## E-1.2 Studies on the potential growth and photosynthesis capacity of tropical tree seedlings

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**Key words:** Disturbed Forest, Seedling Establishment, Pteriophyte, Species Diversity, Tropical rain forest

**Abstract** In order to understand potential growth of tropical tree species in a disturbed ecosystem, seedlings of 21 species with 1200 individuals were transplanted in an abandoned rubber plantation. The seedling survivorship of early succession species was not always higher in open sites than in closed canopy sites. The initial size of seedlings is important for the subsequent growth under the open site but not under the closed canopy sites. A long-term observation was also made on the morphological characteristics during the growth of a common pioneer species *Macaranga gigantea* (Eupholobiaceae). Light environment has been found to be a potentially important factor to control the morphological plasticity of the species. Seedlings of *M. gigantifolia* Merr. and *M. triloba* (bl.) also continuously produce huge leaves on an erect stem like *M. gigantea*. Monoaxial growth with huge leaves and continuously growth is a common growth habit of juvenile pioneer trees. It is very likely that these species avoid mutual shading by elongation of petiole and change in petiole angle. Moreover, photosynthetic measurements were carried out to examine the physiological effects on growth of some tropical tree species. Air humidity was found to affect both steady and dynamic photosynthesis in *Shorea parvifolia*. Sunflecks contributed largely to the carbon gain in the species under a closed canopy, while the combination of low air humidity and the high photon flux density reduced largely the photosynthetic carbon gain of seedlings growing in gaps. Leaf photosynthetic characteristics were compared among trees under different light environments. Saturated photosynthetic rate was much higher in canopy or emergency trees as compared to those under canopy.

**Key words**: Tropical rain forest, Tree Seedlings, Gap, Photosynthesis, Morphological plasticity, Light environment.

## 1. Introduction

The tropical forests, especially the tropical rain forests are of great importance in maintenance of bio-diversity and in global $CO_2$ balance. However, tropical rain forests are facing various ecological and environmental problems due to deforestation. The total area of tropical forest was 24.5 x $10^6$ $km^2$ or 16% of terrestrial area of the earth, but in 1990 it decreased to 16 x $10^6$ $km^2$ or to 7% of the total terrestrial forest. It is, therefore, necessary to regenerate the tropical rain forest with the massive species of tropical trees. After the deforestation, the effort to regenerate tropical forests is particularly appreciated for ameliorating the global environment. In the present study, we therefore focused our attention on the development of the measure to for plantation of tropical forest with high diversity of tree species.

To lead to a successful plantation and management of tropical rain forest, it is important to have some basic and necessary knowledge for growth, morphological and physiological characteristics of various tree seedlings. Growing tree seedlings of different species in nature environments would be one of approaches to determine the growth response to various environmental factors and then to obtain some basic information for the plantation of those tree species. We therefore investigated the growth performance for tree seedlings grown under natural conditions in Malaysia.

Morphological plasticity is always an important determinant for growth performance in tree seedling growth. A crown of trees consists of leaves with different age and size. The effective display of these leaves is of paramount importance to all trees. Mutual shading of leaves within a crown reduces the light capture efficiency and thus lessens the potential of growth and survival of trees. Therefore, trees must have been subjected to strong selection pressure for minimizing the mutual shading. Many scientists have analyzed the crown structure in terms of the effective display of leaves and successfully showed the morphological adaptation to reduce the mutual shading [1,2,3,4,5]. However, majority of the prior investigations focus merely on the species with flushing type leaf emergence. Little is understood how the continuous leafing species reduce it. We therefore carried out a long-term observation on the crown structure of seedlings of a common pioneer species in the Far Eastern tropics, *Macaranga gigantea* (Rub. f. et. Toll.) M. A. (Eupholobiaceae) , which continuously produce huge leaves on an erect stem with short leafing interval to quantitatively analyze the developmental processes of leaves and evaluate the contribution of the developmental process of petiole to avoiding the mutual shading within a crown.

Photosynthesis is another important determinant for growth. We examined also photosynthetic characteristics of *Dipterocarpus sublamellatus*, which is an apparently component of the emergent tree layer, being regarded as an emergent tree species [6,7]. As *D. sublamellatus* can regenerate naturally from seeds, the seedlings or saplings should suffer to extremely low light intensities, while the leaves of mature trees should expose to high light intensities. Therefore, we hypothesize here that differences between long-term understory and canopy-acclimated plants responses to changes in environments may be significant within the specific species. In this study, we examine this hypothesis by evaluating the photosynthetic characteristics of *D. sublamellatus* from in situ field measurements. The study concentrated in particular on photosynthetic characteristics as functions of irradiance intensities and air $CO_2$ concentrations. The objective of this investigation was to assess the degree of acclimation to the micro-environmental gradients from the understory to the over-canopy that occurs in leaves of *D. sublamellatus* across the whole natural regeneration processes from seedling phase to canopy-emergent phase.

## 2. Research Objectives

1) To examine growth and survivorship of tree seedlings in different species from tropical rain forests.

2) To investigate the morphological plasticity of tropical tree seedings.

3) To know the effects of physiological performance of tree seedling in relation to the microclimatic factors including light, water, $CO_2$ availability.

## 3. Research Methods

1) Intra- and Inter-specific differences in growth and mortality of tropical seedlings grown under different light environment

In 1996, mass flowering was observed in South East Asian areas and many trees put fruits after that event in September of the same year. This event was lucky for us to start the field experiment for the regeneration of tropical environmental forest. In that year, many fruits were dropped from trees in the Pasoh Forest Reserve. We collected those seeds at that area in September, 1996. After germination, seedlings were cultivated under the shade condition for three months and were transplanted into the abandoned experimental rubber plantation situated in the UPM farm in December, 1996. The area of the study site is ca. 1 ha and weeds were clear cut before transplanting but trees higher than 2 m were remained. Seedlings were separated into two groups; one group was for an open area (open plots) and another for the area covered with trees (shade plots). Planted seedlings were composed of 21 species and 2400 individuals including early successional species, such as *Macaranga*, and late successional species, such as *Dipterocarpus* and *Shorea*.

2) Leaf developmental process and crown structure of a tropical pioneer tree, *Macaranga gigantea* in Peninsular Malaysia

A destructive sampling was performed in an experimental farm in Universiti Putra Malaysia (UPM), Selangor, Malaysia According to the meteorological records of precipitation at UPM for 3 years from 1994 to 1996, the average annual precipitation is 1,986 mm. Many *M. gigantea* trees naturally invade the experimental farm and varioussizes of trees can be found there. Twelve monoaxial *M. gigantea* trees without any evidence of past breakage ranging from 5 to 210 cm in height were collected from the experimental farm on May 5 and 14, 1997. The sample trees were dug out of the ground and measured by following dimensions immediately after collection:tree height (cm),height of the lowest petiole base (cm),stem diameter at a height of 1/10 of height (cm),two crown diameters for right angle (cm),petiole length (cm),leaf-blade width (cm),leaf-blade length (cm).

All sample trees were divided into root, stem, petiole, and leaf-blade, dried at 70℃ for a week in an electric oven, and weighed. Leaf area ($cm^2$) was measured by an electric leaf-area meter (Licor model 3100). The observed maximum leaf length including petiole and leaf-blade was 153 cm on a sapling of 210 cm in height.

3) Physiological response of *Dipterocarpus sublamellatus* as correlate of the natural regeneration

Gas exchange measurements were performed during September 1998 in the field with a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Neb., USA). *In situ* measurements of seedlings and saplings were carried out by setting the leaf chamber to a tripod. Whist the assays to main-canopy and emergent trees were made possible by employed a canopy walkway. The canopy walkway is constructed by the NIES/FRIM/UPM Joint Research Project supported by the Japanese Environment Agency. Two 34 m-high towers and one 52 m-high tower were connected to each other with three 20-m long walkways at 30-m height. There is a 3-m long measurement stage set to the 52 m-high tower at 36-m height. Leaves were measured at ambient relative humidities, which were 80.7%, 79.2%, 78.0% and 77.7% for seedlings, saplings, main-canopy and emergents, respectively. Leaf temperatures were controlled at 26.5 ± 0.2°C for seedlings and saplings, 28.5 ± 0.2°C and 30.5 ± 0.2°C for main canopy and emergent trees, respectively, according to vertical distribution of air temperature in Pasoh forest (Aoki et al. 1975).

