RAPD genotypic) were compared using the Mantel matrix-correspondence test (Mantel 1967). In order to obtain estimates of the magnitudes of differences among dendograms, cophenetic values were computed for each dendogram, followed by the construction of a cophenetic matrix for each marker type and approach. The cophenetic matrices were then compared using the Mantel matrix-correspondence test.

Results and discussion

Characteristic of RAPD markers

From the 40 different decamer primers evaluated, 7 primers (OPB06, OPB08, OPB12, OPB15, OPB20, OPD02 and OPD08) with good quality of amplification products, and consistent fragment patterns were chosen for this study. The 7 primers evaluated on the 7 *S. leprosula* populations generated a total of 66 reproducible amplification fragments, with the size ranging from 0.1 to 1.7 kb and varying from 2 (OPB06) to 16 (OPB20) fragments per primer. Amplification fragments of the same length were assumed to be homologous. However, lack of homology through hybridisation to cloned RAPD fragments has been demonstrated (Smith *et al.* 1994). Furthermore, with arbitrarily amplified loci, there is no reason to believe that different fragment positions represent different loci. Since these two assumptions are the main criteria for the statistical analysis, one of the fragments from each of the primers was eluted, labelled and hybridised to Southern blots of some RAPD gels. If the amplification fragments were homologous and non-allelic, then one would expect the labelled fragment to hybridise with fragments of similar mobility. Out of the 7 amplified fragments tested (OPB06-01, OPB08-02, OPB12-05, OPB15-01, OPB20-03, OPD02-12 and OPD08-05), all except OPB20-03 showed homology and non-allelic behaviour. The labelled fragment of OPB20-03 hybridised to the fragment of corresponding size and also with most of the fragments present in the blot. Thus, the amplification fragments of OPB-20 (16 fragments) could be allelic or might be of repetitive sequences and were excluded for the data analysis. Of the remaining 50 fragments, 47 were polymorphic in at least one population.

Genetic diversity

Estimation of genetic diversity within populations using RAPD and allozyme markers is summarised in Table 2. For RAPD phenotypic method, simple measures of intrapopulation variability based on the number of polymorphic fragments scored in a single population over the total number of polymorphic

fragments (P), ranged from 58% (SM) to 78% (PA). Similarly, SM exhibited relatively lower levels of Shannon's diversity (H'_o = 1.799), whereas PA exhibited the highest (H'_o = 2.329).

Comparisons of Shannon's diversity index between studies are problematic, due to the qualitative behavior of the parameter and the estimations are dependent on the number of markers being used. Nevertheless, by taking into consideration the number of fragments being used in this study, estimations of H'_o based on 47 polymorphic fragments are higher (1.799 to 2.329, $\bar{x}$ = 2.007) than the results obtained by Russell *et al.* (1993) for *Theobroma cacao* (1.175 to 2.218, $\bar{x}$ = 1.494) and by Chalmers *et al.* (1992) for *Gliricidia sepium* (0.416 to 1.754, $\bar{x}$ = 1.194), which were respectively estimated using 60 and 63 polymorphic RAPD fragments. These observations may indicate that *S. leprosula* maintained a high level of genetic diversity and were in agreement with the results of the allozyme studies using the same set of individuals (Lee *et al.* 2000).

Table 2. Comparison of two genetic diversity parameters for seven populations of *Shorea leprosula* from Peninsular Malaysia based on RAPD marker and allozyme analyses reported by Lee *et al.* (2000). Values in parentheses are standard deviations.

