

## Ti: Genetic Diversity Assessment of *Koompassia malaccensis*

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### ABSTRACT

A genetic diversity study of *Koompassia malaccensis* based on 19 populations from 18 forest reserves in Peninsular Malaysia is reported. The genetic diversity assessment was based on six polymorphic microsatellites. Overall, all the populations showed high levels of genetic diversity. The allelic richness ranged from 6.0 (Pekan) to 9.3 (Lenggong) whereas the gene diversity ranged from 0.683 (Pekan) to 0.859 (Lenggong). The estimated coefficient of population differentiation ( $R_{st}$ ) was 0.07, implying that 93% of the genetic diversity was partitioned within populations, with only 7% distributed among populations. From the cluster analysis among the populations, the two peat swamp populations (Pekan and Kuala Langat Selatan) formed a tight cluster even though they are not adjacent to one another. Further analysis including more populations and applying more microsatellites will generate more comprehensive genetic information.

**Keywords:** Kempas, genetic diversity, population genetics, simple sequence repeats

### INTRODUCTION

*Koompassia malaccensis* Maingay ex Benth. (Leguminosae) is an important tropical timber species distributed in Sumatra, Peninsular Malaysia, Singapore and Borneo (Hou, 2000). It is locally known as kempas and grouped under medium hardwood. It is a very tall tree, easily reaching 55 m in height and has a diameter of 200 cm. It is found in lowland, hill, peat and freshwater swamp forests up to 800 m, but often favouring an altitude not exceeding 150 m. It flowers and fruits regularly and the main flower visitors are bees, *Apis* sp. (Appanah and Weinland, 1993). It produces flowers and fruits all year round with fruits surrounded by a papery wing that spins down. Under the IUCN (1994) version 2.3 criteria, kempas was assigned as lower risk/ conservation dependent (LR/cd) (IUCN, 2006). However, as the demand of its timber is high due to shortage of hardwood, proper conservation measures are crucial to ensure sustainable harvesting.

Knowledge of the distribution of genetic diversity within and among populations of tropical trees is essential in the development of conservation strategies (Hamrick, 1983). Microsatellite markers have been used in population genetic studies for a wide array of timber species (Al-Rabab'ah and Williams, 2002; Novick *et al.*, 2003; Wyman *et al.*, 2003; Lee *et al.*, 2006) due to their ability to detect and describe genetic differences between populations.

This study was carried out using a set of microsatellite loci newly developed for *K. malaccensis* (Lee *et al.*, 2006). The aims were, i) to estimate the genetic diversity levels of *K. malaccensis* in Peninsular Malaysia and, ii) to survey the distribution of genetic diversity within and among populations, and iii) to investigate whether *K. malaccensis* from the peat swamp forests are genetically distinguishable from those of the non peat swamp forests.

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## MATERIALS AND METHODS

Leaves or inner bark tissues were collected from *K. malaccensis* trees from 19 natural forest reserves throughout Peninsular Malaysia with an average sample size of 24 (Table 1). Of all the populations surveyed, two were from the peat swamp forests (Pekan and Kuala Langat Selatan) while the rest were from the non peat swamp forests. *Fig. 1* shows the location of the sampling sites. Genomic DNA was extracted using modified Murray and Thompson (1980) method.

A total of six polymorphic microsatellite loci (*Kma089*, *Kma125*, *Kma127*, *Kma141*, *Kma156*, *Kma157*) were applied on all the 460 samples. Previous characterization showed no linkage among these six loci with a Bonferroni correction ( $\alpha = 0.05/276 = 0.0002$ ) (Lee *et al.*, 2006). Polymerase chain reaction (PCR) amplifications were carried out according to the protocols described in Lee *et al.* (2006). The PCR products were subjected to fragment analysis using ABI PRISM 377 DNA sequencer. Allele sizes were assigned against GeneScan Rox 400 (Applied Biosystems) internal size standard using GENESCAN v3.7.1 and genotyped using GENOTYPER v3.7 software (Applied Biosystems).

Genotypic data generated were analysed using FSTAT v2.9.3 (Goudet 2001). Genetic diversity parameters measured include the average number of alleles per locus ( $A_a$ ), allelic richness  $R_s$ , (Petit *et al.*, 1998) and gene diversity ( $H_e$ , Nei, 1987). Population differentiation coefficient was quantified using R-statistics ( $R_{st}$ ; Slatkin, 1995; Goodman, 1997), an analogue of Nei's genetic diversity statistics ( $G_{st}$ ; Nei, 1987) developed for microsatellite loci under the assumption of a stepwise mutation model, which is likely at many microsatellite loci (Jarne and Lagoda, 1996).

