Journal of Tropical Forest Research 1 (1) : 11-22 (2017)

Original article

Isozyme Variation among 22 Neem (*Azadirachta indica* A. Juss.) Provenances

Wathinee Suanpaga[1*] **Suree Bhumibhamon[1]**
Damrong Pipatwattanakul[1] **Sranya Vojrodaya[2]**
Suchitra Changtragoon[3]

[1]Faculty of Forestry, Kasetsart University, Bangkok, 10900 THAILAND
[2]Faculty of Science, Kasetsart University, Bangkok, 10900 THAILAND
[3]Forest Genetic Conservation and Biotechnology Research Group, Forest and Plant Conservation Research Office, National Parks, Wildlife and Plant Conservation Department, Bangkok, 10900 THAILAND
*Corresponding Author, E-mail: wathinee.s@ku.ac.th

Received: Apr 3, 2016 Accepted: May 31, 2016

ABSTRACT

Neem is one of the most popular multi-purpose tree species that has been planted throughout tropical countries because it grows well on various sites with poor soil and low annual rainfall. Genetic data among Neem provenances is necessary for tree improvement program. Thus this study examined the genetic variation among 22 Neem provenances from the International Provenance Trial in Kampheng Phet province using eight isozyme systems: diaphorase (DIA), format dehydrogenase (FDH), glutamate oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G-6PDH), isocitrate dehydrogenase (IDH), malate dehyfrogenase (MDH), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKDH). The 12 gene loci from these enzyme systems were found in 22 provenances. DIA-A, DIA-B, GOT, MDH-C, and SKDH showed variations between Thai and Indian Neems. GOT, G-6PDH, IDH, MDH-A, MDH-B, PGM-A, and PGM-B showed moderate to high levels of provenance variation. The average expected heterozygosity (*He*=0.226) and Wright's F_{ST} (0.58) were high, which indicated wide genetic variation among provenances. The genetic distances (D) between Thai and Indian Neems were high (D>1.00). However, D values within Thai Neem provenances and within Indian Neem provenances were low. This can be used in future breeding work.

Keywords: Isozyme, *Azadirachta indica* A. Juss., Provenance

INTRODUCTION

Neem (*Azadirachta indica* A. Juss.) belongs to the family Meliaceae. *A. indica* var. *siamensis* (Thai Neem) is native to Thailand (Santisuk, 1993), but *A. indica* (Indian Neem) was introduced to Thailand. Thai Neem is distributed throughout the country, while Indian Neem is seldom found in Thailand (Bhumibhamon and Kamkong, 1997). A common method for identifying the species in Thailand is to observe the leaves (Boontawee *et al*., 1993).

Natural hybrids between Indian Neem and Thai Neem can be found in Thailand where both species grow together and are intermediate regarding the shape and consistency of the leaflets (Schmutterer, 1995).

Neem is one of the most popular multi-purpose tree species planted in various areas because of its potential uses. It is an outstanding example of a species which is highly efficient in restoring soil productivity and simultaneously providing fodder, fire wood, and other products to meet basic needs in rural households like medicines, pesticides, mosquito repellants, fertilizers, diabetic food, soaps, lubricants, gums, agricultural implements, tooth paste, tooth brush sticks, and even contraceptives. Neem oil is used against stomach ulcers, worm infections, and rheumatism. It is a general practice to store grains using Neem leaves to repel insect pests (Tewari, 1992; Gupta, 1993; Puri, 1999).

Although, the Neem tree can grow in unfertile and droughted areas, severe dieback syndrome that appeared on Neem grown in Sahelian Africa was the initial stimulus for Neem improvement. The International Neem Network, thus, was established in 1993 with the long term objective to improve the genetic quality by broadening the existing gene pool and adaptability of Neem and to improve its utilization. Thailand cooperated in this project by establishing the International Provenance Trials of Neem in 1997 using 24 provenances from India, Lao P.D.R., Myanmar, Nepal, Pakistan, Sri Lanka, Senegal, and Thailand. The genetic data among Neem provenances is useful for further tree improvement procedure. Thus, this study aimed to examine the genetic variation among Neem provenances using an isozyme technique.

MATERIALS AND METHODS

Seed Sources

Seeds for the trial establishment were collected from 22 provenances from India, Lao P.D.R., Myanmar, Nepal, Pakistan, Sri Lanka, Senegal, and Thailand (Table 1).

