ALLOZYME DIVERSITY OF *KOOMPASSIA MALACCENSIS* (LEGUMINOSAE) IN PENINSULAR MALAYSIA

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LEE, C. T., LEE, S. L., NG, K. K. S., SITI SALWANA, H., NORWATI, M. & SAW, L. G. 2007. Allozyme diversity of *Koompassia malaccensis* (Leguminosae) in Peninsular Malaysia. Population genetic study of *Koompassia malaccensis,* an important tropical timber tree species, was carried out using allozyme markers. Six natural populations, namely, Bukit Bandi, Bukit Lagong, Jerangau, Pasoh, Sungai Lalang and Sungai Menyala were investigated. The results showed that *K. malaccensis* harboured a higher level of allozyme diversity (mean expected heterozygosity, H_e = 0.253, mean number of alleles per locus, A_a = 2.8 and effective number of alleles per locus, A_e = 1.27), compared with other regionally distributed tropical long-lived tree species. Majority of the diversity was partitioned within-population (F_{st} = 0.045). No correlation was detected between geographical distance and genetic distance (Mantel test, r = -0.0031, p = 0.5130). Only one private allele was detected in Sungai Lalang population.

Keywords: Kempas, tropical tree species, population genetics, isozymes

LEE, C. T., LEE, S. L., NG, K. K. S., SITI SALWANA, H., NORWATI, M. & SAW, L. G. 2007. Kepelbagaian alozim *Koompassia malaccensis* (Leguminosae) di Semenanjung Malaysia. Kajian populasi genetik bagi *Koompassia malaccensis* yang merupakan salah satu spesies kayu balak tropika yang penting telah dijalankan menggunakan penanda alozim. Sebanyak enam populasi semula jadi (Bukit Bandi, Bukit Lagong, Jerangau, Pasoh, Sungai Lalang and Sungai Menyala) telah dikaji. Hasil kajian menunjukkan bahawa *K. malaccensis* mempamerkan tahap kepelbagaian alozim yang lebih tinggi (min keheterozigotan jangkaan, H_e = 0.253, min bilangan alel setiap lokus, A_a = 2.8 dan bilangan efektif alel setiap lokus, A_e =1.27) berbanding dengan spesies pokok tropika berjangka hayat panjang di daerah yang sama. Majoriti daripada kepelbagaian tersebut ialah kepelbagaian dalam populasi (F_{st} = 0.045). Tiada korelasi didapati antara jarak geografi dengan jarak genetik (ujian Mantel, r = -0.0031, p = 0.5130). Hanya satu alel unik dikesan dalam populasi Sungai Lalang.

INTRODUCTION

Koompassia malaccensis (Leguminosae: Caesalpinioideae), locally known as Kempas, is a tropical timber species distributed in Sumatra, Peninsular Malaysia, Singapore and Borneo (Hou 2000). It is a very tall tree, easily reaching 55 m in height and has a diameter of 200 cm (Appanah & Weinland 1993). It is found in lowland, hill, peat and freshwater swamp forests up to 800 m, but often favouring an altitude not exceeding 150 m. In peat swamp forests, the bole is cylindrical with steep plant-like buttresses. It flowers and fruits regularly and the main flower visitors are bees, *Apis* sp. (Appanah & Weinland 1993).

Kempas is a medium hardwood with moderate durability. The timber when treated with preservatives is suitable for all heavy constructional works like railway sleepers, fence posts, beams and bridges, and is used for structures under cover like parquet and strip flooring and panelling when untreated (Wong 2002). It is a protected species under Sarawak's Wildlife Protection Bill, 1990 and is assigned as lower risk/ conservation dependent (LR/cd) (IUCN 2006) under the 1994 IUCN Red List Categories and Criteria version 2.3. However, owing to the high demand of its timber, it is expected that the species might become threatened in the near future if proper conservation measure is not in place.

