โครงสร้างพันธุกรรม และความผันแปรตามกาลของเคี่ยม (*Cotylelobium melanoxylon* Pierre) ในแหล่งพันธุกรรมทุ่งค่าย จังหวัดตรัง

Genetic Structure and Temporal Allozyme Variation of *Cotylelobium melanoxylon* Pierre in Thung Khai Genetic Resource Area, Trang, Southern Thailand

โกวิท ฉายสุรีย์ศรี[1] **Kowit Chaisurisri**
พวงพรรณ ยงรัตนา[1] **Phuangphan Yongratana**
เชิดศักดิ์ ลิ่วลักษณียนาวิน[1] **Cherdsak Liewlaksaneeyanawin**
อับดุลซาลัม อูดราโก[2] **Abdou-Salam Ouedrago**

บทคัดย่อ

ได้ประเมินระดับ และโครงสร้างความผันแปรทางพันธุกรรมของเคี่ยม (*Cotylelobium melanoxylon* Pierre) ในแหล่งพันธุกรรมทุ่งค่าย จังหวัดตรัง เปรียบเทียบกับป่าธรรมชาติอื่นอีกสามประชากร โดยใช้เครื่องหมายยีนไอโซเอนไซม์เป็นเครื่องช่วยประเมิน ผลการศึกษาพบว่าแหล่งพันธุกรรมทุ่งค่ายมีความหลากหลายพันธุกรรมอยู่ในระดับที่สูงกว่าป่าธรรมชาติอื่น โดยมีค่ากลางจำนวนแอลลีลต่อตำแหน่งบนโครโมโซม 2.5 ต่อ 2.4 มีค่าโพลีมอร์ฟิคโลกัส ร้อยละ 83.33 ต่อ 75.00 และค่าความหลากหลายพันธุกรรมที่สำรวจพบ มีค่า 0.20 ต่อ 0.09 จากการวิเคราะห์ความแตกต่างพันธุกรรมระหว่างประชากรป่าธรรมชาติทั้ง 3 ประชากร มีค่าร้อยละ 9.9 เมื่อนำแหล่งพันธุกรรมมาร่วมวิเคราะห์ด้วยทำให้ความแตกต่างพันธุกรรมมีค่าเพิ่มขึ้นเป็นร้อยละ 21.1 ในการวิเคราะห์ระยะห่างพันธุกรรมพบว่าแหล่งพันธุกรรมมีความแตกต่างพันธุกรรมห่างจากป่าธรรมชาติอื่นมาก โดยค่าเฉลี่ยระยะห่างพันธุกรรมระหว่างประชากรธรรมชาติทั้งสามมีค่าเพียง 0.044 เมื่อนำแหล่งพันธุกรรมมาร่วมวิเคราะห์ด้วยผลปรากฏว่าค่าเฉลี่ยระยะห่างพันธุกรรมมีค่า 0.199 แสดงให้เห็นถึงความแตกต่างที่เด่นชัดมากขึ้น

การเพิ่มขึ้นของความหลากหลายพันธุกรรมจากกลุ่มกล้าไม้ (H_e=0.1554) จนถึงไม้ใหญ่ (H_e=0.2655) แสดงให้เห็นว่าเกิดความผันแปรตามกาลในหมู่ประชากรเคี่ยมในแหล่งพันธุกรรม ซึ่งอาจจะมีสาเหตุมาจากเครือข่ายสืบพันธุ์ของไม้ชนิดนี้มีการเชื่อมต่อและรวมตัวกันอยู่ในลักษณะที่หลวม ซึ่งเป็นภาวะปกติของประชากรสิ่งมีชีวิตขนาดใหญ่

[1] ส่วนวนวัฒนวิจัย สำนักวิชาการป่าไม้ กรมป่าไม้

[2] เจ้าหน้าที่อาวุโสแหล่งพันธุกรรมไม้ป่า สถาบันแหล่งพันธุกรรมพืชนานาชาติ กรุงโรม ประเทศอิตาลี

ผลการศึกษาดังกล่าวแสดงให้ถึงประสิทธิภาพของวิธีการอนุรักษ์ในรูปแหล่งพันธุกรรม อย่างไรก็ตามการอนุรักษ์แหล่งพันธุกรรมนี้ได้จำกัดอยู่เฉพาะตอนใต้เขตกระจายพันธุ์ตามธรรมชาติเคี่ยมเท่านั้น ดังนั้น ควรดำเนินกลยุทธการอนุรักษ์ที่มีประสิทธิภาพกว่านี้ เพื่อให้ครอบคลุมตลอดเขตการกระจายพันธุ์ตามธรรมชาติของเคี่ยม

