



Genetic Variation of Six *Azadirachta excelsa* (Jacks) Jacobs Populations

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Abstract

The study of the extent and pattern of genetic variation of six populations of *Azadirachta excelsa* (Jacks) Jacobs, i.e. Manong (Perak), Pokok Sena (Kedah), Sik (Kedah), FRIM (Selangor), Merchang (Terengganu) and Sg. Caru (Pahang) was carried out using starch gel electrophoresis. The analysis on eleven enzyme systems was found to be coded by 25 and 26 loci. The mean of observed heterozygosity ranged from 0.08 to 0.11 and the percentage of proportion of polymorphic loci varied from 61.54% to 73.08%. The extents of genetic identities ranged from 0.81 to 0.98.

Keywords: *Azadirachta excelsa*, Isozyme, Observed heterozygosities, Polymorphic loci

1. Introduction

The ecosystem of tropical rainforest of Malaysia is extremely complex and rich with tree species. In Peninsular Malaysia alone, the flora is estimated to comprise of 7,500 species of seed plants of which 4,100 are woody species (Whitmore 1975). However, its species richness may be threatened due to rapid destruction of the forest. Globally, an estimation of indiscriminate deforestation of tropical forest at 10.4 million hectares a year in the period of 2000-2005 (FAO 2005) has caused the genetic erosion of many valuable forestry species. In Malaysia alone the rate of change in total deforestation is about 85.7% between 2000-2005 and 1990-2000 period (FAO 2005). This has resulted in the disappearance of many valuable genetic traits that gives cause for much apprehension. Forest plantation is one of the approaches which is not only catering the problem of timber shortage but can also be served as a conservation mean.

In Malaysia, most of plantation forestry involved exotic species such as *Acacia mangium*, *Tectona grandis*, *Khaya ivorensis* and *Pinus caribaea* but no major successful outputs were recorded to date. However, the approach of establishing plantation forestry should not be stopped with that hiccup. The idea by shifting the attention to the fast growing indigenous is highly acceptable because these species are readily available at vast in our region and well adapted to our physical and biological environment. The cost of establishing and managing plantations can be reduced by this movement and conservation works can also be done at the same time. Nevertheless, careful plans should be initiated first together with extensive and thorough researches to avoid any unnecessary outcome. One of the important scientific information that should be gathered is about genetic variation of selected indigenous species. However, the knowledge about genetic traits of promising indigenous species is still lacking. This information is actually needed which is can help forest managers to identify the appropriate indigenous species for plantation and conservation purposes.

In this study, we used *Azadirachta excelsa* which is said to be one of the fast growing indigenous species. *A. excelsa* has the potentials to be considered as a plantation species to this region. *A. excelsa* is a tree of lowland monsoon forests from sea level up to an altitude of approximately 300 m and is of scattered distribution. It inhabits a vast region in Southeast Asia extends from Peninsular Malaysia, Sumatra, Borneo to northward in Myanmar, the Philippines, Papua New Guinea and the Aru Islands (Schmutterer and Doll 1993). It is a multipurpose valuable species that has been reported to produce good quality timber for use in construction, panelling, partitioning, flooring, plywood manufacture and packing cases, house and boat building, cigar boxes, shop fitting and production of piano (Jacobs 1960, Burgess 1966, Wong 1976, Corner 1988 and Schmutterer and Doll 1993). The young shoots can be eaten as vegetables or salad while the intensely bitter old leaves have been used in traditional medicines for the treatment of dysentery and diarrhea

(Anon 1995). In addition, chemicals such as azadirachtin and marragin extracted from seed kernels and leaves were more efficacious as an insecticide and growth regulator (Schumutterer 1989, Ermel *et al.* 1991, Mordue and Blackwell 1993).

Isozyme analysis has been used widely to detect the genetic variation of plant species especially using starch gel electrophoresis procedure. With the fast technology advancements, several other reliable markers have been introduced such as RAPD, RFLP, AFLP and SSR. However, isozyme technique still stands by its own advantages such as cheap, fast and not affected by environmental changes. This analysis is one of the multiple forms of an enzyme that produce similar or identical catalytic activities (Feret and Bergmann 1976) and the assessment of single genetic markers is reliable when compared with morphological markers such as colour, tree form and size.

