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——Proceeding——

Construction of a gene-data bank of tropical rainforest tree species in Sarawak, Malaysia

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ABSTRACT A "gene-data bank" of tropical rainforest tree species in Sarawak was constructed. This work was initiated to deposit DNA materials from tree species in the whole area of Sarawak and found the information gathered to be effective tools for the sustainable management of bio-resources. For this study leaf samples, mainly from dipterocarp species, were collected from eight natural populations in national parks mainly and also several man-made forests. We collected nearly 5,000 samples including 127species in 27 families. DNA extraction was done in the Forest Research Centre (FRC) in Kuching, Sarawak using a modified CTAB method. These DNA samples were deposited along with field sampling data and DNA extraction data. Genetic analysis using RAPD, AFLP, microsatellite and DNA sequencing is now under progress to clarify the genetic constitution of the tree populations. Genetic information obtained by these methods will be useful in specifying the individual trees and will be incorporated into the gene-data bank.

Key words: gene-data bank, Sarawak, Dipterocarpus

INTRODUCTION

Several forest types are recognized in Sarawak; they are the peat swamp forests facing the lowland coastline, mixed dipterocarp forests from the plains to gentle-sloping hills, mangrove forests at the mouth of rivers, mountain forests where Fagaceae dominate and kerangas forests (Baillie, 1978). Differentiation between forest types depends primary on altitude and soil type. Many tree species, more than 2,500, are found in the Sarawak area of 12.3 million hectares (Anderson, 1980). Among them, mixed dipterocarp forest occupies 57% of the whole Sarawak area (Baillie, 1978) and is estimated to contain over 2,000 tree species. Lowland tropical rainforests is known to have the most diverse vegetation in the world (Whitmore, 1984). Extensive speciation of Dipterocarpaceae, which includes major canopy trees, has occurred in this region. 267 dipterocarp species are known in Borneo and 155 of them are endemic (Ashton, 1982) suggesting that Borneo have been the center of the speciation of these species. Despite worldwide knowledge of the diverse biota in forests, forest areas have been reduced and fragmented rapidly by human activities such as machinized logging and nontraditional 'Slash and Burn' (Lee, 1997). Reforestation research has been going on in Sarawak since 1965 when a reforestation research program was drawn up for implementation in the first Malaysia Plan (1965-1970) (Lee, 1997). In spite of the efforts of the program, they encountered many problems. For example, restoration and reforestation using tropical exotic conifers and fast-growing exotic hard wood trees have been largely unsuccessful after fifteen years. The importance of indigenous species plantations is realized again (Lee, 1997). Evaluation of both genetic variation accumulated in forest tree populations and the genetic differentiation among the populations is an important issue for the appropriate use of indigenous tree species for future plantations and also for the conservation of forest ecosystems.

This work planned to deposit DNA material from tree species from the whole area of Sarawak. The findings of this study will be used for effective management of biodieversity resources. The genetic variation and genetic relationships of tree populations in Sarawak using molecular markers, such as RAPD, AFLP, microsatellite and DNA sequencing were studied (Harada *et al*., 1994; Kamiya *et al*., 1998). Because DNA is the basic substance that carrying the genetic information

of organisms, the genetic constitution of tree populations could be objectivity determined if proper molecular makers are used, that is, the analysis of DNA variations could provide more conclusive results for studies on phylogeny and population genetics of organisms, than indirect observation of genetic variation using morphology and proteins (Nei, 1987). Moreover, it is also mentioned that because much of the research today uses PCR (polymerase chain reaction) DNA material should be qualified for the method (Mullis & Faloona, 1987). DNA material is thus first analyzed by RAPD. Part of the samples has been examined further by AFLP, microsatellite and DNA sequencing. These data will be put in the DNA-bank to facilitate increasing genetic information. Here, genetic information is arranged into three parts: (1) genetic variation among species, (2) genetic variation within species, and (3) genetic variation in man-made forests. Consequently, sampling is organized to collect these three classes of materials and some effort was made to maximize sampling efficiency.