ABSTRACT

The level and structure of genetic variation, as determined by allozyme genetic markers, in a genetic resource area (GRA) of *Cotylelobium melanoxylon* Pierre were assessed and compared to that of three natural populations in Thailand. The GRA harbored higher level of genetic variation than that observed in the other three natural populations (means number of allele per locus 2.5 vs. 2.4, percentage polymorphic loci 83.33 vs. 75.00, and observed heterozygosity 0.20 vs. 0.09). Genetic differentiation among the three natural populations was 9.9 this value has increased to 21.1 after the GRA was included in the analysis. Genetic distance analysis indicated that the GRA was genetically different from the other populations. The mean genetic distance among the three natural populations was 0.044 this value has increased to 0.199 after the inclusion of the GRA in the analysis (i.e., greater differentiation).

Increase in genetic diversity among cohorts in the GRA, from seedling cohort (H_e= 0.1554) to mature cohort (H_e=0.2655), indicated temporal genetic variation in GRA which might cause by loosely connection of temporal breeding web that ensure genetic flow throughout a population, which is common to a large population.

In summary, the present study has indicated that the establishment of GRA is an effective conservation method. It should be emphasized that this study is restricted to the species southern range. A general and more effective conservation strategy should, however, consider the entire species natural distribution.

Keywords: gene conservation, genetic resource areas (GRAs), allozyme variation, cohort, *Cotylelobium melanoxylon*

INTRODUCTION

Despite tropical forests has diminished so that the remaining forests could no longer sufficiently support human basic need to forest dependants and buffer environmental change, continuous depletion of tropical forests is still on-going. This loss has stimulated the awareness on conservation of tropical forests. Among the efforts in maintaining tropical forest habitats, two well-known conservation methods, i.e., *in situ* and *ex situ* conservation, have played a significant role (FAO, 1993). The role of these methods has shared a common goal in maintaining biodiversity of forest habitats by i) protection, particularly of domesticated plants, from genetic vulnerability- a uniformity that leaves them vulnerable to new environmental conditions; ii) protection of endangered species; and iii) preservation of genes for future use (Ledig, 1988). In practice, *ex situ* conservation is a collection of plants out of their original habitats in various forms such as multiplication garden, seed bank, pollen storage and cryopreservation etc. However, this method is rather suited the term "preservation". *In situ* conservation, on the other hands, is a more logical term for

"conservation". This practice allows plants to grow and evolve along changing environment in their original habitats. Recently, the term "protected area" has been introduced. It was assumed that the establishment of "protected areas" would permit conservation of the forest, but it has become clear that in circumstances of high population pressure, poverty, and relatively weak regulatory authority - common to much of the tropics - protected areas are rarely effective (Boyle and Sayer 1995).

ASEAN Forest Tree Seed Centre realized that conservation of tropical forests is urgently needed for sustainable usage. And knew that unless the gap of conflict between need of forest-dependants and strict regulation enforcement on protected areas was solved, sustainable conservation of forests will never come to reality. The Centre has established a pilot project on "Genetic Resources Areas" in several ASEAN countries to harmonize forest conservation mandatory and the right of local people to utilize protected forests. Thung Khai Genetic Resources Area (Thung Khai GRA) is one site of the pilot project in Thailand located in Trang, a southern province of Thailand. In this GRA, primary plant association survey was conducted and the result indicated that this area is dominated by *Cotylelobium melanoxyon*, local name Kiem, one of a keystone species in the GRA. Kiem is one of the most commercially valuable species in the GRA as well as many southern provinces of Thailand. Kiem distributes in evergreen forests from Chumporn, Surat Thani, Phattalung to Trang (**Figure 1**.) (Smittinand *et al*., 1976). Though it becomes clear that sound management of a species requires an understanding of genetic structure of the species (Templeton, 1991; Wyatt, 1992), little is known about this species, especially genetic variation and structure. In the strategic planning of genetic resource areas, the genetic architecture of the major species in the areas should be well established as well as the relationship between genetic variation and environmental conditions (Nakasaka *et al*., 1997).

In this study, isoenzyme electrophoresis was applied to estimate genetic structure of *C. melanoxylon* population and temporal variation of cohort in the popluation. This technique have eased genetic survey of plant population by providing a rapid assessment with less time and intensive labour consumption while reducing personal bias than convention morphological trait-based method (Lewontin and Hubby, 1966; Nevo, 1978; Hamrick *et al.*, 1979; Hamrick, 1982).