The objective of this study was to assess the amount and distribution of genetic variation of six populations of *A. excelsa* in Peninsular Malaysia by means of isozymes analysis.

2. Materials and methods

2.1 Plant materials

Thirty matured leaves of *A. excelsa* were collected randomly from six populations namely Manong (Perak), Pokok Sena and Sik (Kedah), Bukit Lagong Forest Reserve of Forest Research Institute of Malaysia (Selangor), Merchang (Terengganu) and Sg. Caru (Pahang). Populations from Manong, Pokok Sena and Sik were collected from natural forest stands while populations from FRIM and Sg. Caru were from artificial stands. Seedlings of Merchang, Terengganu origin were obtained from a trial plot established at Pasoh (Negeri Sembilan).

2.2 Sample preparation

0.6 g of each leaf sample was homogenised with liquid nitrogen before adding to 1200 µl of leaf extraction buffer. The samples were then centrifuged at 10 000 rpm for 5 minutes. Supernatant was removed and stored at -70 °C before being used.

2.3 Isozymes analysis

Starch gel was prepared using 10.5 % of potato hydrolysed starch. The resulting homogenate was absorbed onto filter paper (Whatman No. 3) and being inserted gently onto the slice gel. This gel was placed between two electrodes containing 500 ml of electrode buffer. Both the gel and bridge wicks were covered with a polythene sheet to prevent the gel from drying up during the electrophoretic run. Eleven enzymatic proteins were used to stain and they were Alcohol dehydrogenase (ADH), Esterase (EST), Glutamate oxaloacetate transaminase (GOT), α-Glycerophosphate dehydrogenase (α-GPDH), Isocitrate dehydrogenase (IDH), NADP Nothing dehydrogenase (NDH), 6-Phosphogluconic dehydrogenase (6-PGD), Phosphoglucose Isomerase (PGI), Phosphoglucose mutase (PGM), Shikimate dehydrogenase (SDH) and Tetrazolium oxidase (TO).

3. Results

Genetic variation was assessed based on allelic frequencies, observed and expected heterozygosities. It was found that they were coded by 25 and 26 loci. Generally for all loci were polymorphic except 4 loci namely 6-Pgd-1, Pgi-1, To-1 and To-3 where they were monomorphic (Table 1). The mean of observed heterozygosity was found to vary from 0.0800 to 0.1115 while the expected heterozygosity ranged from 0.1474 to 0.1902. The observed heterozygosities were found to show lower values than the expected heterozygosity. The percentage of polymorphic loci among population produced varied from 62% to 73% with the highest polymorphic loci proportion achieved by Manong population and the lowest by Sg. Caru population (Table 2).

Table 3 shows the Nei's coefficient of genetic identities for all possible combinations among six populations of *A. excelsa*. Genetic identities ranged from 0.815 to 0.984 with the mean of 0.889. The combination of Pokok Sena (Kedah) - Sik (Kedah) gave the highest value of genetic identity which is 0.984 and the combination of FRIM (Selangor) - Sik (Kedah) gave the lowest value of genetic identity with 0.815 (Figure 1).

4. Discussion

4.1 The extent of intraspecific variation

The results obtained in this study especially on the range of observed heterozygosity is similar to the ones obtained by Kueh (unpublished). In fact the value of heterozygosities are comparatively similar to Nor Aini and John Keen (2003) on *Acacia crassicarpa* and Moran *et al.* (1989) on *Pinus radiata* but are lower than values obtained in other indigenous species like *Shorea acuminata* by Kong (unpublished), *S. curtisii* by Daim (unpublished) and *S. leprosula* by Daisy (unpublished). However, these values are noted to fall within the range suggested by Hamrick and Loveless (1986) and Loveless and Hamrick (1987) for tropical trees, i.e. between 0.00 to 0.216.