MATERIALS AND METHODS

Sampling sites

Natural forests: For the natural forests, six national parks, Tanjung Datu, Gunung Gading, Kuba, Bako, Similajau, Niah and Lambir Hills were selected. Additional samples from Semengok Arboretum in a suburb of Kuching, and seeds from areas surrounding Forest Department Nurseries of Sibu and Kapit were collected. Long term ecological research (LTER) in Sarawak was initiated in March 1989 as a joint project with Japan, the United States and Malaysia. A 52 ha LTER plot was established in Lambir Hills in 1990 to conduct baseline vegetation studies of the natural forests (Lee, 1997). In parallel with the LTER program was the Canopy Biology Program (CBP) and a 8 ha plot in northeast of the LTER plot (Inoue and Hamid, 1994). All the trees above 1.0cm diameter were identified and mapped on the LTER plot (Yamakura, 1995). We set two one ha subplots in the LTER plot for extensive sample collection.

Man-made forests: We collected samples from experimental reforestation sites in Bakam and Niah which were established in 1995 under a joint project with Japan and the Forest Department of Sarawak aiming to rehabilitate the once degraded forests (Sakurai *et al*., 2000). We also collected samples from the Landeh planting site, where Dipterocarp species and Sarawak Iron trees (*Eusideroxylon zwageri*) have been planted since the 1930s. Sampling sites for both natural and man-made forests are shown in Fig. 1 and the location is listed in Table 1.

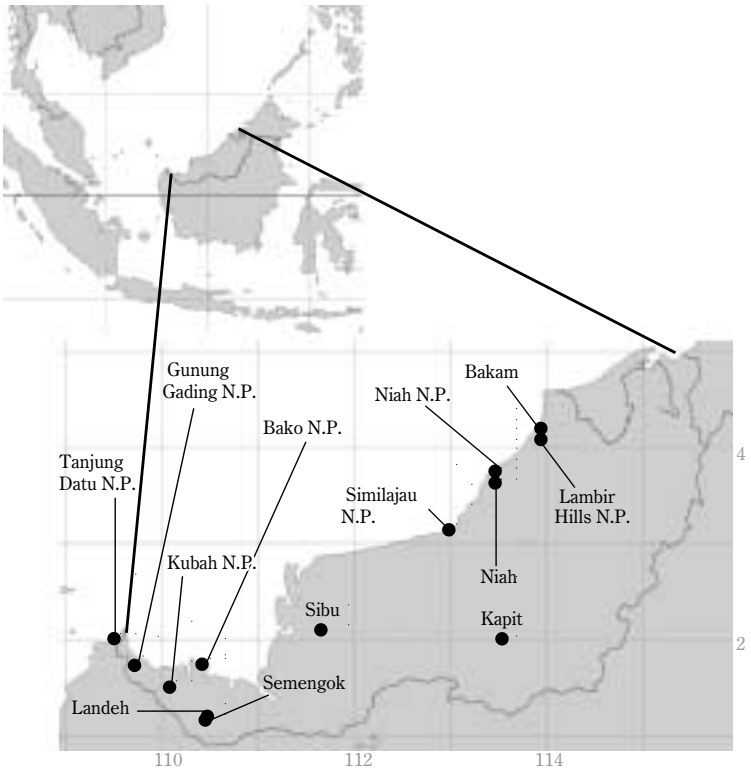

Fig. 1. The study sites in Sarawak. The X axis is longitude and the Y axis is latitude.

Table 1. Collection sites in Sarawak, Malaysia

Collection site	Latitude / Longitude	Status
Tanjung Datu	2°13'N / 109°03'E	National park
Gunung Gading	1°42'N / 109°48'E	National park
Kubah	1°35'N / 110°09'E	National park
Semengok	1°23'N / 110°17'E	Arboretum
Bako	1°43'N / 110°28'E	National park
Similajau	3°27'N / 113°15'E	National park
Niah	3°47'N / 113°46'E	National park
Lambir Hills	4°12'N / 114°01'E	National park
Landeh	1°24'N / 110°18'E	Forest Reserve
Bakam	4°25'N / 113°92'E	Artificial forest
Niah	3°40'N / 113°41'E	Artificial forest
Kapit	1°50'N / 113°00'E	Nursery
Sibu	2°00'N / 112°00'E	Nursery

Sampling methods

Usually the sampling team consisted of one Forest Department officer, one to three tree climbers, two technical staff members and one or two drivers in addition to the researchers. A cutting stick and catapult was used to collect leaves. For tall trees, tree climbers were employed. Five dipterocarp species, *Dryobalanops lanceolata* (Kapur paji), *D. aromatica* (Kapur peringgi), *D. beccarii* (Kapur bukit), *Shorea beccariana* (Meranti langgai) and *S. macrophylla* (Engkabang jantong), which commonly grow in Sarawak and used for planting were selected as the target species for this population study. Thirty to eighty samples were collected for each species where they were found. We also collected other dipterocarp species and other economically important non-dipterocarp species as far as they are identified in the field. Two to five leaves were collected for each tree and kept in a plastic bag with an ID card (Fig. 2). Samples were divided into two groups for DNA extraction and drying. The samples for DNA extraction were carried back to FRC in a cooler box with dry ice and kept in a refrigerator or a freezer. The samples for drying was kept in a plastic bag with silica gel and then transferred to an envelope. Species names were confirmed by referring our specimens to the specimens deposited in the botany section of FRC by T.K.