Photosynthetic light response (*A*/PFD) curves were determined at both atmospheric $CO_2$ concentration ($pCO_2$) of 350 and 700 μmol $mol^{-1}$, respectively, for leaves from each regenerating phase. The desired $CO_2$ concentration of the measurement air was adjusted automatically with a LI-6400-01 external $CO_2$ source assembly (LI-COR Inc.) by injecting pure $CO_2$ into the $CO_2$-free breathing air. PFD was provided by a LI-6400-02B blue-red LED light source (LI-COR Inc.). The leaf was first fully induced at a PFD of 2000 μmol $m^{-2}$ $s^{-1}$, would above the saturation point, and then photosynthesis was measured in response to programmed step-wise decreases in PFD until darkness was reached. Again, the $A/C_i$ response curves were quantified at both low PFD of 50 μmol $m^{-2}$ $s^{-1}$ and saturated light intensities, respectively. Saturated light intensities read from the *A*/PFD response curves were chosen as 500 μmol $m^{-2}$ $s^{-1}$ PFD for seedling and sapling leaves, 1000 μmol $m^{-2}$ $s^{-1}$ for main-canopy and canopy-emergent leaves, respectively. The leaf was first allowed to reach steady-state in $CO_2$-free air. Photosynthesis was then measured as the steady-state rates achieved following programmed step-wise increases in $CO_2$ concentration to 1600 μmol $mol^{-1}$. For both *A*/PFD and $A/C_i$ responses, five replicates were presented for each regenerating phase from the data only obtained during the period 08:00-12:00 hours, in order to avoid a bias from diurnal effects [8].

Photosynthetic light-use efficiency ($\phi$, mol $mol^{-1}$) was determined from the initial slope of the response of photosynthesis to PFD. Photosynthetic capacity, or light saturated photosynthesis ($A_{sat}$) and the corresponding stomatal conductance ($g_{s,sat}$), were means of the specific values at PFD above the saturation point for each measurement, and mean values were calculated for each regenerating phase. Maximum *in vivo* carboxylation activity of Rubisco ($V_{c,max}$, μmol $m^{-2}$ $s^{-1}$) and maximum rate of whole-chain electron transport contributing to $RuP_2$ regeneration ($J_{max}$, μmol $m^{-2}$ $s^{-1}$), which determine the initial slope of the response of $A_{sat}$ to $C_i$ and the $A_{sat}$ at saturation $C_i$, respectively, were calculated from $A/C_i$ curves. Dark respiration ($R_d$) was *in situ* measured value at ambient $pCO_2$. Photosynthetic light compensation point ($Q_{lcp}$), when $A$=0, was calculated from $-R_d/\phi$.

Subsequent to the gas exchange measurements nine of the respective leaves were harvested, three leaves in each sample. The area of the fresh leaves was determined by means of a LI-3100-C leaf area meter (LI-COR Inc.). A section of determined area (540 $mm^2$ each) was punched from each leaf for chlorophyll determination. The leaf section

was cut into small strips and ground with 10 ml of 80% acetone. The samples were then centrifugalized at 800 dm with a Tomy RL-101 refrigerated centrifuge (Tomy Seiko Co. Ltd., Tokyo, Japan) for 2 min at 5°C. The clear supernatant was removed and diluted to 50 ml by 80% acetone and chlorophyll was determined spectrophotometrically using U-3000 spectrophotometer (Hitachi Ltd., Tokyo, Japan).

## 4. Results and Discussion

1) Intra- and Inter-specific differences in growth and mortality of tropical seedlings grown under different light environment

Judging from the survival rate, 21 tree species cultivated in the UPM farm could be categorized into shade tolerant species and sun favorite ones.The survival rate of every species determined at ca. 300 days after transplanting is shown in Table 1. In *Macaranga gigantea*, the early succssional species, the survival rate in an open area was only 19% while that in a closed site was 62% (Table 1). In the present study, we planted three *Macaranga* species, however, only *M. gigantea* showed the typical sun favorite behavior. Other two *Macaranga* species, such as *M. triloba* and *M. lowii*, the survival rate under the open condition was almost comparable with that under the shade condition.

In contrast to the species of *Macaranga*, the survival rates of Dipterocarpaceae species were shade tolerant. The typical shade tolerant Dipterocarpaceae was *Dipterocarpus crinitus*, the survival rate of this species under the shade condition was 100% while that under the open site was 0%. However, not every Dipterocarpaceae species was not survived under the shade condition. the survival rate of *Neobalanocarpus heimii* was comparable between the open and shade areas, 86% and 80% in the open and shade areas, respectively. Beside the species of Dipterocarpaceae, the survival rate of *Elateriospermum tapos*, canopy making species, cultivated under the open condition was comparable with that cultivated under the shade condition.

2) Leaf developmental process and crown structure of a tropical pioneer tree, *Macaranga gigantea* in Peninsular Malaysia.

During the experimental period of 154 days, 10 seedlings studied produced 81 leaves and shed 83 leaves. Figure 1 shows the time trend of the number of at tached leaves and the cumulative numbers of emerged and fallen leave. These seedlings did not show any indications of the rhythmic pattern of growth and continuously produced and fall their leaves. Cumulative numbers of emerged leaves were linearly increased with time ($r^2 = 0.988$) and that of fallen leaves also increased with time ($r^2 = 0.971$). The rate of emerged and fallen leaves was 0.0533 and 0.0597 seedling$^{-1}$ day$^{-1}$, respectively. From this estimation, a seedling produces and falls a leaf every 18.86 and 16.75 days, respectively. Since these values are similar, seedlings can keep dynamic equilibrium status of numbers of the attached leaves (Fig. 1). The arithmetic mean of number of attached leave was 6.88 seedling$^{-1}$.

Table 1. Survival rate of seedlings transplanted in the farm of UPM. The survival rate is the percent of seedlings detected on December 1996 to that detected on October, 1997 (%).

| Species | Open | | Closed | |
|---|---|---|---|---|
| | Days after transplanting | | | |
| | 300 | 660 | 300 | 660 |
| *Dipterocarpus cornutus* | 74 | 43 | 87 | 68 |
| *Dipterocarpus crinitus* | 10 | 0 | 100 | 80 |
| *Dipterocarpus sublamellatus* | 56 | 23 | 69 | 53 |
| *Shorea lepidota* | 56 | 33 | | |
| *Shorea macroptera* | 46 | 17 | 79 | 54 |
| *Shorea multiflora* | 65 | 37 | 62 | 43 |
| *Shorea maxima* | 33 | 11 | | |
| *Shorea paucifolia* | 57 | 36 | 85 | 73 |
| *Neobalanocarpus heimii* | 89 | 74 | 80 | 66 |
| *Elaeocarpus nitidus* | 68 | 61 | 80 | 81 |
| *Elateriospermum tapos* | 85 | 85 | 70 | 20 |
| *Endospermum malaccense* | 0 | 0 | 30 | 0 |
| *Palaquim maingayi* | 60 | 20 | 90 | 60 |
| *Sapium baccatum* | 30 | 15 | 35 | 15 |
| *Scaphium macroppodum* | 50 | 33 | 48 | 28 |
| *Vatica bella* | 32 | 8 | 68 | 52 |
| *Xanthophyllum amoenum* | 66 | 47 | 70 | 56 |
| *Macaranga gigantia* | 60 | 60 | 15 | 5 |
| *Macaranga lowii* | 67 | 46 | | |
| *Macaranga hypoleuca* | | | 81 | 64 |
| *Macaranga triloba* | 51 | 40 | 78 | 65 |
| *Caesalpinia sappan* | 84 | 78 | 88 | 79 |

Figure 2 shows patterns of leaf expansion and petiole elongation. The leaf area quickly expanded in a few weeks after leaf emergence and afterward became constant. The arithmetic means of days for full leaf expansion was 20.8 days ($n$ = 60). On the contrary, petiole elongated for a longer period. The arithmetic means of days for full petiole elongation was 91.8 days ($n$ = 29).

Figure 3 depicts the time trend of petiolar angle. Petioles showed progressive increment in the petiolar angle with time. The petiole toward zenith at the leaf emergence and then eventually stooped with time.

The horizontal distance between stem and petiole-tip increased with insertion level of leaf (Fig. 4). Since petiole elongated (Fig. 2) and was toward from zenith to horizon (Fig. 3) with time, the horizontal distance from stem to the petiole-tip lengthen with time. Thus, a leaf-blade deploys further distance from a stem with time.

The average crown had 11.5% of the whole crown level DMS. The leaves on the average crown had lower degree of mutual shading than 10% except for leaf No. 7. Especially, leaves No. 2, 3, and 4 had no mutual shading. On the other hand, leaf No. 7, which was the oldest leaf in the crown had severely shaded by the upper leaves and c. 79% of leaf area was shaded.

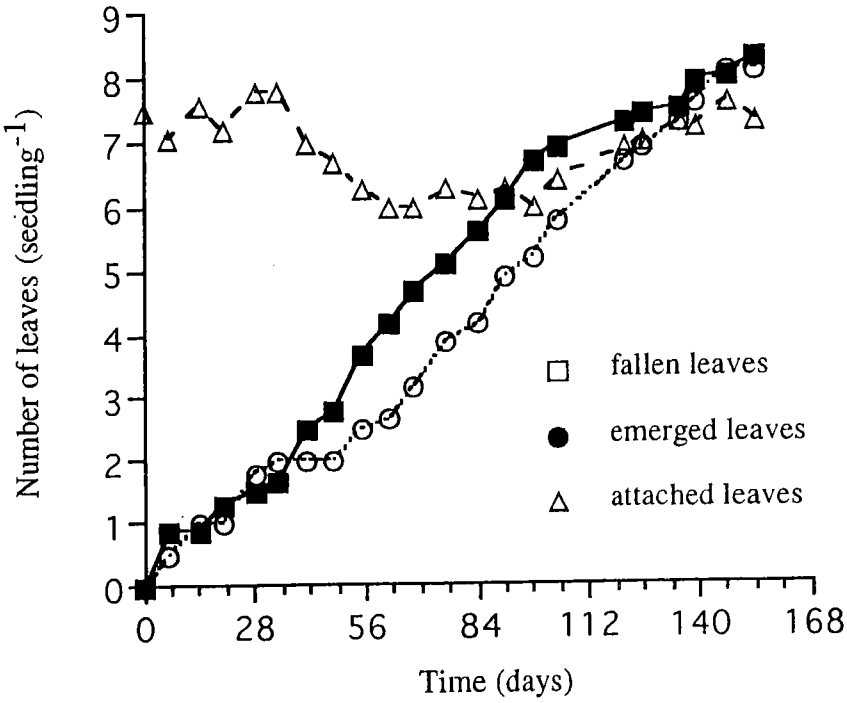



Fig. 1 Time trend of the number of attached leaves and cumulative number of emerged leaves and fallen leaves of 10 seedlings.

