Plantation Technology in Tropical Forest Science

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(Eds.)

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With 46 Figures

Springer

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Preface

This book is intended to be a record of the Biotechnology-Assisted Re/Afforestation Project in the Asia-Pacific Region (BIO-REFOR) since 1992, conducted in cooperation with the International Union of Forest Research Organizations (IUFRO). The purpose of the project is to promote exchanges of information of fundamental research on indigenous species in the Asia-Pacific Region in order to restore natural forests.

The production, cultivation, and maintenance of forest tree species provide highly sustainable production systems that conserve soils, the microenvironment, and biodiversity The key technology for biomass production of forests is propagation via micropropagation or traditional propagation. However, there are many recalcitrant species among useful forest trees to be propagated in large numbers. Recent advances in mycorrhizal technology and in vitro culture have made it possible to commercially propagate useful trees for re/afforestation.

In this book, comprehensive information is provided on propagation, mycorrhizal inoculation, and reforestation of economically and environmentally important forest trees, information that usually is available only in widely scattered resources. Here, we include a wide area of the ecology and physiology of dipterocarps as a general overview, and then cover propagation techniques, mycorrhizal symbiosis, man-made forests, and biodiversity in the Asia-Pacific region.

Our purpose is to provide information on the progress being made in biotechnology-assisted re/afforestation. In vitro culture combined with mycorrhizal inoculation has become a powerful tool for large-scale increases in forest trees, conservation of biodiversity, and reforestation—comprehensive information that until now has been difficult to find in one volume. This compilation provides a valuable resource for scientists, students, policy makers, and industrialists concerned with reforestation.

We would like to express our sincere thanks for the financial support of Japan's ODA for the BIO-REFOR project as well as support for this book by a Grant-in-Aid for Publication of Scientific Research Results from the Japan Society for the Promotion of Science. Finally, we are very grateful to Dr. Masaya Masumori of the University of Tokyo for his work in the preparation of this book.

The Editors

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Part I

General Overview

1
Ecology and Physiology of *Dipterocarpaceae*

SATOHIKO SASAKI

Dipterocarpaceae is a plant family of large tree species that is widely distributed in the tropical rain forests of Southeast Asia. In Borneo and Peninsular Malaysia, natural forests consist of dipterocarp timber, such as Meranti, Kapur, Keruing, Mersawa, and Resak. The trees in *Dipterocarpaceae* are large, exceeding 50 m in height and 1 m in diameter, and they spread large, dense canopies over the jungle. The forest is very dark because of the thick canopy layer and there is little ground vegetation. However, these dense natural forests have become scarce due to exploitation and the pressure to convert them into other land uses. In order to maintain and rehabilitate these dipterocarp forests, and also to establish sustainable timber production, it is necessary to understand the fundamental characteristics of dipterocarp species.

1.1 Distribution of Dipterocarpaceae

The taxonomy, distribution, and basic characteristics of species in *Dipterocarpaceae* were comprehensively studied by Symington (1943). He ended his life in a state of depression without seeing the publication of his book, *Malayan Forest Records No. 16, Forester's Manual of Dipterocarps*. However, his ideas, comprehensive studies, and observations are still valid. This chapter begins with a discussion of the distribution of *Dipterocarpaceae*, mainly following Symington's theory (Table 1).

1.1.1 Monotoideae

There are two sub-families in Dipterocarpaceae, Monotoideae and Dipterocarpoideae. Monotoideae is found only in Africa, with a wide distribution from East Africa through Central Africa to Nigeria. This sub-family is composed of two genera, *Marquesia* and *Monotes*. Although Symington (1943) did not realize the presence of Dipterocarpaceae in Madagascar, a species of *Monotes* has recently been reported

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Table 1. The distribution of Dipterocarpaceae

Genus	Africa	Ceylon	India	Burma	Thai.	Indoch.	Malay	Sumatra	Borneo	Java	Phil.	Sulaw.	Maluk.	N. Gui.	Species
Marquesia	4														4
Monotes	35														35
Anisoptera				2	4	2	7	4	5	1	4	1	2	1[a]	13
Balanocapus							1								1
Cotylelobium		1			1	1	2	2	3						5
Dipterocarpus		5	2	11	17	11	32	22	40	5	11				75
Doona		12													12
Dryobalanops							2	2	6						7
Hopea		4	7	6	12	7	30	11	40	1	9	3	2	7[a]	114
Parashorea				2	1	1	3	3	4		1				10
Pentacme				1	1	1	1								3
Anthoshorea			1	6	5	4	10	6	10	1	2	1	2		25
Richetia					1		10	5	24		1				39
Rubroshorea					4		23	12	55		5				70
Eushorea		5	2	3	5	3	15	4	21		8		1		45
Stemonoporus		14													14
Upuna									1						1
Vateria		3	2												5
Vateriopsis	1[b]														1
Vatica		3	1	5	6	5	25	10	35	3	8	3	1	1	85
Genus/Species	3/40	8/47	6/15	8/36	11/57	9/35	13/162	11/81	12/244	5/11	10/51	4/8	5/8	3/9	20/563

[a]Each one species of Anisoptera and Hopea is present at Louisade Arch, Papua New Guinea

[b]Distributed only in the Seychelle Islands

there. The existence of Dipterocarpaceae in Madagascar may connect Monotoideae in Africa and Dipterocarpaceae in Asia. Most Monotoideae species are small trees that grow in Savanna, but one *Marquesia* is a large tree species found in tropical rain forests.

1.1.2 Dipterocarpatoideae

Dipterocarpatoideae consists of tall and large trees distributed in Asia and the Pacific Islands, including most of the important timber species in Tropical Asia. This subfamily has a wide distribution, from the Seychelles in the west to the Louisaide Arch of Papua New Guinea at its eastern limit, covering India, Sri Lanka, Myanmar, Laos, Vietnam, Hainan, South China, the Philippines, Thailand, Malaysia, Indonesia, and Papua New Guinea (Table 1). Such a wide distribution of Dipterocarpoideae may be related to the development of local species and genera adapted to specific environments of the region.

In Dipterocarpoideae, the 15 genera *Anisoptera*, *Balanocarpus*, *Cotylelobium*, *Dipterocarpus*, *Doona*, *Dryobalanops*, *Hopea*, *Parashorea*, *Pentacme*, *Shorea*, *Stemonoporus*, *Upuna*, *Vateria*, *Vateriopsis*, *and Vatica* have been identified, if *Shorea* is classified as a genus. When *Shorea* is divided into the four genera *Eushorea* (*Shorea*), *Richetia* (*Richetoides*), *Anthoshorea*, and *Rubroshorea*, the total number of genera is 18. The number of species in Dipterocarpoideae may vary, but it is certain that the total number exceeds 520.

Certain genera have specific and limited distributions: *Doona* and *Vateria* in Sri Lanka; *Upuna* in Borneo; *Balanocarpus* in Peninsular Malaysia; and *Vateriopsis* in the Seychelles. In contrast to the genera with a limited distribution, there are widely distributed genera. *Anisoptera*, *Hopea*, and *Vatica* are distributed from Indochina to New Guinea. In particular, *Hopea* and *Vatica* are found in Hainan and South China. Similarly, *Dipterocarpus* is widely distributed from India and Andaman Islands to Borneo, but does not cross Wallace's Line.

Shorea as a whole shows a distribution pattern similar to that of *Dipterocarpus*. However, *Shorea* has a large number of species, with more than 180 identified. These species are not homogeneous in character and should be separated into four different genera or subgenera: *Anthoshorea*; *Richetia*; *Rubroshorea*; and *Eushorea*. Among these subgroups, *Anthoshorea* and *Eushorea* have a wide distribution, similar to that of *Dipterocarpus*. In contrast, *Richetia* and *Rubroshorea* are limited in distribution, confined to Peninsular Malaysia, Borneo, Sumatra, and the Philippines. No species of *Richetia* and *Rubroshorea* are listed from Java Island. The center of the distribution for *Richetia* and *Rubroshorea* is in Borneo and Peninsular Malaysia.

The dipterocarp species are the most abundant in Borneo, with 12 genera and 244 species, if *Shorea* is counted as four genera. Peninsular Malaysia has 13 genera and 161 species. The numbers of genera and species are relatively small in Sumatra, with only 11 genera and 81 species recorded. In the Philippines, Thailand, Myanmar, and India, there are even fewer genera and species. The most interesting phenomena are found in Java. Although Java is close to Borneo and Peninsular Malaysia, Dipterocarpaceae is poorly represented in Java, with only five genera and 11 species recorded.

Wallace's Line is a biological dividing line between Borneo and Sulawesi. Beyond Wallace's Line to the east, the distribution of Dipterocarpaceae is extremely limited. Sulawesi has four genera and eight species, and New Guinea has three genera and eight species. According to Symington (1943), Dipterocarpaceae originated in Southeast Asia in a period from the late Mesozoic to the early Tertiary, and towards the end of the Tertiary Period the family was established within the present distribution limits. Because the seeds cannot be dispersed over a long distance via the sea or air, land bridges are necessary for Dipterocarpaceae species to expand their territories.

About 70 million years ago, when the Dipterocarpaceae were evolving, Borneo, Sumatra, Java, and Peninsular Malaysia were connected, forming Sunda Land as a part of the Asian Continent. To the east, the Australian continental shelf existed, including New Guinea. Between these two continental shelves, orogenetically active and unstable areas such as Sulawesi, Timor and the Philippines were present.

Symington and others believe that Dipterocarpaceae developed in the western part of Borneo during the period when Sunda Land was connected to the Asian Continent. Because Sunda Land and other continental shelves were separated by sea early in the geological-time scale, the numbers of dipterocarp genera and species remain markedly low in the orogenetically unstable areas. Moreover, the eastern side of Wallace's line, near the Philippines, is considered to be a key to the distribution of Dipterocarpaceae. Migration may have occurred across land bridges from Borneo to the Philippines, as these areas were connected to Palawan Island and the Sulu Archipelago at times. The dipterocarp species further migrated to Sulawesi and New Guinea through the other land bridges from the Philippines to the unstable areas and New Guinea.

Although these assumptions are reasonable for the migration of dipterocarp species to the east, the poor development of dipterocarp species in Java requires another explanation. Compared with other Sunda Land areas, such as Peninsular Malaysia, Borneo, and Sumatra, Java has developed and maintained very few dipterocarp species, with only five genera and 11 species recorded. To explain these interesting phenomena, Symington assumed that Java Island had been separated from the Asian Continent in the early period of geological formation. Also, he observed that the forests of Java Island below 1200 m in altitude were completely converted to agricultural uses. Therefore, there is no room for dipterocarp trees to grow in Java. However, it is unbelievable that almost all dipterocarp species have been exterminated in a short time by land development. The limited dipterocarp species present in Java may be a key factor in building a comprehensive theory for dipterocarp evolution.

In the same period that the dipterocarp species expanded their territories to the east, they also expanded their distributions to the north and west. From the present distribution of dipterocarp species, they must have at least reached India, Seychelles, Sri Lanka, and the Andaman Islands to the west. Also, to the north, dipterocarps reached Nepal, Vietnam, Hainan Island, South China, and Myanmar.

There are significant changes in vegetation at the country border between Malaysia and Thailand. These changes may have a good correlation with the evolution-

ary processes of dipterocarp species. At the border, the numbers of species and genera of Dipterocarpaceae on the Thailand or Myanmar side are not only reduced, but also show marked changes in species composition. Judging from the composition of the present vegetation, such a clear floristic demarcation exists at the line from Alor Setar in Kedah State, due north to Hat Yai and Sonkhla in Thailand. Therefore, Perlis and Langkawi in Malaysia belong to the Myanmar side, whereas Pattani in Thailand and Gunong Jerai in Kedah belong to the Malaysian side. Gunong Raya on Langkawi has only five genera and 11 species, and Gunong Jerai has nine genera and 29 species. Also, there are no *Rubroshorea* or *Richetia* species present on the Myanmar side, on the west and north side of the line. *Anthoshorea* and *Dipterocarpus* are the dominant floristic type on the Myanmar side. These differences in vegetation imply that there was a certain geological separation in Peninsular Malaysia at the border.

The glaciation of the Pleistocene Period was probably responsible for the floristic division. The melting of ice in the Quaternary Period (Pleistocene Period) caused a rise in sea level and separated Peninsular Malaysia from the main continent of Asia. This theory is supported by evidence such as a clear demarcation in sea levels, now 60 m elevation at Langkawi, and the presence of a pyrite deposit layer in a vast lowland area of Hat Yai. The pyrite is formed by a reduction of sulfate in seawater in the presence of organic matter.

The distribution and development of dipterocarp species are more complex than was expected. In order to understand the distribution and development of dipterocarps, it is important to incorporate the physiological characteristics, genetic traits, and developmental morphology of the species.

1.2 Chromosome Numbers in Dipterocarpaceae

Judging from chromosome studies on Dipterocarpaceae, two fundamental types are noted: one with $2n = 14$ and its tetraploid $2n = 28$; the other with $2n = 20$ or 22 (Table 2). The species with tetraploid chromosomes ($2n = 28$) have been found in *Hopea* and *Rubroshorea*. The chromosome number $2n = 20$ or 22 is often miscounted as a triploidy of 21. Somego (1978) noted that the chromosomes in $2n = 20$ or 22 plants can be counted as 20, 21, and 22, depending on the mitotic stages from late prophase to metaphase. He speculated that one or two dyad chromosomes separate prematurely into monad chromosomes in early metaphase, resulting in the different chromosome counts. In another study, Somego (1978) noted that three pairs of chromosomes are relatively long, 4 μm in length, whereas four other pairs are short. He suggested that these long chromosomes were cleaved to make six pairs of short chromosomes in $2n = 20$ plants. All the chromosomes in $2n = 20$ plants are observed to be short. Therefore, the $2n = 20$ or 22 group is very likely to have $2n = 20$ chromosomes.

The genera with wide distributions, such as *Anisoptera*, *Vatica*, and *Dipterocarpus*, are classified into the $2n = 20$ group. *Hopea* and *Eushorea* have two species with this chromosome number. *Hopea odorata*, which is distributed in the north, and

Table 2. Number of chromosomes in various dipterocarp species

Genera with 2n = 14 or 2n = 28 chromosomes	Genera with 2n = 20 or 22 chromosomes
Richetia *Shorea multiflora*	
Rubroshorea *Shorea acuminata, S. curtisii, S. leprosula, S. macroptera, S. parvifolia, S. paucifolia, S. platyclados, S. palembanica, S. argentiflora, S. martiniana, S. mecistopteryx, S. ovalis* 2n = 28	
Anthoshorea *Shorea assamica, S. bracteolata, S. hypochra, S. talura*	
Eushorea *Shorea glauca, S. guiso, S. maxwelliana*	*Eushorea* *Shorea obtusa*
Parashorea *Parashorea densiflora*	
Balanocarpus *Balanocarpus heimii*	
	Anisoptera *Anisoptera laevis, A. scaphula*
	Dipterocarpus *Dipterocarpus cornutus, D. oblongifolius, D.-I, D. -II.*
	Vatica *Vatica cinerea, V. odorata*
Hopea *Hopea nervosa, H. sangal, H. nutans* 2n = 28	*Hopea* *Hopea beccariana, H. odorata, H. subalata*
Dryobalanops *Dryobalanops aromatica, D. oblongifolia*	
	Upuna *Upuna borneensis*

Hopea Beccariana, found at a high elevation of 1200 m, are identified as 2n = 20. One *Eushorea*, *Shorea obtuse*, has the chromosome number 2n = 20. This species occurs under dry conditions in Thailand and Myanmar. Another species in the same *Eushorea* group, *Shorea robusta*, is also known as a drought and low-temperature-tolerant species. It is very important to identify the chromosome number of *Shorea robusta*. If *Shorea robusta* is classified as 2n = 20, *Eushorea* must be regrouped into two or three groups. Another species with 2n = 20 is *Upuna Borneensis*, which is distributed only in Borneo.

Although *Anthoshorea* species have a wide distribution, they have 2n = 14 chromosomes. *Anthoshorea* species are known to be adapted to drought and low temperatures. It is likely that *Anthoshorea* has fundamental genes for these tolerances. All *Rubroshorea* species showed stable 2n = 14 chromosomes, with the exception of tetraploid *Shorea ovalis*.

1.3 Physiological Characteristics of Dipterocarpaceae

1.3.1 Flowering and Fruiting

It is generally believed that dipterocarp trees flower in 5- to 6-year intervals, but the actual interval is irregular and erratic. Furthermore, the timing of flowering and fruiting depends on the species and location. Therefore, it is difficult to predict the flowering of dipterocarp trees. In a good flowering year, most dipterocarp trees tend to flower, resulting in gregarious flowering. In particular, *Rubroshorea* has a tendency to flower gregariously. On the other hand, *Anthoshorea*, *Hopea*, *Dryobalanops*, and *Dipterocarpus* tend to flower more or less regularly. For example, *Shorea Talura* and *Hopea odorata* flower every 6 years or every other year. Generally, the species that are distributed on the Asian mainland tend to flower regularly. Smitinand et al. (1980) reported that most of these species flower every year in Thailand and Indochina. This implies that the flowering patterns of the continental species differ from those of *Rubroshorea* and *Richetia*. Flowering also depends on the physiological characteristics of individual trees. Individual trees of *Rubroshorea*, *Hopea*, *Dryobalanops*, *Balanocarpus*, and *Dipterocarpus* have been identified as flowering regularly.

1.3.2 Morphological Characteristics of Seeds

Most dipterocarp seeds develop wings from sepals. The seeds are disseminated by rotating the wings. Sometimes, an updraft of air lifts the seeds high in the air and carries the seeds to a distant place.

Dryobalanops and *Parashorea* generally have five wings, but some species do not develop wings. *Pentacme* and *Shorea*, including *Eushorea*, *Anthoshorea*, *Richetia*, and *Rubroshorea*, develop three wings, whereas *Hopea*, *Dipterocarpus*, *Anisoptera*,

Upuna, *Vatica*, and *Cotylelobium* have two wings. However, some species in *Rubroshorea*, *Eushorea*, *Parashorea*, *Dryobalanops*, *Balanocarpus*, *Vatica*, and *Hopea* do not develop wings at all.

1.3.3 Food Reserves in Dipterocarp Seeds

In dipterocarp seeds, food reserves are stored in their cotyledons. Depending on the genera, the forms of these food reserves differ markedly. Some species develop starch grains in the seed, whereas other seeds store oil bodies for seed reserves (Table 3). Physiological and biochemical responses are useful indices for the classification of a species. Differences in seed reserves reflect changes in metabolic pathways that are the direct expressions of specific genes. Therefore, the species with oil bodies and those with starch grains should be separated into different groups. *Parashorea*, *Dryobalanops*, and *Balanocarpus* develop typical oil bodies in their seeds. The seeds of *Dipterocarpus* and *Vatica* contain starch grains. Among *Shorea*, *Anthoshorea* seeds develop starch grains in their cotyledons, but *Rubroshorea*, *Richetia* and *Eushorea* seeds form oil bodies in their cotyledons. Similarly, some *Hopea* develop starch as seed reserves, whereas others have oil bodies in their cotyledons. As shown by their chromosome numbers, the characteristics of *Hopea* are diverse, and perhaps the genus needs to be reclassified into several groups.

1.3.4 Survival and Storage of Seeds at Low Temperatures

Dipterocarp seeds lose viability at moisture contents below 20%, and all dipterocarp seeds must be stored with controlled humidity. Temperature is one of the most critical conditions for seed storage. There are substantial differences in response to temperature among genera and species (Tables 4 and 5). The seeds of *Rubroshorea* show chilling injury at temperatures below 15°C (Table 4). Some *Rubroshorea* seeds lose viability within 4 h in a refrigerator. Some of the *Eushorea* group also lose viability below 15°C. *Shorea sumatrana* seeds lose viability at 4°C within a week. However, *Shorea robusta*, distributed in India and Nepal, is reported to be tolerant to low temperatures. Similarly, *Shorea obtusa*, found in Thailand and Indochina, may be tolerant to low temperatures. Further studies are needed for the species of *Eushorea*.

Richetia seeds show slightly better performance at low temperatures than *Rubroshorea* seeds, with most *Richetia* seeds surviving for 1 month at 4°C. However, the slow development of chilling injury deteriorates the seeds and eventually they lose viability.

Compared with other *Shorea* groups, the *Anthoshorea* group is highly tolerant to low temperatures (Table 4). Among *Anthoshorea*, *Shorea Talura* is the most tolerant to low temperatures, and the seeds survived for more than 6 months at 4°C. Furthermore, after the seeds were stored in a refrigerator at 4°C, they germinated in a few days (Fig. 1), indicating that cold stratification treatment is effective. *Shorea assamica* and *Shorea hypochra* also demonstrated some tolerance to low temperatures.

Table 3. Differences in morphological and physiological characteristics among Dipterocarpaceae

Species	Cotyledon characteristics			First leaf arrangement and stipules	
Anthoshorea					
S. Talura	starch	hypogeal	no chlorophyll	a pair of opposite leaves	stipules persistent
S. assamica	starch	epigeal	no chlorophyll?	a pair of opposite leaves	stipules persistent
S. hypochra	starch	epigeal	no chlorophyll?	a pair of opposite leaves	stipules persistent
S. sericeiflora	starch	epigeal	no chlorophyll	a pair of opposite leaves	stipules persistent
S. resinosa	starch	epigeal	no chlorophyll	a pair of opposite leaves	stipules persistent
S. baracteolata	starch	epigeal	no chlorophyll?	a pair of opposite leaves	stipules caducous
Eushorea					
S. robusta	?	hypogeal	?	a pair of opposite leaves	?
S. obtusa	?	?	?	?	?
S. glauca	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules caducous
S. guiso	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules caducous
S. leavis	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules caducous
Richetia					
S. faguetiana	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules caducous
S. multiflora	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules caducous
S. resina-nigra	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules caducous
Rubroshorea					
S. acuminata	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules persistent
S. curtisii	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules persistent
S. dasyphylla	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules persistent
S. leprosula	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules caducous
S. ovalis	oil	epigeal	chlorophyll	a pair of opposite leaves	stipules persistent
Parashorea					
P. densiflora	oil	epigeal	chlorophyll	cataphyll-like stipules formed	before leaves develop
P. lucida	oil	epigeal	chlorophyll	cataphyll-like stipules formed	before leaves develop
Hopea					
H. nervosa	starch?	epigeal	?	three leaves develop like pseudo-whorl?	
H. odorata	oil	epigeal	chlorophyll	three leaves as whorl	
H. nutans	starch?	epigeal	?	three leaves as whorl	
Dipterocarpus					
D. oblongifolius	starch	hypogeal	no chlorophyll	a pair of opposite leaves	
Dryobalanops					
D. aromatica	oil	epigeal	chlorophyll	two pairs of opposite leaves expand crosswise	
D. oblongifolia	oil	epigeal	chlorophyll	two pairs of opposite leaves expand crosswise	
Balanocarpus					
B. heimii	oil	epigeal	chlorophyll	two pairs of opposite leaves expand crosswise	
Vatica					
V. cinerea	starch?	epigeal?	no chlorophyll	?	
V. wallichii	starch?	hypogeal?	no chlorophyll	?	
Anisoptera	starch?	hypogeal or epigeal		two pairs of a pair?	

Table 4. Survival and storage of *Shorea* seeds at various temperatures

Species	original		25°C			21°C			17°C			4°C[a]		
	% germ.	% moist.	Days	% germ.	% moist.	Days	% germ.	% moist.	Days	% germ.	% moist.	Days	% germ.	% moist.
Rubroshorea														
S. platyclados	86	59.0				40	20	46.3	40	10	36.2			
S.Curtisii	90	64.1	35	5	21.6	30	78	72.7	14	15	73.1			
						67	83	70.8						
	100	54.8				(60	13	38.1)						
	95	52.8	75	13	31.6	75	7	44.2						
	100	59.1	30	17	54.3	67	14	54.5						
			37	67	56.4									
S. accuminata	95	75.3				30	70	61.6	14	24	92.9			
S. pucifolia	100	103.6	45	67	61.8	45	55	76.5	30	25	91.8			
S. argentifolia	100	84.0	51	30	50.2	45	60	64.1	45	10	68.9			
S. parvifolia	100	40.1				21	43	34.5						
	95	94.3				30	24	70.8						
	100	66.7	21	14	60.1	45	53	51.5	45	14	46.0			
	100	83.0				30	35	56.7	27	5	64.3			
S. dasyphlla	65	166.6	18	0	126.6	35	0		14	35		2	63	118.7
	83	125.5	14	0	88.8	14	0		14	20	93.8	3	15	
	97	86.9	8	48	55.3	14	24	68.0	8	48	68.0			
	100	44.5	21	20	30.4				21	5	30.3			
S. leprosula	100	49.5				30	47		27	17	31.4	4 h		
	100	53.2				30	45	40.4						

S. ovalis	100	138.7	18	53	150.6						
	100	144.9	44	95	174.6						
	100	55.2	25	37	40.9	42	80	58.6			
	100	53.2	30	50	34.4	120	10				
Eushorea											
S. sumatrana[b]	100	50–60	15	germinated					7	no germination	
Richetia											
S. resina-nigra									Survived a month, but slowly developed chilling injury. No germination observed after a month		
S. muliflora											
S. faguetiana											
S. hopeifolia											
S. maxima[b]	100	50.0	14	50–100							
Anthoshorea											
S. Talura	93	56.7	90	64	29.5	220	90	45.9	150[c]	58	55.7
	100	55.0							120[c]	79	46.8
	95	68.1							180	69	68.5
S. assamica	86	38.1							100	50	
	100[b]	70–80							70	50?	70–80
S. hypochra									60	10	
S. bracteolata	50	48.2							60	2	

germ, germination; moist, moisture

[a]All tested seeds of *Rubroshorea* died at 4°C. Seeds were kept in a sealed plastic bag to maintain seed moisture content above 20% of seed weight

[b]Data from Mori 1982

[c]Mechanical failure caused termination of the experiments

Table 5. Survival and storage of *Hopea* and other dipterocarp seeds at various temperatures

Species	original		25°C			21°C			17°C			4°C		
	% germ.	% moist.	Days	% germ.	% moist.	Days	% germ.	% moist.	Days	% germ.	% moist.	Days	% germ.	% moist.
H. latifolia												80	10	32.6
H. odorata	81	113.0										45	5	114.0
			30[a]	≥ 50								30	≥ 50	
H. wightiana	100											60	5	100.5
H. subalata	100	77.4										50	40	46.8
H. ferrea	90	54.7										60	2	49.2
H. nervosa	100	95.0				300	Survived					20	15	76.0
H. beccariana	95	58.7										Survived		
H. helferi			30[a]	≥ 50								30	≥ 50	

[a]Data from Mori 1982

Survival of other dipterocarp seeds at 4°C:

Dipterocarpus oblongifolia: seeds survived for more than 2 months

Dipterocarpus sp.: seeds survived for more than 2 months

Vatica lowii: seeds survived for 2 months

Vatica cinerea: seeds survived for 2 months

Vatica umbonata: seeds survived for about 2 weeks

Other seeds showing chilling injury at 4°C:

Balanocarpus heimii: seeds survived for a month, but chilling injury killed the seeds

Dryobalanops aromatica: seeds develop the chilling injury symptoms within 2 weeks

Dryobalanops oblongifolia: seeds showed slightly faster symptom development than *D. aromatica*

Parashorea sp.: within 1 or 2 weeks, chilling injury symptoms developed

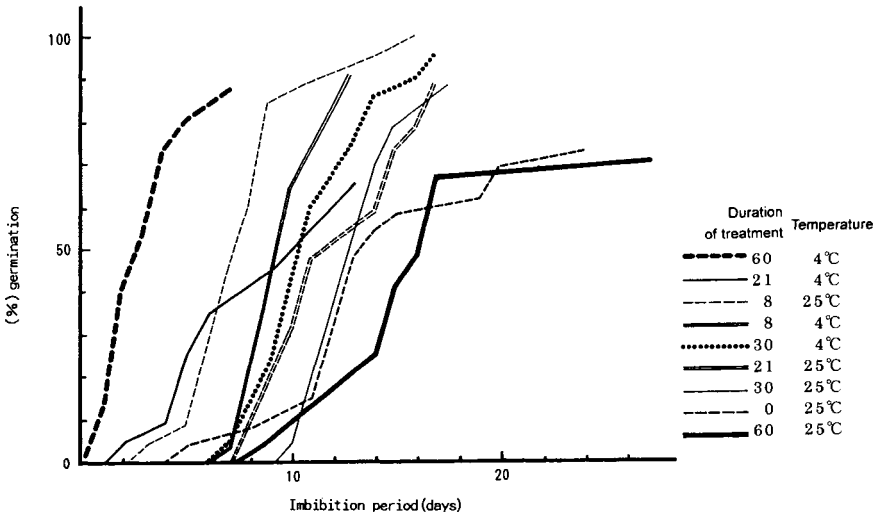

Fig.1. Effect of prechilling on *Shorea Talura* seed germination

Some *Hopea* seeds also show tolerance to low temperatures (Table 5). *Hopea odorata* seeds survived for more than 2 months at 4°C. Other species of *Hopea*, such as *Hopea subalata*, *Hopea wightiana*, and *Hopea ferrea* also show tolerance to low temperatures.

Dipterocarpus and *Vatica* seeds survive at 4°C for about 2 months. These genera are also distributed in India and Indochina. *Anisoptera* has a similar distribution to these genera, but such an experiment has not been conducted on this genus. Detailed studies are needed for these three genera.

1.3.5 Seed Germination

During seed germination, the cotyledons may be epigeal or hypogeal, depending on the species (Table 3). In epigeal species, the cotyledons emerge from the seed coat and expand as the coat is shed. Generally, cotyledon petioles are short in the epigeal seed. Conversely, the petioles of cotyledons in hypogeal species are extremely elongated after the radicle and hypocotyl protrude from the seed coat. With these long petioles, their cotyledons are kept folded under the seed coat. An epicotyl develops at the joint of the pair of cotyledons. Most of the *Shorea* group are epigeal, but *Shorea Talura* in *Anthoshorea* and *Shorea robusta* in *Eushorea* are known to be hypogeal. In addition, the cotyledons of *Anthoshorea* species mostly lack chlorophyll, although they expand during germination. This indicates that the advantages of epigeal cotyledons are not fully exhibited in *Anthoshorea*. *Anthoshorea* species probably belong to an intermediate group between hypogeal and epigeal characters. *Shorea robusta*, which extends its distribution even to Nepal, also has hypogeal cotyledons.

Shorea Talura and *Shorea robusta* are interesting species that require further study. *Shorea obtuse* is known to have 20 chromosomes in somatic cells and it grows in the dry deciduous forests of Thailand, Laos, Cambodia, and Vietnam. No report is available for the cotyledon behavior of *Shorea obtusa*. The physiology of this species needs to be analyzed in order to understand the development of dipterocarp species.

There are other hypogeal genera. All *Dipterocarpus* and most *Anisoptera* and *Vatica* show a hypogeal tendency. All of these genera have a wide distribution, from India to New Guinea. All species of *Rubroshorea*, *Richetia*, *Parashorea*, *Hopea,* and *Dryobalanops* have epigeal cotyledons.

Multi-embryos are commonly formed in dipterocarp seeds, where all embryos within a seed germinate normally, resulting in twins or triplets. The chromosomes in these twins or triplets are normal and no haploid plants are found. This indicates that multi-embryos are not initiated by parthenogenesis.

1.3.6 Tolerance of Dipterocarp Seedlings to Low Temperatures

Various dipterocarp seedlings were wrapped in a polyethylene bag to avoid desiccation and placed in a refrigerator at 4°C for 5 days. They were then transplanted to a nursery. A trend for tolerance to low temperature was observed, although the degree of low-temperature tolerance was comparable to that of the seeds (Table 6). All *Rubroshorea* seedlings were killed by the cold treatment. Similarly, the seedlings of *Shorea glauca* (*Eushorea*), *Shorea multiflora* (*Richetia*), and *Parashorea multiflora* did not survive at 4°C. In contrast, the *Anthoshorea* group showed tolerance to the low temperature. The most tolerant species was *Shorea Talura*, with 85% survival. However, a later experiment demonstrated that even in *Shorea Talura*, chilling injury developed from prolonged exposure to a temperature less than 15°C. *Hopea* showed two types of reactions, depending on the species. The species distributed at an altitude of 1300 m, *Hopea beccariana*, showed 100% survival after the cold treatment, whereas *Hopea nervosa*, the species with a distribution limited to lowland areas, did not survive at 4°C. *Hopea* appears to be a heterogeneous group, and may need reclassification. Also, as anticipated, *Vatica* showed some tolerance to cold temperature.

1.3.7 Adaptations to Drought and Flooding Conditions

Responses to drought conditions differ among the species. *Shorea Talura* can be deciduous in dry countries, as it sheds its leaves in the dry season. Also, it can grow on dry sites, such as limestone ridges and sandy plains. *Shorea Talura* has very few lenticels on its bark, limiting transpiration and protecting the cambium from desiccation. Other *Anthoshorea*, such as *Shorea assamica*, *Shorea hypochra,* and *Shorea*

Table 6. The effect of low temperature on survival of various Dipterocarpaceae seedlings

Species	Stage of seedlings	% Survival	Remarks
Rubroshorea			
Shorea accuminata	First opposite leaves developed	0	
Shorea macroptera	First opposite leaves developed	0	
Shorea parvifolia	First opposite leaves developed	0	
Shorea curtisii	First opposite leaves developed	0	
Eushorea			
Shorea glauca	First opposite leaves developed	0	
Richtea			
Shorea multiflora	First opposite leaves developed	0	
Anthoshorea			
Shorea Talura	First opposite leaves developed	85	Leaves develop chilling injury
Shorea bracteolata	5–6 internodes developed	40	
Shorea hypochra	2 internodes developed	5	
Hopea (2n = 20)			
Hopea beccariana	First opposite leaves developed	100	
Hopea nervosa (2n = 14)	First opposite leaves developed	0	
Parashorea			
Parashorea densiflora	Scale leaves	0	
Vatica			
Vatica cinerea	First opposite leaves developed	10	

The seedlings were kept in a refrigerator at 4°C for 5 days and then transplanted to the nursery

lamellata, also show tolerance to desiccation. These species have a high percentage of seedling survival following transplanting. Similarly, *Hopea odorata* is tolerant to drought, and can be transplanted to bare lands. From distribution patterns, it is expected that some *Vatica*, *Anisoptera*, and *Dipterocarpus* can be used as culture planting stock. Although the distribution is limited, some *Dryobalanops* may be good species for culture plantations, judging from leaf and stem morphology. In contrast, *Shorea ovalis* (*Rubroshorea*) has abundant lenticels on the bark, and loses substantial amounts of water from the bark, causing damage to the cambium.

Some dipterocarp species grow under flood and anaerobic conditions. For example, *Vatica Wallichii*, *Dryobalanops rappa*, *Shorea albida*, and a few species of *Diptercarpus* are known to grow in freshwater swamps. It is possible that these species may be adapted to drought conditions as well as extremely wet conditions. In many cases, extremely wet conditions inhibit root growth and induce physiological drought conditions. *Shorea albida* grows in swamps and also in dry sandy soil called "kerangas." The thickness of leaves and the development of a cuticle layer in *Shorea albida* may be good indicators that the species is adaptable to dry conditions. It is interesting that *Shorea albida* is taxonomically classified as *Anthoshorea*.

1.3.8 Species Suitable for Propagation by Cutting

Recently, cutting techniques have advanced and various species of dipterocarps can now be propagated by cutting. Even some *Rubroshorea* are now successfully propagated by cutting. However, generally speaking, the species that accumulate starch grains in their stems are suitable for cutting. Similar to the seed characteristics, the genera distributed in Thailand, India, Indochina, and Hainan (*Anthoshorea*, *Vatica*, *Anisoptera*, *Dipterocarpus* and some species of *Hopea*) accumulate starch in the stem.

The accumulation of starch in the stem is important not only for the success of cutting, but also for the survival of transplants. Also, in stump planting, species with an abundant carbohydrate reserve perform better. Another propagation technique was developed for the species that accumulate carbohydrates. In this method, after all the leaves were removed, the seedling was buried horizontally in soil. Within a few weeks, axillary buds developed into seedlings. Ringing with wire on both sides of a leaf scar helped to promote root formation.

1.3.9 Response of Dipterocarp Seedlings to Light

It is generally believed that dipterocarps cannot grow under strong light, as they are shade-tolerant species. However, strong light is required for good growth. Seedlings of *Anthoshorea*, *Vatica*, *Anisoptera*, *Dryobalanops*, *Dipterocarpus* and some species of *Hopea* are able to grow under strong sunlight, sometimes even on bare land, provided that the soil is fertile and rich in mineral nutrients. However, as strong sunlight tends to create water-deficient conditions, seedlings of *Rubroshorea* often suffer from desiccation. For species susceptible to drought, the seedlings can be hardened to adapt to dry environments by exposing them to light. Hardened seedlings develop a good root system, a thick stem, and sun leaves with thick cuticle layers. In addition, to protect the leaves from sun scorching, the application of fertilizers is helpful. Sun-scorching symptoms can be minimized. More nutrients are required for open-grown seedlings.

In most nurseries, heavy shading is used in raising dipterocarp seedlings. This is partly because the nutrient content of potting mixtures is poor and unsuitable for open-grown seedlings. Sun-scorching can be avoided under heavy shading, even if poor soil is used. However, the seedlings developed under heavy shade have characteristics typical of shade plants. These seedlings are slender in appearance as a result of the excessive elongation of shoot internodes. The thin tender leaves scarcely develop cuticle layers. Shaded seedlings typically have poor root systems and elongated large shoots. In particular, dipterocarp species are extremely responsive to weak light conditions. For example, *Shorea platyclados* and *Dryobalanops aromatica* responded excessively to weak light conditions, and seedlings with an internode elongation of 30 cm or more were commonly observed. These seedlings looked like vines. When transplanted, such seedlings have a serious water deficiency problem due to excessive transpiration from their shoots and leaves, and inadequate water

supply from their poor root systems. In addition, the shade leaves will suffer from extreme sun scorching when they are exposed to the open sunlight and be shed. Therefore, seedlings grown under shade performed poorly when transplanted.

1.4 Evolution and Development of *Dipterocarpaceae* in Relation to Distribution and Physiological Characteristics

We have been discussing the distributions, chromosome numbers, seed reserves, germination behaviors, and tolerance to low temperatures and drought of dipterocarp species. It appears that certain relationships exist between species distribution and physiological characteristics. As these findings are closely related to evolutionary processes, an attempt is made to elucidate the evolution and development of Dipterocarpaceae. Most people believe that Dipterocarpaceae originated in the western part of Borneo close to Peninsular Malaysia. The main reason for this theory is that the central place for dipterocarp evolution should have the largest numbers of genera and species. Western Borneo has 12 genera and 244 species, with the most genera and species represented in this area. However, in Borneo and Peninsular Malaysia, the numbers of *Rubroshorea*, *Richetia*, and *Eushorea* are disproportionately large compared to the other genera. Also, considering its tolerance to low temperatures, its chromosome numbers, and other physiological characteristics mentioned previously, *Eushorea* may have at least two different groups. Attention should be paid to the geological land formation in order to explain the discrepancy in the "Borneo origin theory" for dipterocarp development.

Rubroshorea, *Richetia,* and *Eushorea* are not represented in Java, which was supposedly separated from Sunda Land at an early stage of the orogenic movement in this region. If this is so, Java must have been separated before *Rubroshorea*, *Richetia*, and *Eushorea* evolved. Similarly, the Myanmar side of the floristic division at the border of Thailand and Peninsular Malaysia does not have *Rubroshorea* and *Richetia*. Also, *Eushorea* is poorly represented on the Myanmar side. Evidence suggests that this division was created by a rise in sea level as a result of the melting ice during the Quaternary Glacial Period. At that time, the Malaysian side was separated from the Myanmar Continental side by the sea. This suggests that *Rubroshorea*, *Richetia*, and some of *Eushorea* evolved after Peninsular Malaysia had been isolated from the Asian Continent. Therefore, *Rubroshorea*, *Richetia*, and some *Eushorea* may have developed in Borneo and Peninsular Malaysia in the Quaternary Period.

These assumptions suggest that the Dipterocarpaceae probably originated from a genus or genera other than *Rubroshorea*, *Richetia*, and *Eushorea*. It is likely that such a genus would have the chromosome number $2n = 14$, because the species with $2n = 20$ may have developed from the species with $2n = 14$. Also, the original genus should be distributed in both Java and the Asian mainland. There are two candidates of genera that satisfy these requirements, *Anthoshorea* and *Hopea*. In Java, only two

species, *Shorea javanica* and *Hopea Sangal*, are represented. *Hopea Sangal* is classified into the *Euhopea* subgroup, which has a wide distribution from India to New Guinea. *Hopea Sangal* itself is distributed in Peninsular Malaysia, Java, and Borneo. Symington (1943) observed that *Hopea* is composed of heterogeneous groups of the species, and he recommended rearrangements of the *Hopea* classification. This is also supported by genetic and physiological studies, such as those of chromosome numbers, reserve food in seeds, and tolerance of seedlings to low temperatures.

In contrast, *Anthoshorea* species are remarkably stable and homogeneous in species characteristics, except that *Shorea talura* is the only species with hypogeal cotyledons. These *Anthoshorea* have wide and even distributions from India and Myanmar to the Maluku Islands. Among *Anthoshorea*, *Shorea Talura* has a wide distribution from India to Peninsular Malaysia. The distribution suggests that the species was present before Peninsular Malaysia was separated by the rise of sea level. Similarly, *Shorea hypochra* and *Shorea assamica* are represented on both the Myanmar and Peninsular Malaysia sides. It is interesting that these *Anthoshorea* species are present on both sides of the Myanmar and Malaysia floristic division. This suggests that these species were present before the sea separated the Peninsula.

Although more studies are needed to speculate on the origin of Dipiterocarpaceae, *Anthoshorea* or a group of *Hopea* are likely to be good candidates. Among *Anthoshorea*, *Shorea Talura* needs specific attention as one of the original species, because it developed many different forms in various regions. Also, it is the most adaptable species to diverse conditions. In particular, among the dipterocarp species, tolerance to low temperatures and desiccation is remarkable in this species.

Physiological traits, such as tolerance to adverse conditions, are considered to be acquired, and it is believed that these traits were developed as the species advanced into the northern territories. However, from the discussion above, it seems natural that these traits are inherent in the original dipterocarp species, such as *Anthoshorea* and *Hopea*. *Rubroshorea* and *Richetia* probably lost these traits early in their evolution, as they do not possess the physiological tolerances. If so, the evolution and development of Dipterocarpaceae must have originated somewhere else, probably somewhere on the Asian mainland but not in western Borneo.

In India, *Shorea robusta* is known as a holy tree and is called "Sal." This species has a distribution up to Nepal. Another species, *Shorea obtusa,* is also distributed in Myanmar, Laos, and Vietnam. Both species are supposed to have tolerance to low temperatures, although they belong to the *Eushorea* group. None of the *Eushorea* group tested previously showed tolerance to low temperatures. To synthesize different sources of information, it is important to study the morphological, genetical, and physiological characteristics of *Shorea robusta* and *Shorea obtusa*. These two species appear to be the keys to understanding the distribution and development of Dipterocarpaceae. It may be possible to separate these two species from *Eushorea*, as *Eushorea* is often divided into three subgroups. *Shorea robusta* and *Shorea obtusa* are classified into the same subgroup. Therefore, it would be interesting to know the chromosome number of *Shorea robusta*, since $2n = 20$ chromosomes are reported for *Shorea obtusa*. Also, *Shorea robusta* is the only species in the *Eushorea* group with hypogeal cotyledons. *Shorea robusta* may be physiologically similar to *Shorea Talura*.

1.5 Dipterocarp Species Suitable for Cultured Planting and Propagation

As dipterocarp trees grow in moist, dark, natural forests, it has been a common belief that dipterocarp species are shade plants that grow under poor light conditions and that they cannot tolerate sudden changes in environment. Therefore, it has been a common practice to raise dipterocarp seedlings under heavy shade. In temporary nurseries in forests, some large-standing trees are left for extra shade in addition to the roofing over nursery beds. The seedlings raised in these nurseries have characteristics typical of shade plants, with elongated shoots and small root systems. Also, following transplanting, canopies of large trees usually shade the planting site and the growth of the transplants is arrested. In other cases, weak seedlings raised in the shade are transplanted directly to bare land. These planting trials show disastrous results, with few seedlings surviving. Therefore, cultured planting of dipterocarp species appears to be extremely difficult.

As discussed in this chapter, there are certain dipterocarp species tolerant to adverse conditions, and species tolerant to desiccation are needed for cultured planting. If the plants are tolerant to desiccation, they can grow in open sunlight. It is most probable that tolerance to low temperatures has a strong correlation with resistance to desiccation. For example, *Anthoshorea*, *Hopea*, *Vatica*, and *Anisoptera*, with wide distributions from India or Indochina, have a tendency to adapt to adverse conditions, particularly under drought conditions with heat and strong sunlight. Among these plants, *Shorea Talura*, *Shorea hypochra*, *Shorea assamica*, and *Hopea odorata* are suitable for transplanting to bare land.

Even among the species susceptible to desiccation, such as *Rubroshorea*, it is important to harden seedlings to survive under drought conditions. In the Forest Research Institute, Malaysia, there are good examples of cultured plantations of dipterocarps. The Institute has long maintained the cultured forests of *Dryobalanops aromatica*, *Shorea hypochra*, *Shorea platyclados*, and *Shorea macroptera*.

Species characteristics may influence the preferred cultured propagation techniques, such as by micropropagation and cutting. Species that accumulate carbohydrate reserves in their stems may be suitable for cultured propagation. More attention should be paid to the species distributed on the Asian mainland. In particular, *Anthoshorea*, *Hopea*, *Dipterocarpus*, and *Anisoptera* should be included for tissue-culture experiments.

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2
Forest Genetics for Sustainable Forest Management

JEFFERY BURLEY

2.1 Introduction

The International Union of Forest Research Organizations (IUFRO) comprises over 700 member institutions in 112 countries, with 15,000 scientists working collaboratively and voluntarily in 280 Divisions, Research Groups and Working Parties. Throughout the twentieth century, the Union stimulated and supported excellent research in a wide range of scientific topics. However, IUFRO has now established Task Forces to encourage the integration of such research and to foster better understanding between researchers and policy makers. At its quinquennial Congress in Malaysia during August 2000, IUFRO scientists produced state-of-knowledge reports on many major issues to indicate both the currently available information and any need for new research. One of IUFRO's Working Parties specifically addresses the potential benefits and risks of molecular technologies in transgenic plantations.

The organizers of the Bio-Refor workshop in Nepal asked me to address particularly the role of forest genetics in sustainable forest management. To some extent I did this in a keynote address to the Queensland Forest Research Institute's conference in Caloundra during November 1996, and some of the points here are repetitions or expansions of issues I raised at that meeting (Burley 1996). At the outset, I should state that I consider forest tree improvement to include enhancements in silviculture, forest management, and product processing; forest genetics must be seen as one component of tree improvement interacting with these elements in the search for sustainable forest management.

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2.2 Sustainable Forest Management

Sustainable forest management is one part of the overall concept of sustainable development. There have been innumerable definitions of this, but a good working definition, provided by the Forestry For Sustainable Development Programme of the University of Minnesota, is: "Development involving changes in the production and/or distribution of desired goods and services which result, for a given target population, in an increase in welfare that can be sustained over time." In order to achieve sustainable development, there has to be a concordance of the three major sets of factors: biological (environmental), economic, and social.

A commonly accepted working definition of sustainable forest management emerged from an Inter-Ministerial Conference on European Forests in Helsinki during 1993: "Sustainable management means the stewardship and use of forest lands in a way, and at a rate, that maintains their biodiversity, productivity, regeneration capacity, vitality, and their potential to fulfil now, and in the future, relevant ecological, economic and social functions at local, national and global levels; and that does not cause damage to other ecosystems."

Sustainable forest management is a major concern for a large number of international and national institutions, including: International Tropical Timber Organization; Helsinki Process; Montreal Process; Tarapoto Proposal; African Timber Organization; Lepaterique Process; UNEP/FAO Expert Meeting; FAO/UNEP Expert Meeting; FAO/ITTO Expert Meeting. All of these are seeking to develop for different forest types, or at different levels, some criteria and indicators of sustainable forest management. Although they differ in indicators, there is a high level of agreement in the criteria, and broadly these include biodiversity, productivity, soil conservation, water conservation, forest ecosystem health and vitality, contribution to global ecological cycles, and fulfilment of socio-economic needs.

2.3 Changes in Forestry

In the second half of the last century, there were major changes in the objectives of forestry. Clearly, these differ significantly between regions and countries, but in summary we might consider the 1950s as a period of concern to produce large volumes of industrial wood. During the 1960s, attention began to focus on the quality of industrial wood, particularly from plantations. In the 1970s, intensive research was conducted on the quality of pulp and paper, while during the 1980s and 1990s, globally there was an expansion of interest in the role of trees and forests in supporting agriculture and human welfare. Throughout the half century, there was a growing interest in the use and improvement of non-wood products.

2.4 Changes in Techniques of Tree Breeding

2.4.1 The Traditional or Classical Programme of Tree Breeding

Classical tree breeding has been practised in many countries and organizations, particularly in the 1950s and 1960s. The programme involved several stages, including species and provenance evaluation, establishment of pilot plantations and eventually commercial plantations, creation of seed stands (seed-production areas), mass selection of superior phenotypes, and the establishment of progeny trials to evaluate genotypes (using a range of mating systems and environmental designs that estimated genetic and environmental parameters with varying levels of precision). These stages were accompanied by the creation of clonal or seedling seed orchards.

In the 1970s and 1980s, considerable attention was given to the needs of recurrent selection over multiple generations and the inclusion of multiple traits. Various programmes used tandem selection, independent culling levels, total score, multiple-trait index, and genetic-selection index methods for the selection of parents of subsequent generations.

By the early 1980s, clonal techniques (cuttings and tissue culture) had been developed for many industrial species, and large areas of single or multi-clone plantations were established, accompanied by considerable debate on the number of clones and their management. The Marcus Wallenberg Prize in 1984 was awarded to four members of the Aracruz company in Brazil for their work on integrating selection, breeding, and clonal propagation into a major commercial plantation eucalypt programme for the production of pulp and paper.

By the 1990s, it became clear that the advances made by genetic selection brought with them risks to biodiversity, conservation, and future selection gains; the concept of the multiple-population breeding strategy was developed and refined, for which another Marcus Wallenberg Prize was awarded to Professor Gene Namkoong in 1994 (Namkoong et al. 1984). Multiple population breeding strategies permit us to improve several traits simultaneously: incorporate new material; minimize inbreeding; maintain genetic diversity; respond to changing management, environment or markets; and use genotype-environment interactions. In parallel with this strategy, the concept of breeding seedling orchards was developed to combine both seed production and genetic evaluation (Barnes 1995).

2.4.2 The Specific Case of Molecular Technology

Possibly the techniques with the greatest potential benefits, but also some potential risks, are those based on molecular methods. Almost daily, new methods or refinements of molecular biotechnologies are developed and published. They have different costs and applications, and a broad summary is given in Table 1 (from Rendell 1999); the most immediate applications of molecular methods in ecology, genetics, and tree breeding are listed in Table 2.

Table 1. Comparison of molecular techniques (From: Rendell 1999)

			Genetic diversity	Population structure	Phylogeny	Hybrid-ization	Intro-gression	Genotype identification	Poly-ploidization	Mating system
Isozymes			+++	+++	+/– [a]	++	++	+/++ [a]	++	+++
RAPDs			+++	++ [b]	– [c]	++	– [d]	+++	+ [e]	– [d]
Microsatellites			+++	++ [f]	– [g]	–	–	+++	–	? [h]
RFLPs	nDNA	Coding	+	+	+ [i]	++	++	++	++	–
		Non-coding	+++	+++	+++	+++	+++	+++	+++	+++
	cpDNA	Coding	–	–	+	+ [j]	+ [j]	–	+ [j]	+ [j]
		Non-coding	+++	+++	+++	+++	+++	++	+++	– [j]
	mtDNA		+	+	–	–	–	++	–	–
DNA sequencing	nDNA	Coding	++ [k]	++ [k]	+++	+++	+++	+++	+++	+++
		Non-coding	++ [k]	++ [k]	+++	+++	+++	+++	+++	+++
	CpDNA	Coding	++ [k]	++ [k]	+++	+++	+++	+++	+++	+++
		Non-coding	++ [k]	++ [k]	+++	+++	+++	+++	+++	+++
	mtDNA		++ [k]	++ [k]	+++	+++	+++	+++	+++	+++

[a] Depends on study taxa

[b] Less appropriate for within-population structure

[c] Problems with homoplasy due to comigration of non-homologous bands

[d] Problems because pattern of inheritance of bands is poorly understood

[e] Problems with interaction between bands in polyploids

[f] Particularly appropriate for within-population structure

[g] Problems because most microsatellites are specific to single species or genera

[h] Few studies to date

[i] Better for taxonomic groups above the species level

[j] Would need to combine with nDNA data

[k] Would need to consider cost and sample size

Table 2. Application of molecular methods to problems of ecology, genetics, and tree breeding

1. Taxonomy, systematics, evolution, and identification of species and individuals.
2. Genecology and habitat-related genetic variation between populations of a given species.
3. Population genetic structure (for both breeding and conservation).
4. Identification of breeding systems (for artificial breeding, conservation, studies of gene flow, and habitat fragmentation).
5. Evaluation and possibly prediction of genetic differences at the levels of species, population (provenance), individual, and clone.
6. Identification ("finger-printing") of pedigree of clones and progenies in breeding populations and for protection of plant breeders' rights and/or traditional resource rights.
7. Identification of the physiological basis for tree resistance to (or tolerance of) drought, heat, cold, salinity, alkalinity, radiation, pests, and pathogens.
8. Determination of genetic control of wood chemical components, such as lignin, and the enhancement of ligninase enzymes in fungal species, with potential application to pulp and paper manufacture.

In developed countries particularly, but increasingly at a global level, there is great concern about the applications of molecular technologies to human and veterinary medicines, and to agricultural crops and foods. Most recently, the concern has spread to genetic modification of forest trees. In response to public and political concerns, IUFRO Working Party 2.04.06 (coordinated by Professor S. Strauss and Dr. M. C. Campbell), at a meeting in Oxford during 1999, developed a position statement on the benefits and risks of transgenic plantations. This position statement expressed a majority opinion on matters of science and technology policy, based on the perspective of a group of professional experts from 21 countries; its main targets were government regulators, scientists and professionals in biological fields, and citizens with scientific backgrounds in biology and natural resources.

While the position statement summarized the background to the debate and defined various technical terms, the principal component statements included the following:

1. The economic benefits of transgenic crops to society, as well as to industries, can be great.
2. Transgenic crops can provide important environmental benefits.
3. The risks of genetically engineered crops should be considered not only in themselves, but in relation to the risks and benefits of all other candidate systems.
4. Excessive restrictions on the use of transgenic organisms in research, breeding, and in international commerce can obstruct opportunities for new knowledge, improved production systems, and environmental benefits.
5. Scientific claims about the benefits or risks of transgenic organisms should address specific genes, traits, environments, and management systems, not their method of introduction.

In developing this position statement, the Working Party members identified some underlying principles applicable to forest plantations, including the following:

1. Intensification of tree-fibre productivity can reduce pressure on native forests for wood, fibre, chemicals, and energy.
2. Transgenic forest plantations promise a number of significant environmental benefits.
3. While transgenic traits pose some risks for plantations and associated ecosystems, many options exist to mitigate their impacts.
4. Field trials, wisely designed and carefully monitored, are an important part of safety evaluations.
5. For transgenes or species whose sexual spread causes concerns, genetic technology is under development that should render trees unable to produce viable seeds and pollen.

2.5 Challenges for Tree Breeding

Broad changes in the objectives of forestry have been accompanied by changes in policies and institutions that affect forests and forestry. These have offered considerable challenges for genetic conservation and tree breeding.

2.5.1 Changing Policies and Institutions

Throughout the world, there has been a growing trend for governments to hand over the management of production forestry to private organizations. International agencies and governments have sought to reduce the emphasis on industrial forestry in favour of rural development forestry; where industrial forestry has been supported, there has been encouragement for outgrower schemes to support central saw mills or pulp mills. Overall, there has been a growing recognition that forestry and forests are global concerns and resources, even though they are under the sovereign rights of nations.

2.5.2 Changing Natural Environment

Tree breeders, like all forest managers, must be aware of the global concerns for changing natural environments, particularly the climate. The changes in the mean and extreme values of temperature, rainfall, wind, and ultraviolet light are predicted to occur in most parts of the world, with a consequent need to reconsider the optimum species, populations, and genotypes that are suitable for given objectives.

2.5.3 Changing Sites

Throughout the world, the types of land available for forestry are changing, and there is a growing need to develop genotypes for the remediation of degraded sites and the use of extreme sites. Land-tenure systems are also changing, and the likely users of improved genetic material are thus altering.

2.5.4 Changing Management

Throughout the world, often under pressure from environmental groups, there is increasing demand for change from exotic species to indigenous species, and from high-input technology to low-input systems. As new biotechnologies develop, clonal-industrial forestry is expanding and yet there is strong public pressure, particularly in developed countries, for the use of mixed-species plantations. Further, in developing countries and some developed countries, intimate mixtures of crops, animals, and trees in agroforestry systems are required.

2.5.5 Changing Uses and Processes

Wood-using technologies are changing rapidly and there are now many ways of using solid wood from small trees; jointing, gluing, and lamination techniques allow large constructional components and furniture to be made from small pieces of wood. Increasing demands for reconstituted wood have caused refinements of techniques for the manufacture of chipboard, fibreboard, pulp, and paper. Over half the wood that is deliberately cut each year is used for energy, principally for domestic heating and cooking, while in many countries there is an expanding demand for non-wood products.

2.5.6 Changing Ownership and Management of Gene Resources

The Convention on Biological Diversity considers that gene resources should be available to all, but it recognizes that intellectual property rights must be duly recompensed. There is a chain of development of genetic resources from the wild type through to advanced generations of breeding and the resultant propagules. While the intellectual property rights to some of the stages can be recognized and rewarded, the pedigree control and rights tend to become lost when improved material is passed on to farmers and local communities who thenceforward do their own selection and propagation.

All of these environmental, political, and technological changes require different ideotypes of trees to fit different management systems and to provide a range of benefits. The traditional concept of genotype–environment interaction has to be expanded to include these issues.

2.6 Specific Tropical Challenges to Tree Breeding

Many of the principles of forest genetics and tree breeding apply equally to tropical and temperate species and conditions, but there are some practices that are specifically influenced by the species and political/social/environmental conditions of tropical and developing countries.

As forestry becomes more widely privatized, breeders must face more rigorous cost-benefit analysis of their efforts, and at the same time play a significant role in determining breeding economics and the value of forests in national accounts. Breeding strategies must be appropriate to the resources and objectives of the various stakeholders who are dependent on the breeders' efforts. Increasingly, genotypes and ideotypes of trees must be manipulated for mixed plantations, producing multiple benefits and with genotypic stability. Forests and trees are to be established on difficult or degraded sites that will require resistance or tolerance to climatic and edaphic extremes, changing climates, and pests. In common with forest managers, tree breeders must learn to collaborate in participatory research and development efforts.

As tree breeding focuses increasingly on species for rural development, including agroforestry, breeders must be aware of the need for appropriate allocation of intellectual property rights and the loss of pedigree control once farmers and local communities learn to select and propagate their own material. There is increasing concern that forests should be naturally and eugenically regenerated, and equal concern that selective logging may lead to habitat fragmentation and the restriction of population genetic variation.

In many countries, both developed and developing, there are hundreds of examples of species, provenance and progeny trials that have not been recently assessed, analysed, and interpreted. There are great opportunities for gaining new information on genetic structure and change, particularly with appropriate molecular appraisal, while also providing material for future selection in such trials. Without such analyses, all the initial costs of establishing, maintaining and assessing these trials will be wasted, and future generations of researchers and breeders may repeat the experiments in an attempt to "re-discover the wheel."

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3
Recent Developments in Vegetative Propagation Techniques and Their Application for Tropical Forest Trees

KATSUAKI ISHII

3.1 Introduction

Vegetative propagation, in contrast to propagation by seeds, enables the capture and transfer of improved material from the donor tree to the asexually obtained offspring. Cutting, grafting and air-layering are conventionally used for vegetative propagation. Recently, micropropagation and plant-tissue culture have been exploited in the propagation of ornamental and horticultural plants by commercial companies. Tissue culture of forest trees has been applied to (1) clonal propagation, (2) viral elimination, (3) gene conservation, (4) in vitro fertilization, (5) mutation induction for genetic diversity, (6) genetic transformation, (7) protoplast culture and somatic hybridization, (8) secondary-metabolite production, and so on. Recent tissue culture is a basic technology for genetic engineering that offers an important regeneration step. Here, I review the development of recent vegetative propagation techniques, including cuttings and tissue culture for application to tropical-forest trees.

3.2 Clonal Propagation

3.2.1 Rooted Cuttings

Vegetative propagation by rooted cuttings has been most frequently used for clonal propagation of forest trees. *Eucalyptus* plants are mainly produced by cuttings, using rigid-tube containers filled with vermiculite-organic compound substrates, in the industrial plantations of Brazil (Stape et al. 2002). Several thousand plants of teak have been produced by rooted cuttings in Malaysia (Monteuuis 1995). Newly-developed sprouting shoots emerging from a stump, cut about 20–30 cm above ground

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level, were used as the stock plant. Expanding axillary shoots, 3–6 cm in length from the basal cut to the terminal bud, were set in rooting beds filled with wet sand. The environmental conditions consisted of 50% shade with intermittent mist sprays provided during the day by a mist system. The mature genotypes selected could be propagated with average rooting rate of 70% (Monteuuis 1995).

Dipterocarps have acquired the reputation of being difficult to root. However, there has been considerable success with some dipterocarp species (Dick and Aminah 1994). The production scale of vegetative propagation by cuttings is being applied by the PT Inhutani in East Kalimantan, Indonesia (Smits 1993). However, there is a need for comparison of the different methods used to propagate cuttings (mist, non-mist, and aerated water system) for recommendation of the best technique for vegetative propagation of dipterocarps (Dick and Aminah 1994). Inducing roots from cuttings requires conditions to control their transpiration and to promote photosynthesis.