MATERIALS AND METHODS

Sample Collection

Fresh vegetative shoots of *C. melanoxylon* were collected from four natural populations namely: Thung Khai Genetic Resource Area, Trang province, and three natural populations in Chumporn province (Khao Srej, Lamae district; Khao Khiew, Sawee district; and Khao Din, Sawee district) for genetic structure analysis (**Figure 1** and **Table 1**).

For the assessment of genetic variation among cohorts in Thung Khai GRA, fresh vegetative shoots were collected from individuals in the population based on five classes of girth at breast height (GBH) according to the following classification: class 1 = gbh ≥ 100 cm, class 2 = GBH between 60-75 cm, class 3 = GBH between 35-50 cm, class 4 = GBH between 10-25 cm, and class 5 = seedlings.

During sample collection and transportation, all the samples were kept cool by ice in a container. And the identities of all vegetative samples were maintained through out from collection to laboratory work.

Table 1. Geographic locations of *Cotylelobium melanoxylon* sample collection

No.	Population Name	# of Sample	Latitude (°N)	Longitude (°E)	Elevation (m)
1	GRA	101	7°27′	99°38′	10
2	Khao Srej	40	9°45′	99°05′	150
3	Khao Khiew	38	10°11′	99°10′	40
4	Khao Din	31	10°13′	99°09′	154

Isoenzyme Analysis

Isozyme procedure used was similar to that described by Liengsiri *et al.* (1990). Approximately four gram of inner bark of each individual sample was ground in 400 ml of grinding buffer (a mixture of 0.1 M Tris-HCl (pH 8.0), 0.2% Ascorbic acid, 5.41mM Cystine-HCl, 0.5 M Sucrose, 1% Tween 80, 10% $MgCl_2$, 10% Calcium chloride, 1% PEG (20M), 1% Tergital 15-S-9, 0.1 M Phosphate buffer pH 6.8 (Na_2HPO_4), 2% PVP (40M), and 0.3% β-mercaptoethanol, adjusted to pH 7.5 with Hydrochloric acid). The extract was then assayed for allozyme variation at eight enzyme systems (**Table 2**). Enzyme systems with multiple loci, the most anodal migrating band (fastest locus) was assigned as 1 and the other loci were assigned by increasing numbers with decreasing migrating distance. At each locus, the most common allele was assigned as 1 and the fast migrating alleles were assigned as even numbers while the slow alleles were assigned as odd numbers.

Table 2. Enzyme system assayed in *C. melanoxylon*[a]

Enzyme System	Abbrev.	EC Code	No. of Loci
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	1
Glutamate dehydrogenase	GHD	1.4.1.3	1
Malate dehydrogenase	MDH	1.1.1.37	2
Malic enzyme	ME	1.1.1.40	1
Menadione reductase	MR	1.6.99.2	1
Phosphoglucose isomerase	PGI	5.3.1.9	2
Shikimic acid dehydrogenase	SDH	1.1.1.25	1
6-Phosphogluconic dehydrogenase	6PGD	1.1.1.44	2

[a]Extraction buffer no. 12 and H buffer system as described by Liengsiri *et al.* (1990) were used. Initial current was 30 mA for approximately 40 min. and re-run current after dewicking was 40 mA until dye-front migrating to 7 cm, 12.5% starch gel was used.

Data Analysis

Genetic data analysis was conducted using the computer program, POPGEN (Yeh, 1997). Level of genetic variation was estimated using four genetic diversity parameters: 1- percentage of polymorphic loci (*P*) at the 0.99 criteria, 2- average number of allele per locus (*A*), 3- observed heterozygosity (H_o), and 4- expected heterozygosity (H_e). The genetic structure was determined using Wright's *F*-statistics (Wright, 1965). Additionally, Nei's (1972) pair-wise genetic distance was calculated to perform a hierarchical cluster analysis of populations using UPGM (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Genetic Structure

Allozyme variation was detected in 11 out of the 11 loci investigated (**Table 3**). The 6PGD-2 locus monomorphic in the three natural populations, but was not resolved for the GRA. Additionally, GDH-2 showed variation in the GRA but was not resolved in the three natural populations (**Table 3**). This probably indicates the difference in source of the GRA and natural populations which they are approximately 260 km apart (**Table 1**). The GRA represents the southernmost of the species range while the other three populations represent the northern range of the species distribution**.** Unfortunately, we are unable to sample natural populations in the middle of the species distribution range to include in this study. However, it can be seen that sampling breadth may be responsible for this difference (Chaisurisri and El-Kassaby, 1994).