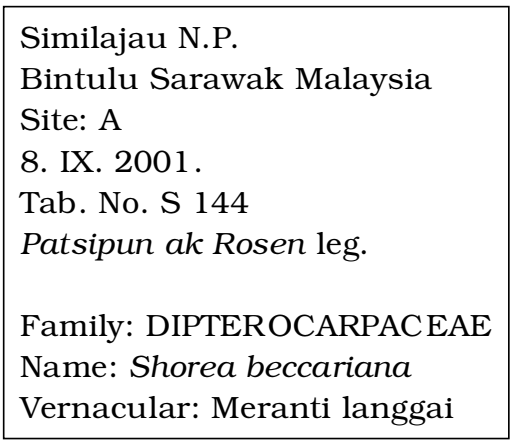

Fig. 2. An example of the ID cards attached to leaf samples.

DNA extraction

DNA was extracted using a modified CTAB method (Murry & Thompson 1980). 0.5 gm of leaves were cut into small pieces and put in a motor with liquid nitrogen and ground into a fine powder. Five ml of cold isolation buffer I (50mM Tris-HCl;pH8.0, 5mM EDTA, 350mM sorbitol, 0.5% 2-mercaptethanol, 0.1% bovine serum albumin, 10% polyethylene glycol) was added and mixed well. They were centrifuged at 3,500 rpm for 5 minutes. The pellet was re-suspended in 5 ml of cold isolation buffer I and mixed thoroughly. The washing process was repeated when necessary. They were again centrifuged at 3,500 rpm for 5 min. The pellet was re-suspended in 3 ml of cold isolation buffer II (50mM Tris-HCl;pH8.0, 5mM EDTA, 350mM sorbitol, 0.5% 2-mercaptethanol, 1% sodium sarkosyl), and then mixed thoroughly. They were kept at room temperature for 30 minutes and then 3 ml of 2X CTAB solution (2% CTAB, 0.1M Tris-HCl;pH8.0, 20mM EDTA, 1.4M NaCl, 2% 2-mercaptethanol) was added and mixed well. After incubation at 60℃ for 30minutes, 3 ml of CIA (chloroform-isoamyl alcohol) was added and let to sit for 15 minutes. Then they were centrifuged at 3,500 rpm for 15 minutes. The aqueous layer was transferred to a new tube, and two-thirds volume isplopanol was added. Samples were then incubated for at least one

night in the refrigerator. Then they were centrifuged at 3,500 rpm for 5 min. and the residual liquid was discarded. Air dried pellets were dissolved into 200μl of TE (10mM Tris-HCl; pH8.0, 1mM EDTA).

Extracted DNA was further purified by Binding Matrix (Bio 101). Fifty μl of extracted DNA solution and 150μl of TE, and 200μl of Binding Matrix were mixed gently and incubated for 5 minutes at room temperature. They were spin for 1minute at 7,000 rpm. Pellets were resuspended gently with 160μl SEWS-M (salt/ethanol wash solution) and spun for 1 minute at 7,000 rpm. The pellets were then resuspended gently with 120μl SEWS-M and spun for 1 minute at 7,000 rpm. They were spun briefly and the residual liquid was removed. They were dried overnight. DNA was eluted from the Binding Matrix by gentle resuspending in 50μl DES (DNA elution solution) followed by two to three minutes of incubation. They were spun for 1 minute at 15,000 rpm and transferred into a new 1.5 ml tube. One microliter of the DNA solution was electrophoresed into a mini-gel apparatus (Mupid-2, ADV). The presence of high-molecular-weight DNA was checked by agarose gel with λ/HindIII as a size maker under UV illumination. We checked the DNA material by RAPD for their applicability to PCR. RAPD analysis was done for each species (one or two samples) and whenever the extraction method was modified.