Cluster analysis using  $D_A$  genetic distances (Nei *et al.*, 1983) and neighbour-joining (NJ) method (Saitou and Nei 1987) was performed using the program PowerMarker (Liu and Muse, 2005). Relative strength of the nodes was determined using bootstrap analysis of 1000 replicates.

## RESULTS AND DISCUSSION

High levels of genetic diversity were observed in most of the populations surveyed (Table 2). The mean number of alleles per locus per population ( $A_a$ ) was 10.2. Gene diversity ( $H_e$ ) of the six loci

TABLE 1  
Details of the *Koompassia malaccensis* populations investigated in this study

Forest Reserve	State	Compartment No./ status
Labis	Johor	C9
Lenggor (VJR)	Johor	C231, VJR (unlogged)
Lenggor	Johor	C238, C241 (tagged for logging)
Mersing	Johor	C71 (unlogged, but tagged for logging)
Panti	Johor	C39, C41 (logged over)
Pasoh	Negeri Sembilan	50 ha ecological plot (unlogged)
Sungai Menyala	Negeri Sembilan	C9, C10, VJR (unlogged)
Pekan	Pahang	VJR (unlogged)
Air Cepam	Perak	C5 (tagged for logging)
Chikus	Perak	C44
Pangkor Selatan	Perak	Permanent Forest Reserve, Gazette No. 119
Pondok Tanjong	Perak	C10, C11
Ulu Kenas	Perak	Recreational Forest
Bukit Lagong	Selangor	C15, VJR (unlogged)
Kuala Langat Selatan	Selangor	C26, VJR (unlogged)
Semangkok	Selangor	C6 (unlogged)
Sungai Lalang	Selangor	C24, VJR (unlogged)
Bukit Bandi	Terengganu	Proposed as VJR (unlogged)
Jerangau	Terengganu	C10, VJR (unlogged)