The polymorphic loci among populations produced an average of 67.5%, which is higher than Kueh (unpublished) on the same species and other *Acacia* species (Nor Aini and John Keen 2003) but is lower than *Shorea* species (Table 4).

The results produced were also higher than the one reported for tropical trees i.e. 11.1% to 54.2% according to Loveless and Hamrick (1987) and is also higher than the Australian tree species according to Moran (1992).

Generally, this species showed low values for both heterozygosities and proportion of polymorphic loci. This could be due to the limited gene exchange within and between populations as seedlings from these populations were raised under uniform environments. Furthermore the limited gene exchange could be due to seeds and seedlings from similar mother trees as a result of their poor occurrence in their natural population. This is evident from population Sik (Kedah), where the seeds and seedlings collected for a provenance trial were actually from only 14 mother trees that were separated 10 m away (Jusoh *pers. comm.*).

Population from Manong (Perak) exhibited low level heterozygosity, i.e. 0.105 although it possessed the highest level of proportion polymorphic loci i.e. 73% (Table 2). Low level of heterozygosity inferred that there is a possibility of related mating between few mother trees. On the other hand, the highest proportion of polymorphic loci of 73% suggests this population has undergone competition and selection within species in the natural stand. Polymorphism is required as part of the adaptive strategies of population in a heterogeneous environment of the forest (Feret and Bergman 1976).

Also, the overall low heterozygosity in these populations indicated that these populations undergo genetic drift where isolation with a small population size often increased the possibility of genetic drift which will reduce the genetic variability as a result of bottlenecks. This phenomenon has been well discussed by other researchers such as Hamrick (1983), Kimberly and Constance (1991) and Liengsiri *et al.* (1995).

Based from both criteria, Manong (Perak) is the most potential population to be conserve or to be sources for future plantation programme purposes. However, values on polymorphism tend to fluctuate depending on the enzyme systems used. Variability could also be increased further if genetic variation is assessed directly from actual mother trees from natural population.

4.2 Genetic identity between population

The mean genetic identity found in this study (0.889) was found to be slightly higher than the one recorded by Kueh (unpublished) on the same species, i.e. 0.820. Figure 1 shows a dendrogram constructed based on genetic identities using un-weighted group analysis (UPGMA). These populations were clustered into two main groups suggesting that they are closely related genetically and may share a common ancestor.

The dendrogram also suggest a clear linkage with the historical and geographical factors. FRIM (Selangor) and Manong (Perak) populations were found to cluster closely together because based on the history, population from FRIM is originated from Perak according to Abd-Ghani (*pers. comm.*). Moreover, the results of clusteration showed a clear association with geographic linkages. Populations from Pokok Sena (Kedah) and Sik (Kedah) might be related to each other because they are from the same geographic region. Pokok Sena area is located not far away from Sik area which is about 110 km away from each other (Jusoh *pers. comm.*). Similar reason can be true also to explain the phylogenetic relationship between Merchang (Terengganu) and Sg. Caru (Pahang) populations. According to Yeh and O'Malley (1980), if geographic distance between population decreases, the genetic similarity between them increases.

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Table 1. Allelic Frequencies of 25 and 26 loci in 6 populations of *A. excelsa*.