RESULTS

Sampling from natural populations

Ten national parks and three wildlife sanctuaries are in Sarawak (Lee, 1997). We collected samples for natural populations mainly from these national parks. Additional samples were collected from Semengok arboretum and nurseries in Sibu and Kapit. Among them the long term ecological research (LTER) plot has been established in Lambir Hills National Park since 1990 and a periodical census has been done to monitor tree populations over time, especially the dynamics of regeneration of key species (Lee, 1997). Two 1 ha subplots which shows high species diversity were selected for extensive sampling. Trees of ten commonest species in these sites which had a DBH over 10 cm were collected. Besides, one to three samples were collected for all dipterocarp species identified in the LTER and CBP plots. Eighty-seven dipterocarp species were collected in these areas.

For the population survey, *D. aromatica* was collected from two populations (Lambir Hills and Similajau) and *D. beccarii* from three populations (Bako, Gunun Gading and Kubah). Morphologies of *D. aromatica* and *D. beccarii* are similar making it difficult to distinguish them, although the species in the southwest part of Sarawak is considered to be *D. beccarii* and that found in the northeast is considered to be *S. aromatica*. *Dryobalanops lanceolata* were sampled only from Lambir Hills, although this species has been recorded in the central to the northeast part of Sarawak. *Shorea macrophylla* was collected from two populations (Lambir Hills and Kubah), while *S. beccariana* (Meranti langgai) was collected from five populations (Lambir Hills, Similajau, Bako, Kubah and Semengok).

Sampling from man-made populations

Since the life cycle of trees is long it is difficult to investigate the genetic composition changes in manmade forests. Consequently, planting sites with different histories could show genetic change by planting. In Landeh, samples of Sarawak iron tree planted in the 1930s together with mother trees of *S. macrophylla* and *S. pinanga* planted in the 1970s as well as seedlings were collected. *Dryobalanops lanceolata* was collected in Niah and Bakam experimental sites. *Shorea beccariana* was also collected from the Niah and Bakam experimental sites. The seedling of these trees were collected from the Lambir Hills area and planted in Bakam in 1996 and 1997, and in Niah in 2000 (Sakurai, personal communication).

Construction of gene-data bank

A total of 126 species representing 27 families was collected by the end of 2002 (Table 2). Among them, 102 were dipterocarp species belonging to 8 genera. The total number of samples was nearly 5000 and DNA extraction from these samples is now being undertaken at the FRC laboratory. Each DNA sample includes: (1) field sampling data including field ID, sampling date, species and family name, vanacular name and sampler, (2) DNA extraction data including tube number, DNA content, date of extraction, extraction method and (3) genetic information obtained by RAPD, AFLP, microsatellite and DNA sequencing. The data set (1) and (2) were combined and put into Excel (Microsoft) data sheets linked with scanned images of the dry leaf samples. The data for category (3) is still being collected and these will be linked to the primary data sets to construct a gene-data bank.

Table 2. Number of samples collected by the end of 2002

Tanjung Datu NP		
Species Name	Family	No. of samples
1 *Goniothalamus* sp.	ANNONACEAE	1
2 *Durio* sp.	BOMBACACEAE	1
3 *Dipterocarpus* sp.	DIPTEROCARPACEAE	1
4 *Hopea* sp.	DIPTEROCARPACEAE	6
5 *Shorea exelliptica*	DIPTEROCARPACEAE	1
6 *Shorea leprosula*	DIPTEROCARPACEAE	2
7 *Shorea* sp.	DIPTEROCARPACEAE	9
8 *Garcinia* sp.	GUTTIFERAE	2
9 *Eusideroxylon* sp.	LAUREACEAE	2
10 *Dialium laurinum*	LEGUMINOSAE	1
11 *Ficus* sp.	MORACEAE	1
12 *Xanthophyllum* sp.	POLYGALACEAE	1
13 Species unknown	RUBIACEAE	1
14 *Scaphium macropodum*	STERCULIACEAE	1