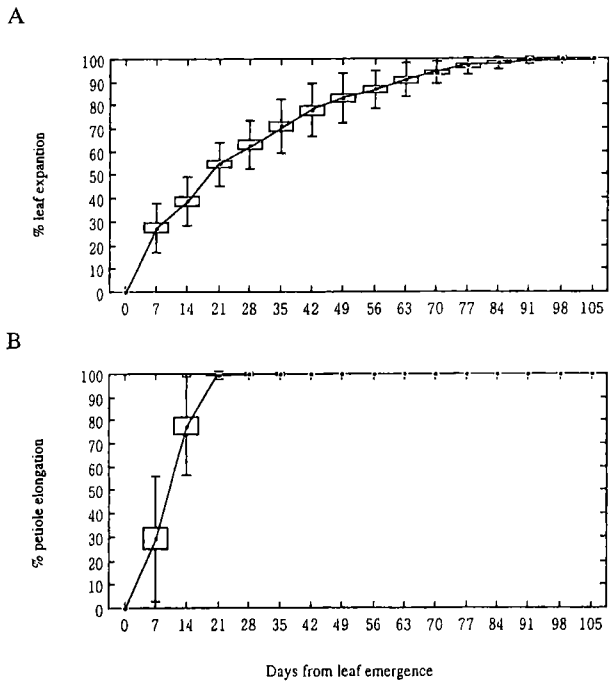



Fig. 2 Patterns of leaf expansion (A) and petiole elongation (B). Circles, vertical bars and boxes indicate the arithmetic mean, ±SD and ±SE, respectively. Sample number is 61 for leaf expansion and 29 for petiole elongation.

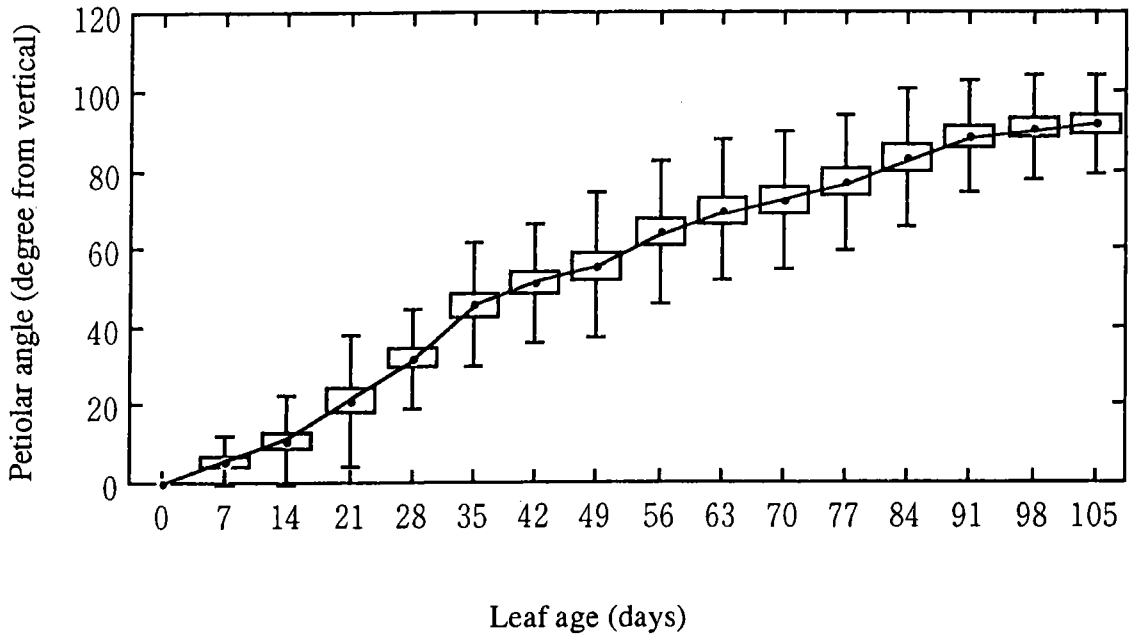



Fig. 3 Change in petiolar angle with time. The petiolar angle are 0 and 90 degrees when petioles are toward zenith and horizon, respectively. Circles, vertical bars and boxes indicate the arithmetic mean, ±SD and ±SE, respectively. Sample number is 29.

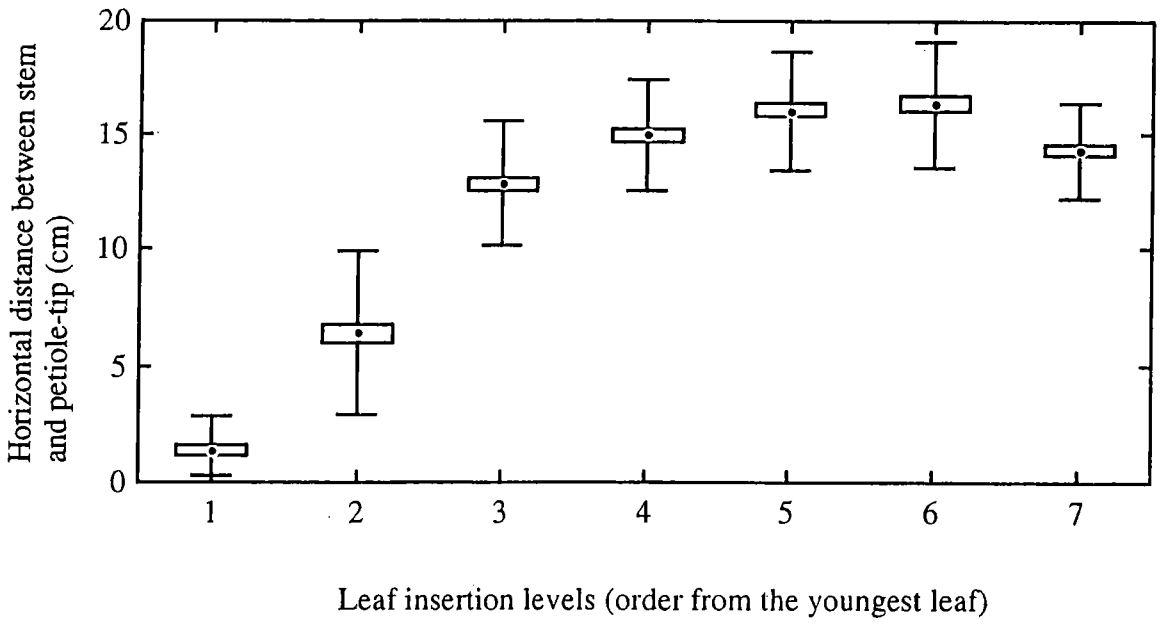
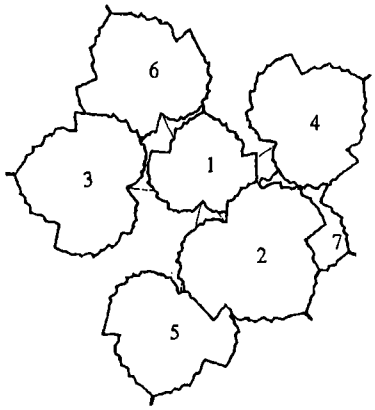
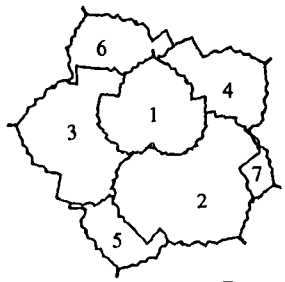


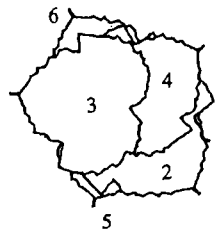

Fig. 4 Horizontal distance between stem and petiole-tip with respect to leaf insertion level. Circles, vertical bars and boxes indicate the arithmetic mean ±SD and ±SE, respectively.