Population (Pop. code)	RAPD phenotypic		RAPD genotypic		Allozyme	
	H'_o	P_o	H_e	P	H_e	P
Bukit Perak (BP)	2.003	64.0	0.182 (0.026)	54.0	0.326 (0.080)	77.8
Segari Melintang(SM)	1.799	58.0	0.205 (0.028)	56.0	0.332 (0.062)	77.8
Bangi (BA)	2.216	74.0	0.232 (0.028)	68.0	0.386 (0.063)	88.9
Sungai Menyala (SG)	1.802	74.0	0.192 (0.027)	52.0	0.400 (0.067)	88.9
Pasoh (PA)	2.329	78.0	0.244 (0.025)	72.0	0.387 (0.065)	88.9
Beserah (BE)	1.885	74.0	0.207 (0.026)	60.0	0.392 (0.057)	88.9
Panti (PT)	2.017	68.0	0.218 (0.028)	62.0	0.356 (0.079)	77.8
Mean	2.007	70.0	0.211 (0.022)	60.6	0.368 (0.030)	84.1

H'_o - Shannon's index of diversity
P_o - Percentage of polymorphic fragments, no criterion
H_e - Nei's (1978) unbiased expected heterozygosity
P - Percentage of polymorphic loci, 0.95 criterion

The P and H_e derived from the RAPD genotypic data (mean P = 60.6% and mean H_e = 0.211) were consistently lower than those values derived from allozyme (mean P = 84.1% and mean H_e = 0.368). This is in accordance with the studies by Szmidt *et al.* (1996) and Roesel *et al.* (1996) who reported that the genetic diversity parameters derived from RAPD allele frequencies (estimated with the assumption of HWE) were lower than those based on allozyme. It is argued that P and H_e derived from RAPD allele frequencies corrected for HWE deviation using fixation index derived from allozyme analysis may be underestimated. This could be due to the fact that the degree of deviation from HWE for RAPD loci and allozyme loci may not be the same. In fact, Szmidt *et al.* (1996) reported that the deviation from HWE for the RAPD loci was generally higher than that for allozyme loci.

The assumption that each fragment represents one RAPD locus with only two alleles might not always be true. Deletion and insertion events that occur in the region between the primers would result in an amplified fragment migrating to a different position, causing it to be scored as a separate locus rather than an additional allele at the first locus. A homology study was carried out to verify this assumption using one representative fragment for each of the primers. The possibility of the remaining fragments to be allelic would lead to biased estimates of P (can be either overestimated or underestimated) and its effect on H_e would depend on the allele frequencies.

Coefficient of population differentiation

The coefficients of population differentiation estimates based on the RAPD phenotypic data, RAPD genotypic data and allozyme are listed in Table 3. The average coefficients of population differentiation from RAPD phenotypic and genotypic data (0.145 and 0.114 respectively) were generally higher than that obtained from allozyme (0.085). The relatively higher G'_{ST} value obtained from RAPD phenotypic data was mainly attributed to four primers: OPD08 (0.235), OPB15 (0.182), OPB12 (0.165) and OPB08 (0.163) whereas for RAPD genotypic data, it was attributed to two primers: OPB15 (0.179) and OPD08 (0.171). Direct comparison between RAPD phenotypic G'_{ST} and allozyme G_{ST} is difficult and is likely to be biased as the coefficients were estimated using different indices (RAPD phenotypic using Gower's coefficient whereas allozyme using Nei's coefficient). As expected, comparison between RAPD phenotypic G'_{ST} and allozyme G_{ST} revealed significant difference (t = 3.65, n = 12, p = 0.003). However, RAPD genotypic G_{ST} was not significantly different from G_{ST} derived from allozyme (t = 0.927, n = 12, p = 0.372).

Table 3. Comparison of levels of population differentiation for 7 populations of *S. leprosula* from Peninsular Malaysia based on 8 polymorphic allozyme loci (Lee *et al.* 2000) and genotypic and phenotypic analyses of 47 polymorphic RAPD fragments

