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Genetic variation of *Pinus Merkusii* jungh et de vriese in Indonesia

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ABSTRACT

The genetic structure and variation of *P. merkusii* from two natural populations in Sumatra and one artificial population in Java was evaluated using isoenzymes as genetic markers. A genetic basis for rational conservation of this only native pine species has not been available, although its conservation efforts was initiated more than a decade ago. Method of dynamic conservation was carried out by selecting some stands of different geographical characteristics in natural forests and in a national park as well as the establishment of seed orchard. Morphological differences between and within the populations are noted. A moderate level of genetic variation was found in a conservation stand population in Aceh (Sumatra) and a seedling seed orchard population in Java, while no variation was observed in a population of Kerinci-Seblat National Park (Sumatra). Genetic variable measures of the seed trees, progenies and pollen in these populations were determined using 8 isoenzyme loci (GOT-B, GOT-C, GOT-D, PGM-A, PGM-B, SKDH-A, NDH-A and FDH-A). In both variable populations 80.0 % of the loci were polymorphic. Analysis of the seed trees showed that effective numbers of alleles per locus (A/L), allelic diversity (v), gametic diversity (V_{gam}) and total population differentiation (δ_T=H_e) in the population of Aceh (Sumatra) were 2.0, 1.544, 40.953 and 0.361 respectively, while in the population of Java were 2.4, 1.630, 62.516 and 0.395 respectively. At the progeny level, the above measures for the population of Aceh were 2.0, 1.565, 46.023 and 0.362 respectively, while in the population of Java were 2.4, 1.636, 63.448 and 0.389 respectively. A higher level of genetic variation of *P. merkusii* in Indonesia was noticed when comparing to the populations of Thailand. The result of this study, especially for the non-variable population of Kerinci, implies that the genetic conservation of this species should not only be based on the results of surveys of genotypes at marker gene loci but requires also information on the expression of genetically controlled adaptive phenotypic traits.

Keywords: *Pinus merkusii*, isoenzymes, genetic structure and variation, genetic conservation

INTRODUCTION

Pinus merkusii is a tropical pine of Southeast Asia. It occurs naturally in Myanmar, Thailand, Laos, Cambodia, Vietnam, Indonesia and Philippines (CRITCHFIELD and LITTLE, 1966). *P. merkusii* does not occur on the Malay Peninsula. It can also be found in an area extending from north-eastern India to southern Tibet, but not enough is yet known about its characteristics there (LAMPRECHT, 1989). In Indonesia, it has natural occurrence 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* occurs mainly in twelve well-studied localities, occupying about 130.000 ha. of savanna-like terrain, where

pines are scattered over repeatedly burned areas (MIROV, 1967). In central Aceh alone *P merkusii* can be found in 10 main locations with a total area of around 70.000 ha (NN, 1988).

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 (NN, 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 a plantation of a shade tolerant species (HARAHAP, 1995).

Due to its present and future ecological and economic importances, attempts to conserve this species, especially in its natural habitats, have been initiated. In Aceh (Sumatra) and Tapanuli, deforestation occurred due to logging to supply woods for paper mills. Therefore, in 1994 conservation efforts were taken up in the Aceh region by choosing 13 natural stands as *in situ* conservation stands. The stands rang from 20 to 425 ha amounting to 900 ha in area. These stands are located in a logging concession of Alas Helau Ltd. at elevations varying from 500 to 1350 m above sea level (HARDIYANTO, 1994). Appropriate management of the stands including silvicultural treatments was also proposed and based on my own observation in 1997, the management to the stands are not regularly and properly conducted. The Kerinci population is also threatened due to very low population size.

Urgent conservation action for *P. merkusii* in Indonesia was highlighted by HARAHAP (1995). He and his team have 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. However, up to now, conservation measures have not yet been carried out nor has the rational basis of conservation been available. Surveying the patterns of genetic variation of a tree species is necessary in order to provide basic information for future activities in conservation of genetic resources. This has now become a fundamental principle of the conservation ethics just as the conviction that genetic variation or diversity is an important consideration when managing forest stands, ecosystems and landscapes. A common example is the investigation on the spatial distribution of genetic variation which can be carried out by analysing the variation at gene marker loci.