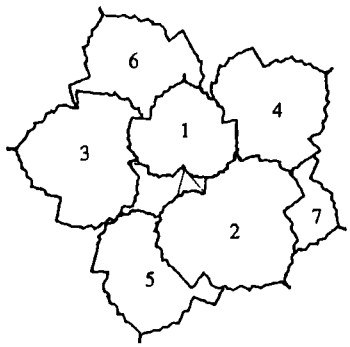

Averege crown



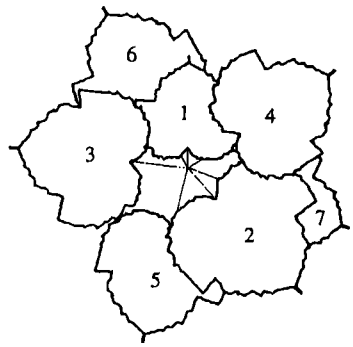

Simulated crown I



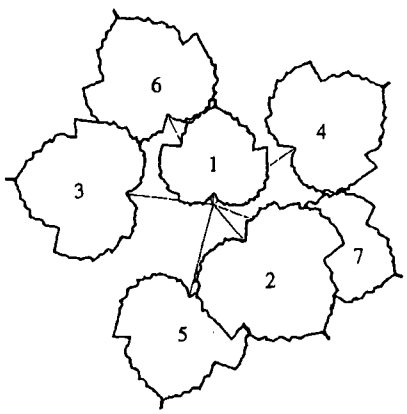

Simulated crown IV



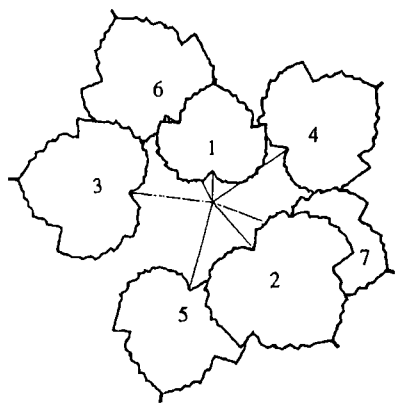

Simulated crown II




Simulated crown V




Simulated crown III




Simulated crown VI

Fig. 5 Polar-view projections of an average crown and six simulated plants of *M. gigantea* seedlings. Numerals on leaf-blades indicate order of leaf age in each crown.

The simulated crowns I, II, and III were constructed to examine the effect of petiole elongation with time on the DMS. Using the total crown-projection area of the average crown as the base line, the simulated crowns I and II had smaller and simulated crown III had higher total crown-projection area than the average crown (Table 2). Simulated crown III yields 5.8% increase in total crown-projection area compare to the average crown. However, on the other hand, the simulate crown III had 42.8% larger total petiole dry mass than the average crown (Table 2). Thus, this crown requires disproportionately energy for constructing petioles of a non-productive organ to gain 5.8% increase in total crown-projection area.

**Table 2.** Total crown-projection area, total petiole dry mass, and the height of the highest leaf-blade from the apex of stem calculated for an average crown and six simulated crowns.

| Type of Crown | % total crown-projection area of the average crown | % total petiole dry mass of the average crown | % the height of the highest leaf-blade from the apex of stem of the average crown |
|---|---|---|---|
| Average crown | 100.0 | 100.0 | 100.0 |
| Simulated crown I | 62.7 | 13.2 | 80.3 |
| Simulated crown II | 91.6 | 58.7 | 150.3 |
| Simulated crown III | 105.8 | 142.8 | 217.7 |
| Simulated crown IV | 37.3 | 100.0 | 142.5 |
| Simulated crown V | 89.0 | 100.0 | 71.8 |
| Simulated crown VI | 102.1 | 100.0 | 0.0 |

To determine the effect of increase in petiolar angle with time on the DMS, the simulated crowns IV, V, and VI were constructed. The simulated crowns IV and V had smaller and simulated crown VI had slightly larger total crown-projection area than the average crown despite having equivalent total petiole dry mass to the average crown. The percentage of total crown-projection area of Simulated crown VI are 102.1% that of the average crown. However, since the leaves on this crown oriented to the horizon, this plant can not gain height by producing leaves. Thus, it is disadvantageous in terms of height competition among other trees.

Some are known about the morphological adaptations of the species with flushing type leaf emergence to reduce mutual shading [1,6,7], but little is understood how the continuous leafing species reduce it. Trees with flushing type leaf emergence do not produce any leaves after a flush of leaves. Therefore, their crowns keep a static condition for a period of growth dormancy. Hence, they can minimize the mutual shading by optimizing leaf arrangement in a cluster of leaves which represent in one flushing and by keeping the crown geometry until the following flushing event. Namely, their morphological adaptation to reduce mutual shading are rather static. On the other hand, the crown of the trees with continuous leafing is constantly under a dynamic condition.

Seedlings of *M. gigantea* formed leaves continuously (Fig. 1). The rate of leaf fall was estimated as 16.85 days seedling-1. Since number of leaves attached on a seedling was dynamic equilibrium around 6.88 (Fig. 1), the leaf longevity can be estimated as 115.9 days. [8] described the leaf longevity is c. 3 to 15 months for evergreen species forming leaves continuously. Ogawa et al. [9] reported that the leaf longevity is 133 days for *Durio zibethinus* Murray which is a evergreen species forming leaves continuously.

Leaves quickly expanded, whereas petiole elongated longer period (Fig. 2). The

**Table 3** Summaries of daily microclimate data for over-canopy (52 m high), a tree-fall gap (*ca.* 800 $m^2$) and understory of lowland primary dipterocarp forest of Pasoh, Peninsular Malaysia throughout September 1998. PFD, air temperatures ($T_{air}$, °C) and relative humidities (RH, %) are presented for daily values over the ranges of 06:30-18:30 hours. Rainfall is presented for total value of the month

| Site | PFD (μmol $m^{-2}$ $s^{-1}$) | | $T_{air}$ (°C) | | RH (%) | | Rainfall (mm) |
|---|---|---|---|---|---|---|---|
| | Max | Mean | Max | Min | Max | Min | Mean |
| Over-canopy | 1258±468 | 635±155 | — | — | — | — | — |
| Gap | 1160±309 | 32±24 | 30.6±0.5 | 23.5±0.5 | 100 | 67 | 102±26 |
| Understory | 180±368 | 6±2 | 27.7±0.5 | 23.4±0.2 | 100 | 67 | 87±19 |

**Table 4.** Leaf morphological characteristics from different regenerating phases of *D. sublamellatus*.

| Characteristics | Regenerating phase | | | |
|---|---|---|---|---|
| | Seedlings | Saplings | Main canopy | Emergents |
| Leaf water potential (bars) | -2.12±0.26 | -2.35±0.52 | -5.07±0.70 | -7.85±0.95 |
| Chlorophyll a+b (g $m^{-2}$) | 1.85±0.04 | 1.92±0.02 | 2.14±0.03 | 2.01±0.02 |
| Chlorophyll a/b ratio | 1.56±0.01 | 1.59±0.01 | 2.60±0.01 | 2.47±0.01 |
| Leaf nitrogen content (g $m^{-2}$) | 0.754±0.047 | 1.008±0.044 | 2.253±0.064 | 2.450±0.089 |
| Leaf area ($cm^2$ $leaf^{-1}$) | 33.01 ± 4.50 | 101.78 ± 9.72 | 65.49 ± 5.03 | 45.42 ± 2.93 |
| LSM (g $m^{-2}$) | 50.30 ± 2.43 | 69.54 ± 3.01 | 141.46 ± 5.34 | 159.47 ± 7.34 |

**Table 5.** Photosynthetic characteristics (Mean ± SD) for the leaves from different regenerating phases of *D. sublamellatus*. The means of maximum photosynthesis ($A_{sat}$) and the corresponding stomatal conductance ($g_{s,sat}$) and photosynthetic light-use efficiency ($\phi$• were estimated from the photosynthetic light response curve either at an ambient or elevated $pCO_2$ of 35 or 70 Pa. Dark respiration ($R_d$) was *in situ* measured value at ambient $pCO_2$. Photosynthetic light compensation point ($Q_{lcp}$) was calculated from $-R_d/\phi$. Rubisco activity ($V_{c,max}$) and $RuP_2$ regeneration capacity ($J_{max}$) were calculated from $A/C_i$ curves.