As genetic diversity is the basis for short-term adaptation and long-term evolution, information on genetic diversity is essential for effective conservation and management of the species. Hence, this study examined

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the population genetics of *K. malaccensis* using allozyme markers. The specific objectives were to estimate the genetic diversity levels of *K. malaccensis* in Peninsular Malaysia and to survey the distribution of genetic diversity within and among populations.

MATERIALS AND METHODS

Sample collection

Six natural populations of *K. malaccensis* in Peninsular Malaysia were identified for this study. These were Bukit Bandi, Bukit Lagong, Jerangau, Pasoh, Sungai Lalang and Sungai Menyala (Figure 1). All the populations investigated are located within virgin jungle reserves (VJRs) except Bukit Bandi, which is currently proposed to be gazetted as VJR (Table 1). About 40 adult trees were sampled from each population using the transect-line sampling method (Lee *et al.* 2000a). Young leaves were collected for enzyme extraction.

Enzyme extraction and allozyme electrophoresis

Enzyme extraction and starch gel electrophoresis were carried out according to Lee *et al.* (2000b). Approximately 0.2 g leaf was ground with liquid nitrogen into fine powder before 0.6 ml cold leaf extraction buffer was added. More than 20 enzyme systems were screened but most of them were either lack of activity, monomorphic or showed complex banding patterns. Only two enzyme systems with scorable and polymorphic banding patterns were obtained. These were peroxidase (PER; EC number 1.11.1.7) and uridine diphosphogluconate pyrophosphatase (UGP).

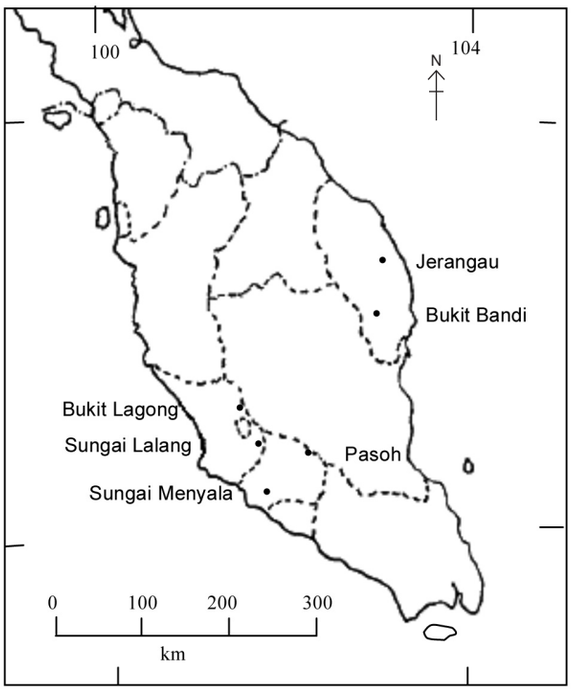

Figure 1 Map of Peninsular Malaysia showing the locations of the six populations of *Koompassia malaccensis* surveyed

Table 1 Details of the study sites

Population	State	Compartment No.	Year declared as VJR	Size (ha)
Bukit Bandi	Terengganu	na	Proposed to be gazetted	86
Bukit Lagong	Selangor	15	1947	161
Jerangau	Terengganu	10	1963	114
Pasoh	Negeri Sembilan	51	1995	108
Sungai Lalang	Selangor	24	1962	82
Sungai Menyala	Negeri Sembilan	9, 10	1948	48

na = information not available

Statistical analysis

Allozyme data were analysed using the program BIOSYS-1 (Swofford & Selander 1981) for the following genetic diversity parameters: mean number of alleles per locus (A_a), effective number of alleles per locus (A_e) and Nei's (1978) expected heterozygosity (H_e). Differentiation among populations was quantified using Wright's (1951) *F* statistics. The fixation indices, F_{is} (inbreeding within individual in population; inbreeding coefficient) and F_{st} (inbreeding due to population subdivision, an indicator of the degree of differentiation among populations), were calculated based on Weir and Cockerham's (1984) estimators, *f* and θ respectively, using the FSTAT program (Version 2.9.1, 2000). The standard error of F_{st} was calculated using unbiased jackknife analysis and the probability of $F_{st} > 0$ was determined using bootstrap analysis with a 95% confidence interval.