Analysis of variation at several markers such as RFLP, RAPD or isozyme do seem useful in providing rough approximations to the total amounts of genetic variation within most forest tree species. On the other hand, analysis of genetic variation can be done also by observing phenotypes at quantitative traits in specifically designed trials. Comparison of patterns of variation of these two traits has been very rare to compare. However, HAMRICK et al. (1993) demonstrated that the proportion of the total genetic variance found among populations for four quantitative traits was quite similar to that observed for 19 polymorphic allozyme loci (DUBA, 1985).

The aims of this study was to determine the patterns of genetic structure and variation of *P. merkusii* found in natural and artificial stands in Indonesia. The results were aimed at providing basic information for future activities in tree breeding and genetic conservation.

MATERIALS AND METHODS

Seed samples

Seeds were harvested in two natural populations and one artificial population of *P. merkusii*, covering the most important populations in Indonesia. The two natural populations are protected in a conservation stand and within a national park respectively, while the artificial stand is regarded as the most productive seedling seed orchard in Java. Details of the investigated populations are given in Table 01 and their approximate locations are indicated in Figure 01.

Table 01. Geographic origin of the *P. merkusii* populations sampled for electrophoresis.

No.	Locality	Altitude (m a.s.l.)	Long. (E)	Lat. (N)	Forest type
1	Aceh (A)	900	96°51’	4°30’	Natural pure forest
2	Kerinci (K)	900	101°26’	2°02’	Natural mixed forest
3	Java (J)	600	113°52’	7°67’	Seedling seed orchard

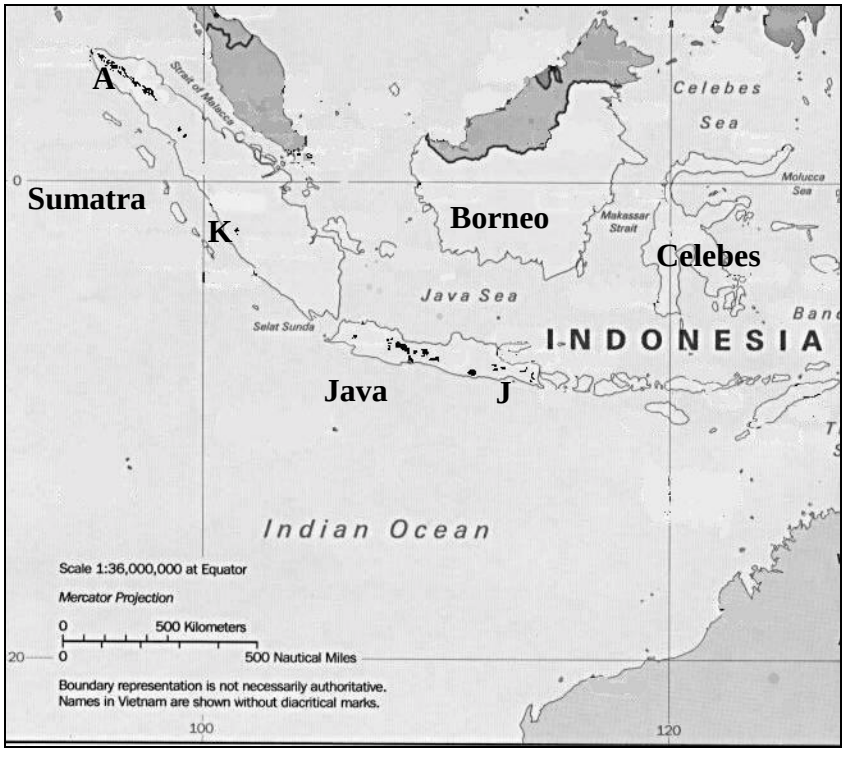


Figure 01. Geographic distribution of *P. merkusii* natural populations in Sumatra and plantations in Java. Sampled populations are marked with **A**=Aceh, **K**= Kerinci and **J**=Java.

The seeds were collected from 21 seed trees in a conservation stand of Aceh, 25 seed trees in a national park of Kerinci and 30 seed trees in a seedling seed orchard in Java. The variation was studied in megagametophytes and their corresponding embryos. At least eight seeds per seed tree was investigated to reveal its genotype.

Electrophoresis

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 H_2O, pH 7.3), respectively. Paper wicks saturated with the homogenate were inserted into starch gels. Extracts from the endosperm 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 GOT and PGM as well as Tris-Citro pH 7.4 for SKDH, NDH and FDH were used as electrode and gel buffers.