Isozyme Study

The mature leaves of 20 trees from 22 provenances of Neem were collected from the International Provenance Trials in Kamphaeng Phet. These leaves were extracted in buffer pH 7.3 containing 0.132 M Tris, 0.003 Triplex II, 5% PVP, 0.003 M DTT, and 1% merceptoethanol. The crude extracts were applied on 12% starch gel and separated for 4-5.5 hrs under five different buffer conditions. Eight isozyme systems were analyzed: diaphorase (DIA, EC 1.6.4.3), format dehydrogenase (FDH, EC 1.2.1.2), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), glucose-6-phosphate dehydrogenase, (G-6PDH, EC 1.1.1.49), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehyfrogenase (MDH, EC 1.1.1.37), phosphoglucomutase (PGM, EC 2.7.5.1), and shikimate dehydrogenase (SKDH, EC 1.1.1.25). The electrophoresis and staining procedures were modified from Feret and Bergmann (1976), Conkle *et al*. (1982), Vallejos (1983), and Changtragoon and Fikeldey (1995).

Data Analysis

The allelic frequency, genetic distance, and cluster analysis among provenances were performed using the GeAlEx software. The Euclidean distance and clustering using the UPGMA method were used to analyze provenance groups.

Table 1 Seed sources for the International Provenance Trials of Neem.

Provenance code	Seed source	Latitude	Longitude	Altitude (m)	Mean annual rainfall (mm)
03/IND/Man	Mandore, Jodhpur, India,	26°18′N	73°01′E	224	373
04/IND/Chi	Chitradurga, Karnataka, India	14°02′N	76°04′E	615	417
05/IND/All	Allahabad Town, Uttar Pradesh, India	25°28′N	81°54′E	320	910
06/IND/Ann	Annur, Tamil Nadu, India	11°17′N	77°07′E	360	875
07/IND/Gha	Ghaati Subramanya, Karnataka, India	13°22′N	77°34′E	950	741
08/IND/Sag	Sagar, Chanatoria Madhya Pradesh, India	21°51′N	78°45′E	527	1405
09/IND/Bal	Balharshah, Maharashtra, India	19°51′N	79°25′E	250	Approx. 1000
10/IND/Ram	Ramannaguda, Orissa, India	19°05′N	83°49′E	250	1100
11/LAO/Vie	Vientiane, Lao P.D.R.	18°00′N	102°45′E	180	1540
12/MYA/Mye	Myene, Myanmar	22°03′N	95°13′E	76	809
13/MYA/Yez	Yezin, Myanmar	19°51′N	96°16′E	100	1269
14/NEP/Lam	Lamahi, Nepal	27°52′N	82°31′E	350-440	1500
15/NEP/Get	Geta, Nepal	28°46′N	80°34′E	170	1725
16/PAK/Tib	Tibbi Laran, Rahimyar Khan, Pakistan	28°24′N	70°18′E	115	140
17/PAK/Mul	Multan, Cantonment Area, Pakistan	30°11′N	71°29′E	>150	276
18/SRL/Kul	Kuliyapitiya, Sri Lanka	07°08′N	80°00′E	-	1397
19/THA/Tun	Tung Luang, Thailand	09°09′N	99°07′E	4	1755
20/THA/Non	Ban Nong Rong, Thailand	14°05′N	99°40′E	40	1145
21/THA/Bo	Ban Bo, Thailand	16°17′N	103°35′E	150	1400
22/THA/Doi	Doi Tao, Thailand	17°57′N	98°41′E	300	1250
23/GHA/Sun	Sunyani, Ghana	07°21′N	02°21′W	950-1000	1270-1400
24/SEN/Ban	Bandia, Senegal	14°30′N	17°02′W	50	436

RESULTS AND DISCUSSION

Genotypic Structure

The genotypic structure of 12 gene loci was detected from 22 Neem provenances (Figure 1). In the gene loci DIA-A and DIA-B, genotype 1x1 was detected only in Indian Neem provenances and more frequently than genotype 2x2 which was presented only in Thai Neem provenances. FDH was found in only one genotype, GOT had two genotypes in which genotype 1x2 was detected in Thai Neem, but genotype 2x3 appeared in Indian Neem. G-6PDH had six genotypes of which the most frequent was 2x2 and a rare genotype was 1x3. However, the six genotypes of IDH appeared with the rare genotype being 3x3 and the most frequent was 2x2.

The gene locus MDH-A had six genotypes of which the genotype 1x1 was the most frequent and genotype 1x3 was rare. However, only five genotypes were detected

in the gene locus MDH-B of which the most frequent was 2x2 and a rare genotype was 1x3. On the other hand, the gene locus MDH-C had only one genotype in Indian Neem provenances. Four genotypes presented in PGM of which genotype1x2 was the most frequent and genotype 1x3 was rare. In contrast, in the gene locus PGM-B, the genotype 1x3 was the most frequent and genotypes 1x2 and 3x3 were rare in the six genotypes. The gene locus SKDH genotype 1x1 was detected only in Thai Neem provenances and was less frequent than genotype 2x2 which presented only in Indian Neem provenances.

