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A Preliminary Study of Genetic Variation of Selected Species of a Lowland Forest at Ayer Hitam Forest Reserve, Selangor

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ABSTRAK

Kajian awal mengenai corak variasi genetik ke atas 10 spesies pilihan (Shorea parvifolia, Shorea macroptera, Shorea acuminata, Shorea leprosula, Hopea beccariana, Dipterocarpus crinitus, Endospermum malaccensis, Artocarpus elasticus, Palaquium gutta dan Macaranga gigantea) dari Hutan Simpan Ayer Hitam telah dijalankan menggunakan kaedah elektroforesis gel kanji. Analisis ke atas 8 enzim menunjukkan yang mereka dikawal oleh 9 ke 12 lokus.Tahap polimorfik dan purata heterozigositi jangkaan bagi spesies-spesies ini berjulat daripada 0.9 ke 1.0 dan daripada 0.454 (S. leprosula) ke 0.602 (H. beccariana) masing-masing.

ABSTRACT

A preliminary study of the extent and pattern of genetic variation of 10 selected species (Shorea parvifolia, Shorea macroptera, Shorea acuminata, Shorea leprosula, Hopea beccariana, Dipterocarpus crinitus, Endospermum malaccensis, Artocarpus elasticus, Palaquium gutta and Macaranga gigantea) at Ayer Hitam Forest Reserve was carried out using the horizontal starch gel electrophoresis. Analysis of eight enzymes indicated that they were coded by 9 to 12 loci. Levels of polymorphism and mean of expected heterozygosities of these species ranged from 0.9 to 1.0 and from 0.454 (S. leprosula) to 0.602 (H. beccariana) respectively.

INTRODUCTION

Tropical forest is rich in genetic resources. High species diversity is the most peculiar feature of tropical rainforest. For instance, the dipterocarps are the most dominant canopy component and represent the richness of tropical taxa (Ashton 1988). The complex community process and reproductive process of these diversified species are challenged by recent tropical ecology (Ashton 1988). Although the genetic studies of tropical species are expected to provide important information on the community, organisation and reproductive ecology, studies on native tree species in tropics are relatively scarce (Hamrick and Loveless, 1986; Loveless 1992 and Kijkar 1992).

Effective forest management needs a thorough understanding of many aspects including biology, ecology and genetics. Information on the genetics of species would be useful in designing appropriate tree breeding programmes. Lewontin (1974), Endler (1977) and Loveless (1992) stated that the presence of variability within a population is responsible in generating and maintaining a population sustainably. Genetic variation is the fundamental requirement for the maintenance and long term stability of forest ecosystem since the amount and pattern of genetic variation would determine the ability of forest tree species to adapt to variable environmental conditions (Bergmann *et al.* 1989). In fact, many studies of genetic variation showed that it is correlated with life history characteristics, breeding system and population dynamics (Loveless and Hamrick 1984). Genetic studies can also help in the identification of superior populations.

Among the many reliable and practical methods used in the study of genetic variation is by isozyme analysis. Isozyme markers have been found to be the cheapest and reliable when compared to the traditional morphological

markers. Its utilisation enables the separation of products of the same genes, regardless of environment. Individuals may be characterised by their genotypes, composed of a sample of gene, comparison of individuals or groups of individuals could be made using specific genetic markers.

Thus, the objective of this study is to assess the genetic variation (inter and intra specific) of 10 selected lowland forest species at Ayer Hitam Forest Reserve, Selangor.

MATERIALS AND METHODS

Samples of cambial tissues of 10 selected species (*Shorea parvifolia, Shorea macroptera, Shorea acuminata, Shorea leprosula, Hopea beccariana, Dipterocarpus crinitus, Endospermum malaccensis, Artocarpus elasticus, Palaquium gutta* and *Macaranga gigantea*) were collected from compartment 15, Ayer Hitam Forest Reserve, Selangor. Cambial tissues were collected from 30 trees per species to determine the total variation of the species. The samples were kept in eppendorf tubes and placed in a cooling box during the transportation. They were then filtered and mixed with extraction buffer prior to electrophoretic run.