The fog-cooling system keeps the temperature inside a box of cutting beds below 30°C, at about 5000 lux of sunlight, due to heat removal by evaporation of the fog, and at a relative humidity of about 95% (Sakai et al. 1994). By using the fog cooling system, the percentage of root formations were 90% in *Shorea selanica* and *S. leprosula* (Sakai et al. 1994). Usually, rooting ability differs between species, genotypes and cutting material (example with *Ficus* species, Danthur et al. 2002b). In an experiment of non-mist leafy cuttings, using 100 tropical rain forest species that are economically less important but ecologically important, species in the family Dipterocarpaceae and Lauraceae had a low rooting ability, while those in Euphorbiaceae, Rubiaceae and Annonaceae had a high rooting ability (Itoh et al. 2002). Species of smaller mature sizes and faster diameter growth rates showed better rooting ability. Species whose habitats were on lower elevations, concave slopes, and/or clay-rich soils rooted significantly better (Itoh et al. 2002). For restoration of rooting competence, using material of suckers from root cuttings and repeated micrografting was effective with *Faidherbia albida* (Danthur et al. 2002a). Scions from mature elite trees were grafted first onto the unselected rootstock for restoraton of rooting competence, then they were used for the production of rooted cuttings with *Prosopis alba* (Felker et al. 2001). Physiological and genetical research is beneficial for understanding the process of root formation in the cuttings of tropical forest trees.

3.2.2 Tissue Culture

More than 600 million micropropagated plants are produced every year in the world (Maes et al. 1998). Micropropagation is one of the few areas of plant tissue culture in which the techniques have been applied commercially. The micropropagation of woody plants is more difficult than that of herbaceous species. Surface and within-explant contaminants are problems. Root initiation, its development, and habituation are also problematic with some forest-tree species. Some species secrete phenolics into the growth medium, which may inhibit growth or promote abnormal patterns of development.

Multiple shoots arising from axillary and/or adventitious budding are the common method of micropropagation. Micropropagation rates are 5–10 times per culture cycle. *Eucalyptus* (Le Roux and van Staden 1991) and *Pinus radiata* (Smith 1997) are advanced in micropropagation.

In Brazil, 250 000 micropropagated plants of 12 different clones were taken to the field over a 2-year period about 10 years ago (Grattapaglia et al. 1990). However, practical clonal propagation is mainly done by rooted cuttings; for example, one company in Brazil plants nearly 30 million stecklings per year (Zobel 1993). About 170 superior trees of *E. grandis*, selected from a 5-year-old progeny test, have been micropropagated by organ cultures for a breeding orchard establishment in Aracruz, Brazil (Ikemori et al. 1994).

Mondi Forests in South Africa uses tissue-culture techniques to establish microcuttings of *Eucalyptus* clones (Jones and van Staden 1997).

Acacia species have been given due importance in tropical-tree tissue culture, owing to their ecological and economic significance. The proper selection and collection of explants with the judicious incorporation of plant growth regulaors, antioxidants, additives, and adsorbents during in vitro culture have greatly contributed to developing successful regeneration protocols for many *Acacia* species, which were recently well reviewed (Vengadesan et al. 2002).

Tasman Forestry in New Zealand has operated 2–3 million micropropagated *Pinus radiata* per annum from selected control-pollinated seed (Gleed 1993). The cost of micropropagated *Pinus radiata* is about six times more expensive than the cost of seedlings (Smith 1997).

It is said that two million plantlets of teak have been produced by tissue culture in Thailand (Ishii and Maruyama 1994). The tissue-culture protocols of teak used by Innoprise Corporation, in Sabah, Malaysia, enable the mass micropropagation of any genotype by axillary shoot, with exponential rates of three to four new shoots every two months (Monteuuis 1995). The rooting-acclimatisation phase was achieved under nursery conditions using a mist system, with 95% success. More than 50,000 microshoots have been produced by that unit that have developed rapidly into true-to-type vegetative offspring of superior quality (Monteuuis 1995).

Recently, somatic embryogenesis has attracted attention for the large-scale production of emblings (somatic seedlings). Somatic embryogenesis in woody plants, such as *Picea*, *Pinus*, *Santalum album*, *Citrus*, and *Mangifera indica* have been reported (Jain et al. 1995).

Scale-up in vitro clonal propagation of oil palm (*Elaeis guineensis*) through somatic embryogenesis has revealed that a five times production cost and epigenetic variation (ca. 5%) were the major bottlenecks that limited commercial development (Rival et al. 1997).

Artificial-seed production in forest trees using somatic embryos has been reported in *Eucalyptus citriodora*, *Santalum album*, *Pinus lambertiana*, *Pinus taeda* and *Picea abies*, usually with a very low regeneration rate (Gupta and Kreitinger 1993). This technique has also be used by the author's group on tropical-forest trees in which somatic embryos have not been produced. In this case, the encapsulation of shoot-tip and/or axillary buds provides an alternative to producing artificial seeds. In an attempt to improve the plant regeneration rate from artificial seeds, two types

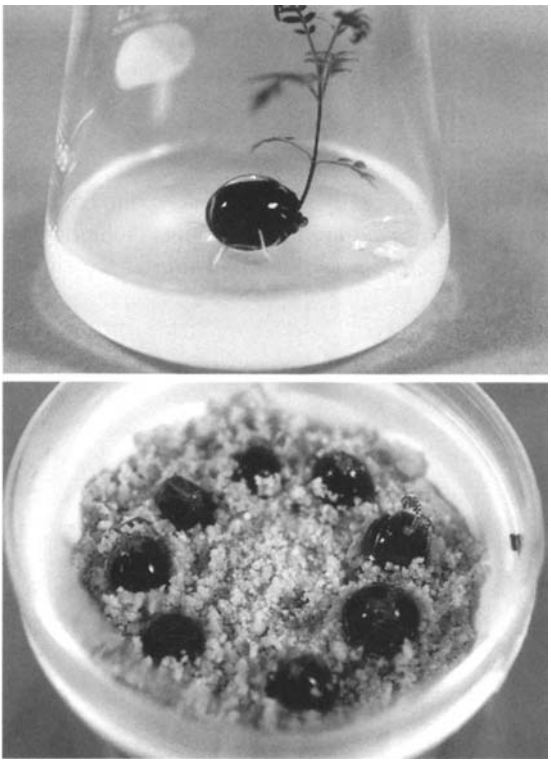

Fig. 1. Germination of artificial seeds of *Jacaranda mimosaefolia* under in vitro **(a)** and ex vitro **(b)** conditions

of beads with a single or double layer were tested. The best result was obtained with double-layered beads containing a medium with a concentration 10 times that of normal and supplemented with 0.5% (w/v) activated charcoal in the inner layer, and a normal concentration in the outer layer. High rates of bud emergence and shoot growth were achieved (Fig. 1): 60% and 60% for *Cedrela odorata*, 100% and 80% for *Guazuma crinita*, and 100% and 100% for *Jacaranda mimosaefolia*, respectively (Maruyama et al. 1997a). The development of a suitable coating for artificial seeds that permits plant conversion under non-aseptic conditions is required for practical implementation.

3.3 Viral Elimination

The technique of virus elimination by meristem-tissue culture is based on the uneven distribution of viruses or mycoplasmas. A shoot's apical meristem contain very little or no viruses. Morel and Martin (1955) successfully regenerated virus-free dahlia plants from infected-donor plants. The technique has been used to eradicate viruses from many horticulturally-important species, including woody perennials like apples (*Malus* sp.) and raspberry (*Rubus ideus*) (Hu and Wang 1984).

Viral elimination of forest trees using tissue culture is scarce. One example used in my laboratory is presented here. Princess tree (*Paulownia tomentosa*) is a fast-growing tree and a first-class material for furniture. However, witches' broom disease, which is ascribed to a mycoplasma-like organism (MLO), has recently been spreading in China and Japan. The apical meristem, where plant cells divide vigorously, is not polluted with this pathogen. Thus, if we can propagate the plant using the apical meristem, we will be able to obtain healthy plantlets. We employed a BT medium with BAP added at a rate of 1 mg/l to culture the shoot tips, including the apical meristem of a 0.4-mm long cut from a diseased *Paulownia.* Of the shoots obtained from the culture, 47% still had the symptoms of witches' broom disease. We heat treated these shoots for two weeks at a temperature of 38°C. When we cultured the shoot tips taken from the newly grown portions of the shoots during the heat treatment we could get healthy plantlets. These healthy plantlets were propagated in BT medium in vitro, and the propagation rate of shoots reached 20 times over a one-month culture. From these shoots, we were able to obtain rooting plantlets after culturing them in a rooting medium with 1 mg/l of IBA. When these plantlets were transplanted into vermiculite soil and the humidity was gradually lowered, more than 85% of them were successfully habituated.

After 9 years of plantation, no evidence of infection with witches' broom disease has been observed. The existence of a ribosomal-RNA gene of a MLO associated with *Paulownia* witches' broom in the leaves of planted-out trees was checked using PCR amplification. No band related to a MLO organism was detected from healthy planted-out trees after 5 years of cultivers (Ishii et al. 1995).

Other than meristem culture, shoot tips of about 0.2 mm in length, isolated aseptically from a disease plant, were grafted onto young rootstock seedlings grown in vitro. This technique, known as shoot-tip grafting, has been extensively applied to *Citrus*. Micrografted plants are free of the citrus-viral disease which infected the shoot tips of donor plants (Navarro 1984). Viral elimination has not been applied to tropical forest trees so far. However, viruses and virus-like diseases of tropical forests are yet to be elucidated in the 21st century.

3.4 Gene Conservation

In vitro storage is now routinely used for germplasm storage of some crops, such as cassava in Colombia (Escober et al. 1992). Once plant tissues have been established in vitro, long-term storage can be achieved by regular subculturing on fresh media. However, there are problems of microbial contamination, equipment failure, labor cost, somaclonal variation, and loss of morphogenic capacity of the materials. For eliminating these problems, slow growth and cryopreservation are usually adopted (FAO 1993).

Useful tropical forest-tree species (*Cedrela odorata* L., *Guazuma crinita* Mart., and *Jacaranda mimosaefolia* D. Don.) were cryopreserved using shoot-tip or root-tip explants from in vitro-grown plantlets (Maruyama et al. 1996). The best results were achieved when the shoot-tips were cooled by slow pre-freezing before immer-

sion in liquid nitrogen. Survival and plant recovery rates of 50% and 20%, and 50% and 15% were obtained in the cryopreserved shoot-tips of *C. odorata* and *G. crinita*, respectively. In vitro-cultured adventitious bud clusters of *G. crinita* were also successfully cryopreserved by the simple one-step vitrification method (Maruyama et al. 1997b). Small segments (1.0–1.5 mm^3), cut from adventitious bud clusters, were exposed to a cryoprotectant-mix solution containing (w/v), 25% glycerol, 15% sucrose, 15% ethylene glycol, 13% dimethyl sulfoxide, and 2% polyethylene glycol, at 25°C for 15-60 min prior to storage in liquid nitrogen. After rapid warming (37°C), the segments were treated with a woody-plant medium, containing 40% (w/v) sucrose, for 20 min at 25°C, and then transferred to a recovery-growth medium. High survival rates (about 80%) were achieved without any cold hardening and/or preculturing treatments, and about 30% of the surviving cryopreserved explants regenerated plants. Furthermore, encapsulated apical meristems of *Eucalyptus* were cryopreserved and used for germplasm conservation (Poissonnier et al. 1992). Germplasm conservation of the tropical trees *Cedrela odorata* L., *Guazuma crinita* Mart., and *Jacaranda mimosaefolia* D.Don., was attempted at above-freezing temperature using artificial seeds. Shoot tips excised from in vitro plantlets were encapsulated in calcium-alginate beads and stored on different substrates at 12°, 20°, and 25°C. The percent viability when encapsulated shoot tips were stored on a substrate containing only water solidified with 1% (w/v) agar was 80% after 12 months at 12°C for *C. odorata*, 90% after 12 months at 25°C for *G. crinita*, and 70% after 6 months at 20°C for *J. mimosaefolia* (Maruyama et al. 1997c). There should be inexpensive tissue-culture protocols for tropical-tree species that are suitable for long-term conservation. The callus tissue or shoots from 700 tropical tree species were maintained in vitro at the National Center for Genetic Engineering and Biotechnology in Bangkok, Thailand (Kirdmanee et al. 1998). The cryopreservation of callus tissue was studied there.

3.5 Genetic Transformation

The genetic transformation of several forest trees has become more or less routine in recent years, as has been reported for many poplars (Sellmer and McCown 1989), *Liquidambar styraciflua* (Sullivan and Lagrimini 1993), *Liriodendron tulipifera* (Wilde et al. 1992), *Larix decidua* (Huang et al. 1991), and *Picea glauca* (Ellis et al. 1993). For the introduction of foreign genes to target trees, *Agrobacterium*-mediated transfer, direct-gene transfer, using bombardment with DNA coated particles, microinjection, and electroporation of protoplast are applied.

The *Agrobacterium* method can usually be used on all broad-leaved trees and on some conifers. Host genotypes differ in susceptibility to *Agrobacterium* infection. The biolistic methods are relatively simple and can be used for transformation of even conifers, but a stable transformation rate is not very high. The protoplast methods are rather difficult because most forest-tree species are not developed by regeneration from a proptoplast.

More than 20 forest broad-leaved tree species have been transformed, mainly using the *Agrobacterium* method (Ishii 1998). Genera include *Populus*, *Fragraea*, *Liriodendron*, *Liquidambar*, *Eucalyptus*, *Betula*, and *Casuarina*.

Introduced characters are herbicide resistance, resistance to crown-gall disease, insect resistance, early flowering, good growth, good-wood property, change of wood color, morphological change, and the presence of several marker genes, such as glucuronidase and antibiotics. In the case of conifers, nine species were stably transformed, mainly using somatic-embryogenic cells. *Larix decidua*, *Picea glauca*, *Pinus radiata*, *Larix lariciana*, *Pinus halepensis*, *Picea mariana*, *Chamaecyparis obtusa*, *Larix kaempferi x L. decidua*, and *Pinus taeda* are among them. The methods of introducting genes are by Ri plasmid, Ti plasmid, and a particle gun. Introduced genes are nos, Bt, uidA, npt, aroA, rolB, rolC, and virC. They express nopalin synthesis, insect resistance, glucuronidase, kanamycin resistance, glyphosphate resistance, geneticine resistance, and good rooting character.

The introduction of foreign DNA techniques has raised issues about the biosafety of transformants and the genetic-transformation process. These have resulted in a number of national and international recommendations, and the formulation of guidelines, regulations, and legislations (Harada 1997). However, national regulations still need to be made in some developing countries. To improve the public acceptance of this technology, it is important to develop international rules for the safety of recombinant DNA applications to tropical-forest trees.

3.6 Conclusion

Vegetative propagation techniques can be used in the wide area of clonal forestry, conservation, genetic engineering, and the utilization of tropical-forest trees (Haines 1994). Major constraints on the application of biotechnology to tropical-forest trees are the shortage of trained researchers, poor facilities, and a lack of research and investment funds. We need to help promote international cooperation and ask advanced biotechnology-research units of industrialized countries to transfer techniques to tropical regions. Also, tropical countries need to establish regulations and guidelines for biosafety to get public acceptance of genetic engineering, and intellectual property protection for newly-produced techniques and products.

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4
The Significance of Mycorrhizae in Forest Ecosystems

KAZUO SUZUKI

4.1 Introduction

In forests, various organisms live by interaction with other species. Fungi, in particular, have various modes of life such as those of saprophytes, parasites, and symbionts. For example, the honey mushroom (*Armillaria* spp.) is known as "a fungus shrouded in a mystery," because it goes through various modes of life. It was recognized as the largest organism in the world by *Nature* in 1992 (Smith et al. 1992; Suzuki 1996). In recent years, it has been revealed that ectomycorrhizal fungi play a significant role in production of substances in forests. For example, the current amount of ectomycorrhizal fungi in the forest biomass of a 180-year-old fir stand in the United States is only 0.3%, but when rootlets are included, the ratio of ectomycorrhizal fungi to net primary production (NPP) goes up to 75%. In the case of a 50-year-old Douglas fir stand, the ratio is estimated to be 50% (Fogel and Hunt 1979; Vogt et al. 1982). Research on fir stands in Japan has shown that the ratio of mycorrhizal biomass to nonmycorrhizal biomass (rootlets) is 4:6 (Nara et al. 1992). Based on the results of these studies, it can be observed how significant a role ectomycorrhizal fungi play in forest ecosystems. It is expected that active methods based on symbiosis with mycorrhizae will be established for enhancement of forest functions in the future.

Species of the pine family such as pines, firs, spruces, and Douglas fir are dominant components of the forests of the northern hemisphere. The origin of the pine trees can be traced to the Bering Strait in the Mesozoic era. They expanded their area of distribution widely into the northern hemisphere to become the circumpolar plant species of the Tertiary period of the Cenozoic era, adapting to various environments around the world, and formed forests (Suzuki 1991). Recently, forest decline has become increasingly apparent in the United States and in European countries, and it

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has emerged as a serious issue (Fukuda et al. 1997). This forest decline can be attributed to complex interplays of biotic and abiotic factors. Therefore, the development and preservation of healthy forests, which, to a high degree, can fulfill their functions of producing substances and establishing an environment, are of critical importance in taking measures against global-scale environmental changes, such as global warming, which are expected to occur in the future. Of particular importance are most of the species of Pinaceae dominant in the northern hemisphere that form ectomycorrhiza. Their ability to produce substances is enhanced by symbiosis with ectomycorrhiza.

As described above, measures for allowing forests to fulfill their functions of producing substances and preserving environments will be of critical importance in coping with problematic environmental issues such as declining forests, changes in forest environments, and the worldwide spread of epidemic diseases such as a pine wilt disease. In particular, measures for enhancing the functions of pine forests and making effective use of them are critical to forests not only in Asia, but also in the northern hemisphere. This is because pine trees are the most important tree species for forest formation and the timber industry, and they are also vital environmental resources in Japan as well as in the northern hemisphere.

It can be considered that the symbiotic relationships between trees and fungi play an important role in the mechanism of maintaining forest ecosystems. Achievement of the following objectives is important to understand forest ecosystems: (1) elucidation of physiological and ecological characteristics of the symbiotic system between trees and ectomycorrhizal fungi, (2) evaluation of the function of trees in resisting environmental stress, which is based on the use of the symbiotic relationships, (3) elucidation of the symbiotic function of ectomycorrhizal fungi such as *Tricholoma matsutake*, and (4) contribution to the preservation of pine forests through effective use of the symbiotic function.

This chapter clarifies the phenomenon of symbiosis of ectomycorrhizal fungi such as *T. matsutake* by focusing on their structure and functions. First, a method for identifying strains of *T. matsutake* and its closely related species around the world is established by determining their genetic characteristics via polymorphic DNA analysis. Next, the morphogenesis of *Pinus densiflora* trees and matsutake mycorrhizal roots is examined. The following methods and technologies are developed: (1) methods for synthesizing artificial mycorrhizae, which can be used as ectomycorrhizal fungi such as *T. matsutake*, (2) methods for encouraging development of artificial shiros of *T. matsutake*, and (3) techniques for enabling *T. matsutake* to colonize and become established. Attempts were then made to make effective use of pine forests via the use of ectomycorrhizal fungi.

One of the features of this research is its focus on interactions between trees and fungi in forest ecosystems, a topic not fully addressed until now, and the study associated experimental systems in vitro with the ones in the field. The results of this research were presented at international symposiums such as *Ectomycorrhizal Ecophysiology and Its Applications in Pine Forests* (Tange et al. 1999; University of Tokyo 2001; Aga et al. 2004).

4.2 The Puzzle of Ectomycorrhizal Fungus *Tricholoma matsutake* (matsutake)

The Japanese authoritative dictionary of Kojien (Shinmura 1991) explains that matsutake (*Tricholoma matsutake*) is parasitic on *Pinus densiflora* trees and grows wild on the ground of *P. densiflora* forests in the autumn months, and sometimes in cold regions it grows in Yezo spruce and hemlock forests. It has a fragrant aroma and is delicious. Stories related to matsutake date back to the Yayoi Era (300 BC–300 AD). As for comprehensive reference materials, there are several documents available. In addition to the documents, reports on recent trends have been published by Wang et al. (1997).

Matsutake have been produced as a special forest product in Japan. The total production volume reached the record-breaking level of 12 000 tons in 1941. Since then, production had hovered around 3000 to 6000 tons/year, but it declined to a level of about 1000 tons/year in the 1960s. Since the 1970s, production continued to fall to the level of several hundred tons per year (Onodera and Suzuki 1998). Matsutake produced in its season in Japan is expensive. Usually, a piece of domestically produced *T. matsutake* is priced at several hundreds US$, and it trades at an average price of around 400 US$/kg (five to six pieces). With the aim of increasing the production of edible mycorrhizal mushroom as a special forest product, the Japan Forestry Agency has been engaged in the following experimental research projects on improvement and utilization of forest resources: (1) Development of cultivation techniques for edible mycorrhizal mushroom (1986–1990), which did not include matsutake; (2) Development of artificial inoculation techniques for mycorrhizal fungi (1991–1995), which included matsutake; and (3) Development of stable production techniques for mycorrhizal mushroom (1996–2003). At present, imports from China and South Korea account for more than 90% of Japan's total consumption of matsutake, and the country is estimated to consume about 3000 tons/year. Based on these facts, it is estimated that matsutake production has the potential to create a billion US$ market. In the mushroom markets of the world, the sales of mycorrhizal mushrooms such as *Tuber melanosporum* called "truffles," *Boletus edulis* called "porcini," and *Cantharellus cibarius* called "girolle" or "chanterelle" exceed 3 billion US dollars (Wang et al. 1997).

Research on mycorrhizae has made little progress due to poor objectivity of experimental results, even though the symbiosis between the roots of plants and fungi has been known for more than 100 years. The preface of *Methods and Principles of Mycorrhizal Research* (Schenck 1982), which was the first book on mycorrhizal research published by the American Phytopathological Society, states:The mycorrhizal research has made progress for the past 15 years thanks to the efforts by researchers in many study fields. At present, however, there is no comprehensive education on mycorrhizae, and most of the past research is not handed down to younger scientists. Given this fact, the American Phytopathological Society publishes this book as the first textbook on mycorrhizae for the purpose of providing reliable knowledge on it."

Almost a decade later, *Techniques for the Study of Mycorrhiza* (Norris et al. 1991) was published. By that time, the whole situation had changed drastically as the preface of the book states:

> Ten years have passed since the publication of *Methods and Principles of Mycorrhizal Research*. During the period, the interest in the study of mycorrhizae has increased explosively. And mycorrhizae have been recognized as a common phenomenon in the natural world by experts in a wide range of fields such as plant physiology, ecology, and plant–parasitic interactions. In addition, new sophisticated scientific techniques such as the ones based on NMR, RFLP, and DNA have been widely applied to research on mycorrhizae.

As described above, the clarification of the symbiotic relationship, which is based on the mycorrhizal symbiosis, required the advent of new scientific techniques that could overcome the problems of handing down research results to a new generation of researchers on an individual basis. Even so, Read (2002) commented, "We have just started understanding the effects of mycorrhizae on the functions of plants and the rhizosphere in the natural world."

As for the study of matsutake, which is famous for its mystery, there are a series of unsolved issues. They are as follows: (1) identification among *T. matsutake* and its closely related species, and intraspecific variations; (2) physiological and biological characteristics based on the differences in the mode of the nutrient system, such as saprophytes, parasites, and symbionts; and (3) issues related to applied studies of artificial cultivation of *T. matsutake*, such as morphogenesis of matsutake mycorrhizae, dynamics of matsutake shiro, and fruit body formation. The findings obtained regarding these unresolved issues are discussed.

4.3 Ectomycorrhizal Ecophysiology of Matsutake

4.3.1 Diversity of *Tricholoma matsutake*: Establishment of a Method for Identification

Species related to *Tricholoma matsutake* in Japan include *Tricholoma robustum* in *Pinus densiflora* forests, as well as *Tricholoma fulvocastaneum* and *Tricholoma bakamatsutake*, which exist in broadleaf forests of beech such as *Quercus serrata*. *Tricholoma robustum* and *T. fulvocastameum* have no aroma like that of *T. matsutake*. *Tricholoma bakamatsutake* does have the aroma, but it is small in size. Therefore, distinguishing the three species is considered to be easy achieved by examining the morphological characteristics of their fruiting bodies. In other parts of the world, *Tricholoma magnivelare* is distributed widely in North America, and *Tricholoma caligatum* grows widely in Europe and North Africa. In addition, there is a species of mushroom in Europe called *Tricholoma nauseosum* that bears a close resemblance to *T. matsutake*. Their similarities and differences have been discussed for the sake of categorization (Kytovuori 1988; Bergius and Danell 2000). Similarly, in

order to study *T. matsutake*, it is necessary to identify species related to it. Furthermore, clarification of intraspecific variation of *T. matsutake* is necessary for the elucidation of its physiological characteristics and the establishment of culture techniques.

Remarkable progress has been made in the recent development of molecular biological techniques, which are used for analysis of genetic variation of fungi and have also been adopted as definitive methods for determining various fungi. rRNA coding regions (rDNA) comprise a single unit by sandwiching noncoding regions called the internal transcribed spacer (ITS) and the intergenic spacer (IGS). About 200 copies of the unit exist on a genome and these have been used frequently for molecular phylogenetic systematics by selecting relevant regions. Generally, intraspecific variation is smaller in the ITS regions, so the regions are useful for determining fungi at the species level (Gardes and Bruns 1993). On the other hand, the variation is larger in the IGS regions. Therefore, the IGS regions are useful for examining intraspecific variation (Bruns et al. 1991).

In general, when a method for identifying species based on DNA analysis is used, it is often difficult to perform direct sequence analysis, which reads base sequences directly. In the case of mycorrhizae or mycelia in soil, DNAs derived from various organisms such as other plants and microorganisms in the soil can be present in a test sample. Thereupon, a primer specific to *T. matsutake* was designed based on the results of the examination of the ITS regions of its rDNA. Using the primer, polymerase chain reaction (PCR) analysis was performed on cultured mycelium of 30 strains of 16 species of ectomycorrhizal fungi, including *T. matsutake*, *T. robustum*, *T. fulvocastaneum*, *T. bakamatsutake*, *T. magnivelare*, and *T. caligatum*. As the result, amplification products were found only in *T. matsutake*. In addition, amplification products were also discovered in the DNAs extracted from fruiting bodies of *T. matsutake*, which were collected from *Pinus densiflora* forests, as well as from the ectomycorrhizal fungi and the soil in *T. matsutake* shiro (soil located directly below fruiting bodies) (Kikuchi et al. 2000). These findings proved that this primer specific to *T. matsutake* was effective for analysis of samples that were collected from forests and fields. At present, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is a commonly used method for identifying species of ectomycorrhizal fungi through DNA analysis. In the case of determining whether a sample is the species being searched for, identification by PCR using a specifically designed primer makes it easier to interpret analysis results and does not require restriction enzyme processing, which is a cumbersome and time-consuming operation. Therefore, it is considered that PCR analysis is superior to PCR-RFLP in the case of species identification. Based on these findings, it can be concluded that the primer specific to *T. matsutake*, which is designed on the basis of the base sequences of the ITS regions, is the best tool for identifying the species related to *T. matsutake*, because it allows its users to reliably determine whether matsutake mycelia exist by analyzing a small amount of a sample in a quantity of less than several milligrams.

For the purpose of clarifying the intraspecific variation of *T. matsutake* in Japan, 84 strains collected from all over the country were compared by analyzing the IGS1 regions of their rDNA. The results indicated no variation among the strains, because

all of their molecular lengths were identified as about 460 bp. The RFLP patterns obtained by analysis of restriction enzyme Cfr131 in the amplified IGS1 regions were categorized into eight types from A to H (Guerin-Laguette et al. 2002). It was also revealed that *T. matsutake* belonging to RFLP type A is widely dominant throughout Japan. In addition, it was proved that *T. matsutake* that grows in East Asia and Europe is identical to the type A species, which is dominant in Japan.

One of the species of *Tricholoma* that grow in other countries, Swedish *T. nauseosum*, is very similar to Japanese *T. matsutake*. It is reported that the degree of similarity of base sequences between them is 99% to 100%, based on comparison of the ITS regions of their rDNA (Bergius and Danell 2000). Consequently, the ITS regions of *T. matsutake* and *T. nauseosum* in Asia and Europe were compared. As the result, it was found that the degree of similarity of base sequences between the species of *T. matsutake* in the *Pinus densiflora* forests of Japan and South Korea and that in the *Quercus* spp. and *Castanopsis orthacantha* forests of China was 99.7% to 100%. It was also revealed that the degree of similarity of base sequences among the species of *T. nauseosum* in the *Picea abies* forests of Switzerland, the *Pinus sylvestris* forests of Sweden, and the *Picea abies* and *Castanea setiva* forests of Italy was 98.4% to 100% (Matsushita et al. 2005). The degree of similarity of base sequences between *T. matsutake* in Japan and *T. nauseosum* was 98.1 to 100% (Matsushita et al. 2005). Judging from these results, it can be concluded that according to the DNA-based classification, *T. matsutake* in the coniferous forests of Japan and South Korea can be included in the same species *as T. matsutake* in the broadleaf forests of China, and that *T. nauseosum* in the coniferous forests and the broadleaf forests of Europe can also be categorized as the same species. However, even if the hypothesis that *T. matsutake* and *T. nauseosum* are the same species cannot be denied by DNA investigations, in order to verify the identification it is necessary to examine them from the aspect of biological species (Suzuki 1996). Until these points are clarified experimentally, it is reasonable to consider that *T. matsutake* is still shrouded in mystery (*Tricholoma matsutake* sensu lato).

4.3.2 Is *Tricholoma matsutake* an Ectomycorrhizal Fungus?: Morphogenesis of *Tricholoma matsutake* Mycorrhizae on *Pinus densiflora*

In response to the basic question "Is *T. matsutake* an ectomycorrhizal fungus?," photos of the mycorrhizae have been presented in a number of studies; however, the anatomical details of *T. matsutake* have not been properly revealed because the photos have been unclear and illegible (Masui 1927; Ogawa 1975; Wang et al. 1997). Determination of whether *T. matsutake* is an ectomycorrhizal fungus is essential in clarifying its nutritional mode throughout its entire life cycle and establishing artificial culture.

Examination of various mycorrhizae of *T. matsutake* on *Pinus densiflora*, which were collected from forests and fields, under stereoscopic microscope and by chlorazol black E (CBE) staining revealed their following characteristics: (1) while the amount

of mycelia in the fungal sheaths declines during the growing period, the amount of phenolic compounds increases, and then their color turns black, which suggests that the fungal sheaths go through many changes (Gill and Suzuki 2000); and (2) a Hartig net that spreads to the endodermis has multibranched hyphal structures, which are characteristic of the *T. matsutake* ectomycorrhizal fungus (Gill et al. 1999, 2000). Examination of the mycorrhizae by electron microscopy confirmed that cell walls and mycelia cell walls existed apart from each other on the contact surface between host cells and Hartig nets. In some cases, examination confirmed the existence of progressive inclusion layers, which were buried in the matrix of the layers between cells, and the existence of mature inclusion layers that were formed while cell walls and mycelia cell walls were in contact and merging gradually into each other (Gill et al. 2000). All of the samples were proved to be *T. matsutake* by DNA analysis. As stated above, although the mycorrhizae of *T. matsutake* on *P. densiflora* grow through various morphological stages, they have Hartig nets, which are typical structures of ectomycorrhizal fungi. It was also revealed that there were two structures for the contact surface between host cells of *P. densiflora* and the mycelia of *T. matsutake*: progressive and mature.

To clarify the physiological significance of the *T. matsutake* mycorrhizae, the distribution and localization of ATPase, which is involved in the active transportation of ions between cells, was examined. The ATPase of the mycelia grown in Hartig nets is located on both sides of the septum of mycelia cells. It was revealed that ATPase is highly active in the cell membranes of host cells and mycelia cells on the contact surface between the two kinds of cells, especially on the areas that Hartig nets invaginate and are considered as regions that nutrition moves through. On the other hand, activation of ATPase was not observed in the mycelia of the fungal sheaths. Therefore, it can be assumed that nutrition is not exchanged between mycelia. Based on these findings, it was revealed that transfer of nutrition, which is one of the basic functions of *T. matsutake* mycorrhizae, is actively carried out between *P. densiflora* and *T. matsutake*.

A variety of discussions have been conducted on whether *T. matsutake* is an ectomycorrhizal fungus. Wang et al. (1997) proposed that *T. matsutake* can be positioned as a species that goes through the triangle of saprophyte, parasite, and symbiont, and each of the characteristics become more dominant or less dominant as the season changes. The reasons why the basic question of the mode of nutrition of *T. matsutake* has not been answered are a lack of scientific data and absence of concomitant use of molecular biology methods. In this research on the structure of *T. matsutake* mycorrhizae, multibranched hyphal structures, which are the distinctive features of *T. matsutake* ectomycorrhizal fungus, were observed. Therefore, decisive anatomical evidence was obtained. Based on the distribution mode of ATPase, it was confirmed that *P. densiflora* and *T. matsutake* form mycorrhizae that have a distinctive function of symbiosis, and it was proved for the first time that *T. matsutake* is a typical ectomycorrhizal fungus.

Given that *T. matsutake* was identified as a typical ectomycorrhizal fungus, attempts were made to synthesize the mycorrhizae between *T. matsutake* and *P. densiflora*. As a result, a method for rapidly synthesizing the *T. matsutake* mycor-

rhizae was established forming which the mycorrhizae formed without fail within 1 to 2 weeks after inoculation using seedlings of *P. densiflora* on artificial substrates. The method was based on techniques that addressed the following requirements: (1) induction of the growth of vigorous mycelia, (2) preparation of inoculum of mycelia, and (3) effective inoculation of mycelia (Guerin-Laguette et al. 2000; Vaario et al. 2000; Suzuki et al. 2001). A similar case of successful research, in which Hartig nets were formed on *P. densiflora* seedlings 3 months after their inoculation, was reported by Yamada et al. (1999).

The effect of *T. matsutake* infection on its host, *P. densiflora*, was examined. It was found that 10 weeks after inoculation of *T. matsutake* mycelia, the weight of *P. densiflora* seedlings increased by 71%–98% (Guerin-Laguette et al. 2004). Previously, it had been believed that *T. matsutake* was parasitic to young seedlings of the host plant (Wang et al. 1997), even though scientific evidence was lacking. This idea was disproved experimentally, and it was shown that *T. matsutake* is a typical symbiotic fungus.

4.3.3 Shiro of *Tricholoma matsutake*: Dynamics of Shiros and Its Artificial Formation

To produce fruiting bodies of *Tricholoma matsutake* under the condition of in vitro culture, it is necessary to quantitatively examine its shiros in the field. Accordingly, to clarify the dynamics of *T. matsutake* shiro, the subterranean part was examined by using the root window technique (Egli and Kalin 1991), which enables nondestructive constant observation. In addition, research on the development of fruiting bodies, which has been conducted for a long time, is also of value. This research was carried out after confirming that the mycelia in shiro soil were the same species as *T. matsutake* by DNA analysis (RFLP of the IGS1 regions) (Kikuchi et al. 2000). The mycelia in *T. matsutake* shiro and the roots of *Pinus densiflora* in the *P. densiflora* natural forests were also examined in a case study. The findings were as follows: (1) the mycelia and the roots grew actively during the period from May to July; (2) the color of the mycelia in *T. matsutake* shiro started to turn from white to light brown from August; and (3) the roots started to grow again in September; and the shiros expanded by about 10 cm width within a year (Suzuki 2005). The major axis of the shiros measured 4.5 m, and their minor axis was 3.7 m. At the site where fruiting bodies had produced over 4 years, the areas where they sprouted moved outward by 10–15 cm/year. Although the results of observation of the dynamics of *T. matsutake* mycelia based on the root window technique vary in some degree, they are almost consistent with the expansion speed of the shiros, which is measured by the conventional method based on the movement of the sites where fruiting bodies sprout. The expansion speed of shiros is not calculated based on the direct measurement of the growth of mycelia in soil. However, although the results obtained by recording the movement of the sites where fruiting bodies sprout were not constant, they showed an expansion of 10–15 cm/year on an average, which was consistent with the estimated length (Ogawa 1978). Based on these findings, it can be concluded that in

general the active site of *T. matsutake* shiros has a ring-like structure that is 10–15 cm wide and 10 cm deep.

It is considered that the amount of mycelia in *T. matsutake* shiros is closely related to the amount of emerged *T. matsutake* fruiting body. Further information is necessary to understand the formation of *T. matsutake* fruiting bodies. Accordingly, the amount of ergosterol in the soil located directly below fruiting bodies was measured, and the total amount of mycelia in a *T. matsutake* shiro was also measured. The measurements showed that ergosterol was present in shiro soil at a concentration of about 64.4 μg/cm^3, and the amount of ergosterol in cultured mycelia was about 1.5 μg/mg. Therefore, the amount of mycelia in shiro soil was calculated to be about 42.9 mg/cm^3. Based on the results, the total weight of mycelia in a shiro was estimated to be 5.6–7.3 kg (Suzuki 2005). Based on the measured amount of emerged *T. matsutake* fruiting bodies in the experiment, the amount of mycelia that one fruiting body requires to grow can be estimated to be about 100 g (90–120 g). Until now, there has been no report of direct measurement of the total amount of mycelia in a shiro. The volume of a shiro was estimated by Ogawa (1978) to be 1500–2000 cm^3 based on the surface area of the soil where one fruiting body emerges (a mass of 64–86 g when calculated based on the above-mentioned amount of mycelia in the shiro soil). The results of this study revealed the details of the relationship between the dynamics of a *T. matsutake* shiro and the biomass of *T. matsutake* mycelia in the field.

It was proved that *T. matsutake* is a typical ectomycorrhizal fungus, and it is possible to artificially synthesize the mycorrhizae between *T. matsutake* and *P. densiflora*. In addition, the dynamics of the *T. matsutake* shiro were clarified. As a result, the quest to develop artificial *T. matsutake* shiros and fruiting bodies has become more interesting. The research on artificial cultivation of *T. matsutake* has been conducted for a long time in Japan. In addition to measures such as improvement cutting and land plowing (Kake et al. 2000), reports have been published on the following subjects: (1) incubation of *T. matsutake* mycelia (Inaba et al. 1993); (2) formation of primordium for *T. matsutake* fruiting bodies by using pure cultures (Ogawa and Hamada 1975); (3) promoting of seedlings infected with *T. matsutake* (Ogawa et al. 1978); (4) formation of shiros by using seedlings infected with *T. matsutake;* and (5) formation of *T. matsutake* fruiting bodies on soil collected from shiros (Inaba et al. 1995). However, it is still practically impossible to artificially develop a shiro by incubating *T. matsutake* mycelia on a culture substrate or by inoculating *T. matsutake* mycelia into the soil of a forest. Indeed there is no case where the success of artificial shiro formation has been proved scientifically by molecular biology techniques.

In general, one of the effective ways to grow a large quantity of mushrooms is to propagate their mycelia in liquid culture. However, under natural conditions it is difficult to form shiros due to the poor growth of *T. matsutake* mycelia. The poor growth is attributable to the dominant growth of saprophytic fungi in *P. densiflora* forests. Therefore, focusing on the extremely slow growth of *T. matsutake* mycelia as one of the factors that hinder artificial cultivation of *T. matsutake*, substances that stimulate the growth of the mycelia were examined. The examination of the

saprophytic ability of *T. matsutake* revealed that all of its strains had an amylolytic ability. It has also been confirmed that *P. densiflora* bark and beech sawdust can be nutrition sources for *T. matsutake* mycelia, based on research on the activities of cellulolytic enzymes (β-glucosidase, D-nitrophenyl -β-D-lactopyranosidase) and the amount of ergosterol (Vaario et al. 2002, 2003). A study of ways to stimulate the growth of *T. matsutake* mycelia revealed that the growth of *T. matsutake* mycelia could be accelerated 15 times by incubating them on culture substrates that contain surfactants such as Tween80 and Tween40, which control the cell membrane permeability of the mycelia, or natural vegetable oils such as olive oil that increase the hydrophilicity of the mycelia (Guerin-Laguette et al. 2003; Suzuki 2004). In this case, it may be considered that the growth was accelerated by the secretion of degradative enzymes from *T. matsutake* mycelia, which was induced by the increased hydrophilicity of the mycelia. Therefore, it can be said that the path toward the artificial induction of *T. matsutake* shiros, which is the first step toward the artificial cultivation of *T. matsutake*, has been paved by the rapid cultivation of large amounts of *T. matsutake* mycelia.

4.4 Concluding Remarks

The examination of the effects of ectomycorrhizal fungi on forest ecosystems has belatedly started in vitro. The existing theories on the functions of forests and trees, which were established by focusing on the aboveground part, need to be reexamined in vivo by taking into consideration the new concept of ectomycorrhizal symbiosis in the rhizosphere.

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5
Plantation Forestry in the Tropics

SHOBU SAKURAI

5.1 Introduction

There are several tree species that were once called "miracle trees" or "wonder trees," which suggests that people wanted such trees to meet their demands. Unfortunately, as Dr. Julian Evans pointed out in *Plantation Forestry in the Tropics*, (Evans 1992) some environmentalists insist that pine, eucalypts, and wattle, considered miracle trees by some, are "green cancer."

Please imagine, however, vast areas of dry, bare, or eroded land, where no tall trees have been able to develop for a long time. Only a few species can survive and cover the damaged land (Table 1), just as a "scab", which covers a wound, is not real skin but the first important stage in the recovery of a wound. I hope to call these precious trees "curing vegetation for the earth", not "green cancer".

After World War II, especially during the 1970s, the demand for wood in Japan increased substantially, and tropical countries met the demand. At first the Philippines, then Indonesia, and later Malaysia exported wood materials to Japan. As a matter of course, not only Japan but many other countries also consume a great deal of wood resources.

Advances in medical technology have lengthened the life span of humans and the population has increased. This has also accelerated the depletion of forests by overuse and overexploitation of the land to meet these changes. Fuel-wood consumption for everyday use has increased and caused the disappearance of woodlands in many areas of the world. Furthermore, it has become clear that forest depletion has strongly influenced the global environment. Consequently, the recovery of destroyed forests is a very important issue in the world. Many people, not only foresters and scholars but also mere citizens, have an interest in nature conservation. As a matter of course, people who live on land where there is a severe shortage of wood resources are also deeply concerned. We, who are concerned with forests and forest science, must try to meet the demand on forests from outside in order to keep a sound forest and to be able to hand our descendants a fine forest.

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Table 1. All assessed tree species and their characteristics (After BFD and JICA 1987)

Fast growing species	Survival	Growth	Remarks
Acacia auriculiformis	++	++	Wide range of adaptability
A. mangium	++	+	Die back in dry land
Paraserianthes falcataria	–	–	
Anthocephalus chinensis	+	+	Die back in dry land
Casuarina equisetifolia	+	+	Die back in dry land
Gliricidia sepium	++	+	Regeneration on slope face
Gmelina arborea	++	++	Wide range of adaptability, fire resistant
Eucalyptus camaldulensis	++	++	Slightly fire resistant, drought resistant
E. citriodora	+	+	Drought resistant
E. deglupta	–	–	Sensitive to site conditions
E. tereticornis	–	–	
E. torrelliana	–	–	
Leucaena leucocephala	+	–	Sensitive to site conditions
Pine species			
Pinus caribaea	+	+	Shoot moth
P. kesiya	++	++	High elevation, shoot moth
P. oocarpa	+	+	Shoot moth
Long-rotation species			
Anisoptera thurifera	+	+	Under/inter planting
Shorea guiso	+	+	Under/inter planting
Vitex parviflora	+	+	Inter planting
Pterocarpus indicus	+	+	Under/inter planting
Swietenia macrophylla	+	+	Under/inter planting
Tectona grandis	++	–	Dieback, fire resistant

++ Generally good, + good depending site conditions, – not good

Planting trial was mainly done from 1979 to 1986 in a degraded grassland at Pantabangan Project site, about 185 km north of Manila, the Philippines

5.2 Why Have the Forests Degraded?

There are various causes and many reasons for forest degradation. The conversion of a forest stand to a cash-crop plantation, such as oil-palm plantations, coconut plantations, pepper fields, fruit orchards, etc., is common. Overexploitation, illegal logging in particular, also damages the sustainability of natural forests. An important cause of damage in Southeast Asia is burning, which typically results in the degradation of forest to grassland. Open land after heavy erosion is also included in this category. It is very common that grassland is used for cattle grazing. Farmers usually set fires to maintain the grassland during the dry season.

We had a similar experience in Japan, especially along the Pacific Ocean side, where scattered pastures had been maintained for more than 1000 years. Year by year, in early spring, when a short dry season occurred, farmers set fire to the grass-

land. When they did not have enough knowledge about the environment, they believed that burning kept the pasture healthy. Pests and diseases were excluded by burning and the quality of grass became good. This practice continued until the 1950s in several parts of rural Japan.

We now know that modern science denies such a theory. Furthermore, burning is dangerous as fire can easily spread to neighboring forests. Consequently, farmers have now stopped burning. Burning is now strictly limited. We also know that forests produce various properties, so farmers never set fires.

Once ground cover is removed, strong raindrops in the area hit the surface and flush the precious thin soil downstream. Fertile soil is lost rapidly. Subsequent grazing compacts the ground harder.

Usually, fire and grazing will produce *Imperata cylindrica* (cogon, alan-alan, lalang) grassland, and heavier fire and grazing will change the vegetation from *I. cylindrica* to *Saccharum spontaneum* or *Themeda triandra*, finally resulting in bare land. The site quality of *I. cylindrica* land is not so poor, but sites supporting the other two species are poor (Sakurai and de la Cruz LU 1993).

5.3 How Do We Prevent Fire?

If grassland changes into forest or tree plantations, farmers lose their pasture. So it is very difficult to stop a fire. As I already mentioned, however, if farmers know the advantage of the forest, they may stop it. I will introduce several trials to recover the forest by Japanese re/afforestation activities.

Japanese experts who engaged in re/afforestation activities under the Republic of the Philippnes (RP) – Japan forestry development project of the Pantabangan area by Bureau of Forest Development (BFD), the Department of Environment and Natural Resources (DENR), and Japan International Cooperation Agency (JICA) gave a lot of attention to fire prevention. They constructed two or more watch-towers to detect fire by a triangular surveying technique as soon as they started the project, and then set up wide firelines. They organized the fire brigade with a fire engine if they had the budget. Sometimes, they held a poster-drawing contest at rural schools during fire prevention week and gave prizes to the students who participated in the contest. They planted fruit trees for the community and gave many seedlings of useful trees to the villagers. They expected that these activities would bear the fruits of fire prevention in the minds of the future (BFD and JICA 1987).

The forest that gives no benefit to the people or their community, or is believed to bear no benefit, is easily destroyed. The forest that does not produce continuous profit for the people is also easily destroyed for momentary gain.

Advanced countries like the United Kingdom (UK) have a severe history of forest degradation. Oak forests were formerly very important in the UK. They produced fuel wood, charcoal, barrels, furniture, construction, and so on. In particular, they supported the strongest navy, being used for making warships. Until the 18th century, much wood went into Thames river as the British warships that commanded every sea in the world. The industrial revolution occurred in the 19th century. Coal

became the leading actor instead of wood, and steel made warships. Then, forests were destroyed rapidly, and most of the forests in the UK were lost. The forest area in the UK in the early 20th century was only 7% of the total land. At that time, the existence of forestland was evidence of a lack of civilization. The other European countries also lost vast amounts of forestland. Recently, environmental issues have changed the trend. People now realize the value of forests and the percentage of forest to total land area in the UK has increased to 11.6% (FAO 2001).

In Japan, forests were also very important for everyday use until the early 1960s. They provided energy, and litter for agricultural fields, children's playfields, and nature education material. However, the modern industrialized atmosphere changed the value of the forest. The brilliance of industrial products, such as stainless ware and plastic ware attracted people. Japanese believed at that time that natural ware was inferior to industrially-produced wares. Science was better than tradition. Peoples' labor for managing forests decreased, and the demands/interests on forests became small. No one wanted the shrubs and litter in the forest. No one wanted the dead trees, which, for example, were attacked by pine nematode diseases. Forests around the urban areas changed into housing areas or dumping grounds.

Efficiency is required in the modern age. So, natural-hardwood forests in Japan were converted to highly productive coniferous plantations. Certainly, such coniferous-tree species also hold the capacity for environmental conservation. However, serious problems, such as insect attack, pollen allergy, loss of biodiversity, and imbalance of wildlife habitat, causing serious damage to the forest, became apparent. Recently, we gradually noticed that efficiency often damages the environment. When the problem has become clear, every measure against it is too late to correct the failure. Usually, the damage costs more to restore than that earned by earlier increased efficiency. This may teach us that endurance/tolerance may be important in some cases. These experiences are applicable to tropical forest management.

5.4 Afforestation Trial in *Imperata cylindrica* Grassland in Benakat, South Sumatra Island

I. cylindrica grasslands are widely distributed throughout Indonesia. The total area of grassland in Indonesia was about 24 million ha in 1978 (Tanimoto 1981) and 35 million ha before 1985 (Ishi 1985). These data show the rapid incremental increase of grassland in Indonesia.

A Japan-Indonesia afforestation project was started in Benakat, about 130 km west of Palembang city, Sumatra Island, in December, 1980. Desiccation during the dry season in this area is not so severe. Various species' planting trials were undertaken, using mechanical land-cultivation techniques by tractor that had been examined and developed by the Pantabangan Project in the Philippines (BFD and JICA 1987). This project clarified that the productivity of the site occupied by tall *I. cylindrica* (more than 1 m in height) was not so poor. I measured the size of planted trees in April, 1992 (Sakurai et al. 1994). The height of 8-year-old *Acacia mangium* exceeded 20 m, with a diameter of around 20 cm. Eleven-year-old *Swietenia*

Table 2. Description of forests in Benakat, surveyed in April, 1992 (After Sakurai et al. 1994)

Examined tree species	Age (year)	Average dbh (cm)	Average Height (m)	Per hectare No. (m^2)	Per hectare BA (m^3)	Per hectare Volume (m^3)	Per hectare Total volume
Swietenia macrophylla	11	20.0	16.4	640	21.2	188	209
		10.8	11.7	300	3.0	21	
Acacia mangium	8	19.2	21.3	664	20.1	232	255
		11.1	14.5	248	2.6	23	
Peronema canescens	11	11.7	11.8	856	14.1	99	102
		6.0	8.4	160	0.5	3	

Upper rows indicate dominant trees and lower rows suppressed trees

dbh: diameter at breast height

BA: Total basal area at 1.3 m above ground surface

Stem volumes were estimated from the volume table for Japanese natural Akitasugi

macrophylla was 16 m tall and had a diameter of 20 cm. The annual increment in stem volume per hectare for these species was as follows: *S. macrophylla*, 19 m^3; *A. mangium*, 32 m^3; and *Peronema canescens*, 9 m^3 (Table 2). We could see monkeys playing on the planted trees. Grassland afforestation recovered the wildlife habitat.

5.5 Influence of the Private Sector

The results of the afforestation trial conducted at Benakat strongly influenced others. A private enterprise started to establish an industrial plantation of *Acacia mangium* around the project site in 1990s. They established more than 150 000 ha of *A. mangium* plantation in less than 5 years (Kato 1995). This work was led by former staff of the early period of the project. We must know that when the private sectors began to plant as a business, the power of re/afforestation was very large. When the private sector notices that forest farming produces enough profit, re/afforestation might be strongly implemented. Therefore, I want to point out that if people begin to develop forests for their own interests, the result will be a huge area of forestland. The Japan International Cooperation Agency (JICA) project planted only 3,100 ha over 8 years in south Sumatra (Kato 1993) and about 10,600 ha in the Pantabangan area in the Philippines over 16 years (Masuko 1998), but the private sector established more than 150 000 ha in less than 5 years (Kato 1995).

Certainly, severe problems can occur in such a wide area of monocultural forestland. I worry about diseases, like heart rot, insect attack, and decline in soil productivity due to short rotation, in addition to the decline in biodiversity. To avoid these problems, mixed forests and indigenous-species plantings should be examined. We must develop sustainable forest-management techniques through experimentation, because we are yet to have techniques that keep widespread-monocultural forests healthy.

Table 3. Description of man-made forests in the experimental forest of UPLB (After Sakurai et al. 1994)

Age is about 70 years	Average		Per hectare			
Planted tree species in each stand	dbh (cm)	Height (m)	No.	BA (m^2)	Volume (m^3)	Total volume (m^3)
Swietenia macrophylla	43.4	29.1	281	44.6	636	
	18.5	16.8	521	16.1	158	795
Swietenia macrophylla	52.3	31.7	224	52.0	764	
	18.2	17.1	181	5.7	56	897
Parashorea malaanonan	44.8	30.1	188	31.0	444	
and	23.3	18.9	200	9.2	94	
Anisoptera thurifera	46.8	30.4	50	9.1	131	
	20.0	17.3	131	4.9	50	800
Parashorea malaanonan	42.9	28.7	81	13.9	200	
and	18.5	15.9	30	1.1	11	
Dipterocarpus grandiflorus	26.9	23.8	200	12.2	153	
	13.4	14.2	390	6.6	58	440

Upper rows in each stand indicate dominant trees and lower rows indicate suppressed trees

Total volume includes the other tree species in the stand

dbh: diameter at breast height

BA: Total basal area at 1.3m above ground surface

Stem volumes were estimated from the volume table for Japanese natural Akitasugi

I have another anxiety, which is the friction among such vast monocultural-planting activities, inhabitants, and government. The people in rural areas/forests who gain non timber benefits from forests, and the enterprises who want wood resources, are not same. The administration sector of the country may often give critical regulations to the weak rural people; sometimes they are the first nations in the forest. If trouble happens, re/afforestation activities will be greatly damaged. Consequently, it is very important to build up a consensus among inhabitants.

5.6 Man-Made Forests of Long-Rotation Species and Indigenous Species

Fast-growing species are widely employed as plantation species due to their economic advantages. However, I worry about the degradation of soil productivity and environmental conservation by the monoculture of a fast-growing species. If enough valuable long-rotation species forest or indigenous-species forest is established, its economic value and ability to conserve the environment may be higher than a fast-growing species forest. For instance, *Tectona grandis* forests in Thailand, Malaysia, Indonesia, and elsewhere are good examples of such forests.

Table 4. Description of man-made forests in the Dramaga experimental forest (After Sakurai et al. 1994)

Examined tree species	Age (year)	Average dbh (cm)	Average Height (m)	Per hectare No.	Per hectare BA (m^2)	Per hectare Volume (m^3)	Per hectare Total volume (m^3)
Dipterocarpus retusus	35	43.7	40.0	196	30.4	563	
		23.1	22.4	232	11.2	151	714
Shorea seranica	34	52.8	43.3	184	42.3	829	
		32.6	29.7	60	5.2	81	910

Upper rows indicate dominant trees and lower rows suppressed trees

dbh: diameter at breast height

BA: Total basal area at 1.3 m above ground surface

Stem volumes were estimated by the volume table for Japanese natural Akitasugi

There are several such forests in the experimental forests of the University of the Philippines at Los Baños (UPLB), and Haurbentes and Dramaga experimental forests of the Nature Conservation and Forest Research and Development Centre (Psat Penelitan dan Pengembangan Hutan; NCFRDC), Bogor, Indonesia. We can see fine stands of *Swietenia macrophylla*, *Parashorea malaanonan*, *Anisoptera thurifera*, and *Dipterocarpus grandiflorus* in UPLB. *Shorea stenoptera*, *S. seranica*, *D. retusus* and many other such species are found in the experimental forest of NCFRDC, Bogor. Since the Centre of International Forestry Research (CIFOR) was founded in the Dramaga experimental forest in 1993, visiting this forest has become easier.

These trees in UPLB reach more than 30 m in height and are 40 to 50 cm in diameter. The ages of the stand in the UPLB forest are not clear, so I assumed that they were about 70 years old when I measured them in 1991, because the University was established in 1909 (Brown 1919), and afforestation might go well after 10 years. On this assumption, the average yearly stem-volume increment of *S. macrophylla* ranged from 11.5 to 13 m^3 per hectare (Table 3). This range is almost equivalent to the value of a 70-year-old fine *Cryptomeria japonica* stand (12.6 m^3), which has very high productivity in Japan.

Heights exceeding 40 m and diameters of 40 to 60 cm were found in the experimental forests of NCFRDC, Bogor (Table 4). The annual stem volume increment ranged from 20 to 27 m^3 per hectare. Those well-developed forests contain many other tree species in them.

These results show the marvelous future of forestry. If these trees endure without cutting for 50 to 100 years they will grow big enough to use, and the forest can be sustainably managed. High-quality wood can also be harvested, while wildlife and biodiversity can coexist.

5.7 Another Aspect of Forest Decline and Multi-Storied Forests

Decline in the species components of a forest by selective cutting is another form of degradation. Even if the composition of the remaining forest declines, its role in environmental conservation and wildlife habitat maintenance will remain. Fuel-wood supply will also be kept. This type of degradation is caused by logging. If suitable treatments are applied to such a forest, the quality of the forest will be recovered. Appropriate tending techniques, derived from a shelterwood system, are valuable. For that purpose, multi-storied forest trials are useful.

One of the multi-storied forest experiments started in 1992, as a joint project between the Forestry Department of Peninsular Malaysia and JICA, near Ipoh city, Malaysia (FDPM et al. 1999). The experimental site near Ipoh was established in an approximately 4-year-old *Acacia mangium* forest, where lowland dipterocarp forest was clear-cut in 1988 and 1989.

Experimental plots were established using five different strip widths in the 4-year-old *A. mangium* plantation. Five treatments were employed for this experiment: (1) cutting and retaining one row; (2) cutting and retaining two rows; (3) cutting and retaining four rows; (4) cutting and retaining eight rows; and (5) cutting and retaining sixteen rows. Thirteen species were planted. Among the species measured in 1992, *Shorea leprosula*, *S. parvifolia*, *and Neobalanocarpus heimii* were reported (Iwasa et al. 1993). Favorable values of relative illuminance on top of the planted seedlings were 30% to 70% (one-, two-, and four-row cut sites). *S. leprosula* and *S. parvifolia* showed better height growth than *N. heimii*. I observed this experimental forest in 1994 and 2001, and found that the height of these planted trees reached more than 4 m in 1994, and was 7.2 m for *S. leprosula*, 6.7 m for *S. parvifolia* and 3.8 m for *N. heimii* after 6 years. Indigenous long rotation tree-species forests can be established easily by multi-storied forest techniques in a fast growing tree-species plantation.

5.8 Activities of NGOs/NPOs for Plantation Making

There are many Japanese NGOs/NPOs engaged in re/afforestation in the tropical countries.

The Research Association for Reforestation of Tropical Forest (RETROF) is a unique organization among them. This association is composed of private companies. The Government of Japan offers 50% of the research budget to the member companies and a secretariat. The members are Komatsu, Kansai Environmental Engineering Center, Sumitomo Forestry, Ishinomaki Plywood, Toyoboseki, Mitsui-Norin, Toyota, Gifu-Serakku, Oji Paper, and Nissho-Iwai Corporation.

The members contribute the research reports of their activities to the Bio-Refor Program and exchange information. Bio-Refor is a program of IUFRO/SPDC, and is financially supported by the Japanese Ministry of Foreign Affairs. The program

was started in 1992 under an agreement among Southeast Asian researchers in the Philippines, Taiwan, Malaysia, Indonesia, Thailand, and Japan. The objectives of Bio-Refor are to provide the opportunity for information exchange and a discussion table to foresters and forest science researchers that enable and accelerate re/afforestation in Southeast Asia and the Pacific region through biotechnology development. The members of RETROF are important members of Bio-Refor. They join the Bio-Refor Workshop and present their research.

Toyoboseki constructed a simple and cheap pond to preserve water for the dry season in Borneo Island. Komatsu staff developed a tissue culture of Dipterocarpaceae spp. (*Shorea leprosula*, *S. laevis*, *S. parvifolia*, *S. pauciflora*, *Dryobalanops lanceolata*, *and D. beccarii*) in Bogor with the Nature Conservation and Forest Research and Development Centre, and established an experimental plantation, using cuttings of *Shorea* spp., near the campus of CIFOR, to clarify the result of the experiment. Ishinomaki Plywood developed propagation techniques for *S. albida* by cuttings, and they obtained a high survival ratio of cuttings of *S. albida* in their trial. The study was done with the staff of the Forest Department of Sarawak. Kansai Environmental Engineering Center developed an effective usage of mycorrhizal fungi with the staff of Gadjah Mada University, Indonesia. Mycorrhizal fungi have also been studied by Sumitomo Forestry in Borneo with PT. Kutai Timber, Indonesia. This group is also developing methods for the propagation of many indigenous-tree species by cuttings and out-planting trials. Their objective is to recover the dipterocarp forest that was lost by the big fire in the early 1980s. Other companies are also advancing forest-related activities and are developing techniques for the sustainable management of forests (RETROF 1997; Ministry of Forestry, Republic of Indonesia et al. 1993, 1994).

The activities of the private sector are very important for the future of forest resources and environmental conservation. During the implementation of such re/afforestation works, some companies became involved with social forestry trials, and they noticed that good human relations were very important to the success of the experiment. Everywhere there is forest, there are people who live and work in it. If they want to keep their activities for a long time, the private sector must get their agreement and cooperation. Such experiments are very important for future coexistence.

5.9 Conclusion

Plantation forestry has become a worldwide concept for increasing the stock of timber resources. Many plantation activities have been instigated. Many of them choose fast-growing species for their industrial plantations. Through many trials, suitable fast-growing tree species and silvicultural techniques for plantation establishment have been developed, and such trees are important planting stock now. Wood characteristics have also been clarified, but some problems still remain. Heart rot disease of *Acacia mangium* and life-span control for many fast-growing species are important objectives that require clarification.

To conserve the environment, including wildlife habitats and biodiversity, reforestation by indigenous species has attracted a great deal of attention. Some private companies are becoming involved in setting up plantations and taking account of environmental effects. Even if the purpose is not for a timber-resource forest, mature and well-tended forests produce enough good-wood resources. Even if the species employed are exotic, fast-growing species are important for the begining of rehabilitation of degraded-forest land. Once the degraded land has changed into forest, a second generation of fast-growing tree species forest can be changed into the advanced phase of the forest, such as long-rotation species forest and indigenous-tree species forest. So, such a trend is very important.

We saw that private enterprise has established huge fast-growing species plantations in Sumatra. If the private sector realizes that plantations produce big benefits, they will start to plant trees. If many people know this, planting power will increase. We must manage forests sustainably, recover the degraded forest, and hand down fine forests to our descendants.

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Part II

Propagation Technique for Tropical Forest Trees

A

Cutting Technology

6
Vegetative Propagation of Dipterocarp Species by Stem Cuttings Using a Very Simple Technique

DARUS HAJI AHMAD

6.1 Introduction

In tropical countries, there are several problems with the production of quality planting stocks of important dipterocarp species, such as irregularity of seed supply due to irregular flowering and fruiting, short viability period of seeds, poor-quality seeds, and lack of seed storage and handling facilities (Ng 1977; Sasaki et al. 1978). These problems have hampered the development of forest plantations and the enrichment planting activities of over-logged forests. When dipterocarp trees do fruit, they sometimes produce poor-quality seeds due to frequent insect and fungal attacks (Sasaki 1980; Tompsett 1987). Therefore, it is very difficult to predict the yield of quality seeds and seedlings for reforestation programs.