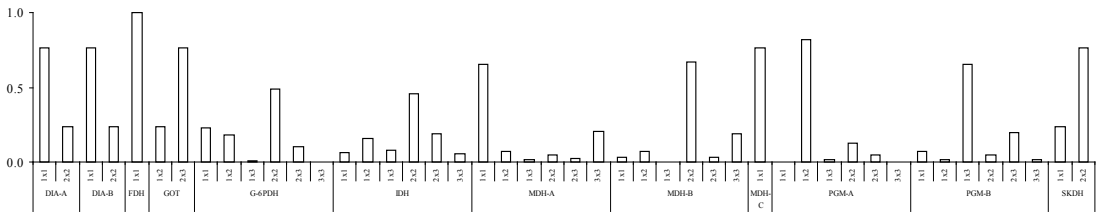

Figure 1 Genotypic frequency of22 Neem provenances.

Allelic Frequency

The results of the present study showed that DIA-A, DIA-B, FDH, MDH-C, and SKDH were monomorphisms, while GOT, G-6PDH, IDH, MDH-A, MDH-B, PGM-A, and PGM-B were polymorphisms with these gene loci showing moderate to high levels of variation on allele frequency as presented in Table 2.

In the gene loci DIA-A and DIA-B, allele 1 presented only in Indian Neem provenances and allele 2 appeared only in Thai Neem provenances. In the gene locus GOT, all provenances possessed allele 2, but allele 1 presented only in Thai Neem provenances while allele 3 occurred only in Indian Neem provenances. The gene locus G-6PDH was variable with allele 1 presented in all Thai Neem provenances and in some Indian Neem provenances except Provenances 08/IND/Sag, 09/IND/Bal, 03/IND/Man, 16/PAK/Tib, 17/PAK/Mul, and 18/SRL/Kul, but allele 2 was detected in all Indian Neem provenances and in two Thai Neem provenances (20/THA/Non and 19/THA/Tun). Moreover, allele 3 was rare and appeared in some Indian Neem provenances (10/IND/Ram, 13/MYA/Yez, 12/MYA/Mye, 08/IND/Sag, 07/IND/Gha, 04/IND/Chi, 06/IND/Ann, 05/IND/All, and 15/NEP/Get).

However, IDH was the most variable enzyme gene locus with allele 1 occurring in all provenances except Provenance 14/NEP/Lam, and all provenances possessed allele 2, but allele 3 presented in all provenances except Provenances 10/IND/Ram and 23/GHA/Sun. Variations were found in the allele structure of MDH-A and MDH-B but not in MDH-C. In gene locus MDH-A, allele 1 was detected in all provenances except Provenances 22/THA/Non, 11/LAO/Vie, and 19/THA/Tun, and allele 2 did not present in Provenances 19/THA/Tun, 12/MYA/Mye, 06/IND/Ann, 05/IND/All, 16/PAK/Tib, 18/SRL/Kul, and 24/SEN/Ban, but allele 3 was the most frequent in all Thai Neem provenances and rarely found in

two Indian Neem provenances (03/IND/Man and 06/IND/Ann). In gene locus MDH-B, allele 1 occurred in all provenances except Provenances 20/THA/Non, 11/LAO/Vie, 19/THA/Tun, 08/IND/Sag, 17/PAK/Mul and 24/SEN/Ban, while allele 2 was most frequent in Indian Neem provenances but was rarely detected in Thai Neem provenances. On the other hand, allele 3 was most frequent in Thai Neem provenances but rarely presented in Provenances 06/IND/Ann, 16/PAK/Tib, and 17/PAK/Mul. The allele 1 of gene locus MDH-C appeared only in Indian Neem provenances.

PGM-A and PGM-B were variable enzyme gene loci. In gene locus PGM-A, all provenances possessed alleles 1 and 2 but allele 3 was only present in Provenances 20/THA/Non, 19/THA/Tun, 10/IND/Ram, 07/IND/Gha, 14/NEP/Lam, 15/NEP/Get, 17/PAK/Mul, and 18/SRL/Kul. In gene locus PGM-B, allele 1 was detected in all provenances except Provenance 11/LAO/Vie but allele 2 occurred in all Thai Neem provenances and in some Indian Neem provenances such as Provenances 10/IND/Ram, 09/IND/Bal, 07/IND/Gha, 14/NEP/Lam, 17/PAK/Mul, and 18/SRL/Kul. Furthermore, all provenances possessed allele 3. In gene locus SKDH, allele 1 presented only in Thai Neem provenances, while allele 2 was found only in Indian Neem provenances.