Primer (No. of fragments/loci)	RAPD phenotypic			RAPD genotypic			Allozyme locus	Allozyme		
	H	H_{pop}	G'_{ST}	H_T	H_S	G_{ST}		H_T	H_S	G_{ST}
OPB06 (2)	0.583	0.567	0.027	0.411	0.386	0.061	*Gpi-2*	0.569	0.533	0.063
OPB08 (7)	1.379	1.154	0.163	0.164	0.147	0.104	*Pgm*	0.383	0.321	0.162
OPB12 (9)	3.163	2.641	0.165	0.296	0.269	0.091	*Idh*	0.638	0.514	0.194
OPB15 (13)	3.639	2.977	0.182	0.229	0.188	0.179	*Mdh-2*	0.359	0.351	0.022
OPD02 (12)	3.872	3.502	0.096	0.308	0.284	0.078	*Sdh-1*	0.174	0.170	0.023
OPD08 (7)	1.569	1.201	0.235	0.187	0.155	0.171	*Sdh-2*	0.394	0.366	0.071
							Ugp-1	0.502	0.487	0.030
							Ugp-2	0.609	0.538	0.117
Weighted mean	2.367	2.007	0.145	0.266	0.238	0.114		0.454	0.410	0.085

RAPD and allozyme gave comparable estimates of differentiation when populations of black spruce from a contiguous region were studied (Isabel *et al.* 1995). As the G_{ST} values were calculated as 1 - (H_S/H_T), if H_S is reduced proportionately with the reduction of H_T, one will expect the G_{ST} value to remain unchanged. As shown in Table 3, the H_S and H_T derived from RAPD allele frequencies ($\bar{x}$ = 0.238 and 0.266 respectively) were proportionately smaller than those derived from allozyme allele frequencies ($\bar{x}$ = 0.410 and 0.454 respectively, in the ratio of 0.580 for H_S and 0.586 for H_T). This may suggest that the degree of bias affecting both H_S and H_T is approximately equal, resulting in the reduction ratio to remain the same. Thus, the estimation of genetic diversity based on RAPD genotypic method can be biased, but this method is still able to provide an acceptable estimate of the degree of population differentiation, if the degree of bias applies proportionally to both the H_S and H_T.

Genetic identity and cluster analysis

Table 4. Genetic identity between pairs of populations of *S. leprosula* using RAPD marker and allozyme data reported by Lee *et al.* (2000)

Population	BP	SM	BA	SG	PA	BE
	a0.527					
SM	b0.953					
	c0.905					
	a0.604	a0.639				
BA	b0.960	b0.970				
	c0.890	c0.951				
	a0.586	a0.623	a0.728			
SG	b0.951	b0.967	b0.986			
	c0.898	c0.957	c0.980			
	a0.547	a0.599	a0.628	a0.595		
PA	b0.948	b0.963	b0.975	b0.965		
	c0.951	c0.946	c0.921	c0.915		
	a0.478	a0.539	a0.674	a0.673	a0.563	
BE	b0.947	b0.954	b0.980	b0.962	b0.970	
	c0.877	c0.969	c0.986	c0.978	c0.909	
	a0.616	a0.613	a0.655	a0.608	a0.615	a0.652
PT	b0.966	b0.947	b0.970	b0.961	b0.967	b0.966
	c0.979	c0.944	c0.896	c0.910	c0.979	c0.907

a: RAPD phenotypic method [Gower's (1985) phenotypic coefficient], mean pairwise genetic identity among the populations is 0.608 ± 0.056.

b: RAPD genotypic method [Nei's (1978) genotypic coefficient], mean pairwise genetic identity among the populations is 0.963 ± 0.011.

c: Allozyme data [Nei's (1978) genotypic coefficient], mean pairwise genetic identity is 0.936 ± 0.035 (Lee *et al.* 2000).