| Characteristic Measurement | | Regenerating phase | | | |
|---|---|---|---|---|---|
| | Condition | SeedlingsSaplings | Main-canopy | Emergents |
| $A_{sat}$ (•mol $m^{-2}$ $s^{-1}$) | Amb $CO_2$ | 2.54 ± 0.81 | 3.11 ± 0.39 | 5.88 ± 0.52 | 4.21 ± 1.58 |
| | Ele $_{CO2}$ | 3.52 ± 0.49 | 4.98 ± 0.401 | 6.66 ± 2.7 4 | 11.40 ± 1.09 |
| $g_{s,sat}$ (mmol $m^{-2}$ $s^{-1}$) | Amb $CO_2$ | 32.07 ± 4.60 | 54.25 ± 8.59 | 75.77 ± 7.82 | 70.05 ± 11.63 |
| | Ele $CO_2$ | 29.61 ± 7.96 | 31.45 ± 8.22 | 63.34 ± 6.37 | 56.76 ± 9.37 |
| $\phi$•(mol $mol^{-1}$) | Amb $CO_2$ | 0.034 ± 0.007 | 0.037 ± 0.001 | 0.062 ± 0.004 | 0.056 ± 0.003 |
| | Ele $CO_2$ | 0.042 ± 0.005 | 0.053 ± 0.004 | 0.091 ± 0.007 | 0.087 ± 0.009 |
| $V_{c,max}$ (•mol $m^{-2}$ $s^{-1}$) | Sat PFD | 8.86 ± 0.51 | 10.75 ± 1.24 | 26.39 ± 5.43 | 24.97 ± 4.70 |
| $J_{max}$ (•mol $m^{-2}$ $s^{-1}$) | Sat PFD | 23.57 ± 3.08 | 27.82 ± 7.14 | 37.69 ± 7.17 | 35.85 ± 5.33 |
| $R_d$ (•mol $m^{-2}$ $s^{-1}$) | Amb $CO_2$ | 0.16 ± 0.07 | 0.29 ± 0.16 | 0.91 ± 0.04 | 1.42 ± 0.33 |
| $Q_{lcp}$ (•mol $m^{-2}$ $s^{-1}$) | Amb $CO_2$ | 2.3±1.0 | 4.4±1.8 | 9.2±1.4 | 12.0±2.1 |

mean period of leaf expansion was 20.8 days. Ogawa et al.[9] reported that the period of leaf expansion was c. two weeks for *D. zibethinus*. Coley and Barone [10] described that the most tropical trees complete leaf expansion within a few weeks. Younger leaves are higher in nitrogen and water content than mature leaves because of an avoidable consequence of cell division and/or growth. Due to the higher nutritional quality, they stand in the breach of herbivores. For tropical shade-tolerant species, 68% of lifeti me damage occurred during the short leaf expansion [10]. Reducing the expansion period would lead to lower overall damage of leaf. The rapid leaf expansion would be a constraint underling leaf development.

Unlike leaf-blade, petiole elongated for a longer period (Fig. 2). The mean period of petiole elongation was 91.8 days. Since petiolar angle increased with time (Fig. 3), the horizontal distance between stem and petiole-tip lengthen with time (Fig. 4). Namely, leaf-blade deploys at a further position from stem with time. Leaves on a vertical shoot are arranged in several rows along the stem. The number of rows varies among the phyllotaxis. *Macaranga gigantea* has 13 rows. Takenaka [11] discussed that shading among leaves on a shoot can be divided into two components, shading among leaves within a row and that among leaves of different rows. He demonstrated that longer petioles reduce between row shading by increasing the distance between rows of leave. Therefore, M. gigantea can reduce mutual shading within a crown by the petiole elongation and increasing petiolar angle. The degree of mutual shading of leaves no. from 1 to 6 was lower than 10% (Table 3). Photosynthetic capacity rapidly increases during leaf development, reaches a maximum shortly after full leaf expansions and decreases with aging[12]. Accordingly, the effective display of younger leaves of which photosynthetic capacity is high is important to increase whole plant carbon gain.

3) Physiological response of *Dipterocarpus sublamellatus* as correlate of the natural regeneration

The microenvironments differed considerably across the canopy gradients with respect to median PFD, $T_{air}$, rainfall and RH (Table 3). The greatest differences occurred in the sunny days (06:30-18:30 hours), when there was a substantial increase in direct irradiance over the canopy. Mean PFD received was 635 μmol $m^{-2}$ $s^{-1}$ for the emergent leaves (periphery), 32 μmol $m^{-2}$ $s^{-1}$ for gap area, and 6 μmol $m^{-2}$ $s^{-1}$ for seedling or sapling leaves in the understory. Value of median PFD for understory was 0.9% over the canopy. The maximum PFD of 180 μmol $m^{-2}$ $s^{-1}$ attaining the understory was around 13:40 on a sunny day, as some strong sunflecks occurred (ranging from 6 to 1045 μmol $m^{-2}$ $s^{-1}$).

Leaf morphological traits varied significantly along the regenerating phases from seedlings to emergents (Table 6). Main-canopy plants have the highest total chlorophyll content followed in order by canopy-emergents, saplings and seedlings, but the differences were not statistically significant ($p > 0.05$). Again, chlorophyll *a/b* ratios had the same rank as total chlorophyll contents, the values for leaves from main-canopy and emergents were significantly ($p < 0.01$) higher than that of seedlings and saplings.

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Leaf area, LSM and nitrogen contents were also associated with regenerating phases (Table 4). Single leaf area decreased dramatically with regeneration developed from saplings to emergents. Saplings had markedly larger single leaf area, *i.e.*, 2.25 times of that of canopy-emergents. However, seedlings had smallest single leaf area, *i.e.*, just one third of that for saplings. In contrast, LSM increased steadily and significantly along the regeneration processes. In particularly, leaf nitrogen content on area basis increased statistically significant with the regeneration processes, *i.e.* nitrogen content of emergent leaves was more than three-fold of that of seedling leaves

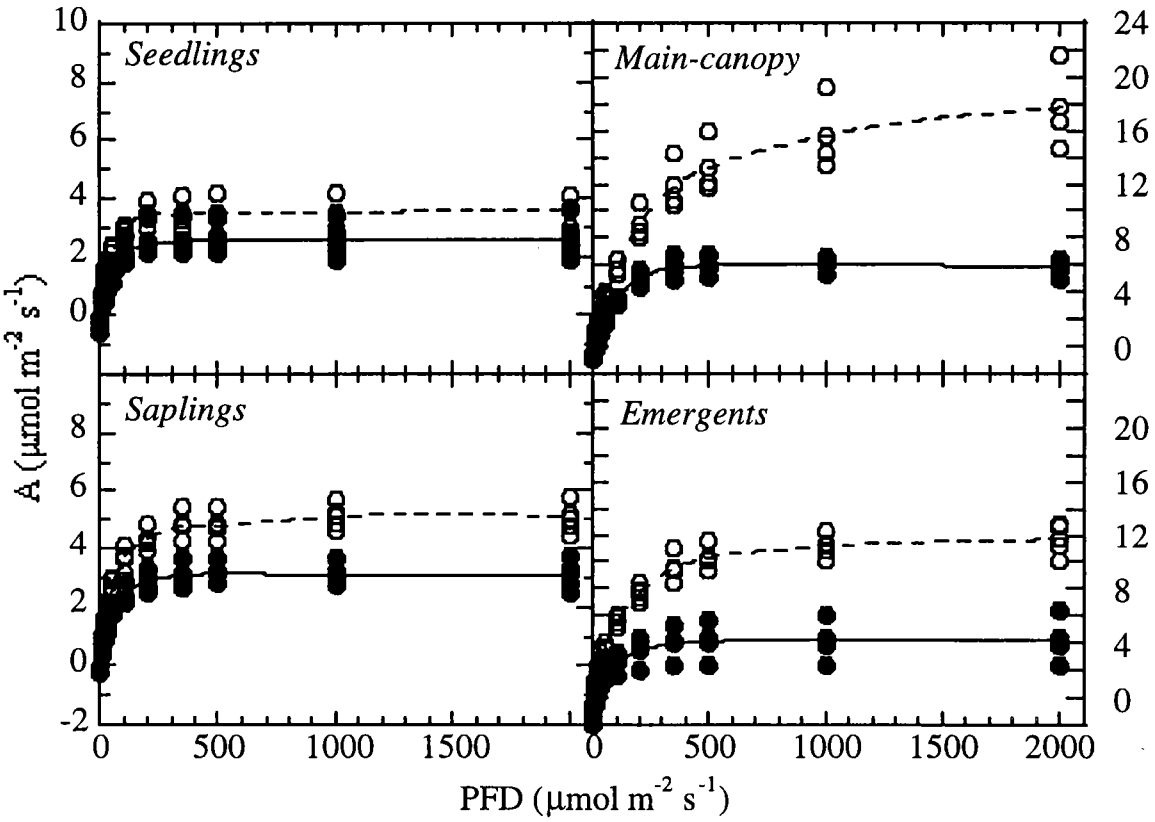


**Fig. 6.** Photosynthetic light responses for a tropical forest tree species, *D. sublamellatus*, from different regenerating stage. Measurement was performed in a primary lowland dipterocarp forest during September 1998 either at ambient $CO_2$ of 350 μmol $mol^{-1}$ (●) or elevated $CO_2$ of 700 μmol $mol^{-1}$ (○). Solid line and broken line indicate the mean value of *A* obtained at ambient and elevated $pCO_2$, respectively. Note that the scales on the axes are different.

Regenerating phases were also associated with a decrease in leaf water potentials (Table 6), being resulted from the significant increase in height of main-canopy and emergent plant as compared to seedlings and saplings. Leaf water potentials were -2.12 and -2.35 bars for seedling and sapling leaves, and decreased to -5.07 and -7.85 bars for those of main-canopy and emergent leaves, respectively.

When measurements were conducted with elevated $CO_2$ air of 700 μmol $mol^{-1}$, photosynthesis increased more dramatically with increasing PFD for all regenerating phases as compared to the measurements with ambient $CO_2$ air (Fig. 6). Furthermore, $A_{sat}$ and $\phi$ increased more significantly for main-canopy and emergent leaves than that for seedling and sapling leaves (Table 5). Results reflected similar trends among the regenerating stages as those measured with 350 μmol $mol^{-1}$ $CO_2$. However, $g_{s,sat}$ decreased for leaves from all regenerating stages.