Gunung Gading N.P.		
Species Name	Family	No. of samples
1 *Durio* sp.	BOMBACACEAE	1
2 *Dipterocarpus* sp.	DIPTEROCARPACEAE	3
3 *Dryobalanops beccarii*	DIPTEROCARPACEAE	36
4 *Hopea* sp.	DIPTEROCARPACEAE	9
5 *Shorea dasyphylla*	DIPTEROCARPACEAE	1
6 *Shorea leprosula*	DIPTEROCARPACEAE	1
7 *Shorea patoiensis*	DIPTEROCARPACEAE	1
8 *Shorea* sp.	DIPTEROCARPACEAE	20
9 *Vatica* sp.	DIPTEROCARPACEAE	1
10 Species unknown	EUPHORBIACEAE	1
11 *Castanopsis hypophoenicea*	FAGACEAE	1
12 *Eusideroxylon zwageri*	LAUREACEAE	1
13 *Artocarpus* sp.	MORACEAE	2
14 *Scorodocarpus borneensis*	OLACACEAE	1
15 *Rinorea* sp.	VIOLACEAE	1

Kubah N.P.		
Species Name	Family	No. of samples
1 *Cotylelobium burckii*	DIPTEROCARPACEAE	3
2 *Dipterocarpus nudus*	DIPTEROCARPACEAE	1
3 *Dipterocarpus sarawakensis*	DIPTEROCARPACEAE	1
4 *Dipterocarpus* sp.	DIPTEROCARPACEAE	1
5 *Dryobalanops beccarii*	DIPTEROCARPACEAE	58
6 *Dryobalanops oblongifolia*	DIPTEROCARPACEAE	4
7 *Hopea kerangasensis*	DIPTEROCARPACEAE	3
8 *Hopea micrantha*	DIPTEROCARPACEAE	3
9 *Hopea* sp.	DIPTEROCARPACEAE	3
10 *Shorea beccariana*	DIPTEROCARPACEAE	66
11 *Shorea brunnescens*	DIPTEROCARPACEAE	1
12 *Shorea coriacea*	DIPTEROCARPACEAE	3

13 *Shorea dasyphylla*	DIPTEROCARPACEAE	1
14 *Shorea exelliptica*	DIPTEROCARPACEAE	3
15 *Shorea hopeifolia*	DIPTEROCARPACEAE	3
16 *Shorea kunstleri*	DIPTEROCARPACEAE	1
17 *Shorea longiflora*	DIPTEROCARPACEAE	1
18 *Shorea macrophylla*	DIPTEROCARPACEAE	52
19 *Shorea maxwelliana*	DIPTEROCARPACEAE	3
20 *Shorea myrionerva*	DIPTEROCARPACEAE	1
21 *Shorea ovata*	DIPTEROCARPACEAE	1
22 *Shorea parvifolia*	DIPTEROCARPACEAE	3
23 *Shorea pauciflora*	DIPTEROCARPACEAE	1
24 *Shorea pubistyla*	DIPTEROCARPACEAE	1
25 *Shorea quadrinervis*	DIPTEROCARPACEAE	1
26 *Shorea richetia*	DIPTEROCARPACEAE	3
27 *Shorea scaberrima*	DIPTEROCARPACEAE	1
28 *Shorea sdealbata*	DIPTEROCARPACEAE	3
29 *Shorea slootenii*	DIPTEROCARPACEAE	3
30 *Shorea* sp.	DIPTEROCARPACEAE	6
31 *Vatica coriacea*	DIPTEROCARPACEAE	1
32 *Vatica sarawakensis*	DIPTEROCARPACEAE	1
33 *Vatica* sp.	DIPTEROCARPACEAE	1
34 *Eusideroxylon* sp.	LAURACEAE	1
35 *Eusideroxylon zwageri*	LAURACEAE	1

Semengok Arboretum

Species Name	Family	No. of samples
1 *Dipterocarpus* sp.	DIPTEROCARPACEAE	1
2 *Hopea* sp.	DIPTEROCARPACEAE	4
3 *Shorea parvifolia*	DIPTEROCARPACEAE	1
4 *Shorea beccariana*	DIPTEROCARPACEAE	75
5 *Shorea macroptera*	DIPTEROCARPACEAE	1
6 *Vatica* sp.	DIPTEROCARPACEAE	6

Bako N.P.