The relationship among populations was also calculated using Nei's genetic distances (Nei 1973) for pairwise comparison of divergence between populations, and cluster analysis on genetic distances via the unweighted pairwise groups with arithmetic averaging (UPGMA) (Sneath & Sokal 1973). Relative strength of the nodes was determined using bootstrapping analysis (1000 replicates) with the program PowerMarker (Liu & Muse 2005). Relationship between geographical distances and genetic distances between populations were analysed using Rousset's (1997) isolation-by-distance procedure and Mantel test in matrices comparison.

RESULTS AND DISCUSSION

Two enzyme systems were obtained, namely, PER and UGP which yielded a total of five scorable loci (*Per*-1, *Per*-2, *Per*-3, *Per*-4 and *Ugp*-1). In peroxidase, the multiple loci were designated numerically starting from the one which migrated most anodally. Similarly, the most anodal allozyme of a gene (i.e. allele) was assigned 1 followed by 2, 3 and so on. Figure 2 shows the banding

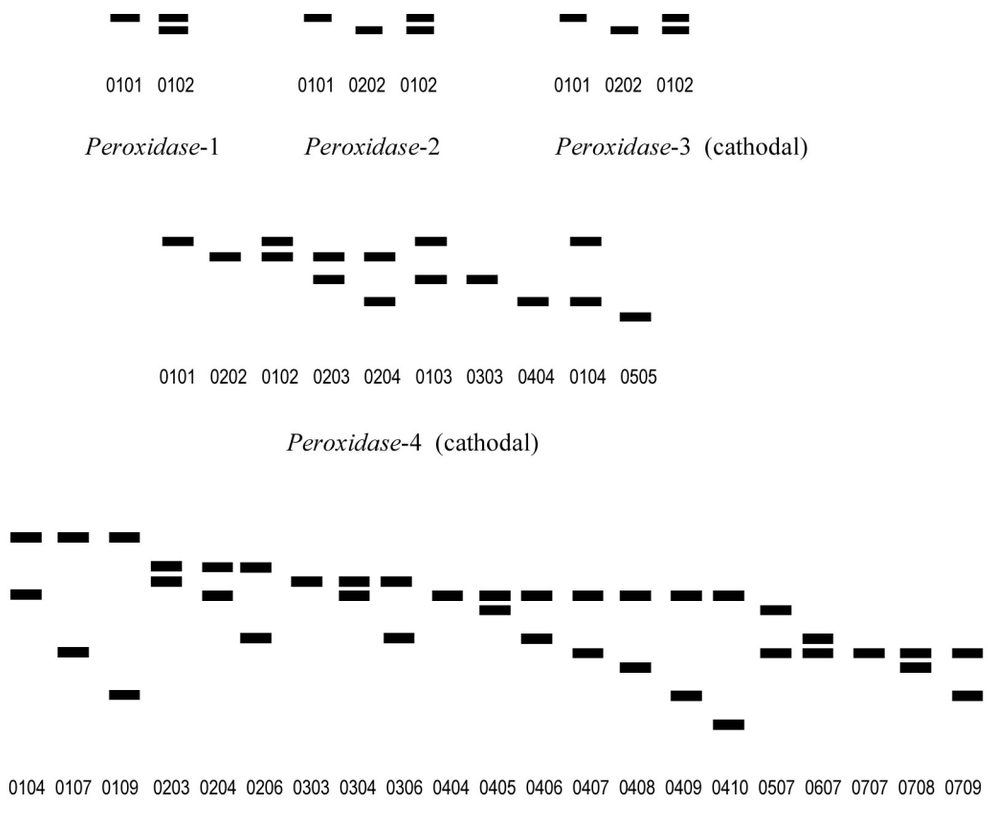

Figure 2 Schematic representation of various genotypes observed in respective polymorphic loci assayed in *Koompassia malaccensis* with peroxidase enzyme giving two cathodal loci