The measures of genetic diversity suggest that when analysis was made without GRA, percentage of loci polymorphic (*P*), means number of allele per locus and observed heterozygosity are 75, 2.4 and 0.093, respectively (**Table 4**). Inclusion of GRA in the analysis resulted in an increase in genetic diversity: percentage of loci polymorphic, means number of allele per locus and observed heterozygosity are 91.7, 2.6 and 0.142, respectively (**Table 4**). The result suggests that the GRA captures large amount of genetic variation in terms of percentage of loci polymorphic (*P*=83.33), means number of allele per locus (*A*=2.45), and observed heterozygosity (H_o=0.20) (**Table 4**). The observed heterozygosity (H_o) is lower than expected heterozygosity (H_e) in all populations indicating the occurrence of related mating in these populations. Genetic diversity of *C. melanoxylon* is lower than that reported in legumes, *Pterocarpus macrocarpus* Kurz (Liengsiri *et al.*, 1995) and *Dalbergia cochinchinensis* Pierre (Soonhuae *et al.*, 1995) and a dipterocarp (*Hopea ferrea*: *P*=68.75, *A*=2.2 and H_o=0.205) (Chaisurisri unpublished data). However, it is higher than that reported for another dipterocarp (*Hopea odorata*: *P*=40.7, *A*=1.5 and *Ho*=0.293) (Wickneswari *et al.*, 1994).

F-statistics estimation was made in two groups, i.e., without and with GRA (**Table 5).** The estimate of F_{IS} indicates that only three loci (MDH-2, MR and PGI-2) showing excess heterozygosity, each of the remaining eight loci exhibits heterozygote deficiency. The F_{ST} estimates with and without GRA indicate that the genetic differentiation among populations are 10 and 21.1 percent, respectively. The remaining 90 (without GRA) and 78.9 (with GRA) percent of genetic variation resides within population (**Table 5**). Genetic differentiation among populations of *C. melanoxylon* is higher than other studies reported for Angiosperms, i.e., *Pterocarpus macrocarpus* Kurz (12.1%) (Liengsiri *et al.*, 1995) and *Dalbergia*

cochinchinensis Pierre (12.7%) (Soonhuae *et al.*, 1995). However, this estimate is lower than that observed in *Acacia auriculiformis* (27%) (Wickneswari and Norwati, 1993).

Table 3. Allele frequentcies of 12 loci of *C. melanoxylon* in Thung Khai GRA and other three populations

Locus	Allele	Population			
		GRA	Khao Srej	Khao Khiew	Khao Din
G6PD-1	1	0.6584	0.8750	0.8289	0.8226
	2	0.2277	0.1125	0.1579	0.1452
	3	0.1139	0.0125	0.0132	0.0323
GDH-1	1	0.9802	0.8250	0.9677	0.9800
	2	0.0198	0.1250	0.0323	0.0200
	3		0.0500		
GDH-2	1	0.9455			
	2	0.0248			
	3	0.0297			
MDH-1	1	0.8812	1.0000	1.0000	0.8800
	2	0.1188			0.1200
MDH-2	1	0.8317	0.8750	0.8710	0.8350
	2		0.0125	0.0323	
	3	0.1683	0.1125	0.0968	0.1650
ME	1	0.9406	0.7000	0.4839	0.9400
	2	0.0594	0.3000	0.5161	0.0600
MR	1	0.8069		0.9194	0.8100
	2	0.1683		0.0654	0.1650
	3	0.0248		0.0161	0.0250
PGI-1	1	0.7871	0.8375	0.9032	0.7900
	2	0.1287	0.0750		0.1300
	3	0.0842	0.0875	0.0968	0.0800
PGI-2	1	0.7673	0.9000	0.9516	0.7650
	2	0.1089	0.1000	0.0484	0.1100
	3	0.1238		0.0000	0.1250
SDH	1	1.0000	0.7500	0.8387	1.0000
	2		0.2500	0.1613	
6PGD-1	1	0.5990	1.0000	0.9677	0.6000
	2	0.3812			0.3800
	3	0.0198		0.0323	0.0200
6PGD-2	1		1.0000	1.0000	1.0000

When two populations possess identical diversity, it does not necessarily mean they have a single gene in common (Gregaoius, 1978). The amount of difference can be obtained from genetic distance analysis (**Table 6**). The GRA provides the highest genetic distance pairing with the three natural populations (genetic distance ranges from 0.177 to 0.261). Khao Khiew and Khao Srej give the smallest genetic distance, 0.015. It is interesting to report that the genetic distance among populations is significantly correlated to geographic distance *(r*= 0.973, P<0.002). The GRA is the most isolated population than the three natural ones in term of both genetic and geographic distances. This result might indicate that the relationship

between genetic differentiation as the result of microclimatic differences as it was observed in *Abies sachalinensis* (Nagasaka *et al.*, 1997). The other three populations are very close. Relationship between genetic distance and geographic distance was also observed in *Pterocarpus macrocarpus* Kurz. (Liengsiri *et al.*, 1995) and *Pinus radiata* (Moran *et al*., 1988). The relationship between genetic distance and geographic distance of this species can also be confirmed by the genetic distance phenogram (**Figure 2**) which indicates that the GRA is isolated from the others.