Species Name	Family	No. of samples
1 *Mangifera* sp.	ANACARDIACEAE	1
2 *Durio* sp.	BOMBACACEAE	1
3 *Anisoptera* sp.	DIPTEROCARPACEAE	1
4 *Cotylelobium* sp.	DIPTEROCARPACEAE	3
5 *Dipterocarpus borneensis*	DIPTEROCARPACEAE	1
6 *Dipterocarpus* sp.	DIPTEROCARPACEAE	4
7 *Dryobalanops beccarii*	DIPTEROCARPACEAE	55
8 *Hopea* sp.	DIPTEROCARPACEAE	5
9 *Shorea beccariana*	DIPTEROCARPACEAE	43
10 *Shorea dealbata*	DIPTEROCARPACEAE	1
11 *Shorea macroptera*	DIPTEROCARPACEAE	1
12 *Shorea ovata*	DIPTEROCARPACEAE	1
13 *Shorea* sp.	DIPTEROCARPACEAE	4
14 *Vatica* sp.	DIPTEROCARPACEAE	4
15 *Lithocarpus* sp.	FAGACEAE	1

16 *Garcinia* sp.	GUTTIFERAE	1
17 Species unknown	LAUREACEAE	1
18 *Dialium* sp.	LEGUMINOSAE	1
19 *Aglaia* sp.	MELIACEAE	1
20 *Artocarpus* sp.	MORACEAE	2
21 *Rhizophora* sp.	RHIZOPHORACEAE	38
22 *Eurycoma longifolia*	SIMAROUBACEAE	1
23 *Sonneratia* sp.	SONNERATIACEAE	43
24 *Aquilaria* sp.	THYMELAEACEAE	1
25 *Avicennia alba*	VERBENACEAE	37
26 *Avicennia marina*	VERBENACEAE	34

Similajau N.P.

Species Name	Family	No. of samples
1 *Dipterocarps* sp.	DIPTEROCARPACEAE	6
2 *Dryobalanops* sp.	DIPTEROCARPACEAE	64
3 *Hopea miclantha*	DIPTEROCARPACEAE	3
4 *Hopea* sp.	DIPTEROCARPACEAE	3
5 *Shorea beccariana*	DIPTEROCARPACEAE	61
6 *Shorea hopeifolia*	DIPTEROCARPACEAE	1
7 *Shorea* sp.	DIPTEROCARPACEAE	9
8 *Vatica* sp.	DIPTEROCARPACEAE	6

Niah N.P.

Species Name	Family	No. of samples
1 *Dipterocarpus* sp.	DIPTEROCARPACEAE	3
2 *Hopea* sp.	DIPTEROCARPACEAE	2
3 *Shorea macroptera*	DIPTEROCARPACEAE	1
4 *Shorea ochracea*	DIPTEROCARPACEAE	1
5 *Shorea parvifolia*	DIPTEROCARPACEAE	1
6 *Shorea* sp.	DIPTEROCARPACEAE	5
7 *Vatica* sp.	DIPTEROCARPACEAE	1
8 *Eusideroxylon* sp.	LAUREACEAE	1

Lambir Hills N.P.

Species Name	Family	No. of samples
1 *Gluta laxiflora*	ANACARDIACEAE	83
2 *Parishia maingayi*	ANACARDIACEAE	124
3 *Polyalthia glabrescens*	ANNONACEAE	65
4 *Dacryodes rostrata*	BURSERACEAE	74
5 *Anisoptera grossivenia*	DIPTEROCARPACEAE	3
6 *Anisoptera* sp.	DIPTEROCARPACEAE	1
7 *Cotylelobium melanoxylon*	DIPTEROCARPACEAE	2
8 *Cotylelobium* sp.	DIPTEROCARPACEAE	2
9 *Dipterocarpus acutangulus*	DIPTEROCARPACEAE	3
10 *Dipterocarpus caudatus*	DIPTEROCARPACEAE	1
11 *Dipterocarpus caudiferus*	DIPTEROCARPACEAE	1
12 *Dipterocarpus confertus*	DIPTEROCARPACEAE	2
13 *Dipterocarpus crinitus*	DIPTEROCARPACEAE	3
14 *Dipterocarpus geniculatus*	DIPTEROCARPACEAE	2