Allelic frequencies among provenance can become different because of random processes (random genetic drifts) as well as by natural selection with complications from migration among the subpopulations (Hartl and Clark, 1997).

Genetic differentiation

Table 3 shows that the average Proportion of Polymorphic Gene Loci (PPL) of Neem was 57.02%. The PPL of Indian Neem (57.22) was higher than that of Thai Neem (56.36). The highest value of PPL was 63.64% in Provenances 20/THA/Non, 10/IND/Ram, 13/MYA/Yez, 23/GHA/SUN, 07/IND/Gha, 04/IND/Chi 06/IND/Ann, 14/NEP/Lam, and 15/NEP/Get, while the lowest value was 45.46% in Provenances 16/PAK/Tib, 18/SRL/Kul, and 24/SEN/Ban. The mean Alleles per Locus (A/L) value of Neem in the present study was 1.76. Indian Neem had a higher average A/L than Thai Neem (1.77 and 1.74, respectively). Provenance 07/IND/Gha had the highest A/L value (2.00); on the other hand, the lowest A/L value was 1.545 in Provenances 11/LAO/Vie and 24/SEN/Ban. The mean gene pool diversity (v) of Indian Neem was greater than that of Thai Neem (1.44 and 1.38, respectively). The highest v was detected in Provenance 07/IND/Gha, followed by Provenances 14/NEP/Lam, 03/IND/Man, 20/THA/Non, and 10/IND/Ram (1.53, 1.51, 1.50, 1.49, and 1.49, respectively). The lowest v was found in Provenance 11/LAO/Vie (1.29).

Table 2 Allelic frequency among 22 Neem provenances.

Locus	Allele	21/THA/Bo	20/THA/Non	22/THA/Doi	11/LAO/Vie	19/THA/Tun	10/IND/Ram	13/MYA/Yez	23/GHA/Sun	12/MYA/Mye	08/IND/Sag	09/IND/Bal	07/IND/Gha	04/IND/Chi	03/IND/Man	06/IND/Ann	05/IND/All	14/NEP/Lam	15/NEP/Get	16/PAK/Tib	17/PAK/Mul	18/SRL/Kul	24/SEN/Ban
DIA-A	1	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DIA-B	1	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FDH	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GOT	1	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
	3	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
G-6PDH	1	1.00	0.92	1.00	1.00	0.79	0.26	0.05	0.50	0.12	0.00	0.00	0.18	0.02	0.00	0.02	0.21	0.52	0.02	0.00	0.00	0.00	0.22
	2	0.00	0.08	0.00	0.00	0.21	0.66	0.93	0.50	0.62	0.68	1.00	0.77	0.97	1.00	0.86	0.61	0.48	0.88	1.00	1.00	1.00	0.78
	3	0.00	0.00	0.00	0.00	0.00	0.08	0.02	0.00	0.27	0.32	0.00	0.05	0.02	0.00	0.12	0.18	0.00	0.10	0.00	0.00	0.00	0.00
IDH	1	0.16	0.29	0.58	0.07	0.55	0.05	0.13	0.17	0.23	0.15	0.14	0.05	0.17	0.35	0.05	0.18	0.00	0.10	0.10	0.09	0.05	0.20
	2	0.21	0.32	0.09	0.54	0.05	0.95	0.85	0.83	0.73	0.69	0.73	0.73	0.63	0.48	0.93	0.71	0.82	0.62	0.77	0.88	0.75	0.73
	3	0.63	0.39	0.33	0.39	0.40	0.00	0.02	0.00	0.03	0.16	0.13	0.23	0.20	0.17	0.02	0.11	0.18	0.28	0.13	0.03	0.20	0.07
MDH-A	1	0.04	0.08	0.00	0.00	0.00	0.68	0.83	0.91	1.00	0.86	0.84	0.86	0.90	0.75	0.98	1.00	0.90	0.98	1.00	0.53	1.00	1.00
	2	0.06	0.03	0.02	0.17	0.00	0.32	0.17	0.09	0.00	0.15	0.16	0.15	0.10	0.20	0.00	0.00	0.10	0.02	0.00	0.47	0.00	0.00
	3	0.90	0.89	0.98	0.83	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MDH-B	1	0.02	0.00	0.06	0.00	0.00	0.03	0.30	0.16	0.05	0.00	0.04	0.16	0.07	0.10	0.14	0.03	0.12	0.02	0.10	0.00	0.07	0.00
	2	0.09	0.07	0.30	0.06	0.03	0.97	0.70	0.85	0.95	1.00	0.96	0.84	0.93	0.90	0.83	0.97	0.88	0.98	0.87	0.94	0.93	1.00
	3	0.90	0.94	0.64	0.94	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.06	0.00	0.00
MDH-C	1	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGM-A	1	0.29	0.44	0.16	0.20	0.19	0.45	0.50	0.50	0.50	0.48	0.48	0.37	0.37	0.50	0.50	0.50	0.48	0.50	0.50	0.06	0.50	0.50
	2	0.71	0.50	0.84	0.80	0.67	0.50	0.50	0.50	0.50	0.52	0.52	0.53	0.63	0.50	0.50	0.50	0.50	0.47	0.50	0.50	0.48	0.50
	3	0.00	0.07	0.00	0.00	0.14	0.05	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.44	0.02	0.00
PGM-B	1	0.09	0.16	0.38	0.00	0.24	0.44	0.50	0.50	0.50	0.50	0.54	0.37	0.50	0.50	0.67	0.50	0.43	0.50	0.75	0.13	0.22	0.50
	2	0.38	0.37	0.19	0.48	0.45	0.07	0.00	0.00	0.00	0.00	0.23	0.15	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.53	0.55	0.00
	3	0.53	0.47	0.44	0.52	0.31	0.50	0.50	0.50	0.50	0.50	0.23	0.48	0.50	0.50	0.33	0.50	0.42	0.50	0.25	0.34	0.23	0.50
SKDH	1	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 3 Genetic parameters among 22 Neem provenances.

