

GENETIC CONSERVATION OF *Pinus merkusii* IN INDONESIA:

Present Status of Conservation Stands and Genetic Diversity

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INTRODUCTION

An understanding of a wide array of topics such as the extent and diversity of forest ecosystems, the impact of forest management practices on biological diversity and tactics and strategies for gene conservation is necessary for the development of successful forest management strategies. In the tropics, the rate of forest degradation has been estimated at more than 20 million hectares per year (Soonhuah and Sziklai, 1994). Therefore, conservation of forests to retain biodiversity and maintain a balance of ecosystems is urgently needed. Conservation of genetic diversity will enable a species to adapt to changing environmental conditions, and will be of immense benefit to society.

Pinus merkusii is a tropical pine of Southeast Asia. In Indonesia, it occurs naturally in three disjunct locations on the island of Sumatra, namely Aceh, Tapanuli and Kerinci. In the northern parts of Sumatra, precisely in Aceh and Tapanuli. *P. merkusii* has been planted quite extensively in Java with a total area of approximately 900.000 ha, of which 570.000 ha serve as production forests and 330.000 ha as protection forests (Anonymous, 1998). Nowadays, it is the second-most extensively planted species after teak (*Tectona grandis*), making up more than 30% of the total plantations in Java. The species was proven also to be satisfactory for reforestation and afforestation of critical land and can be used as a pioneer species in plantations of a shade tolerant species (Harahap, 1995).

Due to its present and future ecological and economic importance, attempts to conserve this species, either in its natural habitats or plantations, have been initiated. However, the rational basis of conservation has not been available. Surveying the patterns of genetic diversity of a tree species is necessary in order to provide basic information for future activities in the conservation of genetic resources.

OBJECTIVES

The aims of this study were : 1) to find out the present status of conservation stands of *P. merkusii* in Indonesia and 2) to determine the patterns of genetic variability and diversity of *P. merkusii* found in natural and artificial stands in Indonesia. The results were aimed at providing basic information for future activities in genetic conservation and in tree breeding.

METHODOLOGY

Literature Review:

Review of literature was carried out to record the status of conservation stands of *P. merkusii* as well as its genetic studies from the available publications or reports.

Laboratory Work:

P. merkusii seeds were harvested in two natural populations: Aceh dan Kerinci; and one artificial population in Java, covering the most important populations in Indonesia. Seeds were immersed overnight in water, dissected and the embryo carefully separated from the megagametophyte. The embryo and the megagametophyte were ground in one and two drops of homogenising buffer (0.97 g Tris-HCl, 30 mg DTT and 2.5 g PVP in 100 ml H₂O, pH 7.3), respectively. Paper wicks saturated with the homogenate were inserted into starch gels. Extracts from the megagametophyte and the embryo of the same seed were positioned adjacent to each other. Horizontal starch gel electrophoresis of seeds (10.5% starch concentration plus 2.5-3.5% sucrose) was performed as described by Feret and Bergmann (1976), Conkle et al. (1982) and Liengsiri et al. (1990). The buffer system of Ashton pH 8.7 for glutamate oxaloacetate transaminase (GOT) and phosphoglucumutase (PGM) as well as Tris-Citro pH 7.4 for shikimate dehydrogenase (SKDH), nicotinamide adenine dinucleotide dehydrogenase (NDH) and formiate dehydrogenase (FDH) were used as electrode and gel buffers. A total of eight polymorphic enzyme gene loci was recovered by analysing the segregation of megagametophytes. The methods were largely those used by Changtragoon and Finkeldey (1995). The results on the genetic control of enzyme systems was published in Siregar (2000).

Analysis of Electrophoretic Data:

Data of seed trees, embryos and pollen contributions were utilised in the statistical analysis. Genetic variability of seed trees and embryos/progenies were calculated using the following measures: number of alleles per locus and (A/L); percentage of polymorphic loci (PPL); genetic diversity (v); gametic diversity (v_{gam}) and observed or expected heterozygosity (H_a and $H_e = \delta_T$). On the other hand, allelic diversities (v) were calculated for seed trees, embryos and pollen contributions according to Gregorius (1978). The computer programs of GSED version 1.1. (Gillet, 1998) and BIOSYS-2 (Swofford and Selander, 1997) were used to calculate the above measures. No software was required in order to determine the following genetic multiplicity measures of seed trees and embryos, namely number of alleles per locus and (A/L) and percentage of polymorphic loci (PPL).