Table 4. Measures of genetic variability of *C. melanoxylon* in Thung Khai GRA and three natural populations, percentage of loci polymorphic (*P*, at 99% criterion), means number of alleles per locus (*A*), observed heterozygosity (H_o) and expected heterozygosity (H_e) average over all loci

Population	*P*	*A*	H_o	H_e
Thung Khai GRA	83.33	2.4545	0.1998	0.2560
Khao Srej	58.33	2.1000	0.0925	0.2030
Khao Khiew	50.00	1.9000	0.0947	0.1888
Khao Din	75.00	2.0909	0.0909	0.1704
Means				
Without GRA	75.00	2.4	0.0930	0.1902
With GRA	91.67	2.6	0.1422	0.2300

Table 5. Summary of *F*-statistics at 11 loci of *C. melanoxylon*, analyses were made in two groups, 4 populations (with GRA) and 3 populations (without GRA)

Locus	With GRA			Without GRA		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
G6PD-1	0.2389	0.2619	0.0303	0.2442	0.2469	0.0037
GDH-1	0.9016	0.9085	0.0699	1.0000	1.0000	0.0701
GDH-2	0.3365	0.9816	0.9723	-	-	-
MDH-1	0.5272	0.5706	0.0918	-	-	-
MDH-2	-0.1414	-0.1218	0.0171	-0.1105	-0.0983	0.0110
ME	1.0000	1.0000	0.1692	1.0000	1.0000	0.0501
MR	-0.0269	0.8510	0.8549	0.3564	0.9644	0.9447
PGI-1	0.5437	0.5588	0.0331	0.5926	0.6104	0.0436
PGI-2	-0.0128	0.0389	0.0511	-0.0778	-0.0622	0.0144
SDH	0.2663	0.3390	0.0990	0.2663	0.2848	0.0252
6PGD-1	0.4499	0.6078	0.2871	1.0000	1.0000	0.0217
Means	0.4045	0.5178	0.2114	0.4972	0.5504	0.0988

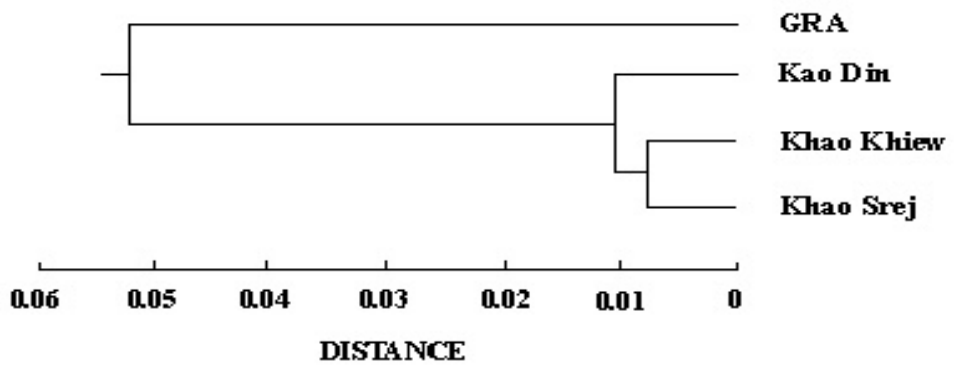


Figure 2. Phenogram of *C. melanoxylon* differentiation based on Nei's (1972) genetic distance coefficients and UPGMA.