Light availability varies more dramatically than any other single plant resource across the complex superposed strata of Pasoh forest. The relative PFD is 0.4-0.9% on the forest floor, 1-2% at breast height, 10-15% and 30-34% at 21-25 m and 30 m canopy height, respectively, as compared to that incident above the canopy (Table 3; also see [13,14,15]). The variation of light intensity in turn drive air temperature and humidity different between understory, gap and over-canopy, but the temperature and humidity dependence of photosynthesis is identical for understory and canopy leaves. Therefore, light response characteristics of net photosynthesis are of considerable the most important resource determining the growth, survival, and reproduction of seedlings [16,17,18]

Changes in photosynthetic characteristics with regeneration development are accompanied by changes in morphological traits. Total chlorophyll and leaf nitrogen contents increased from seedlings to matures might be a result of substantial increase in LSM with increasing height in canopy or with increasing light intensity. Fetcher et al. [17]demonstrated that in the vast majority of tropical tree species, seedling leaves are smaller and thinner, with low mass per unit leaf area than adult leaves, until they reach a certain size or developmental stage. Leaf thickness, LSM and stomatal density increase to a considerable extent with higher height in the canopy [19]. Total leaf chlorophyll and chlorophyll *a/b* ratios were different between leaves from different canopy positions and showed the same rank as $A_{sat}$, consisting with the results for other tropical tree species [19,20].

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E-1 DETERMINING INDICATORS FOR SUSTAINABLE MANAGEMENT OF TROPICAL FOREST

(3) Study on genetic relationship between individuals in tropical rainforest.

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**Abstract**

Microsatellite markers were developed from *Shorea curtisii* using. Analysis of 40 individuals of *S.curtisii* from natural forest in Malaysia revealed that all SSR loci were polymorphic. Four SSR markers, *Shc*01, *Shc*04, *Shc*07, and *Shc*09 showed highly polymorphic. We have also compared the genetic diversity between three populations with the different generattions with DBH>5cm, 1cm>DBH>5cm and seedlings of 1 year old. The genetic diversity of seedling population was not high comaring to those of the other generations. The limited number of parent trees, therefore, might be contributed to the next generations. We have also tested the applicability of these SSR primers to other Dipterocarpaceae species using PCR amplification. Since the flanking region sequences of the *S.curtisii* SSR were well conserved within this family, the SSR primers for *S.curtisii* can be applied to almost all Dipterocarpaceae species. The average of outcrossing rate in the trees of natural forest was 86.4 % and showed similar tendency of the other dipterocarps species. We also investigated five individuals in logged forest to clarify the genetic diversity and inbreeding rate. The inbreeding rate was quite different in each mother tree and ranged from 21.2 to 85.7 %. with the average of 53.6 %. The inbreeding rate between natural and logged forests was also quite different.

**Key Words** Dipterocarpaceae, microsatellites, *Shorea curtisii*, Genetic diversity, outcrossing rate

## introduction

*Shorea curtisii* is a species of the Dipterocarpaceae: a family that includes major component species of the tropical forests of South East Asia. Although the family is important in tropical forestry and ecosystems, genetic information about member species is limited. Therefore, to determine genetic diversity and structure in *S.curtisii* and related species, we planned to develop a series of SSR markers.

Several improvements in methodology have been reported recently for efficient development of SSR markers (Ostrander *et al.*, 1992; Karagyozov *et al.*, 1993; Lyall *et al.*, 1993; Cifarelli *et al.*, 1995; Kirkpatrick *et al.*, 1995; Takahashi *et al.*, 1996), one of which is the vectorette PCR strategy (Lench *et al.*, 1994). The procedure relies on PCR amplification using a vectorette specific primer in combination with anchored dinucleotide repeat primers. in this case, we used genomic library DNA sequences as templates. Anchor primers consist of tandem repeat sequences and possible 3' anchor nucleotide sequences. If an SSR region occurs in the insert DNA, it can be amplified when the 3' nucleotide sequence flanking the SSR locus matches the anchor nucleotide of the primer. This allows specific SSR primers to

be designed, which can be used to re-amplify the vectorette libraries in combination with the other side of the vectorette primer.

In this study, SSR markers of *S.curtisii* were developed using a vectorette PCR method and a general method involving screening by colony hybridization. The applicability of these primers to other species of the Dipterocarpaceae was also examined.

## Materials and Methods

### *Plant materials*

Leaves of *S.curtisii* were collected at the research plot at Semangkok Forest Reserve in Serangor, Malaysia (Niiyama et al. in press). The leaves of various other Dipterocarpaceae species were collected from the aboretum of the Forest Research institute of Malaysia (FRIM). Total DNA was extracted from leaves of each individual by a slightly modified CTAB method (Tsumura *et al.*, 1996).

### *Study plots*

Research plots have been constructed in Semangok forest reserve. This forest is a typical hill forest in Peninsula Malaysia, and *Shorea curtisii* is dominat species in this reigion. Two kinds of research plots have been constructed in this forest, which are 6 ha natural and 4 ha logged forests. Selective cutting was conducted in 1988 and the proportion was 30 % of the total wood volume of *S. curtisii*. Species identification and locations of all trees more than 5 cm diameter of breath hight (DBH 1.2m) in both plots have been confirmed. We collected leaves or inner barks from individuals of *S. curtisii* with 20 cm DBH in the both plots (Niiyama et al. 1993). General flowering have occurred at this forest in 1998, and *S. curtisii* also had a lot of fruits on August to September in this year. We also collected the seeds from ten and five individuals of natural and logged plots, respectively to survey the inbreeding rate in each tree.

### *Screening the Genomic DNA library*

The genomic DNA samples were digested with *Sau*3A I, *Hae*III, *Alu*I, and *Rsa*I, and fragments of 300 to 500bp were fractionated. The DNA fragments were ligated into pUC18, and the ligation mixtures were then transformed into *E.coli*, JM109. Colony hybridization was carried out using a DIG detection kit (Boehringer Co. Ltd.) according to the manufacturer's instructions, except that 6×SSC was used in the hybridization buffer. Two oligonucleotides, (CA)15 and (CT)15, were used as probes to survey SSR sequences. The hybridization temperature was 64º C for each probe. Positive clones were isolated and were subjected to DNA sequencing, using an ABI 377 automatic sequencer, according to the manufacturer's instructions (Perkin-Elmer ABI Co. Ltd.).

### *Vectorette PCR*

This procedure was mainly based on the protocol described by Lench et al (1994). Genomic DNA of *S.curtisii* was digested with a mixture of restriction enzymes including *Eco*RI, *Eco*RV, *Xba*I, and *Xho*I. Digested fragments between 500bp and 2500bp in size were fractionated. Fragments were then repaired by the Klenow method to make the ends blunt, and were ligated to pUC18. The ligation mixtures were transformed into *E.coli* JM109. The transformants were incubated on LB agar plates overnight at 37º C, after which five ml of LB medium was added to the surface of each plate. The plates were shaken gently for 30 min to recover cell suspensions. The cell suspensions were incubated at 37º C for a further 30 min. The DNA mixture of this genomic DNA library was prepared from the cell suspensions by the alkaline lysis procedure.

Vectorette PCR was performed in 25ul reaction volumes containing 10ng DNA from the genomic DNA library, and 0.128uM universal or reversal vectorette primer for pUC18 (primer 1; CCCAG TCAGC AGCTT GT or primer 3; GGAAA CAGCT ATGAC CATG, Nippon Gene Co.) in combination with each one (separately) of the 12 anchored dinucleotide repeat primers; (CT)10A, (CT)10T, (CT)10G, (CT)10CA, (CT)10CG, (CT)10CC, (CA)10A, (CA)10T, (CA)10G, (CA)10CT, (CA)10CG, (CA)10CC. Reaction mixtures were denatured at 94º C for 3min, followed by 34 cycles of amplification consisting of: 94º C for 30s, 55º C for 30s, and 72º C for 30s. Amplified fragments were then fractionated in 2% agarose gel. When a single fragment was observed, the PCR products were purified by Suprec-02 columns (TAKARA Co). When multiple fragments were observed, individual fragments were picked up from the gel by pipette tip. The tips were washed in PCR reaction mixture directly, and a single fragment was obtained through one more PCR cycle. The isolated fragments, including SSR flanking regions were sequenced. Specific upward primers were designed for the flanking regions of each SSR based on the sequence data with the aid of the Oligo program (version 4.0 National Bioscience). Using the forward primers in combination with the other side of the vectorette primers, vectorette libraries were re-amplified by PCR. Amplified fragments were sequenced, and downward primers were also designed.

***SSR polymorphism of* S.curtisii**

PCR was performed in 25ul reaction volumes containing 25ng genomic DNA. The PCR annealing temperature was 50 or 55º C, as appropriate for each pair of primers. Amplified fragments were fractionated on 3.5% agarose gels, and were sequenced to investigate their polymorphism.