RESULTS

Previous Genetic Studies and Conservation Stands:

Studies on the genetic variation at gene marker loci of *P. merkusii* have been carried out since 1995. A genetic inventory based on patterns of variation at 17 isozyme gene loci was performed in 11 populations of Thailand (Changtragoon and Finkeldey, 1995). Using samples of four populations in Thailand and one population in Vietnam, Szmidt et al. (1996) compared the patterns of genetic diversity of *P. merkusii* and *P. kesiya*. Meanwhile, the first publication of genetic studies at seven polymorphic loci of isozymes in Indonesia was written by Na'iem and Indrioko (1996) using artificial populations in Java. Very low genetic variation within populations (average $H_e = 0.058-0.073$) was observed in Thailand and Vietnam. This low genetic variation of *P. merkusii* may be explained by previous bottlenecks and reduced gene flow among populations. On the contrary, high level of genetic variation at seven polymorphic loci ($H_e = 0.259$) within four artificial populations was found in Java respectively. Up to now, reports on the genetic inventories of natural populations of *P. merkusii* in Indonesia are not yet available. Urgent conservation action for *P. merkusii* in Indonesia was highlighted by Harahap (1995). This author has surveyed and identified in detail the important localities where *P. merkusii* naturally occurs. The stands suggested to be conserved are in the region of Aceh (17 localities), Tapanuli (7 localities), Kerinci (4 localities). The areas to be conserved range

from 10 to 20 ha or at least 50 trees per stand. In Central Aceh, attempts have been made to design 13 natural stands for *in situ* conservation as shown in Table 1. The population to be conserved are located at various elevations ranging from 500 to 1350 above sea level. The area to be conserved also varies from 20 to 425 ha.

Table 1. Conservation Stands in Aceh (Sumatra)

No.	Conservation Stands	Size (ha)
1	Blang Kuyu	30
2	Baleq Rajawali	120
3	Burni Telong	32
4	Bidin Timur	35
5	Danau Laut Tawar	425
6	Isaq	70
7	Gelampang/Batu Belah	38
8	Penarun Simpang Umo	22
9	Serule/Uyem Ratus	20
10	Pantan Nangka/Manggis	31
11	Lumut	42
12	Ise Ise	25
13	Blang Kejeren	50
Total		940

Source : Tusam Hutani Lestari Ltd. (1999)

It is known that *P. merkusii* in Java was introduced from a population of natural forests in Aceh (Sumatra). *Ex situ* conservation was conducted in Java in forms of seed orchards and seed production areas. By the year 2000, the total area was around 700 ha as shown in Table 2 (anonymous, 2000). In the seedling seed orchards, the *ex situ* conservation is being extended for populations of Aceh, Tapanuli and Kerinci. Unfortunately, reports have been unavailable as to the success of the programme.

Table 2. Seed Production Areas (SPA) and Seedling Seed Orchards (SSO) in Java

No.	Province	Size (ha)	
		SPA	SSO (ha)
1	West Java	65	88
2	Central Java	234	117
3	East Java	100	96
Total		399	301

Source : Anonymous (2000)

In Kerinci National Park, sub-populations of *P. merkusii* are scattered over a number of locations and are small in size, sometimes less than 1 ha with low stocking (Na'iem, 1980). The current size of the Kerinci population is not known; however, the major part of it, which had previously been estimated to be around 3000 ha, seems to have disappeared (Hardiyanto, 1994)

Present Study on Genetic variability and diversity:

Measures of genetic variation of three investigated populations were based on seed trees and their progenies (see Table 3). The population of Kerinci National Park showed no variability at all eight gene loci investigated. Comparisons of the measures of genetic variability were then made only between populations of Aceh and Java. In general, the

population of Java had higher variability than that of Aceh. Only one measure, that is the percentage of polymorphic loci, showed no difference.

Table 3. Genetic variation measures of seed trees and progenies in the investigated populations of *P. merkusii* based on a survey of 10 enzyme gene loci.

Population	Sample		Genetic Multiplicity		Allelic Diversity	Gametic Diversity	Heterozygosity	
	Material	Size (N)	A/L	P	v	v_{gam}	H_a	$H_e=\delta_T$
Aceh	Seed trees	21	2.0	80.0	1.544	40.953	0.369	0.361
	Embryos	253	2.0	80.0	1.565	46.023	0.348	0.362
Java	Seed trees	30	2.4	80.0	1.630	62.516	0.433	0.395
	Embryos	797	2.4	80.0	1.636	63.448	0.355	0.389
Kerinci	Seed trees	25	1.0	00.0	1.000	1.000	0.000	0.000
	Embryos	200	1.0	00.0	1.000	1.000	0.000	0.000

A/L=Number of allele per locus; P=Percentage of polymorphic loci (among 10 loci studied); δ_T =Total population differentiation

The allelic diversity was given for each locus and averaged over all gene loci in Table 4. The gene pool data showed slightly different patterns of diversity. In the Aceh population, the allelic diversity of the effective pollen cloud was higher than in the seed trees and embryos, while in the Java population the allelic diversities were similar in all samples. The single-locus diversities were similar and low at the gene loci GOT-B, PGM-A and PGM-B. At GOT-C, allelic diversity of the pollen cloud was considerably higher ($v=1.817$) than that of the seed trees ($v=1.250$) and embryos ($v=1.288$).