Temporal Variation

Genetic variation of five cohorts of individuals in GRA was estimated using girth at breast height (GBH), class 1 = gbh ≥ 100 cm, class 2 = GBH between 60-75 cm, class 3 = GBH between 35-50 cm, class 4 = GBH between 10-25 cm, and class 5 = seedlings, to represent different in age groups of individuals in the population and the result showed expected heterozygisity (H_e) increases from seedling to mature tree (class 1 = gbh ≥ 100 cm), 0.1554 and 0.2655, respectively (**Table 7**). The percentage of loci polymorphic (*P*) was maintained at 83.33% through all size classes. However, means number of allele per locus (*A*) was the same in class 2 (60-75 cm) and class 5 (seedlings), 2.556 while in other classes was smaller (class 1= 2.333, class 3= 2.222, and class 4=2.444) (**Table 7**). The increase in expected heterozygosity (H_e) indicates variation in genetic diversity among cohorts of population in the GRA, which implies temporal genetic variation in GRA. Temporal genetic variation in GRA might be caused by loosely connection of temporal breeding web that ensure genetic flow throughout a population, which is common to a large population (Hamrick and Nason, 2000). Presents and absent of alleles in some locus show differences in contribution of gene flow among cohorts in the population that confirmed the temporal variation indicates loosely connection in the breeding web of the population (**Table 8**). However, genetic differentiation among cohorts is only 2.7% (**Table 9**). The result of this study showed the same trend as sugar maple (Plessas and Strauss, 1986). However, in Monterey pine demonstrated an opposite trend (Fore *et al.*, 1992).

Implications for Conservation of Genetic Resources

Current genetic resources are the basis for success or failure of forest renewal today and in the immediate future. It is vital that those species and populations best suited, genetically and economically to support reforestation be recognized, managed, and utilized (Krugman, 1984).

Table 6. Geographic distance (above diagonal) and Nei's (1972) genetic distance (below diagonal) between populations of *C. melanoxylon* in Thung Khai genetic resource area (GRA) and three natural populations

Population	Khao Khiew	Khao Din	Khao Srej	GRA
Khao Khiew	-	10	60	320
Khao Din	0.0581	-	50	310
Khao Srej	0.0153	0.0586	-	260
GRA	0.2612	0.1765	0.2280	-

Table 7. Measures of genetic variability (*P* = percentage of loci polymorphic, *A* = means number of allele per locus, H_o = observed heterozygosity and H_e = expected heterozygosity) of *C. melanoxylon* in Thung Khai Genetic Resources Area comparing differences among five classes, class 1 = dbh ≥ 100 cm, class 2 = 60-75 cm, class 3 = dbh 35-50 cm, class 4 = dbh 10-25 cm, and class 5 = seedlings

Size Class	*P*	*A*	H_o	H_e
1	88.89	2.333	0.1936	0.2655
2	88.89	2.556	0.1954	0.2122
3	88.89	2.222	0.1667	0.1641
4	88.89	2.444	0.1453	0.1604
5	88.89	2.556	0.1356	0.1554
Overall	100	3.000	0.1671	0.2015

The GRA is different from the other three natural populations in term of genetic diversity and number of alleles presents in the populations. While the GRA has a more diverse genetic variation than the other three natural populations studied, allele frequencies demonstrated that the GRA represents the southernmost populations of the species distribution range. Genetic distance between these populations also confirms these differences. Though Thung Khai genetic resources area harbors sufficient amount of genetic diversity of *C. melanoxylon*, it represents only the populations at the southernmost of the species distribution range. Gaining and losing alleles in GRA imply that viable conservation of this species should be implemented so that most of the alleles represent in the species be conserved within their natural distribution range. In this context, one genetic resource area is not sufficient to conserve the genetic diversity of the species. A viable method is, therefore, to set up several genetic resource areas scattering along the range of the species' natural distribution breadth. The low genetic diversity in the other three populations in Chumporn indicates the presence of a reduced genetic variability in the three areas especially Khao Khiew. And also indicates that the genetic variability of these populations is vulnerable. Consequently, conservation of these populations, especially Khao Khiew, is urgently needed to prevent further loss of genetic variability and to provided a wide genetic base for future improvement of the species. Analysis of genetic diversity among cohorts in the population has shown that this techniques can be used as a tool to monitor pattern of change in genetic diversity over time or temporal genetic variation.

Table 8. Allele frequentcies of 10 loci of *C. melanoxylon* at different tree size class in Thung Khai GRA