***Application of SSR primers to other Dipterocarp species***

The PCR was carried out using seven primer pairs: *Shc*01, 04, 07, 08, 09, 11, and *Shc*14, using the same conditions as for *S.curtisii* DNA, except that the annealing temperature was set at 50º C for three cycles for each locus. All amplified fragments of *Shc*07 and *Shc*11 were sequenced, and randomly selected fragments obtained using the other five SSR loci were sequenced to confirm the SSR sequences in the various species.

## Results and Discussion

***Analysis of polymorphism of the SSRs***

Length polymorphism of 12 SSR loci among 32 *S.curtisii* specimens was investigated using sequence analysis. Eleven SSR loci were polymorphic, but *Shc*15 was monomorphic. *Shc* 08 seemed to show more than three alleles on 3.5% agarose gels, but their sequence data were not sufficiently clear, to allow its allele number to be determined exactly. These results are summarized in Table 1.

For simple repeat loci such as *Shc*04 and *Shc*09, allele polymorphism was found to depend mainly on differences in the number of CT repeats. However, in some cases, short compound repeats were found in the SSRs, especially at the *Shc*11, *Shc*13, *Shc*14, and *Shc*17 loci. The allelic polymorphism at these loci was found to depend on insertions or deletions in the flanking region. *Shc*01 and *Shc*07 were also compound repeats, and highly polymorphic. Their polymorphism depended not only on differences in the repeat number of each repeat unit, but also on the combination of different repeat units. Therefore, we observed many alleles per locus for *Shc*01 and *Shc*07.

In this study, eleven polymorphic SSR markers were developed using two different strategies. Comparing the two strategies, a vectorette PCR approach may generally be a more

useful strategy than the commonly used method, such as screening by colony hybridization, because it allows more rapid surveys of numerous clones. However, as shown in Table 1, some SSR loci isolated by the vectorette PCR method included short or compound repeats. Using the commonly used, SSR loci which have short or compound repeats can be discarded before primers are designed, but for the vectorette primer method, it was necessary to design forward primers for all possible SSR loci to obtain SSR core sequences. This problem could be overcome by optimization of PCR conditions.

The CT/AG motif in *Shorea curtisii* is as abundant as it is in other plants (Lagercrantz *et al.*, 1993; Wang *et al.*, 1994). Simple CA repeats, known to be common in animals, were not isolated in this study.

The allelic polymorphism of *Shc*04 and 09, which have simple repeats, depends mainly on differences in CT repeat number, but the polymorphism of alleles involving compound repeat SSR loci is much more complex (Table 1). Such complexity may lead to errors in designating genotypes. in other words, even if amplified fragments are the same in size, they may not always have the same sequences. The complex compound repeats like *Shc*01 and *Shc*07 were found to be highly polymorphic, but it may be better to develop SSR markers only for simple repeat loci to limit errors in genotype identification.

The frequency of dinucleotide repeats in the genome of several woody plants has been assessed, the estimates ranging from one repeat every 64 to 1105 kbp (Condit & Hubbell 1991). in our study, two SSR loci were isolated out of 6000 clones from a genomic DNA library in which the average insert size was 400bp, and eleven SSR loci were isolated out of 104 clones from a genomic library with an average insert size of 1500bp. Thus, according to these results, although the frequency of the dinucleotide repeats in the *Shorea* genome could not be determined exactly, they seem to occur approximately once every 1200 kb. in *S.curtisii*, therefore, dinucleotide repeats are apparently more widely separated than in most other species.

The SSR primers developed for *S.curtisii* analysis are also useful for analyzing other Dipterocarp species, therefore they are potentially powerful tools in genetic analysis of the tropical forest. The results also indicate that affinities among these species are relatively close.

***Conservation of SSR loci within Dipterocarpaceae***

The primers developed for analyzing seven of the SSR loci were used to assess conservation of the loci among 30 species from 10 genera of the Dipterocarpaceae. Within the genus *Shorea,* all seven SSRs were well conserved (Table 2), but *Shc08* amplification was weak for *S.macroptera* and *S.singkawang*, and multiple amplification products were obtained from *S.lepidota, S.macrophylla, S.ovalis*, and *S.scaberrima* DNA using *Shc*01 primers, from *S.scaberrima* DNA using *Shc*07 primers, and from *S.lepidota* DNA using *Shc*09 primers. For species in the genus *Hopea*, *Shc*01, 07, 11, and 14 were well amplified by PCR. However, *Shc*04, 08 and 09 were all amplified weakly from the DNA of certain *Hopea* species: *Shc*04 from *H.latifolia*, *H.nervosa*, *H.sangal*, and *H.subalata* DNA, *Shc*08 from *H.latifolia, H.odorata, H.subalata*, and *H.wightiana* DNA, and *Shc*09 from *H.dyeri* DNA. All loci in *Neobalanocarpus heimii, Parashorea lucid* and *Dryobalanops aromatica*, were well amplified except *Shc*04 in *Parashorea*. Among the other seven species (*Dipterocarpus baudii, D. kerii, D. oblongifolius, Anisoptera oblonga, Vatica odorata, Cotylelobium malayanum*, and *Upuna bornensis*), the sequences of the flanking SSR region were found to be better conserved than they were in *Shorea* and *Hopea*.

*Shc*07 and 11 were selected from the seven loci, for sequence analysis of the 30 test species. *Shc07* was chosen because the PCR amplification lengths for this marker

differed on 3% agarose gels: *Hopea* species, in particular, giving longer lengths than other genera. *Shc*11 was selected because this locus was well amplified in all species except for *Upuna bornensis*. We observed some base substitutions between species at this locus, but the locus was well conserved within the family.

Recently, a molecular phylogeny of the same 30 species of Dipterocarpaceae was constructed using PCR-RFLP analysis of chloroplast genes by Tsumura et al. (1996). According to the molecular phylogeny, there are two major groups of Dipterocarpaceae; Shoreae which includes *Shorea, Hopea, Parashorea, Neobalanocarpus,* and *Dryobalanops*, and Dipterocarpeae which includes *Dipterocaropus, Anisoptera, Upuna,* and *Cotylelobium*. The molecular phylogeny analysis also revealed close affinities among *Shorea, Hopea, Parashorea, Neobalanocarpus*, and *Dryobalanops*.

PCR Amplification patterns of these 30 species, using the seven SSR loci markers developed for *Shorea curtisii,* do not show clear correlations with the molecular phylogeny. However, the amplification patterns of *Shc*04, *Shc*08, and *Shc*09 suggest that the sequences of the flanking regions of these SSR loci are not well conserved in relatively distant species. These loci were well amplified in Shorea, but not in several species from other genera.

The average size of *Shc*07 amplification length also seems to correlate well with the molecular phylogeny. Based on the sequence data for *Shc*07, the average amplification length is 200 bp in *Hopea*, 130bp in *Neobalanocarpus heim*, 168bp in *Shorea* and *Parashorea lucida*, and 143bp in the other seven species included in the tribe Dipterocarpeae. According to the sequence data, these differences mainly depend on CATA repeats in core sequences. in the tribe Dipterocarpeae, CATA repeats do not seem to exist.

In contrast, although base substitutions in *Shc*11 were detected in several species, it is not clear whether or not this reflects genetic distance.

Further study is required in order to evaluate the generality of the conservation phenomenon, and is likely to lead to greater understanding of the evolution of SSR markers during speciation, and the genetic mechanisms involved.

***Genetic diversity of mother trees in both plots***

The genotypes of three microsatellite loci for all individuals were determined, and the genetic parameters were estimated (Table 3). The *He* is ranged from 0.663 for seedling population of natural forest to 0.865 for sapling population of logged forest. The smallest *Na* was 6.00 for mature tree population for logged forest and the highest one was 11.50 for sapling population of natural forest. There were some differences between mature tree, sapling and seedling, but these parameters were affected by investigating population size. Thus, we compared the unbiased *He* and *Ne* between the categories, which are not affected by the population size. The five populations of the unbiased *He* were similar, 0.769 to 0.786, and the Ne between them were also very similar, 4.33-4.59. Consequently, there were no significant difference between them. The area of study sites were 6 ha for natural forest and 4 ha for logged forest, therefore, we can estimate the genetic diversity per area but the number of alleles per ha was also very similar between natural and logged forests.

***Gene flow and outcrossing rate***

In 1998, general flowering had occurred in this region, and S. curtisii also had lots of fruits in this time. We collected seeds from each individuals of natural and logged plot to survey the mating system and the genetic diversity of next generation. The number of detected alleles in natural plot for each mother tree in three loci was ranged from 16 to 22. The inbreeding rate also had quite wide range, 0.0 - 38.7 % and its average was 13.6.(Table 4) The average inbreeding rate is similar as other reports. Murawski and Bawa (1994) reported that the

outcrossing rate of *Stemonoporus oblongifolious* which is another genus of Dipterocarpacea was 84 % using isozyme data. Kitamura et al also estimated the outcrossing rate of *Dryobanops aromatica* in primary and secondary forests and the estimates was ranged from 79.4 to 85.6 %. Our estimates was 86.4 % as the average and showed similar tendency of the other dipterocarps species (Table 4).