VJR = virgin jungle reserve

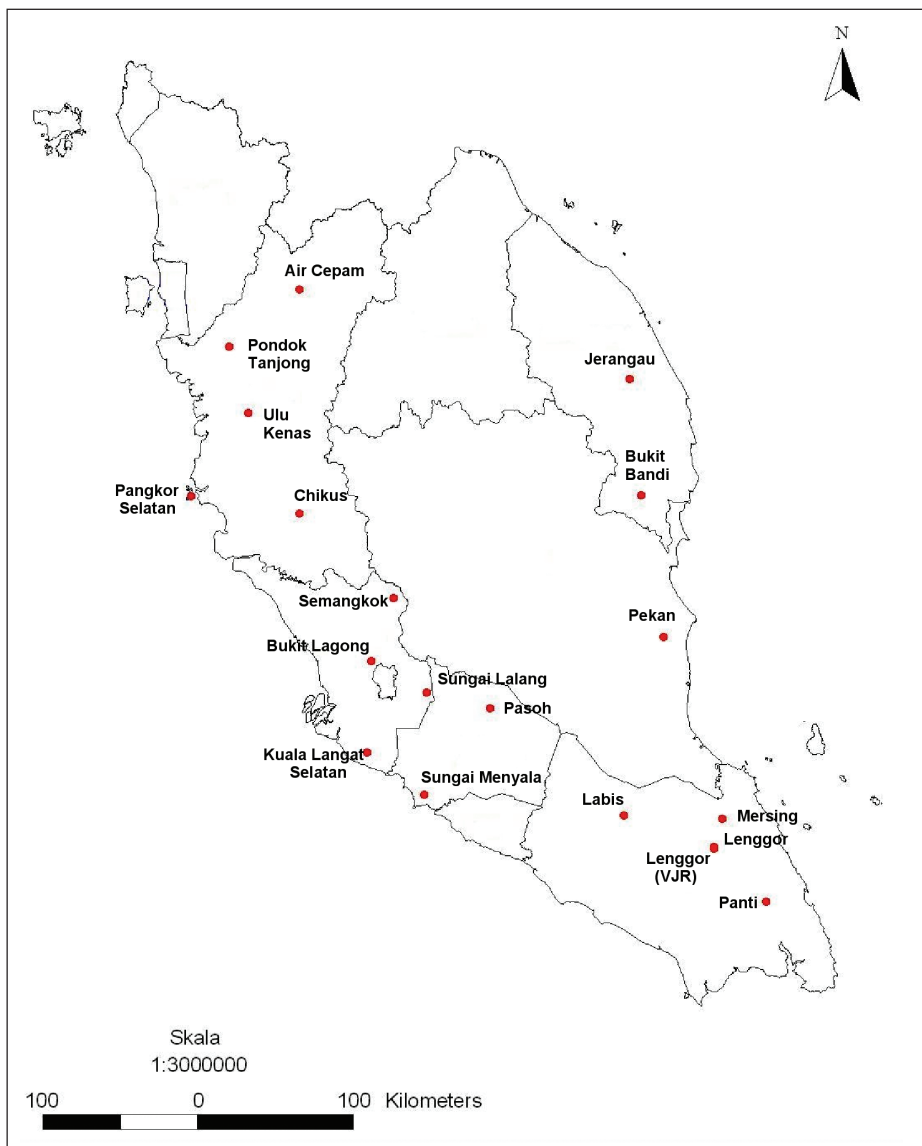


Fig. 1: Map of Peninsular Malaysia showing the sampling sites of *Koompassia malaccensis*

ranged from 0.683 (Pekan) to 0.859 (Lenggorg), with a mean of 0.798, whereas the mean allelic richness,  $R_s$  was 9.7, ranging from 6.0 (Pekan) to 9.3 (Lenggorg). Gene diversity ( $H_c$ ) obtained is comparable to *Swietenia macrophylla* (Lemes *et al.*, 2003) and *Jacaranda copaia* (Jones and Hubbell, 2003), but slightly higher than *Santalum austrocaledonicum*, an insular tree (Bottin *et al.*, 2005) and *Shorea lumutensis*, a rare dipterocarp (Lee *et al.*, 2006). Notably, the two peat swamp populations (Pekan and Kuala Langat Selatan) exhibited relatively low genetic diversity compared

with the non peat swamp populations. In fact, Pekan showed the lowest values for all the three genetic diversity parameters estimated ( $A_a = 6.8$ ,  $R_s = 6.0$  and  $H_c = 0.683$ ) and Kuala Langat Selatan is the second lowest in  $A_a$  (7.0) and  $R_s$  (6.3) (Table 2).

The population differentiation coefficient  $R_{st}$  was 0.07, i.e., 93% of the genetic diversity was partitioned within populations, with only 7% distributed among the populations. A previous study based on six populations using isozyme markers reported 4.5% of  $F_{st}$  (Lee *et al.*, 2007).

TABLE 2  
Average number of alleles per locus ( $A_a$ ), allelic richness ( $R_s$ ) and gene diversity ( $H_e$ ) of *Koompassia malaccensis* from 19 populations surveyed based on 6 microsatellite loci

Population	No. of samples analysed	$A_a$	$R_s$	$H_e$
Air Cepam	19	10.1	8.8	0.847
Bukit Bandi	20	8.0	6.7	0.707
Bukit Lagong	20	9.8	8.7	0.839
Chikus	19	9.0	8.0	0.806
Jerangau	20	10.8	8.9	0.795
Kuala Langat Selatan	20	7.0	6.3	0.713
Labis	30	12.5	9.0	0.834
Lenggor (VJR)	20	10.3	8.9	0.815
Lenggor	25	12.0	9.3	0.859
Mersing	19	9.3	8.2	0.802
Pasoh	40	12.3	8.6	0.801
Panti	26	11.0	8.3	0.798
Pekan	20	6.8	6.0	0.683
Pangkor Selatan	37	10.0	7.4	0.785
Pondok Tanjung	34	10.3	7.9	0.806
Semangkok	16	10.3	9.2	0.829
Sungai Lalang	20	11.2	8.9	0.811
Sungai Menyala	43	14.3	9.0	0.813
Ulu Kenas	13	8.8	8.7	0.827
Mean	24	10.2	9.7	0.798

In comparison, Hamrick (1993) reported 13.5% of  $G_{st}$  for tropical woody species. According to Hamrick *et al.* (1992), woody species with large geographic range, outcrossing breeding systems, and wind or animal-ingested seed dispersal have more genetic diversity within species and populations but less variation among populations than those with other combination of traits. *Koompassia malaccensis* is wind dispersed, with fruits of twisted papery pods. To date, there is no reported study on the mating system of *K. malaccensis*, however Appanah and Weinland (1993) reported that bees, *Apis* sp. are the main flower visitors, which are generally long distance pollinators. Recent studies revealed that most tropical tree species are predominantly outcrossing (Doligez and Joly, 1997; Lee *et al.*, 2000; Loveless, 2002; Ward *et al.*, 2005).