To solve these problems, the collection of wildings has been carried out, but dependency on such supplies could not be sustained due to erratic seed years, high mortality rate, and difficulty in collecting seeds in bulk from natural forests. Beset with these drawbacks in the production of sexually propagated planting materials, efforts have been made to massproduce planting materials of commercially important dipterocarp and non-dipterocarp species through stem cuttings. It is an important alternative method to producing high-quality and uniform planting stocks for large-scale reforestation programs (Aminah 1991; Muckadell and Malim 1978; Smits et al. 1993; Srivastava and Manggil, 1981; Hamsawi, 1981; Kondo et al. 1986; Mohamad Lokmal et al. 1992; Monteuis 1992; Yahaya 1979).

Stem cuttings offer several advantages over seeds. They save time and labor, and produce genetically superior and uniform planting materials from superior parent stocks. Stem cuttings are also inexpensive and easier to practice than other vegetative-propagation methods, such as tissue culture. In addition, stem cuttings can continuously supply planting stocks throughout the year for reforestation activities.

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In Malaysia, as well as in other tropical countries, interest in using vegetatively propagated clones of timber species for forest-plantation establishment and enrichment planting activity has been increasing. This has been further encouraged by the success of *Eucalyptus* forest plantations in Aracruz, Brazil, using rooted cuttings as planting materials, and the mass production of dipterocarp planting materials via stem cuttings for commercial plantations and enrichment planting activities, which is successfully carried out in Kalimantan, Indonesia (Smits and Daud Leppe 1991, 1992; Smits 1992; Zobel et al. 1983). Therefore, research activities on stem cuttings of commercially important dipterocarp species should be continuously conducted in order to understand some of the important factors affecting the rooting of cuttings and, subsequently, to refine the existing method for mass production of dipterocarp species by stem cuttings. This report documents important steps and cheaper techniques used for stem cuttings of commercially-important dipterocarp species carried out in Malaysia.

6.2 Materials and Methods

6.2.1 Species Used for Stem Cuttings

Seven species were selected for stem cutting activities. They were: (1) *Shorea parvifolia*, (2) *S. leprosula*, (3) *S. acuminata*, (4) *S. bracteolata*, (5) *S. ovalis*, (6) *S. roxburgii*, and (7) *Hopea odorata*.

S. parvifolia is a large tree, frequently exceeding 90 cm in diameter at breast height, with short and sharp buttresses, a fairly open crown, relatively light foliage, and a well-shaped bole. The species occurs in the lowlands and hill forests up to 800 m above sea level. It is classified under the red-meranti group and its timber is used for furniture making, interior finishing, paneling, partitioning, molding, skirting, veneer, and plywood.

S. leprosula is a tall, large-buttressed tree with a well-shaped bole. It is a fast-growing dipterocarp species and has good self-pruning capacity. It is easily recognized in the forest by its yellowish-brown crown. It is classified under the red-meranti group and its timber is used for interior joinery, domestic woodworks, furniture, interior fitting, plywood, door and window frames, stair stringers, railing, ceilings, framing, and lorry-body works.

S. acuminata can be easily identified in the forest by its drooping branchlets, large semi-persistent stipules and asymmetrical leaves. It is a fast-growing dipterocarp species with an average annual-diameter increment of about 1.4 cm, and in 15 years a tree with a 21 cm diameter can be easily obtained. The timber is used for general-utility purposes.

S. bracteolata is a medium to large tree exceeding 240 cm in girth, with small buttresses and a clear and well-shaped bole. It grows fast and has strong apical dominance and is geotropic. It is in the white-meranti group. The wood is used for general utility purposes, such as furniture, high-class interior finishing, flooring, paneling, fancy doors, molding, skirting, and veneer. The wood fetches good prices on the international market.

S. ovalis is a large tree. Its early growth is slower but later taller than many of other common red-meranti species. It has a good self-pruning capacity and the branches are horizontal to slightly upward. It is a reasonably fast- growing species, which can reach a diameter of about 70 cm in 50 years. Its timber is used for general utility purposes.

S. roxburgii is a large timber tree and is considered one of the fastest-growing dipterocarp species. It has been strongly recommended for reforestation programs. Its timber is a light hardwood and it is classified under white-meranti group. It is suitable for general-utility purposes, particularly for plywood.

H. odorata is a medium-sized tree and grows up to 40 m tall and 75 cm in diameter at breast height. The bole is cylindrical with small buttresses. The timber is classified as a light hardwood and the wood is a yellow to yellowish-brown color. It is used for construction, such as for flooring for pedestrian walks and light industrial floors, joinery, boat making, cart wheels, deck planks, bridges, and other general purposes.

6.2.2 Stock Plants

Stock plants were permanently planted in a clonal multiplication garden. They originated from both (1) healthy seedlings and (2) healthy, recently-germinated wildings collected from natural forests. The normal planting distance of stock plants within and between rows was about 0.5 m and 1.0 m, respectively. For dipterocarp species, such as *Shorea parvifolia*, *S. ovalis*, *S. bracteolata*, *S. leprosula*, *S. acuminata*, *S. roxburgii*, *and Hopea odorata*, a temporary sarlon netting shade was needed during the initial 3 to 4 months after planting.

The plants were regularly watered at least once a day, preferably between 9:00 to 10:00 a.m. They were also regularly pruned to about 15–25 cm from ground level, depending on the species, in order to induce continuous production of juvenile coppices for cutting materials. A commercial compound fertilizer, such as NPK Blue (12:12:17:2-N: P_2O:K_2O:MgO: TE-trace element) was applied every 2 months to ensure the healthy growth of stock plants. The rate of application was about 50 gm per plant. Fungicide and insecticide were applied when necessary to protect these plants from fungal diseases and insect attack. The planting site was tilled regularly to avoid soil compaction around the stock plants and, at the same time, manual weeding was also carried out when necessary.

6.2.3 Rooting Containers

Normally, for stem cuttings of difficult-to-root species, such as dipterocarps, we should have a proper infrastructure to induce faster rooting. This set-up is considered very important to keep planted cuttings, as well as the rooting medium, moist during the rooting process. The sprinkler system should be fitted along the centre of the rooting bed and operated over 24 hours at hourly spray intervals, with each spray for 1 min. Drainage holes are made around the rooting bed to drain out excess water.

The bed is then covered with a plastic sheet in order to ensure higher air humidity around the cuttings. If the bed is not covered with a plastic sheet, the sprinkler system should be operated more frequently.

Based on these requirements, I developed a simple portable propagator for tropical timber species. A white polyurethane box, 49 cm long × 38 cm wide × 32 cm high, normally used for transporting and exporting fresh fish and vegetables from cold storage to wet markets and supermarkets in Malaysia, was used for the stem cutting container. The box was filled with clean river sand to about half of its volume, and water was poured into the box to about half of the sand's volume. The cuttings were planted into the rooting medium at 1–2 cm deep, depending on the length of the cuttings. They were planted in rows so that no overlapping occurred between them. About 20–30 cuttings were planted in the box, depending on the species and number and size of the leaves. After planting, the box was tightly closed with its cover so that the loss of water through transpiration and evaporation could be minimized, as well as to maintain higher air humidity inside the box. In order to ease daily observations and to provide enough sunlight to the cuttings, the box's cover was cut and replaced with a transparent plastic sheet.

6.2.4 Rooting Medium

River sand, comprising about 60% of 2-mm diameter particles and 40% of about 2–5-mm diameter particles, was used for the stem cuttings. This sand has been successfully used for rooting the cuttings of many dipterocarp and non-dipterocarp species (Darus 1982; Aminah 1990). Freshly collected river sand was cleaned of debris, large stones, rubbish, and mud before it was placed into the boxes.

6.2.5 Preparation of Cuttings

Cutting scions were obtained from healthy juvenile stock plants planted in the clonal multiplication garden, where they should have been free of fungal disease and insect attack. A sharp blade was used when preparing cutting scions. The scion was cut smoothly with a sharp knife or secateurs at a slanted angle in order to prevent rotting. For these species, only orthotropic shoots were used for cuttings and the middle part of the stem was chosen to ensure that the new plants grew orthotropically. Branch cuttings were avoided because some of dipterocarp and non-dipterocarp species, such as *H. odorata* (Aminah 1991), *Araucarai hunstenii* (Darus 1982), and *Agathis dammara* (Smits 1992) retain their plagiotropic growth.

The length of a cutting was 3–8 cm long, depending on whether it was a single node or two nodes . The diameter of the cutting material was 1–6 mm, depending on the species, node position, and age of the stock plants. However, the soft-terminal shoot/apex of the seedlings or coppices was not used for cutting.

The presence of a leaf or leaves on a cutting is important for root formation and development. For species with big leaves, such as *S. bracteolata, S. ovalis, and S.*

Table 1. Number of leaves per stem cutting of the seven dipterocarp species

Species	Number of leaves per cutting
Shorea bracteolata	One third of the leaf
S. ovalis	One half of the leaf
S. parvifolia	One leaf
S. acuminata	One to two leaves
S. leprosula	One half of the leaf
S. roxburgii	One third of the leaf
Hopea odorata	One leaf

roxburgii, the leaf was cut transversely to one half or one third of its original size, in order to reduce water loss through transpiration and to save space in the rooting container (Table 1).

6.2.6 Planting of Cuttings

Before planting, the sand medium was rinsed with clean water to keep it moist. The cuttings were treated with the hormone rooting powder, Seradix 3 (0.8% IBA), just before planting, and were then planted in the medium at about 1–2 cm deep, depending on the length of the cuttings.

6.2.7 Harvesting and Potting of Rooted Cuttings

Stem cuttings were regularly inspected, and dead cuttings and dried leaves were removed from the box to avoid the spread of diseases. Fungicides were sprayed on the cuttings when there was any sign of fungus attack.

The time at which cuttings should be harvested from the boxes varied between species. For example, the harvesting of *S. roxburgii*, *S. bracteolate*, and *H. odorata* rooted cuttings was carried out much earlier than the other species, at 3–5 weeks after planting. For *S. ovalis*, *S. leprosula*, *S. acuminate*, and *S. parvifolia*, some cuttings started to root at 6–8 weeks after planting. Higher rootings of 70%–100% were obtained from the *S. bracteolata*, *S. roxburgii*, and *H. odorata* cuttings (Table 2).

Rooted cuttings were immediately transplanted into polybags when the roots showed some lignification (brown in color) and the axillary buds had developed into shoots. Before the lignification process, the young roots were soft and easily broken, and this would cause the death of recently-transplanted cuttings. Rooted cuttings were potted in a mixture of river sand and forest topsoil at a ratio of 1:3. Inorganic fertilizer, such as triple superphosphate (NPK), was added to the mixture at a rate of 1.2 kg/m^3 of soil mixture.

Table 2. Rooting percentage of the stem cuttings of the seven dipterocarp species

Species	% Rooting	Duration (weeks)
Shorea bracteolata	70–80	3–5
S. ovalis	30–40	8–12
S. parvifolia	30–40	8–12
S. acuminata	40–50	8–12
S. leprosula	40–50	6–10
S. roxburgii	80–90	3–5
Hopea odorata	90–100	3–5

6.2.8 Maintenance of Rooted Cuttings

The potted cuttings were placed under 70%–80% shade for 2–3 months before being transferred to transplanting beds. The application of fertilizer was conducted every 2 months, starting 1 month after potting, in order to maintain the healthy growth of rooted cuttings. Compound fertilizer, NPK Blue, was applied at a rate of 10–20 g/plant. Watering was carried out twice a day, in the morning and late afternoon, to prevent wilting. Weeding, insecticide, and fungicide applications were carried out whenever necessary. The rooted cuttings were planted out in the field 8–10 months after potting.

6.3 Discussion and Conclusion

Tropical hardwoods, particularly dipterocarp species, which are considered "difficult to root" species, have been successfully propagated using a very simple technique and an unsophisticated rooting container. In fact, this technique produced similar rooting percentages to a sophisticated infrastructure and expensive sprinkler system. However, in order to get a high rooting percentage, it is very important to note that cutting materials should be taken only from young seedlings or juvenile stock plants, the humidity in the rooting container must be kept high and the light intensity should be reduced during the rooting process. The other important factor that influenced the rooting of stem cuttings of the seven dipterocarp species was the presence of leaves on a cutting. When the stem cuttings dropped their leaves 1 or 2 days after planting, they failed to root. Therefore, the presence of leaves on a stem cutting is very important for root formation of dipterocarp species.

The technique is now being used for the large-scale production of ornamental and flowering plants by commercial nurseries and the Forestry Department of Peninsular Malaysia to mass produce quality-planting stocks of dipterocarp and non-dipterocarp species for reforestation programs. It is easy to implement, particularly for forest nurseries where electricity is not available. In the case of private nurseries, the technique will reduce electricity bills.

Appendix

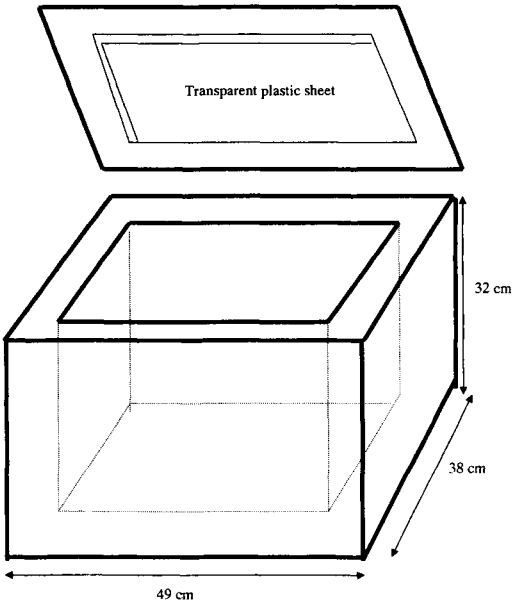

Fig. 1. Polyurethane box used for stem cuttings of dipterocarp species

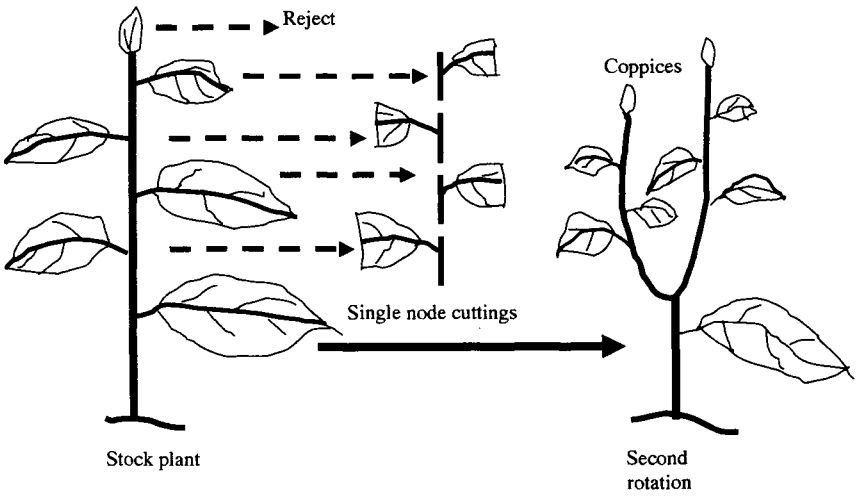

Fig. 2. Schematic drawing of a stock plant, planted in a clonal-multiplication garden, and cutting scions

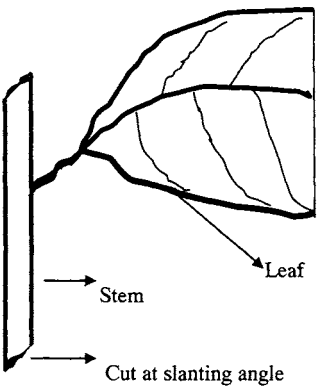

Fig. 3. A stem cutting of a dipterocarp species, showing a slanted cut at the end of the stem, and the leaf cut in half

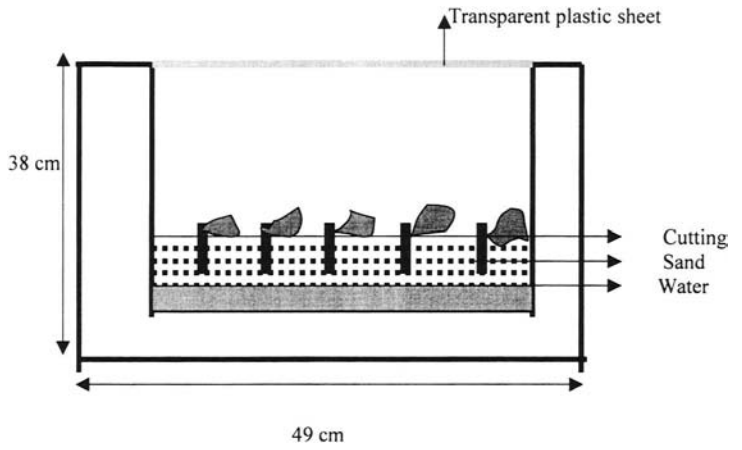

Fig. 4. Polyurethane box with planted stem cuttings, covered tightly with a transparent plastic sheet

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7

Assessment of the Relative Amenability to Vegetative Propagation by Leafy Cuttings of 14 Tropical and Subtropical *Eucalyptus* and *Corymbia* Species

AMANDA BAKER and STEPHEN WALKER

7.1 Introduction

The decline of Queensland's native forest resources, the state's primary source of hardwood timber (Lee et al. 2000), has resulted in a very high requirement for establishing commercial plantations of tropical and subtropical hardwood species for harvest within the next 25 years. The long rotation of native hardwood species necessitates the production of high-quality planting stock within a short period.

An effective tree-improvement program incorporates both a breeding strategy, by means of which the ongoing genetic improvement of a species is affected, and a propagation strategy, in which the resultant gains are captured in operational planting programs (Haines and Walker 1996).

One of the most important factors influencing the choice of a propagation strategy is the reproductive features of the species in question; in particular, the ability of the species to be propagated vegetatively. A propagation strategy based on vegetative propagation provides a means by which to capture genetic gain rapidly, enhance uniformity, overcome shortages of improved seed, and match genotypes to specific sites. If the species under consideration for commercial plantations in Queensland were amenable to vegetative propagation, it would be an ideal tool for the provision of high-duality planting stock within a short period.

In South America, India, South Africa, and the Congo, tested clones of *Eucalyptus grandis*, *E. camaldulensis*, *E. tereticornis* and the *E. grandis* × *E. uropyhylla* and *E. grandis* × *E. camaldulensis* hybrids are being mass-propagated successfully from

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cuttings (Sachs et al. 1988; Eldridge et al. 1994). In Brazil (Aracruz) and the Congo (Pointe Noire), the growth rate of eucalypt plantations established in the 1980s from cuttings was about double that of plantations established from the unimproved seed available before 1975, even after allowing for improvements in establishment practices (Eldridge et al. 1994).

At the Queensland Forestry Research Institute (QFRI), work is being conducted on the vegetative propagation of 14 tropical or subtropical *Eucalyptus* and *Corymbia* species considered to have potential for establishment in commercial plantations. This paper assesses the relative amenability of the following species to vegetative propagation from leafy cuttings: *E. acmenoides* (Schauer), *E. argophloia* (Blakely), *E. cloeziana* (F Muell.), *E. dunnii* (Maiden), *E. grandis* (W Hill ex Maiden), *E. microcorys* (F Muell.), *E. pellita* (F Muell.), *E. pilularis* (Smith), *E. resinifera* (Smith), *E. tereticornis* (Smith), *E. urophylla* (ST Blake), *C. citriodora* (Hook), *C. henryi* (ST Blake) and *C. variegata* (F Muell.) (Hill and Johnson 1995). This is the first report of research on the response to vegetative propagation by *E. argophloia*.

7.2 Methods and Materials

The procedure for the assessment of the relative amenability to vegetative propagation from leafy cuttings of the 14 species is summarized in Table 1.

Seedling hedge plants of the 14 species were established at Beerburrum Nursery (60 km north of Brisbane) in late 1996, and were periodically decapitated to a height of 15–20 cm to promote the development of shoots suitable for harvesting as cuttings.

For 11 of the species (Table 2), eight clones from each of five seedlots were randomly selected to represent (as far as possible) the genetic range of the species. For three of the species, fewer seedlots were available (Table 2). Two clones from each of the seedlots were randomly allocated to each of four replicates. Rows of seven cuttings were set for each clone.

The experiment was established across a four-day period in late March, 1997, with one replicate being collected and set each day. The shoots were collected in the morning and placed into moistened-plastic bags immediately after severance. These bags were sealed and then stored in a cooled, insulated box until the time of setting.

Two- to three-node cuttings were used, with the leaf area of each cutting being reduced by approximately 50%. Prior to setting, the base of each cutting was freshly cut perpendicular to the main stem and dipped into a commercial rooting hormone powder (0.8% IBA). Clones were set in rows of seven cuttings in trays filled with pots containing a 3:2 vermiculite to perlite mixture.

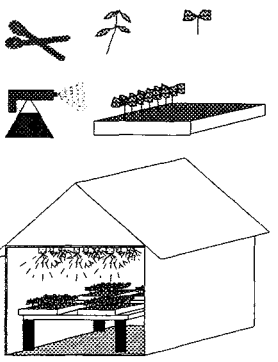
Trays of set cuttings were placed in a shade house under a fogging regime of 60 s every 20 min during daylight hours. A backup misting system, activated by a leaf-balance mechanism, was also in use to ensure that there were water droplets on the leaves at all times.

Initial rooting assessments were conducted 5 and 9 weeks after setting. A final, comprehensive rooting assessment was completed at 15 weeks. Rooting data was

Table 1. Experimental procedure

Activity	Details
Sow seeds 	June 1996 July 1996 August 1996
Plant seedlings 	July 1996* October 1996 December 1996 February 1997
Hedge to 20cm 	November 1996 to January 1997

Species	No. Toppings
C. citriodora	1
C. henryi	1
C. variegata	3
E. acmenoides	1
E. argophloia	1
E. cloeziana	3
E. dunnii	1
E. grandis	3
E. microcorys	1
E. pellita	5
E. pilularis	3
E. resinifera	1
E. tereticornis	3
E. urophylla	1

Collect shoots 	March 1997
Propagate cuttings 	2–3 nodes 0.8% IBA 3:2 Vermiculite–Perlite medium 50% Shade Cloth 60 s fog/20 min during daylight hours Weekly fungicide
5 week assessment	April 1997
9 week assessment	May 1997
15 week assessment	July 1997

**E. pellita* seedling supplied from North Queensland, Seeds of Australian Trees Project

Table 2. Number of seedlots, clones, and cuttings set for each species

Species	Seedlots	Clones	Cuttings
C. citriodora	5	40	280
C. henryi	1	8	56
C. variegata	4	32	224
E. acmenoides	5	40	280
E. argophloia	5	40	280
E. cloeziana	5	40	280
E. dunnii	5	40	280
E. grandis	5	40	280
E. microcorys	5	40	280
E. pellita	5	40	280
E. pilularis	5	40	280
E. resinifera	5	40	280
E. tereticornis	5	40	280
E. urophylla	4	32	224

analyzed using an Analysis of Variance on arcsine-transformed percentages. The statistical analysis of differences between species was assessed using least significant differences ($\alpha = 0.05$).

7.3 Results and Discussion

Rooting success was highly variable between species; with mean rooting success ranging from 83.2%, for *E. pellita*, to 0.4%, for *C. citriodora*, 15 weeks after setting. The mean rooting successes for each species and the statistical differences among species, at 5, 9, and 15 weeks after setting, are presented in Table 3.

E. pellita had significantly higher rooting success at 5, 9, and 15 weeks than any other species. Sachs et al. (1988) refer to *E. camaldulensis* as one of the most easily rooted *Eucalyptus* species, as its rooting success often exceeds 60%. Using this definition, the rooting success of *E. pellita* at 15 weeks (83.2%) is very impressive.

E. grandis, E. tereticornis, and E. urophylla are already the subjects of well-established plantation programs and were primarily included in this experiment as controls. These species will not be discussed in detail, as the results achieved in this experiment were comparable to the results of other trials on non-selected genetic material: *E. grandis* (Valle 1978; Bouvet and Andrianirina 1990; Campinhos and Ikemori 1983; Wignall et al. 1992), *E. tereticornis* (Ivashchenko 1939; Campinhos and Ikemori 1983; Chandra and Yadava 1986; Jagadeesh and Adkoli 1987; Gurumurti et al. 1988), and *E. urophylla* (Valle 1978; Campinhos and Ikemori 1983; Huong 1993).

E. resinifera, E. pilularis, E. microcorys, and *E. acmenoides* demonstrated moderate rooting success in this experiment. Campinhos and Ikemori (1983) and McComb and Wroth (1986) have also described the successful rooting *E. resinifera* cuttings. Campinhos and Ikemori (1983) successfully produced rooted cuttings of *E. pilularis*

Table 3. Mean rooting successes for *Eucalyptus* and *Corymbia* species, five, nine, and 15 weeks after setting

Species	Rooting Success (%)					
	5 weeks*		9 weeks*		15 weeks*	
E. pellita	73.2	a	82.1	A	83.2	a
E. urophylla	29.5	b	43.8	B	51.8	b
E. resinifera	30.0	b	39.6	Bc	42.9	bc
E. tereticornis	18.6	bcd	33.9	Bcd	38.9	bcd
E. grandis	23.2	bcd	31.8	Bcde	38.6	bcde
E. pilularis	29.3	bc	33.2	Bcde	34.6	bcde
E. acmenoides	26.1	bcd	28.9	Bcde	31.4	bcde
E. argophloia	10.4	bcd	18.2	Bcde	23.6	bcde
E. microcorys	12.1	bcd	17.1	Bcde	20.4	cde
E. cloeziana	6.4	bcd	9.6	cde	11.4	cde
C. henryi	1.8	cd	3.6	De	3.6	de
E. dunnii	0.7	d	3.2	de	3.2	de
C. variegata	1.3	d	1.8	de	1.8	e
C. citriodora	0.4	d	0.4	e	0.4	e

*Means with the same letter are not significantly different at $\alpha = 0.05$. Significant differences were determined using arcsine transformed data

and *E. microcorys*. Hodgson (1974) described the vegetative propagation of *E. microcorys*, and Niccol et al. (1994) successfully induced rooting of this species using in vitro micropropagation techniques. Campinhos and Ikemori (1983) successfully produced rooted cuttings of *E. acmenoides*.

Many of the eucalypt species can be propagated easily, or moderately easily, from leafy stem cuttings (Leakey et al. 1992). However, some species are recalcitrant, including *C. citriodora*, *C. henryi*, and *C. variegata*. Cuttings of the three *Corymbia* species began dropping their leaves 5–8 days after setting, and the rooting success of each of these species was very low. Other researchers also report low rooting success for cuttings of these species. Cutting root-formation tests, run at Aracruz, failed to produce rooted *C. citriodora* or *C. maculata* cuttings, but a *C. citriodora* × *C. torelliana* hybrid rooted successfully (Campinhos and Ikemori, 1983). In Brazil, TF de Assis (personal communication) also described *C. citriodora* and *C. maculata* as very difficult to root from cuttings, with success only obtained using mini cuttings taken from very juvenile plants. Better rooting individuals of *C. torelliana* and its hybrids, e.g. *C. citriodora* × *C. torelliana* and *C. torellinna* × *C. citriodora*, are being propagated on a commercial scale in Brazil (TF de Assis personal communication). Recent research conducted by State Forests of New South Wales has assessed the propagation success of *C. vaiegata*, *C. maculata*, *C. citriodora*, and *C. henryi* by cuttings and in vitro micropropagation. *C. variegata* and *C. citriodora* failed to produce rooted cuttings, and a maximum of 0.05% of *C. maculate* and *C. henryi* cuttings rooted. In vitro micropropagation was extremely successful for *C. variegata*, *C. citriodora*, and *C. maculate*, with some clones exhibiting 70% rooting success. *C. henryi* multiplied in vitro but failed to produce roots (H Smith pers comm).

Gupta et al. (1981) also reported that conventional propagation methods for *C. citriodora* were unsuccessful, and described a successful in vitro micropropagation technique.

Although the results of the present study indicate that *E. dunnii* also has very poor rooting success, researchers from State Forests of New South Wales have reported rooting successes of up to 40% for this species (H Smith pers comm).

In addition to the variation in rooting ability between species, there was considerable variation of rooting success within species. For this reason, selection of good-rooting clones is very important for commercial production, even in species that propagate easily, as consistency and small gains in rooting percentage can be of considerable economic value (Leakey et al. 1992). The operationally acceptable limit for rooting success in eucalypt vegetative propagation programs in Aracruz (Brazil) and Smurfit Carton de Colombia (Colombia) is 70% and above (Zobel et al. 1983; Wright 1992). The distribution of clonal rooting successes for each species, indicating the percentage of clones within the operationally acceptable limit, is shown in Table 4.

E. pellita again demonstrates considerable advantage over the other species, with 85% of clones having commercially acceptable rooting success; 50% of *E. pellita* clones exhibited 100% rooting success. The primary focus of a breeding strategy for *E. pellita* can be the selection of clones for growth and wood-quality, because the selection required for rooting success is limited.

Most previously known attempts to propagate *E. cloeziana* by cuttings have been unsuccessful, including the root formation tests at Aracruz (Campinhos and Ikemori 1983). However, Catesby and Walker (1997) reported for this species a mean overall rooting success of 20.6%, 8 weeks after a December (summer) setting. There was considerable within- and between-family variation in rooting success, and four clones from the best rooting family had a rooting success of 100%. Similarly, in the March (autumn) setting, 10% of clones had commercially acceptable rooting success, and the mean overall rooting success at 15 weeks was 11.4%. Therefore, rooting success for this species can be improved with selection.

In this study, 97.5% of autumn-collected *E. dunnii* clones had a rooting success of < 30% at 9 and 15 weeks. These results concur with a study reported by Cooper and Graca (1987), in which 85% of *E. dunnii* cuttings collected from 645 mother trees showed a rooting success of < 30% in spring collected shoots, with those collected in autumn demonstrating even poorer rooting ability. A preliminary requirement for a vegetative propagation strategy for this species would be wide screening for individual clones with enhanced rooting success.

This research on the response to vegetative propagation of *E. argophloia* is the first reported. The results of this study indicate that although rooting of leafy cuttings is only moderately successful for *E. argophloia* (23.6%), there is some scope for the selection of high-rooting genotypes. This species has a very small natural range of 26°30'–26°40'S, with an altitudinal range of between 300–340 m. *E. argophloia* generally grows with excellent form, naturally occurring in a region with a mean annual rainfall of 700 mm (Boland et al. 1984). With the use of cuttings technology, there is potential for the identification of high rooting, drought resistant, clones of *E. argophloia* for use in low rainfall areas.

Table 4. Distribution of clonal rooting successes for *Eucalyptus* and *Corymbia* species, 15 weeks after setting

Species	Proportion of clones with rooting success of:		
	≤ 30%	> 30% and < 70%	≥ 70%
E. pellita	0.08	0.08	0.85
E. urophylla	0.41	0.09	0.50
E. resinifera	0.55	0.13	0.33
E. tereticornis	0.50	0.20	0.30
E. pilularis	0.53	0.25	0.23
E. acmenoides	0.60	0.23	0.18
E. grandis	0.55	0.30	0.15
E. argophloia	0.75	0.15	0.10
E. cloeziana	0.88	0.03	0.10
E. microcorys	0.83	0.10	0.08
E. dunnii	0.98	0.00	0.03
C. henryi	1.00	0.00	0.00
C. variegata	0.97	0.03	0.00
C. citriodora	1.00	0.00	0.00

7.4 Future Directions

The outline for future directions for the vegetative propagation of hardwoods in Queensland, outlined below, is based on discussions with Drs Russell Haines, Garth Nikles and David Lee. QFRI aims to:

Conduct research to develop clonal lines of *Ramularia*-tolerant *C. variegata* using tissue culture, in collaboration with State Forests of New South Wales.

Examine the potential for tissue culture of other recalcitrant species and hybrids, such as *E. cloeziana* and *C. torelliana* × *C. variegata* hybrid.

Refine protocols for the grafting of clonal seed orchards of *Ramularia*-tolerant *C. variegate* and *E. cloeziana* and of *E. grandis* clone banks for hybrid production.

Refine protocols for the rooting of species and hybrids amenable to propagation by cuttings, including *E. pellita*, *E. urophylla*, *E. grandis*, *E. pilularis*, and *E. grandis* hybrids.

Refine protocols for the rooting of more recalcitrant species of commercial interest, including *Corymbia* spp., *E. cloeziana*, *E. argophloia*, and some hybrids, in conjunction with developing high-rooting populations.

Develop high-rooting populations of *C. variegate*, *E. cloeziana*, *E. argophloia* and some hybrids by sowing large quantities of seed of each species and setting a single cutting collected from the resultant seedlings. Only the rooted cuttings will be used to establish the cutting progeny trials.

Refine hedge management protocols.

Coppice field selects for propagation.

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8
Production of Cuttings in a Peat Swamp Species from Sarawak, Malaysia

AKIRA KITAOKA, YOICHI UCHIMURA, and HIDEAKI ASAISHI

8.1 Introduction

The site of this study is shown in Fig. 1. *Shorea albida* Sym., called *ALAN* in local Sarawak, Malaysia, is a kind of Dipterocarpaceae. *S. albida* grows on low-peat swamp areas as pure forest, and is distributed throughout Sarawak, Brunei, and a part of Kalimantan. Normally, the harvesting of *S. albida* involves thorough clearance, because of its formation as pure forest (Yamada 1984). It is a very useful species for wood-based industries, such as sawn timber, plywood, etc., due to its straightness, uniformity of color, easy processing, etc. The deforestation site is not suitable for agricultural purposes, due to its soil acidity. If *S. albida* could be reforested, it would be an effective forest resource, due to:

1. *S. albida* can grow under severe conditions such as peat swamp.
2. *S. albida* is very useful for wood-based industries.

However, like other dipterocarp species, sexual propagation of *S. albida* is difficult because of its irregular seeding years and the short viability of its seeds. Consequently, many investigations into vegetative propagation have been undertaken, such as producing planting stocks by cuttings and/or tissue culture (Kondo 1989; Kondo et al. 1992; Momose 1976). However, not much success has been reported regarding the vegetative propagation of dipterocarp species. As seedlings are rare in a pure forest, it is difficult for *S. albida* to regenerate naturally. The purpose of this study was to establish techniques for enabling the clonal propagation of *S. albida* from a cutting.

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Fig. 1. Rough map of Sarawak and location

8.2 Materials and Methods

8.2.1 Materials

In the preliminary experiment, the stems for cuttings were collected on the day before the cutting trial from seedlings 2 to 3 years old growing in the forest. The seedlings were about 100 cm long. After that, the stems were cut into eight to ten nodal segments, each with one or two leaves and sound petioles. The top part of a seedling was not used because it was so soft that it could mold easily. The cut-end of each stem was set under a node or between nodes for the preparation of the cutting. The leaves of the cuttings were cut in half. The preparation of the cuttings is shown in Fig. 2.

8.2.2 Planting of Cuttings

Cuttings were planted as slants in the culture media. After planting, a wound sealer was applied on the top end of each cutting to prevent it from drying out. The cuttings were then irrigated and the rooting bed was sealed immediately with a clear plastic sheet to keep cuttings moist (Machida 1974). A shading net was put over the plastic sheet to prevent the temperature from rising inside the cutting bed.

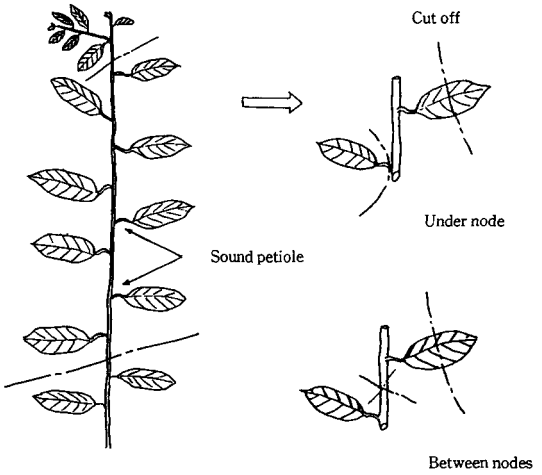

Fig. 2. Preparation of a cutting

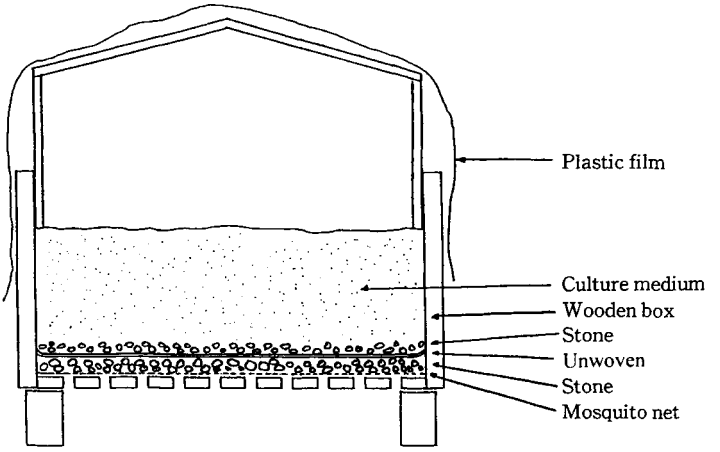

Fig. 3. Closed sutting box

8.2.3 Cutting Bed

At the beginning of this study, a wooden box (60 × 60 × 30 cm) sealed with a clear plastic sheet was used as a cutting bed. An outline of the box is shown in Fig. 3. The bed could be kept at a high relative humidity. A mosquito net and unwoven cloth were set on the bottom of the box for water drainage and to prevent the culture medium from falling. Later, a concrete rooting bed (100 × 200 cm) was built.

8.2.4 Culture Medium

Graded river sand (fine and large) was used for the culture medium. Rice-husk charcoal was mixed with fine-grain sand to improve the porosity. River sand was washed with rainwater to remove as much silt and pieces of any organic material as possible.

8.2.5 Rooting Hormone

In the preliminary experiment, IBA was used. Solutions of IBA with concentrations of 1, 2, 5, 10 and 100 ppm were applied to the treatment. In the comprehensive experiment, *Seradix* (containing IBA, for semihardwood, MAY & BAKER LTD, UK) was used. *Seradix* powder was applied to the cut end of the cuttings.

8.2.6 Irrigation

In the preliminary experiment, the irrigation frequency was twice a day, morning and evening. The quantity was approximately 2 l each time, which was controlled with a simple flow meter. The water source was preserved rainwater.

In the mist-spray experiment, computer-controlled spraying was done twice a day, at 10:00 and 14:00 hours, with 1 l over 5 s for each time per concrete cutting bed (100 cm × 200 cm). In the comprehensive experiment, the irrigation frequency and quantity were greatly reduced. Irrigation was implemented according to the moisture content of the culture medium. Therefore, the irrigation was, in some cases, necessary only once or twice a month.

8.3 Results and Discussion

8.3.1 Preliminary Experiment

8.3.1.1 Preliminary Experiment Schedule

The preliminary experiments were carried out in nursery house No. 1, using the following schedule.

No. 1	21 Aug 1992–30 Sep 1992	62*
No. 2	13 Oct 1992–28 Apr 1993	83
No. 3	15 Dec 1992–15 Sep 1993	285
No. 4	20 Jan 1993–15 Sep 1993	116
No. 5	03 Apr 1993–15 Sep 1993	200
No. 6	17 Apr 1993–15 Sep 1993	96
No. 7	24 Nov 1993–23 Apr 1994	234

*Number of cuttings

8.3.1.2 Results of Preliminary Experiment

The results of the preliminary experiment are summarized in Table 1. Rooting was observed on 30 of the 842 cuttings. While the rooting rate was low, this result suggests that clonal propagation is possible for *S. albida*. The rooting rate in experiment Nos. 3 and 4 were higher than those in the other experiments. In experiment No. 1, all cuttings dried out and died, due to the incomplete sealing of the cutting bed. The maximum temperature inside the cutting bed was 32°C in the rainy season, and the temperature reached 36°C in the dry season. The temperature of 36°C seemed to be too high for the cuttings to survive, although some of the cuttings could grow under this condition.

Differences Among Culture Media

The results of the cutting experiment with different culture media are shown in Table 2. The rooting rates on the fine-grain sand washed with water were higher than those on the unwashed fine-grain sand. In the experiment with washed fine-grain sand, a cutting with a callus and roots was observed after 2.5 months of planting. The underground part of this cutting was sound and had not turned brown, probably due to an improvement in permeability of the culture medium by washing.

Table 1. ALAN (*Shorea albida*): Results of the cutting experiment (Preliminary Nos. 1 to 6)

Trial No.	No. of cuttings examined	No. of cuttings rooted (%)
1	62	0 (0.0)
2	83	2 (2.4)
3	285	14 (4.9)
4	116	8 (6.9)
5	200	4 (1.9)
6	96	2 (2.1)
Total	842	30 (3.6)

Table 2. ALAN (*Shorea albida*): Results of the cutting experiment using different culture media (Preliminary Nos.1 to 6)

Culture medium	No. of cuttings examined	No. of cuttings rooted (%)
Fine-grain sand (unwashed)	83	2 (2.4)
Fine-grain sand (washed)	294	15 (5.1)
Fine-grain sand (washed) + rice-husk charcoal	345	11 (3.2)
Large-grain sand (washed)	21	2 (9.5)
Fine-grain sand (washed) + medium-grain sand (washed)	37	0 (0.0)
Total	780	30 (3.8)

Table 3. ALAN (*Shorea albida*): Results of the IBA treatment on cuttings (Preliminary Nos. 1 to 6)

IBA Concentration (ppm)	No. of cuttings examined	No. of cuttings rooted (%)
Control	232	14 (6.0)
1	203	10 (4.9)
2	80	2 (2.5)
5	60	0 (0.0)
10	115	2 (1.7)
100	90	2 (2.2)
Total	780	30 (3.8)

IBA, Indolbutyric Acid

Table 4. ALAN (*Shorea albida*): Results of the cutting experiment comparing cut-end positions

Trial No.	Cut-end position	Nos. of cuttings examined	No. of cuttings (%)	
			Survived	Rooted
7	Between node	113	27 (23.8)	17 (15.0)
	Under node	121	10 (8.2)	6 (4.9)
Total		234	37 (15.8)	23 (9.8)

The cuttings on the large-grain sand had sound roots, and showed better root growth after rooting compared with those on the fine-grain sand. These phenomena can be explained by the differences in porosity between the culture media. The underground part of some cuttings on the washed fine-grain sand or on the same sand mixed with rice-husk charcoal had turned brown, but rooted on the surface of the media. The cause of this phenomenon seemed to be because the porosity and permeability of the culture media were poor. The effects of the IBA treatment on rooting are shown in Table 3. The cuttings of the control (no treatment) and the treatment with 1 ppm of IBA showed similar rooting rates. The effects of the IBA treatment on rooting were not observed in this experiment.

Cut-end Position

The results of the cutting experiment for the comparison of cut-end positions are shown in Table 4. The rooting rate of the stems prepared by cutting between nodes was higher than that of those cut just under a node. After this experiment, all cuttings were prepared by cutting between the nodes.

Table 5. List of classified conditions for the comprehensive experiment

Trial	Culture medium		Rooting hormone		Stock habitat		Shading net		Cutting part	
No.	Large	Fine	Seradix	Control	Rassau	Lassa	With	Without	Ordinary	2nd Top
1	—	○	—	—	—	—	○	—	○	—
2	—	○	○	○	○	—	○	—	○	○
3	○	—	—	○	○	—	○	—	○	—
4	○	—	○	○	○	—	○	—	○	—
5	○	—	○	○	—	○	○	—	○	—
6	○	—	○	○	○	—	○	—	○	—
7	○	—	○	○	○	○	○	—	○	—
8	—	○	○	○	○	—	—	○	○	○
9	○	—	○	○	○	○	—	○	○	—
10	○	—	○	○	○	○	—	○	○	—
11	○	—	○	○	—	○	○	—	○	—
12	○	—	○	○	○	○	○	—	○	○
13	○	—	○	○	○	—	○	—	○	—
14	—	○	○	—	○	—	○	—	○	—
15	—	○	○	○	○	—	—	○	○	—

Culture medium: Large = large-grain sand (washed), Fine = fine-grain sand (washed)
Watering: planting day and once a month after checking rooting

8.3.2 Comprehensive Experiment

8.3.2.1 Comprehensive Experiment Schedule

These experiments were carried out to investigate the effects of culture media and watering regimes on the rooting and growth of the cuttings.

No. 1	05 Feb 1994–07 Sep 1995	590	Nursery house 1
No. 2	18 Sep 1995–10 Jan 1996	540	Nursery house 1
No. 3	01 Aug 1995–21 Dec 1995	200	Nursery house 2
No. 4	17 Aug 1995–21 Dec 1995	170	Nursery house 2
No. 5	08 Aug 1995–21 Dec 1995	160	Nursery house 2
No. 6	23 Aug 1995–21 Dec 1995	180	Nursery house 2
No. 7	03 Aug 1995–22 Dec 1995	370	Nursery house 2
No. 8	03 Oct 1995–26 Dec 1995	230	Nursery house 3
No. 9	27 Sep 1995–26-Dec 1995	500	Nursery house 3
No. 10	09 Oct 1995–26 Dec 1995	320	Nursery house 3
No. 11	10 Oct 1995–27 Dec 1995	200	Nursery house 3
No. 12	28 Sep 1995–27 Dec 1995	410	Nursery house 3

8.3.2.2 Synthetic Experiment

Wooden beds were set in nursery house No. 1, and concrete beds with a mist-spray system were set in nursery house Nos. 2 and 3. The list of classified conditions for the comprehensive experiment are shown in Table 5. The results of the comprehen-

Table 6. ALAN (*Shorea albida*): Results of the cutting experiment (Comprehensive experiment Nos. 1 to 12)

Trial No.	Nos. of cuttings examined	No. of cuttings (%)		
		Survived	Callused	Rooted
1	590	488 (82.7)	79 (13.6)	409 (69.3)
2	540	477 (88.3)	59 (10.9)	418 (77.4)
3	200	84 (42.0)	39 (19.5)	45 (22.5)
4	170	113 (66.4)	43 (25.2)	70 (41.1)
5	160	135 (84.3)	38 (23.7)	97 (60.6)
6	180	104 (57.7)	52 (28.8)	52 (28.8)
7	370	151 (40.8)	66 (17.8)	85 (22.9)
8	230	168 (73.0)	40 (17.3)	128 (55.6)
9	500	237 (47.4)	94 (18.8)	143 (28.6)
10	320	236 (73.7)	112 (35.0)	124 (38.7)
11	200	127 (63.5)	98 (49.0)	29 (14.5)
12	410	196 (47.8)	68 (16.5)	128 (31.2)
Total	3,870	2,516 (65.0)	788 (20.3)	1,728 (44.6)

sive experiment are shown in Tables 6, 7, 8, 8A, 9, 9A, 9B, and 9C. Because nursery house Nos. 1, 2, and 3 were under different conditions, the results are shown separately.

Nursery House No. 1

As shown in Table 6, the rooting rates of the cuttings in experiment Nos. 1 and 2, which were carried out in nursery house No. 1 with airtight wooden cutting beds, were highest among the results of the all experiments. In experiment No. 1, the irrigation was done only when the experiment was started, with no more irrigation for 3 months. This means that the humidity inside the rooting bed could be kept at a high level for a long time with this method. No cuttings turned brown underground in experiment Nos. 1 and 2.

The effects of rooting hormones on cuttings (at house No. 1) are shown in Table 7. Rooting of the cuttings was observed 33 days after planting. Because on average it took more than 8 weeks for rooting in the previous experiments, the rooting condition was considered to be much improved in this experiment. The effects of rooting hormones were not clear for the cuttings planted on the fine-grain sand in the airtight wooden boxes.

Nursery House No. 2

The results of the cutting experiment at nursery house No. 2 are shown in Tables 8 and 8A. The light intensity in house No. 2 was lower than that of house No. 1. The effects of rooting hormones were clear in this experiment. It seemed that the light intensity in house No. 2 was insufficient for rooting of the cuttings.

Table 7. ALAN (*Shorea albida*): Results of the cutting experiment at House No. 1

Culture medium	Rooting hormone	Stock habitat	Shading net	No. of cuttings examined	No. of cuttings (%) Survived	Callused	Rooted
Fine sand	Seradix	Rassau	present	250	211 (84.4)	19 (7.6)	192(76.8)
Fine sand	Control	Rassau	present	250	228 (91.2)	31 (12.4)	197(78.8)
Total				500	439 (87.8)	50 (10.0)	389(77.8)

Table 8. ALAN (*Shorea albida*): Results of cutting experiment at House No.2

Culture medium	Rooting hormone	Stock habitat	Shading net	No. of cuttings examined	No. of cuttings (%) Survived	Callused	Rooted
Large sand	Seradix	Rassau	with	250	156 (62.4)	45 (18.0)	111(44.4)
Large sand	Seradix	Lassa	with	150	132 (88.0)	35 (23.3)	97(64.6)
Large sand	Control	Rassau	with	470	210 (44.6)	112 (23.8)	98(20.8)
Large sand	Control	Lassa	with	210	89 (42.3)	46 (21.9)	43(20.4)
Total				1,080	587 (54.3)	238 (22.0)	349(32.3)

Table 8A. ALAN (*Shorea albida*): Effects of stock habitat on cuttings

Habitat	No. of cuttings examined	No. of cuttings (%) Survived	Callused	Rooted
Rassau	720	366 (50.8)	157 (21.8)	209 (29.0)
Lassa	360	221 (61.3)	81 (22.5)	140 (38.8)
Total	1,080	587	238	349

Table 9. ALAN (*Shorea albida*): Results of the cutting experiment at House No. 3

Culture medium	Rooting hormone	Stock habitat	Shading net	No. of cuttings examined	No. of cuttings (%) Survived	Callused	Rooted
Fine sand	Seradix	Rassau	without	100	65 (65.0)	9 (9.0)	56(56.0)
Fine sand	Control	Rassau	without	100	79 (79.0)	16(16.0)	63(63.0)
Large sand	Seradix	Rassau	without	250	168 (67.2)	35(14.0)	133(53.2)
Large sand	Seradix	Rassau	with	130	58 (44.6)	16(12.3)	42(32.3)
Large sand	Seradix	Lassa	without	150	105 (70.0)	51(34.0)	54(36.0)
Large sand	Seradix	Lassa	with	150	81 (54.0)	51(34.0)	30(20.0)
Large sand	Control	Rassau	without	300	115 (38.3)	61(20.3)	54(18.0)
Large sand	Control	Rassau	with	130	82 (63.0)	37(28.4)	45(34.6)
Large sand	Control	Lassa	without	120	85 (70.8)	59(49.1)	26(21.6)
Large sand	Control	Lassa	with	150	68 (45.3)	50(33.3)	18(12.0)
Total				1,580	906 (57.3)	385(24.3)	521(32.9)

Table 9A. ALAN (*Shorea albida*): Effects of the culture medium on cuttings

Culture medium	No. of cuttings examined	No. of cuttings (%) Survived	Callused	Rooted
Fine sand	200	144 (72.0)	25 (12.5)	119 (59.5)
Large sand	1,380	762 (55.2)	360 (26.0)	402 (29.1)
Total	1,580	906 (57.3)	385 (24.3)	521 (32.9)

Table 9B. ALAN (*Shorea albida*): Effects of the rooting hormone and stock habitat on cuttings

Rooting hormone	Stock habitat	No. of cuttings examined	No. of cuttings (%)		
			Survived	Callused	Rooted
Seradix	Rassau	480	291 (60.6)	60 (12.5)	231 (48.1)
Seradix	Lassa	300	186 (62.0)	102 (34.0)	84 (28.0)
Control	Rassau	530	276 (52.8)	114 (21.5)	162 (30.5)
Control	Lassa	270	153 (56.6)	109 (40.3)	44 (16.2)
Total		1,580	906 (57.3)	385 (24.3)	521 (32.9)

Table 9C. ALAN (*Shorea albida*): Effects of the shading net on cuttings

Culture medium	Shading net	No. of cuttings examined	No. of cuttings (%)		
			Survived	Callused	Rooted
Large sand	without	820	473 (57.6)	206 (25.0)	267 (32.5)
Large sand	with	560	289 (51.6)	154 (27.5)	135 (24.1)
Total		1,380	762 (55.2)	360 (26.0)	402 (29.1)

Nursery House No. 3

The results of the cutting experiment at house No. 3 are shown in Tables 9, 9A, 9B and 9C. The rooting rates of cuttings planted on fine-grain sand were higher than those on large-grain sand (Table 9A). The rooting rates of cuttings under these conditions were lower than those in the airtight wooden boxes. It was impossible to keep the relative humidity higher than 95% inside the cutting beds with the mist-spray system, due to incomplete sealing of the beds. The effects of rooting hormones on rooting were also clear in this experiment (Table 9B). The effects of shading on the rooting rate of cuttings on large-grain sand are shown in Table 9C. The rooting rate of cuttings grown without shade was higher than that with shade. It shows that light intensity is a very important factor for making rooted cuttings. As shown in Tables 8A and 9B, the differences in rooting between the provenance tests were not clear.

8.4 Conclusions

According to the results of the investigations, the following conditions are recommended for the clonal propagation of *S. albida* by cutting.

1. Good permeability of water is required for the cutting bed.
2. The cutting bed should be sealed with a clear plastic film to keep high relative humidity inside.
3. Washed large-grain river sand is suitable as a culture medium, due to its good porosity and permeability for developing a sound root system.

4. Irrigation frequency should be kept to a minimum, due to the sensitivity of *S. albida* against excessive water.
5. Cutting stems should be prepared by cutting between nodes.
6. Although the most suitable light intensity has not yet been found for rooted cuttings, the inside of the nursery house should have a bright-light intensity.
7. The effect of rooting hormones is clear in the case of insufficient conditions for rooting, such as incomplete sealing and/or insufficient light intensity etc.

Further studies on nursing, hardening and planting experiments are required to establish the series of reforestation techniques for rooted cuttings of *S. albida* in the field.

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9
Developments in Dipterocarp Propagation Research in the Philippines

MITZI POLLISCO

9.1 Introduction

Dipterocarps are the dominant species and the skeletal backbone, providing the structure, of lowland tropical rainforests. The contribution of dipterocarps to national welfare, in terms of foreign exchange generation, is historically significant. Their ecological importance is unparalleled: the home of the Philippine eagle and other wildlife; the stronghold that regulates water releases from the uplands; climatic influence; and aesthetic value. The threat of dipterocarps becoming just one of the entries in the list of extinct species is always there unless something is done to protect the remaining stands and to propagate them.

In 1993, the Philippine government, through the Department of Environment and Natural Resources (DENR), issued a directive to establish at least 10 hectares of dipterocarp plantations and to restock second growth forests annually throughout the country. The directive was implemented through Department Administrative Order (DAO) # 21, of Series 1996. The first option was wildling collection, which is a ready source of planting materials after a fruiting season. Despite the irregular occurrence of seed years, 25,000–100,000 wildlings per hectare germinate under the forest canopy (Revilla 1978, as cited by Pollisco 1991). However, recovery of wildlings in the nursery has been very low, with some regions having 100% mortality.

9.2 The Tree Propagation Complex

Prior to the 1990s, techniques for the mass propagation of dipterocarps were limited to seeds and wildlings. Towards the mid 1990s, propagation by cuttings from juvenile stockplants became a useful addition to the propagation of dipterocarps, specifically between seed years. This technique paved the way for the development of a

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new nursery set up, the Tree Propagation Complex, which is comprised of different facilities to fully utilize technologies on dipterocarp propagation. The Ecosystems Research and Development Bureau (ERDB), of the Department of Environment and Natural Resources (DENR), was able to construct the propagation complex from funds provided by the FORTIP project in 1991. This eventually led to full Philippine government support from 1994-2000, during the implementation of the project entitled "Macro-propagation of dipterocarps through seedlings, wildlings and rooted cuttings."

The nursery for dipterocarp seedlings is now just a part of the complex. Propagation by cuttings requires non-mist propagation chambers, a small tissue-culture laboratory, and a hedge garden. The wildlings require a recovery chamber to increase their chances of survival.

The non-mist system component of the Tree Propagation Complex is now adopted nationwide to generate planting stocks of valuable timber species for reasons of biodiversity conservation, protected/watershed-areas rehabilitation, and reforestation. The salient features of these technologies are that they (1) are simple, (2) do not require large capital and, (3) can be used in areas where electricity and piped water are unavailable.

9.2.1 Seedlings

Seeds of dipterocarps are normally collected from the ground, because mother trees are tall and inaccessible. However, quality seeds can only be collected directly from trees that have been selected based on a set of criteria that can be strictly followed if there is gregarious fruiting, where numerous trees bear fruit at the time of collection. The criteria are that the trees must: (1) be free from pests and diseases; (2) be greater than 100 in apart; (3) have a well-balanced crown; (4) have a straight, cylindrical bole with a 40–80 cm diameter at breast height. The distance between trees is critical to avoid collecting from individuals that are most likely to be related by common maternal descent.

The collected seeds can be mixed into a bulked seedlot, comprising equal numbers of seeds from each represented tree. The resulting seedlings are morphologically graded, and the vigorous ones selected for hedge garden and plantation establishment.

9.2.2 Vegetative Propagation by Cuttings Using The Non-Mist System

Pollisco (undated) reported detailed methodologies and results on the macropropagation of dipterocarps through vegetative propagation using the non-mist system, as well as their performance after outplanting.

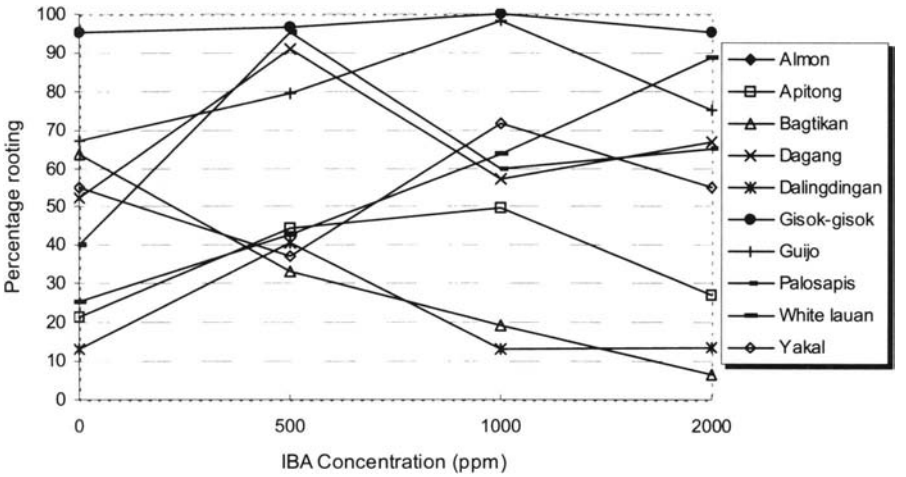

Fig. 1. Effects of different Indole butyric acid (IBA) concentrations on the rooting performance of dipterocarp cuttings 3 months after treatment

9.2.2.1 Sand-Rooting System

Rooting was observed as early as 28 days after planting of dagang (*A. aurea* Foxw.), apitong (*Dipterocarpus grandiflorus* Blanco), guijo [*S. guiso* (Blanco) Blume], and bagtikan [*Parashorea malaanonan* (Blanco) Merr.]. For white lauan (*Shorea contorta* Vid.), rooting was observed after 45 days. Variation in rooting responses, as well as the effects of different hormone concentrations, was noted among and within the species tested (Fig. 1).

The Propagation System

Pollisco (1994a, b, c) described the simple propagation system and how it works. The basic requirements for the success of this method are: (1) the rooting medium must be watered to saturation at the start of the propagation process; (2) propagules must be mist watered once or twice a week, and thereafter depending on climatic conditions; and (3) plastic bags must be intact and free of openings or holes during the propagation period.

Species Propagated

A striking feature of the yakal-saplungan (*H. plagata* Blanco Vid.), gisok-gisok (*H. philippinensis* Dyer), and bagtikan experiments was the rootability of the species, even without the application of rooting hormones. Successful rooting without applied auxins has been reported in a number of tropical tree species, such as *Shorea bracteolata* (Srivastava and Manggil 1981), *Shorea macrophylla* (Lo 1985), *Milicia excelsa* (Ofori et al. 1996), and *Nauclea dedirichii* (Leakey 1990). Such results could reflect the availability of high endogenous auxin contents at the time of severance from the stockplant (Hartmann and Kester 1990).

Yakal-saplungan, gisok-gisok, and bagtikan belong to the easy-to-root group of species. In these species, quiescent pre-formed-root primordia are present in stems that root so readily that the most simple facilities and care result in high rooting success (Hartmann and Kester 1975). Pre-formed roots develop naturally on the stems while they are still attached to the parent plant, but they do not emerge until the stem piece is severed (Dick and Aminah 1996). Other dipterocarp species, which are difficult-to-root, belong to the wound-adventitious roots group. Wound-adventitious roots develop only after the cutting is made, as a direct response to the wound made in preparing the cuttings. These generally take longer to develop than pre-formed roots because more anatomical changes are necessary (Dick and Aminah 1996).

Smits et al. (1989) stated that the genus *Shorea* is the easiest to root, while several *Vatica* and *Dipterocarpus* species are quite difficult to root.

Rooting Hormones

The marked positive effect of IBA on the rooting of palosapis, guijo, and dalingdingan can be attributed to the enhanced transport of carbohydrates to the base of the cuttings (Hartmann and Kester 1990). Srivastava and Manggil (1981) also stated that treatment with IBA promoted heavier rooting in *S. bracteolata* at 500 ppm IBA and in *A. scaphula* and *S. leprosula* at 2,000 ppm IBA.

Time of Collection

The season in which cuttings are taken from difficult to root species can be very important, while cuttings from easy-to-root species can be taken at any time of the year (Menzies 1992). It was observed that dipterocarp cuttings taken during dry season (April-May) do not root as easily as those taken during the rest of the year.

9.2.2.2 Bubble-Bath System

The bubble-bath system is a modified hydroponic system of non-mist clonal propagation being used by TROPENBOS, Indonesia. The system depends on electricity (de Fraiture et al. 1992), wherein an aerator continuously supplies oxygen (bubbles) to the solution that touches the base of the suspended cuttings throughout the duration of propagation, that is, until the cuttings are ready to be potted into the soil-rooting medium.

All of the species tested with different concentrations of IBA (0–10 ppm), namely white lauan, dagang (*Anisoptera aurea*), apitong, palosapis, almon (*Shorea almon*), guijo, and bagtikan, had a very low rooting success rate (< 50%), even after three months. Cuttings taken from the tip portion of the stockplant consistently had a higher rate of rooting. Smits et al. (1989) reported the same results with *A. marginata, S. smithiana, S. laevis,* and *S. blanco*, using the bubble-bath method.