We also investigated five individuals in logged forest to clarify the genetic diversity and inbreeding rate. The number of detected alleles was 17 except for No. 7 tree which was only 11. The inbreeding rate was quite different in each mother tree and ranged from 21.2 to 85.7 %. with the average of 53.6 %. The inbreeding rate between natural and logged forests was also quite different, and the inbreeding rate was about 40 % high at the average values. The inbreeding rate is generally affected by self-incompatibility, biparental inbreeding and apomixis. Self-incompatibility was reported in some diptercarps species such as *S. hemsleyana, S.,acroptera, S. lepidota, S. acuminata, S. macroptera, S. aplendida, D. oblongifolius* and inferred to five dipterocarps species (Chan 1981). Apomixis also occurred in some dipterocarps species such as *S. agami, S. ovalis , S. sericea* and inferred to *S. macroptera, S. resinosa* and three *Hopea* species (Kaur et al. 1978). But there is no evidence of self-incompatibility and apomixis in *S. curtisii*.

The outcrossing rate is also influenced by the pollinator behavior, flowering density, the spatial positioning of flower and the density of mature trees. In 1998, mass-flowing, following by mast fruiting had occurred in the Semangok region. *S. curtisii* also had lots of fruits in this period, therefore, the flowering density was quite high in each tree and we don't think the spatial positioning of flower affected the outcrossing rate in this study because each tree had lots of flowers in a whole branches. However, the density of mature tree is six times different between natural and logged plots, which are 31.5 trees per ha and 5 trees per ha (DBH>30cm), respectively. The pollinator of *S. curtisii* thought to be small beetle and thrips which are not long distant pollinator. Their behavior range is not so wide comparing to *Apis*, small social bee, *Xylocopa, Amegilla, Halictidae* and *Megachille*. Momose et al. (1998) reported that characteristics of he pollinator community in a lowland dipterocarps forest at Lambir. According to their results, the main pollinator of dipterocarps are *Apis* in *Dipterocarpus* and *Dryobalanops* species and Chrysomelidae and Curculionidae in many *Shorea* species. Sakai et al. (1999) also reported beetle pollination of *S. parvifolia* at Lambir, and thrip also visited the flowers but the density of thrip was much lower than that at Pasoh of Peninsula Malaysia. The density at Pasoh (2.4 thirps per flower) was eight times higher than that of Lambir (0.31) in six *Shorea* species sect. *Mutica* (Appanah and Chan 1981). *S. curtisii* also is included same sect. *Mutica* and flower shape also very similar, therefore, the pollinator of this species must be a kind of thrip or small beetle. If the main pollinator was thrip, their move to tree to tree by wind. They can not move to the long distant mother tree exactly. In our study, the most important factor affected the inbreeding rates of *S. curtisii* between natural and logged forests might be the density of mature trees. Murawski and Hamlick (1992) reported that the outcrossing rate of *Cavanillesia platanifolia* associate positively with flowering-tree density. They compared the outcossing rate between three different flowering-tree densities, and the outcrossing rate was reduced depending on their flowering-tree density. Unfortunately, they don't discuss the pollinator of this species. The behavior and quantity of pollinator is one of the most important factor to the outcrossing rate. Kitamura et al. (1994) compared outcrossing rate between primary and secondary forests for two years in *D. aromatica*. The flowering density of primary and secondary (2nd year) forests were 14.0 and 6.7 (6.7) tree per ha, respectively and the outcrossing rates were 0.856 and 0.794 (0.787), respectively. But there were no significant different statistically. The pollinator of *D. aromatica* is *Apis dorsata* in Lambir (Momose et al. 1998), therefore, the

outcrossing rates between primary and secondary forests is not significant difference as the behavior range is rather wider than that of thrip or beetle. The reason of the difference in our study is the density of flowering-tree and the behavior of pollinator.


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## Research Output

### Publications


Ujino, T., T. Kawahara, Y. Tsumura, T. Nagamitsu, Wickneswari R. and H. Yoshimaru (1998) Development and polymorphism of simple sequence repeat DNA markers for Shorea curtisii and other Dipterocarpaceae species. *Heredity* 81:422-428

Kajita, T.,K.Kamiya, K. Nakamura, R. Wickneswari, H. Yoshimaru, Y.Tsumura, H.Tachida and T. Yamazaki (1998) Molecular phylogeny of Dipterocarpaceae in Southeast Asia based on nucleotide sequence of matK, trnL intron and trnL-trnF intergenic spacer region in chloroplast DNA. *Molecular Phylogenetic Evolution* (in press)

Tsumura, Y., Kawahara, T., Wiekneswari, R. and Yoshimura, H. (1996). Molecular ohirogeny of dipterocarpaceae in Southeast Asia using RFLP of PCR-amplified chloroplast genes. *Theor Appl Genet* **93**: 22-29.


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Table 1. SSR allele number, and observed allele types based on sequence data.

| Locus | Core sequence* | Number of allele | Heterozygosity ($H_e$) |
|---|---|---|---|
| *Shc*01 | $(CT)_n(CA)_mCT(CA)_lCTCA$ | 20 | 0.922 |
| *Shc*02 | $(CT)_2CA(CT)_nGC(AT)_2$ | 2 | 0.180 |
| *Shc*03 | $(CT)_n$ | 3 | 0.591 |
| *Shc*04 | $(CT)_n$ | 12 | 0.844 |
| *Shc*07 | $(CT)_nCA(CT)_mCACCC(CTCA)_lCT(CA)_k$ | 11 | 0.810 |
| *Shc*09 | $(CT)_n$ | 9 | 0.818 |
| *Shc*11 | $(CT)_m(A/T)T(CT)_n$ | 4 | 0.640 |
| *Shc*17 | $(CT)_5AT(CT)_n$ | 2 | 0.304 |
| | Average | 7.9 | 0.639 |

Table 2, Application of SSR primers developed for *Shorea curtissi* to other Dipterocarpaceae species.

| Species | Locus | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Shc*01 | *Shc*04 | *Shc*07 | *Shc*08 | *Shc*09 | *Shc*11 | *Shc*14 |
| *Anisoptera oblonga* | S | W | S | W | S | S | M |
| *Cotylelobium malayanum* | S | W | W | W | W | S | S |
| *Dipterocarpus baudii* | S | W | S | W | W | S | S |
| *Dipterocarpus kerrii* | S | W | M | W | W | S | S |
| *Dipterocarpus oblongifolius* | S | W | S | W | S | S | S |
| *Hopea dyeri* | S | S | S | S | W | S | S |
| *Hopea latifolia* | M | W | S | W | S | S | S |
| *Hopea nervosa* | S | W | S | S | - | S | S |
| *Hopea odorata* | S | S | S | W | S | S | S |
| *Hopea sangal* | S | W | S | S | S | S | S |
| *Hopea subalata* | S | W | S | W | S | S | S |
| *Hopea wightiana* | S | S | S | W | S | S | S |
| *Neobalanocarpus heimii* | S | S | S | S | S | S | S |
| *Shorea atrinervosa* | S | S | S | S | S | S | S |
| *Shorea bracteolata* | S | S | S | S | S | S | S |
| *Shorea kunstleri* | S | S | S | S | S | S | S |
| *Shorea lepidota* | M | S | S | S | M | S | S |
| *Shorea macrophylla* | M | S | S | S | S | S | S |
| *Shorea macroptera* | S | S | S | W | S | S | S |
| *Shorea multiflora* | S | S | S | S | S | S | S |
| *Shorea ovalis* | M | S | S | S | S | S | S |
| *Shorea parvifolia* | S | S | S | S | S | S | S |
| *Shorea scaberrima* | M | S | M | S | S | S | S |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Shorea singkawang* | S | S | S | W | S | S | S |
| *Parashorea lucida* | S | - | S | S | S | S | S |
| *Vatica odorata* | S | W | S | S | S | S | S |
| *Upuna bornensis* | M | - | S | W | S | W | M |

[a] S: strong amplification, W: weak amplification, -: amplification failed M: multiple bands amplified.

Table 3 Genetic diversity between natural and lohhed forests.

| Population | n | *He* | unbiased *He* | *Na* | *Ne* |
|---|---|---|---|---|---|
| Natural DBH>30cm | 45 | 0.800 | 0.786 | 9.00 | 4.63 |
| Natural sapling | 171 | 0.739 | 0.782 | 11.50 | 4.59 |
| Natural seedling | 58 | 0.663 | 0.769 | 7.25 | 4.33 |
| Logged DBH>30cm | 10 | 0.850 | 0.779 | 6.00 | 4.52 |
| Logged sapling | 26 | 0.865 | 0.768 | 7.75 | 4.44 |

Table 4 Comparison of outcrossing rate and heterozygosity between natural and logged forests.

| | No. of investigated mother trees | No. of analyzed seeds | No of detected alleles | Outcrossing rate (%) | *He* |
|---|---|---|---|---|---|
| Natural Forest | 10 | 326 | 31 | 86.4 | 0.761 |
| Logged Forest | 5 | 169 | 26 | 46.4 | 0.736 |