Fig. 2 shows the neighbour-joining tree (Saitou and Nei, 1987) based on the  $D_A$  genetic distances (Nei *et al.*, 1983). Three clusters were observed with Pangkor Selatan, the only island population in this study as the outlier. The biggest cluster comprised of the western and southern populations. The other two clusters (Jerangau – Bukit Bandi – Bukit Lagong and

Pekan – Kuala Langat – Lenggor – Lenggor VJR) did not correspond to the geographical locations. More stable dendrograms could be obtained by increasing the number of loci utilized (Koskinen *et al.*, 2004).

The cluster analysis also revealed close relationship between the two peat swamp populations, which formed a tight cluster with 100% bootstrap support value (Fig. 2). As they are not adjacent to one another (Fig. 1), the underlying factor for the high genetic similarity could be due to selection that might have had taken place in the process of adaptation to the habitat. In fact, morphologically, the boles of *K. malaccensis* from the peat swamp forests are generally cylindrical with steep plank-like buttresses. However, this speculation is not conclusive as microsatellite markers are generally selectively neutral. The application of more loci and/ or other molecular markers would further elucidate whether *K. malaccensis* of the two ecotypes (peat swamp and non peat swamp) are genetically distinguishable. Morgan-Richards and Wolff (1999) studied the genetic structure and differentiation of *Plantago major* and found a pair of sympatric sister species of two different ecotypes.

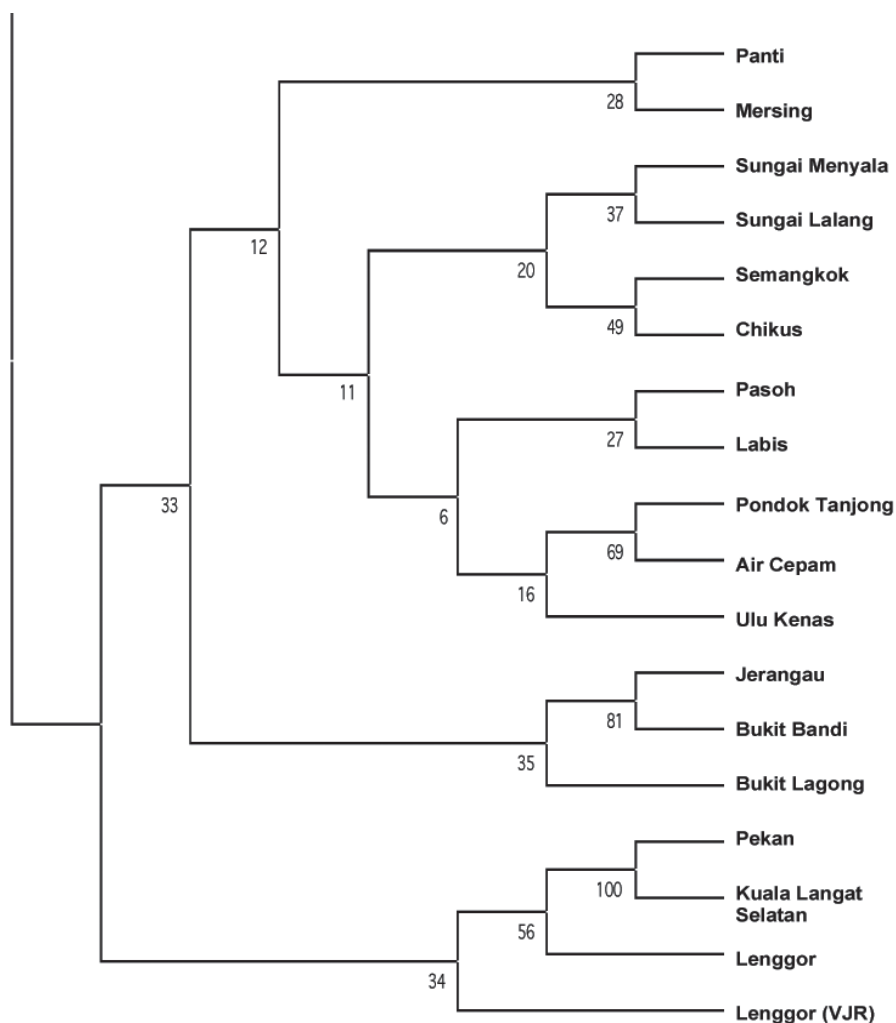


Fig. 2: Cluster analysis based on the genetic distances among the populations of *Koompassia malaccensis* surveyed (bootstrap values were estimated based on 1000 replications)

## CONCLUSIONS

The application of more microsatellite markers and expansion of the study sites will generate more comprehensive genetic information for the planning of effective conservation and management programs for *K. malaccensis*.

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