Coefficients of genetic similarity computed from RAPD phenotypic data (Gower 1985), RAPD genotypic data (Nei 1978) and allozyme data (Nei 1978) are presented in Table 4. Mean genetic similarity coefficient derived from RAPD genotypic data (0.963) was slightly higher than that based on allozyme (0.936) and was much higher than that based on RAPD phenotypic data (0.608). The similarity matrices obtained were compared by the Mantel statistics test for matrix comparisons. This test gives the product moment correlation (r) and a statistic test, Z, to measure the degree of relationship between two matrices. The similarity matrix generated from RAPD phenotypic data was significantly correlated with that of RAPD genotypic data ($r = 0.740$, $p = 0.001$). However, no significant correlation was observed on different marker types ($r = 0.389$, $p = 0.076$ for RAPD phenotypic vs. allozyme and $r = 0.330$, $p = 0.111$ for RAPD genotypic vs. allozyme, see Figure 1), suggesting that RAPD and allozyme markers provided different estimates of genetic relationship among the populations tested.

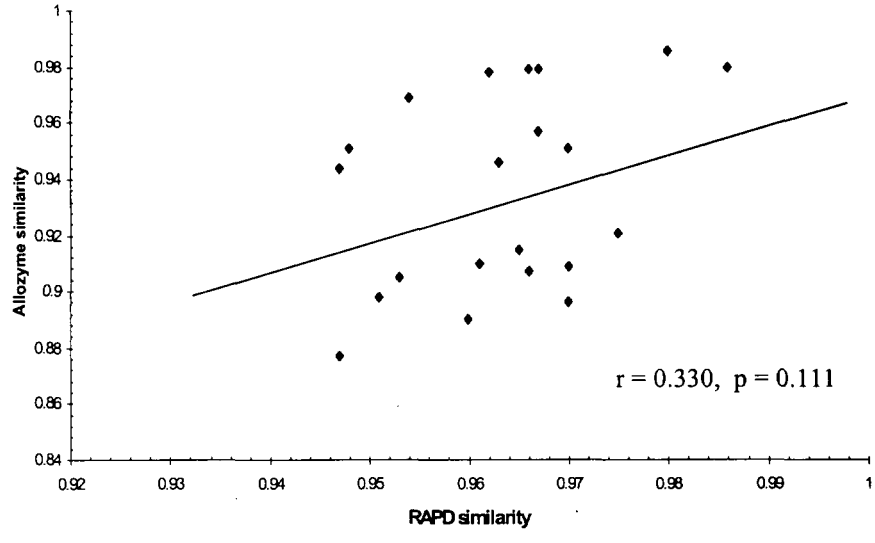

Figure 1. Plot of genetic similarity based on RAPD genotypic data set and allozyme data set reported by Lee *et al.* (2000). Each dot in the graph corresponds to a pairwise genetic similarity estimated using Nei's (1978) similarity coefficient. The values were used in the Mantel test for matrix correlation.

Cluster analyses were carried out for the three sets of similarity measured using UPGMA. The dendogram based on RAPD phenotypic data poorly discriminated the populations. Dendogram based on RAPD genotypic similarity was comparable to that based on allozyme, with the exception of the placement of PA population (Figure 2).