Based on the results, bagtikan cuttings taken from different parts of the stockplants (base, middle, tip) treated with different IBA concentrations (control, 5 ppm, and 10 ppm) showed that the application of hormones induced early shoot development and a higher rate of rooting. Cuttings taken from the tip portions of the stockplants consistently had a higher rate of rooting, even without hormone treatment (Fig. 2).

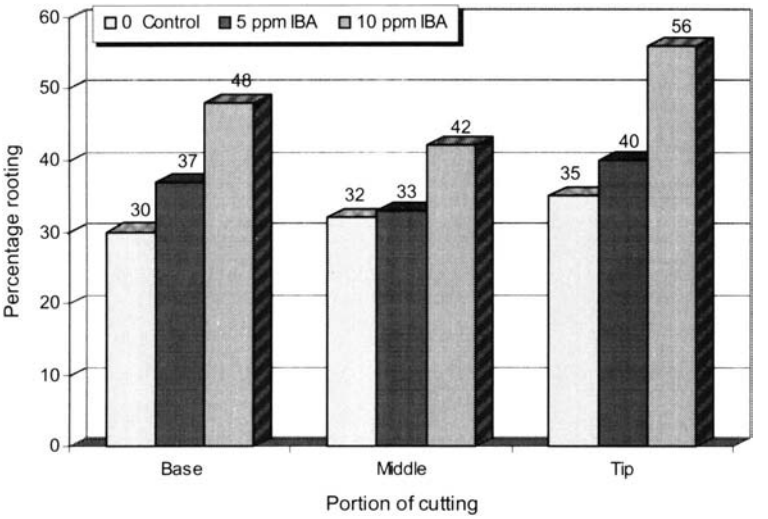

Fig. 2. Percentage rooting of 3-month old bagtikan *(Parashorea malaanonan)* cuttings using the bubble-bath system

9.2.2.3 Tissue Culture

Plantlet production using tissue culture is a far more difficult alternative to the macropropagation of dipterocarps from cuttings (Pollisco 1994). The successful sterilization and callus initiation of white lauan (Lapitan 1983), and nodule-like formation from juvenile nodal explants of palosapis *(A. thurifera)* (Pollisco 1994c), have been reported, but there have been no reports to date on the successful production of dipterocarp plantlets.

9.2.3 Wildlings

The care of dipterocarp wildlings in the nursery is more complicated than the care of seedlings. Wildlings require a different set of conditions in order to survive (Pollisco 1994). The survival rate of dipterocarp wildlings with a recovery chamber is generally higher than without a recovery chamber (Fig. 3). After 2 months in a recovery chamber, the dipterocarp species with the highest survival rates were panau (*D. gracilis*), white lauan, yakal-saplungan, guijo, gisok-gisok, and dalingdingan (*H. foxworthyi*).

Wildlings are nursed longer than seedlings due to their shade leaves and poorly developed root systems. The lateral roots are left in the soil due to the bareroot system of collection. With the provision of a recovery chamber, wildlings survive regardless of their height at the time of collection, ranging from those with their cotyledons still attached up to those 18.90 cm tall. These wildlings also develop new root systems and, therefore, should undergo the same process of hardening or acclimatization as with cuttings, to allow the newly rooted wildlings to adjust to the

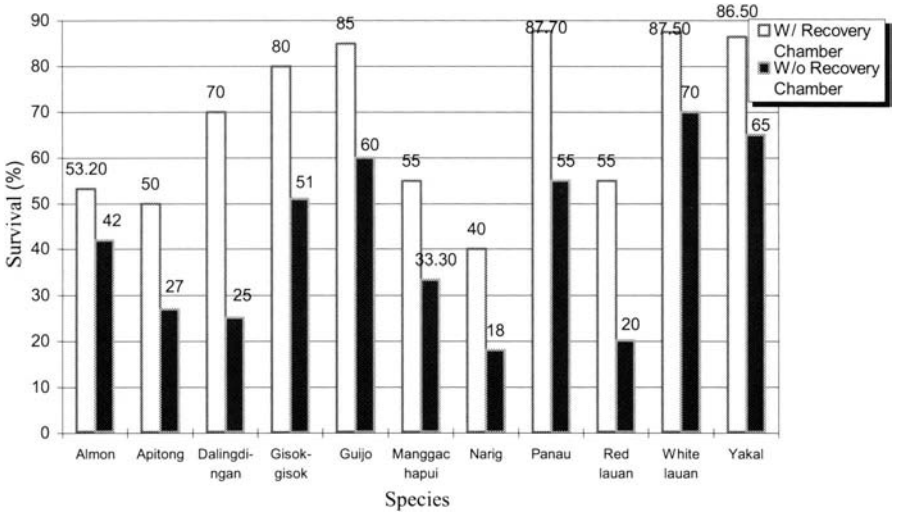

Fig. 3. Percent survival of dipterocarp wildlings with and without a recovery chamber after 2 months in the nursery

lower relative humidity conditions outside the recovery chamber. During this time, functional stomates and normal leaf cuticles develop to allow the wildlings to control water loss. This is achieved by the gradual reduction of relative humidity and increase in light intensity during the last 3–6 weeks of the recovery process after root formation.

The recovery chamber consists of two layers of black nets and polyethylene plastic that fully cover (including the sides) the structure, simulating an almost airtight condition. The wildlings are kept inside the chamber for 2–3 months, prior to new growth. The removal of the covers is done gradually, at intervals of 2 weeks, to allow the new leaves to develop mechanisms by which they can eventually adapt to normal conditions.

9.2.4 The Hedge Garden

To ensure the availability of planting stocks, hedge gardens established near the propagation facility are a must. When there is a shortage of planting stocks, it is best to plant any available material when starting a multiplication area. Seedlings and wildlings can be utilized. However, when vegetative propagation is part of a tree-improvement program, it is important to separate the genetically different material. These could be seedlings or wildlings from desirable trees (Kantarli 1995c).

9.2.4.1 Reiteration

Artificial induction of reiteration in stock plants needs to be done to include stump sprouts and to release buds on leaning or bent stems. Reiteration is the replication of the original architectural model from one or more active or inactive buds (Tomlinson 1983). Not all young dipterocarps (2–3 years old) have the inherent ability to reiterate. Most species usually have only one or two shoots, even after five times of regular harvesting at intervals of 3–4 months. Exceptions to these are *S. contorta* and *A. thurifera*, where after 4 months, or even earlier, harvest of multiple orthotropic shoots and stump sprouts could be made. Ashton (1988) stated that with the exception of dry dipterocarps, the species are remarkable in their poor capacity for reiteration. As seedlings, however, they have a high capacity for shoot reiteration, often through the formation of accessory buds.

To maintain juvenility in *Hopea odorata*, Kantarli (1995a, b) recommended detopping at 20 cm above ground level. In addition, Longman (1993), reported that coppice shoots about 1 m above the ground are usually juvenile (like shoots from seedlings), often producing vigorous shoots with a main-stem structure that root easily.

9.2.4.2 Apical Dominance

Cline (1996) found that different intact plants have differences in apical dominance: weak, medium, and strong. According to this author, "strong apical dominance" signifies little or no lateral-bud outgrowth, "medium" implies some bud growth and "weak" indicates substantial and continuing lateral-bud growth in intact plants. Wareing and Naser (1961), as cited by Zimmerman and Brown (1980), and Kantarli (1995b) said that apical dominance was exerted by the terminal shoot only when it was in a vertical position.

Pollisco and Rodriguez (1998) reported the results of hedge-garden management for some Philippine dipterocarps. The first detopping of stockplants is at 20 cm above ground level to encourage the development of multiple shoots. This disruption of apical dominance typically induces a shorter, bushier growth form (Cline 1991 as cited by Lortie and Aarssen 1997). After the diameter of stockplants reaches 0.75–1.25 cm, the treatments of detopping at 1 m or combined detopping and bending at 1 m can be done to induce reiteration.

The emergence of orthotropic shoots in *A. thurifera*, primarily at the tip of detopped stockplants, was attributed to the removal of apical dominance of the intact shoots, which released dormant buds located at the lower nodes. Cline (2000) stated that decapitation at any location on the stem releases lateral buds at the node, below the point of decapitation. Kantarli (1995) reported that the distance from the decapitated portion included about three to five nodes for *Hopea odorata*. However, in *S. contorta*, orthotropic shoots also developed at the mid-portion, and basal stump sprouts developed.

Shoots arranged in a scattered manner throughout the stem have a more or less uniform growth as a result of the absence of competition for light. Changing the orientation of stockplants from vertical to horizontal exposes them to increased irradiance, which according to Cline (1996) can greatly weaken apical dominance.

9.2.4.3 Light Exposure

Dipterocarps require some shade, but it should not exceed 50%, since they reach light saturation at about 30%–50% full sunlight (Kantarli, 1995).

9.2.4.4 Maintenance Activities

Cuttings of white lauan and bagtikan harvested from fully fertilized stockplants at the Los Baños Experiment Station did not root. Since fertilization encourages rapid vegetative growth, application is done after the harvest of stem cuttings. Tender, juvenile shoot tips that extend to the midportion are observed if plants are fertilized towards harvesting. When harvesting is done at this stage, the high endogenous nitrogen levels that are low in stored carbohydrates result in inhibited root formation.

9.3 Summary

Pollisco (undated) provided the following summary.

1. Dipterocarp species are amenable to vegetative propagation by cuttings using the non-mist system.
2. Not all dipterocarps are difficult to root.
3. There is an effective range of hormone concentrations for each species, not just a single concentration.
4. The use of a Wildling Recovery Chamber increases the survival rate of dipterocarp wildlings.
5. *S. contorta* and *A. thurifera* are species with weak apical dominance, due to their substantial and continuing lateral-bud growth in intact plants. The other dipterocarp species producing solitary or double orthotropic shoots are those with strong apical dominance. Therefore, more stockplants are needed for sustained vegetative propagation by cuttings.
6. Combined hedging and bending treatment, or manipulating the vertical orientation of shoots/stems to the horizontal, produces more orthotropic shoots compared to detopping/decapitation alone, due to their better advantage in capturing more light.

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Tissue-Culture Technology

10
An Updated Overview of Advances in Somatic Embryogenesis in Forest Trees

S. MOHAN JAIN

10.1 Introduction

Continuous human population growth is one of the major causes of concern for meeting the demands of feeding new mouths. However, the situation could further worsen with the increase in purchasing power of people in the developing world, by creating an imbalance between supply and demand, environmental degradation, deforestation, global warming, water shortages, and a halt to the scope for arable land expansion (Jain 2002a). Plant breeders and available infrastructure will come under sustained pressure for continuous food supply and sustainable agriculture production. Moreover, conventional methods of tree improvement and selection offer limited possibilities of meeting the rapidly growing demands of the industry. Therefore, new innovative and cost-effective technologies are needed to develop new hybrids, early selection and testing of desirable genotypes, and rapid vegetative propagation of selected genotypes.

Recent advances in plant cell and tissue culture have facilitated plant regeneration via somatic embryogenesis, organogenesis, etc., in seed and vegetatively propagated crops. As a result, there is great potential for improving the technology for the rapid multiplication of elite genetic material for large-scale production over a short time (Jain and Ishii, 1998). This technology is important for woody plants that have a long life cycle and are difficult to multiply by conventional methods. Several important woody-plant species, such as legumes, conifers etc., cannot be improved through the selection of elite trees for seed production, owing to self incompatibility or low seed viability.

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10.2 Somatic Embryogenesis

Somatic embryogenesis (SE) is suitable for large-scale clonal propagation, and has made tremendous progress in all commercially-important trees belonging to both angiosperms and gymnosperms (Jain et al. 1995, 1999). It is a process of forming embryos from somatic cells without going through sexual cycles; it is similar to zygotic embryogenesis (see Von Arnold et al. 2002). SE was first described in *Daucus carota* (see Jain et al. 2000), and then in *Santalum album*; the first gymnosperm to undergo the process was *Picea abies* (see Jain et al. 1995).

Polyembryony is a common phenomenon in conifers that results from in vivo multiple-embryo formation within the seed. Gupta and Grob (1995) described two types of polyembryony: (1) simple polyembryony, which results from the fertilization of many eggs per female gametophyte to form multiple embryos, and (2) cleavage polyembryony, whereby one embryo cleaves to form several genetically-identical embryos. Mostly, SE originates from a single cell, as demonstrated in *Pinus elliottii* (Jain et al. 1989). This origin type is suitable for mutation induction with either physical or chemical mutagens for preventing or minimizing chimerism. It takes three to four generations of plant or seed multiplication to dissociate chimerism (Predieri 2001).

10.2.1 Establishment of Embryogenic Cultures

Embryogenic cultures of forest trees are established by culturing female gametophytes or mature zygotic embryos under in vitro conditions (Jain et al. 1995). These cultures include immature embryos of important conifier trees, including *Pinus, Picea, Larix*, etc., mature embryos of *Picea, Pinus*, and others, and thin cell-layer method induced somatic embryogenic cultures (Jain et al. 1995, 1999, 2000; Tran Thanh Van and Le 2000). In conifer trees, embryogenic suspensor masses (ESM) consist of early-stage embryos at varying developmental stages, which contain an embryonal head and suspensor system (Jain et al. 1989). Gupta and Durzan (1987) developed a double-staining method for the confirmation of embryogenic cells.

The advantages of using mature zygotic embryos are: (1) it is easier to extract seeds; (2) embryos of most conifers in mature seeds remain viable longer in storage, enabling the availability of suitable material throughout the year; and (3) their prominent genotype influences on embryogenesis (Bonga and Von Aderkas 1992).

Ruaud et al. (1992) reported the induction of ESM from the needles of 14-month-old somatic seedlings growing in a greenhouse. Rohr et al. (1997) induced SE in *Cephalotaxus harringtonia* on an MS medium (Murashige and Skoog 1962) with 5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), by culturing together embryos and megagametophyte halves, sectioned longitudinally, taken from a 60-year-old tree. Low percentages of sucrose and gelrite, mostly under darkness, were better for producing more ESM (Finer et al. 1989). Both cytokinin and auxin are needed for the initiation of embryogenic cultures (Radojevic et al. 2002). Watt et al. (1999) induced SE from seedling explants of *Eucalyptus citriodora* and *E. grandis*. Somatic plants were regenerated in *E. dunnii* without any difficulties.

10.2.2 Maintenance of Embryogenic Cultures

The maintenance of somatic-embryogenic cultures is usually done on a medium similar to the initiation medium, which is either a semi-solid or liquid medium (Lu et al. 1991; Gupta and Durzan 1987; Durzan and Gupta 1987). Gupta and Grob (1995) failed to maintain *Pinus taeda* embryogenic cultures for longer on a semi-solid medium. Jain et al. (1988) noticed that a delay in subculture on a fresh medium resulted in the loss of embryogenic potential of *Picea abies* embryogenic cultures that could not be reversed naturally. This is apparently due to the cultures undergoing an aging process and often showing a decline in embryogenic potential.

Cheliak and Klimaszewska (1991) separated embryogenic outgrowth from the primary explant and other types of outgrowth as soon as tissue reached a sufficient mass; otherwise the embryo could be overgrown by a callus and lost. It is desirable to subculture embryogenic cultures on a fresh medium at 2–3 week intervals. *Picea abies* somatic-embryogenic cultures require both auxin and cytokinin for the proliferation of embryos (Von Arnold et al. 1995), and their growth pattern does not change. However, when auxin alone is added to the medium, embryogenic cultures remain white and translucent.

Bellarosa et al. (1992) noticed that embryos increased in size and developed extremely large embryogenic regions within 40 days, and the cultures turned brown when cytokinin alone was in the culture medium. Muralidharan and Mascarenhas (1995) maintained *Eucalyptus citiridora* embryogenic cultures by subculturing regularly, at 4-week intervals, on a fresh medium without loss of regeneration potential, and maintained them for more than 9 years without loss of embryogenic potential or competence. The maintenance of embryogenic cultures is very much dependent on the subculture interval of the fresh medium, type of culture medium, and tree species. For preventing somaclonal variation or loss of embryogenic potential, it is necessary to determine the number of subcultures as well as the duration of subculture interval, depending on the plant species.

10.2.3 Maturation and Germination of Somatic Embryos

Several factors are involved in the maturation and germination of somatic embryos, such as osmotic potential of the culture medium, abscisic acid (ABA), carbohydrates, and plant growth regulators. Gupta and Grob (1995) suggested that osmotic potential of the culture medium and 0.1–25 mg/l ABA are essential for the development and maturation of conifer somatic embryos. Polythene glycol (PEG 4000–8000) was used as an osmoticum, together with ABA, in a culture medium for conifer somatic embryo maturation (Attree et al. 1991; Gupta and Pullman 1991). Abscisic acid treatment alone inhibits cleavage polyembryony and allows embryo singulation and further development, depending on the size of the embryonal head. While somatic embryos undergo a maturation process, embryos stop proliferating, increase in size, start accumulating storage material, including carbohydrates, proteins, and lipids (Von Arnold et al. 1995). Norgaard (1997) found maltose superior

to sucrose in terms of the number of mature somatic embryos in *Abies nordmanniana*. The addition of indole-butyric acid (IBA) or benzyl-aminopurine (BA) to an ABA-containing medium also improved maturation (Von Arnold et al. 1995). Furthermore, the maturation of somatic embryos resulted in high levels of triglycerides, which is similar to that of zygotic embryos (Attree et al. 1992). However, ABA was ineffective in *Popolus* and *Eucalyptus grandis*, and caused better desiccation tolerance in sandalwood (Jain and Ishii 1998).

One of the limitations of SE is a low germination rate of somatic embryos, which is also genotype dependent. Therefore, culture-growing conditions, light regime, sucrose concentration, or strength of the culture medium need to be manipulated. Gupta and Grob (1995) synchronized the germination of mature conifer somatic embryos by reducing the sucrose concentration and the activated charcoal, without having plant-growth regulators in the culture medium. Carrier et al. (1997) observed that the exclusion of sucrose from the culture medium resulted in a retardation of embryo growth or in necrosis, and linolenic acid content was barely detectable in the fatty-acid profile. The accumulation of lipid reserves and, subsequently, their utilization, is essential during the germination of somatic embryos.

Jain et al. (1988) germinated 11%–15% of *Picea abies* somatic embryos in the darkness by reducing the strength of the culture medium. Muralidharan and Mascarenhas (1995) found that an auxin-free medium was effective in the germination of *Eucalyptus citridora* somatic embryos. In *Quercus robur*, Chalupa (1995) reported that the germination rate of somatic embryos was either low or absent in the culture medium containing cytokinin. The influence of plant-growth regulators on the germination rate of somatic embryos is very much dependent on tree species and genotype.

10.2.4 Field Trials and Genetic Fidelity of Somatic Seedlings

It is desirable to conduct field trials of somatic seedlings and critically evaluate their performance before commercialization. So far, limited numbers of field trials on somatic seedlings have been conducted due to the low germination rate of somatic embryos. Field trials of *Picea abies* and Douglas fir somatic seedlings, conducted by Weyerhauser, US, show striking uniformity within a clone compared to the uniform seedlings during the first year. Roberts et al. (1993) conducted field trials of *Picea glauca engelmannii* emblings from 71 genotypes in Canada. At the end of the first season, the embling survival rate was 80%–100% and genotype dependent. Height, shoot, and root morphology, and the cold hardiness of emblings did not differ from the seedlings. Currently, field trials are being conducted by several paper companies in the US, Canada, and New Zealand. The preliminary results indicate morphological variation in somatic seedlings of conifers.

Since trees have a long-life cycle, it is important to maintain the genetic fidelity of somatic seedlings. Some genetic changes cannot be observed at the morphological or physiological level because the structural difference in the gene product may

not sufficiently alter its biological activity to produce an altered phenotype. Molecular marker analysis is needed to identify genetic variation at the early stage of plant development, rare-point mutations, and duplications of genes or chromosomes (Jain et al. 2002). Molecular analysis (RAPDs) indicated identical clonal-somatic seedlings in several conifer species (Jain and Ishii 1998), and Thakur et al. (1999) did not find somaclonal variation in *Quercus serrata* somatic seedlings using RAPD markers. Some of these variations are hardly detectable with current methods of DNA analysis. Feuser et al. (2003) provided evidence of detecting somaclonal variation with isozymes and RAPD markers in pineapple. However, morphological and cytological approaches are valuable complementary tools for identifying chromosomal variation (Fourre et al. 1997).

10.3 Major Applications of Somatic Embryogenesis

Somatic embryogenesis has several advantages and can be put to practical applications in forestry (Jain and Ishii 1998), such as cryopreservation, bioreactors, encapsulation, genetic transformation, mass clonal propagation, and induced mutations. There are some disadvantages of SE, however. For example, the low germination rate of somatic embryos has limited large-scale plant multiplication and commercialization (Jain and Ishii 1998).

10.3.1 Cryopreservation

Somatic embryos are cryo-stored for the long-term storage of elite germplasm to be used in the future. The water content in somatic embryos is crucial for subsequent survival in liquid nitrogen, and desiccation before freezing enhances their survival rate (Percy et al. 2001). Cyr et al. (1994) cryopreserved embryogenic cultures of interior spruce, derived from 12 full-sib families, and obtained a 97% success rate for 357 genotypes without any adverse affect on regenerative potential. There was no evidence of somaclonal variation. The risk of somaclonal variation can be reduced by the cryopreservation of newly-established somatic embryogenic cultures, reducing the risk of culture contamination by subcultures, infrequent subculturing, and the storage of a large population of genetic material. The company Silvagen, Canada, has clone banks that have been initiated for blister-rust-resistant western white pine (seven families, 50 genotypes) and high yield coastal Douglas fir (12 families, 220 genotypes). For three southern pine species, 400 genotypes, representing 27 families, have been stored (Cyr 1999).

10.3.2 Bioreactor Technology

Somatic embryogenic cultures can be grown in a liquid medium, including bioreactors of various sizes (Jain et al. 1995; Ingram and Mavituna 2000; Ibaraki and Kurata 2001), and the temporary immersion system (Akula et al. 2000; Etienne and Berthouly 2002). Bioreactors are part of the pilot plant for large-scale somatic seed production. The quality control of somatic embryos is done with a computer-aided image analysis system (Ibaraki and Kurata 2001), and undesirable somatic embryos are recycled in the bioreactor for improving quality. The recycling of somatic embryos may not work well for every forest tree, and perhaps the role of genotype variation in somatic embryo size (Barry-Etienne et al. 2002) will become crucial. There are challenges to improving the yield of ESM and, ultimately, somatic-embryo production in a liquid-culture medium. The whole process of somatic-embryo production can be automated, including quality evaluation, and ultimately could reduce labor costs and commercialize low-cost somatic embryo production.

10.3.3 Encapsulation

Well-developed single somatic embryos are encapsulated in alginate gel or artificially developed seed coatings for making somatic seeds. Each encapsulated embryo behaves like a normal zygotic seed. The principal idea behind this technology is the automation of somatic-seed production in an integrated pilot plant under controlled conditions. This technology is still at the developmental stage for most forest trees.

10.3.4 Genetic Transformation

Mostly genetic-transformation systems have been developed for agronomically important crop plants because woody plants are usually recalcitrant to regenerate. Somatic-embryogenic cultures have been used for genetic transformation in *Picea* spp., *Pinus* spp., *Larix* spp., *Populus* spp., and others (Newton et al. 2001; Cerda et al. 2002). So far, a few important genes, including *rolC* and those for lignification, insect resistance, reproductive sterility, drought and herbicide resistance have been introduced in forest trees using several transformation methods, including biolistics and *Agrobacterium*-mediated transformation (Newton et al. 2001). Gradual progress is being made in producing transgenic trees due to the monitoring of transgene expression during the long-life cycles of forest trees. Transgenes may cause immediate abnormality, remain inactive for a long time and become active under favorable conditions, or could even be lost during the long-vegetative phase of the trees. Therefore, it is rather difficult to predict the behavior of transgenes in the future.

10.3.5 Mass Clonal Propagation

Somatic embryogenesis appears to be a feasible approach for mass-clonal propagation. For example, the hybrid sweet gum (Vendrame et al. 2001) is essential for commercial exploitation and has two advantages:

1. The commercial exploitation of unique combinations of invaluable genetic traits that can not be reproduced through conventional breeding.
2. The harvesting of uniform material, leading to many opportunities for increasing the efficiency of wood-based manufacturing (Timmis 1998).

10.3.6 Induced Mutations

Genetic variation is helpful in crop improvement, which can be induced with physical and chemical mutagens, and somaclonal variation in seed and vegetative-propagated crops. Gamma radiation is commonly used for mutation induction. The selection of experimental material for irradiation is important for preventing chimerism in regenerated plants. Irradiation of seeds, budwoods, axillary buds, or shoot tips will result in chimeric plants. Several generations, M1V1–M1V4, are needed to dissociate chimerism for the selection of pure desirable mutants, which is time consuming and costly.

Embryogenic cell-suspension cultures are well suited for gamma irradiation because they originate from a single cell (Jain et al. 1989). This approach prevents chimerism and obtains pure-mutant lines. FAO/IAEA Joint Division has initiated several projects on tropical and subtropical fruits, including banana and date palm. The objectives are to improve these fruit crops by induced mutations and biotechnology (Jain 2002b).

10.4 Conclusion

Somatic embryogenesis has the vast potential to produce plants in their millions, and has now become a routine protocol for many trees. However, the use of SE in a wide range of woody plants is yet to be utilized due to several limitations facing the process, including poor germination of somatic embryos, genotypic influences, a limited number of explants inducing SE, and somaclonal variation. These limitations have hindered the commercialization of SE in a wide range of forest trees. Further research is required at both the biochemical and molecular levels to understand the mechanism of SE, enabling the induction of SE in other recalcitrant woody species. A sharp focus is needed to increase the rate of ESM establishment, improving yield from established ESM, and increasing the germination rate of somatic embryos for commercialization, without which this technology will have very limited scope (Timmis 1998). Automation of somatic-seed production is the ultimate goal of commercial seed industries. The combination of high technology with tissue

culture, including computer-aided image analysis, robotics, bioreactors (including temporary immersion systems) somatic-embryo encapsulation, development of appropriate somatic-embryo coating material, etc., require further investment for producing millions of somatic seeds in a short time and to cut down the cost of seed production. The genetic fidelity of somatic seedlings is important for all of this, which can be tested with molecular markers, such as AFLPs or microsatallites. Although several molecular markers are available, a reliable molecular diagnostic kit is still needed for the early detection of genetic variability during plant development.

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11
Comparative Studies on the Survival and Growth of Seedlings and In Vitro-Raised Plants of *Shorea robusta* and *Dipterocarpus turbinatus*

SHYAMAL ROY

11.1 Introduction

Dipterocarp trees (family Dipterocarpaceae) of South and Southeast Asia provide valuable hardwood timber. *Shorea robusta* and *Dipterocarpus turbinatus* are the two major dipterocarp species in Bangladesh. *Shorea robusta* Gaertn. f. is locally known as "Sal" in Bangladesh (FAO 1985). The species has a fairly wide but interrupted distribution in Bangladesh, extending from Panchagar in the north, Sherpur in the east and Comilla in the south. The total area of Sal forest in Bangladesh is about 110,000 ha, with 86% in the central region and 14% in the northern region (Ghani et al. 1990). *Dipterocarpus turbinatus* is distributed in the forests of Chittagong and Chittagong Hilltracts, in the southeast of Bangladesh.

Due to indiscriminate logging, many of the dipterocarp forests are being depleted. Seeds of these species exhibit loss of viability after 4 days of maturity due to a reduction in moisture of less than 37%, and become nonviable on the eighth day. Thus, due to a very short viability period, seeds cannot be preserved in conventional seed banks. Therefore, concerted efforts must be made to develop methods for both the mass multiplication of these forest trees, and the conservation and cloning of superior genotypes for the use in tree-improvement programs. As tissue-culture technologies can provide methods for both large-scale propagation and the improvement of tree species, a program was undertaken to establish efficient and reproducible methods for in vitro propagation of these two species. Side by side, seedlings produced from viable seeds were compared with in vitro-produced plants for their survival and growth.

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11.2 Materials and Methods

Seeds of *Shorea robusta* and *Dipterocarpus turbinatus* were collected, and seedlings were raised in the nursery under tissue-culture laboratory conditions. Shoot tips and nodal segments were taken from 1–4-month-old seedlings and used as explant material for in vitro propagation. The explants were washed thoroughly under running tap water. Surface sterilization was achieved with 0.2% $HgCl_2$ for 5 minutes, followed by 3–5 washings with sterile distilled water. Explants were cultured in a 0.7% agar-gel MS (Murashige and Skoog 1962) medium with 3% sucrose, and different concentrations and combinations of auxin and cytokinin were used for shoot proliferation.

For root induction, different concentrations and combinations of auxins were used with half strength MS nutrient medium. The effects of casein hydrolysate (CH) and coconut milk (CM) on shoot multiplication and growth were also studied. The pH of the medium was adjusted to 5.8 before autoclaving. The cultures were incubated at 24 ± 2°C under a 16 h photoperiod, with a light intensity of 5,000 lux under white fluorescent tubes. The cultures were subcultured on fresh media at 4-week intervals. All treatments consisted of 20 replicates. The in vitro-regenerated plantlets were removed from the culture flasks, washed to remove the nutrient agar medium from the roots, and transplanted into small pots containing sterile soil and compost. The plantlets were subsequently transferred to larger pots, and gradually acclimatized to outdoor conditions when their length was 20 cm.

To get seedling-raised plants, seeds were sown in polyethylene bags with alluvial soil and compost. The percent of seeds that germinated and the survival rate of seedlings at the age of 6 months were recorded. When the seedlings were 20 cm in length they were transplanted in the field. All categories of plants were planted in three blocks of 25 plants. The distance between plants at their base was 150 cm. The height and base diameter of seedlings and in vitro-raised plants were measured every 6 months up to 2 years, and then every 12 months up to 5 years.

11.3 Results

11.3.1 In Vitro Regeneration

Explants grown under in vitro conditions showed different responses when cultured in full strength and half strength MS-nutrient media with various concentrations and combinations of cytokinin and auxin. For both species and all experiments, half strength MS medium was found to be most suitable for shoot multiplication. Within 2 days of culture, the gelled medium absorbed the oxidized phenolic compounds from the explants and turned brown. To overcome the browning, the explants were initially cultured in a liquid medium with 0.25% activated charcoal (AC) and 0.01% ascorbic acid. Explants were transferred to a fresh medium every 24 h and agitated in a rotary shaker at 60 rpm. After 7 days, the explants were transferred to an agar medium with 0.3% aqueous polyvinyl pyrrolidone.

Table 1. The effect of growth regulators on shoot proliferation and number of shoots per culture, established from seedling explants of two species of dipterocarps

Growth regulators (mg l^{-1})	*Shorea robusta*		*Dipterocarpus turbinatus*	
	% of shoot-proliferating cultures	Average number of shoots per culture	% of shoot-proliferating cultures	Average number of shoots per culture
BA + NAA				
0.50 + 00	16	2.1	24	2.4
1.00 + 00	21	3.1	22	2.6
1.50 + 00	21	3.4	31	7.3
2.00 + 00	49	4.1	29	9.6
2.25 + 00	37	4.0	67	18.4
2.50 + 00	63	8.7	41	5.4
1.00 + 0.5	26	3.6	36	3.3
2.00 + 1.0	32	3.8	37	3.8
2.25 + 1.0	32	4.1	35	4.6
2.50 + 1.0	43	4.5	37	4.6

Data are from the fourth subculture, and results are the average of three experiments with 20 replications

BA: Benzyladenine

NAA: α-naphthalene acetic acid

11.3.1.1 *Shorea robusta*

Of the two cytokinins, Benzyladenine (BA) was superior to kinetin (Kn) for shoot regeneration. Explants responded best for shoot regeneration on half strength MS medium containing 2.5 mg l^{-1} BA, and were excised and reinoculated into fresh medium with the same combination of hormones (Table 1). In each subculture, axillary and adventitious shoots grew from each shoot and lateral branch. A clump of six to ten shoots was produced after three to four subcultures. CH (50–150 mg l^{-1}) showed no effect on shoot multiplication and growth, but the addition of 15% CM enhanced the growth of regenerated shoots. Consequently, the best medium determined for shoot multiplication and growth was ½MS with 2.5 mg l^{-1} BA + 15% CM.

11.3.1.2 *Dipterocarpus turbinatus*

The cotyledonary node of a germinated seed was found to be the best explant for shoot multiplication of *D. turbinatus*. The explants became swollen and produced four to five shoots within 3–4 weeks of inoculation in half strength MS medium supplemented with 2.25 mg l^{-1} BA. The number of shoots increased with the number of subcultures, and after five subcultures number of shoots was 10–5 shoots per culture. For further development of the medium, CH (50–150 mg l^{-1}) and CM (5%–20%) were added individually, and it was found that 10% CM was effective for satisfactory growth. Thus, the medium determined was ½ MS + 2.25 mg l^{-1} BA + 10% CM for shoot multiplication and growth.

Table 2. Effect of auxin(s) on root induction in regenerated shoots cultured in half-strength MS medium

Auxin (mg l^{-1})	*Shorea robusta*	*Dipterocarpus turbinatus*
	% of shoots rooted (± SE)	% of shoots rooted (± SE)
IBA 0.5	12 (3.1)	19 (6.5)
IBA 1.0	22 (6.4)	18 (4.1)
IBA 1.0	21 (6.3)	17 (3.6)
IBA 2.0	22 (5.6)	19 (4.1)
IBA 2.5	28 (3.2)	18 (3.2)
IBA 0.5 + NAA 0.5	69 (4.5)	66 (7.2)
IBA 1.0 + NAA 0.5	81 (2.1)	64 (5.3)
IBA 1.0 + NAA 1.0	90 (6.2)	93 (7.4)
IBA 1.5 + NAA 1.0	70 (5.6)	77 (8.3)
IBA 2.0 + NAA1.0	70 (4.1)	89 (4.9)
IBA 2.0 + NAA 2.0	61 (5.1)	79 (6.2)
IBA 2.5 + NAA 2.0	42 (4.3)	71 (3.6)
IBA 0.5 + IAA 0.5	33 (3.1)	63 (4.7)
IBA 1.0 + IAA 0.5	31 (2.8)	69 (5.8)
IBA 1.0 + IAA1.0	35 (4.1)	43 (5.9)
IBA 1.5 + IAA 1.0	20 (5.1)	39 (7.2)
IBA 2.0 + IAA 1.0	20 (6.3)	31 (5.3)
IBA 2.0 + IAA 2.0	20 (7.3)	42 (6.9)
IBA 2.5 + IAA 2.0	20 (3.4)	33 (7.1)

Data were recorded after 4 weeks of culture

IBA: Indole-3-butyric acid

NAA: α-naphthalene acetic acid

IAA: Indole-3-acetic acid

SE: Standard error

11.3.1.3 Rooting of In Vitro-Regenerated Shoots

For rooting, the in vitro-regenerated shoots were excised and implanted on half strength MS medium in combination with 1.0 mg l^{-1} each of Indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA). Stout roots with laterals emerged in 90% of regenerated shoots within 4 weeks of culture (Table 2).

The in vitro-regenerated plantlets were removed from the culture vessels, washed thoroughly of the nutrient medium and transplanted into small pots containing sterile soil and compost. The plantlets were reared in a chamber at a semi-controlled temperature (25°–32°C) and at 2,000 lux. After 25–30 days, the plantlets were transferred to an open room and gradually acclimatized to outdoor conditions. About 55% mortality occurred following transplantation of the plantlets.

11.3.2 Seedlings Raised From Seeds

Seed germination was 70% for *S. robusta* and 85% for *D. turbinatus*. Mortality of seedlings due to leaf necrosis, up to the age of 6 months, was 55% in *S. robusta* and 20% in *D. turbinatus*.

11.3.3 Growth of Transplanted Plants

At 6 months, the mean height and base diameter of the seedlings were 36 cm and 0.6 cm, respectively, in *S. robusta* and 38 cm and 0.9 cm in *D. turbinatus*. The in vitro-raised plantlets were much smaller, with a mean height and base diameter of 18 cm and 0.3 cm in *S. robusta* and 23 cm and 0.4 cm in *D. turbinatus*.

At the age of 2 years, the mean height, base diameter, and diameter at breast height of the seedling-raised plants were 210 cm, 4.1 cm, and 1.6 cm in *S. robusta* and 240 cm, 6.8 cm, and 3.0 cm in *D. turbinatus*. For the in vitro-raised plants, these values were 226 cm, 4.0 cm, and 1.5 cm in *S. robusta* and 235 cm, 6.3 cm, and 2.3 cm in *D. turbinatus* (Figs. 1, 2).

The mean height, base diameter and diameter at breast height of 5-year-old seedling plants was 750 cm, 15.5 cm, and 9.4 cm in *S. robusta*, and 1050 cm, 20.2 cm, and 14 cm in *D. turbinatus*. For the in vitro-raised plants, the values were 902 cm, 18.6 cm, and 13.7 cm in *S. robusta* and 1200 cm, 24 cm, and 16.4 cm in *D. turbinatus* (Figs. 1, 2).

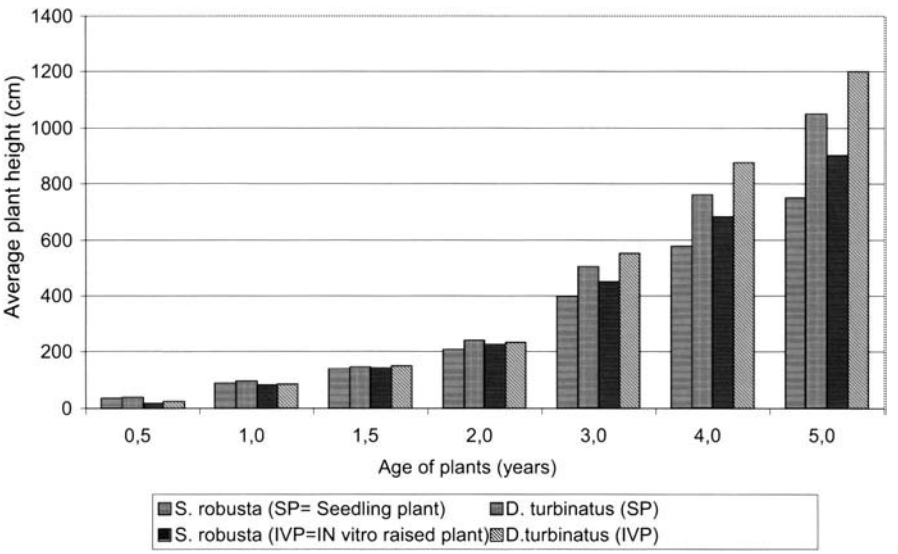

Fig. 1. Average height of the seedlings and in vitro-raised plants of *S. robusta* and *D. turbinatus*

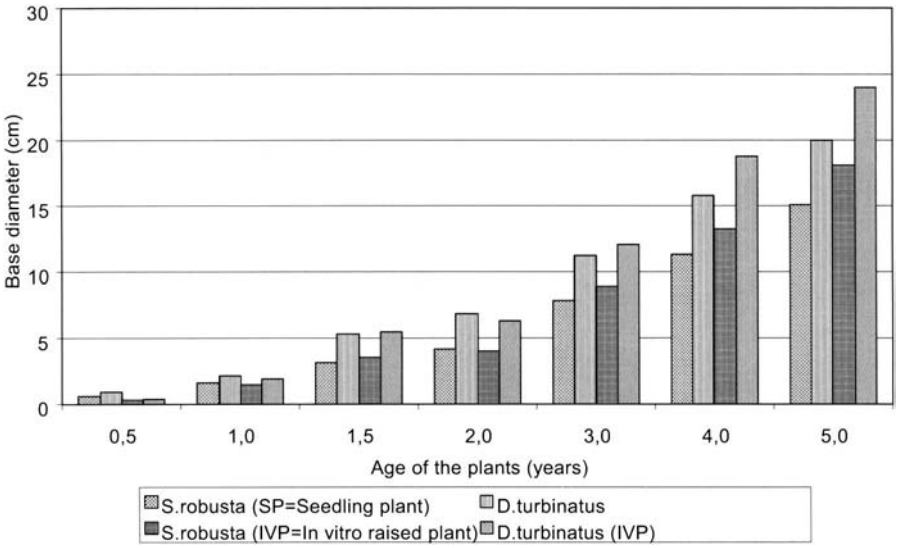

Fig. 2. Average base diameter of the seedlings and in vitro-raised plants of *S. robusta* and *D. turbinatus*

11.4 Discussion

Mature trees demonstrating superior genotypes are ideal for mass propagation. However, it is usually more difficult to establish shoot cultures from mature trees than from juvenile plants (Bonga 1987, Hackett 1987). We tried repeatedly for shoot multiplication from mature plants of different species of dipterocarps but were unsuccessful, whereas in other tree species, such as *Techtona grandis* (Gupta et al. 1980), *Albizia lebbek* (Gharyal and Maheshwari 1982), *Artocarpus heterophyllus* (Roy et al. 1990), and *Mitragyna parvifolia* (Roy et al. 1988), shoot proliferation in explants of mature trees occurred. According to Bonga (1982), multiplication of shoot-bud explants of both tropical and temperate trees is easier if the explants are derived from germinated seedlings. However, in the case of dipterocarps, the explants turned brown and ultimately died, due to excessive exudation of phenolic compounds. The addition of activated charcoal, ascorbic acid, and polyvinyl pyrrolidone into the medium could be effective for overcoming this problem. Linington (1991) also used activated charcoal to neutralize the effect of phenolic compounds in the medium of *D. alatus* and *D. intricatus* cultures.

Claims have been made that tissue-culture systems would enable large-scale, rapid propagation through cloning, and prove useful in commercial forestry (Bonga and Durzan 1987), but this has not eventuated because such techniques are still tedious, time consuming, and expensive. Plants grown from seedlings grew faster than in vitro-raised plants in the first 6 months, although after 2 years the tissue-cultured plants had similar growth and survival.

For large-scale planting of the two species of dipterocarps discussed here, seed collection at the proper maturation time and seedling management should be the strategies used until a low-cost tissue culture technique for mass propagation is developed (Mascarenhas et al. 1988, Moura-Costa 1994). From the results obtained through the present experiments, it can be concluded that for a large-scale plantation program of these two dipterocarp species, the collection and sowing of seeds at the proper maturation time, and the management of seedlings is superior to the in vitro-regeneration method.

Acknowledgment

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12
Tissue Culture of *Swietenia macrophylla* King (Big-Leaf Mahogany)

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12.1 Introduction

Swietenia macrophylla King (big-leaf mahogany), which grows as natural stands in tropical America with a distribution from the Peninsula of Yucatán in México to the Amazonian region of Brazil, Perú, and Bolivia (Encarnación 1983), is one of the most valued timber trees in the world. Big-leaf mahogany wood (in tropical America also named "caoba," "aguano," "mogno," "acajou," and "mara") is greatly valued for its esthetic appeal, ductility, and durability and is used for high-quality furniture manufacture, general joinery work, fine decorative-veneer production, boat construction, and so on (Chichignoud et al. 1990).

Due to continuous selective exploitation, this species has deteriorated in terms of abundance and genetic resources. Germplasm conservation has become necessary for a future sustainable management system and as a means to maintaining genetic diversity for preventing genetic erosion. In the tropical regions of Central and South America, *S. macrophylla* and other Meliaceae have been used for reforestation. However, they have been damaged severely by the shoot-borer (*Hypsipyla grandella* Zeller), the most harmful insect pest against Meliaceae (Maruyama et al. 1989a). For resistant-tree breeding, the selection and propagation of resistant clones are essential.

Tissue culture can be used as a technique for the propagation and conservation of selected clones. However, to my knowledge, only a few studies in tissue culture (Venketeswaran et al. 1988, Lee and Rao 1988, Maruyama et al. 1989b, Kondo and Okamura 1994) and no studies on somatic embryogenesis have been documented on big-leaf mahogany.

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This chapter describes shoot-tip culture and somatic embryogenesis from seedlings and young trees as the basis for the further application of tissue culture to propagation and conservation of selected clones, or as a tool for the genetic engineering of this species.

12.2 Materials and Methods

12.2.1 Multiplication and Plant Regeneration

Seeds of *S. macrophylla* were surface sterilized on a clean bench by agitating them in 70% (v/v) ethanol for 2 min and then in 1% (w/v available chlorine) sodium hypochlorite solution for 15 min. After surface sterilization, seeds were washed three times in sterile double distilled water and placed individually onto a 0.8% (w/v) agar medium (15 ml), containing 2% (w/v) sucrose, in 1.8- × 18-cm test tubes.

About 20-mm long apical shoots from aseptically germinated seedlings were used for initial explants. Explants were cultured on plant growth regulator-free WPM (Lloyd and McCown 1980), containing 0.5% (w/v) activated charcoal, for 2–3 months. Then, shoot-tips were excised (about 20 mm long) and cultured on WPM supplemented with 10 μM BAP (6-benzylaminopurine) or ZEA (trans-zeatin).

For rooting experiments, shoots were transferred to half-strength WPM supplemented with IBA (indole-3-butyric acid) alone or in combination with NAA (naphthaleneacetic acid), or to plant growth regulator-free medium.

Proliferated shoots that were less than 15 mm long were transferred to a medium supplemented with a low concentration of cytokinin (0.2 μM BAP or ZEA), alone or in combination with a low concentration of IBA (0.1 μM), or to a plant growth regulator-free medium containing activated charcoal, to induce elongation.

Culture media were adjusted to pH 5.8 before autoclaving for 15 min at 121°C (1.1 kg cm^{-2}). Agar (Wako Pure Chemical Industries, Osaka, Japan) was used as the gelling agent. Cultures were kept in a culture room at 25°C under white fluorescent light of about 65 μmol m^{-2} s^{-1} photon flux density (400–700 nm), with a regime of 16 h light and 8 h dark.

Rooted shoots were transferred into pots filled with vermiculite and acclimatized in a growth cabinet at 25°–30°C under a photon flux density of about 35 μmol m^{-2} s^{-1}, with a 16-h photoperiod. Plantlets were acclimatized to high relative humidity (about 90%–95%) during the first two weeks, in plastic boxes with transparent covers. After that, the cover was opened gradually over the next two weeks, and removed completely about one month after transplanting. Acclimatized plantlets were irrigated with water for the first two weeks, and then with 0.1% (v/v) Hyponex 5-10-5 plant-food solution (Hyponex, Osaka, Japan).

12.2.2 Somatic Embryogenesis

Embryogenic cultures were initiated from shoot-tip explants taken from aseptically germinated seedlings. Explants were cultured at 1–2 month intervals on WPM, containing 1–10 µM ZEA, at 25°C under white fluorescent light of about 65 µmol m^{-2} s^{-1} photon flux density, with a 16-h photoperiod.

Fresh solid or liquid media of the same composition, in combination with plant growth regulator-free medium containing activated charcoal, were used for the proliferation and maintenance of embryogenic calli. Cultures were kept under a photon flux density of about 35 µmol m^{-2} s^{-1}, or 25 µmol m^{-2} s^{-1} for liquid culture, at 70–80 rpm.

Plant growth regulator-free medium or media containing a low concentration of ZEA, supplemented with glutamine, asparagine, arginine, proline, and lysine, were used for the maturation and germination of somatic embryos.

12.3 Results and Discussion

12.3.1 Multiplication and Plant Regeneration

After 1–2 months of culture, a five to seven-fold shoot multiplication rate was achieved with subcultured shoot-tips on WPM containing 10 µM ZEA (Fig. 1D). However, a large variation in the shoot multiplication rate was observed between clones from shoot-tips of aseptically-germinated seedlings. The shoots per explant varied from 1 to 16. BAP at the same concentration was also effective for inducing multiple shoots, but resulted in less than the ZEA-induced shooting rate.

Multiple shoots (Fig. 1C) were also obtained from coppice shoot-tips of 3-year-old (6–10-m tall) greenhouse-grown trees (Fig. 1A). However, serious problems regarding culture contamination with the clones tested did not permit efficient propagation. I tried several sterilants, such as ethyl alcohol, hydrogen peroxide, mercuric chloride, sodium or calcium hypochlorite, and fungicides and antibiotics, alone or in succession, at different concentrations and length of times to obtain pathogen-free cultures for a long time, but without success. In some cultures, where the contaminant was not expressed during the initial culture or subcultures, contamination was observed after the fourth, fifth, or later subculture routines. This result suggests that the difficulty to obtain pathogen-free cultures was related to the presence of endogenous contaminants in donor plants. Shoot-tips of sprouts from cut branches put in water inside a growth cabinet (Fig. 1B) also failed to produce pathogen-free cultures.

For rooting, shoots longer than 15 mm were transferred to rooting media. The results of rooting experiments are shown in Table 1. After about 2 months of culturing, more than 50% of explants formed roots in all treatments (Fig. 1E). Although a

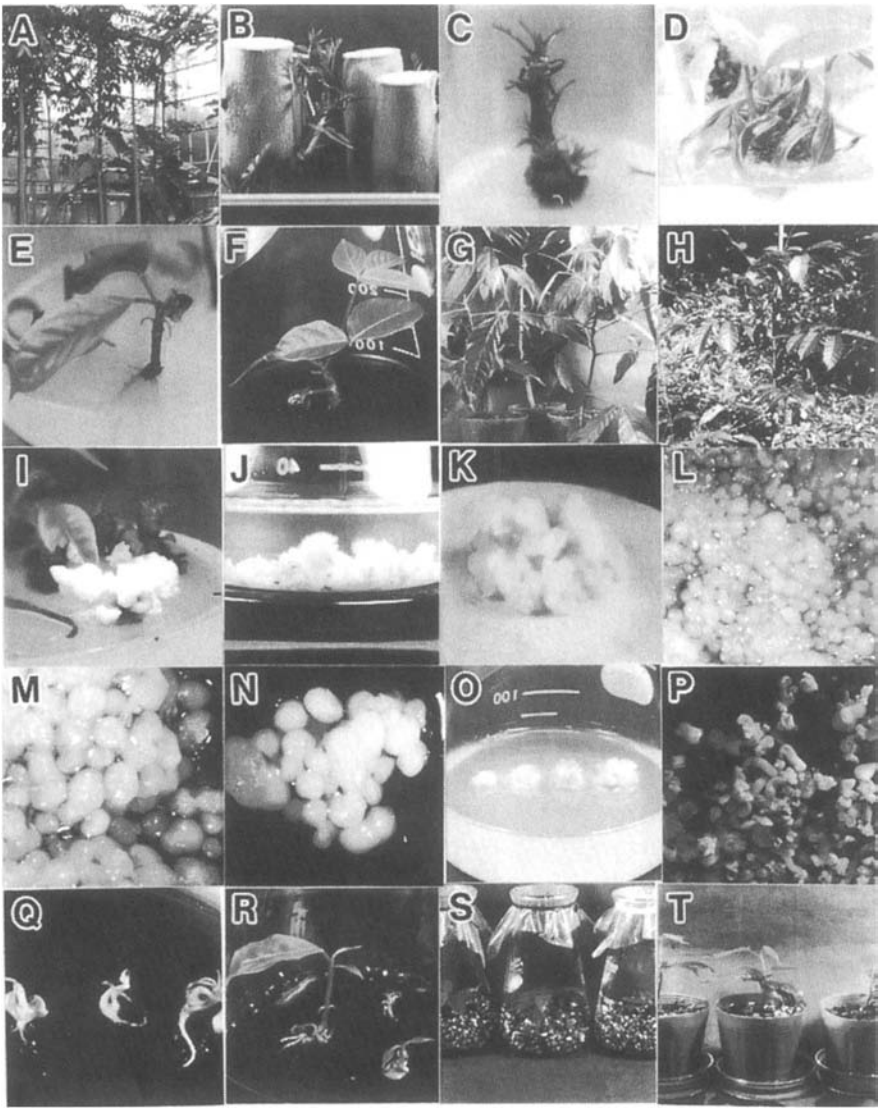

Fig. 1. Tissue culture of *Swietenia macrophylla*. **A** 3-year-old (6–10 m tall) greenhouse-grown trees. **B** Sprouts from cut branches put in water. **C** Multiple shoots from coppice shoot-tips. **D** Multiple shoot formation on subcultured shoot-tips from aseptically germinated seedlings. **E** Rooting of proliferated shoots. **F** Root development on plant growth regulator-free medium containing activated charcoal. **G** Successfully acclimatized plantlets. **H** Plant growing in the field. **I** Induction of primary embryogenic tissues at the bases of shoot explants. **J** Proliferation of embryogenic tissues in liquid medium. **K** Proliferation of embryogenic tissues on solid medium. **L-O** Formation of embryo-like structures. **P** Maturation of somatic embryos. **Q** Germination of somatic embryos.**R** Regenerated emblings. **S** Growth of emblings in vitro. **T** Acclimatized emblings

Table 1. Effects of IBA and NAA on the rooting of proliferated shoots of *Swietenia macrophylla*

IBA-NAA concentration (μM)	Explant tested	Explant rooted	Rooting rate (%)	Number of primary roots per explant		Number of secondary roots per explant	
				Mean	Range	Mean	Range
0.0–0.0	20	12	60	1.2	1–2	1.2	1–4
2.5–0.0	20	12	60	1.5	1–2	1.3	1–5
2.5–0.25	20	11	55	2.0	1–3	6.7	1–19

Data were taken after 2 months of culturing on half-strength WPM

IBA, indole-3-butyric acid

NAA, naphthaleneacetic acid

large difference regarding the rooting rate between treatments was not observed, the root system of rooted shoots was better in media containing IBA in combination with NAA, increasing the mean number of secondary roots per explant by more than five times.

After root development on the plant growth regulator-free medium containing activated charcoal (Fig. 1F), plantlets were transferred into pots with vermiculite, and successfully acclimatized in a growth cabinet by the method described above. All plants survived and grew well (Fig. 1G). Then, the acclimatized plantlets were transferred to the field (Fig. 1H).

12.3.2 Somatic Embryogenesis

After several consecutive subcultures of shoot-tips on WPM containing 1–10 μM ZEA, primary embryogenic tissues were obtained at the bases of explants in contact with the medium (Fig. 1I). Embryogenic cultures were subcultured to fresh liquid or solid medium of the same composition every 4-6 weeks. Proliferation and maintenance of embryogenic cultures were possible in both liquid (Fig. 1J) and solid media (Fig. 1K). However, the solid medium was better for long-term culture. I have been maintaining embryogenic cultures in a solid medium for more than 2 years without the loss of embryogenic potential.

The formation of embryo-like structures and development of different stages (Fig. 1L–O), including abnormal or aberration structures, were observed. Recurrent embryogenesis was frequent and numerous secondary small embryoids were formed, mostly on cotyledonary tissues forming a new generation of embryoids.

Maturation (Fig. 1P) and germination (Fig. 1Q) of somatic embryos were obtained in a plant growth regulator-free medium or that containing a low concentration of ZEA (0.1 μM), supplemented with glutamine (0.5 g/l), asparagine (0.3 g/l), arginine (0.1 g/l), proline (0.04 g/l), and lysine (0.04 g/l), or in combination with media of the same composition supplemented with 0.5%–1% (w/v) activated charcoal. Although the germination of somatic embryos occurred frequently, the majority of germinated embryos did not have a well-developed epicotyl. Subsequently, the normal plant conversion rate was very low. Regenerated emblings (Fig. 1R)

were transferred to flasks containing vermiculite, irrigated with 0.1% (v/v) Hyponex solution (Fig. 1S), and kept in a culture room for about 1 month before acclimatization (Fig. 1T).

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13
Micropropagation of *Shorea roxburghii* and *Gmelina arborea* by Shoot-Apex Culture

KENTARO NAKAMURA

13.1 Introduction

Shorea roxburghii G. Don is a tropical rainforest tree species belonging to the white-meranti group of Dipterocarpaceae, and is commonly used for plywood, construction timber, and furniture. It is distributed from Eastern India to Southeast Asia, and grows to over 40 m in height and 1 m in trunk diameter (Soerianegara and Lemmens 1994). This species is useful for silviculture in the tropics, because of its tolerance to heavy drought and high survivorship after forest fire. Dipterocarp species have been propagated mostly from seeds and cuttings, but seed production is erratic and infrequent, and seeds deteriorate rapidly in storage. In addition, the number of mother trees has decreased rapidly due to deforestation and disorderly-shifting cultivation. Propagation by cutting from mature trees is difficult, and the plagiotropic growth of shoots is also a problem.

In recent years, many researchers have designed tissue-culture techniques in an effort to solve these problems (Scott and Rao 1988, 1989; Scott et al. 1995; Vaario et al. 1995). They have used embryos or germinated seedlings under aseptic conditions as explants. However, there have been only a few reports of clonal propagation of aged seedlings by tissue culture, as follows. *Shorea roxburghii* was propagated by axillary-bud culture from 3-year-old seedlings raised in a greenhouse (Nakamura et al. 1994). Roy (1997) propagated *Shorea robusta* and *Dipterocarpus turbinatus* by shoot-apex and axillary-bud culture from 1- to 4-month-old seedlings raised in a greenhouse. *Hopea odorata* was also propagated by shoot-apex and axillary-bud cultures from 15–20-year-old trees (Roy et al. 1997). However, the productivity of clonal stocks produced by these methods is not high enough for practical stock production, and a more efficient culture method for the rapid propagation of stocks is urgently needed. Here, I describe a stable method for the clonal propagation of *Shorea roxburghii* by shoot apex culture.

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Gmelina arborea Roxb. is a multipurpose and fast growing tree. It is commonly used for pulp, fiberboard, particleboard, and plywood. Because of its multiple uses and vigorous growth, it is widely planted as commercial plantation in some tropical areas. *Gmelina* is propagated by both seed and cutting. In recent years, many researchers have reported on vegetative-propagation efforts for the multiplication of superior trees. However, most of the research is based on explants from young seedlings. In woody plants, rooting-success rate declines as trees become older. When we tested cuttings of *Gmelina*, a similar tendency was found (K Nakamura et al. unpublished); the rooting rate of 1-year-old seedlings was 100%, 2-year-old seedlings was 70%, and 3-year-old seedlings was 60%. This limitation of decreasing rooting success with age adversely affects operational-plantation programs. To maximize plantation growth and uniformity, techniques need to be developed to clone mature trees.

Tissue-culture techniques may be useful in the propagation of superior individuals from mature trees. Tissue culture on *Gmelina* has been attempted by several researchers (Yang 1993; Crizaldo 1980; Roy et al. 1992; Kannan and Jasrai 1996; Naik et al. 2003). The culture from a shoot apex is useful for the micropropagation of stock, since many explants are available from a mature tree, and sterilization of the explants is easy. In addition, there is an advantage to using shoot-apex culture, in that it contains less polyphenolic compounds that often inhibit explant growth, although some woody plants contain large amounts of these compounds in the shoot. Therefore, shoot-apex culture is useful for studying tissue culture in tropical areas where contamination occurs frequently. In the paper, we report on the clonal propagation of plantlets of *Gmelina* by shoot-apex culture from 4-year-old mature trees.

13.2 Materials and Methods

13.2.1 *Shorea roxburghii*

13.2.1.1 Preparation and Sterilization of Explants

Secondary branches, about 2 cm long and with apices, were cut from 5-year-old seedlings raised in a greenhouse. The branches were washed in a 2% neutral household-detergent solution with a brush, and rinsed in running tap water for 30 min. For surface sterilization, the branches were soaked in 70% ethanol for 30 sec and then 1% NaClO solution with 4 drops (1 drop/50 ml) of Tween 80 for 7 min. The branches were rinsed five times in sterile distilled water, and then dried on sterile filter paper on a clean bench. The effect of plant-growth regulators on multiple-bud induction and propagation was examined.

13.2.1.2 Shoot Apices Excised from Branches Under a Dissecting Microscope

The branches were cultured in 16- × 150-mm test tubes with 10 ml of half MS (½MS) liquid medium, in which the concentrations of inorganic elements were reduced to half of those in standard MS medium (Murashige and Skoog 1962). The

medium contained 30 g l^{-1} of sucrose, and was supplemented with 0, 0.02, 0.1, 0.2, 1.0, 2.2, or 4.4 mg l^{-1} of 6-benzylaminopurine (BAP), zeatin, or 1-phenyl-3-(1,2,3-thiadiazol-5-yl)-urea (thidiazuron). The pH of the medium was adjusted to 5.7 before autoclaving at 121°C for 15 min. Twenty explants were cultured in each treatment.

13.2.1.3 Effect of Carbon Source on Multiple-Bud Induction and Propagation

Five types of carbon sources were examined: 30 g l^{-1} sucrose; 60 g l^{-1} dextrose; 30 g l^{-1} maltose; 30 g l^{-1} trehalose; and a combination of 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose. From the results of the preliminary test in 13.2.1.2-examinations, I chose a ½MS liquid medium containing 1.0 mg l^{-1} BAP or 1.0 mg l^{-1} zeatin as a basal medium. Multiple buds induced from shoot apices in the medium after 3 months of culture were divided into single buds. Each single bud was then subcultured into the same medium. Explants were maintained under a 24-h photoperiod at 25 ± 2°C, with horizontal rotation at 1 rpm. Light intensity was between 1000 lux (at the bottom of the rotation) and 12000 lux (at the top of the rotation). Twenty explants were cultured in each treatment, and the experiment was repeated three times. Cultures were subcultured every 20 days and maintained for 6 months. After 6 months of culture, masses with more than five buds formed from single buds, and were counted as multiple buds.

13.2.1.4 Effect of Carbon Source on Shoot Elongation from Multiple Buds

Multiple buds formed in the previous culture were transferred into 300-ml conical flasks with 40 ml ½MS liquid medium containing 5.2 g l^{-1} of dextrose plus 5.2 g l^{-1} of maltose, and 1.0 or 4.4 mg l^{-1} of zeatin. To determine the best carbon source for shoot elongation, buds were also cultured in ½MS liquid medium containing 1.0 mg l^{-1} zeatin and different concentrations and combinations of carbon sources. These were: 30 g l^{-1} sucrose; 60 g l^{-1} dextrose; 30 g l^{-1} maltose or 60 g l^{-1} fructose; 15 g l^{-1} dextrose and 15 g l^{-1} fructose; 20 g l^{-1} dextrose and 10 g l^{-1} maltose; or 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose. Ten multiple buds were cultured in each treatment. Cultures were kept for 1 month under 3000-lux fluorescent light with a 16-h photoperiod at 25 ± 2°C in a 60-rpm gyratory culture.