The buffers used in this study were based on the ones recommended by previous studies on *Shorea* species (Daisy 1995 and Noridah, 1996). The three buffers used were Histidine (H), Lithium (L) and Morpholine Citrate (MC). Electrophoresis was done on a 10.5% potato hydrolysed starch. After electrophoresis, the gels were stained for 8 different enzymes namely *Esterase* (EST), α *Glycerophosphate dehydrogenase* (αGPDH), *Isocitrate dehydrogenase* (IDH), *Malate dehydrogenase* (MDH), *Phosphoglucomutase* (PGM), 6- *Phosphoglucose Dehydrogenase* (6 PDGH), *Phosphoglucose isomerase* (PGI) and *Shikimate Dehydrogenase* (SDH). The genetic interpretation of the enzyme was based on the phenotypes obtained according to the mobility of the isozyme bands. When an enzyme revealed more than one zone of activity, the fastest migrating (most anodal) was designated as locus 1, the next, 2 and so on. In addition, the most anodal allele was labelled as fast(F), medium (M) and the least migrated allele as slow (S). Clear banding patterns were obtained for all 9 loci and the results of the electrophoretic phenotype variation were analysed for allelic frequencies and expected heterozygosities.

RESULTS AND DISCUSSION

Interpretation of the enzyme phenotypes was based on patterns of variability in 10 species. Direct verification of genetic control of the electrophoretic patterns of the enzymes examined was not carried out. It was assumed based on the consistency of the electrophoretic patterns of the monomorphic and polymorphic enzymes from each individual of the species analysed. A total of 9-12 loci were scored from the eight enzyme systems. Almost all of the loci scored were polymorphic except for Sdh-1 in *S. acuminata* and Pgi-2 for *P. gutta*. The average number of alleles per locus ranged from 2.4 to 2.9 (Table 1).

The interspecific genetic variation is quantified by measuring the mean heterozygosity, the percentage of polymorphic loci and the percentage of alleles per locus. The mean observed heterozygosities (H_O) of these species ranged from 0.383 in *S. acuminata* to 0.608 in *P. gutta* while the mean of expected heterozygosities ranged from 0.454 to 0.602. On the other hand, level of polymorphic loci ranged from 0.9 to 1.0 (Table 1). Values of heterozygosities and polymorphisms were found to be similar to those reported by Kong (1994) on *Shorea acuminata* (H_O=0.604, P=1.0); Daim (1993) and Daisy (1995) on *S. leprosula* (H_O=0.565, and H_O=0.457, P=0.9 respectively) (Table 2). However, these values were higher than the values given by Hamrick and Loveless (1986), Loveless and Hamrick (1987), John (1996) and Hazandy (1997) for other tropical tree species (Table 2).

The higher values of heterozygosities and polymorphism in this study indicated that these species have undergone effective competition and selection within and between species for survival in natural stand. The natural forest, which normally portrays heterogeneous ecological condition, would justify them to possess high genetic variability. Thus, the heterozygotes being the fitter genotypes would survive better due to these effects as being indicated by the higher values of H_O over H_e (Feret and Bergmann, 1976) (Table 1). Hamrick and Loveless (1984) also found that the genetic variation of tree species in natural stand higher almost double that of other plant species. In addition, Hamrick (1989) and Hamrick *et al.* (1992) also reported that long-lived woody perennials would show high levels of genetic diversity. Hamrick and Godt (1989) and Hamrick *et al.* (1992) showed that H_e

TABLE 1
Summary on the Mean Observed Heterozygosity (H_o), Mean Expected Heterozygozity (H_e) and Proportion of Polymorphic Loci.

	S. parvifolia (30)	*S. macroptera* (30)	*S. acuminata* (30)	*S. leprosula* (30)	*H. beccariana* (30)	*D. crinitus* (30)	*E. malaccensis* (30)	*A. elasticus* (30)	*P. gutta* (30)	*M. gigantea* (30)
Range										
H_o	0.000	0.200	0.000	0.000	0.100	0.200	0.000	0.100	0.300	0.100
H_o	0.800	1.000	0.900	0.800	1.000	0.900	0.900	0.900	1.000	0.800
Mean	0.446	0.562	0.383	0.510	0.473	0.467	0.422	0.558	0.608	0.440
Range										
H_e	0.255	0.255	0.420	0.255	0.445	0.180	0.322	0.460	0.000	0.255
H_e	0.825	0.743	0.990	0.620	0.695	0.906	0.655	0.665	0.640	0.790
Mean	0.512	0.506	0.582	0.454	0.602	0.471	0.536	0.509	0.482	0.537
L	11	12	12	10	11	9	9	12	12	10
P	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	0.9	1.0
A	2.7	2.6	2.4	2.5	2.9	2.6	2.7	2.8	2.6	2.7