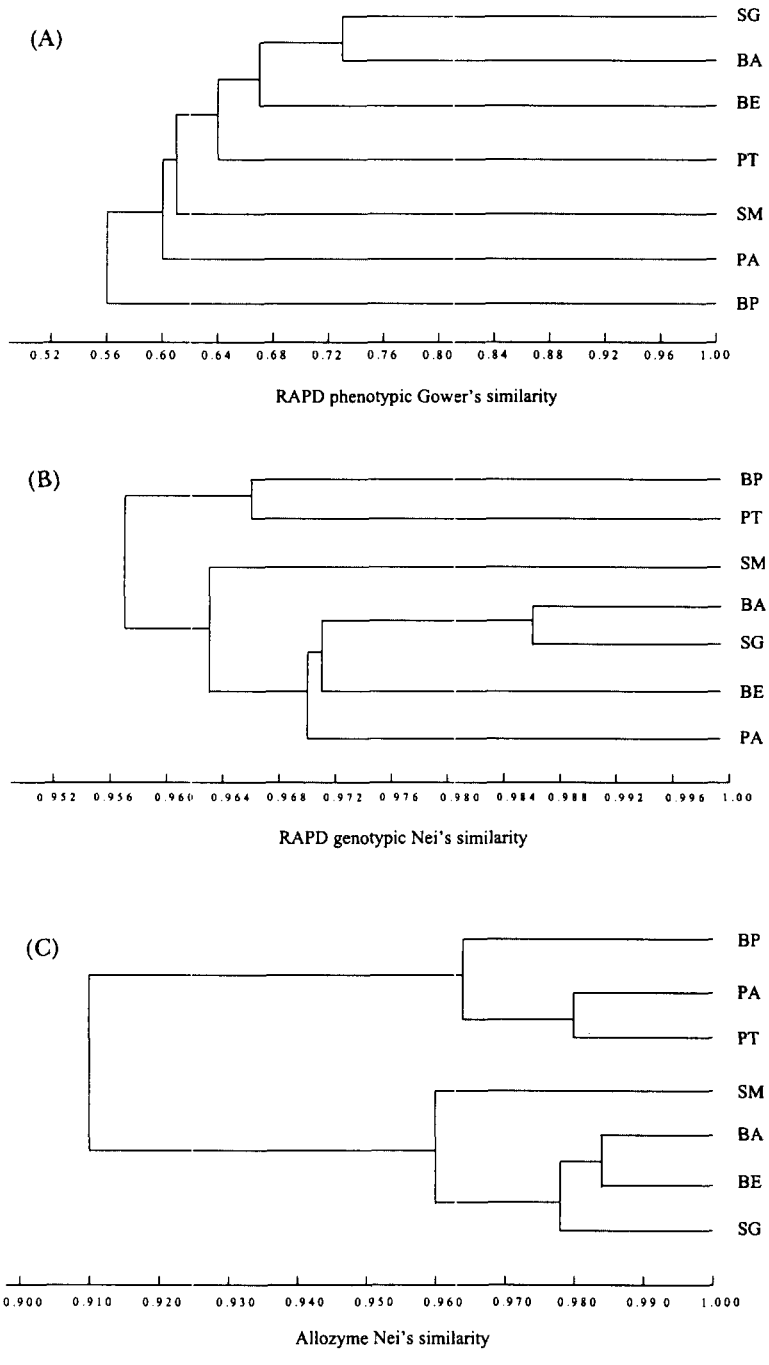

Figure 2. Dendrogram of *Shorea leprosula* populations generated by UPGMA method. (A) RAPD data analysed phenotypically based on Gower's (1985) quantitative similarity coefficient. (B) RAPD data analysed genotypically based on Nei's (1978) similarity coefficient. (C) Allozyme data analysed based on Nei's (1978) similarity coefficient. Comparisons between dendrograms were carried out based on the cophenetic values using Mantel test for matrix correspondence.

Despite these initial observations, it is quite difficult to visually ascertain how significant the differences are between these dendograms. In order to estimate the similarity between dendograms, a new set of matrices based on the cophenetic values were constructed and compared using the Mantel test for matrix correspondence. The correlations between matrices of cophenetic values were not significant for RAPD phenotypic versus RAPD genotypic and RAPD phenotypic versus allozyme ($r = 0.611$, $p = 0.058$ and $r = 0.194$, $p = 0.120$ respectively), but significant for RAPD genotypic versus allozyme ($r = 0.839$, $p = 0.01$). In fact, RAPD genotypic method resulted in a more definitive separation of populations than allozyme. Unlike the dendogram derived from allozyme, PA population was clustered with BA, SG and BE (Figure 2). At the similarity coefficient level of 0.964, two main clusters were discerned: populations in the central region (BA, SG, BE and PA) were more closely related, while a representative population each from the southern (PT) and northern (BP) regions formed another cluster. Segari Melintang (SM), located between the central and northern region populations, was placed intermediate between these two main clusters. The greater discriminatory power of RAPD compared to allozyme may be due to its larger coverage of the genome.

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