13.2.1.5 Effect of Rooting Medium

Elongated shoots were excised from multiple buds and transplanted into rooting media in 70 mm wide × 121 mm high plant-culture jars (Agripot, Asahi Techno Glass, Tokyo, Japan). For the rooting medium, we used Florialite (Nisshinbo Industries, Tokyo, Japan), which is a compressed compound of vermiculite and cellulose fiber, vermiculite, peat moss, a mixture of vermiculite and peat moss (1:1 v/v), or a mixture of vermiculite and perlite (1:1 v/v), with a supplement of ½MS liquid medium containing 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose. As the control medium, we used ½MS medium containing 5.2 g l^{-1} dextrose, 5.2 g l^{-1} maltose and 3.0 g l^{-1}

Gelrite (Merck, Rahway, NJ, USA). Shoots were maintained under 3000-lux a fluorescent lights with a 16-h photoperiod at 25 ± 2°C. Thirty explants were cultured in each treatment, and the treatments were repeated three times.

13.2.1.6 Acclimatization

Two hundred plantlets regenerated in Florialite were transplanted into pots (diameter 10.5 cm, height 9.3 cm), which were filled with vermiculite and covered with clear-plastic boards (diameter 9 cm, height 12 cm) in a greenhouse. One week after transplanting, the boards were removed.

13.2.2 *Gmelina arborea*

13.2.2.1 Induction of Multiple Shoots and Regeneration of Plantlets

To study the effects of age, explants (terminal buds about 4 cm in length) were excised from 4-year-old *Gmelina* trees, about 10 m in height, and 1 year-old seedlings, about 2 m in height. Explants were washed in a neutral detergent with a brush and rinsed with tap water for 5 min. They were surface sterilized with 70% ethanol for 30 sec and 2% sodium hypochlorite with 6 drops of Tween 80 (1 drop/50 ml) for 6 min successively, and then rinsed five times in sterile distilled water. After surface sterilization, the explants were dried in dishes for 5 min. Stipules were removed, and a 4-mm-long shoot apex with four leaf primordia was excised under a dissecting microscope.

Shoot apices were cultured on half strength Gamborg's B5 (½B5) solid medium, solidified by 3.2 g l^{-1} of Gelrite or in a liquid ½B5 medium containing 0.0 and 0.002 mg l^{-1} of indole-3-butyric acid (IBA) and 0, 0.01, 0.03, 0.1, 0.3 and 1.0 mg l^{-1} of benzylaminopurine (BAP) with 2% sucrose. Explants were cultured in the liquid medium with continuous horizontal rotation at 1 rpm and under a 16 h photoperiod at 12,000 lux. Explants on the solid medium were maintained under a 16 h photoperiod (3,000 lux). All cultures were incubated at 25 ± 2°C in a culture room. Ten explants were used in each treatment. Induced multiple shoots were subcultured into the same medium at intervals of 30 days for the shoot elongation. For rooting, shoots over 3 cm in height were excised from multiple shoots and planted on the same medium, or on the hormone-free ½B5 medium.

13.2.2.2 Acclimatization of Plantlets

Two experiments were conducted to investigate acclimatization: (1) three plantlets were transplanted into 500 ml conical flasks one by one, which were filled with 200 ml of sterilized vermiculite, and incubated for 2 weeks; and (2) two plantlets were directly transplanted into pots, which were filled with sand, and raised in a cuttage box for 2 weeks. The cuttage box was made from Ulin (*Eusideroxylon zwageri* Teijsm et Bin) wood and filled with sand to a depth of 10cm. The box was placed in a green house and covered with plastic film to maintain ambient humidity. A shadecloth was placed 2 m above the box to maintain the light intensity at 5,000 lux and to reduce temperature.

13.2.2.3 Plantation of Plantlets

After 2 weeks of raising in a conical flask or cutting box, the plantlets were transferred to the nursery and raised for 1 month. Following this, the plantlets were planted in an experimental field. Field-testing was undertaken in East Kalimantan, Indonesia.

13.3 Results and Discussion

13.3.1 *Shorea roxburghii*

13.3.1.1 Success of the Preparation and Sterilization Procedures

In a study of tissue culture in tropical regions, surface sterilization is an important step. We used shoot apices as explants and sodium hypochlorite solution as a sterilizing solution, and the rate of contamination was 5% (Table 1). In small-stem twigs of some tropical trees, the asepsis percentage has been reported as 95%, but mercuric chloride solution was used as a sterilizing agent in these studies (Roy 1996). The use of mercuric chloride should be avoided, as it is toxic. The result from this study suggests that shoot-apex culture is a useful tool in the micropropagation of tropical trees.

13.3.1.2 Effects of Plant-Growth Regulators on Multiple-Bud Induction and Propagation

After 1 month of culture, the shoot apices sprouted and elongated. The shoot apices on the medium containing thidiazuron formed only a callus, but multiple buds were induced in the media containing BAP or zeatin. Some axillary buds appeared after 2 months of culture. Twenty to 50 axillary buds were formed on some branches, and multiple shoots formed after 3 months of culture (Fig. 1). In *S. robusta*, clumps of six to ten shoots were formed on a solid medium after 3–4 months of culture (Roy 1997). By comparison, the method here produces enough buds to be of practical value.

13.3.1.3 Effect of Carbon Source on Induction and Propagation of Multiple Buds

In the axillary-bud and callus cultures of this species, the type of carbon source greatly affects growth (Sukartiningsih et al. 1994), and is presumably a crucial factor in maintaining the life of the culture.

The combination of 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose was the most effective of all the carbon sources for the induction and propagation of multiple buds. In the medium containing 1.0 mg l^{-1} zeatin and only a single carbon source, sucrose or maltose was effective for the induction of multiple buds, whereas maltose or dextrose was effective for the propagation of multiple buds. However, the mortality rate of multiple buds was very high in media containing a single-carbon source. All shoot apices in the medium containing trehalose died after 1 month of culture. Zeatin

Table 1. Effects of plant-growth regulators on the formation of multiple buds after 3 months of culture in half MS liquid medium

Plant growth regulators (mg l^{-1})			Induction rate of	Mortality	Callus formation	Contamination
BAP	Thidiazuron	Zeatin	multiple buds (%)	(%)	(%)	(%)
0	0	0	0	95	0	5
00.2	0	0	0	85	15	0
0.1	0	0	20	70	0	10
0.2	0	0	5	75	20	0
1.0	0	0	40	45	15	0
2.2	0	0	5	60	30	5
4.4	0	0	10	60	30	0
0	00.2	0	0	60	35	5
0	0.1	0	0	65	35	0
0	0.2	0	0	45	55	0
0	1.0	0	0	20	80	0
0	2.2	0	0	25	75	0
0	4.4	0	0	75	20	5
0	0	00.2	0	85	5	10
0	0	0.1	20	75	0	5
0	0	0.2	15	80	5	0
0	0	1.0	75	5	20	0
0	0	2.2	5	85	10	0
0	0	4.4	50	30	20	0

Shoot apices sprouted and elongated after 1 month of culture

Twenty explants were cultured in each treatment

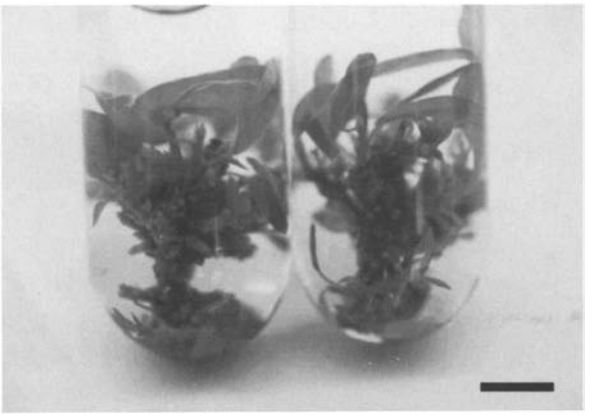

Fig. 1. Formation of multiple buds after 3 months of culture in half MS liquid medium containing 1.0 mg l^{-1} zeatin, 5.2 g l^{-1} dextrose, and 5.2 g l^{-1} maltose. *Bar* 1 cm

was more effective than BAP for induction, propagation, and survival of multiple buds when 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose were used in combination for proliferation. However, there was no difference between zeatin and BAP for induction and multiple-bud proliferation when sucrose was used as the carbon source

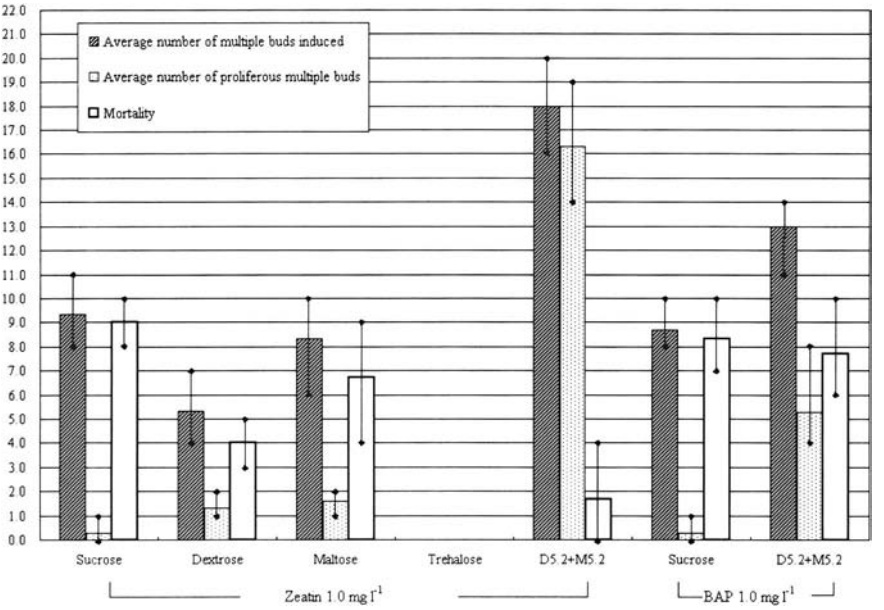

Fig. 2. Effects of carbon sources on the induction and propagation of multiple buds. Twenty shoot apices were cultured in each treatment, and tests were repeated three times. All data are the average of three tests. Average number of multiple buds induced: the average number of multiple buds induced from shoot apices after 3 months of culture. Average number of proliferous multiple buds: the average number of multiple buds after 6 months of culture. Sucrose: 30 g l^{-1} sucrose; Dextrose: 60 g l^{-1} dextrose; Maltose: 30 g l^{-1} maltose; Trehalose: 30 g l^{-1} trehalose; D5.2 + M5.2: 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose. *error bar* maximum and minimum

(Fig. 2). These results indicate that the ½MS medium containing 5.2 g l^{-1} dextrose, 5.2 g l^{-1} maltose, and 1.0 mg l^{-1} zeatin was the most effective for the induction and propagation of multiple buds.

13.3.1.4 Effects of Plant-Growth Regulator and Carbon Source on Shoot Elongation from Multiple Buds

Many shoots elongated from multiple buds in the medium containing 1.0 mg l^{-1} zeatin, but none elongated on the medium containing 4.4 mg l^{-1} zeatin. The use of two carbon sources in combination was more effective than the use of a single carbon source (Fig. 3). Of the treatments, the combination of 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose was most effective, followed by 20 g l^{-1} dextrose and 10 g l^{-1} maltose, and 15 g l^{-1} dextrose and 5.2 g l^{-1} fructose. In the single carbon-source treatments, sucrose was the most effective for the number of shoots induced, followed by maltose, fructose, and dextrose. These results suggest that supplementation with two carbon sources in combination was effective for shoot elongation from multiple buds. Normal leaves were formed in all treatments except that with 60 g l^{-1} fructose. Malformed leaves that were rounded and shrunken were observed in the medium

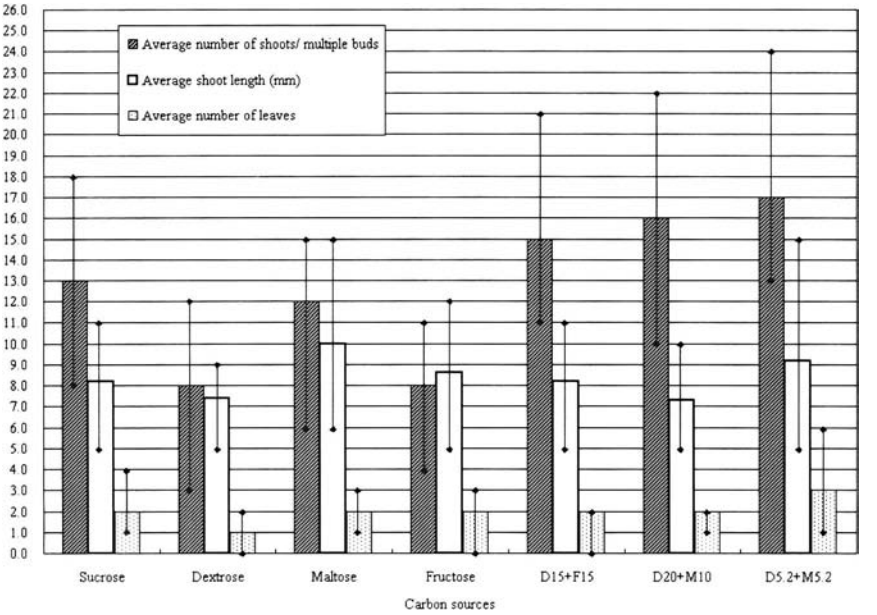

Fig. 3. Effects of carbon sources on shoot elongation from multiple buds after 1 month of culture. Ten multiple buds were cultured in each treatment. Average number of shoots/multiple buds: the average number of shoots that elongated from 10-multiple buds Average shoot length: the average length of shoots that elongated from 10-multiple buds. Average number of leaves: the average number of leaves that were formed on all shoots. Only shoots that elongated to more than 5 mm were counted. Sucrose: 30 g l^{-1} sucrose; Dextrose: 60 g l^{-1} dextrose; Maltose: 30 g l^{-1} maltose; Fructose: 60 g l^{-1} fructose; D15 + F15: 15 g l^{-1} dextrose and 15 g l^{-1} fructose; D20 + M10: 20 g l^{-1} dextrose and 10 g l^{-1} maltose; D5.2 + M5.2: 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose. *error bar* maximum and minimum

containing 60 g l^{-1} fructose, but normal leaves appeared in the medium containing the combination of 15 g l^{-1} dextrose and 15 g l^{-1} fructose. These results indicate that supplementation with high concentrations of fructose resulted in the malformation of leaves. Further, a combination of 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose as a carbon source, and 1.0 mg l^{-1} zeatin as a plant growth regulator, was most effective for the induction and propagation of multiple buds and for the shoot elongation from multiple buds of *S. roxburghii*. Notsuka et al. (1987) reported that the most suitable carbohydrate was different in each variety. In Nostuka et al.'s study, sucrose was an effective carbohydrate for culture growth, but a combination of two carbohydrates was more effective than sucrose alone.

13.3.1.5 Effects of Rooting Medium

Shoots planted in the Florialite rooted well (Fig. 4) and formed many rootlets. The rooting rate and shoot height increment were 98% and 38 mm, respectively, in Florialite; both of these values were the highest among the media, followed by those

Fig. 4. Regeneration of plantlets after 1 month of culture in Florialite supplemented with half MS liquid medium containing 5.2 g l^{-1} dextrose and 5.2 g l^{-1} maltose. *Bar* 3 cm

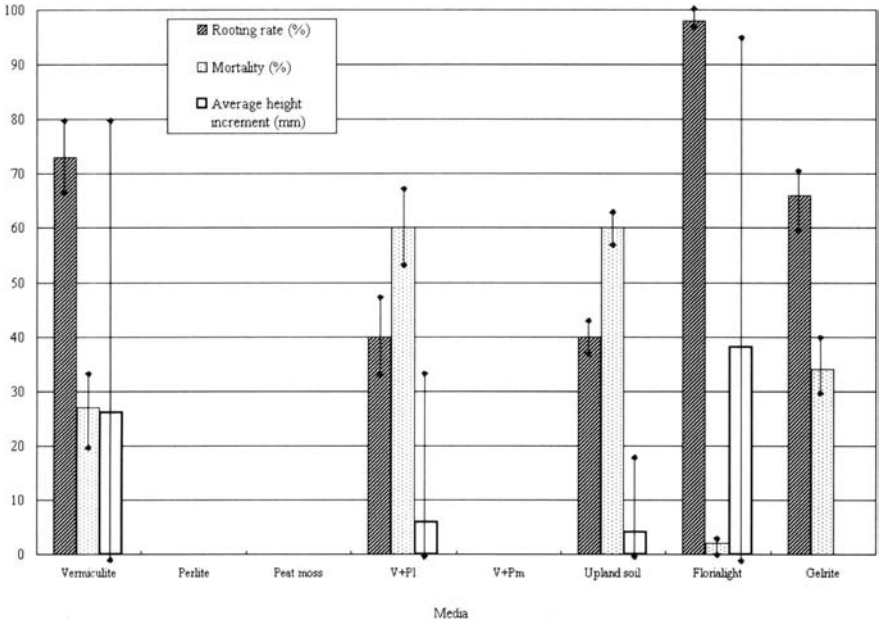

Fig. 5. Effects of rooting media on the rooting of shoots after 1 month of culture. Thirty shoots were cultured in each treatment and tests were repeated three times. All data are the average of three tests. V + Pl: mixture of vermiculite and perlite; V + Pm: mixture of vermiculite and peat moss. *error bar* maximum and minimum

for vermiculite. Rooting and survival rates in the other media were low. In particular, shoot elongation was not observed in Gelrite (Fig. 5). Afreen-Zobayed et al. (1999) reported that plantlets of *Ipomoea batatas* exhibited greater growth of shoots and roots on Florialite than on gellan gum, vermiculite, or Sorbarod. We conclude that the balance of air space and water content in Florialite is suitable for the formation of rootlets.

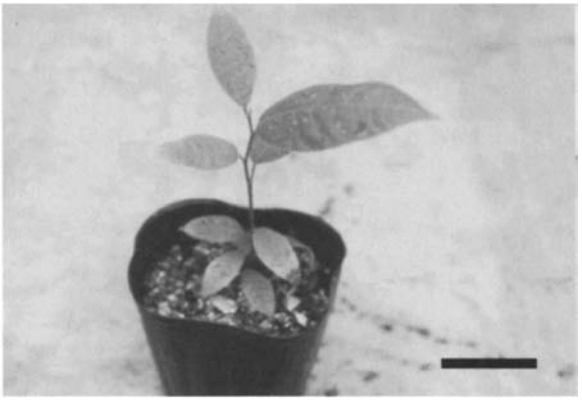

Fig. 6. A plantlet after 1 month of acclimatization in a pot filled with vermiculite. *Bar* 5 cm

13.3.1.6 Acclimatization

The acclimatized plantlets grew well (Fig. 6), and the survival rate was 98%. Although we used branches to provide apices, all plantlets grew vertically. This indicates that clonal propagation through multiple shoots was effective in avoiding plagiotropic growth.

13.3.2 *Gmelina arborea*

13.3.2.1 Induction of Multiple Shoots and Regeneration of Plantlets

At the commencement of this study we investigated axillary-bud culture, which is popular and easiest among the tissue-culture techniques. However, all explants died because *Gmelina* has some substance that caused the explants to wither. Therefore, the shoot apex of *Gmelina* was used. Multiple shoots did not develop after 3 months in the liquid medium. On the solid media, multiple shoots were induced, except for the medium containing 1.0 mg l^{-1} BAP and 0.002 mg l^{-1} IBA, or 1.0 mg l^{-1} BAP alone (Table 2). The maximum average numbers of buds per multiple shoot that were excised from mature trees (4 years old) and from seedlings (1 year old) were 5.3 and 5.4, respectively, on the medium containing 0.03 mg l^{-1} BAP and 0.002 mg l^{-1} IBA (Fig. 7). These results indicate that the age of the mother plant did not affect the induction of multiple shoots. Induced multiple shoots were subcultured on the same medium as that used for shoot elongation, and were incubated for 3 months. After 3 months of culture, shoots elongated and roots were formed directly at the base of multiple shoots. New shoots were cut from multiple shoots and transplanted onto the medium containing 0.03 mg l^{-1} BAP and 0.002 mg l^{-1} IBA for the rooting. The results of rooting are shown in Fig. 8. The rooting rate on the medium containing 0.03 mg l^{-1} BAP and 0.002 mg l^{-1} IBA was 50% (from 4-year-old mature trees) and 20% (from 1-year-old seedlings). On the hormone-free medium, the rooting rate was 33.3% (from mature trees) and 20% (from seedlings). On the medium contain-

Table 2. Effects of indole-3-butyric acid (IBA) and benzylaminopurine (BAP) after 3 months of shoot-apex culture from mature trees

IBA (mg l^{-1})	BAP (mg l^{-1})	No. of shoot elongations	No. of explants flushing	No. of multiple shoots	No. of explants formed of roots	No. of explants formed of callus	Died
0	0	0	0	0	0	0	
10							
0	0.01	1	3	1	0	1	4
0	0.03	2	6	1	0	0	1
0	0.1	1	5	1	0	2	1
0	0.3	1	2	1	0	4	2
0	1.0	0	1	0	0	7	2
0.002	0	0	0	0	0	0	10
0.002	0.01	4	2	1	2	1	2
0.002	0.03	2	1	3	1	3	1
0.002	0.1	4	0	1	0	3	2
0.002	0.3	6	1	1	0	0	2
0.002	1.0	0	1	0	0	6	3

ing 0.03 mg l^{-1} BAP and 0.002 mg l^{-1} IBA, shoots elongated well and had many roots (Fig. 9). These results suggest that explants from mature trees rooted better than those from seedlings. A similar result has been obtained on cuttings of *G. arborea* by Surendran (1990).

13.3.2.1 Acclimatization of Plantlets

(1) After 2 weeks of rearing, one plantlet died and two plantlets grew. The two plantlets were transplanted into pots and raised in the nursery for 1 month. One seedling died after 1 month, but the other grew well at the nursery (Table 3). After 3 months of rearing at the nursery, the one remaining seedling was transplanted into the experimental field.

(2) The two regenerated plantlets that were planted directly into the pot in the cutting box showed more vigorous growth than the plantlets in the conical flasks. After 1 month of rearing in the cutting box, they were transplanted into pots and raised under shadecloth in the nursery. The two plantlets had good growth at the nursery. After 1 month of rearing in the nursery, they were transplanted into the experimental field.

13.3.2.2 Plantation of Plantlets

Four seedlings were transplanted into the experimental field. They grew well and the tallest tree was about 250 cm in height after 8 months.

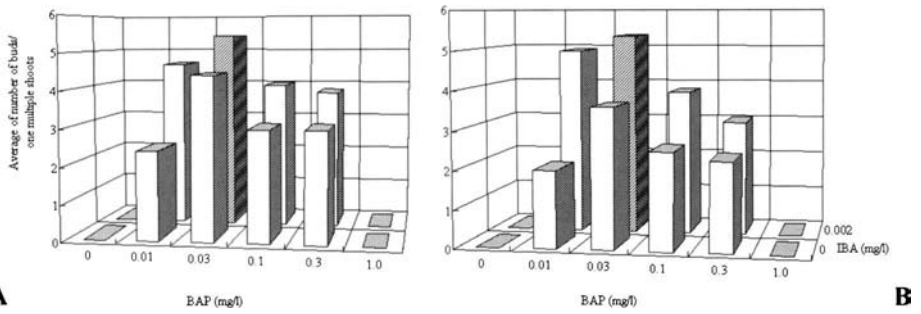

Fig. 7. Effects of indole-3-butyric acid (IBA) and benzylaminopurine (BAP) on the induction of multiple shoots by shoot-apex culture. **A** Shoot apexes collected from mature trees. **B** Shoot apexes collected from seedlings. *Shaded bar* maximum data

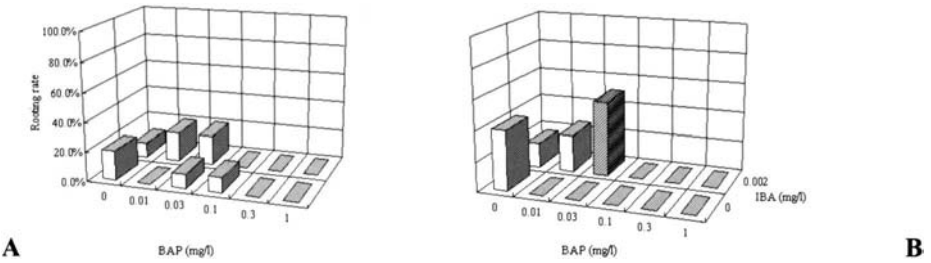

Fig. 8. Effects of indole-3-butyric acid (IBA) and benzylaminopurine (BAP) on rooting. **A** Explants were collected from mature trees. **B** Explants were collected from seedlings. *Shaded bar* maximum data

13.4 Conclusion

The method described here is of practical use for the production of stock. Rejuvenation occurred in all of the multiple buds that were cultured. This is the first report of efficient mass-clonal propagation of *Shorea roxburghii* and *Gmelina arborea* by shoot-apex culture. We have already produced stocks of these species using this method and have started an experimental plantation in East Kalimantan, Indonesia.

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Fig. 9. Regeneration of plantlets from shoot apices. *Bar* 1 cm

Table 3. Growth of plantlets at the nursery and the test reforestation land

Plant No.	Height (cm)		
	0 month	3 months	8 months
1[a]	7.0	Died	
2[a]	8.0	12.0	131
3[b]	11.0	18.3	154
4[b]	12.0	27.2	250

[a]Plantlets were transplanted from conical flasks to the nursery

[b]Plantlets were transplanted from the cutting box to the nursery

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14
Tree Tissue Culture and Ex Vitro Sand Rooting for Reforestation

H.K. Saiju

14.1 Introduction

Micropropagation has been routinely used for the clonal multiplication of plants. However in vitro-produced shoots often fail to root, or die, due to contamination in the field. The problem to be solved for the mass-scale production of plants through micropropagation is how to induce roots that can survive in the field (Nemeth 1986).

Conventionally, microshoots are rooted in vitro in an auxin-rich medium. This is followed by the hardening process and finally plantation to the field. The conventional method of in vitro rooting develops weak roots with low survival and growth rates under natural conditions (Kozai 1991). In vitro-proliferated potato shoots developed roots in nonsterile sand and dried leaves mixed in an equal proportion by volume (Manandhar and Rajbhandary 1986). In vitro-produced plant species have rooted in nonsterile sand (Rajbhandary and Bajaj 1991). Tissue-cultured microshoots of forestry species also develop roots ex vitro in sand. These microplants can easily survive in field plantations.

14.2 Materials and Methods

Explants (shoot tip, young leaf, node, cotyledon node, seed) from 21 forest-tree species and two forest-bamboo species were used for tissue culture (Table 1).

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Table 1. Forestry species used for tissue culture

Species	Explant
Tree	
Acacia auriculiformis	Cotyledonary node
Artocarpus heterophyllus	Cotyledonary node
Artocarpus lakoocha	Cotyledonary node
Citrus aurantifolia	Cotyledonary node
Citrus limon	Cotyledonary node
Citrus sinensis	Cotyledonary node
Dalbergia sissoo	Cotyledonary node
Elaeocarpus sphaericus	Nodal segment
Eucalyptus camaldulensis	Shoot tip/Young leaves
Eucalyptus citriodora	Nodal segment
Eucalyptus terecticornis	Nodal segment
Ficus auriculata	Cotyledonary node
Ficus carica	Shoot tip
Ficus elastica	Shoot tip
Ficus lacor	Shoot tip
Ficus neriifolia	Shoot tip
Ficus semicordata	Shoot tip
Fortunella sp.	Shoot tip/Cotyledon node
Morus alba	Shoot tip/Cotyledon node
Poncirus trifoliata	Shoot tip/Cotyledon node
Populus ciliata	Shoot tip/Cotyledon node
Bamboo	
Dendrocalamus hamiltonii	Seed
Dendrocalamus striactus	Seed

14.3 Methods

Explants were cultured on Murashige and Skoog (1962) medium supplemented with various concentrations of kinetin, benzylaminopurine (BAP), napthaleneacetic acid (NAA) and/or 2,4-dichlorophenoxyacetic acid (2,4-D). The pH of the medium was adjusted to 5.8 before autoclaving. The culture flasks were incubated in a room maintained at 25°C and 3 kilolux light. The established explants were kept in the same medium for 6 weeks, and then the microshoots were recultured in a cytokinin-enriched medium. Subculturing was done at 8-week intervals. The maximum number of shoots was produced after the sixth subculture, after which the flasks with microshoots were incubated for 3 months for the elongation of microshoots. The flasks with microshoots were transferred to a glasshouse for 2 weeks before rooting, for acclimatization, hardening, and change to an autotrophic mode of nutrition. Sand was cleaned with water, dried in sunlight for 2 days, sieved through a 2 × 2 mm-pore sieve, put in a box and wetted evenly with water.

The leafy, single node, 2 cm-long microcuttings were dipped in 1 ppm IAA for 10 min and then transplanted in the sand. The sandbox was covered with a polythene

sheet. The maximum and minimum temperatures in the box were 30°C and 20°C, respectively. The sunlight intensity varied from 4–15 kilolux. Watering was done using a sprayer to maintain 80% humidity.

14.4 Results

The tree and bamboo species cultured in the nutrition medium produced healthy microshoots that developed roots ex vitro in sand within 3 weeks. The microshoots were left in the same box for 3 more weeks to develop healthy roots. The rooted microplants were transferred to a soil sand mixture (1:1) in polythene bags. When the plants were 10–25 cm long, they were ready for field plantation. The sand-rooted plants established easily in the field.

14.5 Discussion

In vitro-produced microshoots of 23 tree and bamboo species transplanted in sand for ex vitro-root induction produced healthy roots. These plants were successful as part of a reforestation program.

Glasshouse conditions of 20°–30°C, a sunlight intensity of 4–15 kilolux, and 80% humidity were good for ex vitro-root development in *Ficus carica* (Saiju et al. 1995). These conditions were also suitable for the plant species trialed in the present study (Table 1). Optimal temperature, light, and humidity under natural or greenhouse conditions will have a positive affect on growth (Kozai 1991).

The factor limiting the use of micropropagation of forest-tree species is the high cost of production involved in tissue culture (Chu 1989). Ex vitro-rooting technique can lower the production cost of tissue-cultured plants. During this process, a nutrient medium and incubation room are not needed. This rooting technique may be helpful for taking plants produced in the laboratory to the field. The roles of temperature, humidity and light for ex vitro-root development in in vitro-produced shoots need further detailed study.

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Part III

Use of Mycorrhizae Symbiosis for Dipterocarp Forests

15
Mycorrhizal Research in Malaysian Plantation Forestry

SU-SEE LEE

15.1 Introduction

The role of mycorrhizal symbiosis gained prominence in the early decades of the twentieth century, with the pioneering research of the Swedish scientist, Elias Melin. The importance of the mycorrhizal association was reaffirmed by subsequent evidence, which showed that the failure of exotic pines introduced as plantation species to many tropical countries was due to the absence of suitable symbiotic fungi, and that this could be corrected by introducing mycorrhizal infection. The aim of inoculating seedlings with mycorrhizal fungi is to provide seedlings with adequate mycorrhizas for planting in man-made forests. In world forestry, ectomycorrhizal fungi have been shown to be very important to artificial regeneration, not only in logged-over forests but also in the reclamation of adverse sites, such as mine spoils.

Over 90% of the 300,000 species of vascular plants in the world form arbuscular mycorrhizas, and the remaining 10% form ectomycorrhizas. The most important family of timber trees in Malaysia, the Dipterocarpaceae, are ectomycorrhizal, although a few species have been recorded to form both ecto- and arbuscular mycorrhizas (Lee 1998). Other important families of timber trees in Malaysia are mainly arbuscular mycorrhizal, and many of the species selected for forest plantations are known to be arbuscular mycorrhizal.

Mycorrhizal research in Malaysian forestry began fairly recently, with the first report of ectomycorrhizas in roots of some dipterocarp seedlings made by Singh (1966). A year earlier, Wastie (1965) reported the presence of endomycorrhizas in the roots of rubber (*Hevea brasiliensis*) trees. Mycorrhizal research on various indigenous and exotic forest trees in Malaysia intensified in the 1970s, with the aim of applying mycorrhizas for better plant growth and survival in plantations and reforestation efforts, and in the rehabilitation of degraded sites. Recent research has concentrated on the dipterocarps.

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This chapter discusses the more significant results of mycorrhizal research conducted in the context of Malaysian forestry, and identifies priorities for future mycorrhizal research in relation to plantation forestry. While the species discussed in the following sections have, at one time or another, been considered suitable for plantation establishment, many have not been planted for a variety of reasons.

15.2 Plantation Species in Malaysia

15.2.1 *Agathis* and *Araucaria*

There are three native species of *Agathis* in Malaysia: *A. borneensis* (formerly known as *A. alba* = *A. dammara*); *A. flavescens*; and *A. kinabaluensis*; but *A. macrophylla*, a native of Australasia, was introduced as potential plantation species in 1962 (Ivory 1975). *A. macrophylla* plants in the Kepong nursery were found to be mycorrhizal (Ivory 1975), but it was not stated whether these were ecto- or arbuscular mycorrhizal.

Species of *Araucaria* were introduced much earlier: *Araucaria cunninghamii* in 1924 and *A. hunstenii* about 30 years later as potential species for plantation establishment. Both species have been found to form arbuscular mycorrhizas (Griffiths 1965; Ivory 1975; Hong 1978). In experiments carried out to investigate the effect of commonly used prophylactic fungicides on the mycorrhizas of both species, it was found that frequent applications of Daconil reduced mycorrhizal root development on young plants, whereas Colliodox and Difolatan did not (Ivory 1975).

Further research on *Agathis* and *Araucaria* mycorrhizas was not pursued, in view of the decision to embark on the Compensatory Forest Plantation Project in the early 1980s, where plantations of fast-growing exotic hardwood species were to be established.

15.2.2 Acacias

There are approximately 100,000 ha of *Acacia mangium* plantations in Malaysia, with small trial plantings of other *Acacia*, such as *A. aulacocarpa*, *A. auriculiformis* and *A. crassicarpa*. Recently, the *A. auriculiformis* × *A. mangium* hybrid has gained popularity, but areas planted with the hybrid are still relatively limited. These *Acacia* are known to form arbuscular mycorrhizas (Reddell and Warren 1987), and spores of *Gigaspora* sp. and *Glomus* sp. have been recovered from the soil in nursery-potted *A. mangium* plants (Lee 1990). Fruiting bodies of *Thelephora ramarioides* are commonly found growing around *A. mangium* plants (Gibson 1981; Lee 1990) but anatomical studies show that it is unlikely that this fungus forms ectomycorrhizas with the host (Lee 1990). More recently, the ectomycorrhizal fungus *Pisolithus tinctorius* was found in *A. mangium* plantations on the peninsula (Patahayah et al. 2003), and *P. tinctorius* ectomycorrhizas were found on roots of the associated 2-year-old trees (S Lee unpublished). In a sandy tin-tailing site at Bidor, three

ectomycorrhizal types were found on roots of planted one-year-old *A. mangium* trees (S Lee unpublished) and samples have been sent to France for identification using molecular techniques.

A. mangium was reported to be mycorrhizal-dependent in the Philippines, i.e. that it would not survive in soils of marginal fertility without arbuscular mycorrhizas (Tambalo-Zarate and de la Cruz cited in de la Cruz and Yantasath 1993), but this has not been observed in Malaysia. In Malaysia, *A. mangium* has been successfully planted on degraded grasslands in Sabah and on compacted log-landing sites on the peninsula. Recently, we also successfully established a small plantation of uninoculated *A. mangium* in a sandy tin-tailing area in Bidor, Perak, with minor soil amendments and minimal fertilizer application. It would appear that native mycorrhizal fungi and rhizobia were able to form successful and functional associations with the host, as demonstrated by the survival and growth of the plants, and the presence of mycorrhizas and nodules on the roots.

Locally produced commercial arbuscular-mycorrhizal inoculants are now available in Malaysia and used for oil-palm seedlings. However, in view of the cost involved, the low-capital investment in forest plantations, and the relatively satisfactory growth rates of forest-tree species, even on marginal sites and poor soils it appears unlikely that such inoculants will become widely used in Malaysian plantation forestry in the near future.

15.2.3 Dipterocarps

The Dipterocarpaceae, one of the few tropical tree taxa to form ectomycorrhizas, has been the main focus of Malaysian forestry research on mycorrhizas; the state of knowledge on dipterocarp mycorrhizas was reviewed by Lee (1998). It has only been fairly recently that research into applied aspects of the dipterocarp-mycorrhizal association, in particular its role in plant growth, survival, and nutrition, has intensified. Results from such research are important in view of current interest in the establishment of plantations of indigenous species, especially in the region.

Dipterocarp-mycorrhizal research in Malaysia has concentrated on two main aspects, both of direct relevance to the establishment of dipterocarp plantations. The first deals with the ecology and biology of dipterocarp mycorrhizas, while the second focuses on inoculation and planting. Studies have been ongoing to collect, identify, and isolate putative ectomycorrhizal fungi associated with dipterocarp hosts (Watling and Lee 1995, 1998) and to investigate changes in mycorrhizal populations due to logging (Lee et al. 1996, 2003; Watling et al. 2000). Information from such studies is essential for developing an understanding of the relationship between mycorrhizal fungi, fungal succession, and forest function and regeneration.

Controlled mycorrhizal inoculation of *Hopea helferi* and *H. odorata* with *P. tinctorius* has resulted in improved growth and phosphorus uptake of seedlings in nursery trials (Yazid et al. 1994). However, subsequent small-scale field trials showed that this fungus did not persist on the roots of inoculated seedlings when outplanted in the field (Chang et al. 1996). My colleagues and I have also observed that diptero-

carp seedlings inoculated with this ectomycorrhizal fungus do not always successfully form ectomycorrhizas (Aminah et al. 2003). However, infection by naturally occurring, unidentified dipterocarp-mycorrhizal fungi has been shown to improve phosphorus uptake and growth of *H. odorata* seedlings in the nursery (Lee and Alexander 1994). An indigenous *Tomentella* sp., isolated from *Shorea parvifolia* seedlings in a nursery, has also shown promising results, being able to infect both dipterocarp and *A. mangium* seedlings (Lee and Patahayah 2003; Lee et al. 2002). This fungus is also able to persist on the roots of inoculated dipterocarp cuttings for at least up to six months after outplanting into a harsh, degraded tin-tailing site (S Lee unpublished). In addition, a technique for production of the inoculum on a relatively-large scale has also been developed (Lee and Patahayah 2002). This fungus is a very promising candidate for ectomycorrhizal inoculation of seedlings and cuttings for plantation establishment, especially on degraded sites. Studies have also been conducted on the ability of various ectomycorrhizal-fungal extracts and IAA antagonists to enhance rooting of cuttings of four dipterocarp species, with mixed results (Aminah et al. 2000, 2002).

Although there are some encouraging results emerging from dipterocarp mycorrhizal research in Malaysia, much research still needs to be conducted before controlled inoculation of dipterocarps can be assured of producing productive plantations in the future. Priorities for dipterocarp mycorrhizal research have been identified elsewhere (Lee 1998), but it is worth reemphasizing some points here. More species of indigenous mycorrhizal fungi that have the potential to boost dipterocarp-seedling growth and which are also competitive under field conditions still need to be identified and isolated into culture. Tests also need to be conducted to determine whether selected mycorrhizal fungi are better able to stimulate the growth of dipterocarp plants compared to naturally occurring mycorrhizas introduced through the standard potting mix.

15.2.4 *Hevea brasiliensis*

Over the last two decades, rubberwood has become a very important resource for the local furniture industry. This has led to the development of specific rubber-timber clones targeted at timber rather than latex production, and some plantations of these clones have already been established. Mycorrhizal research on rubber has been conducted since the 1960s by researchers at the Rubber Research Institute of Malaysia. Results showed that the response of *H. brasiliensis* to arbuscular-mycorrhizal inoculation was variable and unpredictable, the response being modulated by soil characteristics, introduced mycorrhizal-fungal species, the presence of indigenous mycorrhizal fungi, and their interactions (Ikram et al. 1990). The improved growth response in *H. brasiliensis* from introduced arbuscular-mycorrhizal fungi was suggested to be, in part, due to the ineffectiveness of indigenous mycorrhizal fungi, and the importance of testing selected arbuscular mycorrhizal-fungal inoculants over a range of field sites and soils was emphasized (Ikram et al. 1993a).

The creeping legume *Calopogonium caeruleum* is planted in rubber plantations as a ground cover, not only to protect the soil but also to encourage proliferation of rubber roots, improve soil structure, fix atmospheric nitrogen, and rapidly return nutrients to the soil, thus contributing to better growth of the rubber trees. Ikram et al. (1993b) concluded that arbuscular-mycorrhizal inoculation of *C. caeruleum* in field soils was unlikely to be worthwhile if the indigenous arbuscular-mycorrhizal endophyte population was effective. No reports are available on further research on mycorrhizas of rubber in Malaysia, and presently mycorrhizal-inoculated planting material is not used when planting new rubber plantations.

15.2.5 *Pinus* species

Several species of pines were introduced as potential long-fiber plantation species in the 1950s. One of these early introductions, *Pinus caribaea* var. *hondurensis*, was selected for plantation establishment under the Pilot Plantation Project of the early 1970s (Mohd Afzal and Zakaria 1985). Early growth of pines in the Malaysian highlands was good, and poor-seedling growth in the nursery at Kepong was alleviated by the introduction of surface litter from the established stands of *P. insularis* in the Cameron Highlands (Griffiths 1965).

The effect of applying several common nursery fungicides on ectomycorrhizal development and the growth of *P. caribaea* was the subject of several studies, with conflicting results. Lim and Anthony (1970) found that the application of Daconil stimulated mycorrhizal development, while Hong (1976) reported that this fungicide suppressed mycorrhizal development. Ivory (1975), on the other hand, reported that Daconil had no apparent deleterious effects. Hong's (1976) comprehensive study concluded that mycorrhizal development on *P. caribaea* seedlings was suppressed by 0.3% Daconil and 0.2% Thiram, and that the best mycorrhizal development was obtained in the 0.2% Difolatan treatment in comparison to the untreated control. Such results are important in nursery-management programs, but further research on pines ceased with the launching of the Compensatory Plantation Project and subsequent change in the choice of species for plantation establishment in the early 1980s.

15.2.6 Rattans

Rattans are an important forest product and there was a lot of interest in the establishment of rattan plantations during the 1990s. In support of such efforts, some preliminary studies on the benefits of mycorrhizas to Malaysian rattans, in particular *Calamus manan*, were conducted. The application of a mixed indigenous arbuscular-mycorrhizal inoculum at the time of transplanting improved the survival of micropropagated *C. manan* plantlets from 2.6% to 61.8% after 12 months (Maziah 1991). Mycorrhizal inoculation was thought to confer some measure of resistance

against collar rot caused by the soil-borne fungus *Fusarium oxysporum*. Mycorrhizal inoculation also stimulated plantlet height growth. However, no field trials were conducted and, as Maziah (1991) noted, response to mycorrhizal inoculation in pot trials is no guarantee of continued response in the field. No further reports of mycorrhizal research on rattans are available. Interest in the establishment of rattan plantations declined towards the end of the 1990s due to a number of problems, among them the low yield of mature canes on a short rotation of 15 years. Some plantations are known to be harvesting their rattan and replacing the plantations with other crops (RS Raja Barizan, 2003, personal communication). Presently, the Forestry Department of Peninsular Malaysia records about 20,000 ha of rattan plantations in Peninsular Malaysia (Forestry Department Peninsular Malaysia 2003) but these do not include rattan plantations established by smallholders and private plantation companies. Extensive areas of rattan plantations have been established in Sabah, but actual figures are not available.

15.2.7 Other Timber Tree Species

Mycorrhizal pot experiments in the nursery and shadehouse have been carried out with *Gmelina arborea*, *Intsia palembanica*, *Leucaena leucocephala*, *Paraserianthes falcataria* (Norani and Maziah 1987) and *Parkia speciosa* (Norani 1988). *Eucalyptus* spp., *G. arborea*, and *P. falcataria* have been planted quite extensively in Sabah. While there is a wealth of literature on the mycorrhizas of *Eucalyptus* spp., especially from Australia, relatively little is known about the mycorrhizas of *G. arborea* and *P. falcataria*. From Malaysia, there are no known reports of mycorrhizal research on these species, apart from the preliminary studies, mentioned above, for *G. arborea* and *P. falcataria*.

A number of species are presently recommended for forest plantation establishment in Malaysia, and include both indigenous and exotic species. Among the more popular species are *A. mangium*, an *Acacia* hybrid (*A. mangium* × *A. auriculiformis*), *Azadirachta excelsa* (Meliaceae, indigenous), *Dyera costulata* (Apocynaceae, indigenous), *Hopea odorata* (Dipterocarpaceae, indigenous), *H. brasiliensis* (Euphorbiaceae, exotic), *Khaya ivorensis* (Meliaceae, exotic), and *Tectona grandis* (Verbenaceae, exotic). Other high value, indigenous timber tree species have also been planted in Sabah and Sarawak, some strictly for their timber and others more for their valuable fruit, e.g. the illipe nut trees (Dipterocarpaceae, *Shorea* spp.) and *Durio* spp. (Bombacaceae). With the exception of the dipterocarps, there is presently no active mycorrhizal research on any of the other species.

15.3 Application of Research Results

Despite the encouraging results with various plantation species, mycorrhizal inoculation is not a routine practice in Malaysian forest plantation management. This is due to a number of reasons, as follows.

15.3.1 Frequent Changes in the Choice of Species for Reforestation

Over the last three decades, the choice of tree species for reforestation has changed a number of times and for a variety of reasons (Lee and Maziah 2003). The frequency of such changes has disrupted the accumulation of silvicultural and genetic knowledge for any species. Only with the indigenous dipterocarps has there been a sustained, though low-level of research, but problems with the supply of seeds and planting material, and their relatively slow growth/long rotation have discouraged planters from establishing plantations.

15.3.2 Compartmentalization of Research

Present day forestry research in Malaysia is narrowly compartmentalized into botany, ecology, physiology, tree improvement, in-vitro propagation, mycorrhizas, etc. Silviculture has been reduced to the monitoring of tree growth, and this is detrimental because successful reforestation must integrate many diverse areas of knowledge. It is crucial, for example, that fertilizer trials and the role of soil microorganisms, in particular mycorrhizal fungi and nitrogen-fixing bacteria, be examined together so that fertilizers can provide the correct balance of nutrients conducive to mycorrhizal and rhizobium development and function. The integration of such studies requires close cooperation between many scientists, but this is easier said than done. In practice, individual scientists have their own ideas and it is difficult to achieve any real integration. The best way to break out of such mindsets would be to allow all researchers to cross their job boundaries and pursue their research until they get results.

15.3.3 Lack of Support for Fundamental Research

The trend in many countries, including Malaysia, is to fund and support applied research at the expense of fundamental research. This trend is understandable, but applied research is held up if fundamental research is too weak. In the case of fungi, it is estimated that 70% of the fungi in Malaysia are yet to be discovered and described (the late EJH Corner, 1991, personal communication). Thus, it is not surprising that we still have much to learn about the mycota, their biology and ecology, in particular of the symbiotic mycorrhizal fungi, before they can be optimally and effectively applied in the field.

15.3.4 Limitations to Mycorrhizal Application

There is a general lack of taxonomic knowledge of the indigenous mycorrhizal fungi because very little is known of our indigenous mycota, as explained above. In addition, few field studies have been conducted, in particular on the competitiveness,

efficacy, and persistence of the inoculant fungi. Integrated studies on mineral nutrition and mycorrhizal infection are also lacking, and an effective and efficient mycorrhizal inoculation protocol has yet to be fully realized.

15.3.5 Oversell of "Magic" Solutions

Many field managers, while highly competent and experienced with agronomy, generally know little about symbiotic microorganisms. The over-zealous marketing of products, such as the so-called 'biofertilizers' and mycorrhizal inocula, combined with scanty technical information, has resulted in the general belief that "magic" solutions can be applied to enhance tree establishment, growth, and survival. However, the usage of such products has often led to disappointment, disillusion, and disbelief. There is a need for researchers and the vendors of such products to clearly define the conditions under which the potential of mycorrhizas can be fully realized, and to make known these conditions to the public who are utilizing the technology.

15.4 Conclusion

The application of mycorrhizas in afforestation, reforestation and plantation establishment in Malaysia has largely remained in the experimental and trial stages. This has been due to a combination of factors, ranging from frequent changes in the choice of desired plantation tree species and the demand for fast-growing, short-rotation species, to the oversell of so-called "magic" solutions. There also has to be a significant increase in the number of mycorrhizal researchers to tackle the many aspects of mycorrhizal application so that some of the areas where data are lacking or insufficient may be overcome. It is clear that a considerable amount of fundamental as well as integrated-multidisciplinary research needs to be carried out before some of the already well-established biotechnological techniques, such as mycorrhizas, can be usefully applied to assist reforestation and plantation establishment in Malaysia.

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16
Remarks on the Mycorrhizae of Some Tree Species in Vietnam

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16.1 Introduction

Mycorrhizae play an important role in the growth of vegetation, including the majority of timber-tree species, and to forest ecology. This has been shown by the work of scientists from many countries for over a century, particularly in the last fifty years (e.g., Boullard 1968; Bjorkman 1970; Mikola 1973; Bakshi 1974; Le Tacon 1978; Marx 1980).

Mycorrhizal research was formerly sponsored by the International Foundation For Science (IFS-Sweden). The Mycorrhiza Network Asia was founded, and the BIO-REFOR's mycorrhizal work has confirmed the significance of this subject. Various international and national organizations and private associations (IFS, IUFRO/SPDC, DCR, etc.) have promoted mycorrhizal research by grants and sponsorships.

16.2 The Status of Mycorrhizal Research in Vietnam

Mycorrhizae have been recognized in Vietnam since the early 1960s, through foreign literature and from field studies. The first field application of mycorrhizas, with pine-forest soil as a natural inoculum, was carried out by the Silviculture Department of the Forest Research Institute (Dinh 1977). For many years, research concentrated on pine mycorrhizae: mycorrhizal classification and identification; morphology and anatomical structure; influence of mycorrhizae on seedling growth; and the impacts of soil characteristics, fertilizer performances, and seasonal climate on mycorrhization (Giao 1976a, 1976b, 1977, 1978, 1979). From early 1970 to 1981, a wide range of fungal carpophores and mycorrhizal soils have served as mycorrhizal inocula, and have been tested in uncontrolled and semi-controlled mycorrhizal-inoculation experiments. At the same time, the FRI worked on fungal isola-

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tion and pure cultures. Some isolated strains have been tested in sterile soil with indigenous and exotic-pine species. In 1982, the first semi-controlled field experiment was performed. Selected strains were tested further in the centre of Vietnam during 1985 and 1986. These experiments ceased after that, due to financial and material supply difficulties.

No official mycorrhizal research has been done for timber-tree species, apart from pine. However, there have been personal studies and occasional preliminary observations on mycorrhizae of *Eucalyptus*, dipterocarps, and *Casuarina equisetifolia*. Generally, the research was carried out on ectomycorrhizae; endomycorrhizal research is still lacking.

Mycorrhizal research was formerly executed at the FRT and is currently done at the FSI with one or two researchers. The work has been supported by the LFS (Grant D477), and its funding was recently extended. It has also had support from Mycorrhiza Network Asia, India and the BIO-REFOR.

Mycorrhizal research in Vietnam is currently facing many difficulties: the deficiency of specialized researchers; financial and technical conditions; and a lack of awareness of the benefits of mycorrhizae. Actually, we are further behind in this scientific area than many other Asian countries.

16.3 Remarks on Mycorrhizae of Some Timber-Tree Species

16.3.1 Pines

For many years, the yellow-stunted disease of *Pinus merkusii* and the violet-stunted disease of *P. massoniana* occurred frequently in the nursery, and its harmfulness to pine seedlings is second only to brown needle-blight disease of Cercosporiose (Giao 1969, 1976a). These plant diseases cause serious damage to pine plantations. A field study and man-made disease test showed that the lack of seedling mycorrhizae, caused by a deficiency of appropriate mycorrhizal associations and unsuitable nursery practices, is the main cause (Giao 1979, 1981). The Silviculture Standard for out-planting pine seedlings, including mycorrhizal-formation criteria and mycorrhizal-soil inoculating indication, which was achieved and approved by the Forestry Ministry in 1983. The damage is diminishing now, but not everywhere and at all times. The first semi-controlled mycorrhizal inoculation field experiment was carried out on a slightly sloping hill site near Hanoi City, from 1982 to 1984. Six pure strains were employed, including five local strains and one exotic strain, and raised on a sterile peat + sawdust + forest-soil mixed substrate. Three of the strains showed good symbiosis, and the stem volume of 9-month-old *Pinus merkusii* seedlings increased 150%–350% compared to the control. The most effective was the exotic strain M15 (*Amanita muscaria*) (Le Tacon 1978). The pine forest soil inoculum, at a 5% rate, increased the dry weight of 15-month-old *Pinus caribaea, P. elliottii, P. kesiya,* and *P. oocarpa* seedlings by 7–10 times that of the controls. The two most

effective strains (M15, M4) were further tested in plain and mountain sites in the centre of Vietnam, at Quynhon City, from 1985 to 1986, with *Pinus merkusii* and *P. caribaea*. A good result was obtained (Giao et al. 1987, 1988).

Mycorrhizal soil inoculum was used in the production of sampling stock for three pine species in the coastal zone in the south of Vietnam, in Vungtau City, from 1991 to 1995. The superficial layer of *Pinus kesiya* forest soil and a wide range of fungal carpophores collected there, + peat + mixed sawdust, served as the mycorrhizal inoculum. The inoculum was brought 400 km from Dalat City. The seed of *P. kesiya, P. caribaea,* and *P. merkusii* were sown in a bag containing 1 kg of a mixture of 40%–80% local-mountain soil +15%–35% red-basalt soil, +1% superphosphate, +2%–5% mycorrhizal inoculum. Sowing bags were placed on sandy soil mixed with red-basalt soil (50:50 ratio) in an unshaded and windy-adverse site. The test was repeated four times over three years, with a total of 25,000 sowing bags; two-thirds of them *P. kesiya*. In the fourth month, rare mycorrhizal formation occurred on seedling root-tips. Using three good-testing formulas, the rates of mycorrhizal infection of 6-month and 10-month-old seedlings were about 20%–40% and 70%–80%, respectively. About 30% of the 3–5-month-old seedlings died. Most of them were sown in unsuitable testing formulas and were non-mycorrhizal infecting. *Amanita* sp. sporocarps occurred rarely in sowing bags in the first year. After 1–2 years, *Pisolithus tinctorius* and *Scleroderma* sp. carpophores occurred occasionally in bags and frequently in the outplanting plot. Most of the pines samples were *Pisolithus* infected. Thus, perhaps *Pisolithus tinctorius* is the most effective and adaptive fungi to new adverse-site conditions among the introduced mycorrhizal associations.

The responses of different pine species in such new sites were different. The high growth of 34-year-old trees of *Pinus caribaea, P. kesiya,* and *P. merkusii* are about 3–4 m, 1.5–2 m, and 0.3–0.5 m, respectively. Consequently, it seems that *Pisolithus tinctorius* did not affect growth by much in *P. merkusii* under the above-mentioned conditions, although its high effectiveness on the same conifer species in other adverse sites in the north was recorded. Marx (1980) and other authors have published distinguished works on *Pisolitus tinctorius*. This well-known mycorrhizal fungus has also been investigated by researchers in Thailand (Bonyuen 1989, cited in Sangwanit 1995), Malaysia (Lee 1994), and Indonesia (Suhardi 1988; Setiadi 1995). The propagation of mycorrhizal fungi in the coastal sandy zones of Vietnam should be further studied.

Former field observations have revealed a strange phenomenon in *P. merkusii* seedlings, where the growth of some seedlings was enhanced by 200%–250% and their appearance was substantially changed compared to the majority of seedlings in the nursery. This led to discussions among researchers and the formulation of different hypotheses. Our semi-controlled mycorrhizal inoculation indicated that several mycorrhizal strains could result in analogous phenomena (NS Giao unpublished).

The carpophores of over 10 mycorrhizal fungi have been observed in *Pinus merkusii* plantations. These fungi include *Amanita rubescens, Amanita* sp., *Amanitopsis vaginata, Boletus* sp., *Cantharellus tubaeformis, C. cibarius, Rhizopogan luteolus, Russula xerampilena, Russula* sp., *Scleroderma bovista, Scleroderma* sp., *S. auratium, Xerecomus* sp., and *Pisolithus tinctorius* (Giao 1979b, Giao and Nham 1987).

The decline of pine forests in many zones of the country was recorded at the Pine Forest Plantation National Symposium, 1978, in Hue City. This decline has been followed by the degradation of soil fertility and forest microflora, including mycorrhizal associations. The main recognized causes of the decline are the pine larva, *Dendrolimus punctatus* Walk., excessive employment of insecticides, forest fire, human activities, and other unstudied factors.

16.3.2 *Eucalyptus*

Eucalyptus spp. (including *E. camaldulensis, E tereticornis, E. exerta, E. robusta, etc.*) are the most common tree species formerly used for silviculture in a wide range of denuded hills, highland and wasteland in Vietnam. However, for a long time, *Eucalyptus* plantations in many zones did not give the expected results, to such a degree that in some years they were excluded from silviculture. The failure of *Eucalyptus* plantations, mainly in poor, arid soil, is due to insufficient knowledge of their ecological characteristics, including mycorrhizal symbiosis, and unfavorable plantation-site conditions. A range of published data mentions the ectomycorrhizae on *Eucalyptus* (Bakshi 1974; Malajczuk et al. 1982 as cited in Thapar 1987; Pryor 1956). Bakshi showed that the development of mycorrhizae in *Eucalyptus* is particularly significant when the species is raised on submarginal or poor sites. Pryor noted that the chlorosis and lack of seedling vigor of renantherous species of *Eucalyptus* was caused by the lack of mycorrhizae (cited by Bakshi 1974). Our field observations are in accordance with the above-mentioned data. *Boletus* sp. (preliminary identification) occurred widely under mature *Eucalyptus* plantations on sites with shallow soil. It seems that this is a frequent *Eucalyptus* mycorrhizal symbiont. The carpophores of *Rhizopogon* sp., *Scleroderma* sp., and *Pisolithus* appeared there rarely. An experiment conducted outside of Vietnam, where *Pisolithus tinctorius* and *Rhizopogon luteolus* were inoculated on *Eucalyptus maideni*, gave good results (Chen Ceke Xuan Yu, 1988). Actually, *Eucalyptus* silviculture in Vietnam has been rehabilitated and promoted by the use of fast-growing species. However, whether *Eucalyptus* mycorrhizae are a beneficial bio-factor is still unknown or omitted, and *Eucalyptus* plantations do not do well everywhere.

16.3.3 Dipterocarps

The Dipterocarpaceae include many dominant timber-tree species of the tropical-rain forest. Dipterocarp species are distributed from the north to the south of Vietnam, but mainly in the centre and south of the country. Their timbers are classified as high-quality woods and are over consumed. Therefore, most dipterocarp forests have been over-exploited and are exhausted. The spraying of toxic chemicals during the war still causes harm in places. Some precious dipterocarps had their root-systems dug up for ornamental and handicraft uses, and became extremely rare. Therefore, the rehabilitation and reforestation of dipterocarp forests, especially the con-

servation and propagation of these precious species, are urgently needed for both economical and ecological reasons. Dipterocarp ectomycorrhizae have been studied, and results published by many researchers, particularly in the South and Southeast Asian countries. All researchers working on this topic confirmed the role of mycorrhizae on the growth of dipterocarp species. There has been a preliminary and interrupted study on this subject in Vietnam. A brown dichotomous ectomycorrhiza of *Dipterocarpus tonkinensis* has been observed. The transplantation of 3–5-year-old *D. tonkinensis* trees along the pavement of a principal street of Hanoi City did not result in the expected success, in spite of special care for them. An investigation of the root system of almost-dead trees revealed that their mycorrhizal roots were wasted. Slow growth in dipterocarp plantations in many sites in the south of Vietnam can be observed. It seems that this is due to the absence of appropriate mycorrhizal associations and the lack of effective strains. It is regrettable that until now there has been no research in Vietnam on this important topic. There have recently been attractive studies on the artificial mycorrhization of dipterocarp species with some mycorrhizal fungi, such as *Scleroderma columnae* (Kikuchi and Ogawa 1995), *S. dictyosporium* (Supriyanto et al. 1994), and *Pisolithus tinctorius* (Lee 1994). The results are promising.

16.3.4 *Casuarina equisetifolia*

Casuarina equisetifolia plantations have been established in many regions, mainly in coastal zones for wind-break use and the prevention of sandy-dune movement. Its other common utilizations are for shade and for firewood in rural areas. This species tolerates poor sandy soil, high temperatures and dry-windy conditions. Therefore, it is a popular tree species in Vietnam. However, there are many locations where *Casuarina* plantations have been clear felled for firewood and the preparation of construction areas. Many large coastal-sandy zones are still wastelands, and plantation establishment under such extreme conditions has not always been successful. The *Rhizobium* of *Casuarina equisetifolia* has been well known for a long time. The actinomycete root nodule andophyte, *Frankia*, has been studied (Tan Sai Tee 1987), but there is little literature related to the mycorrhizae of this tree species. It seems that our recent observations are in accordance with this. An extremely high density of brown, coralloid mycorrhizae has been observed on the root system of mature *Casuarina equisetifolia,* which grows well on coastal-sandy zones, even dunes, in the south of Vietnam, observed at Vungtau City from 1991 to 1995. *Clavaria* sp. and *Ramaria* sp. sporophores were found to occur frequently on sandy ground, even in unshaded sites, while *Amanita* sp. and *Clitocybe* sp. carpophores appeared there rarely. Thus, it seems that some species belonging to Clavariaceae are effective mycorrhizal fungi for *Casuarina equisetifolia* in different adverse sites. This also indicates the extraordinary adaptive capacity of these fungi to hard ecological conditions. Certainly, this fungal performance has enhanced the capacity of the tree host to tolerate such plantation-site conditions. This could be a beneficial silviculture application and needs further study.

16.4 Conclusion

Vietnam is, like all nations, facing up to the global problem of the deterioration of the environment, provoked by many unreasoned human activities. However, the main cause is the destruction of forests, our common house roof. Therefore, rehabilitation and reafforestation is urgent work nowadays. In this difficult and heavy task, mycorrhizae could help as an effective tool in afforestation. Mycorrhizae are a bio-factor giving multiple advantages as a water-nutrient supplier to vegetation, soil fertility and structure ameliorator, contributor to ecosystem equilibration, etc. Scientists are searching to manage this bio-tool. Both fundamental and applied aspects of mycorrhizal science have been studied by researchers in many countries. It is expected that in years to come, especially in the 21 Century, mycorrhizology could contribute greatly to forestry plantations and production. This depends on mycorrhizologists' endeavors, and their cooperation among themselves and with biologists and foresters. Important factors for ensuring success are: expert help from advanced researchers; financial aid from developed countries, international and national organizations, and private associations concerned with rehabilitation of the global ecosystem; and concrete reafforestation in the region and the world.