patterns of the five polymorphic loci. Taking into consideration all six populations, the highest number of alleles (10) was observed in *Ugp*-2. Allele frequencies for the five loci are given in Table 2. *Per*-4 and *Ugp*-2 were polymorphic across all populations; however, *Per*-1, *Per*-2 and *Per*-3 only showed variations in some populations. In Bukit Lagong population, *Per*-1, *Per*-2 and *Per*-3 were monomorphic. Overall, only one private allele was detected, i.e. in Sungai Lalang population (*Per*-4, allele-5).

Genetic diversity parameters for the six populations of *K. malaccensis* in Peninsular Malaysia are summarized in Table 3. The mean H_e was 0.253, ranging from 0.208 (Sungai Menyala) to 0.307 (Bukit Bandi). A_a ranged from 2.4 (Bukit Bandi and Jerangau) to 3.6 (Sungai Lalang), with a mean of 2.8. Values of A_e (mean = 1.27) were consistently lower than A_a, ranging from 1.21 (Sungai Menyala) to 1.34 (Bukit Bandi). This may indicate the presence of rare alleles.

Generally, the present study showed that *K. malaccensis* harboured a higher level of allozyme diversity compared with other regionally distributed tropical long-lived tree species (e.g. H_e = 0.125, A_a = 1.51 and A_e = 1.16 in Hamrick *et al.* 1992). However, the mean H_e for this legume species is comparable with *Intsia palembanica* (H_e = 0.242 in Lee *et al.* 2002) of the same family. On the other hand, it is lower than some dipterocarps, for example, *Dryobalanops aromatica* (H_e = 0.459; Lee *et al.* 2000c), *Shorea leprosula* (H_e = 0.369; Lee *et al.* 2000a) and *S. parvifolia* (H_e = 0.324; Siti Salwana *et al.* 2003), but higher than *Hopea odorata* (H_e = 0.190; Wickneswari *et al.* 1995).

Fixation indices (F_{is}) ranged from 0.132 (Sungai Lalang) to 0.317 (Sungai Menyala) with a mean of 0.205 (Table 3). The positive values of F_{is} across all six populations indicated excess of homozygotes. This may be due to some extent of selfing and/or occurrence of biparental mating across all six populations surveyed.

The proportion of genetic variation distributed among populations (F_{st}) was estimated at 0.045 and this value was significantly greater than zero (F_{st} within 95% confidence interval = 0.006–0.087; 95% confidence interval did not overlap with zero). This meant only 4.5% of the genetic variability was distributed among populations compared with 13.5% reported by Hamrick (1993) for tropical woody species.

Cluster analysis among populations showed that Bukit Bandi, Sungai Menyala, Bukit Lagong and Sungai Lalang formed a common genetic cluster, while Pasoh and Jerangau were the two outliers (Figure 3). Mantel test using isolation-

Table 2 Allele frequencies for the polymorphic loci assayed in the six natural populations of *Koompassia malaccensis*