Locus	Allele	Tree Size Class (gbh in cm)				
		≥ 100	60-75	35-50	10-25	Seedling
G6PD-1	1	0.6584	0.7241	0.8667	0.8654	0.9050
	2	0.2277	0.1724	0.0333	0.0673	0.0150
	3	0.1139	0.1034	0.1000	0.0673	0.0800
GDH-1	1	0.9802	0.9914	0.9917	0.9904	1.0000
	2	0.0198	0.0000	0.0000	0.0000	0.0000
	3	0.0000	0.0086	0.0083	0.0096	0.0000
MDH-1	1	0.8812	0.8966	0.9917	0.9615	0.9450
	2	0.1188	0.0948	0.0000	0.0096	0.0200
	3	0.0000	0.0086	0.0083	0.0288	0.0350
MDH-2	1	0.8317	0.8879	0.9083	0.9135	0.8900
	2	0.0000	0.0000	0.0000	0.0000	0.0200
	3	0.1683	0.1121	0.0917	0.0865	0.0900
ME	1	0.9406	1.0000	1.0000	1.0000	0.9950
	2	0.0594	0.0000	0.0000	0.0000	0.0000
	3	0.0000	0.0000	0.0000	0.0000	0.0050
MR	1	0.0000	0.8966	0.9750	0.9231	0.9300
	2	0.0000	0.0776	0.0250	0.0769	0.0450
	3	0.0000	0.0259	0.0000	0.0000	0.0250
PGI-1	1	0.7871	0.8362	0.8000	0.8077	0.8850
	2	0.1287	0.0259	0.0250	0.0577	0.0100
	3	0.0842	0.1379	0.1750	0.1346	0.1050
PGI-2	1	0.7673	0.8103	0.7750	0.8654	0.8300
	2	0.1089	0.0259	0.0000	0.0192	0.0050
	3	0.1238	0.1638	0.2250	0.1154	0.1650
SDH	1	1.0000	0.9828	0.9917	0.9904	0.9650
	2	0.0000	0.0086	0.0083	0.0096	0.0000
	3	0.0000	0.0086	0.0000	0.0000	0.0350
6PGD-1	1	0.5990	0.7155	0.7917	0.7885	0.7950
	2	0.3812	0.2500	0.2000	0.1442	0.1250
	3	0.0198	0.0345	0.0083	0.0673	0.0800

Table 9. Summary of *F*-statistics at 9 loci of *C. melanoxylon* of among group of tree at different size classes

Locus	F_{IS}	F_{IT}	F_{ST}
G6PD-1	0.1295	0.1717	0.0485
GDH-1	-0.0137	-0.0071	0.0066
MDH-1	0.2570	0.2825	0.0343
MDH-2	-0.1338	-0.1234	0.0091
ME	0.9178	0.9214	0.0437
PGI-1	0.1881	0.1988	0.0131
PGI-2	0.1046	0.1169	0.0138
SDH	-0.0235	-0.0109	0.0123
6PGD-1	0.1287	0.1607	0.0367
Means	0.1202	0.1468	0.0269

ACKNOWLEDGMENT

We are grateful to various people and organizations contributing to the success of this study namely: ASEAN Forest Tree Seed Centre, International Plant Genetic Resources, and Royal Forest Department provided financial support; Mss. Karuna Klinsukhon, Sunan Kaewkla, and Mr. Sommart Saengdao provided technical assistance and sample collection; staff of Southern Botanic Gardens (Thung Khai), Mr. Chana Promdej, and Mr. Prapot Sajthaporn; Mr. Phanom Phudpetchkaew, a former staff of Chumporn Provincial Forest Office, provided all means of support form sample survey to sample collection; Dr. Y.A. El-Kassaby for his helpful comment on the manuscript. Our gratitude also goes to Mr. Somyos Kijkar, a former AFTSC director and Mr. Jim Coles, former Canadian Project manager, for their supports to this study.

REFERNCES

Boyle, T.J.B. and J.A. Sayer. 1995. Measuring, monitoring and conserving biodiversity in managed tropical forests. *Commonwealth Forestry Review 74*: 20-25.

Chaisurisri, K. and Y.A. El-Kassaby.1994. Genetic diversity in a seed production population vs. natural populations of Sitka spruce. *Biodiversity and Conservation 3*: 512-523.

Collins, N.M., J.A. Sayer and T.C. Whitmore. 1991. The conservation atlas of Tropical forests: Asia and the Pacific. Macmillan Press Ltd., Singapore, p. 224.

El-Kassaby, Y.A. 1990. Genetic variation within and among conifer populations; review and evaluation of methods. Pages pp. 59-74 In: *Biochemical markers in population genetics of forest trees* (H.H. Hattemer, S. Fineschi, F. Cannata and M.E. Malvolti, eds.). SPB Academic Publishing, The Hague.

FAO, 1993. Conservation of genetic resources in tropical forest management: Principles and concepts. *FAO Forestry Paper 107*, 105 p.

Fore, S.A., R.J. Hickey, S.I. Guttman, J.L. Vankat. 1992 Temporal differences in genetic diversity and structure of sugar maple in an old-growth forest. *Can. J. For. Res. 22*:1504-1509.