Variety	Provenance	N	A/P	PPL, %	A/L	v	Ho	He	F
Thai	21/THA/Bo	34	20	54.55	1.82	1.33	0.28	0.217	-0.06
	20/THA/Non	31	21	63.64	1.91	1.49	0.35	0.255	-0.12
	22/THA/Doi	32	19	54.55	1.73	1.39	0.32	0.225	-0.18
	11/LAO/Vie	27	17	54.55	1.55	1.29	0.30	0.206	-0.15
	19/THA/Tun	29	19	54.55	1.73	1.41	0.28	0.233	-0.03
Indian	10/IND/Ram	31	21	63.64	1.91	1.49	0.31	0.244	-0.03
	13/MYA/Yez	30	20	63.64	1.82	1.42	0.32	0.235	-0.13
	23/GHA/Sun	29	18	63.64	1.64	1.45	0.40	0.246	-0.29
	12/MYA/Mye	30	19	54.55	1.73	1.45	0.36	0.231	-0.24
	08/IND/Sag	31	18	54.55	1.64	1.45	0.41	0.242	-0.33
	09/IND/Bal	28	19	54.55	1.73	1.43	0.29	0.216	-0.14
	07/IND/Gha	31	22	63.64	2.00	1.53	0.39	0.271	-0.21
	04/IND/Chi	30	20	63.64	1.82	1.40	0.35	0.215	-0.28
	03/IND/Man	30	19	54.55	1.73	1.50	0.39	0.245	-0.29
	06/IND/Ann	29	21	63.64	1.91	1.34	0.28	0.195	-0.10
	05/IND/All	31	19	54.55	1.73	1.46	0.38	0.233	-0.28
	14/NEP/Lam	30	20	63.64	1.82	1.51	0.39	0.256	-0.21
	15/NEP/Get	30	21	63.64	1.91	1.42	0.36	0.212	-0.30
	16/PAK/Tib	30	18	45.46	1.64	1.32	0.29	0.182	-0.24
	17/PAK/Mul	16	20	54.55	1.82	1.46	0.30	0.225	-0.11
	18/SRL/Kul	30	19	45.46	1.73	1.39	0.27	0.194	-0.08
	24/SEN/Ban	30	17	45.46	1.55	1.39	0.32	0.205	-0.22
Thai	Mean	31	19.20	56.36	1.75	1.38	0.31	0.227	-0.11
Indian	Mean	29	19.47	57.22	1.77	1.44	0.34	0.226	-0.20
Mean		30	19.41	57.03	1.76	1.42	0.33	0.226	-0.18

Remarks: N = Sample size, A/P = No. of alleles per population, PPL = Proportion of polymorphic gene loci, A/L = Average no. of allele per gene locus, *v* = Genetic diversity, *He* = Expected heterozygosity, *Ho* = Observed heterozygosity, F = Fixation indices

The mean observed heterozygosity (*Ho*) of Neem in the present study was 0.33. Indian Neem had a greater *Ho* than Thai Neem (0.34 and 0.30, respectively). The mean expected heterozygosity (*He*) of Neem in the present study was 0.226. The average *He* value of Thai Neem was slightly more than that of Indian Neem (0.227 and 0.226, respectively). Provenance 07/IND/Gha had the greatest *He*, followed by 14/NEP/Lam, 20/THA/Non, 23/GHA/Sun, and