Measures of variability

Data of seed trees, embryos and pollen were utilised in the statistical analysis. Genetic variability of seed trees and embryos/progenies were calculated using following measures : number of alleles per locus and (A/L); percentage of polymorphic loci (PPL); genetic diversity (ν); gametic diversity (V_{gam}) and observed or expected heterozygosity (H_o and $H_e=\delta_T$). On the other hand, allelic frequencies and diversities (ν) were calculated for seed trees, embryos and pollen. The degree of differentiation between and among populations was analysed with the genetic distance measures of d_o (GREGORIUS, 1978) and D (NEI, 1972) as well as the genetic differentiation measures of D_j (GREGORIUS AND ROBERDS, 1986) and F_{ST} statistics (WRIGHT, 1965). 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 used 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

Genetic variability of seed trees and progenies within populations

Measures of genetic variability of three investigated populations were based on seed trees and progenies (see Table 02). 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 02. Genetic variability measures of seed trees and progenies in the investigated populations of *P. merkusii* based on 10 enzyme gene loci.

Population	Sample		Genetic Multiplicity		Allelic Diversity	Gametic Diversity	Heterozygosity	
	Source	Size (N)	*A/L*	*P*	*V*	V_{gam}	H_a	$H_{e=}\delta_T$
Aceh	seed tree	21	2.0	80.0	1.544	40.953	0.369	0.361
	embryo	253	2.0	80.0	1.565	46.023	0.348	0.362
Java	seed tree	30	2.4	80.0	1.630	62.516	0.433	0.395
	embryo	797	2.4	80.0	1.636	63.448	0.355	0.389
Kerinci	seed tree	25	1.0	00.0	1.000	1.000	0.000	0.000
	embryo	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 (from 10 loci)
δ_T = Total population differentiation

Allelic structures among seed trees, embryos and pollen

Allele frequencies in different populations for the loci studied in each population are presented in Table 4-02. Of 10 loci examined, eight were polymorphic (0.95 criterion) in at least one population. The population of Kerinci showed surprisingly no polymorphisms at all loci and all sample sources, i.e. seed tree, embryo and pollen, investigated. The existing alleles of monomorphic loci found in Kerinci population were GOT-B_2, GOT-C_2, GOT-D_1, PGM-A_2, PGM-B_2, SKDH-A_2, NDH-A_2 and FDH-A_1.

The most polymorphic loci found in the other two populations were GOT-D, PGM-B, SKDH-A, FDH-A and NDH-A. Only one GOT-C locus were moderately polymorphic, while the remaining two loci, GOT-B and PGM-A, were found to have a low polymorphism. Of the five most polymorphic loci, NDH-A and PGM-B loci were found to have alleles (NDH-A_2 and PGM-B_2) predominating in all sample sources across populations examined. This allele was also re-found in a fixed population of Kerinci population. At other four loci (GOT-D, PGM-B, SKDH-A, FDH-A), no single allele predominated in all sample sources of two investigated populations. However, there was a tendency that alleles existing in Kerinci population was also predominantly found in the other two populations.

Rare alleles (p < 1%) were found at GOT-B and NDH-A loci and they were GOT-B_1 and NDH-A_3 alleles with frequencies of 0.3% and 0.4% respectively. These alleles were found more in pollen samples than that found in embryos. It seemed also that alleles with low frequencies found more in pollen than that of other sample sources. The common alleles of loci showing moderate and low polymorphisms, GOT-B, GOT-C and PGM-B, were also re-found as the fixed alleles in Kerinci population.

Table 4-02. Allelic structures of seed tress, their embryos and pollen at the polymorphic gene loci in the investigated populations of *P. merkusii*