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17
Mycorrhizal Formation and Growth of *Shorea leprosula* in Bukit Suharto After Using Charcoal and Rockphosphate

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17.1 Introduction

East Kalimantan and some other parts of Indonesia face the great problem of fire, not only during the long, dry season but also during the rest of the year as well. Low soil-fertility and small numbers of mycorrhizae in burnt and open areas of East Kalimantan cause the biggest problems for reforestation and afforestation. Soil improvement is needed, and so it is necessary to improve the soil using phosphate fertilizer, mycorrhizal inoculation, and other methods, whenever possible and practicable, in dipterocarp plantations. In some soils, high yields can only be obtained by the combination of inoculation and P-fertilization (Howeler and Sieverding 1983). It has already been proven that charcoal product can improve the activities of VA mycorrhizae and increase the resistance of seedlings to disease (Kobayashi 1989; Ogawa 1989a,b; Handojo et al 2001). Therefore, it is very important to observe whether the combination of phosphate, inoculation and charcoal can improve the growth of seedlings in the field.This research aimed at establishing an experimental plantation using charcoal and natural P to promote more practicable research activities for the reforestation and afforestation of Dipterocarpaceae.

The objectives of the research were:

1. To improve soil condition with charcoal and rockphosphate.
2. To utilize mycorrhizal formation for better absorption of nutrients.
3. To establish an experimental plantation.

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17.2 Materials and Methods

17.2.1 Materials

Shorea leprosula seeds were collected from the area in Bukit Suharto, East Kalimantan. Seeds of *S. leprosula* were selected to get healthy and similarly sized seeds to germinate. Other materials prepared were charcoal and mycorrhizal inoculum. The charcoal was bought at a local market in Bukit Suharto and crushed to powder before use. Mycorrhizal inocula were collected in Bukit Suharto forest and inoculated onto the seedlings (for details see Section 18.2.3). Additional materials were used for maintainence and measuring. These were medium plastic pots, aquades, Tween 20, insecticide, ruler, caliper, thermometer, hygrometer, and light meter.

17.2.2 Seed Germination

Seeds were germinated in the soil medium from Bukit Suharto. After germination, the seeds were planted directly into the plastic-pot medium. The seedlings were maintained in the nursery for 6 months (in the first month of germination, inoculation was done to half of all seedlings). Watering, weeding, and the use of insecticide as necessary were part of maintaining the seedlings' growth.

17.2.3 Inoculation Method

Mycorrhizal inoculum of *Scleroderma columnare* was collected from natural and healthy sporocarp in the nursery and under dipterocarp stands near Bukit Suharto. Spores were then extracted from the sporocarps. The liquid from 25 mg of reconstituted dried fungal spores mixed in one liter water was used for inoculating each seedling. Inoculation was done at the pot in nursery about 1 cm from the base at the seedlings in a soil depth of 5 cm. Inoculation was done during the first month of germination.

17.2.4 Planting in the Experimental Field

Five to six months after germination, the seedlings were transferred to the field and then planted in the experimental location. The following additional materials were used as treatments for the seedlings: (1) 100, 50, or 0 g of charcoal (coal); and (2) 50, 25, or 0 g of rockphosphate.

The experiment was conducted in Bukit Suharto experiment field. The seedlings were planted in the experimental layout as follows:

1. A 40 × 40 m square was divided into two blocks, shaded and open. The shade was applied by leaving the mature trees in the block, whereas in the open block all the trees were cut/cleaned. Half of the seedlings were planted in the open

Table 1. Analysis of variance for the height of *S. leprosula*

Source	SS	df	MS	*F*	*P*
Shade	78.975	1	78.975	0.094	
Coal	335.205	2	167.603	0.199	
Shade Coal	3116.746	2	1558.373	1.850	0.1596
Phos	11612.163	2	5806.082	6.892	0.0018
Shade Phos	454.275	2	227.138	0.270	
Coal Phos	2453.288	4	613.322	0.728	
Shade Coal Phos	1331.978	4	332.994	0.395	
Block	419.175	1	419.175	0.498	
Shade Block	830.048	1	830.048	0.985	
Coal Block	14249.523	2	7124.762	8.457	0.0006
Shade Coal Block	1778.018	2	889.009	1.055	0.3521
Phos Block	4511.701	2	2255.851	2.678	0.0710
Shade Phos Block	1977.548	2	988.774	1.174	0.3126
Coal Phos Block	2298.376	4	574.594	0.682	
Shade Coal Block	3416.446	4	854.111	1.014	0.4037
Error	106993.703	127	842.470		

Coal: charcoal

Phos: rockphosphate

block and the rest were planted in the shaded block.

2. Each block had two sub-blocks (inoculated and uninoculated seedlings). Each sub-block had one-third of the total seedlings with 0, 50, and 100 g of coal.
3. Each sub-block had one-third of the seedlings with 0, 25, and 50 g of rockphosphate.
4. Each experimental unit consisted of 10 replicate seedlings. The research in the field was conducted for about 6 months. To maintain seedling growth during the field period, weeding was done and insecticide was applied as necessary.

17.2.5 Data Collection and Analyses

Measurements of plant height and diameter were done monthly. After 6 months in the field, the final total height and base diameter of each young tree was measured. The percent mycorrhizal formation of each seedling was also measured. Soil analysis was done before and after the experiment to observe the effect of all treatments on soil structure. The total potassium, available phosphate, available zinc and total nitrogen were analyzed for content. The collected data for the height and diameter of seedlings and mycorrhizal formations during transplanting were analyzed. Data were analyzed as a factorial block design.

Table 2. The effect of rockphosphate on the height of *S. leprosula*

Rockphosphate (g/seedling)	Height (cm)
0	58.805
25	76.554
50	79.979

17.3 Results

17.3.1 Height

The results show that rockphosphate had a significant effect on the height of *S. leprosula* in the field (Table 1). Height was not affected by shading or coal, but was significantly affected by rockphosphate. Height was also not affected by the combinations of shading and rockphosphate, or coal and rockphosphate. The results in Table 1 also show that height was not affected by block, or the combination of block and shading, but was significantly affected by the combination of coal and block. In terms of other combinations, height was affected by the combinations of: shading, coal, and block; phosphate and block; shading, rockphosphate, and block; coal, rockphosphate, and block; and shading, coal; and rockphosphate.

Height was significantly affected by rockphosphate. Table 2 shows that height was strongly affected by adding 25 g of rockphosphate per seedling. Furthermore, seedlings supplied with 50 g of rockphosphate had the highest growth, but were not significantly different to those with the 25 g/seedling treatment.

Data presented in Table 3 show that a combination of coal and block had a significant effect on plant height. The seedlings of shade block (II) with 0 or 25 g of coal per seedling grew better than the seeds planted in open area block (I) with 0 or 25 g of coal per seedling. An exception was observed between seedlings of the open and shaded areas in combination with 50 g of coal per seedling.

17.3.2 Diameter

The results show that rockphosphate had a significant effect on the diameter of *S. leprosula* in the field (Table 4). Diameter was not affected by shading or coal treatment. Diameter was also not affected by a combination of shading and phosphate, coal and rockphosphate, or shading, coal, and rockphosphate. In addition, the results show that diameter was not affected by block or a combination of block and shading, but was significantly affected by a combination of coal and block.

In terms of other combinations, diameter was not affected by the combinations of: shading, coal, and block; phosphate and block; shading, rockphosphate, and block; coal, rockphosphate and block; or shading, coal, and rockphosphate.

Table 3. The effect of the combination of charcoal and block on the height of *S. leprosula*

Charcoal (g/seedling)	Block	Height (cm)
0	I	61.97
	II	83.42
25	I	69.22
	II	72.75
50	I	84.34
	II	60.11

Table 4. Analysis of variance for the diameter of *S. leprosula*

Source	SS	df	MS	*F*	*P*
Shade	5.290	1	5.290	1.158	0.2837
Coal	5.652	2		2.826	0.619
Shade Coal	20.939	2	10.470	2.292	0.1033
Phos	96.244	2	48.122	10.535	0.0002
Shade Phos	6.643	2	3.327	0.727	
Coal Phos	17.924	4	4.481	0.981	
Shade Coal Phos	5.596	4	1.399	0.306	
Block	7.031	1	7.031	1.539	0.2147
Shade Block	0.252	1	0.252	0.055	
Coal Block	61.899	2	30.950	6.775	0.0020
Shade Coal Block	20.011	2	10.005	2.190	0.1142
Phos Block	16.563	2	8.281	1.813	0.1655
Shade Phos Block	3.434	2	1.717	0.376	
Coal Phos Block	15.180	4	3.795	0.831	
Shade Coal Block	23.405	4	45.851	1.281	0.2802
Error	580.133	127	4.568		

Coal: charcoal

Phos: rockphosphate

Table 5. The effect of rockphosphate on the diameter of *S. leprosula*

Rockphosphate (g/seedling)	Diameter (mm)
0	6.070
25	7.678
50	8.008

Table 6. The effect of the combination of coal and block on the diameter of *S. leprosula*

Coal (g/seedling)	Block	Diameter (mm)
0	I	6.552
	II	7.836
25	I	6.721
	II	7.371
50	I	8.517
	II	6.687

Table 7. Analysis of variance for mycorrhizal formation on *S. leprosula*

Source	SS	df	MS	*F*	*P*
Shade	3377.793	1	3377.793	6.096	0.0142
Coal	2646.692	2	1323.346	2.388	0.0938
Shade Coal	130.283	2	65.142	0.118	
Phos	5116.069	2	2558.043	4.617	0.0115
Shade Phos	535.549	2	267.774	0.483	
Coal Phos	2714.327	4	678.582	1.225	0.3028
Shade Coal Phos	2773.663	4	693.416	1.251	0.2917
Block	6903.634	1	6903.634	12.460	0.0009
Shade Block	1453.029	1	1453.029	2.622	0.1038
Coal Block	886.829	2	443.415	0.800	
Shade Coal Block	380.730	2	190.365	0.344	
Phos Block	1758.953	2	879.476	1.587	0.2067
Shade Phos Block	77.500	2	38.750	0.070	
Coal Phos Block	388.375	4	97.094	0.175	
Shade Coal Block	354.703	4	88.676	0.160	
Error	74800.602	135	554.079		

Coal: charcoal

Phos: rockphosphate

Table 8. The effect of rockphosphate on mycorrhizal formation

Rockphosphate (g/seedling)	Mycorrhizal formation (%)
0	52.273
25	64.655
50	60.345

Table 9. The effect of shading on mycorrhizal formation

Shading	Mycorrhizal formation (%)
Open area (Block I)	53.274
Closed area (Block II)	64.944

Diameter was significantly affected by rockphosphate. Table 5 shows that diameter was strongly affected by the addition of 25 g of rockphosphate per seedling. Furthermore, it can be seen that seedlings supplied by 50 g of rockphosphate had better, but not significantly different, diameter growth than those with just 25 g.

Data in Table 6 show that the combination of coal and block significantly affected diameter growth. The combined treatments of shading, block, and 0 or 25 g of coal per seedling produced better results on seedling diameter growth than the treatments of open block with 0 or 25 g of coal per seedling. An exception was observed for the treatments of 50 g coal per seedling between the shaded and open blocks.

17.3.3 Mycorrhizal Formation

The results presented in Table 7 show that mycorrhizal formation was affected significantly by shading and rockphosphate. However, mycorrhizal formation was not affected by the combinations of shading and phosphate, coal and rockphosphate, or shading, coal and rockphosphate. The table also shows that mycorrhizal formation was significantly affected by block. Mycorrhizal formation was not affected by the combinations of block and shading, or coal and block.

In term of other combinations, mycorrhizal formation was not affected by the combinations of: shading, coal and block; shading, rockphosphate, and block; coal, rockphosphate, and block; or shading, coal, and rockphosphate.

Mycorrhizal formation was significantly affected by rockphosphate. Table 8 shows that the addition of rockphosphate affected mycorrhizal formation. The results show that adding 25 g of rockphosphate per seedling gave the best mycorrhizal formation among the treatments. It can be seen that seedlings supplied with 50 g of rockphosphate had lower mycorrhizal formation than those supplied with 25 g of rockphosphate.

The average of mycorrhizal formation in each seedling differed between blocks. Table 9 shows that mycorrhizal formation was significantly affected by blocks exposed to the sun. Block II, which was in the shade, had higher mycorrhizal formation (64 %) than the open area (Block I) which had only 53% mycorrhizal formation.

17.4 Discussion

17.4.1 Rockphosphate

Based on the results in Tables 1 and 2, it can be seen that rockphosphate has a significant effect on the height of *S. leprosula* seedlings. Rockphosphate is an inexpensive fertilizer and has a good combination with mycorrhizal growth. Primarily, there is a tendency in the long run for rockphosphate to have a beneficial effect on seedling growth. Since it is a slowly released fertilizer, and the growth of the tree needs a long period to absorb fertilizer, the choice of rockphosphate as a tree fertilizer is useful. Normally, phosphate is present in artificial media as inorganic potassium phosphate, but it may also be present as organic phosphate (Harley and Smith 1983). The benefit of rockphosphate on the seedling stage has also been shown by some researchers, such as on *Pinus kesiya* (Fakuara 1984).

Rockphosphate also has a profitable effect on mycorrhizal formation, as it increased mycorrhizal formation (Table 8). Harley and Smith (1983) stated that a supply of phosphate is essential to all mycorrhizal fungi. Their research report shows that phosphate presents on the surface of a fruit-body of some ectomycorrhizae and some vascular-arbuscular mycorrhizae. This phenomenon indicates that phosphate is essential to all mycorrhizal fungi. However contrary result was reported by Suhardi (1998).

17.4.2 Shading

In the early stage of *S. leprosula* seedling growth, there is evidence that growth in a shaded area is better than in an open area. There is one possible explanation that supports this phenomenon. For most *Shorea*, it can be said that the plants need low-intensity light during their early stage (Suhardi 1989; Suzuki and Jacalne 1986). Therefore, it can be understood that the plants in this research are more suitable to the lower-intensity light than the higher one.

It is known that optimum mycorrhizal formation can only occur at a suitable soil temperature e.g. 27°–28°C. The shade areas with low-intensity light can keep the soil temperature low (around 27°–28°C). Therefore, mycorrhizal formation can occur more in the shade than in the open areas with much higher-intensity light. Mycorrhizal formation will bring good effects on plant growth, as mycorrhizae increase the absorption of some important nutrients, especially P-available nutrients. This phenomenon was also found in other species, e.g., *Shorea bracteolate*, until at least 6 months in the field (Suhardi 1990).

17.4.3 Charcoal

Charcoal had no effect on plant height and diameter. There was also no significant difference in mycorrhizal formation. It is possible that the quantity of charcoal used was not enough to influence seedling growth in the field, due to the many factors involved. Another possible factor that might make the charcoal have no effect on seedling growth is its material. It is still not known what the raw material of the charcoal was. In fact, the kind of raw material (kind of wood material) apparently influences the quality of the charcoal itself (Handojo et al 2001). So, as a suggestion for the next research project, it is important to find the best quantity and raw material of charcoal used for improving the growth of seedlings or plants in the field.

17.4.4 Soil Analysis

Based on the nutrient content of the soil, there was also a tendency for the available phosphate to be found more in areas where mycorrhizal formation was high. The result of the nutrient content analysis can be clearly seen in Table 10. From Table 10, it can be concluded that the presence of mycorrhizae in soil can eventually increase the content of some nutrients in that area i.e., P-available, Zn-total and Cu-total. These three nutrients are important for plant growth, as all of them are readily absorbed. However, it can also be seen that the open area had a much higher P-total than the shaded area, but it does not necessarily indicate that plants in the open area can absorb higher phosphate than those in shade area, since plants do not readily absorb nutrients unless it reforms them into P-available nutrients.

Table 10. Nutrient contents near mycorrhizae-associated and non mycorrhizae-associated blocks

Nutrient content (ppm)	Mycorrhizae	Non mycorrhizae
P total	250.52	874.76
P available	5.18	0.00
Zn total	14.08	9.52
Cu total	8.70	6.9

17.5 Conclusions

1. Rockphosphate has a significant effect on the growth of *Shorea leprosula*, both for diameter and height.
2. Rockphosphate has a good relation to mycorrhizal formation.
3. Shaded blocks in the field have a better effect on mycorrhizal formation of seedlings than do open blocks.
4. Mycorrhizal formation could increase the P-available, Cu total, and Zn-total nutrients.

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18
Inoculation Method of *Scleroderma columnare* onto Dipterocarps

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18.1 Introduction

The roles of ectomycorrhizal fungi in the nutrient uptake and root protection of host plants have been well established over years of study of mycorrhizal symbiosis. Many practical methods of using mycorrhizal fungi in raising seedlings and for afforestation have also been developed in various countries. However, when the author started work on the mycorrhizae of Dipterocarpaceae in East Kalimantan, Indonesia, there were few such methods.

Tree species with ectomycorrhizae are limited to several families such as Pinaceae, Fagaceae, Beturaceae, Salicaceae, and Dipterocarpaceae that are mainly distributed in the northern hemisphere. In lowland tropical regions, only Dipterocarpaceae are dominant among the tree species with ectomycorrhizae. Originally, the number of ectomycorrhizal-fungal species seemed to be less in the lowlands than in the north, and the frequency of mycorrhizal formation in nature was also lower. Particularly in devastated areas, after burning or on barren land, the spore numbers of mycorrhizal fungi seemed to be extremely low. Moreover, the climatic condition is too severe for spores to survive in the surface soil. Therefore, it was considered that the inoculation of useful mycorrhizal fungi is essential for afforestation of *Dipterocarpaceae* in this region.

Most tree species, except for those with ectomycorrhizae, bear VA mycorrhizae (VAM) or seem to be free from symbionts in tropical-rain forests. Therefore, different techniques to those used for Dipterocarpaceae should be applied to these species.

Julich (1988) reported the collection list of mycorrhizal fungi in a natural stand of Dipterocarpaceae in Kalimantan. Most species had not been identified, but he described some major species, including *Scleroderma columnare* and *Laccaria* sp. Some people in the JICA project also collected mushrooms in Bukit Suharto experi-

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ment forest, from 1986 to 1988. These species were added to the author's list in 1988. Hadi (1987) reported a trial of inoculation of mycorrhizal fungi to five dipterocarp species.

The present study was conducted from 1988 to 1992 by the author at Mulawarman University, Samarinda, Indonesia, supported by JICA. Also involved were Dr. Suhardi of Gadjah Mada University, and Mr. Agus Darmawan of Mulawarman University.

18.2 Research Results

18.2.1 Mycorrhizal Fungi of Dipterocarpaceae

18.2.1.1 Collection and Identification of Mushrooms

Mushrooms, including mycorrhizal fungi, were collected mainly from the natural stands of dipterocarps in Bukit Suharto experiment Forest, Lempake experiment forest, and the nursery of Mulawarman University, from February to March 1989, March 1990 and February to March 1991. The genera and species names were identified by the author referring to some manuals published in Japan, and they were named temporarily. Some major mycorrhizal species were later identified by Dr. T. Hongo in Japan.

The total number of mushroom species collected was 68, including 34 species of mycorrhizal fungi. As shown in Table 1, among the mycorrhizal fungi the genera *Scleroderma*, *Laccaria*, *Russula*, *Amanita*, and *Boletus* were dominant. Most litter decomposers, like *Mycena*, *Xeromphalina*, *Oudimansiella*, *Marasmius*, and some others belonged to the group that formed smaller fruit bodies. Wood-decaying fungi belonged to *Fomes*, *Polyporus*, *Ganoderma*, *Auricularia*, and so on. Generally speaking, most fruit bodies, except for the hard-fruit bodies of wood-decaying fungi, were produced only in the rainy season and disappeared after being attacked by insects or microbes, soon after their occurrence. The number of fruit bodies collected from a stand in one season was smaller than those in temperate forests.

The genus-level composition of mycorrhizal fungi was very close to those collected from some evergreen broad-leaved forests in Asia. Although most species were unidentified or new ones, several species were the same as species described in Japan.

18.2.1.2 Dominant Mycorrhizal Fungi in a Nursery and Young Stands

Many fruit bodies of *Scleroderma columnare*, named by Berkrey in 1875 (as cited in Guzman 1969), were collected from the nursery and young stands in the campus of Mulawarman University. Those of *Laccaria* sp., which was similar to *L. vinaceoavellanea* and *Inocybe* sp., were found in nursery pots and around the saplings of *Shorea* and *Hopea* in the arboretum. These three species seem to be dominant on the seedlings and also the precursors of succession among mycorrhizal fungi of dipterocarps. The growth of seedlings that were infected with the mycorrhiza

Table 1. Fungal species collected from dipterocarp forest in Bukit Suharto Experiment Forest and Lempake Experiment Forest in the rainy season, from 1989 to 1991

Mycorrhizal fungi	Wood decaying or litter decomposing fungi
1 *Scleroderma columnare*	1 *Oudimansilla rudicata*
2 *Scleroderma flavidum*	2 *Oudimansilla* sp.
3 *Scleroderma verrucosum*	3 *Clitocybe odora*
4 *Scleroderma* sp.	4 *Lepiota cygnea*
5 *Scleroderma* sp.	5 *Xeromphalina tenuipes*
6 *Scleroderma* sp.	6 *Xeromphalina* sp.
7 *Laccaria laccata*	7 *Macrolepiota* sp.
8 *Laccaria vinaceoavellanea*	8 *Marasmius* sp.
9 *Russula virescens*	9 *Marasmius* sp.
10 *Russula nigricans*	10 *Mycena* sp.
11 *Russula senesis*	11 *Mycena* sp.
12 *Russula emetica*	12 *Cymnopilus aeruginosus*
13 *Russula vesca*	13 *Lycoperdon* sp.
14 *Russula cyanoxantha*	14 *Fomes* sp.
15 *Russula foetens*	15 *Fomes* sp.
16 *Russula* sp.	16 *Polyporus* sp.
17 *Russula* sp.	17 *Polyporellus* sp.
18 *Russula* sp.	18 *Polyporellus* sp.
19 *Xerocomus subtomentosus*	19 *Daedaleopsis* sp.
20 *Boletus* sp.	20 *Termitomyces* sp.
21 *Boletus* sp.	21 *Trichaptum* sp.
22 *Boletus* sp. (*B. edulis*)	22 *Bierkandera* sp.
23 *Boletellus* sp.	23 *Fomes* sp.
24 *Amanita sycropyramis f. subannulata*	24 *Panus* sp.
25 *Amanita verna*	25 *Ganoderma* sp.
26 *Amanita spissacea*	26 *Ganoderma* sp.
27 *Amanita vaginata*	27 *Microporus* sp.
28 *Amanita* sp.	28 *Microporous* sp.
29 *Amanita* sp. (*A. virosa*)	29 *Stereum* sp.
30 *Cantharellus* sp. (*C. cibarius*)	30 *Exidia* sp.
31 *Hygrocybe* sp.	31 *Pycroporous coccineus*
32 *Inocybe* sp.	32 *Auricularia auricula*
33 *Xerocomus* sp.	33 *Microstoma* sp.
	34 *Helvella* sp.

Scleroderma columnare was apparently better than those infected by *Laccaria* sp. or *Inocybe* sp. in the nursery. The bulb-shaped fruit body of *Scleroderma* was also suitable for retaining spores for the long term. Spores with small, numerous spines and a thick wall seemed to be suitable for floating in water and surviving under severe conditions. Consequently, this species was selected for the experiments and practical use as an inoculum of mycorrhizal fungi.

18.2.2 Ecological Characters of *Scleroderma columnare* — Fructification, Morphology of Mycorrhizae and Rhizomorphs, and Host Range —

18.2.2.1 Fructification of *Scleroderma columnare*

A description of the fruit body and taxonomical studies of *S. columnare* were reported by Guzman (1969). In the corner of the nursery, 1-year-old saplings of *Shorea leprosula* and *Shorea academica* grew surrounded by boards. Some fruit bodies of *S. columnare* were collected after a heavy shower in February 1989. The fruit-body occurring points were recorded in February 1989, March 1990, and February to March 1991. As shown in Fig. 1, the points moved outward, and the territory expanded about 1 m every year. This fungus stimulates the growth of main roots and branching of lateral roots, forming typical ectomycorrhiza, and forms well-developed rhizomorphs growing next to young roots. So, this fungus occupies a certain area and produces fruit bodies at the margin of its territory.

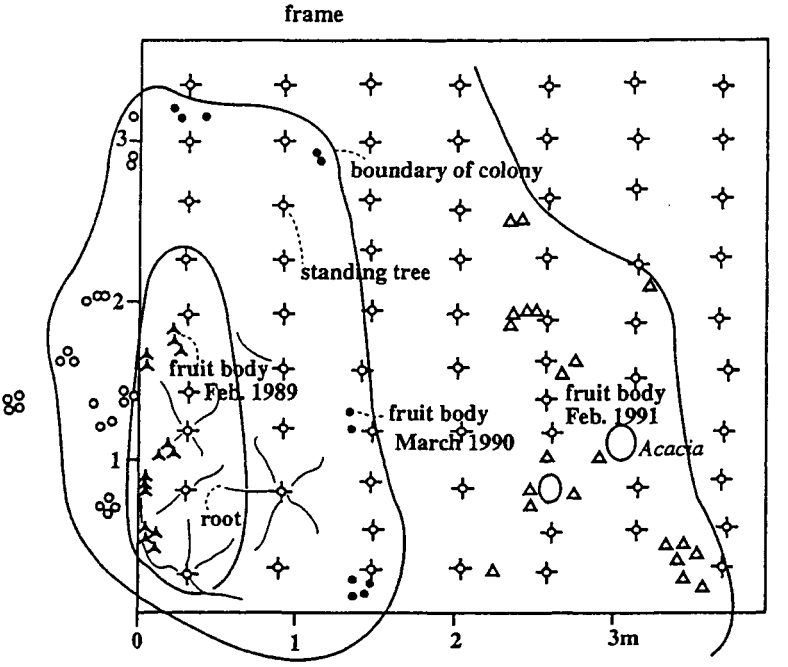

Fig. 1. The colony and fruit bodies of *Scleroderma columnare* in the nursery of 3-year-old seedlings of *Shorea leprosula*

In 1990, primordia formation started 10 days after a heavy shower of about 77 mm in February and continued to March 20, as shown in Fig. 2A. In 1991, the fructification also showed a good response to rainfall (Fig. 2B). It took about 10 days to produce the primordia after enough rainfall, and 10–14 days to mature. From these behaviors, it seems that fruit-body formation can be controlled by watering more than 70 mm in a day.

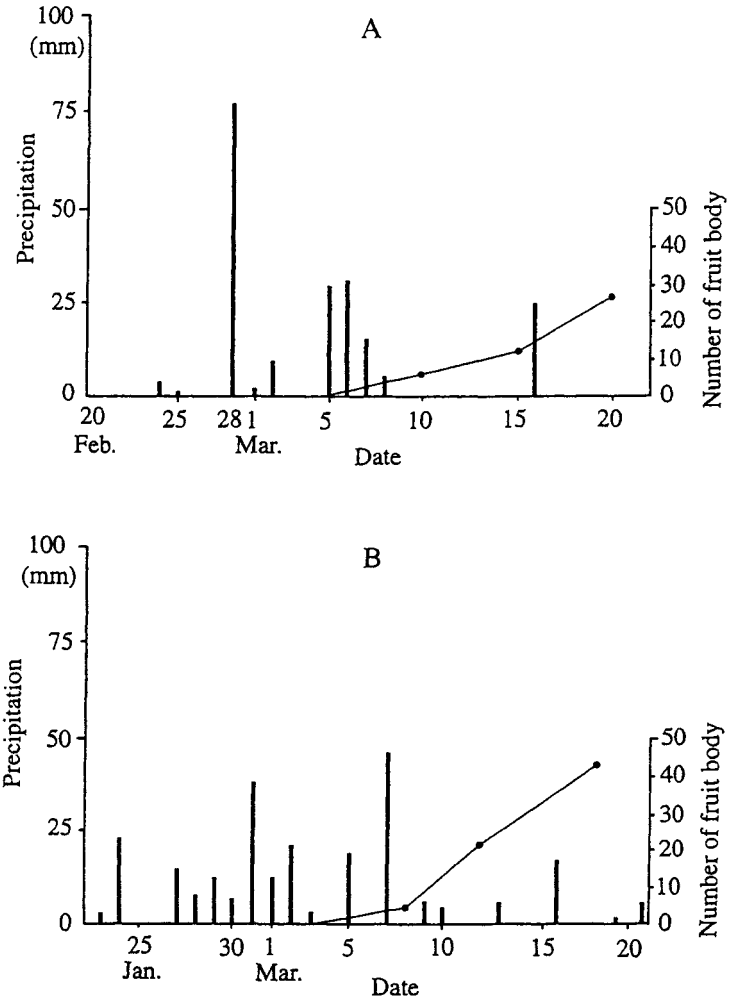

Fig. 2. Fructification of *Scleroderma columnare* in relation to precipitation in 1990 (**A**) and 1991(**B**). *Solid bars* represent precipitation, and *solid lines* represent the number of fruit bodies

Table 2. Host range of *Scleroderma columnare* and *Laccaria vinacecavellanea*

Species name of host plant	Mycorrhizal fungi			
	Scleroderma columnare		*Laccaria vinacecavellanea*	
	Mycorrhiza	Fruit body	Mycorrhiza	Fruit body
Dryobalanops lanceolata	–	+	+	+
Dryobalanops sp.	+	–	–	–
Shorea leprosula	+	+	–	–
Shorea parvifolia	+	+	+	–
Shorea smithiana	+	+	–	–
Shorea lamellata	+	+	–	–
Shorea ovalis	+	+	–	–
Shorea macrophylla	+	+	–	–
Shorea seminis	–	+	–	–
Shorea pinanga	–	+	–	–
Hopea mengarawan	+	+	–	–

Symbiotic relation was confirmed by observation of mycorrhiza and fruit body occurrence under planted trees

18.2.2.2 Rhizomorphs and Mycorrhizae of *S. columnare*

This fungal species forms well-developed rhizomorphs as well as the other species of *Scleroderma*. These white-colored rhizomorphs grow into cracks of soil spreading along young roots and a developing white mycorrhiza. Inside a rhizomorph, several main hyphae, which are five to seven times that of usual hyphae in diameter, ornamental tendril hyphae, and surface hyphae differentiate relatively well.

The main or secondary roots infected by the fungus elongate and branch with high frequency, so that the shape of the mycorrhiza becomes broom or pinnate type. The surface of the main root also shows mycorrhizal structure, but the root tips are free from fungal infection. The fungal sheath of the main root is removed according to the secondary growth of the root.

The surfaces of lateral roots are covered by a white and thick fungal sheath, and glisten a silver color in water because the hyphae of the fungal sheath keeps much air in the vacuole. There is the typical and well-developed Hartig's net in the intercellular spaces of the outer cortical cells. There is neither the accumulation of pigments in the epidermis nor the specific structure of mycelium over the surface of the mycorrhiza. It is easy to identify the *Scleroderma*'s mycorrhiza from morphological features.

18.2.2.3 Host Range and Natural Infection in the Nursery

The host range of the fungus was examined by mycorrhizal formation and fruit-body production around the bases of saplings of Dipterocarpaceae that were planted in the university campus and arboretum. *Scleroderma columnare* formed a white mycorrhiza with 11 species of Dipterocarpaceae, as shown in Table 2. Meanwhile, *Laccaria* sp. formed a mycorrhiza with only two host species. It seems from these facts that *S. columnare* has wide-host range, but *Laccaria* sp. and *Inocybe* sp., on which mycorrhiza is hardly identified in the field, has stricter host specificities.

Table 3. Natural infection of *Scleroderma columnare* in seedlings in the nursery near the dipterocarp forest in Bukit Suharto Experiment Forest

Frequency of mycorrhiza[b]	*Shorea seminis*[a]			*Drybalanops* sp.[a]			*Shorea seminis*[a]	
	40 days	7 months		40 days	6 months		7 months	
	Infection[c] (%)	Infection[c] (%)	Average height (cm)	Infection[c] (%)	Infection[c] (%)	Average height (cm)	Infection[c] (%)	Average height (cm)
–	0	32	23.2	0	65	21.1	3	26.0
+	0	57	27.2	0	19	43.2	36	35.2
+	0	57	27.2	0	19	43.2	36	35.2
++	0	7	29.0	0	16	51.2	42	35.5
+++	0	4	25.0	0	16	—	19	35.2
Total of infection	0	68		0	35		97	

[a]Seeds of *S. seminis* were sown in August, 1990 and February 9, 1991, *Dryobalanops* sp. in September, 1990 and February 1, 1991, and *S. parvifolia* in August, 1990. Pot soil was not sterilized

[b]Frequency of mycorrhiza was checkd at 4 points around the base of each seedling. (–) is non-infected, (+) is 1 point infected, (++) is 2 points infected and (+++) is 3 points infected

[c]Natural infection rate

Table 4. Inoculation test of *Scleroderma columnare* to *Shorea leprosula* seedlings in root box. Spore was immobilized into two kinds of charcoal

Treatment	Hight growth (cm)		No. of leaves		Frequency of Mycorrhiza	
Root box No.	1	2	1	2	1	2
Control						
'91. Sep	21.0	17.0	8	8	—	—
'92. Mar	25.0	22.0	7	7	—	—
Spore suspension	14.5	10.0	5	3	—	—
sprayed	16.0	10.0	2	1	—	—
(A)(a) + Silicagel + agar	32.5	27.0	8	7	+	+
+ spore (ball) buried	51.0	37.0	9	9	+++	+++
(B)(a) + Spore (crust) buried	35.0	23.5	10	7	+	+
	52.0	36.0	11	7	+++	+++
(C)(b) + Spore	24.5	13.0	8	7	+	+
mixed powder	35.0	32.0	17	16	+++	+++
(C)(b) + Spore (crust) buried	21.5	16.0	6	6	+	+
	26.0	20.0	6	7	++	++

18.2.2.4 Natural Infection of *S. columnare* in the Nursery

Natural infections of mycorrhizal fungi were sometimes observed in the nursery near the dipterocarp forest. The mycorrhizal formation rate was checked on the seedlings of three species of dipterocarps that were raised in the nursery of Bukit Suharto experiment forest. Two kinds of seedlings of *Shorea seminis* with different growth terms — 7 months and 40 days — were checked. The seedling ages of *Dryobalanops*

sp. were 6 months and 48 days and that of *S. panvifolia* was 7-months. The unsterilized surface soil of a natural stand was used for the nursery pot, but there was no other treatment to inoculate the fungus.

After washing the seedling roots, mycorrhizal-formation rates were divided into four grades and calculated, as shown in Table 3. Most of the mycorrhizae formed on these roots were *S. columnare*.

Natural infection rates of the seedlings varied, depending on the host species and age. The infection rate of *S. parvifolia* was the highest among them, and the effect on seedling height growth was relatively high. Thinking from the distribution of the mycorrhiza on the root system, the fungal infection probably did not come from pot soil, but from the nursery ground soil through a hole at the bottom. Therefore, so far as is concerned to this species, it is not always necessary to inoculate the fungus artificially, if there are some fungal spores in the nursery bed.

On the other hand, the infection rate of *Dryobalanops* sp. was lower than the others, but the effect of mycorrhizal formation was remarkable. So in this case, artificial inoculation is recommended in nursery practice.

The infection rate of *S. seminis* was higher than that of *Dryobalanops* sp., but the frequency of the mycorrhiza was rather low. The growth-promotion effect was mostly negligible.

Young seedlings of *S. seminis* and *Dryobalanops* sp. had no mycorrhizae, even at 40 and 48 days after sowing. Probably, mycorrhizal formation by natural infection starts 2–3 months later, after the sowing of seeds.

From these results, it is certain that the inoculation of suitable fungal species is essential for raising seedlings, particularly when the tree species has low affinity with the fungal partners. Moreover, the raising term can be shortened and fast growing and healthy seedlings can be obtained by inoculation.

18.2.3 Inoculation of *Scleroderma columnare* onto Dipterocarps seedlings

18.2.3.1 Immobilization of the Spores

Fruit bodies were collected from the nursery and young stands of *Shorea* spp. in 1990. The glebas of the fruit bodies, 15 g in fresh weight, were crushed in 100 ml of sterilized water by a homogenizer and diluted to a 300 ml suspension. Two kinds of charcoal were used for the immobilization, because this fungus showed good growth and mycorrhizal formation on charcoal and cinder. One was a fine powder of white charcoal made from sawdust (a), and the other was a powder made from charcoal purchased in the local market and crushed (b).

Three kinds of inoculum were prepared as follows:

(A) A 60 ml spore suspension was added to the mixture of charcoal powder (a) and silicagel, 2:1 in volume. The suspension was immobilized with 20% agar-agar and kept in a refrigerator for 1 month after desiccation.

(B) A 60 ml spore suspension was mixed with charcoal powder (a) and kept for a month at room temperature after desiccation.
(C) A 60 ml spore suspension was mixed with charcoal powder (b) and kept for a month at room temperature after desiccation.

18.2.3.2 Inoculation and the Effect of Charcoal on the Infection of Mycorrhizal Fungus

Glass cases, 15 cm in width, 40 cm in height, and 2 cm in thickness, were prepared for observation of the mycorrhizal formation process. Top soil, collected from the experiment forest, was sterilized by autoclaving at 120°C and 1.2 atoms for 1 h, after chilling and keeping for 1 week in the laboratory to avoid the contamination of some molds.

Sterilized soils were packed into root boxes and the inoculum was set in the soil. Inoculum (A) was set as a small ball, 1 cm in diameter, sporadically. Inoculum (B) was buried as crusts, 3 cm in diameter, like spots. Inoculum (C) was buried as crusts, 3 cm in diameter, like spots. The powder of inoculum (C) was mixed with soil, 5% in volume. Root boxes without any treatment and those with soil sprayed by spore suspension were set up as controls.

On 25 February 1991, two seeds of *Shore leprosula* were embedded into each root box after washing with sterilized water. Root boxes were covered with black-plastic sheets. Plant growth and mycorrhizal formation were observed and estimated after 6 months and a year.

Apparent differences in growth were not clear after 6 months, but it became remarkable after a year. Inoculation only by spore suspension was ineffective, and the growth was rather inhibited, similar to that of the no-inoculation box. There was no mycorrhizal formation even after a year. Charcoal powder (b) was harmful to plant growth, but somewhat efficient for mycorrhizal formation. It was probable that this was caused by high alkalinity and volatile substances in the uncarbonized charcoal.

Inoculum (B) was most effective for mycorrhizal formation and plant growth. The mycorrhiza of *S. columnare* formed frequently inside and around the inoculum crusts. Inoculum (A) was also efficient in promoting plant growth and mycorrhizal formation. The mycelia growing out of the inoculum crusts formed an abundant mycorrhiza on the growing root. Inoculum (A) promoted high activity in formation of the mycorrhiza, even after a year.

From these results, it is recommended that spores should be immobilized in a porous material, like charcoal, when *S. columnare* is utilized in nursery practice. Charcoal also has remarkable effects on controlling the propagation of soil bacteria, including nitrogen-fixing bacteria in the tropics, and the infection of some symbiotic microorganisms, root-nodule bacteria and VAM fungi. Therefore, the utilization of charcoal or other carbonized materials should be recommended in tropical agriculture and forestry (Ogawa 1994).

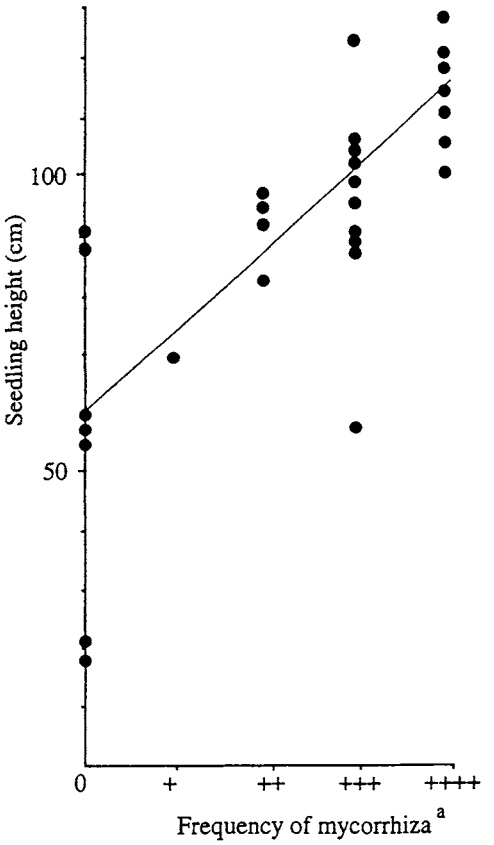

Fig. 3. Growth of *Shorea smithiana* and mycorrhizal formation of *Sclerodemta columnare*. Frequency of the mycorrhiza was checked at four points around the base of each seedling 10 months after planting. On the *x axis*: 0 = no mycorrhiza at the formation points; ++++ = mycorrhiza at all of the points

18.2.4 Growth Response of Dipterocarps to Mycorrhizal Formation

18.2.4.1 Estimation of the Frequency of Mycorrhizal Formation

Seedlings of *Shorea smithinna*, *S. ovalis*, and *S. parvifolia* were raised in the nursery of the experiment forest in 1988. Topsoil of natural stands containing some spores of mycorrhizal fungi was used for the pots. After 3 months of growth, these seedlings were transplanted to the open site. The interval between standing trees was 1 m each. In March 1989, after 10 months of planting, the tree height, base diameter, and number of leaves of 30 plants were measured. The frequency of mycorrhizal formation was checked at four points, 5 cm apart from the stem by digging out the fine roots (Figs. 3 and 4). These observations and measurements were continued for 3 years.

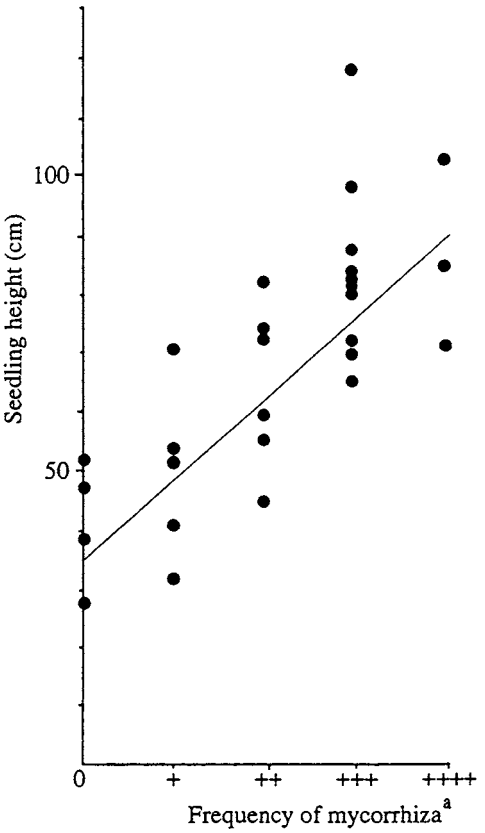

Fig. 4. Growth of *Shorea ovalis* and mycorrhizal formation of *Scleroderma columnare*. Frequency of the mycorrhiza was checked at four points around the base of each seedling 10 months after planting On the *x axis*: 0 = no mycorrhiza at the formation points; ++++ = mycorrhiza at all of the points

18.2.4.2 Tree Growth and Mycorrhizal Formation

In 1989, as shown in Figs. 3 and 4, the growth response to mycorrhizal formation was remarkable in both tree species. Clear linear relationships were found between tree height and the frequency of mycorrhizae. Of the *S. smithiana* plants that had two kinds of mycorrhizae, *S. columnare* and *Laccaria* sp. showed better growth. The mycorrhiza of *Laccaria* sp. was found in deeper soil or at the area covered by litter-like grass and straw, whereas that of *S. columnare* could be found even in an open site without litter. Probably, *S. columnare* has better resistance against high temperature and desiccation. It seems that some seedlings had already been infected by *S. columnare* in the nursery during the 3 months before planting.

In 1990, the differences among individual trees were more obvious than in 1989. *S. columnare* was expanding its territory along roots and had infected the seedlings that had no previous mycorrhiza. Also, this species produced fruit bodies at two points.

The frequencies of *Laccaria* sp. mycorrhizae and others increased gradually. In the stand of *S. parvifolia*, in which tree growth was depressed at the lower level in 1989, the differences in growth probably appeared due to the propagation of mycorrhizal fungi.

In 1991, most of the *S. smithiana* showed good responses to the frequency of mycorrhizal infection by *S. columnare* and *Laccaria* sp., and survived for 3 years. In the case of *S. parvifolia* and *S. ovalis*, which had lower frequencies of mycorrhizae in the nursery, only about 50% of saplings survived and they exhibited good growth. In these stands, the ground surfaces had a light covering of leaf litter and the grass was cut in the third year. The mycorrhizae, except for *S. columnare*, could be found beneath the litter. It is probable that the fungal succession started changing the species composition gradually.

It was certain that the mycorrhizal formation extended over 3 years and, as a result, the survival rate and average tree growth were also promoted. The same results have been obtained in various areas of Southeast Asia. Therefore, if the methods of using mycorrhizal fungi in nursery practice are introduced to tropical forestry, we can expect the development of more successful rehabilitation techniques for tropical rain forest.

18.3 Conclusion

Sixty-eight species of basidiomycetes, including 34 species of mycorrhizal fungi, were collected from dipterocarp stands in Kalimantan, Indonesia. Among these mycorrhizal fungi, *Scleroderma columnare* was selected as the most useful species for a dipterocarp nursery because this fungus keeps its spores long term in bulb-shaped fruit bodies and is specific to seedlings and saplings. Also, this fungus has a wide host range compared to other fungi. The fruit bodies were produced mainly in spring at the end of the rainy season and fructification was stimulated by heavy rain. The fruit bodies occurred normally around the tree. The fungi formed well-developed rhizomorphs along roots and a typical ectomycorrhiza with both a fungal sheath and a Hartig's net. The spores could be immobilized in charcoal powder, with mycorrhizal formation stimulated by the charcoal.

The growth of seedlings of the *Shorea* species was stimulated remarkably by infection with *Scleroderma columnare*. In the field, good responses of tree growth to mycorrhizal formation could be observed in the young stand of *Shorea* saplings.

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19
Competitiveness of Two Strains of *Pisolithus tinctorius* on Seedlings of Three Dipterocarp Species Under Nursery and Field Conditions

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19.1 Introduction

Studies on controlled ectomycorrhizal synthesis have shown that selected strains of *Pisolithus tinctorius* (Pers.) Coker & Couch (Pt) form mycorrhizas with various dipterocarp species in Indonesia (Hadi et al. 1991), Thailand (Sangwanit and Sangthian 1991), and Malaysia (Yazid et al. 1994, 1996; Lee et al. 1995). In the Malaysian studies, Yazid et al. (1994, 1996) showed that a selected strain of Pt was able to stimulate growth of *Hopea odorata* and *H. helferi* under controlled conditions, whereas researchers in Indonesia found that Pt had no significant effect on the growth of *H. odorata* and *Shorea pinanga* (Hadi et al. 1991). Most of the above studies were conducted under controlled conditions in the absence of other fungal competitors. It would, therefore, be useful to find out how selected strains of Pt perform in the presence of indigenous ectomycorrhizal fungi, both in the nursery and in the field.

Studies that examined the field performance of Pt on inoculated plants have been conducted on several pine species (Marx et al. 1977) and eucalypts (Garbaye et al. 1988; Aggangan et al. 1994). However, there are no reports on the competitiveness of Pt on inoculated dipterocarp seedlings, either in the nursery or in the natural forest. This chapter discusses the results of an investigation into the competitiveness of two strains of Pt under both nursery and field conditions.

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19.2 Materials and Methods

19.2.1 Nursery Competition

Seedlings of *Dipterocarpus alatus* and *H. odorata* were grown in rhizotrons and inoculated with a 2-week-old cardboard inoculum of selected strains of Pt, according to the method outlined by Yazid et al. (1994). Five seedlings of *H. odorata* were inoculated with the strain Ptmsn (isolated by Sangwanit from a carpophore collected in Thailand under pines) and two with Pt441 (isolated by Ivory from a carpophore collected in Brazil under a eucalypt), whereas three seedlings of *D. alatus* were inoculated with Ptmsn only. Upon successful mycorrhizal formation with the inoculated fungal strains, a 1-cm-thick layer of non-sterile nursery soil containing short lengths of ectomycorrhizal roots of *S. leprosula* was added to the rhizotron soil surface (Fig. 1). The same soil-root mixture was added to all the non-inoculated control seedlings (two replicate plants of *H. odorata* and four of *D. alatus*) at the same time. The rhizotrons were inclined at an angle of about 70° (Fig. 1) to encourage root growth through the added layer of soil and kept for 9 months in a shade house (30% shade). The roots of both the inoculated and non-inoculated plants were checked for infection under a stereomicroscope before adding the soil layer and at the end of the experiment.

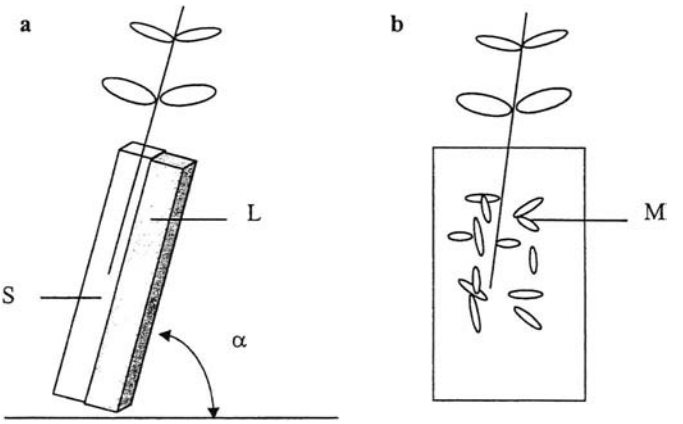

Fig. 1. Diagrammatic representation of the experimental setup for the nursery trial. **a** A layer of nursery soil (*L*) containing naturally infected roots of *S. leprosula* is added to the original layer of soil (*S*) in the perspex rhizotron. 'α' represents the angle at which the rhizotron was inclined to encourage root growth towards the outer surface of the added layer of soil. **b** Mycorrhizas (*M*) of both the inoculated and indigenous ectomycorrhizal fungi reaching the surface of the added layer of soil

19.2.2 Field Competition

Five-month-old seedlings of *Shorea glauca*, grown in perspex rhizotrons, were inoculated with either Ptmsn or Pt441. Both the inoculated and non-inoculated plants remained in the nursery for at least 6 months before outplanting.

Prior to outplanting, all the *S. glauca* plants were examined for mycorrhizal formation by the inoculated fungal strains (Ptmsn and Pt441) and contamination by nursery mycorrhizal fungi. Only root tips on the surface of the rhizotron were examined. Then, netting with a mesh size of 2 × 2 mm (large enough to allow new roots to grow through), reinforced with wire, was used to enclose the original root system of the plant (Fig. 2).

Heavily Pt-infected and non-inoculated control seedlings (eight replicates per treatment) were planted in a logged area in Compartment 52A, Sungai Lalang Forest Reserve, Selangor. The forest was logged in 1991 under the Selective Management System. The vegetation surrounding the planting site was shrubs and young trees, dominated in particular by the palm *Eugeissona tristis*, with remnants of dipterocarps. The canopy was quite open (no measurements were made of the light intensities) and the soil was compacted.

Six months after planting in the field, the plants were excavated and the roots examined. All new roots were examined for the presence of Pt441 or Ptmsn, according to the treatment given, and for colonization by field ectomycorrhizal fungi both inside and outside of the mesh envelope. The field mycorrhizas were typed according to their external morphology. Infection by the inoculated fungal strains as well as by field mycosymbionts was visually estimated.

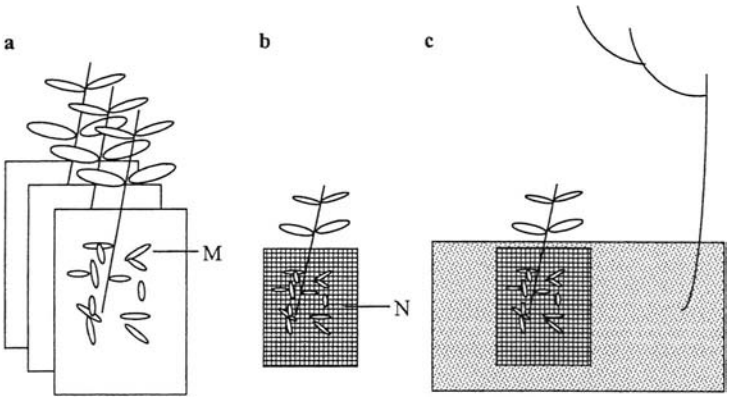

Fig. 2. Diagrammatic representation of the experimental setup in the field trial. **a** Inoculated (*M*) and non-inoculated seedlings in the nursery. **b** Seedling enclosed in a netting or mesh envelope (*N*) with a mesh size large enough for new roots to grow through. **c** Netted seedlings planted out in the field

19.3 Results

19.3.1 Nursery Competition

19.3.1.1 *Dipterocarpus alatus*

Before the addition of the soil layer, all inoculated seedlings were heavily infected by Ptmsn. All of the infected root tips on the rhizotron surface were those of Ptmsn, and no indigenous contaminant mycorrhizas were observed (Table 1). Non-inoculated plants used as a control were free of any mycorrhizal infection (Table 1).

Nine months after the addition of the soil layer, a mean (± SD) of 254 ± 143 ectomycorrhizal root tips were observed on the lower rhizotron surface of inoculated plants. Only 99 ± 94 ectomycorrhizal root tips were observed on the control plants, but this was not significantly different to that of the inoculated plants. Of the ectomycorrhizal root tips on the inoculated plants, 92% were those of Ptmsn, while 8% were those of indigenous ectomycorrhizal fungi (Table 1). In contrast, all of the ectomycorrhizal root tips in the control plants were those of indigenous fungi (Table 1).

Table 1. Relative abundance (%) of different mycorrhizal types on seedlings of *D. alatus* inoculated with *P. tinctorius* strain Ptmsn and on non-inoculated plants in the nursery

Before				After 9 months			
Inoculated		Control		Inoculated		Control	
Ptmsn	Indig	Ptmsn	Indig	Ptmsn	Indig	Ptmsn	Indig
100	0	0	0	92	8	0	100

Infection by Pt and indigenous nursery mycobionts (*Indig*) was recorded before and 9 months after the addition of a nursery soil-root mixture. Mycorrhizal root tips were recorded from the lower surface of the rhizotron

Table 2. Percentage of root tips infected by *P. tinctorius* or indigenous nursery mycobionts (*Indig*) on seedling of *H. odorata* in the nursery

(a)

Before				After 9 months			
Inoculated		Control		Inoculated		Control	
Ptmsn	Indig	Ptmsn	Indig	Ptmsn	Indig	Ptmsn	Indig
+++	0	0	0	traces	+	0	+

(b)

Before				After 9 months			
Inoculated		Control		Inoculated		Control	
Pt441	Indig	Pt441	Indig	Pt441	Indig	Pt441	Indig
+++	0	0	0	0	+	0	+

Seedlings were either uninoculated (*Control*) or inoculated with Ptmsn (**a**) or Pt441 (**b**). Infection was recorded before and 9 months after the addition of a nursery soil-root mixture. Mycorrhizal root tips were recorded from the lower surface of the rhizotron

0, < 10% of root tips infected; +, 10–40% of root tips infected; ++, 40–70% of root tips infected; +++, > 70% of root tips infected

19.3.1.2 *Hopea odorata*

Before the addition of the soil layer, all *H. odorata* plants inoculated with either Ptmsn or Pt441 had > 70% of their root tips infected by the inoculated fungal strain (Table 2). No indigenous ectomycorrhizal fungi were observed on the roots of any of the plants, including the controls at that time. Nine months after the addition of the soil layer, there were traces (< 10%) of root tips infected by Ptmsn at the lower rhizotron surface (Table 2a). However, no Pt441 ectomycorrhizas were observed (Table 2b). On the control plants, 10%–40% of root tips were infected by indigenous ectomycorrhizal fungi (Table 2).

19.3.2 Field Competition

Before planting, > 40% of root tips of the inoculated seedlings of *S. glauca* were infected by the respective strains of Ptmsn and Pt441 (Table 3). The inoculated and non-inoculated (control) seedlings were free of contamination by nursery ectomycorrhizal fungi (Table 3).

On inoculated plants, < 10% (traces) of root tips inside the mesh were infected by either Ptmsn or Pt441 6 months later (Table 3). However, most of the Pt mycorrhizas appeared unhealthy. Outside the mesh, root extension was poor, and no Pt mycorrhiza was observed (Table 3).

Table 3. Infection of seedlings of *S. glauca* non-inoculated (*Control*) or inoculated with *P. tinctorius* strain (**a**) Ptmsn or (**b**) Pt441 in the nursery

(**a**)								
Location	Before				After 6 months			
	Inoculated		Control		Inoculated		Control	
	Ptmsn	Indig	Ptmsn	Indig	Ptmsn	Indig	Ptmsn	Indig
In	++	0	0	0	traces	+	0	+
Out	—	—	—	—	0	+	0	+
(**b**)								
Location	Before				After 6 months			
	Inoculated		Control		Inoculated		Control	
	Pt441	Indig	Pt441	Indig	Pt441	Indig	Pt441	Indig
In	++	0	0	0	traces	+	0	+
Out	—	—	—	—	0	+	0	+

The entire root system of each seedling was enclosed in a mesh envelope before planting out in a logged-over forest. Infection by Pt and indigenous field mycobionts (*Indig*) on root tips was recorded before outplanting and 6 months later inside (*in*) and outside (*out*) the mesh envelope

0, < 10% of root tips infected; +, 10–40% of root tips infected; ++, 40–70% of root tips infected; +++, > 70% of root tips infected

Four different types of indigenous field mycorrhizas were observed on inoculated and control plants. Between 10% and 40% of root tips inside and outside of the mesh in all treatments were colonized by these fungi. None of the field mycobionts was dominant.

Pisolithus tinctorius mycorrhizas (both Ptmsn and Pt441) tended to be found near their original location on the root system, whereas new infection by field mycobionts was found in the region of new root growth.

19.4 Discussion

There has been successful synthesis of ectomycorrhizas between various strains of *Pisolithus tinctorius* and dipterocarps in the nursery in Indonesia (Hadi et al. 1991), Thailand (Sangwanit and Santhien 1991), and Malaysia (Yazid et al. 1994, 1996; Lee et al. 1995). In this study, Ptmsn persisted well on the roots of *D. alatus* but not on *H. odorata* in the nursery. Pt441 also did not persist well on the roots of *H. odorata*. These results suggest that a given ectomycorrhizal fungal strain can react differently with different host plants. Lee et al. (2002) also confirm the irregularity of exotic strains of Pt in forming mycorrhizas with dipterocarps. In their study, Pt failed to form ectomycorrhizas with *H. odorata* seedlings and was outcompeted by nursery ectomycorrhizas.

In the field, both Ptmsn and Pt441 showed poor persistence on the roots of *S. glauca* after outplanting in logged-over forests. Their virtual disappearance after 6 months in the field suggests that they may not be adapted to such biological and/or physical environments or that they may not be aggressive competitors against the indigenous field fungi. However, as < 40% of root tips were actually colonized by indigenous fungi, we tend to favor the first hypothesis.

Pisolithus tinctorius is a widely distributed fungus found in various environments of both the temperate and tropical regions of the world (Marx et al. 1977). In Southeast Asia, it has been reported from dry deciduous forests of North Thailand, on the island of Sumbawa in Indonesia, and in the Philippines, but not from Peninsular Malaysia in association with indigenous tree species. More recently, it has been isolated from a single carpophore associated with introduced species of *Acacia* (Lee et al. 2002).

Although *P. tinctorius* has been successfully introduced into many reforestation sites throughout the world (Marx and Cordell 1989), there are also some reports of poor field performance of this fungus (McAfee and Fortin 1986; Lee and Koo 1992).

Even though Ptmsn was isolated from a carpophore collected in a pine forest that had been established near the original dry dipterocarp forest in northern Thailand, it may not have been able to fully adapt to the wet dipterocarp forest of Malaysia, and was outcompeted by the indigenous ectomycorrhizal fungi that were probably better adapted to this environment. A less apparent reason for the demise of Pt could have been due to preferential grazing by soil animals, particularly soil arthropods. The inability of Pt to spread further along the root system could also have been hindered by poor root extension and slow root growth, and this was probably further compounded by physical damage and loss of fine roots during outplanting.

While Pt appears to be well adapted to the inoculation of selected dipterocarp species in the nursery, its performance in the field has been rather disappointing. At this time, it is not known whether host plants infected with Pt at the time of outplanting have some kind of advantage for better survival or more rapid establishment in the field. Further studies on the field performance of this fungus as a symbiont of dipterocarps are needed.

Field soil-nutrient status could also influence the persistence of Pt in the field. Dell and Malajczuk (1995) demonstrated the effect of balanced nutrients on mycorrhizal eucalypts at the time of planting in China. This is perhaps another aspect for future investigation.

Future experiments should also be conducted with indigenous fungal strains that could be better adapted to the local environmental conditions. A new species of *Pisolithus*, *P. aurantioscabrosus*, was discovered in association with a lowland dipterocarp forest (Watling et al. 1995). However, this species failed to form ectomycorrhizas with the dipterocarps tested (Lee et al. 1995). A more recent study by Lee et al. (2002) demonstrated the competitiveness against nursery contaminant ectomycorrhizas of another strain of local indigenous ectomycorrhizal fungus. This is a species of *Tomentella*, and has persisted well on *H. odorata* seedlings in the field, even 3 months after outplanting (unpublished data). Further studies are being conducted on using this indigenous strain of ectomycorrhizal fungus.

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20
Ectomycorrhizas of Dipterocarps in Logged-Over Forests and Plantations

JUNICHI KIKUCHI

20.1 Introduction

Dipterocarps, the main tree species of tropical rain forests in Southeast Asia, are mostly ectomycorrhizal (Alexander and Hoegberg 1986; Lee and Alexander 1996), whereas many of the other tree species in tropical forests are VA mycorrhizal. It is well known that mycorrhizal formation promotes the growth of host plants by improving the absorption of nutrients and water from soil or by protecting roots from pathogenic fungi (Harley and Smith 1983; Smith et al. 1994). The promotion of dipterocarp seedling growth by the inoculation of mycorrhizal fungi has also been studied (Ogawa 1991; Mori and Marjenah 1994; Smits 1994), Mycorrhizal formation of small natural dipterocarp seedlings has been studied (Lee and Alexander 1996; Lee et al. 1997), but most of these studies have used nursery seedlings and the fate of mycorrhizas on field-planted seedlings has not been studied in detail. The biomass of mycorrhizas of dipterocarp seedlings and trees has not been studied in detail in either plantations or natural forests. The objective of this study was to examine the role of mycorrhizas on dipterocarp seedlings in plantation and forest environments by quantifying the mycorrhizas.