Note : Number in parentheses indicate sample size.
Key : H_o - observed heterozygosity, H_e - expected heterozygosity;
L - loci scored
P - proportion of polymorphic loci
A - average number of alleles per locus

TABLE 2
Genetic diversity parameters of tropical tree species as compared to genetic diversity of ten species obtained in this study.

Species	Heterozygosity Expected	Proportion of Polymorphic Loci (%)	References
Acacia auriculiformis	0.071	39.8	Wickneswari and Norwati (1993)
A. auriculiformis	0.146	n.a.	Moran *et al.* (1989a)
A. crassicarpa	0.086	58.7	John (1996)
A. crassicarpa	0.141	n.a.	Moran *et al.* (1989a)
A. mangium	0.200	67.0	Hamidi (1990)
Artocarpus elasticus	0.508	100.0	Present study
Azadirachta excelsa	0.094	67.5	Hazandy (1997)
Dipterocarpus crinitus	0.471	100.0	Present study
Endospermum malacensis	0.536	100.0	Present study
Gliricidia sepium	0.260	59.9	Chamberlain *et al.* (1996a)
Hevea brasiliensis	0.307	87.5	de Paiva *et al.* (1994)
Hopea beccariana	0.602	100.0	Present study
Hopea odorata	0.190	40.7	Wickneswari *et al.* (1995)
Leucaena shannonnii	0.271	65.7	Chamberlain *et al.* (1996b)
Macaranga gigantea	0.538	100.0	Present study
Palaquium gutta	0.482	91.6	Present study
Pinus kesiya	0.166	54.2	Boyle *et al.* (1991)
Pterocarpus macrocarpus	0.246	82.3	Liengsiri *et al.* (1995)
Shorea acuminata	0.604	100.0	Kong (1994)
S. acuminata	0.582	91.7	Present study
S. leprosula	0.565	72.2	Da im (1993)
S. leprosula	0.457	93.3	Daisy (1995)
S. macrophylla	0.209	47.0	Kanzaki *et al.* (1996)
S. macroptera	0.507	100.0	Present study
S. leprosula	0.454	100.0	Present study
S. parvifolia	0.535	95.2	Kong (1994)
S. parvifolia	0.512	100.0	Present study
Tectona grandis	0.347	79.0	Kertadikara and Prat (1995)
Tropical species	0.111	27.6	Hamrick and Loveless (1986)

Note : n.a. - not available.

depends on the geographic range of the species i.e. species with wider distribution range has higher H_e. The outcrossing nature of most of these species can further support such result. An outcrossed species has been reported to produce a more genetically diverse sample (Moran *et al.* 1989a), especially when samples were taken from individuals originating possibly from only a few mother trees. This phenomenon would be likely to occur for species that experience prevalent a sexual reproduction i.e. by apomixis. For instance Kaur *et al.* (1978);(1986) and Somego (1978) have provided some evidence of apomixis of some Malaysian dipterocarps. Thus detailed reproductive biology and cytological studies should be incorporated to clarify the observed genetic status of these species.

CONCLUSION

Genetic variation among the selected species is relatively high in terms of polymorphism and heterozygosity values. High levels of genetic variability for all species are strongly associated with the life history, reproductive biology and their capability for adaptation. The average genetic variation of the available species with H_e of 0.454 to 0.602 is high and sufficient to support a selective breeding programme. However, further verification of genetic control of enzyme loci assayed is required. In addition, evaluation of these parameters should be conducted on similar species from different compartments to assess for the intraspecific variation before any sampling and breeding strategies could be outlined. In addition, growth performances of

these species should also be evaluated so as to capture maximum genetic gain for the purposes of breeding and conservation.

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