Gene loci	Allele	Population								
		Aceh			Java			Kerinci		
		seed tree	Embryo	pollen	seed tree	embryo	pollen	Seed tree	embryo	pollen
GOT-B	1	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000	0.000
	2	0.950	0.927	0.931	0.850	0.899	0.896	1.000	1.000	1.000
	3	0.050	0.073	0.069	0.150	0.100	0.102	0.000	0.000	0.000
GOT-C	1	0.100	0.057	0.060	0.050	0.067	0.063	0.000	0.000	0.000
	2	0.875	0.826	0.714	0.800	0.759	0.763	1.000	1.000	1.000
	3	0.000	0.022	0.039	0.067	0.107	0.155	0.000	0.000	0.000
	4	0.025	0.059	0.188	0.083	0.067	0.019	0.000	0.000	0.000
GOT-D	1	0.525	0.486	0.518	0.517	0.425	0.420	1.000	1.000	1.000
	2	0.475	0.514	0.482	0.483	0.575	0.580	0.000	0.000	0.000
PGM-A	1	0.071	0.036	0.024	0.017	0.029	0.011	0.000	0.000	0.000
	2	0.929	0.960	0.968	0.983	0.953	0.949	1.000	1.000	1.000
	3	0.000	0.004	0.008	0.000	0.018	0.041	0.000	0.000	0.000
PGM-B	1	0.286	0.340	0.397	0.400	0.422	0.430	0.000	0.000	0.000
	2	0.714	0.660	0.603	0.600	0.578	0.570	1.000	1.000	1.000
SKDH-A	1	0.524	0.518	0.530	0.417	0.372	0.390	0.000	0.000	0.000
	2	0.476	0.482	0.470	0.583	0.628	0.610	1.000	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NDH-A	1	0.381	0.379	0.393	0.333	0.327	0.315	0.000	0.000	0.000
	2	0.619	0.619	0.603	0.617	0.635	0.668	1.000	1.000	1.000
	3	0.000	0.002	0.004	0.050	0.038	0.017	0.000	0.000	0.000
FDH-A	1	0.571	0.486	0.538	0.583	0.526	0.450	1.000	1.000	1.000
	2	0.429	0.514	0.462	0.417	0.474	0.550	0.000	0.000	0.000

Allelic diversities between seed trees, embryos and pollen

The allelic diversity was given for each locus and pooled across locus. In the gene pool data there were slightly different patterns of diversity across sample sources. In Aceh population, the allelic diversity of pollen was higher than that of seed trees and embryos, while in a Java population there were a homogeneity of allelic diversities between sample sources.

In single locus diversities there were apparent heterogeneities at locus GOT-C, PGM-A and PGM-B. At the GOT-C allelic diversity of pollen was considerably higher (v=1.817) than those of seed trees (v=1.250) and embryos (v=1.288).

Table 04. Allelic diversity of seed trees, their progenies (embryos) and their pollen clouds in the investigated populations.

Gene loci	Allelic diversity $\nu = (\sum_i p_i^2)^{-1}$								
	Aceh			Java			Kerinci		
	seed tree	Embryo	pollen	seed tree	embryo	pollen	Seed tree	embryo	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

Genetic distances between populations

Two mostly applied indices of genetic distance were compared and the values are presented in Table 4-04. It is shown that the genetic distance of GREGORIUS (d_o) has clearly higher values than that of NEI distance. The difference of the two was low and comparable when comparing genetic distance between populations of Java and Aceh. However, the differences were considerably high and not comparable anymore when calculating the distances between population Kerinci and Java as well as population Kerinci and Aceh.

Table 05. Matrix of average gene pool distances, according to genetic distance (d_o) of GREGORIUS (1974, below diagonal) and genetic distance of NEI (1972, above diagonal).

Population		Aceh			Java			Kerinci		
		seed tree	embryo	Pollen	seed tree	embryo	pollen	seed tree	embryo	pollen
Aceh	Seed tree	***	0.000	0.000	0.000	0.008	0.012	0.122	0.122	0.122
	embryo	0.036	***	0.001	0.001	0.009	0.010	0.154	0.154	0.154
	pollen	0.042	0.031	***	0.000	0.009	0.011	0.154	0.154	0.154
Java	Seed tree	0.071	0.065	0.047	***	0.000	0.003	0.119	0.119	0.119
	embryo	0.092	0.066	0.060	0.047	***	0.001	0.147	0.147	0.147
	pollen	0.105	0.073	0.077	0.065	0.025	***	0.165	0.165	0.165
Kerinci	seed tree	0.293	0.315	0.321	0.308	0.325	0.337	***	0.000	0.000
	embryo	0.293	0.315	0.321	0.308	0.325	0.337	0.000	***	0.000
	pollen	0.293	0.315	0.321	0.308	0.325	0.337	0.000	0.000	***

Genetic differentiation among populations

The calculation of allelic differentiation (D_j) for each locus and gene pool is presented in Table 06 and Figure 02. The level of differentiation (D_j, δ) found in this study could be classified as moderate (δ= 16%) if compared with the results of other studies which showed low differentiation of only δ=5%.