15 *Dipterocarpus globosus*	DIPTEROCARPACEAE	86
16 *Dipterocarpus kunstleri*	DIPTEROCARPACEAE	52
17 *Dipterocarpus pachyphyllus*	DIPTEROCARPACEAE	4
18 *Dipterocarpus palembanicus*	DIPTEROCARPACEAE	5
19 *Dipterocarpus* sp.	DIPTEROCARPACEAE	2
20 *Dryobalanops aromatica*	DIPTEROCARPACEAE	188
21 *Dryobalanops lanceolata*	DIPTEROCARPACEAE	221
22 *Hopea beccariana*	DIPTEROCARPACEAE	149
23 *Hopea bracteata*	DIPTEROCARPACEAE	3
24 *Hopea dryobalanoides*	DIPTEROCARPACEAE	137
25 *Hopea mesuoides*	DIPTEROCARPACEAE	1
26 *Hopea mesuoides*	DIPTEROCARPACEAE	34
27 *Hopea micrantha*	DIPTEROCARPACEAE	3
28 *Hopea pterygota*	DIPTEROCARPACEAE	2
29 *Parashorea parvifolia*	DIPTEROCARPACEAE	3
30 *Parashorea smythiesii*	DIPTEROCARPACEAE	2
31 *Shorea acuta*	DIPTEROCARPACEAE	3
32 *Shorea agami*	DIPTEROCARPACEAE	3
33 *Shorea almon*	DIPTEROCARPACEAE	2
34 *Shorea amplexicaulis*	DIPTEROCARPACEAE	2
35 *Shorea argentifolia*	DIPTEROCARPACEAE	2
36 *Shorea asahi*	DIPTEROCARPACEAE	3
37 *Shorea atrinervosa*	DIPTEROCARPACEAE	2
38 *Shorea beccariana*	DIPTEROCARPACEAE	56
39 *Shorea biawak*	DIPTEROCARPACEAE	3
40 *Shorea bullata*	DIPTEROCARPACEAE	2
41 *Shorea cf. patoiensis*	DIPTEROCARPACEAE	1
42 *Shorea confusa*	DIPTEROCARPACEAE	2
43 *Shorea curtisii*	DIPTEROCARPACEAE	1
44 *Shorea dasyphylla*	DIPTEROCARPACEAE	2
45 *Shorea domatiosa*	DIPTEROCARPACEAE	1
46 *Shorea exelliptica*	DIPTEROCARPACEAE	3
47 *Shorea faguetiana*	DIPTEROCARPACEAE	3
48 *Shorea falciferoides subsp. glaucescens*	DIPTEROCARPACEAE	3
49 *Shorea fallax*	DIPTEROCARPACEAE	1
50 *Shorea ferruginea*	DIPTEROCARPACEAE	2
51 *Shorea flemmichii*	DIPTEROCARPACEAE	3
52 *Shorea foxworthyi*	DIPTEROCARPACEAE	3
53 *Shorea geniculata*	DIPTEROCARPACEAE	2
54 *Shorea havilandii*	DIPTEROCARPACEAE	3
55 *Shorea hopeifolia*	DIPTEROCARPACEAE	3
56 *Shorea inappendiculata*	DIPTEROCARPACEAE	2
57 *Shorea johorensis*	DIPTEROCARPACEAE	2
58 *Shorea kunstleri*	DIPTEROCARPACEAE	2
59 *Shorea laxa*	DIPTEROCARPACEAE	234
60 *Shorea leprosula*	DIPTEROCARPACEAE	3
61 *Shorea longiflora*	DIPTEROCARPACEAE	3
62 *Shorea macrophylla*	DIPTEROCARPACEAE	81
63 *Shorea macroptera subsp. baillonii*	DIPTEROCARPACEAE	2
64 *Shorea macroptera subsp. macropterafolia*	DIPTEROCARPACEAE	2