03/IND/Man (0.271, 0.256, 0.255, 0.246, and 0.245, respectively). Provenance 16/PAK/Tib had the lowest *He* (0.18). The high values of *He* were probably due to the mating system and adaptive features of Neem such as insect pollination, high fecundities, seed dispersal by birds, facultative selfing and outbreeding, and the large geographical range of the species. However, the gene diversity in Neem in this study was lower than that reported by Kundu (1999) because of the differences in the enzyme systems and the geographical zones of the populations studied.

Wright's *F* coefficients at 11 loci across the 22 provenances of Neem are shown in Table 4. While, the mean F_{IS} of the present study was negative because of the excess of heterozygotes in subpopulations, the mean F_{IT} was positive due to the deficiency of heterozygotes in the mean population. The possible explanation for this result may be attributed to many factors such as natural selection, genetic drift, inbreeding, nonrandom mating, and population subdivision (Halliburton, 2004). The mean F_{ST} was 0.58, indicating the wide genetic differentiation over subpopulations probably because of considerable differences between the geographical zones.

Table 4 Wright's *F* coefficients at 11 loci across 22 Neem provenances.

	H_I	H_S	H_T	F_{IS}	F_{IT}	F_{ST}
DIA-A	0.00	0.00	0.35	0.00	1.00	1.00
DIA-B	0.00	0.00	0.35	0.00	1.00	1.00
GOT	1.00	0.50	0.59	-1.00	-0.70	0.15
G-6PDH	0.28	0.22	0.49	-0.29	0.43	0.56
IDH	0.41	0.42	0.53	0.01	0.22	0.21
MDH-A	0.11	0.17	0.47	0.32	0.76	0.65
MDH-B	0.11	0.16	0.42	0.34	0.75	0.62
MDH-C	0.00	0.00	0.40	0.00	1.00	1.00
PGM-A	0.88	0.49	0.53	-0.80	-0.67	0.07
PGM-B	0.87	0.54	0.62	-0.61	-0.39	0.13
SKDH	0.00	0.00	0.35	0.00	1.00	1.00
Mean	0.33	0.23	0.46	-0.18	0.40	0.58

Remarks: H_I = Observed heterozygosities over all subpopulations, H_S = Expected heterozygosities over all subpopulations, H_T = Expected heterozygosities in total population, F_{IS} = Deviation among individuals relative to their subpopulation, F_{IT} = Deviation among individuals relative to total population, F_{ST} = Deviation among subpopulations relative to the total population

Genetic Distance

Within each variety (Thai and Indian Neems), the D values were differed little. The most and least D values within Thai Neem provenances were 0.012 (Provenance 21/THA/Bo x 20/THA/Non) and 0.060 (Provenance 22/THA/Doi x 19/THA/Tun), while the most and least D values within Indian Neem provenances were 0.002 (Provenances 12/MYA/Mye x 05/IND/All) and 0.104 (Provenances 23/GHA/Sun x 17/PAK/Mul). On the other hand, D values were extremely different between varieties. The D value between Provenances 19/THA/Tun and 06/IND/Ann was the most (1.832) but the least was between the Provenances 22/THA/Doi x 23/GHA/Sun (1.327). The results of high distances between Thai and Indian Neems varieties and low distances within Neem varieties was similar to the results of Changtragoon *et al.* (1996) and Kundu (1999).

Similarities or differences in the type, amount, and pattern of genetic variation between populations can be the result of many factors. If two populations are genetically similar, this may be because: 1) they recently separated into two populations, or 2) gene flow occurred between them, or 3) they were large populations (with little genetic drift), or 4) similar selection pressures affected loci similarly in both populations. Likewise, if two populations are different, then this could be because: 1) they have been isolated for a long time and there has been no gene flow between them, or 2) genetic drift has generated large differences, or 3) there are different selective pressures in the two populations (Hedrick, 2000). More than one or possibly all of these factors may be important in a particular situation of Neem in the present study. These results can be used in future breeding work and the selection of better genotypes.