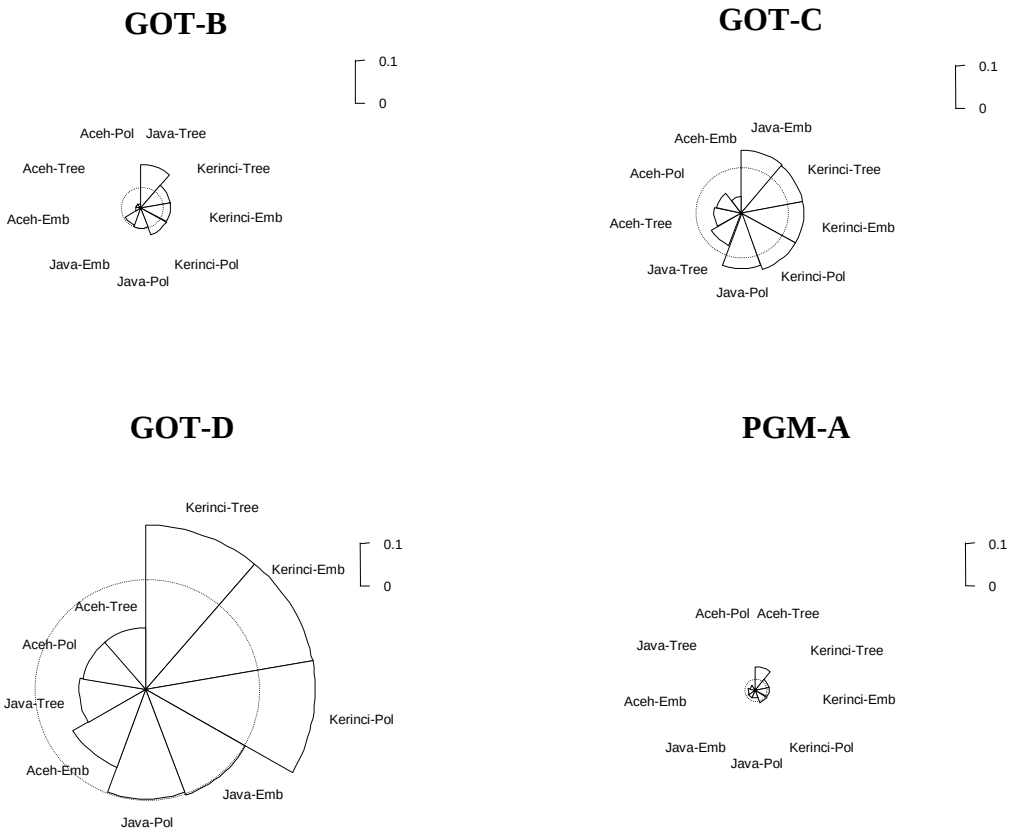
In general, when comparing to F_{ST}, the values of δ are higher. However, across loci, the gene pool differentiation of δ and F_{ST} is still comparable. The significant heterogeneity of allelic frequency was observed at all loci.

Table 06. Allelic differentiation among population samples of *P. merkusii* ; D_j and δ (GREGORIUS and ROBERDS, 1986) and F_{ST} (WRIGHT, 1965) and results of a log likelihood ratio test of homogeneity of the allelic frequency distributions (G_h test).

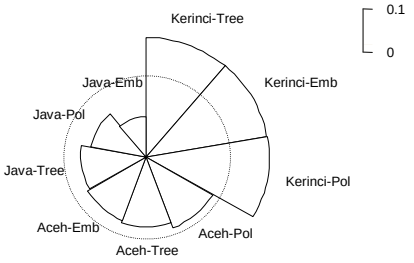
Gene loci	D_j									δ	F_{ST}	G_h
	Aceh			Java			Kerinci					
	St	emb	pol	St	emb	Pol	st	emb	pol			
GOT-B	0.012	0.013	0.010	0.101	0.045	0.049	0.068	0.068	0.068	0.048	0.024	***
GOT-C	0.065	0.037	0.059	0.082	0.132	0.147	0.143	0.143	0.143	0.106	0.049	***
GOT-D	0.146	0.194	0.148	0.155	0.258	0.266	0.389	0.389	0.389	0.259	0.216	***
PGM-A	0.054	0.019	0.004	0.014	0.021	0.021	0.032	0.032	0.032	0.025	0.009	***
PGM-B	0.037	0.106	0.156	0.165	0.190	0.199	0.285	0.285	0.285	0.190	0.132	***
SKDH-A	0.243	0.256	0.251	0.122	0.072	0.093	0.347	0.347	0.347	0.231	0.148	***
NDH-A	0.163	0.157	0.175	0.152	0.131	0.094	0.279	0.279	0.279	0.190	0.100	***
FDH-A	0.128	0.213	0.165	0.114	0.179	0.264	0.354	0.354	0.354	0.236	0.181	***
Gene pool	0.106	0.125	0.121	0.113	0.128	0.142	0.237	0.237	0.237	0.161	0.135	