Table 4. Allelic diversity (Gregorius,1978) of seed trees, their progeny (embryos) and their effective pollen clouds in the investigated populations.

Gene loci	Allelic diversity $v = (\sum_i p_i^2)^{-1}$								
	Aceh			Java			Kerinci		
	seed trees	Embryos	pollen	Seed trees	embryos	Pollen	seed trees	embryos	pollen
GOT-B	1.105	1.157	1.147	1.342	1.222	1.230	1.000	1.000	1.000
GOT-C	1.288	1.250	1.817	1.529	1.676	1.638	1.000	1.000	1.000
GOT-D	1.995	1.998	1.997	1.998	1.956	1.950	1.000	1.000	1.000
PGM-A	1.152	1.084	1.066	1.035	1.100	1.108	1.000	1.000	1.000
PGM-B	1.690	1.814	1.919	1.923	1.952	1.962	1.000	1.000	1.000
SKDH-A	1.995	1.997	1.993	1.946	1.877	1.908	1.000	1.000	1.000
NDH-A	1.893	1.898	1.930	2.024	1.955	1.832	1.000	1.000	1.000
FDH-A	1.960	1.998	1.989	1.946	1.995	1.980	1.000	1.000	1.000
Gene pool	1.544	1.582	1.631	1.630	1.635	1.625	1.000	1.000	1.000

DISCUSSION

Knowledge of the distribution of genetic variation within and between populations is of substantial benefit in tree breeding and in the conservation of plant genetic resources. The results on genetic variation reported in this paper might contribute to the currently available information on the pattern of genetic variation in *P. merkusii*. It was found that *P. merkusii* in Indonesia harbours larger amounts of genetic variation than was found by Changtragoon and Finkeldey (1995) in mainland provenances. A high level of genetic variation within populations is a common phenomenon in most pines as was reported by Ledig (1986). It was found that of 20 conifer species, the mean percentages of polymorphic loci, the numbers of alleles per locus and the expected of heterozygosities are 67.7, 2.29

and 2.07, respectively. The results of the present study indicated that, with the exception of the Kerinci population, the level of genetic variation in a natural population and a seed orchard was moderately high. A previous study on genetic variation of four artificial stands of *P. merkusii* in Java was conducted by Na'iem and Indrioko (1996). They reported that the mean percentages of polymorphic loci, the numbers of alleles per locus and the expected of heterozygosities are 85.7, 0.259 and 2.250, respectively. These values are similar to the values found in the seed orchard population as reported in the present study (PPL= 80.0, H_e =0.395 and A/L =2.40). This evidence, therefore, confirms the findings of the previous study (Na'iem and Indrioko, 1996). Muona and Harju (1989) attributed the high variation in pine species is due mainly to wind-mediated pollination, considerable pollen migration and large effective population size.

The absence of any genetic variation found in Kerinci raises speculations about the possible causes. It may be reasonable to adopt the possible explanation for the low genetic variation found in Thailand and Vietnam. Changtragoon and Finkeldey (1995) as well as Szmidi et al. (1996) attributed the low level of genetic variation in *P. merkusii* of mainland Asia to bottlenecks and reduced gene flow among populations. With regards to the Kerinci population, an additional explanation might be the geological history of Mount Kerinci. Kerinci is a stratovolcano and has erupted at least twenty times since 1838. The most recent eruption was in 1969-1970. An unconfirmed eruption was reported in 1971 (Simkin and Siebert, 1994). It is hypothesised that there have been drastic reductions in the number of trees in Kerinci during this geological age, forming repeated bottlenecks that resulted in low genetic variation. The presumable geological reason for the genetic fixation of *P. merkusii* in Kerinci is similar to that which caused the low genetic diversity in *P. resinosa* in Quebec and *Amentotaxus formosana* Li in Taiwan. These low diversities resulted from passing through a genetic bottleneck during glacial episodes of the Holocene (Fowler and Moris, 1977; Simon et al., 1986; Wang et al. 1996). Also low genetic diversity in Kerinci could then be the result of small population size. There is no direct evidence that the Kerinci population suffers from inbreeding depression. In any event, the protection of the Kerinci population might be only little effective as a gene resource. A final decision should take account of the expression of adaptive phenotypic traits as well as further investigation on increased number of seed samples and enzymes.

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