Cluster Analysis

A dendrogram was constructed from the D values of Neem and is presented in Figure 2. It is clear that the two varieties (Thai and Indian Neems) had considerably different clusters. Within Thai Neem, Provenances 20/THA/Non and 21/THA/Bo had a close genetic distance. Thai Neems were divided into three clusters when D = 0.01: 1) 22/THA/Doi, 2) 19/THA/Tun, and 3) 21/THA/Bo, 20/THA/Non and 11/LAO/Vie. Among Indian Neems, Provenance 17/PAK/Mul had noticeable isolation and it was grouped into four clusters using D = 0.01: 1) 12/MYA/Mye, 05/IND/All, 24/SEN/Ban, 08/IND/Sag, 07/IND/Gha, 04/IND/Chi, 15/NEP/Get, 13/MYA/Yez, 09/IND/Bal, 06/IND/Ann, 16/PAK/Tib, and 03/IND/Man, 2) 10/IND/Ram, 23/GHA/Sun, and14/NEP/Lam, 3) 18/SRL/Kul; and 4) 7/PAK/Mul. The present genetic analysis suggested that the African provenances originated from the Indian subcontinent.

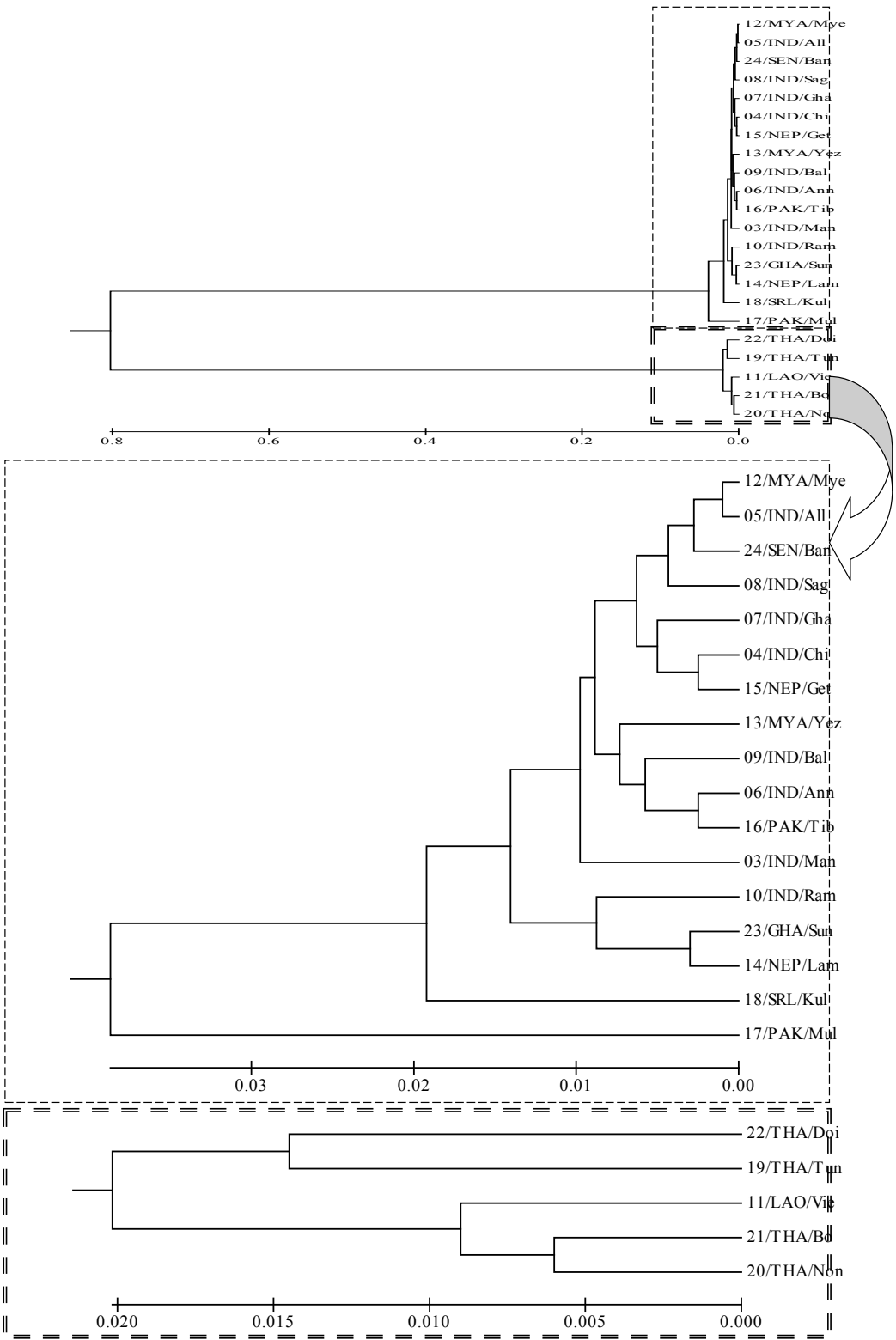

Figure 2 Genetic dendrogram of 22 Neem provenances.