Note:
*** = significant at 0.1% level of significance
st = seed Tree
emb = embryo
pol = pollen

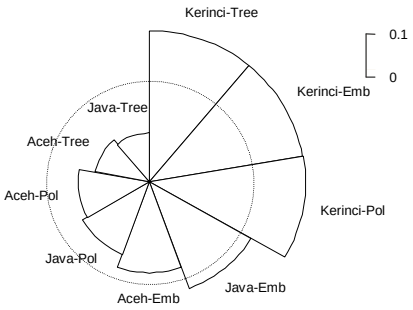
Figure 02. Allelic differentiation (D_j) among population as shown by differentiation snail.



NDH-A



FDH-A



Gene Pool

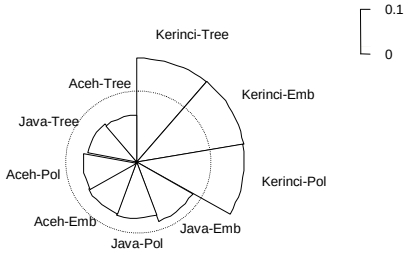


Figure 02 (*continued*..).Allelic differentiation (D_j) among population as shown by differentiation snail.

DISCUSSION

Knowledge of the distribution of genetic variation within and between population is of substantial benefit in tree breeding and in the conservation of plant genetic resources. The genetic variation reported in this chapter might contribute to the currently available information on the pattern of genetic variation in *P. merkusii*. It was clearly mentioned that *P. merkusii* in Indonesia harbours larger amounts of genetic variation than was found in mainland provenances. Such high level of genetic variation within population is a common phenomenon in most pines as was reported by LEDIG (1986). It was found that of 20 conifer species, the means 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 this 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 means 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 still comparable to the values found in the seed orchard population as reported in this study (PPL= 80.0, Ho=0.433 and A/L=2.40). This evidence would therefore confirm the finding of the previous study. MUONA and HARJU (1989)

mentioned that the high variation in pine species is due mainly to wind-mediated pollination, heavy flower production, considerable pollen migration and large effective population size. The genetic differentiation of the Kerinci population was larger than that of the other populations (see Figure 02). It was still only moderate, because this population was fixed to the globally most frequent allele.

The absence of any genetic variation found in Kerinci has raised speculations about the possible causes. It may be reasonable to adopt the possible explanation for low genetic variation found in Thailand and Vietnam. CHANGTRAGOON and FINKELDEY (1995) as well as SZMIDT et al. (1996) mentioned some possible causes in order to explain the low level of genetic variation in *P. merkusii* of mainland Asia which are bottlenecks, reduced gene flow among populations, in breeding due to small population size and asynchronous flowering. With regards to Kerinci population, the additional possible explanation might be due to 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 has been a drastic reduction in the number of trees in Kerinci during this geological age, forming a bottleneck that resulted in low genetic variation. The same geological reason for the genetic fixation of *P. merkusii* in Kerinci may be 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). The low genetic diversity could then be the result of the small population of Kerinci because random genetic drift occurs particularly in small populations (HARTL, 1980) and results in fixation of alleles after many generations.

Low genetic distance between the seed orchard population in Java and the natural population in Aceh is due to historical background of this species introduction. The existence of *P. merkusii* plantations in Java was introduced from a sub population of Aceh natural forest in early 1930. However, the exact origin of this sub-population is still not yet confirmed, presumably of Blang Kejeren population (HARDIYANTO, 1996). Unfortunately, there is currently no information available on patterns of genetic variation within Aceh population. Similarity in gene pool diversities among seed tree, embryo and pollen which were found in the seed orchard population would suggest little gene flow. On the other hand, higher gene pool diversity found in pollen of the natural population of Aceh would suggest extensive gene inflow. This natural population has a total area of around 7000 hectares (NN, 1988).

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