Locus	Allele	Population					
		Manong	Pokok Sena	Sik	FRIM	Merchang	Sg. Caru
Adh-1	F	0.0333	0.0833	0.1500	-	-	-
	M	0.9333	0.8333	0.7833	0.9667	1.0000	1.0000
	S	0.0334	0.0834	0.0667	0.0333	-	-
Adh-2	F	-	-	0.0833	0.0500	-	-
	M	1.0000	1.0000	0.7500	0.9167	1.0000	0.9333
	S	-	-	0.1667	0.0333	-	0.0667
Adh-3	F	0.8500	-	-	0.7833	-	-
	M	0.0833	-	0.1000	0.1000	-	-
	S	0.0667	1.0000	0.9000	0.1167	1.0000	1.0000
Est-1	F	-	-	-	-	-	0.0333
	S	1.0000	1.0000	1.0000	1.0000	1.0000	0.9667
Est-2	F	0.1833	0.3500	0.3667	0.2333	0.1500	0.2167
	S	0.8167	0.6500	0.6333	0.7667	0.8500	0.7833
Est-3	F	-	0.0167	-	-	0.0667	-
	S	1.0000	0.9833	1.0000	1.0000	0.9333	1.0000
Got-1	F	0.1500	0.1667	0.1833	0.0500	0.1000	0.0833
	S	0.8500	0.8333	0.8167	0.9500	0.9000	0.9167
Got-2	F	0.2167	0.1833	0.1500	0.0833	0.2333	0.2167
	M	0.7833	0.7000	0.7500	0.8833	0.7667	0.7833
	S	-	0.1167	0.1000	0.0334	-	-
Got-3	F	0.8500	0.6833	0.8000	-	-	-
	M	0.1500	0.1167	0.1000	0.0667	0.0167	-
	S	-	0.2000	0.1000	0.9333	0.9833	1.0000
α Gpdh-1	F	0.2000	0.1167	0.0833	0.1833	0.1667	0.1500
	S	0.8000	0.8833	0.9167	0.8167	0.8333	0.8500

Table 1 (continued)

Locus	Allele	Population					
		Manong	Pokok Sena	Sik	FRIM	Merchang	Sg. Caru
Idh-1	F	0.9667	0.9333	1.0000	1.0000	1.0000	0.9833
	S	0.0333	0.0667	-	-	-	0.0167
Idh-2	F	0.0333	0.0167	0.0167	0.0833	0.0667	-
	M	0.0500	0.0167	0.0333	0.0500	0.8333	-
	S	0.9167	0.9667	0.9500	0.8667	0.0500	1.0000
Idh-3	F	0.2000	0.0833	0.0833	0.1333	0.1333	0.0500
	M	0.6000	0.8333	0.7833	0.7833	0.7500	0.8333
	S	0.2000	0.0834	0.1334	0.0834	0.1167	0.1167
Ndh-1	F	0.0500	0.0667	-	0.4667	0.0833	0.1500
	S	0.9500	0.9333	1.0000	0.5333	0.9167	0.8500
6 Pgd-1	F	1.0000	-	1.0000	1.0000	-	1.0000
6 Pgd-2	F	0.9667	0.0833	0.0667	1.0000	0.0667	0.1167
	M	0.0333	0.9167	0.9333	-	0.8833	0.8500
	S	-	-	-	-	0.0500	0.0333
6 Pgd-3	F	0.9833	1.0000	1.0000	0.3667	0.9667	1.0000
	M	0.0167	-	-	0.5833	0.0333	-
	S	-	-	-	0.0500	-	-
Pgi-1	F	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Pgi-2	F	0.1167	0.0667	0.0333	0.0167	0.0833	0.0333
	M	0.1333	0.0333	0.1167	0.0167	0.0667	0.0667
	S	0.7500	0.9000	0.8500	0.9666	0.8500	0.9000
Pgi-3	F	0.6500	0.5167	0.6333	0.8333	0.6667	0.7167
	M	0.2500	0.4500	0.3000	0.1167	0.3167	0.2000
	S	0.1000	0.0333	0.0667	0.0500	0.0166	0.0833
Pgm-1	F	0.2167	0.1000	0.0500	0.3167	0.0833	0.1333
	S	0.7833	0.9000	0.9500	0.6833	0.9167	0.8667

Table 1 (continued)

Locus	Allele	Population					
		Manong	Pokok Sena	Sik	FRIM	Merchang	Sg. Caru
Pgm-2	F	0.0833	0.1167	0.1500	0.1333	0.1833	0.0833
	S	0.9167	0.8833	0.8500	0.8667	0.8167	0.9167
Sdh-1	F	0.0333	1.0000	0.9833	0.1833	0.0833	0.1500
	M	0.1333	-	0.0167	0.0833	0.1000	0.1167
	S	0.8334	-	-	0.7334	0.8167	0.7333
To-1	F	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
To-2	F	0.9167	0.8667	0.8667	0.8833	0.9500	0.8333
	M	0.0500	0.0333	0.0500	0.0500	0.0333	0.0167
	S	0.0333	0.1000	0.0833	0.0667	0.0167	0.1500
To-3	F	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Table 2. Mean observed (Ho) and expected heterozygosity (He) and percentage of polymorphic loci of *A. excelsa*.