Hamrick, J.L., Y.B. Linhart and J.B. Mitton. 1979. Relationship between life history of characteristics and electrophoretically detectable genetic variation in plants. *Ann. Rev. Ecol. Syst. 10*: 173-200.

Hamrick, J.L.1982.Plant population genetic and evolution. *Amer. J. Bot.* 69: 1685-1693.

Hamrick, J.L.and M.J.W.Godt. 1989. Allozyme diversity in plant species. *In Differentiation patterns in higher plants* (K. Urbanska, ed.), Academic Press, New York, pp. 53-67.

Hedrick, P.W. 1975. Genetic similarity and distance: comments and comparison. *Evolution 29*: 362-366.

Krugman, S. 1984. Policies, strategies and means for genetic conservation in forestry. *In Plant genetic resources*: A conservation imperative, C.W. Yeatman, D. Kafton and G. Wilkes, eds. Westview Press. Inc.Boulder, Colo., USA, pp. 71-78.

Ledig, F.T. 1986. Heterozygosity, heterosis, and fitness in outbreeding plants. In *Conservation biology* (M.E. Soule, ed.) Sunderland, MA: Sinauer, pp. 77-104.

Ledig, F.T. 1988. The conservation of diversity in forest trees. *BioScience 38*: 471-479.

Lewontin, R.C. and J.L. Hubby. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural population of *Drosophila pseudooscura Genetics 54*:595-609.

Liengsiri, C, C. Piewluang, and T.J.B.Boyle.1990. Starch Gel Electrophoresis of Tropical Trees: A Manual, ASEAN-Canada Forest Tree Seed Centre, Muak-Lek, Saraburi, THAILAND, 52 p.

Liengsiri, C,F.C.Yeh and T.J.B.Boyle. 1995. Isozyme analysis of tropical forest tree, *Pterocarpus macrocarpus* Kurz. in Thailand. *For. Ecol. & Mange. 74*: 13-22.

Mitton, J.B. 1983. Conifers. Pages 443-472 In: *Isozymes in plants genetics and breeding*, Part B (S.D. Tanskley and T.J. Orton, eds.) Elsevier Science Publishers, Amsterdam.

Nagasaka, K. Z.M. Wang and K. Tanaka. 1997. Genetic variation among natural *Abies sachalinensis* populations in relation to environmental gradients in Kokkaido, Japan. *For. Gen. 4*: 43-50.

Nei, M. 1972. Genetic distance between populations. *Am. Nat. 106*: 283-292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA 70*: 3321-3323.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. *Theor. Pop. Biol. 13*:121-177.

Plessas, M.E. and S.H. Strauss. 1986. Allozyme differentiation among populations, stands, and cohorts in Monterey pine. *Can. J. For. Res. 16*: 1155-1164.

Reynolds, J., B.S. Weir and C.C. Crockerham. 1983. Polymorphism in the esterases of Atlantic herring. *trans Am. Fish. Soc. 99*: 147-151.

Sneath, P.H.A. and R.R. Sokal. 1973. *Numeric taxonomy*. W.H. Freeman, San Francisco, CA.

Soonhuae, P., T.J.B. Boyle and F. Yeh. 1995. The population genetics of *Dalbergia cochinchinensis* Pierre and implications for genetic conservation. *In Population genetics and genetic conservation of forest trees*, Ph. Baradat, W.T. Adams and G. M. SPB Academic Publishing, Amsterdam, pp. 371-385.

Templeton, A.R. 1991. Off-site breeding of animals and implications for plant conservation strategies. *In Genetics and conservation of rare plants*, D.A. Falk and K.E. Holsinger, eds., Oxford University Press, New York, pp. 182-194.

Smittinand, T., T. Santisuk and C. Phengkhai. 1979. The manual of Diperocarpaceae of mainland South-East Asia. *Thai For. Bull. No. 12*, 24 p.

Wickneswari, R. and M. Norwati. 1993 Genetic diversity of natural populations of *Acacia auriculiformis*. *Aust. J. Bot. 41*:65-77.

Wickneswari, R., Z. Ismail, S.L. Lee and N. Muhammad. 1994 Genetic diversity of remnant and planted populations of *Hopea odorata* in Peninisular Malaysia. *Proc. Inter. Workshop, BIO-REFOR*, Kangar, Malaysia, pp. 72-76.

Wright, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution 19*: 395-420.

Wyatt, R.1992. Conservation of rare and endangered bryophytes: input from population genetics. *Biol. Conserv. 59*: 99-107.