65	*Shorea multiflora*	DIPTEROCARPACEAE	3
66	*Shorea myrionerva*	DIPTEROCARPACEAE	1
67	*Shorea ochracea*	DIPTEROCARPACEAE	2
68	*Shorea ovalis*	DIPTEROCARPACEAE	3
69	*Shorea ovata*	DIPTEROCARPACEAE	3
70	*Shorea parvifolia*	DIPTEROCARPACEAE	3
71	*Shorea patoiensis*	DIPTEROCARPACEAE	2
72	*Shorea pauciflora*	DIPTEROCARPACEAE	3
73	*Shorea pilosa*	DIPTEROCARPACEAE	3
74	*Shorea pubistyla*	DIPTEROCARPACEAE	3
75	*Shorea quadrinervis*	DIPTEROCARPACEAE	3
76	*Shorea rubella*	DIPTEROCARPACEAE	2
77	*Shorea rubra*	DIPTEROCARPACEAE	1
78	*Shorea sagittata*	DIPTEROCARPACEAE	3
79	*Shorea scaberimma*	DIPTEROCARPACEAE	10
80	*Shorea scabrida*	DIPTEROCARPACEAE	2
81	*Shorea scrobiculata*	DIPTEROCARPACEAE	2
82	*Shorea slootenii*	DIPTEROCARPACEAE	3
83	*Shorea smithiana*	DIPTEROCARPACEAE	12
85	*Shorea* sp.	DIPTEROCARPACEAE	12
86	*Shorea superba*	DIPTEROCARPACEAE	3
87	*Shorea xanthophylla*	DIPTEROCARPACEAE	3
88	*Vatica badiifolia*	DIPTEROCARPACEAE	3
89	*Vatica micrantha*	DIPTEROCARPACEAE	256
90	*Vatica nitens*	DIPTEROCARPACEAE	3
91	*Vatica oblongifolia subsp. crassilobata*	DIPTEROCARPACEAE	3
92	*Vatica oblongifolia subsp. multinervosa*	DIPTEROCARPACEAE	3
93	*Vatica oblongifolia subsp. oblongifolia*	DIPTEROCARPACEAE	3
94	*Vatica sarawakensis*	DIPTEROCARPACEAE	3
95	*Vatica* sp.	DIPTEROCARPACEAE	1
96	*Diospyros decipiens*	EBENACEAE	49
97	*Drypetes mymecophila*	EUPHORBIACEAE	46
98	*Mallotus leucodermis*	EUPHORBIACEAE	42
99	*Hydnocarpus pentagyna*	FLACOURTIACEAE	79
100	*Irvingia malayana*	IRVINGIACEAE	46
101	*Allantospermum borneense*	IXONANTHACEAE	255
102	*Whiteodendron moutlonianum*	MYRTACEAE	69
103	*Rinorea bengalensis*	VIOLACEAE	160

Landeh FR. (Planting)

	Species Name	Family	No. of samples
1	*Shorea macrophylla*	DIPTEROCARPACEAE	120
2	*Shorea pinanga*	DIPTEROCARPACEAE	45
3	*Eusideroxylon zwageri*	LAURACEAE	48

Bakam Experiment Site (Planting)

	Species Name	Family	No. of samples
1	*Dryobalanops aromatica*	DIPTEROCARPACEAE	40
2	*Dryobalanops lanceolata*	DIPTEROCARPACEAE	40
3	*Parashorea smythiesii*	DIPTEROCARPACEAE	40

4 Shorea beccariana	DIPTEROCARPACEAE	40
5 Shorea macrophylla	DIPTEROCARPACEAE	40

Niah Experiment Site (Planting)		
Species Name	Family	No. of samples
1 Dipterocarps tempehes	DIPTEROCARPACEAE	40
2 Dryobalanopus beccarii	DIPTEROCARPACEAE	40
3 Dryobalnopus lanceolata	DIPTEROCARPACEAE	40
4 Hopea dryobalanoides	DIPTEROCARPACEAE	40
5 Parashorea macrophylla	DIPTEROCARPACEAE	40
6 Shorea argentifolia	DIPTEROCARPACEAE	40
7 Shorea beccariana	DIPTEROCARPACEAE	40
8 Shorea isoptera	DIPTEROCARPACEAE	40
9 Shorea leprosula	DIPTEROCARPACEAE	40
10 Shorea macrophylla	DIPTEROCARPACEAE	57
11 Shorea ovalis	DIPTEROCARPACEAE	40
12 Shorea ovata	DIPTEROCARPACEAE	40
13 Shorea parvifolia	DIPTEROCARPACEAE	40
14 Shorea slooteni	DIPTEROCARPACEAE	40
15 Shorea virescence	DIPTEROCARPACEAE	40
16 Eusideroxylon zwageri	LAURACEAE	40

Kapit (Seedling)		
Species Name	Family	No. of samples
1 Shorea beccariana	DIPTEROCARPACEAE	5

Sibu (Seedling)		
Species Name	Family	No. of samples
1 Shorea beccariana	DIPTEROCARPACEAE	5

CONCLUSIONS

Although the number of species and the area covered by the sampling doesn't satisfy the objectives of this experiment, it is anticipated how much of the genetic variation is present in tree populations of tropical rainforests and how they are different from each other from population to population will be clarified. The populations in national parks and forest reserves in Sarawak will be the representative ones which once covered the broad area of Sarawak and connected each other. By using the appropriate molecular markers the genetic constitution of tree populations is expected to be elucidated. These data will be incorporated into the gene-data bank to identify individual trees of certain species at certain locations.

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