20.2 Materials and Methods

20.2.1 Seedlings

Seedlings of *Shorea parvifolia* and *S. macroptera* that grow naturally in small gaps of the logged-over forest in Jambi, Sumatra, were sampled to examine their mycorrhizal formation. A half or a quater, depnding on the seedling size, of the areas of root distribution of 23 and 11 seedlings of *S. parvifolia* and *S. macroptera*, respec-

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tively, were carefully excavated, and mycorrhizas were separated from their roots. The heights of the sampled seedlings ranged from 30 to 400 cm for *S. parvifolia* and 40 to 360 cm for *S. macroptera*. Roots and mycorrhizas were oven dried at 120°C and weighed.

Seedlings of *S. parvifolia* and *S. macroptera* that had been planted in the open area 2 years earlier were also sampled and examined, as for the natural seedlings. I examined 17 seedlings for *S. parvifolia* and 16 seedlings for *S. macroptera*. The heights of the sampled seedlings ranged from 60 to 300 cm for *S. parvifolia* and 45 to 210 cm for *S. macroptera*. Roots and mycorrhizas were oven dried at 120°C and weighed.

20.2.2 Trees

Half the areas of root distribution of two small trees of *S. parvifolia* were sampled. The heights of the trees were about 17 and 21 m. Both trees were more than 10 m away from other dipterocarp trees. A 12 × 6 m plot was established for the sampling of mycorrhizas from each tree. The plot was divided into 2 × 2 m subplots. I sampled all the mycorrhizas and roots of the *S. parvifolia* in 50 × 50 cm sampling plots of each subplot. All mycorrhizas were traced to small lateral roots, which were later traced to the big lateral roots from the trunk.

20.3 Results and Discussion

The importance of mycorrhizas in small dipterocarp seedlings has been well recognized for natural seedlings (Lee and Alexander 1996) and nursery seedlings (Kikuchi and Ogawa 1997). However, the role of mycorrhizas in somewhat larger seedlings has not been clear. Seedlings of 2 to 4 m in height were found to have a lot of mycorrhizas in this study, under both plantation and natural-regeneration conditions. Correlations between the dry weight of mycorrhizas and seedlings of *S. parvifolia* are shown in Fig. 1. The amount of mycorrhizas increased with seedling size in both natural and planted seedlings. If seedlings become less dependent on mycorrhizas when they grow larger, then the proportional mass of mycorrhizas would decrease as seedlings grow. However, abundant mycorrhizas were found in large seedlings, and mycorrhizas seemed indispensable for both small and large seedlings. The types of mycorrhizas found in natural and planted seedlings were quite different. Most of the mycorrhizas found in planted seedlings were those formed by *Scleroderma columnare*, which had colonized most of the nursery seedlings at planting, whereas several other types were dominant in natural seedlings. The percentage of mycorrhizas to total dry weight was higher in planted seedlings than natural seedlings. This is partly due to the different mycorrhizal types between the planted and natural seedlings, because *S. columnare* forms many dense and well-branched mycorrhizas. The percentage of mycorrhizas to total dry weight did not vary among

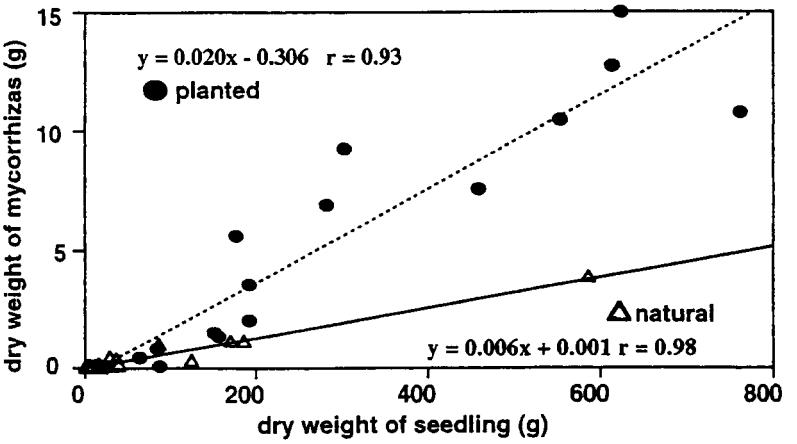

Fig. 1. Correlations between the dry weight of mycorrhizas and *Shorea parvifolia* seedlings from a plantation (planted) and a forest (natural)

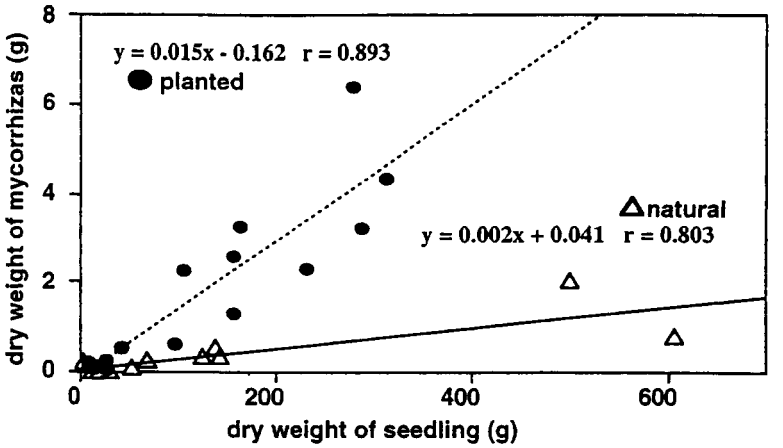

Fig. 2. Correlation between the dry weight of mycorrhizas and *Shorea macroptera* seedlings from a plantation (planted) and a forest (natural)

natural seedlings, but was about 2.3% higher in large-planted seedlings (> 2 m high) than in small-planted seedlings. The difference in height of planted seedlings was due to differences in individual growth rate because these seedlings had been planted at the same time. Therefore, seedlings that grew faster needed relatively more mycorrhizas than slow-growing seedlings. The growth rate was about 1 m/year in planted seedlings and about 50 cm/year in natural seedlings. These differences in growth rate may also explain the different masses of mycorrhizas between natural and planted seedlings.

Correlations between the dry weight of mycorrhizas and seedlings of *S. macroptera* are shown in Fig. 2. The tendency was almost the same as for *S. parvifolia*, although the amount of the mycorrhizas was relatively smaller than that for *S. parvifolia*.

The percentages of mycorrhizas to total tree dry weight for two small trees were less than 0.5% and much less than that of seedlings, although the vertical distribution of mycorrhizas was almost the same as that for seedlings. Many mycorrhizas were distributed in the litter-humus layer, where most nutrients are released by the decomposition process. The low percentage of mycorrhizas in trees is mainly due to the percentage increase in trunk mass with the increase in size. Vogt et al. (1982) reported that the percentages of mycorrhizas in a 23-year-old and an 180-year-old *Abies amabilis* plantation was about 1% and 0.3%, respectively. Dipterocarp trees seem to have less mycorrhizas than *Abies*. Smits (1994) reported that the occurrence of sporocarps of mycorrhizal fungi in dipterocarp forests is rather scarce compared to temperate forests. This may be due to the relatively small amount of mycorrhizas in dipterocarp forests.

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Part IV

Man-Made Forests and Biodiversity in the Asia-Pacific Region

21
The Role of Industrial Forest Plantations in Supporting Pulp and Paper Industries: A Case Study in South Sumatra, Indonesia

BAMBANG HERO SAHARJO

21.1 Introduction

Indonesia had plans to establish 6.2 million hectares of industrial forest plantation (Hutan Tanaman Industri, "HTI") by the year 2000. Based on government regulation no. 7 (Ministry of Forestry 1990) the objectives of the industrial forest plantation were:

1. To produce logs for the domestic forest industry and the production of goods for foreign trade.
2. To improve forest productivity and environmental quality.
3. To promote employment and business opportunities.

According to the Indonesian Ministry of Forestry, the role of plantation forests in Indonesia is very important, especially for conservation purposes. It is believed that high-yielding plantations will meet the increasing wood demand while maintaining or reducing the areas of natural forest that are harvested (Seabright 1995).

One of the reasons why industrial forest plantations are valuable is the high demand for raw materials, especially for pulp and paper, which has increased year by year. For pulp, the 1997 projected product capacity will increase 4.6 million ton/year. In 1998, the estimate is 5.2 million ton/year, and in the year 2010 the capacity will increase to 11 million ton/year. To guarantee sustainability of the raw materials, a million hectares of industrial forest plantation needs to be established. Without this, the natural rainforest will surely be sacrificed.

Many factors affect the success of forest planting: poor maintenance (weeding, pruning, thinning), which increases the risk of fire invasion; tree spacing; pest and diseases; and the invasion of millions of seeds on the forest floor and under the surface soil that germinate in the second rotation.

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Despite rapid internal growth, many tropical-plantation forests have not fulfilled their early promises. In some cases, plantation forests have been established but poorly maintained, or have successfully reached maturity only to find that there were no markets for the species that were grown (ITTO 1993).

This chapter describes the importance of industrial forest plantations in supporting the pulp and paper industries. This includes factors that affect the success of a plantation: increasing forest-fire risk with an increase in fuel load, resulting from poor maintenance; poor growth performance; and high storage of seeds on the forest floor and under the soil surface.

21. 2 Methods

Research was carried out from August 1994 to September 1997 in young *Acacia mangium* plantation stands, aged 1, 1.5, 2, 3, 4, 5, and 6 years, belonging to a forest concession area in South Sumatra, Indonesia. There, a company plans to develop a 300 thousand ha plantation to support a pulp mill with a capacity of 450 thousand tons/year, consuming 2 million m^3 of wood with an 8-year rotation. To date, about 200 thousand ha have been planted.

The mean annual rainfall is approximately 2,800 mm and the monthly rainfall ranges from 92 mm in July to 278 mm in February. According to the Schmidt and Fergusson system (1951), the climate of this area belongs to rainfall type A ($0 < Q < 0.143$). The mean maximum temperature in this area is 32.6°C in August, the mean minimum air temperature is 22.3°C in December, and the mean relative humidity is about 85%. In 1994, however, there was an extremely dry period when rainfall in July was only 3.9 mm, with two rainy days, while in August there was one rainy day, and in September there was 63.5 mm over six rainy days (Saharjo and Watanabe 1997b).

Three factors, forest-fire behavior, seed storage, and growth performance, were checked in the field to confirm their significance in affecting the success of a forest plantation.

21.2.1 Forest-fire Behavior

21.2.1.1 Estimation of Fuel Load

Four quadrats of about 1 ha each were set up in four different age groups (1, 2, 3, and 4 years) of trees in a young *A. mangium* plantation. In each of these quadrats, two subplots of 300 m^2 (15 × 20 m) were chosen for the estimation of flame temperature during burning. Another 15 subplots of 2 m^2 (2 × 1 m) were set aside in order to estimate the fuel load. Fuel-bed depth was estimated by measuring the average height of the associated living and dead plant materials of various sizes and shapes that extended from the soil to the top of the vegetation canopy. All floor vegetation, except for the *A. mangium* trees, was cut, felled, collected, and brought to the laboratory for measuring its weight and fuel-moisture content.

21.2.1.2 Estimation of Flame Temperature

Fires were allowed to spread naturally in the two 300 m^2 subplots. Flame temperatures at 0, 1, and 2 m above the soil were measured at two locations in each subplot, using a temperature-indicating liquid (Tempilaq), which melts at a specified temperature and provides estimates of the maximum temperatures. Each liquid was set in an aluminum pipe, 2 cm in diameter and 30 cm long (Saharjo 1995a). The temperature range at 0 m was 139°–550°C, at 1 m was 59°–302°C, and at 2 m was 38°–302°C.

The rate of spread of fire was estimated by measuring the average distance perpendicular to the moving flame front per time, using a stopwatch and measuring tape. Flame height was estimated from the average height of soot marks on the trees (Pickford et al. 1992). Crown scorch was estimated by measuring the percentage of blackened canopy leaves. Fire intensity was calculated using Byram's equation (Chandler et al. 1983), FI : $273(h)^{217}$, where FI is the fire intensity (kW/m) and h is flame height (m).

21.2.2 Seed Storage

21.2.2.1 Plantation Site

One plot of 30 × 50 m was established for each plantation age class. In each of these plots, the seed storage per m^2 was determined on the forest floor and in the soil at depths of 0–5 cm and 6–10 cm. Seed storage was determined using a ring sample of 400 cm^3 in volume (surface area 100 cm^2 and height 4 cm).

21.2.2.2 Second Rotation Site

The first planting of *A. mangium*, which included the study site, occurred in 1980. In 1994, as the forest concession expanded, about 1 ha of this site was clear cut, but it was replanted with *A. mangium* and managed by the R&D department, the main objectives being to investigate the growth performance in the second rotation. As this plot will not be harvested as a normal plantation site, it is called a second rotation site. In this plot, seeds stored at 0–5 cm and 6–10 cm below the soil surface were measured. In addition to the number of remaining seedlings, their diameters and heights were also measured.

21.2.2.3 Seed Germination

Seeds taken from all samples (0–5 cm and 6–10 cm from the soil surface and on the forest floor), were germinated in a box containing moist sand that had been previously dried for 8 h and cooled for one night. Seeds were immersed in hot water (85°C) for 1 min and then soaked in fresh water for 24 h.

21.2.3 Growth Performance

A 1600 m^2 (40 × 40m) plot was established in each of the *A. mangium* plantation stands, aged 1, 1.5, 2, 3, 4, 5, and 6 years, with various spacings between the trees. The spacings for the stands were as follows: 2 × 4 m at 1 year; 2.5 × 3.5 m, 2.5 × 4 m, 3 × 3.5 m, 3.5 × 4 m, and 3 × 4 m at 1.5 years; 2 × 4 m at 2 years; 3.5 × 2.5 m, 4 × 4 m, 3 × 2 m, and 3 × 4 m at 3 years; 2 × 4 m at 4 years; 2 × 4 m at 5 years; and 4 × 4 m, 3 × 2 m, 3 × 3 m, and 2 × 2 m at 6 years. In these plots, the diameter and height of each tree was measured and the basal area of each tree was then calculated.

21.3 Results

21.3.1 Forest Fire

Table 1 shows that the highest fuel load, 21.2 tons/ha, occurred in the 2-year-old stand while the lowest load, 14.5 tons/ha, occurred in the 4-year-old stand. The maximum flame temperature, 454 °C, occurred in the 2-year-old stand and the minimum flame temperature, 139°C, occurred in the 4-year-old stand.

21.3.2 Seed Storage

Seeds stored in the forest floor ranged from 50 thousand/ha in the 3-year-old stand to 2.2 million/ha in the 6-year-old stand (Table 2). At the 0–5 cm depth, seed numbers ranged from 0.9 million/ha in the 3-year-old stand to 8.5 million/ha in the 6-year-old stand. At 6–10 cm, the density ranged from 0.2 million/ha to 0.7 million/ha. In the second-rotation site, the number of seeds stored at a depth of 0–5 cm ranged from 300 million/ha to 1 billion/ha, and at 6–10 cm ranged from 160 million/ha to 440 million/ha. The density of remaining seedlings ranged from 85,000/ha to 270,000/ha, with average heights of 26 cm to 2.2 m and diameters of less than 1 cm. The germination success of seeds from both the second rotation site and the plantation were 80%–90 %.

21.3.3 Growth Performance

Table 3 shows that, in first year, the average tree diameter was 2.8 cm, with tree height at 3.12 m. After 1.5 years, the range in tree diameter was 7.4–8.0 cm, and in tree height was 5.3–5.8 m. In the second year, the averages were 8.3 cm in diameter and 9.9 m in height. In the third year, the ranges were 9.2–9.7 cm in diameter and 11.6–11.9 m in height. In the fourth year, the averages were 10.8 cm in diameter and 15.2 m in height. In the fifth year, the averages were 11.9 cm in diameter and 17.3 m in height. In the sixth year, the ranges were 11.3–18.7 cm in diameter and 18.1–20.6 m in height. The basal area tended to increase as trees got older, especially in trees that were narrowly spaced.

Table 1. Weather conditions and fire behavior at different plantation ages

Parameter	Age of Plantation (years)			
	1	2	3	4
Weather condition				
Air temperature (°C)	33	35	30	33
Relative humidity (%)	60	60	65	65
Wind speed (m/s)	1.40	1.70	1.32	1.18
Fire behavior				
Fuel load (kg/m^2)	1.62 ± 0.18ab	2.12 ± 0.15c	1.70 ± 0.09b	1.45 ± 0.17a
Fuel bed depth (m)	0.45 ± 0.07a	0.53 ± 0.10b	0.35 ± 0.06c	0.20 ± 0.04d
Rate of fire spread (m/min)	1.50 ± 0.09bc	1.70 ± 0.15c	1.30 ± 0.09ab	1.15 ± 0.05a
Flame height (m)	1.30 ± 0.13a	1.70 ± 0.08b	1.20 ± 0.15a	1.11 ± 0.07a
Flame temperature above soil (°C)				
0 m	302–343	343–454	159–302	139–302
1 m	139–159	159–198	76–159	76–121
2 m	101–121	101–159	~ 121	~ 76
Fire intensity (kW/m)	482.4 ± 105.0a	863.5 ± 93.7b	405.5 ± 107.7c	342.8 ± 45.5d

For a given factor, the means of different years are significantly different when the standard error is followed by the same letter ($P \leq 0.05$)

Table 2. Seed storage in the forest floor and under the soil surface at different plantation ages

Year	Seed storage in the forest floor per m^2	Seed storage under the soil surfaceper 100 cm^2	
		0–5 cm	6–10 cm
1	0 ± 0a	0 ± 0a	0 ± 0a
2	0 ± 0a	0 ± 0a	0 ± 0a
3	5.0 ± 3.0a	0.9 ±.1.0a	0 ± 0a
4	16.5 ± 10.6a	1.2 ± 1.9a	0.2 ± 0.2a
5	31.3 ± 18.0a	3.0 ± 2.8a	0.1 ± 0.5a
6	215.3 ± 135.0b	8.5 ± 7.4a	0.4 ± 0.7a
10	no data	121.8 ± 116.5b	14.0 ± 18.1b
16	no data	67.7 ± 24.2b	16.0 ± 10.5b

For a given factor, the means of different years are significantly different when the standard error is followed by the same letter ($P \leq 0.05$)

21.4 Discussion

Saharjo and Watanabe (1997a) stated that the maximum fuel load permitted in a plantation to save it from fire invasion is 3 ton/ha. Table 1 shows that the entire plantation had a fuel load over that limit, placing it in a high-risk category for fire invasion. In 1994, approximately 20,000 ha of the plantation at the research site in South Sumatra was destroyed by fire over a 3-month period. One year after the fires, no trees had recovered and no natural seedlings remained on the forest floor (Saharjo

Table 3. The diameter at breast height, height, and basal area of *Acacia mangium* trees at different plantation ages and spacings on a 40 × 40 m plot

Spacing	No./ha	Diameter(dbh, cm)	Height(m)	Basal area(m^2/ha)
1 year				
2 × 4 m	1250	2.8 ± 0.78	3.12 ± 0.03	0.8
1.5 years				
3.5 × 4 m	714	7.7 ± 2.6a	5.3 ± 0.7a	3.5
4 × 3 m	833	7.4 ± 2.3a	5.5 ± 0.5a	3.7
3 × 3.5 m	952	8.0 ± 2.5a	5.5 ± 0.7a	4.0
4 × 2.5 m	1000	7.4 ± 2.9a	5.4 ± 0.7a	4.2
3.5 × 2.5 m	1142	7.4 ± 2.4a	5.8 ± 0.9a	5.1
2 years				
2 × 4 m	1250	8.3 ± 3.2	9.9 ± 0.3	7.1
3 years				
4 × 4 m	625	9.6 ± 4.5a	11.6 ± 0.9a	5.2
4 × 3 m	833	9.7 ± 3.7a	11.7 ± 0.8a	6.4
3.5 × 2.5 m	1142	9.2 ± 4.7a	11.8 ± 0.2a	8.0
3 × 2 m	1666	9.3 ± 4.8a	11.9 ± 0.2a	11.9
4 years				
2 × 4 m	1250	10.8 ± 4.2	15.2 ± 0.4	12.4
5 years				
2 × 4 m	1250	11.9 ± 4.2	17.3 ± 0.6	14.6
6 years				
4 × 4 m	625	18.7 ± 5.9a	18.1 ± 0.3a	16.9
3 × 3 m	1111	15.9 ± 4.9b	18.7 ± 0.5b	24.0
3 × 2 m	1666	13.5 ± 4.7c	19.5 ± 0.8c	25.5
2 × 2 m	2500	11.3 ± 3.7d	20.6 ± 0.5d	26.7

For a given factor, the means of different years are significantly different when the standard error is followed by the same letter ($P \leq 0.05$)

and Watanabe 1996). The loss of these natural seedlings was caused by high flame temperatures on the forest floor, as the lethal temperature duration for *A. mangium* seeds was shown to be 150°C for 5 minutes (Saharjo and Watanabe 1997b). Moreover, limited heat penetration below the soil surface failed to provide the temperature (76°C) required to break the dormancy of the seeds. The high-fuel load in the forest plantation was caused by incorrect maintenance (Saharjo 1995b) and a lack of data concerning optimum spacing.

The many different tree spacings now used in the plantation were selected predominantly by trial and error. Table 3 shows that there was no significant difference between the diameter and the height of the trees until the plantation was 3 years old. The basal area increased in approximate proportion to tree density. In the third year, it was suspected that competition for light began between the trees, especially between those with narrow spacings. The crowns of individual trees began to interfere with each other (Long and Smith 1984) and most competition between trees in a stand is for light (Evans 1992). This competition may also indicate unsatisfactory

conditions, especially with close spacing, once a canopy begins to close and weeds become suppressed by shading (Evans 1992), and it is one reason why the living fuel load (*Imperata cylindrica* and shrubs) decreased. At this stage, 19% of trees were dead in the stand with the closest spacing of 3 × 2 m, and 17% were dead at a spacing of 3.5 × 2.5 m. By the sixth year, the effects of competition on tree and stand growth were clear. Many trees died: 18.5% at a spacing of 2 × 2 m and 18.75% at 3 × 2 m, and the quality of both stands was poor. At a spacing of 3 × 3 m, 15% of trees died but only 5% died at 4 × 4 m. The stand quality varied with spacing, reflecting higher levels of competitive stress at the closest spacing. Close spacing reduces tree diameter but increases tree height and stand-basal area, and also results in a poorer-quality stand. Inappropriate tree spacing will affect the production of raw materials for harvesting.

As well as the problems of competition, there is the problem of natural-seed regeneration in the forest floor. Thousands of natural seedlings dominate the second rotation, and even after numerous cuttings new seedlings reemerge. These seedlings become "weeds" and reduce the performance of planted trees by competition. This situation is not good for plant management because it requires time and money to reduce this stock. This risk is also increased by the potential for arson around the plantation. Close spacing and tree maintenance activities are not sufficient in reducing the risk of forest-fire invasion because maintenance activities contribute litter to the forest floor. More must be done to reduce high-fuel load and high-seed storage in the forest floor, and also to improve stand quality. One possible measure might be prescribed burning.

The question remains as to whether it is possible to conduct prescribed burning effectively in *Acacia mangium* plantations. It is not easy to start and control a fire in a plantation. For example, the flame temperature must not go below 150°C, in order to prevent seed germination. To achieve this temperature, the fuel load must be at least 3 tons/ha and the moisture content must be uniformly between 10% and 15%. Also, the fuel-bed depth would have to be 6–8 cm, so that the maximum flame height would not exceed 1 m. This would prevent the fire climbing to the canopy where the lower branches, after pruning, are around 1 m high. These three factors (fuel load, flame temperature, and fuel bed depth) should be taken into consideration when planning prescribed burning.

The present study shows that the mismanagement of plantation forests is costly in terms of pulp and paper production. If this situation continues and the problems cannot be solved quickly, the annual target for a pulp mill will not be achieved. Large plantations do not guarantee survival of the industry and, if the targets are followed relentlessly, more tropical rainforest will be destroyed and sacrificed. High targets of pulp and paper production set for a larger market and higher profit must be balanced with the resource of the forest plantation. Without this balance, the targets are meaningless.

21.5 Conclusion

The role of the industrial forest plantation is very important in order to preserve forest industries, especially pulp and paper. The success of this role is affected by many factors, including plantation maintenance and growth performance and management, and these should be taken into account when pulp mill production targets are decided.

The research shows that a large plantation does not guarantee a supply of raw materials. High-production targets will have no meaning if these sources are not made available through appropriate forest management.

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22
Planting Techniques and Growth of Dipterocarps in an Abandoned Secondary Forest in East Kalimantan, Indonesia

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22.1 Introduction

A large forest fire occurred in East Kalimantan, Indonesia, from 1982 to 1983, and about 3.1 million ha of forest were lost. Degradation of the damaged forest was further accelerated by shifting cultivation and illegal felling. Various efforts have been made to find an effective and realistic remedy for the degradation (Adjjers et al. 1995; Palmiotto 1993; Nussbaum et al.1995; Ang and Maruyama 1995). One solution is to reforest the degraded forest, which has become an urgent matter in this area. Therefore, silvicultural techniques for reforestation and sustainable forest utilization must be developed. The 3,000 ha Sebulu Experimental Forest, located in Sebulu, East Kalimantan, was granted by the Indonesian Ministry of Forestry in 1991, with the aim of developing reforestation techniques and establishing a sustainable management system for the forest. We planted approximately 500,000 seedlings, mainly dipterocarps, on 300 ha of this forest from 1991 to 1997, in order to study and develop the most efficient and cost-effective planting technique. However, in February and March of 1998, the last big forest fire burned 80% of the plantation. In this report the results of various planting methods are described with consideration given to cost savings.

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22.2 Materials and Methods

22.2.1 Survival and Growth of Planted Seedlings by Planting Methods

In 1992, six dipterocarp species (*Shorea leprosula*, *S. ovalis*, *S. seminis*, *S. pauciflora*, *S. johorensis*, and *Dryobalanops lanceolata*) were planted using four planting methods. Seedlings were raised from wildings, and were 25–60 cm in height when planted. The planting methods were as follows:

1. Open-area planting: Planting blocks wider than 30 m were defined as 'Open area.' Preexisting trees were removed before planting. The height of trees around blocks was approximately 8–10 m.
2. Line planting: Planting blocks with a length of 150 m and widths of 6 m, 10 m, or 20 m were tested. Preexisting trees were removed before planting.
3. Gap planting: Planting block sizes of 5 × 5 m, 10 × 10 m, and 20 × 20 m were tested. Preexisting trees were removed before planting.
4. Under-canopy planting: Two types of planting methods were tested in the shade under the canopy.

(i) S1 type. Planting lines, 1 m wide, were made at 5-m intervals. Seedlings were planted at 2-m intervals in each line. Trees between the lines were approximately 5 m in height, and the canopy was tightly closed.
(ii) S2 type. All trees with a diameter at breast height (dbh) < 10cm were felled before planting. The remaining trees were almost all *Macaranga* sp., and were 8–12 m in height. Each planting block was 30 m wide and 150 m long.

The planting density was 2,500 seedlings per hectare in all blocks, except for plants in the S1 under-canopy block. The planting density of S1 type was about 830 seedlings per hectare. The total number of seedlings planted in each experimental block is shown in Table 1. The survival rate and average height of the seedlings for 3 years from 1992 to 1995 were measured. The average height of the tallest 100 seedlings (defined as the "dominant trees") for each hectare was also calculated, in order to avoid the effects of unsound and damaged seedlings. In February and March of 1998, the forest fire burnt 80% of the plantations in the experimental forest.

22.2.2 Timing of Girdling on Upper-Story Trees in Under-Canopy Planting

In March 1994, five dipterocarp species (*S. leprosula*, *S. johorensis*, *S. ovalis*, *S. pauciflora*, and *D. lanceolata*) were planted under the canopy, in spacings of 2 × 2 m, where about 2,000 upper-story trees, 5–10 m tall, remained. Approximately 2,300 seedlings per species were planted. Three girdling plots of 1 ha each and one ungirdled plot were constructed. In one girdling plot the plants were girdled 3 months after planting, and in the other two plots they were girdled at 6 months and 1 year after planting, respectively. Each plot contained the same number of seedlings for each of

Table 1. Number of planted seedlings, average height, average height of the tallest 100 seedlings per hectare, and survival rate of seedlings 3 years after planting, using various methodsw

Species	Parameter	Planting method							
		Open area	Line planting			Gap planting			Under-canopy planting	
			Width (m)			Size (m)				
			6	10	20	5 × 5	10 × 10	20 ×20	S1[b]	S2
S. leprosula	No. planted seedlings[a]	3101	241	415	932	9	25	100	302	
	Av. height (cm)	294.3	272.4	239.3	229.8	280.3	317.0	311.9	196.2	
	Av. height of tallest 100 tallest trees/ha (cm)	526.4	466.0	369.1	379.5			469.0	368.4	
	Survival rate (%)	58.7	68.0	69.2	50.3	77.8	88.0	78.0	71.5	
S. johorensis	No. planted seedlings[a]		278	483	916				315	1323
	Av. height (cm)		192.5	215.8	207.4				108.9	212.9
	Av. height of tallest 100 trees/ha (cm)		392.3	378.3	367.6				217.0	434.9
	Survival rate (%)		51.1	44.3	38.0				59.4	64.6
S. ovalis	No. planted seedlings[a]	581	261		235	9	25	100	351	
	Av. height (cm)	257.7	147.0		182.4	162.7	184.6	212.2	102.4	
	Av. height of tallest 100 trees/ha (cm)	364.3	289.1		319.7			355.0	239.7	
	Survival rate (%)	60.4	56.7		60.4	100.0	68.0	78.0	68.4	
S. seminis	No. planted seedlings[a]	1973	260	454	721				292	1278
	Av. height (cm)	189.6	180.9	200.8	182.1				92.7	238.5
	Av. height of tallest 100 trees/ha (cm)	352.0	342.0	330.7	309.9				192.3	393.9
	Survival rate (%)	40.7	53.1	48.2	35.9				54.1	49.7
S. pauciflora	No. planted seedlings[a]	2584	247	445	849	9	25	100	326	1306
	Av. height (cm)	129.6	171.1	162.3	127.6	90.8	154.3	161.1	97.9	200.4
	Av. height of tallest 100 trees/ha (cm)	265.3	375.1	293.3	238.1			268.3	197.1	421.3
	Survival rate (%)	18.4	60.7	40.0	29.3	44.4	28.0	45.0	57.1	51.3
D. lanceolata	No. planted seedlings[a]	644	273	459	965	9	25	100	362	1088
	Av. height (cm)	253.0	196.7	208.2	284.8	240.6	230.2	253.2	128.8	220.0
	Av. height of tallest 100 trees/ha (cm)	403.3	353.1	361.1	495.6			414.4	260.7	422.0
	Survival rate (%)	51.7	44.3	44.7	49.0	77.8	52.0	48.0	51.7	76.2

[a]Includes the number of seedlings in the supplementary planting, 2 months after the initial planting

[b]Trees were 2½-years old

the five species. Height of about 100 of seeding was measured in June, July, August, and November 1994, and in April and September 1995. Light intensity was measured in the plot with plants girdled 6 months after planting (i) prior to girdling, in October 1994, and (ii) after girdling, in November and December 1994, and February, March, and May 1995. Relative light intensities were expressed as the ratio of daily accumulated-light intensity in the plot to that of the open area.

22.2.3 Planting Methods and Growth of Weeds

After planting, the weeding requirements around seedlings in the under-canopy site were compared with the requirements in the open-area site. Weed dry weight was measured in three 4 m^2 blocks of each of the under-canopy and open-area sites, where dipterocarp seedlings had been planted monthly for 11 consecutive months. The fresh weights of weeds from a total of 66 blocks were measured and the dry weight per hectare was calculated. The stem length of all woody plants in each block was also measured for an indication of weed height. Girdling was not carried out at the under-canopy site.

22.2.4 Survival and Growth of *Shorea leprosula* Plantations for 10 Years

The height, dbh, and survival rate of approximately *S. leprosula* plants that had not been damaged in the forest fire of 1997–1998 were monitored for these 10 years from 1993 to 2002. Plots of *S. leprosula*, used for density examination, had planting densities of 1,000 (3 × 3 m), 2,500 (2 × 2m), 5,000 (1.4 ×1.4 m) and 10,000 (1 × 1 m) trees ha^{-1}. The plot with a planting density of 2,500 trees ha^{-1} was located beside the marsh, and the other plots were located on the hillside.

22.3 Results

22.3.1 Survival and Growth of Planted Seedlings by Planting Method

The number of seedlings, average height, average height of dominant trees, and survival rates are shown in Table 1. The survival rate and height growth were higher for *S. leprosula* than for *S. pauciflora*. Among the planting methods investigated, the under-canopy method (S2) resulted in a higher survival rate than the open-area, gap, and line methods. In the under-canopy site, height growth was accelerated by girdling the upper-story trees. Based on the results, the most suitable time for girdling was 3 months after planting.

The survival rate and height growth of dominant *S. leprosula* and *S. pauciflora* seedlings planted using the various methods are shown in Fig. 1. The survival rate decreased sharply during the 3 months after planting, after which it decreased more slowly. This tendency was also common in the other species. *S. leprosula* seedlings had higher survival rates in the gap and under-canopy (S1) sites than in the open-area site and the 20-m wide line site, where planted seedlings suffered under strong sunlight conditions. Conversely, greater height growth was observed in the open-area site, 20 × 20-m gap site, and 20-m wide line site. For *S. pauciflora*, higher survival rates were observed in the 6 m-wide line-planting site and under-canopy site. Survival rates in the open-area site, 20 × 20-m gap site, and 20 m-wide line site were very low 3 years after planting.

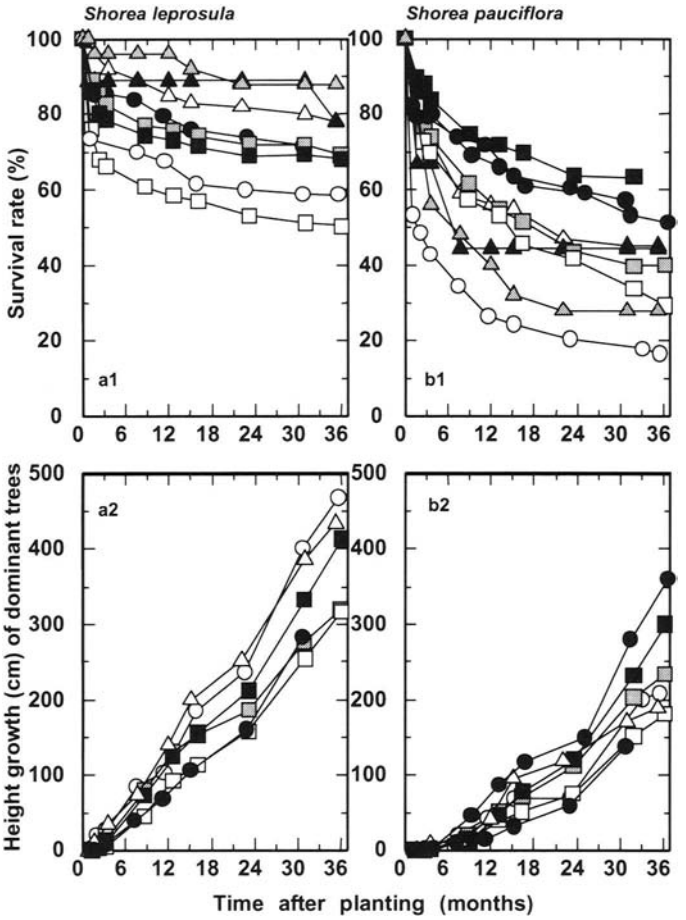

Fig. 1. Survival rate (**1**) and height growth of the 100 dominant seedlings (**2**) of *Shorea leprosula* (**a**) and *Shorea pauciflora* (**b**) seedlings using various planting methods. *Solid squares*, 6-m wide line plantations; *hatched squares*, 10 m wide; *open squares*, 20 m wide; *open circles*, open-area plantations; *solid circles*, under-canopy plantations; *solid triangles*, gap plantations of 5 × 5 m quadrats; *hatched triangles*, 10 × 10 m quadrats

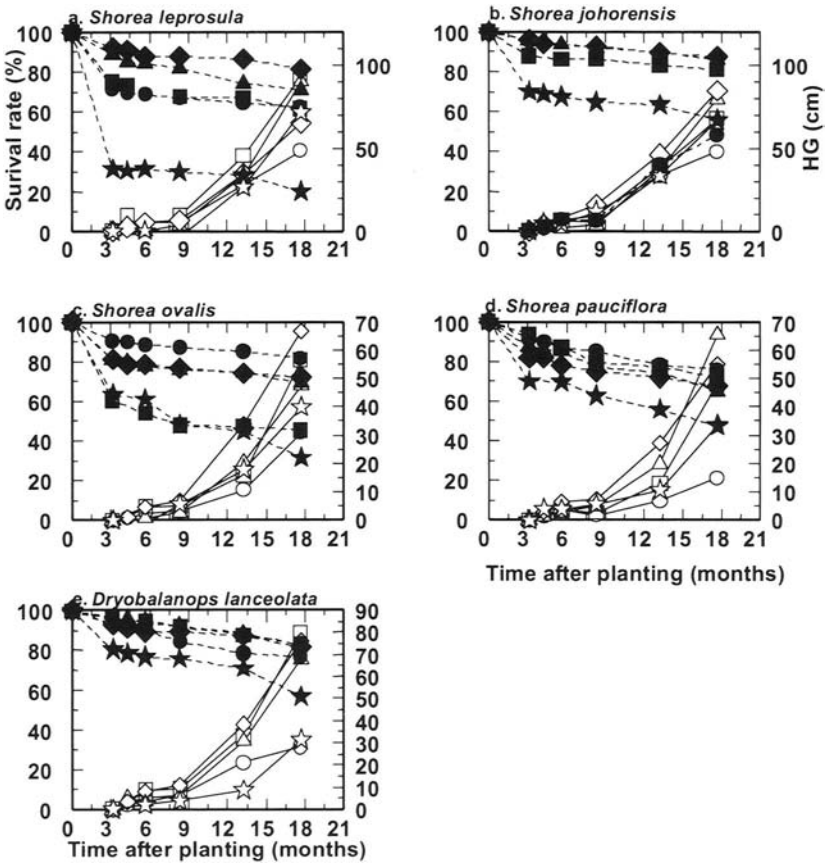

Fig. 2. Survival rate (*filled symbols*) and height growth (HG: *open symbols*) of five dipterocarp seedlings planted in under-canopy conditions in relation to the timing of the girdling of upper-story trees at 3 (*diamonds*), 6 (*triangles*), and 12 (*squares*) months after planting. *Circles* and *asterisks* represent survival rate and height growth, respectively, under open-canopy conditions. **a** *Shorea leprosula*, **b** *S. johorensis*, **c** *S. ovalis*, **d** *S. pauciflora*, **e** *Dryobalanops lanceolata*

22.3.2 Timing of Girdling on Upper-Story Trees in Under-Canopy Planting

The temporal changes in average heights and survival rates of dipterocarp seedlings girdled and ungirdled on upper-story trees in under canopy planting are compared in Fig. 2. It is apparent that the height growth of seedlings in girdled sites was larger than that of plants in girdled sites at about 13 months after seedlings. Difference in the effect of the seedling growth by the timing of girdling was not clear.

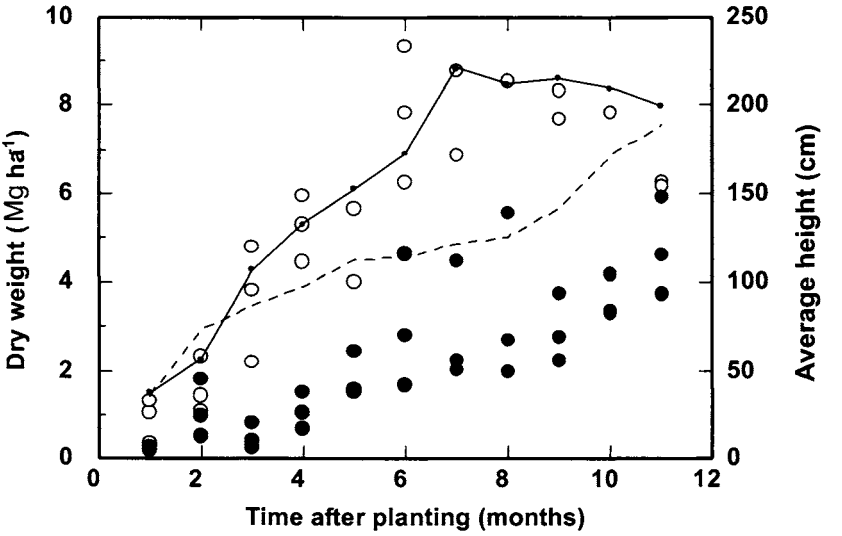

Fig. 3. Temporal changes in the dry weight (*circles*) and average height (*lines*) of weeds in the under-canopy site (*filled circles and dotted line*), and the open-area site (*open circles and solid line*)

22.3.3 Planting Methods and Growth of Weeds

The results of weed growth according to planting method are shown in Fig. 3. In the open-area site, the dry weight of weeds increased to 4 Mg ha^{-1} in the 3 months after planting. In the under-canopy site, it took about 1 year for the weeds to increase to 4 Mg ha^{-1}.

22.3.4 Survival and Growth of *Shorea leprosula* Plantations for 10 Years

The temporal changes in survival rates of *Shorea leprosula* trees are shown in Fig. 4a, and average height and dbh are shown in Figs. 4b and c. The survival rate of the plot with a planting density of 2,500 trees ha^{-1} decreased in the heavy drought of 1997–1998 (arrows in Fig. 4a–c). This plot was located beside the marsh, whereas the other plots were located on the hillside. Soil conditions, especially soil-water content, might have been better in the plot beside the marsh than in the other plots, and *S. leprosula* trees planted in this plot might be less tolerant to water deficiency.

The average heights and dbh of 10-year old *S. leprosula* trees ranged from 8.6–12.8 m and 8.9–15.1 cm, respectively. Trees in the plot with a planting density of 2,500 trees ha^{-1} had the highest average height and dbh (Figs.4b, c).

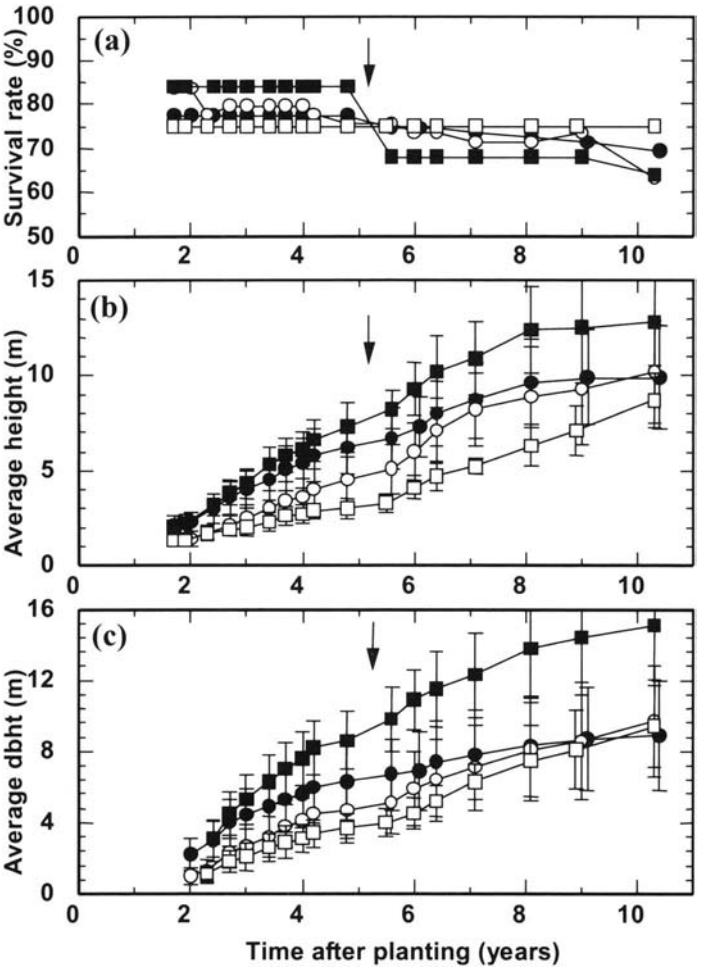

Fig. 4. Changes in of the survival rate (**a**), average height (**b**) and diameter at breast height (dbh) (**c**) of 10-year old *Shorea leprosula* plantations with planting densities of 10,000 trees ha^{-1} (*open circles*), 5,000 trees ha^{-1} (*filled circles*), 2,500 trees ha^{-1} (*open squares*), and 1,100 trees ha^{-1} (*filled squares*). *Arrows* indicate the period of the prolonged, severe drought in 1998

22.4 Discussion

In under-canopy plantings, the height growth of girdling plots was larger than that of the ungirdled plot at about 13 months after planting. The results proved the positive effect of girdling on height growth, and that girdling was most desirable at 3 months after planting, or within a year at the latest.

The data and observations from the open-area site indicated that weeding seemed to be necessary 3 months after planting when the weeds had increased to 4 Mg ha^{-1}. In the under-canopy planting area, it took about 1 year for the weeds to increase to 4 Mg ha^{-1}, suggesting that weeding would be necessary 1 year after planting at the ungirdled site.

The survival and growth of each species differed with silvicultural technique, including planting method and tending by weeding and cutting, which influence the light conditions of planted seedlings (e.g. Sasaki and Mori 1981; Suzuki and Jaceline 1985). Our results suggest that we can reduce reforestation costs by (1) improving the survival rate, by selecting the most suitable planting method for each species in order to decrease the planting density, and (2) controlling the light conditions to increase the growth of seedlings and decrease the times of weeding.

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23
Effect of Enrichment Planting on Restoring the Logged-Over Dipterocarps in a Tropical Rainforest of Central Sumatra

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23.1 Introduction

Indonesian natural forests have been extensively exploited using the selective logging system, but there has been recent debate over the ecological impact of this system. Meanwhile, tree plantations have been established, almost without exception, to restore the logged-over forests (Forestry Department of the Republic of Indonesia 1997). Also, linear-enrichment planting designs have been used for a decade, and gap-planting and belt-shelterwood systems have been recommended for native-forest regeneration (Kobayashi 1992, Evans 1992). Each method has its advantages and/or disadvantages, and the relative efficiencies of methods depend on the objectives of the plantations. The general principle of enrichment planting is to improve the light environment around the planted seedlings by removing overtopping trees, and to reduce competition from the surrounding undergrowth (Appanah and Weinland 1993).

If the aspects of logging operations that affect forest structure can be simplified, the first issue to be addressed should be the effects felling and removing large trees and the associated damage to small- and middle-sized trees, which create unnatural gaps in the forest (Okimori and Matius 2000). The aspect factor is the construction of roads and skid trails to reach and extract the target trees. If logging disturbance is reduced, the recovery process in the gaps could be simplified through the vertical and lateral growth of the residual trees and the enhancement of natural regeneration, including seedling banks and saplings.

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The purpose of the research presented here was to study the effect of releasing trees on the growth of the remaining natural saplings, especially dipterocarp species, and to study the effect of enrichment planting on restoring a disturbed forest.

23.2 Research Site

The research sites were located in the education and research forest of Gadjah Mada University (GMU), located in the middle district of the Batang Hari River basin, Jambi (102°30'E, 1°33'S). The forest occupies one compartment of the company concession forest, where logging operations started in the mid 1970s and terminated in 1990. The annual rainfall is around 2400–3000 mm, and the mean annual temperature is 27.3°C. The climate is characterized by a dry season from July to September and a rainy season from December to April, but the rainfall pattern fluctuates considerably from year to year. The forests in this area belong to the lowland dipterocarp forest type.

23.3 Research Plots and Measurements

Three categories of research blocks were established, as shown in Fig. 1. Of the two blocks used for research on natural saplings, the first was established in a moderately logged forest, where emergent trees were felled but the forest maintained a balanced structure consisting of small, medium and tall trees (Okimori et al. 1996). This block included two smaller plots, designated Plot 1, with 15 ha of treatments, and Plot 2, with 1 ha of natural saplings that were used as a control for the investigation. The second block contained plots within constructed gap plantations that had been established in a heavily logged forest. The natural dipterocarp saplings were left uncut in three constructed gaps, A, B, and C, which were compared with saplings in the tall forest. The saplings were less than 5 cm in stem diameter and more than 1.5 m tall. The sizes of the constructed gaps are described below.

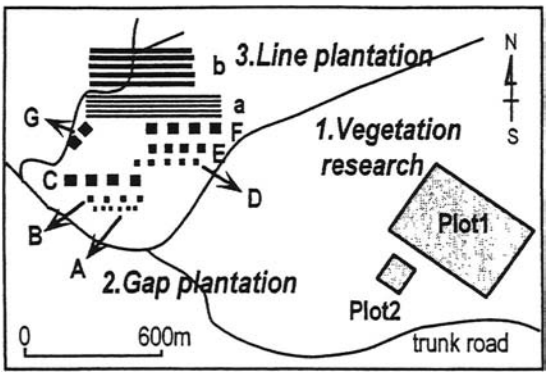

Fig. 1. Plot arrangement of sapling research and trial plantations. Legend is described in the main text

Two blocks were used for the enrichment planting study, including the gap plantation and the line plantation, as shown in Fig. 1. Four gap sizes were selected in 1994, 1995, and 1996. Plots C and F were established in large (40 × 40 m, 1600 m^2), approximately square, gaps. Plots E and G plots were established in middle-sized gaps (30 × 30 m, 900 m^2), Plots B and D were established in middle-sized gaps (20 × 20 m, 400 m^2), and Plot A was established in a small gap (10 × 10 m 100 m^2). Six species were planted: *Shorea parvifolia, S. acuminata, S. leprosula, S. macroptera*, meranti cengal (*Shorea sp.*), and *Parashorea lucida*. To construct the gap, trees below 20 cm in diameter were cut, and larger trees were girdled and left to die gradually. However, the largest trees were retained, and useful species, such as the dipterocarps, were left uncut.

Two types of line-planting transects were established in 1995. One type consisted of narrow transects, from which the vegetation was removed from a 5-m wide and 450-m long strip (labeled *a* in Fig. 1). In the second type, vegetation was removed from a 10-m wide and 200-m long strip (labeled *b* in Fig. 1). The planted trees were of five dipterocarp species: *Shorea acuminata, S.parvifolia, S.pauciflora, S.macroptera*, and *Parashorea lucida*. The saplings were planted at intervals of 2 m, with one line for the narrow transect and two lines, 3 m apart, for the wider transect.

Light intensity was measured beside the planting lines in the 10 × 10-m subplots, using a digital light meter (T1-H, Konica Minolta, Tokyo, Japan) in units of klux. In the line-planting plots, light intensity was measured at intervals of 10 m along the line in the narrow transects and 8 m in the wider transects. Results within the plots were compared with open-area readings made under equivalent conditions.

23.4 Results and Discussion

23.4.1 The Effects of Release Cutting on Light Condition

Relative light intensity (RLI) was an average of 6.3% in the logged forest. The RLI in the small (100 m^2) gaps varied between 6% and 13%, which was slightly higher than that under the forest canopy. The RLI in the large gaps (1600 m^2) was between 18% and 24%, which was higher than the other gaps, but not as high as expected.

23.4.2 The Effects of Release Cutting on Natural Saplings

The stand structure of the natural dipterocarp saplings is shown in Fig. 2, which provides a frequency distribution of sapling height. In the logged forest, the frequency distribution produced an L-shaped curve with a gentle slope, as commonly observed in a natural forest. In the constructed gaps, the number of smaller saplings < 300 cm in height was comparatively less than in the forest, and the mode of height distribution was displaced from the 150–300-cm size class to the larger 300–600 cm size class.

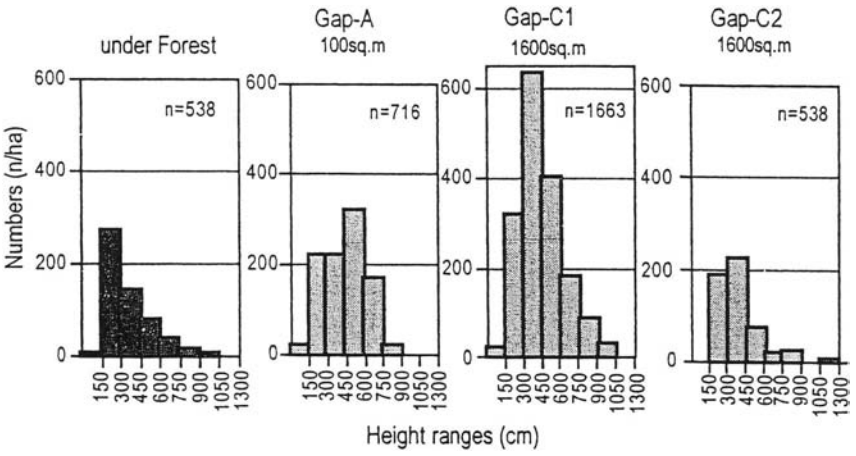

Fig.2. Frequency distribution of height of natural saplings

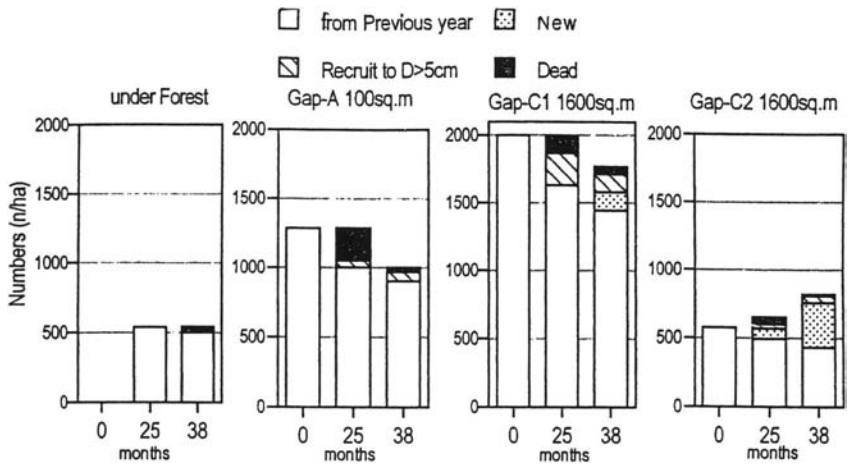

Fig.3. Change of dipterocarp sapling population

Figure 3 shows the variation in sapling numbers, which is limited in the forest plot, but more definite in the various constructed gaps. In the small gaps of A (100 m^2), an average of 18% of the saplings died during the 3 years of the study. The sapling numbers in the large gap of C1 (1600 m^2) also decreased, but this was due to recruitment into the larger-size class of > 5 cm diameter. On the other hand, sapling numbers in the large gap of C2 increased slightly because the recently recruited saplings exceeded the number of dead ones. Sapling numbers had large fluctuations in the constructed gaps.

Frequency distributions of height increment are shown in Fig. 4, with the mode being between 0 and 50 cm/yr, especially in the forest plot where more than 60% of stems were in this class. In the gaps, the mode of height increment was same as that

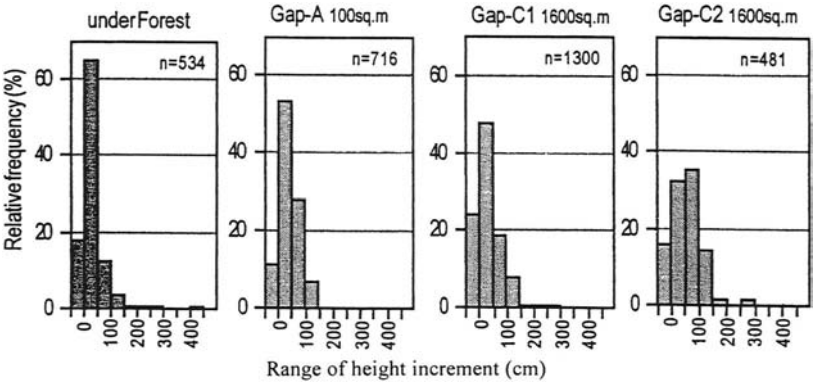

Fig.4. Relative frequency distribution of height increment of natural saplings

Table 1. The mean growth rate of natural saplings

			Forest	Gap		
				A	C1	C2
		Area (ha)	0.5	0.06	0.16	0.16
		No. (n/ha)	466	983	1494	538
		months				
Increment Height	cm/yr	14	14.0	35.4	19.6	46.9
Diameter	cm/yr	13	—	0.12	0.31	0.40
	cm/yr	27	0.09	0.40	0.39	0.54

of the forest plot, but the upper class of 50–100 cm in height increment had relatively more stems. Attention should also be paid to the high percentage of negative growth that accounted for between 10% and 25% of stems throughout the plots. This point is discussed later in relation to damage sustained by the terminal shoots of saplings.

On average, the mode of diameter growth was in the range of 0–0.5 cm/yr throughout the plots. In the forest plot, this class contained nearly 90% of all stems, suggesting that diameter growth in the forest was low compared to that in the gaps.

The mean growth rates are shown in Table 1. Height growth was greatest in the large gap, C2, at 46.9 cm/yr. However, it did not always follow that growth rate increased with the size of the gap, because height growth was 19.6 cm/yr in gap C1 (1600 sq.m) and 14.0 cm/yr in the forest plot. Diameter growth was clearly the lowest in the forest and greatest in the gaps.

Table 2 shows the extent of damage to the leading shoots, which was mentioned previously. Damaged tips were observed in 26%–36% of saplings in any plot. This kind of negative growth in height often occurs because the leading shoots are broken by falling branches and lianas, are withered by drought, or eaten by deer. Usually, if slight damage occurs to a terminal shoot, lateral shoots sprout and grow to

Table 2. Damages of the terminal leading shoot of saplings

		Forest	Gap		
			A	C1	C2
Plot area	(ha)	0.5	0.06	0.16	0.16
Total number of sapings	(n)	233	59	239	86
Broken, dead at leading shoot	(n)	70	21	62	25
	(%)	30.0	35.6	25.9	29.1
Serious damage	(n)	38	4	41	11
(Negative growth < –6.0 cm)	(%)	16.3	6.8	17.2	12.8

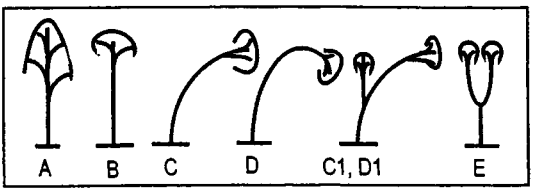

Fig.5. Some sapling types of epinastic growths. The characteristic of each type is described in the main text

replace the terminal one. However, when the terminal shoot is damaged seriously or repeatedly, regrowth seems to be difficult. The extent of serious damage was 6.8% in the small gap, A, which was less than in the other plots, and could have contributed to the relatively high mean rate of height growth in this gap.

Regarding the damage to terminal shoots, some of it seems to have been caused by the abnormal shapes of the saplings. In the natural saplings, several types of pole shapes were observed, as shown in Fig. 5. Type A was a straight pole with a substantial crown, and Type B was a straight pole with a small or very small crown. Both crown types A and B were of the normal shape. Type C was an inclining pole, and Type D was a more distinctively inclining pole with a bent head. Usually, the upper part of the pole in types C and D died, and new shoots sprouted from the base. Type E was a pole with a branching forked crown, and it was supposed to have developed from type C or D crowns. The irregular shapes were Type C, D, and E, which commonly suffered damage in the upper part of the stem. They constituted 15% of stems in the forest plot, 20% in gap C2, but 41% in gap A and 32% in gap C1.

23.4.3 Effects of Light Condition on Enrichment Planting

Relative light intensity (RLI) inside the forest was only a few percent of that in the open, but in the line it was around 15%. RLI in the gaps commonly increased with gap size, but it varied widely, even within the same gap size class. In the logged-over forests, the circumstances were very different with respect to the orientation of the gap, surrounding trees, and position of measurement in the gaps, so that a clear result was not obtained.

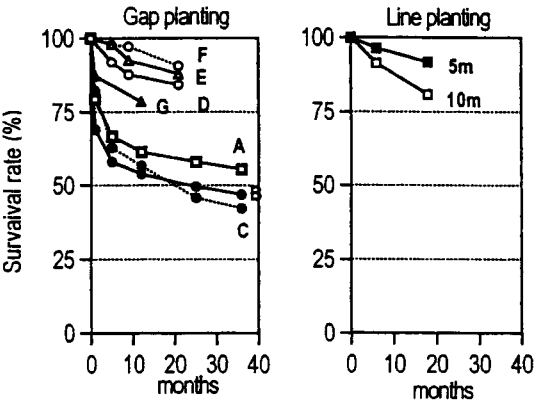

Fig.6. Survival rate of seedlings in the gap and line plantings. Gap sizes: A, 100sq m, B and D, 200sq m, E and G, 300sq m, C and F, 1600sq m

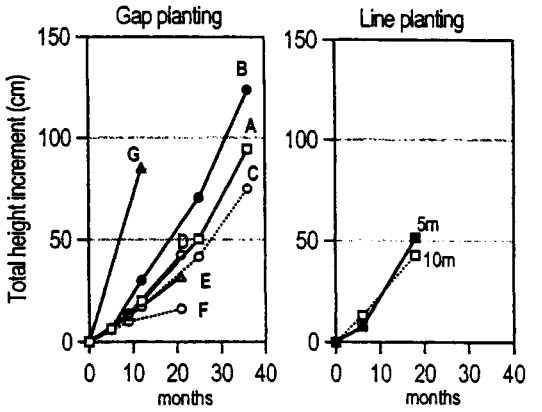

Fig.7. Total height increment of seedlings in the gap and line plantings. Gap sizes: A, 100sq m, B and D, 200sq m, E and G, 300sq m, C and F, 1600sq m

23.4.4 Survival and Growth of Enrichment Planting

Figure 6 shows survival rate of the planted seedlings. The survival rates in the gap plantings are divided into three groups. The first group includes gaps A, B, and C, all planted in 1994 and in which survival rates fell to around 50% at 38 months. The second group includes gaps D, E, and F, planted in 1995, and in which survival rates were around 90% after 20 months. Gap G was planted in 1996. The second group had a shorter observation period than the first group, but the results clearly show their higher survival rate. It is noteworthy that the differences in survival rate were associated with planting year rather than with gap size. The survival rate of line plantings, which were also established in 1995, was 80%–90%, which was almost the same as that of the second group.

Figure 7 shows the height increment of seedlings. The seedlings in gap G (900 m^2) had the highest growth rate. The reasons suggested are (1) the light intensity was higher in this gap than in others, (2) the planting density was greater with a planting space of 1 × 1 m, and (3) the planted species, *Shorea acuminata*, is one of the light-demanding species. The height-growth rate of line-planted trees was similar to that of the gap plantings. Compared with the growth rate of the natural saplings (Table 1), the planted seedlings grew substantially faster than the saplings under the forest canopy.