CONCLUSION

The 12 gene loci from 8 enzyme systems were found in 22 provenances. DIA-A, DIA-B, GOT, MDH-C, and SKDH showed variations between Thai and Indian Neems. GOT, G-6PDH, IDH, MDH-A, MDH-B, PGM-A, and PGM-B showed moderate to high levels of provenance variation. The average expected heterozygosity (*He*=0.226) and Wright's F_{ST} (0.58) were high which indicated wide genetic variation among provenances. The genetic distances (D) between Thai and Indian Neems were high (D>1.00). However, D values within Thai Neem provenances (0.012-0.050) and within Indian Neem provenances (0.002-0.088) were low. This can be used in future tree improvement work.

ACKNOWLEDGEMENTS

Sincerely gratitude is recorded to the Kasetsart University Krabi campus, Thailand and to the Graduate School, Kasetsart University, for a scholarship and financial support. My grateful thanks are given to Mr. Prapai Khaennak for the help and hospitality provided during data collection. I would like to express my thanks to Mr. Thawee Kanhawan and Mr. Sa-nguan Wongsa, laboratory officers in the Forest Genetic Conservation and Biotechnology Research Group for their assistance.

REFERENCES

Bhumibhamon, S. and A. Kamkong. 1997. **Edible Multipurpose Tree Species**. National Research Council of Thailand. [In Thai]

Boontawee, B., C. Kanchanaburagura and S. Boonsermsuk. 1993. Studies on Neem in Thailand, pp. 41-45. In: Read M.D. and J.H. French. **Genetic Improvement of Neem: Strategies for the Future**. Proceedings of the International Consultation on Neem Improvement held at Kasetsart University, Bangkok, Thailand. 18-22 January 1993. Craftsman Press Ltd.

Changtragoon, S., A.E. Szmidt and B. Boontawee. 1996. The study of genetic diversity of Azadirachta spp. by using isozyme analysis. pp. 353-360. In: **Proceedings of the Third Asia-Pacific Conference on Agricultural Biotechnology**. 10-15 November, 1996. Hua Hin, Prachuapkhirikhan, Thailand.

Changtragoon, S. and R. Finkeldey. 1995. Genetic variation of *Pinus merkusii* in Thailand 1. Genetic analysis of isozyme phenotype. **Journal of Tropical Forest Science** 8 (2): 167-177.

Conkle, M.T., P.D. Hodgskiss, L.B. Nunnally and S.C. Hunter. 1982. **Starch Gel Electrophoresis of Conifer Seeds: A Laboratory Manual.** General Technical Report PSW-64. Pacific Southwest Forest and Range Experiment Station, Berkeley, California.

Feret, P.P. and F. Bergmann. 1976. Gel electrophoresis of proteins and enzymes. pp. 49-77. In: Mikshe, J.P. (ed.). **Modern Methods in Forest Genetic**. Springer, Berlin, Heidelberg.

Gupta, R.K. 1993. **Multipurpose Trees for Agroforestry and Wasteland Utilisation**. Oxford & IBH Publishing Co. PVT. Ltd., New Delhi.

Halliburton, R. 2004**. Introduction to Population Genetics**. Pearson Education, Inc., New Jersey.

Hartl, D.L. and A.G. Clark. 1997. **Principles of Population Genetics**. Sinauer Associates, Inc., Massachusetts.

Hedrick, P.W. 2000. **Genetics of Populations**. Jones and Bartlett Publishers, Inc., London.

Kundu, S.K. 1999. Comparative analysis of seed morphometric and allozyme data among four populations of Neem (*Azadirachta indica*). **Genetic Resources and Crop Evolution** 46: 569-577.

Puri, H.S. 1999. **Neem (*Azadirachta indica*): The Divine Tree**. Harwood Academic Publishers, the Netherlands.

Santisuk, T. 1993. The natural distribution and propagation. pp. 44-58. In: **Proceeding on Research and Development of Native Multipurpose Fast-Growing Tree**. National Research Council. [In Thai]

Schmutterer, H. 1995. The tree and its characteristics. pp. 1-34. In: Schmutterer, H. (ed.). **The Neem Tree**. VCH Verlagsgesellschaft, Weinheim.

Tewari, D.N. 1992. **Monograph on Neem (*Azadirachta indica* A. Juss.)**. International Book Distributors, India.

Vallejos, C.E. 1983. Enzyme activity staining. pp. 469-516. In: Tanskley, S.D. and T.J. Orton (eds.). **Isozymes in Plant Genetics and Breeding**. Part A. Elsevier, Amsterdam.