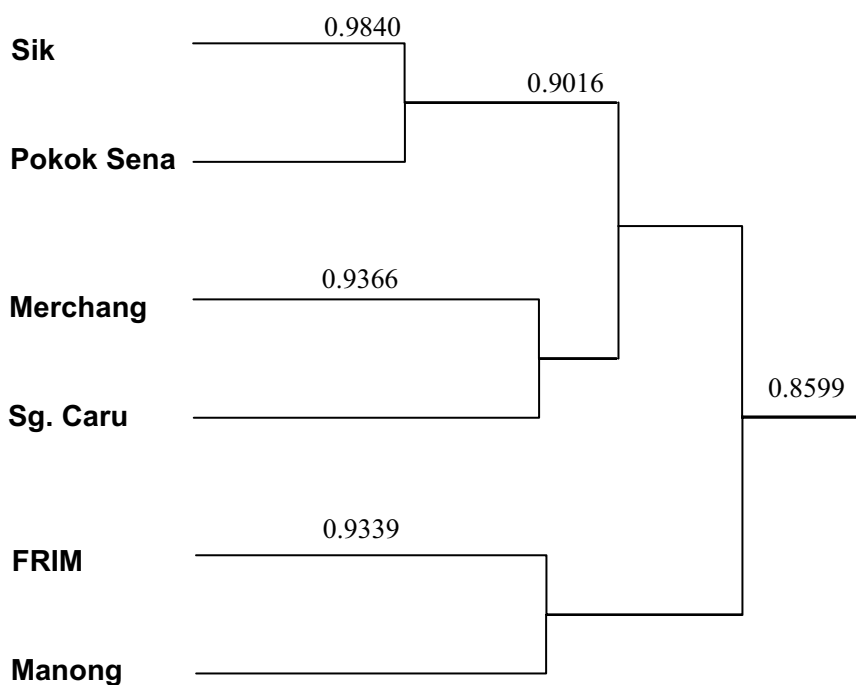
	Population					
	Manong	Pokok Sena	Sik	FRIM	Merchang	Sg. Caru
Ho	0.1051	0.0999	0.1115	0.0846	0.0800	0.0846
He	0.1719	0.1721	0.1759	0.1902	0.1579	0.1474
Proportion Polymorphic Loci (%)	73	68	65	69	68	62

Table 3. Nei's coefficients of genetic identity among six natural populations of *A. excelsa*

	Sik	Pokok Sena	FRIM	Merchang	Sg. Caru
Manong	0.852	0.894	0.934	0.824	0.886
Sik		0.984	0.815	0.861	0.915
Pokok Sena			0.852	0.888	0.943
FRIM				0.845	0.911
Merchang					0.937

Table 4. The mean heterozygosity and percentage of proportion polymorphic loci.

Species	H	P (%)	References
<i>Acacia crassicaarpa</i>	0.086	58.7	Nor Aini and John (2003)
<i>Azadirachta excelsa</i>	0.086	60.8	Kueh (unpublished)
<i>A. excelsa</i>	0.094	67.5	Present study
<i>Shorea acuminata</i>	0.604	100	Kong (unpublished)
<i>S. curtisii</i>	0.480	72.2	Daim (unpublished)
<i>S. leprosula</i>	0.457	93.3	Daisy (unpublished)
<i>S. parvifolia</i>	0.535	95.2	Kong (unpublished)

Figure 1. Dendrogram of Six Populations of *A. excelsa* using Un-Weighted Pair Group Cluster Analysis of Identity Coefficients