Locus	Allele	Bukit Bandi	Bukit Lagong	Jerangau	Pasoh	Sungai Lalang	Sungai Menyala
Per-1	1	1.000	1.000	0.987	1.000	1.000	0.989
	2	0.000	0.000	0.013	0.000	0.000	0.011
Per-2	1	0.867	1.000	0.944	1.000	1.000	0.867
	2	0.133	0.000	0.056	0.000	0.000	0.133
Per-3	1	0.092	0.000	0.000	0.013	0.027	0.000
	2	0.908	1.000	1.000	0.988	0.973	1.000
Per-4	1	0.076	0.243	0.096	0.069	0.225	0.023
	2	0.515	0.608	0.712	0.625	0.575	0.886
	3	0.000	0.149	0.154	0.167	0.075	0.080
	4	0.409	0.000	0.038	0.139	0.075	0.011
	5 *	0.000	0.000	0.000	0.000	0.050	0.000
Ugp-2	1	0.036	0.000	0.000	0.000	0.039	0.012
	2	0.000	0.013	0.000	0.088	0.026	0.023
	3	0.024	0.026	0.000	0.075	0.013	0.000
	4	0.524	0.333	0.500	0.375	0.447	0.430
	5	0.000	0.038	0.000	0.013	0.013	0.023
	6	0.000	0.077	0.000	0.000	0.039	0.000
	7	0.417	0.474	0.438	0.425	0.368	0.500
	8	0.000	0.026	0.000	0.000	0.026	0.000
	9	0.000	0.013	0.063	0.000	0.026	0.000
	10	0.000	0.000	0.000	0.025	0.000	0.012

* *Per*-4 allele 5 is a private allele observed only in Sungai Lalang population.

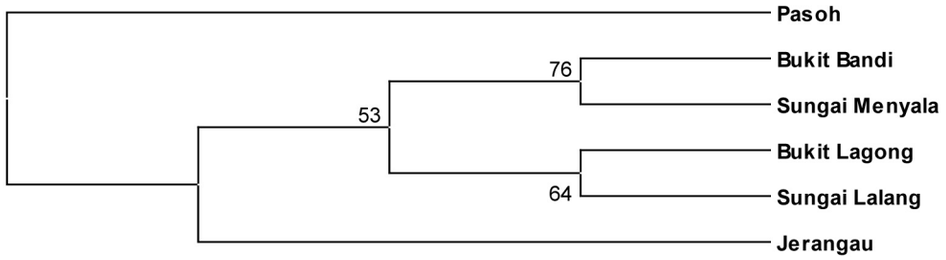

Figure 3 Cluster analysis on genetic distances among six natural populations of *Koompassia malaccensis* (bootstrap values were estimated based on 1000 replications)

Table 3 Estimated genetic diversity parameters and fixation index (F_{is}) of *Koompassia malaccensis* based on six natural populations

Population	A_a	A_e	H_e	F_{is}
Bukit Bandi	2.4	1.34	0.307	0.211
Bukit Lagong	2.8	1.25	0.245	0.236
Jerangau	2.4	1.24	0.235	0.156
Pasoh	2.8	1.27	0.253	0.180
Sungai Lalang	3.6	1.29	0.269	0.132
Sungai Menyala	3.0	1.21	0.208	0.317
Mean	2.8	1.27	0.253	0.205
Standard deviation	0.4	0.05	0.034	0.066

A_a = mean number of alleles per locus, A_e = effective number of alleles per locus, H_e = Nei's (1978) expected heterozygosity, F_{is} = fixation number

by-distance procedure (Rousset 1997) did not detect any significant relationship between genetic distance and geographic distance (r = -0.0031, p = 0.5130). This indicates either extensive gene flow among the populations studied (Gregorius & Namkoong 1983) or that these populations could have originated from a continuous population.

The effectiveness of genetic conservation depends on the appropriate representation and viability of important populations (Thomson *et al.* 2001). Low population genetic differentiation between the *K. malaccensis* populations (F_{st} = 0.045) implied no preference in the identification of populations for *in situ* conservation or in germplasm collection for *ex situ* conservation. However, from the cluster analysis Pasoh and Jerangau are relatively genetically more unique and, thus, should be given proper attention in future planning of conservation strategies. Nevertheless, it should be noted that this study was based on very few allozyme loci which might not involve adaptive variation.

As this study yielded a limited number of allozyme loci, other molecular markers which allow assay of more loci will be deployed in future for genetic diversity assessment as well as the estimation of effective population size. In fact, a more comprehensive study using microsatellite analysis is currently on-going whereby the sampling sites have been expanded to include more populations throughout Peninsular Malaysia, including a few from peat swamp forests.

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