23.5 Conclusion

1. Releasing trees by creating gaps will enhance the growth of natural saplings, but the stand structure within the sapling size classes may become unstable.
2. Enrichment planting leads to faster growth than natural sapling growth in a forest. The species selected for planting and thinning intensity should be considered further.
3. Both of the above methods are applicable to the practical rehabilitation of a forest. Combinations of silvicultural systems, involving enhancement of natural regeneration and enrichment plantings should be investigated.

Acknowledgment

We are grateful for the financial support of Kansai Electric Power.

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24
Effects of Periodic Drought on Gas Exchange and Phyllode Water Status of *Acacia mangium* and *A. auriculiformis* Growing on Sand Tailings

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24.1 Introduction

Tin tailings resulting from mining activities in Malaysia cover about 113,700 ha (Chan 1990), of which 80% is sand (Lim et al. 1981). Afforestation trials initiated since the 1950s showed that some timber tree species are suitable for growing on sand dunes that have a water-table level reaching the root zones (Ang et al. 1993; Ang 1994). However, there is a lack of documentation on afforestation techniques for high sand dunes, which are normally more than 4 m above the standing water-table level (a.s.w.l). Besides low soil fertility, water deficits and high air temperature are the two main factors that limit plant growth in high sand tailings (Mitchell 1957; Ang 1994). Ang and Maruyama (1995) reported that *Acacia auriculiformis* and *Acacia mangium* had greater growth on low-site (< 1.5 m a.s.w.l) than on high-site (>7 m a.s.w.l.) sand tailings. The vegetative growth of *A. auriculiformis* was greater than *A. mangium* on high sand dunes, and was largely due to the greater water use efficiency of *A. auriculiformis* (Ang and Maruyama 1995). Moreover, Ang et al. (1997) showed that *A. auriculiformis* grew better than *A. mangium* on high sands of 8 m a.s.w.l., mainly due to its physiological advantages during water deficit and heat stress. However, a direct relationship between phyllode water potential and gas exchange characteristics was not documented. This paper aims to show, (1) the effect of dry periods on the gas exchange characteristics of *A. mangium* and *A. auriculiformis* growing on sand tailings; and (2) the relationship of phyllode water status to gas exchange characteristics of the two *Acacia* species after a drought period.

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24.2 Materials and Methods

Three-month-old seedlings of *Acacia mangium* and *A. auriculiformis* were planted under shade (30% of full irradiance at 1300 Malaysian Standard Time, MST) or open conditions on levelled barren sand dunes of tin tailings, situated 8 m above the standing water table level (a.s.w.l.), and located in Au's mine, Malim Nawar, Perak (4°22.5'N, 101°7.5'E). Ang et al. (1997) described the study site in detail and showed that the particle-size distribution of sand dunes at 0–30 cm and 1.40–1.45 m depths comprised gravel (12%), medium to coarse sand (76.7%), fine sand (11%), and clay and silt (1%).

24.2.1 Gas Exchange Activities

The gas exchange activities of the first fully expanded leaf of both species were determined by a portable Infrared Gas Analyser (IRGA) (ADC LCA-2, XXX, XXX UK). These data were collected between 0900 to 1130 MST. The photosynthetic photon flux density (PPFD) normally exceeded light saturation point, and midday depression was unlikely during this period (0900–1130 MST), which is hereafter referred to as "at full capacity." Net photosynthesis at full capacity (A_{net}), transpiration (E), and leaf temperature ($T_{phyllode}$) were calculated from the data collected by the IRGA. Due to the unpredictable field conditions, data for net photosynthesis at full capacity in the dry period were not collected. However, data sets collected 1 or 2 days immediately after two dry periods were used to assess the effect of drought on the gas exchange characteristics of the species. Four data sets were selected from both plots in the wet and after-drought periods. Wet periods are defined as those in which the mean daily rainfall over the 2 weeks immediately before the last rainfall exceeded 4.0 mm day^{-1} (Ang 1996). If the mean rainfall during this 2 week period was < 4.0 mm day^{-1}, followed by rain before gas exchange data collection, then it is referred to as "after drought." The last rainfall was the rainfall recorded just before the gas exchange data were measured. All the data sets of photosynthesis at full capacity and diurnal patterns collected after drought and during wet periods were used for the construction of light response curves of photosynthesis for both species in the open plots. The estimated photosynthetic photon flux density at light saturation points of both species in the open plots, either during the dry or wet period, was 800–900 μmol $m^{-2}s^{-1}$. Hence, only data of gas exchange characteristics collected at > 800 μmol $m^{-2}s^{-1}$ were used for the analysis (Ang 1996).

A randomized factorial experiment consisted of eight combined levels, including site (open and shade), species (*A. mangium* and *A. auriculiformis*), preconditioning treatments (with or without) and period (wet or after dry). Each combined factor had nine replicates. The data were analyzed using the statistics program Minitab 10.1 for Windows. The comparisons of means are after Parker (1979).

24.2.2 Gas Exchange and Phyllode Water Potential

Data for transpiration, net photosynthesis and phyllode water potential were collected from 0900 to 1530 MST. The first fully mature phyllode of both *Acacia* species was examined for net photosynthesis and transpiration, and then removed immediately for the determination of phyllode water potential. The measurements of gas exchange and phyllode water potential were determined by the IRGA and a pressure bomb (Soil Moisture, USA), respectively. Only data collected above the light saturation point were used for the analyses.

24.3 Results

24.3.1 Gas Exchange and Water Use Efficiency

The preconditioning treatments did not significantly affect the gas exchange characteristics of either species (Table 1). *A. auriculiformis* had significantly higher A_{net} and WUE than *A. mangium*. Both species had significantly higher A_{net}, and WUE in the wet than the after-drought treatment. There was also a significant difference between the interaction effects of period x site x species on the mean PPFD and A_{net}

Table 1. Main effects of the treatments on mean PPFD, Anet, E, and WUE

Treatment	PPFDμmol $m^{-2}s^{-1}$	A_{net}μmol $m^{-2}s^{-1}$	Emmol $m^{-2}s^{-1}$	WUEMmol mol^{-1}
Period				
After drought	910.9a	10.0a	11.2a	0.90a
Wet	916.8a	11.3b	9.1b	1.19b
$P < 0.05$, $n = 144$	LSD = 53.0	LSD = 0.7	LSD = 0.4	LSD = 0.06
Site				
Open plots	1260.6a	12.90a	10.1a	1.32a
Shaded plots	567.1b	8.42b	10.2a	0.77b
$P < 0.05$, $n = 144$	LSD = 53.0	LSD = 0.69	LSD = 0.4	LSD = 0.06
Species				
A. mangium	909.5a	10.20a	10.1a	0.98a
A. auriculiformis	918.3a	11.12b	10.3a	1.11b
$P < 0.05$, $n = 144$	LSD = 53.0	LSD = 0.69	LSD = 0.39	LSD = 0.06
Preconditioning Treatment				
Yes	916.9a	10.80a	10.23a	1.07a
No	910.8a	10.51a	10.12a	1.02a
$P < 0.05$, $n = 144$	LSD = 53.0	LSD = 0.69	LSD = 0.39	LDS = 0.06

PPFD, photosynthetically active photon flux density; A_{net}, net photosynthesis; E, transpiration; WUE, water use efficiency expressed as a ratio of A_{net}: E; LSD, least significant difference; *n*, sample size

Within a column, the means of a treatment that are significantly different ($P < 0.05$) share the same letter

Table 2. The interaction effects of period × site × species on mean PPFD, E, A_{net}, and WUE

Treatment	PPFD		E	A_{net}		WUE
	μmol $m^{-2}s^{-1}$		mmol $m^{-2}s^{-1}$	μmol $m^{-2}s^{-1}$		Mol mol^{-1}
After drought	Shade	Open	Open	Shade	Open	Open
A. mangium	593.9 a1	1288.4 a2	11.83a	8.40 ac1	10.87 a2	0.96a
A. auriculiformis	553.9 a1	1267.8 a2	10–97a	8.45 ac1	12.34 b2	1.11a
Wet						
A. mangium	560.9 a1	1254.9 a2	8.94b	7.69 a1	13.78 c2	1.53b
A. auriculiformis	559.7 a1	1291.6 a2	8.83b	9.13 c1	14.61 c2	1.66b
$P < 0.05$, $n = 18$	LSD = 106.1		LSD = 0.87	LSD = 1.43		LSD = 0.15

PPFD, photosynthetically active photon flux density, E, transpiration; A_{net}, net photosynthesis; WUE, water use efficiency expressed as ratio of A_{net} : E

Within factors only, letters indicate significant differences ($P < 0.05$) between values in the same column, and numerical numbers indicate significant differences ($P < 0.05$) between values in the same row

($P < 0.001$). Table 2 shows that the A_{net} of the two species in the shade plots did not differ significantly between the wet days after a drought and the wet period, but they did differ in the open plots. Both *Acacia* species had significantly lower A_{net} after a drought. Data sets from the open plots were separated for two-way ANOVA analyses to assess the relationship between the effects of drought and irradiance on the net photosynthesis of the two *Acacia* species. Table 2 shows that there was a significantly higher E but lower A_{net} in both species in the wet days following a drought than in the wet period.

24.3.2 The Relationship of Gas Exchange Characteristics and Leaf Water Status Above Light Saturation Point

Fig.1 shows the relationship between several gas exchange characteristics and leaf water potential above light saturation point. The data were collected on 11 and 12 January 1995 in wet conditions, just after the end of a 2-week drought. Hence, atmospheric drought would be the main factor affecting the leaf water status.

Fig. 1 shows that *A. auriculiformis* had a higher A_{net} and WUE than *A. mangium* at a lower $\psi_{phyllode}$. Generally, *A. mangium* had a lower A_{net} than *A. auriculiformis* from –0.8 MPa to –1.2 MPa on sunny days between 0900 and 1530 MST, while both species had a similar E at the same $\psi_{phyllode}$. The variations in net photosynthesis, transpiration, WUE, and leaf temperature with leaf water potential of both *Acacia* species on sunny days in a wet period show a greater WUE for *A. auriculiformis* than for *A. mangium* at a similar $\psi_{phyllode}$. In addition, the leaf temperature of *A. mangium* was greater than that of *A. auriculiformis* at a lower $\psi_{phyllode}$.

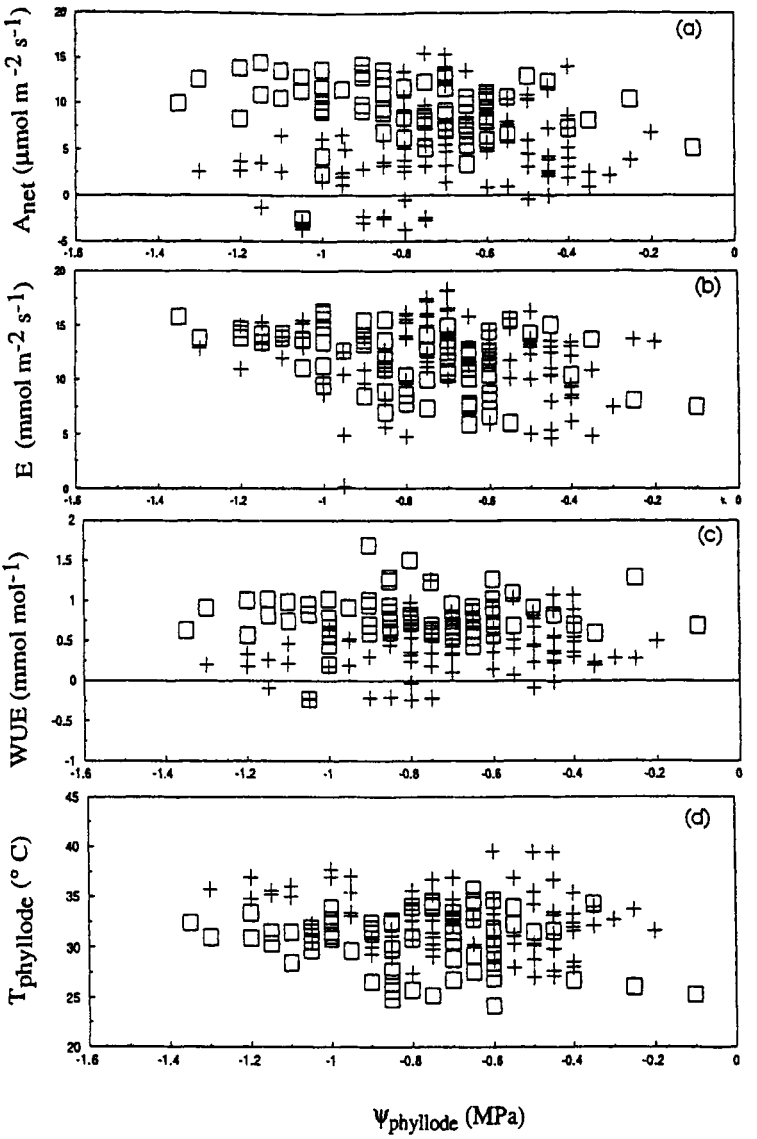

Fig.1. The relationships of phyllode water potential ($\psi_{phyllode}$) and (a) net photosynthesis (A_{net}), (b) transpiration (E), (c) water use efficiency (WUE) and (d) leaf temperature ($T_{phyllode}$) of both species during the sunny days of 11–12 January 1995, which was 1 or 2 days after about 40 mm of rain, on 10 January, 1995. However, prior to the wet day, it was 2 weeks without rain in the study site. The data sets were collected from 0900 to 1530, Malaysian Standard Time, at a photosynthetic photon flux density (PPFD) > 1000 mmol $m^{-2}s^{-1}$ in the open plots. *A. auriculiformis* (*squares*) and *A. mangium* (*plus symbols*) were only examined without preconditioned treatments

24.4 Discussion and Conclusion

Shading significantly reduced A_{net} and WUE but not E in both species (Table 1). The highest A_{net} and the lowest E in the open plots during the wet periods resulted in the highest WUE. The A_{net} and WUE in the shaded plots did not differ significantly between the two periods. Both species had significantly higher A_{net} and WUE in open than in shaded plots. This was due to a significantly higher PPFD in the open plots. The effects of drought significantly reduced the A_{net} on subsequent wet days for the open plots but not for the shaded plots. The shade reduced the adverse effect of drought on A_{net}. However, the reduction in A_{net} for both species in the open plots immediately after the drought indicated that the drought had reduced their photosynthetic efficiency (Table 2). Since the E of the subsequent wet days after the drought was significantly higher than the wet periods, stomatal limitation cannot not be the reason for the reduction of A_{net} on the subsequent wet days after the drought. Likewise, the PPFD, which was similar between assessments periods, is unlikely to be the factor that influenced the A_{net} of both species on the subsequent wet days after the drought. The reduction in A_{net} of both species on the subsequent wet days after the drought could be due to damage of their photosynthetic systems. *A. auriculiformis* had significantly higher A_{net}, at full capacity, and WUE than *A. mangium* in the wet periods.

The results showed that *A. auriculiformis* had significantly higher net photosynthesis and water use efficiency but lower phyllode temperature (data not shown) than *A. mangium* at a similar range of phyllode water potentials on a wet sunny day. This suggests that *A. mangium* maintained a higher leaf temperature than *A. auriculiformis* at the same $\psi_{phyllode}$. The effects of atmospheric drought on light saturation conditions for both *Acacia* species in one experimental series showed that A_{net}, WUE, and phyllode temperature in *A. mangium* were more sensitive to $\psi_{phyllode}$ than in *A. auriculiformis* in the –0.2 to –1.35 Mpa range. *A. auriculiformis* had greater A_{net} and WUE than *A. mangium* within the same $\psi_{phyllode}$ range (Fig. 1). This suggests that *A. auriculiformis* has greater drought tolerance than *A. mangium* because it is physiologically more active at lower phyllode water potentials. This is probably due to its ability to maintain positive turgor. Phyllode temperatures of *A. mangium* were higher than those of *A. auriculiformis* within the same $\psi_{phyllode}$ range. This suggests that the two species differ in phyllode temperature regulation mechanisms, which may account for some of the difference in their response to atmospheric drought. Hence, the results suggest that *A. auriculiformis* has significantly better physiological responses than *A. mangium* to the fluctuations of site and atmospheric water status on sand tailings. This at least partly explains the better growth of *A. auriculiformis* than *A. mangium* in drought-prone sand tailings.

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25
Reforestation and Biodiversity in the Asia-Pacific Region

JIRO KIKKAWA

25.1 Introduction

Since equatorial forests contain the greatest biological diversity among the terrestrial ecosystems on Earth (Richards 1996), tropical forestry has a unique challenge to sustain the biological diversity of the region. Forestry, more than any other form of economic land use, except natural tourism, has the opportunity to conserve regional biodiversity and habitats for wildlife. Thus, the forest industry has a special responsibility to ensure the conservation of biodiversity through its new practices under the International Convention on Biological Diversity (UNEP 1992). This responsibility may be met by such activities as establishing a protected areas system, as in Nepal (Shrestha and Belbase 2000), or minimizing the damage caused by logging operations, as in Malaysia (Muziol et al. 2000). Unfortunately, deforestation and forest degradation continue to threaten the rich biological diversity of forests in many parts of Southeast and Northeast Asia (Lee and Park 2002).

Reforestation has many different purposes, ranging from the rehabilitation of impacted forest landscapes to timber production via plantations of fast-growing commercial species. Reforestation may also be primarily to revegetate mined sites or to generate carbon credits. Whatever the purposes of reforestation, BIO-REFOR has a role not only to provide technology to optimize economic and conservation values of biodiversity in reforestation, but also to enhance the intrinsic values of biological and cultural diversity in the Asia-Pacific region. It is well established that the Asia-Pacific region is extremely rich in both biological and cultural diversity, and that their conservation is essential for the sustainable use of biological resources in the region (Asian Development Bank 1995; Myers et al. 2000).

This chapter explores the criteria for assessing the biodiversity of tropical forests and plantations, then develops indices to compare the potential values of various reforestation sites for biodiversity conservation, and finally makes some suggestions for research to develop biodiversity friendly methods for reforestation.

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25.2 What Criteria Should We Use to Assess the Importance of Reforestation Sites for Biodiversity?

The floral and faunal features of a forest site are characterized by the distribution and abundance of regional biota that forms the forest community and maintains a dynamic state under given ecological conditions. Where these conditions are damaged or destroyed through disturbances, reforestation may aim to restore some or all of these attributes. The main criteria by which to assess the potential value of such sites for biodiversity conservation may be based on the attributes used for selecting sites for the International Biological Program (IBP) in the 1960s (Peterken 1967). In the following sections, I give new interpretations to these attributes and add one other for the present purpose.

25.2.1 Species Diversity

Expressed as species richness (the total number of species), species diversity represents the essence of biodiversity. Within the Asia-Pacific region, two major biogeographic regions are involved, representing (1) the Oriental region of the Asian continent with adjacent islands, and (2) the Australian region, east and south of the Wallace's line, with the Australasian biota of Gondwanaland origin, superimposed by that derived from the post-Miocene Indo-Malayan colonizers. The Australian region has been isolated from the species-rich Oriental region, even after the collision of the Australian plate with the Southeast Asian (Sunda) plate in the Miocene, due to the dry climatic barrier in the Wallacea region throughout the late Tertiary (Whitmore 1982). As the level of diversity differs between the two regions, local species diversity should be assessed in the light of both endemicity and regionally available diversity. Species diversity is further reduced in the Pacific Islands, generally from west to east (Whiffin and Kikkawa 1992), depending on the dispersal ability of colonizing species (Diamond 1984) and environmental disturbances (Müller-Dombois 1995). To the north, the fauna of the Palearctic region in the cold climate blend with that of the Oriental region, and many species of migratory birds breed in the Palearctic region in summer and return to the tropics in winter. To the west, the dry heat of North Africa separates the fauna of the two continents, although they share some elements.

On the continents and large islands, there is a series of core areas where rainforest massif may have acted as epicenters of evolution or refugia in the past. Away from the core areas, the environmental gradients of temperature (both latitudinal and altitudinal), humidity (both seasonal and absolute), and soil fertility and drainage generally reduce the species diversity of vascular plants and birds from the higher to the lower ends of the gradients (Kikkawa 1990; Whiffin and Kikkawa 1992). Between the core areas and along the gradients, unique associations of species, often involving endemic species, may develop to form nodal communities. For example, monsoon forest formations develop under seasonal rainfall, affecting soil moisture

(Whitmore 1984). Strong seasonality in deciduous and fruiting trees moulds the animal community in monsoon forests. Another unique association is found in swamp forests that cover masses of peat, which develop in nutrient-poor areas with impeded drainage (Richards 1996). The species diversity of these nodal communities may be lower (Ashton 1982) than the core areas of humid tropical lowlands on nutrient-rich soil, but the unique assemblages of species contribute significantly to regional biodiversity.

25.2.2 Exceptional Species Associations

The geographic and ecological distributions discussed above produce distinctive communities, depending on the origin and dispersal of biota on the one hand and ecological responses of species on the other. The distributions can be characterized by biogeographic subregions or habitat types that support distinctive communities or unique associations of species. Conservation values of biodiversity will increase with the number of such associations and the number of distinctive forest habitats available regionally.

In the humid tropics, the floristic classification of rainforests becomes not only difficult but also of little use for the description of biodiversity or wildlife habitats. An all-purpose classification has not been successful for tropical rainforests, which are floristically diverse and structurally complex. Thus, hierarchical classifications based on physiognomic-structural features may be used as first approximations to habitat classification (Kikkawa and Webb 1967), and further elaboration may be made by pattern analysis (ordination and classification) of these features to identify important habitat components associated with wildlife guilds (Kikkawa and Webb 1976). For example, associations of species may be predicted by certain habitat components, such as soil moisture for small mammals of lowland rainforests in Malaysia (Kemper and Bell 1985), or densities and types of plant cover for birds of North Queensland rainforests (Kikkawa 1982). It is important to match the scale of habitat classification with the target taxa for the pattern analysis.

25.2.3 Rare, Relict, or Threatened Species

The presence of endemic species, often the phylogenetic or geographic relicts unique to a region, increases the biodiversity value of the region. Both oceanic and habitat islands, numerous in the Asia-Pacific region, contain such species, which are usually rare and qualify for the IUCN categories of threatened species (IUCN 1996). At least for birds, the forest habitat is by far the most important habitat (65%) for the threatened species (Collar et al. 1994). Twelve of the top 20 countries ranked by the number of threatened bird species in the world are in the Asia-Pacific region. Including other countries, the Asia-Pacific region contains 403, or 43.6%, of the world's threatened species of land birds (Collar et al. 1994). These categories include critically endangered (50% probability of going extinct in 5 years), endangered (20% probability of going extinct in 20 years), and vulnerable (10% probability of going

extinct in 100 years) species. These estimates are conservative in view of the rapid changes that have occurred in the environments of forest birds in recent years. In fact, it is also time to consider the near-threatened species (those considered close to qualifying for the threatened categories) among the low-risk group. There are 393 species of land birds identified as such in the Asia-Pacific region (data from Collar et al. 1994). In assessing the reforestation site for biodiversity, it is important to identify the hotspots of threatened and near-threatened flora and fauna from a composite map of the distribution of threatened species. This is particularly important for vascular plants and land vertebrates, as they are better known than other groups and may also indicate the general level of biological diversity for the region.

25.2.4 Abundance of Protected Species

In setting priority areas for conservation, unique and localized populations of plants and animals are often considered. For example, a biodiversity database for Sabah forests aims to identify species and areas of importance for conservation by integrating information from the biological inventory work (Tangah et al. 1997). Some animals live in colonies or breed in groups, congregating in large numbers as they do in bat camps or heronries in the forest environment. Other species may gather around waterholes or hibernate in groups. If the forest area contains wetlands, the congregation of waterbirds may occur. If protected species are involved in these congregations, and if they use the same area traditionally, then the area needs protection. If the reforestation site contains such an area, the sustainable use of the area by the protected species must be assured. The abundance of individuals of otherwise rare species often gives a false sense of security for species recovery, but being abundant at one locality does not guarantee the survival of the species if it occurs nowhere else. Also, abundance needs to be scaled for rating different groups of animals.

25.2.5 Concentration of Migratory Populations

The Asia-Pacific region serves as the wintering ground for many migratory forest birds. Although they are not as conspicuous as migratory waders, which have staging areas in the region, a large number of northern forest birds, including species of owls (*Ninox*), cuckoos (*Cuculus*), rollers (*Eurystomus*), minivets (*Pericrocotus*), thrushes (*Turdus*), warblers (*Phylloscopus*), flycatchers (*Muscicapa*), and shrikes (*Lanius*) winter in Southeast Asia. Also, some Australasian breeders, such as the bee-eater (*Merops*), drongo (*Dicrurus*), metallic starling (*Aplonis metallica*), and long-tailed cuckoo (*Eudynamis taitensis*) winter on Pacific islands. Along the route of migration, some species, such as the Chinese sparrow hawk (*Accipiter soloensis*), congregate in large numbers, but many small forest birds, such as the brown shrike (*Lanius cristatus*), disperse from their flyways and become inconspicuous in their wintering grounds (McClure 1998). In recent years, concern has been expressed that these birds may be losing their wintering habitats due to the deforestation of rainforests in the tropics (Higuchi and Morishita 1999).

25.2.6 Type Localities

The type locality of new species contains biological information on the described species, and both the environment and the original population from which the type specimen was collected should be preserved. For example, as many sites as possible of those involved in the descriptions of dipterocarp species (Ashton 1982) should be protected for future reference. For the study of genetic diversity and reproductive biology of commercial timber species or other forest products, it is important to preserve the forest resources in which the breeding systems have been established. As molecular techniques have advanced, the genetic variability of natural populations has become the subject of study for improved management or conservation (Wickneswari et al. 1996). These are important reference populations for future studies. In early taxonomic work, collecting places concentrated at a few points on the map. If the reforestation site falls on one of these points, the conservation value of the existing resources at the site will be very high, not only for the preservation of the type locality but also for ecological and genetic studies of the populations concerned.

25.3 A Simple Index of Site Values for Biodiversity Conservation

25.3.1 A Rationale for the Biodiversity Index

The above features may be indexed in a regional context so that they can be compared between sites for the selection of reforestation sites. First, a number of qualities, such as the scientific, educational, historical, and aesthetic qualities, may be recognized as values of a site for conservation. Each quality may be expressed numerically and, if necessary, as a series of subindices derived from the terms of subqualities. Although the qualities are intangible, in terms of benefit, the criteria for them may be established scientifically to obtain unit-free indices. Then the important features of biodiversity may be considered as part of the scientific value to arrive at an index for comparison between sites.

An index of site value is proposed, consisting of the significant qualities of the site for nature conservation. It is obtained by a simple root-mean-square method, which can accommodate very large values entered for special qualities, and at the same time can give weight to any particular subindex, which is a primary, rather than ancillary, quality of the reforestation site. If the ideal state of the site with the maximum conservation value is expressed as unity, the site index (Is) may be written as:

$$Is = \sqrt{\frac{aI_{sc}^{\ 2} + bI_{ed}^{\ 2} + cI_{ht}^{\ 2} + dI_{ae}^{\ 2}}{a+b+c+d}}$$

where I_{sc}, I_{ed}, I_{ht}, and I_{ae} represent scientific, educational, historical, and aesthetic indices, respectively, and if no weighting is given to any of the subindices, $a = b = c = d = 1$. If, for example, the scientific value is considered twice as important as the other values, the weighting of $a = 2$ will be given, whereas if the educational value is considered to duplicate the scientific value by half, the weighting of b may be reduced to 0.5.

The hierarchical way that the subindices are arranged permits the construction of an equation pertinent to a particular quality. For example, an index for the scientific value of the site (*Isc*) might be expressed as:

$$Isc = \sqrt{\frac{I_{gsc}{}^2 + I_{psc}{}^2 + I_{asc}{}^2}{3}}$$

where I_{gsc}, I_{psc}, and I_{asc} represent the geological, floral, and faunal value indices, respectively.

For our purposes, the faunal value of the site (I_{asc}) may be expressed as a biodiversity index (I_{bd}), which can be derived from the criteria developed in the previous section:

$$I_{bd} = \sqrt{\frac{I_d^2 + I_x^2 + I_r^2 + I_a^2 + I_g^2 + I_t^2}{0.75 \times 6}}$$

where I_d, I_x, I_r, I_a, I_g, and I_t represent values of species diversity, exceptional species associations, rare or threatened species, abundance of protected species, concentration of migratory populations, and type localities, respectively. A correction factor of 0.75 is applied to adjust the subindices values, with reference to the unattainable ideal state of 1. This index is developed for the comparison of sites and not for the selection of sites for a comprehensive network.

25.3.2 Calculation of the Biodiversity Index

If we have a complete knowledge of the fauna from the region in which the indices are compared, it is easy to give a numerical value to each of the subindices. With the hierarchical arrangement, the maximum value of the index for the entire region is 1 when the indices of subregions are compared, while the maximum value for the subregion becomes 1 when the indices are compared between sites within the subregion. Thus, we can compare the biodiversity values of different regions within a nation, or of different sites within a particular region. In the example below, I use birds of Australia to represent the fauna, as their distribution is well known. Caution is needed, as being species-rich for one group of organisms does not mean species richness for another group (Faith and Walker 1996). For example, using freshwater invertebrates as indicator organisms may produce completely different results to that of birds. Two regions of Australia, Tasmania and Cape York Peninsula, are compared at the regional level, and three sites of Cape York Peninsula are used for a within-region comparison.

Table 1. Indices and subindices of biodiversity (I_{bd}) based on avian diversity features for Tasmania, Cape York Peninsula, and three sites within Cape York Peninsula

Indices*	Tasmania	Cape York Peninsula	Sites		
			Cape York	Cooktown	Archer River
I_d	0.27	0.48	0.72	0.65	0.53
I_x	0.44	0.55	0.50	0.50	0.50
I_r	0.21	0.24	0.32	0.16	0.52
I_a	0.41	0.59	0.10	0.00	0.20
I_g	0.40	0.70	0.29	0.43	0.29
I_t	0.18	0.06	0.87	0.09	0.00
I_{bd}	0.39	0.56	0.62	0.44	0.45

* For explanation, see text

The three sites chosen for comparison within Cape York Peninsula are: Cape York (the northern tip of the Peninsula with a cleared patch of rainforest surrounded by tall woodland); Cooktown (a historical site with a mosaic of vegetation remnants); and the Archer River (a west-flowing river with a narrow belt of riverine vegetation bordered by disturbed dry low-woodland). All indices for these sites are based on the proportions of the site features out of the total for Cape York Peninsula. Hotspots for conservation may emerge and can be identified in between-site comparisons, particularly if the values are high for all features.

Table 1 gives indices and subindices, obtained using the bird fauna for the two regions of Australia and three sites within Cape York Peninsula. The species diversity index (I_d) may be expressed as the ratio of the fauna of the region (or site) to be considered to the total number of species known from the country (or region) (Blakers et al. 1984). Tasmania, with 205 species, would have an I_d value of 0.27 and Cape York Peninsula, with 363 species, would have a value of 0.48. Within Cape York Peninsula, Cape York has the highest value of 0.72.

For exceptional species associations, only the major subdivisions of the biogeographic region and habitats may be considered. The possibility of co-occurrences of species from different subregions or different habitats to form exceptional associations of species depends on the number of such subdivisions available in the region or at the site. Accepting five biogeographic subregions (Kikkawa and Pearse 1969) and four major habitat types (coastal habitats, wet forest, dry forest-savannah, grassland-desert) in Australia, the I_x value would be 0.44 for Tasmania and 0.55 for Cape York Peninsula. Within Cape York Peninsula, eight distinct subdivisions of habitats can be identified within the same biogeographic subregion. Although different combinations of habitat types were available at the three sites, all had four, giving each site an index value of 0.5.

For the index of rare, relict, or threatened species (I_r), all categories of threatened species (critically endangered, endangered, vulnerable, conservation dependent, and near threatened) (Collar et al. 1994) are included. To this list, the relict species endemic to the region being considered are added, if not already included, in the threatened species category. The total number of bird species for comparison will come to

106, of which 22 species occur in Tasmania and 25 in Cape York Peninsula (none shared), giving an index value of 0.21 for the former and 0.24 for the latter. Within the Peninsula, the Archer River had the highest value of 0.52. Changes in status of species would affect the index values. For example, the new systematic treatment of passerine birds by Schodde and Mason (1999) and the conservation status proposed by Garnett and Crowley (2000) would alter these indices somewhat.

Since all native species are protected, the abundance of individuals of protected species was considered only for those that form single or mixed breeding colonies, such as the Torresian imperial pigeon (*Ducula spilorrhoa*), budgerigar (*Melopsittacus undulatus*), white-rumped swiftlet (*Collocalia spodiopygius*), rainbow bee-eater (*Merops ornatus*), fairy martin (*Hirundo ariel*), metallic starling (*Aplonis metallica*), grass-finches (*Taeniopygia, Poephila, Lonchura*), egrets, ducks, and other water birds. There are 17 such groups of birds in Australia, and of these, seven (0.41) are found in Tasmania and 10 (0.59) are found in Cape York Peninsula. Within the Peninsula colony breeders are insignificant.

Similarly, the migratory species or species groups can be identified, which concentrate in particular habitats, such as estuarine habitats and forest refugia. The habitats in which migratory species roost or nomadic species concentrate, are included in 10 categories of habitat-species groups used for comparison. Tasmania has four (0.40) and Cape York Peninsula has seven (0.70) of these categories. Within the Peninsula, Cooktown has the highest value (0.43).

For the index of type localities, the calculation may be based on the number of species that have the type localities within the country rather than the entire avifauna of Australia (RAOU 1926). With high endemism, there are 506 species of birds with type localities in Australia, of which 36 have been described from Tasmania and 22 from Cape York Peninsula. However, if the proportion of the regional fauna that has the type locality within the region is used, Tasmania has 0.18 and Cape York Peninsula has 0.06 of the regional fauna with type localities within the region. Within the Peninsula, this was mostly at Cape York itself.

In this example, we may conclude that Cape York Peninsula has a generally higher biodiversity value of fauna than Tasmania and that, other things being equal, the priority of reforestation for biodiversity conservation among the three sites of Cape York Peninsula should be given to the rainforest remnants of Cape York. In reality, priority setting of large areas for establishing reserve systems is dictated by socioeconomic incentives and may require active adaptive management (Possingham et al. 2001a).

25.4 How Can We Enhance Biodiversity in Reforestation?

At the landscape level, conservation goals extend to areas of both production and protection, and require the systematic planning of reserves (Margules and Pressey 2000). Existing reserves are usually located in remote areas and selected for features that are unsuitable for commercial development at the time of designation. Parks, on

the other hand, contain spectacular sceneries or other features attractive to recreation. Biodiversity conservation has not been the primary consideration for developing a reserve system in the past. A new systematic approach to locating and designing reserves requires a framework, such as the comprehensive one proposed by Margules and Pressey (2000), and practical decision-making procedures, such as the mathematical methods proposed by Possingham et al. (2000), for some minimum representation of the biodiversity features. New reserve systems should incorporate information from different indicator taxa, such that sites would be selected to complement one another with respect to the abundance of these taxa (Faith and Walker 1996).

Although the causes of biodiversity are not fully understood, the evolutionary history of a fauna is reflected in the biogeographic regions and subregions. We also know enough about the environmental correlates of biodiversity in rainforests to be able to suggest measures that can be taken to enhance biodiversity in reforestation (Asian Development Bank 1995). The maintenance of high biodiversity is encouraged nowadays in quality timber plantations, as it not only causes the plantations to approach natural forests in appearance but it also contributes to ecosystem health and enhances environmental sustainability (Weinland and Zuhaidi 1997). Even for intensive silviculture, there is room for coexistence and biodiversity conservation (Kimball and Hunter 1990; Moore and Allen 1999).

For the recovery of threatened species, we need to build a program based on the life history and environmental requirements of the species concerned (Brook and Kikkawa 1998). Unfortunately, the ecology of most threatened species is not well known and they are usually difficult to study in situ. For example, of the 1069 threatened species of mammals in the world, 335 (31.3%) occur in the Oriental region, being by far the richest in threatened species of mammals, but they have been barely studied (Amori and Gippoliti 2000). Reliable population viability analysis (PVA, Possingham et al. 2001b) data simply do not exist. Thus, we cannot depend on the knowledge of threatened species for enhancing biodiversity in reforestation. Here, I will examine some important environmental correlates of faunal diversity and suggest areas of research to enhance biodiversity in reforestation.

25.4.1 Physical Characteristics

The size of the area is an important parameter of biodiversity. Even in the same habitat, larger areas can accommodate more rare species and species with large territories. Although larger areas mean more species, consideration must be given to the minimum size required for the sustainable population of a threatened species. The study of minimum area size requirements of threatened species lead to the effective size for biodiversity conservation. For a first approximation of community size, estimating z (log-scale slope of a regression) in the species-area (S-A) curve ($S = CA^z$) can give us a measure of effectiveness of the size of an area for biodiversity conservation. If fragmented, tropical forests may lose their original ovian species diversity very quickly (Ferraz et al. 2003).

As well as historical factors, the present-day pattern of local biodiversity is influenced by topography, parent rock, soil type, substrate, drainage, and availability of water. Generally speaking, the greater the heterogeneity of the environment the more complex the habitat, and hence the more species, but the scale of heterogeneity is important and should be studied in relation to the taxa targeted for conservation.

25.4.2 Plant Species Diversity

There is a general correspondence between the numbers of plant and animal species in forest environments. For example, the numbers of tree and bird species in the rainforests of Australia and the islands of the Pacific vary in parallel. This could be because both are responding similarly to the same gradients of the environment or because they are limited similarly by the power of dispersal (Whiffin and Kikkawa 1992; Kikkawa and Green 1994).

However, detailed studies show that there are often causal relationships between floral and faunal diversity. It is usually assumed that more plant species make the plant community more complex in both structure and nutritional relationships, hence producing greater heterogeneity for animal habitats and accommodating more animal species. The dynamics of such relationships are best illustrated by the interactions of plants with their pollen carriers, seed dispersers, and other specialized dependants, such as in canopy arthropods (Kitching and Zalucki 1996), social bees (Nagamitsu and Inoue 1997), and fruit-eating vertebrates (Willson et al. 1987; Green 1993; Balasubramanian et al. 1998) in the Asia-Pacific region.

In the past, forestry practices selected exotic species for plantations into which pests and diseases of indigenous trees did not spread. Nowadays, species of *Pinus*, *Eucalyptus*, *Acacia*, and *Tectona* outside their ranges in the tropics have been well established in the absence of pest insects, but they support little local wildlife. Establishing mixed stands and planting food trees for wildlife will generally encourage wildlife to enter plantations. However, we need to understand plant-animal interactions that support regional biodiversity without damage to the forest, if we are to enhance regional biodiversity in plantations of indigenous species.

Biodiversity conservation is an economic problem requiring political decision making. In this sense, it is important to recognize the information content of what is being preserved (Crozier 1997). The opportunity to redeem tropical forest biodiversity exists in small plantations, which target forestry on farms with mixed endemic hardwood species being planted in North Queensland. Unfortunately, socioeconomic limitations are currently preventing this practice (see chap. 26 by NE Stork in this book).

25.4.3 Forest Architecture

It is well established that the structural complexity of forests produced by plants of different ages and life forms promote diversity of wildlife. Reduced Impact Logging (RIL) is one measure to reduce the impact of logging further from selective logging

by minimizing the disturbances to the forest in the logging operation. For example, its effect on biodiversity has been measured in a pilot study on dung beetles (Davis 1999). On the other hand, the past practice of removing the undergrowth in plantations and natural-production forests has contributed significantly to the reduction of regional biodiversity. In the natural forest of Okinawa, the continuing practice of understorey clearing has caused the reduction of insect fauna and the chances for survival of endangered wildlife (Azuma et al. 1997; Itô et al. 2000). Conversely, plantations as wildlife habitats could be improved by increasing the structural diversity of plantations. Even the limited understorey regeneration of local flora in teak plantations or the retention of a sparse overstorey for coffee plantations can support some insectivorous birds in plantations (personal observations).

Ecosystem management concepts may be applied to plantations for improving wildlife habitats (Gepp 1985; Kimball and Hunter 1990; Keenan et al. 1999). The various methods recommended by Spellerberg and Sawyer (1997) for improving wildlife habitats of plantations are applicable to the management of rainforest plantations for the protection of wildlife. These include:

1. Creating complex age structures of plantation trees, including early and late stages of tree growth.
2. Keeping stands of old-planted trees and dying and dead wood
3. Providing some open space.
4. Establishing buffer zones.

All of these require research before application, especially to determine the size and complexity of proposed measures. The effects of such management measures on the abundance of pest organisms and introduced species should also be studied.

25.4.4 Life-History Characteristics of Wildlife

While it is important to study individual species of wildlife, and threatened species in particular, priorities in rainforest biodiversity conservation in reforestation may be given to those that share the life-history characteristics typical of tropical rainforest animals. Recent studies of birds and mammals in tropical rainforests indicate that they maintain very small populations in complex rainforests of humid tropical lowlands (Kikkawa and Dwyer 1992). Compared to the related species in other habitats, these species are characterized by small litter (clutch) sizes, delayed maturity, long life span, unusual breeding systems, small feeding niches, specialized habitat requirements, and limited dispersal and migration. Such life-history characteristics are related to the degree of specialization that they have undergone to survive in the complex rainforest, where resources are scattered and scarce so that young animals must learn the skills to survive and breed (Kikkawa and Dwyer 1992). These species may have evolved in stable and highly predictable environments and are more vulnerable to extinction than others if changes to the environment or the population occur under human impacts. If the reforestation site contains any species with such characteristics, the site should be regarded as important for biodiversity conserva-

tion in the tropics. The number of species sharing such characteristics may also be an indicator of the complexity of the rainforest habitat and its value in biodiversity conservation.

25.4.5 Forest Dynamics

At the landscape level, biodiversity conservation may be part of the new systematic planning (see above), but may also be involved in the restoration of tropical rainforests after clear felling (Lamb 1990), severe cyclones (Webb 1958), or El Niño-induced forest fire (Beaman et al. 1985) over an extensive area. Within production forests, landscapes are increasingly degraded by the fragmentation of old growth or intact forests into isolated patches. Building biodiversity into ecologically sustainable forest management depends on what remains available in fragmented landscapes and ex situ wildlife stocks. Critical thresholds of biodiversity beyond which there is little chance of recovery may be identified by fragmentation indicators developed from spatial and species attributes (McAlpine and Loyn 2000). Thus, species assemblages must be assessed in the light of landscape structure (Stork et al. 1997) and wildlife corridors between patches of fragmented rainforest (Tucker 2000).

Natural regeneration is usually assisted by seed-dispersing animals. In the Nilgiri Biosphere Reserve in India, the seeds of 52% of 115 woody species recorded in a study area were dispersed by birds. Important genera among them were *Ficus*, *Celtis*, *Syzygium*, *Santalum*, *Premna*, *Grewia*, *Ixora*, and *Vitex* in dry mixed deciduous forests, and in many cases seed germination was enhanced by birds (Balasubramanian et al. 1998). In North Queensland rainforests, 105 species of plants belonging to 48 families have been identified in the droppings of the southern cassowary (*Casuarius casuarius*), suggesting the importance of the role of cassowaries in seed dispersal (Crome 1976; Stocker and Irvine 1983). In fact, seedlings are grown from seeds collected from cassowary droppings for the purpose of rehabilitating parts of the wet tropics World Heritage Area. Natural regeneration following clear felling at Gogol-Naru, Papua New Guinea, comprised certain tree species that were a subset of the original flora adapted to local edaphic conditions (Lamb 1990). Similarly, the avifauna of regrowth areas was largely derived from bird populations in unlogged forest. However, many of the species abundant in intact forests were seldom detected in the regrowth area and some additional species of seed eaters and nectar feeders of shrub layers appeared that were normally associated with edge conditions (Driscoll and Kikkawa 1989). Assessment methods of logged forests for biodiversity have been developed in Malaysia, where they are categorized according to the degree of biodiversity loss, which is weighted for species characteristics in terms of scientific, ecosystem, and economic value (Lian et al. 1998).

At a local level, natural tree falls create openings in the canopy and encourage growth of understorey vegetation, and such events occur sufficiently frequently to permit the maintenance of local tree species diversity through compensatory processes (Connell et al. 1984). Each disturbance event leaves a mosaic of conditions responded to by the local populations of plants and animals (Oliver et al. 1998). Thus, ecological succession never reaches a stable "climax" stage in a tropical

rainforest; rather the forest following the pioneer and seral stages will become a mature forest in which a vegetation mosaic is in a dynamic state. If the size of the gap is large, it may attract invasive weed species. As disturbances to extensive forests affect large ground animals and frugivores first (Driscoll and Kikkawa 1989), it is difficult to have colonization of these species in regrowth or reforestation areas once the forest is fragmented, though the role of these species is important in regeneration. Large frugivores, which are crucial in the seed dispersal of trees in mature rainforests, could require a large area to survive in seasonal (wet and dry) climates and prolonged or repeated drought conditions. In West Malaysia, the great majority of bird species are considered to be dependent on the continued presence of undisturbed forests (Medway and Wells 1971). At least for those animals that share the life-history characteristics typical of mature forest species, persistence of undisturbed forests within the range of their dispersal is essential.

25.5 Conclusions

In conclusion, the surviving tropical forest at the onset of the 21st century is a powerhouse of biological diversity, and we have the responsibility to make it sustainable. At the landscape level, we should maintain defined minimum areas of natural forest wherever feasible, and vigorously pursue reforestation in other areas. We should aim at soil, water, and biodiversity conservation, and multiple and sustainable use of forests. It is important that the local people accept the principles of planting native rather than exotic species, and mixed species rather than monoculture, as well as providing structural diversity to the plantation profile. In Southeast Asia and Papua New Guinea, experimental work on reforestation using indigenous tree species has already begun (Sakurai 1997; Tropical Forest Reforestation Technology Research Association 1997). In Australia, the Rainforest Cooperative Research Centre is conducting research into biodiversity values in reforestation in order to assess the patterns, causes, and consequences of biotic changes during reforestation, and to devise restoration methods within multiple-use landscapes (Catterall 2000). The data accumulating at the Long-Term Ecological Research (LTER) sites of intact forests will permit testing of the parameters to be used in sustainability models. Comprehensive baseline data are also expected from the International Biodiversity Observation Year (IBOY), which started in 2001 (DIWPA: DIVERSITAS in Western Pacific and Asia, IUBS). Conservation priorities of biodiversity hot spots have already been identified on a global basis (Myers et al. 2000). It is hoped that future reforestation will be able to incorporate regional biodiversity conservation into its program.

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26
Biodiversity Conservation and Sustainable Management of Forests: Socioeconomic Problems with Farm-Forestry of Rainforest Timber Production in North Queensland

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26.1 Introduction

Although it is now six years since the signing of the Convention on Biological Diversity and Agreement on Forest Principles, there seem to have been few improvements in the sustainable management of biodiversity in forests. In reality, most forest managers have little understanding of biodiversity issues and how they can improve the maintenance of biodiversity. Recognizing this problem, eight priority forest research needs (Box 1) were identified by the Conference of the Parties on Biological Diversity (CBD) at its third meeting in Buenos Aires, in November 1996, based on recommendations from the Subsidiary Body on Scientific, Technical, and Technological Advice (SBSTTA). The first part of this paper discusses some of the advances made towards answering some of these questions, through the use of a number of case studies. The relationship between biodiversity and sustainability in managed forest systems is very poorly understood. Some have looked at how species composition and abundance, or assemblage structure, changes with forest disturbance and use, but often these studies have been based on single taxa. Others are looking at rapid biodiversity assessment techniques for sampling indicator groups. In practice, these studies are often designed with a view to identifying a number of key groups that might act as surrogates for others. A third approach is taken by those who investigate the relationship between biodiversity and forest ecosystem processes. In this paper, we argue that this third approach may hold the greatest utility for those

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Box 1. Research and technological priorities from the 1996 meeting of the Conference of the Parties of the Convention on Biological Diversity

- Building the scientific foundations and methodologies necessary to advance the elaboration and implementation of criteria and indicators for forest quality and biodiversity conservation as part of sustainable forest management
- Analyzing the role of biodiversity in forest ecosystem functioning
- Analyzing measures for mitigating the underlying causes of biodiversity loss
- Advancing scientific and technical approaches to (i) rehabilitating degraded and deforested ecosystems and (ii) enriching biodiversity in forest plantations
- Identifying gaps in knowledge in the areas of fragmentation and population viability to include mitigation options such as corridors and buffer zones
- Assessing ecological landscape models, the integration of protected areas in the ecosystem approach to sustainable forest management, and the representativeness and adequacy of protected areas networks
- Analyzing scientifically the ways in which human activities, in particular forest management practices, influence biodiversity, and assessing ways to minimize or mitigate negative influences
- Developing assessment and evaluation methodologies for the multiple benefits derived from forest biodiversity

concerned with assessing the sustainable management and use of forests, particularly in developing countries where resources for such exercises are extremely limited. In particular, a new approach to assessing sustainable management of forests, criteria and indicators, shows considerable promise and is discussed here.

The second part of this paper discusses some of the socioeconomic issues that are limiting the uptake and growth of farm forestry. In Australia, there has been recognition that forestry practices need to pay greater attention to biodiversity issues, particularly in those areas that are most biologically sensitive, such as the tropical rainforest region of North Queensland. Over the last five years, there has been a major attempt to develop a farm-forestry program in tropical North Queensland based on endemic rainforest tree species — rather than exotic softwoods — that enhance the biodiversity values of plantation forests, while at the same time providing a new revenue source to farmers through timber production. Although many farmers have provided land for such farm forestry and have been satisfied with this investment, the take up of the program has not been as widespread as it could be. Here, we discuss some of the socioeconomic problems that are preventing the development of this industry. We consider that these problems are not unique to farm forestry in North Queensland, but are likely to be similar in other parts of the world where farm forestry has been far less successful or non-existent.

26.2 Biodiversity and Sustainable Management of Forests

26.2.1 Cameroon: Biodiversity and Forest Disturbance

Most studies of the relationship between biodiversity and rainforest disturbance have focused on a few taxa, usually birds or other vertebrates. However, most organisms in forests are invertebrates, rather than vertebrates, and the roles of invertebrates in ecosystems are critical. Lawton et al. (1998) have provided for the first time a broad ranging investigation of the effects of forest disturbance on vertebrates and invertebrates in rainforests in Cameroon. Here, a series of silvicultural treatments and land uses on a scale from intensively managed to unmanaged natural forests were used to examine how the abundance, diversity and biomass of a large number of animal taxa are affected. The implications from this study and the use of indicator groups are discussed below.

Over the last couple of decades, efforts have been made to increase timber productivity from plantations of endemic tropical hardwood trees. The provenances of trees have improved considerably through selection and cloning (Leakey and Newton 1994). In one such large-scale experiment, a partnership between the UK government, through the Overseas Development Administration, the Institute of Terrestrial Ecology, and the Government of Cameroon Office National de Développement des Forêts (ONADEF) established a set of demonstration plots in the Mbalmayo Forest Reserve to the south of Yaoundé, the capital of Cameroon. Several sets of silvicultural plots were established in 1987, 1988, and 1991, thus providing replicate sets of plots with different levels of intensification in a time sequence. Initial studies focused on the relative productivity of these treatments and on the likelihood of insect and other pest problems (Watt et al. 1997a, b). A subsequent broader and separate focus was aimed at how biodiversity (and ecosystem function) is affected by disturbance (Lawton et al. 1998). The study area, the Mbalmayo forest reserve (11°25' 11°31'E, 3°23' 3°31'N), is approximately 50 km south of Yaoundé, at an altitude of approximately 650 m. The six sites selected for the study represent a disturbance gradient from least to most disturbed. The sites were: near primary forest, old-growth secondary forest, partial manual clearance, partial mechanical clearance, complete clearance, and manually cleared farm fallow. A range of taxa were selected for study. Termites are one of the most abundant but most cryptic invertebrate groups in a rainforest; they are usually of low species richness but high biomass. As decomposers, they play a critical role in nutrient cycling in soils. Terrestrial nematodes are abundant, poorly known taxonomically, and are found in almost all terrestrial niches. Beetles are also abundant and species-rich in tropical rainforests, are trophically diverse, and occupy a wide range of habitats. Birds and butterflies are very well known, and as 'flag-ship' taxa are often used as indicators for other groups in environmental studies. Ants are extremely abundant, have high biomass, and play critical roles in the forest ecosystem. Key data for these taxa are shown in Table 1.

Table 1. Key data for the taxa studied in the biodiversity survey of disturbed and undisturbed forest in south Cameroon (from Lawton et al. 1998)

Group surveyed	Total species* recorded in study plots during survey	Index of species turnover (β–2)	Geometric mean body length (range) (cm)	Scientist hours to sample, sort and identify	Morphospecies that cannot be assigned to known species (%)
Birds (Aves)	78 (H)	18.3	26.5 (9–78)	50	0
Butterflies (Lepidoptera)	132 (I)	22.7	4.7 (2.0–11.1)	150	1
Flying beetles (Coleoptera)	358 (malaise)(L) 467 (interception)	45.0 22.1	0.37 (0.06–2.26)	600 (both methods combined)	50–70
Canopy beetles (Coleoptera)	342 (L)	39.4	0.45 (0.05–4.0)	1,000	>80
Canopy ants (Hymenoptera: Formicidae)	96 (I)	30.4	0.32 (0.1–1.0)	160	40
Leaf-litter ants (Hymenoptera: Formicidae)	111 (I)	6.8	0.32 (0.1–1.0)	160	40
Termites (Isoptera)	114 (H)	28.8	0.38 (0.2–0.75)	2,000	30
Soil nematodes (Nematoda)	374 (I)	21.0	0.089 (0.02–0.4)	6,000	>90

* H (high, >90%), I (intermediate 50%–90%), and L (low, <50%) are estimates of the degree of completeness in the species inventory for each group.

The Cameroon study provides a useful indication of some of the problems in understanding the changes in biodiversity across a wide scale of forest use. Correlations in the change of species richness across plots on the disturbance gradient were made between groups. Of these 45 correlations, only five were positive. On average, only about 10% of the variation in the species richness of one group is predicted by the change in another group. It is clear, therefore, that no single group of organisms is representative of another and, as such, cannot be used as a surrogate in assessments elsewhere. In addition, the rate of species turnover between sites is very different among the groups, with beetles having the highest rate of turnover and leaf litter ants the lowest. Finally, the data on the number of hours it took to sort the species, and the fact that for most groups a very high proportion of the morphospecies could not be identified to named species, indicate that a taxon-based approach — even with a package of different groups, such as that used in the Cameroon study — provides an inadequate approach to the rapid assessment of sustainable management of biodiversity in forests.

26.2.2 Biodiversity in the Understorey of Plantations in North Queensland

Forest plantations are being established at an unprecedented rate throughout the tropics (Cohn 1995), in some cases on degraded areas and previously forested grasslands or on areas previously occupied by natural forests. Traditionally, plantation management has focused on wood production, although many planting programs have also recognized potential benefits in reducing soil erosion, provision of habitat for some wildlife species, and maintaining water-catchment values. However, plantations established on degraded sites or fire-maintained grasslands may provide a broader conservation benefit by 'catalyzing' secondary successional processes and providing suitable conditions for the development of native species that are not adapted to exposed microclimatic conditions, degraded soils or competition from grasses. Studies of this phenomenon in Puerto Rico (Lugo 1988, 1992; Parrotta 1993, 1995; Lugo et al. 1993) and China (Brown and Lugo 1994) suggest that recolonization is dependent on the degree of site degradation, proximity to native forest seed sources, characteristics of the planted species, and the age and management intensity of the plantation (Parrotta 1995). However, our understanding of many of the processes affecting recolonization is limited.

Recent work in North Queensland investigated understorey recruitment at four sites in plantations of a range of species and ages (Keenan et al. 1997). Plantation species included the natives *Araucaria cunninghamii* D. Don (6–63 years old), *Flindersia brayleyana* F. Muell. (50–64 years old), and *Toona ciliata* (F. Muell.) Harms (55 years old), and the exotic *Pinus caribaea* Morelet var. *hondurensis* Barrett and Golfari (5–31 years old).

In total, 350 vascular plant species were found in the plantations. Trees species dominated recruitment (176 species) in both young and older plantations but representatives of most life forms were present in most plantations. Epiphytes were absent in *P. caribaea* and in the younger *A. cunninghamii* plantations. Older plantations had appreciably more understorey species overall than younger ones and some of this recruitment had become part of the plantation canopy. There were 14 exotic species, largely herbs and grasses. The proportion of recolonizing tree species in plantations compared with the number found in surveys of rainforest surrounding the plantations ranged from 34% at Kuranda (all *P. caribaea*) to 51% at Danbulla (*P. caribaea*, *A. cunninghamii*, and *F. brayleyana*).

The diversity of understorey recruitment was higher in plantations of the native *A. cunninghamii* and *F. brayleyana* than the exotic *P. caribaea*, and there was a greater diversity of tree species found under the broadleaved species, *F. brayleyana* and *T. ciliata*, than *A. cunninghamii*. On the other hand, the rate of increase in species density and seedling density with plantation age was greater in *P. caribaea* than in *A. cunninghamii*.

Species numbers and seedling densities were slightly lower than those reported for plantations of a similar age in Puerto Rico (Lugo 1992; Parrotta 1993, 1995). This is possibly due to the higher intensity of management in Queensland planta-

tions than those in Puerto Rico, which were largely in hand-tended field trials, had higher species richness in surrounding forests, or had different plantation usage by dispersal agents.

Most recolonizing tree species were primarily dispersed by birds, with wind dispersal of secondary importance, and mammals were only responsible for the dispersal of a small proportion of the species. The proportion of bird-dispersed species was higher in *A. cunninghamii* (80%) than in *P. caribaea* (70%). No primarily mammal-dispersed species were found in the young *P. caribaea* at Kuranda, but a small proportion were found in older stands.

Early pioneer species tended to dominate in the younger plantations, presumably reflecting the larger seed rain of these species. However, species more representative of later successional stages were also present from an early age, perhaps because the dispersal distances were not large and because of the microenvironments provided by the young plantations. Wind-dispersed species were more prominent in younger plantations when the nearby intact forest had a height advantage, but the proportion of these tended to decline as the plantations aged and the height advantage disappeared.

Given that older plantations of a range of species do contain a considerable component of biological diversity, there appear to be four possible alternatives for their management.

1. Maintain the original plantation management objectives, and treat the new understorey diversity as a transitory community to be destroyed at the time of thinnings or clear felling.
2. Change from an even-aged plantation management system to a polycyclic silvicultural system that yields timber and maintains a high degree of stand diversity. This would be achieved by harvesting the forest on a selection basis, using both the plantation trees and new tree species that have regenerated beneath the plantation and that in some cases are now present in the forest canopy.
3. Carefully thin the plantation at an intensity sufficient to generate enough revenue to repay the cost of establishing the plantation; thereafter the forest could be managed for its biodiversity values.
4. Change the land-use objective from production to biodiversity protection, abandon the plantation and manage the new plant community as a conservation stand.

26.2.3 Biodiversity Criteria and Indicators

There has been recognition by many national bodies and international agencies that new means of assessing the management status of forests are urgently required. A major development in this regard has been made by researchers from the Center for International Forest Research and by the Montreal Process on criteria and indicators (C&I).

The conceptual framework for C&I is a relatively straightforward hierarchical system (Box 2). The principle or law that is generally used is the working definition for the project. For example, the principle might be that the forests are sustainably

Box 2. Principles, criteria, indicators, and verifiers (from Stork et al. 1997)

Criteria and Indicators form part of a hierarchy of assessment tools. The four levels of this hierarchy are Principles, Criteria, Indicators, and Verifiers. Each level in the hierarchy is defined as follows.

Principle: *A fundamental truth or law as the basis of reasoning or action.* In the context of sustainable forest management, principles are seen as providing the primary framework for managing forests in a sustainable fashion. They provide the justification for criteria, indicators, and verifiers. Consider that principles embody human **wisdom**, where wisdom is defined as: *a small increment in knowledge created by a person's (group's) deductive ability after attaining a sufficient level of understanding of a knowledge area.* Wisdom, therefore, depends on knowledge.

Example: "Ecosystem Integrity is maintained or enhanced" or "Human well-being is assured"

Criterion: *A standard that a thing is judged by.* A criterion can, therefore, be seen as a 'second order' principle, one that adds meaning and operationability to a principle without itself being a direct measure of performance. Criteria are the intermediate points to which the information provided by indicators can be integrated, and where an interpretable assessment crystallizes. Principles form the final point of integration. In addition, criteria should be treated as reflections of **knowledge**. Knowledge is the accumulation of related information over a long period of time. It can be viewed as a large-scale selective combination or union of related pieces of information.

Example: "Processes that maintain biodiversity are maintained"

Indicator: *An indicator is any variable or component of the forest ecosystem or the relevant management systems used to infer attributes of the sustainability of the resource and its utilization.* Indicators should convey a 'single meaningful message'. This 'single message' is termed **information**. It represents an aggregate of one or more data elements with certain established relationships.

Example: "Landscape pattern is maintained"

Verifier: *Data or information that enhances the specificity or the ease of assessment of an indicator.* At the fourth level of specificity, verifiers provide specific details that would indicate or reflect a desired condition of an indicator. They add meaning, precision, and usually also site-specificity to an indicator. They may define the limits of a hypothetical zone from which recovery can still safely take place (**performance threshold/target**). On the other hand, they may also be defined as procedures needed to determine satisfaction of the conditions postulated in the indicator concerned (**means of verification**).

Example: "Areal extent of each vegetation type in the intervention area relative to area of the vegetation type in the FMU"

managed. A criterion is defined as a 'principle or standard that a thing is judged by.' A criterion is therefore a second order principle, and identifies the critical components that make sustainability operational. Criteria are therefore more practical than principles in the context of C&I. Indicators are the variables or components of the forest ecosystem or the relevant management systems used to infer attributes of the sustainability of the resources and their utilization. Verifiers have the broad definition as the data or information that enhances the specificity or the ease of assessment of an indicator (definitions after Prahbu et al. 1996). In practice, it is the indicators and verifiers that give the C&I process a real cutting edge towards assessing sustainability. Examples of biodiversity indicators and verifiers (from Stork et al. 1997) are shown in Box 3.

Previous attempts to provide means of assessing the sustainability of forest management have usually either been too academic and hence impractical, or have been too costly and difficult to implement. Prabhu et al. (1996) examined more than 1100 socioeconomic and ecological C&I in a pre-fieldwork phase. After field trials in Cote D'Ivoire, Brazil, Germany, Austria, and Indonesia, they reduced this number to around 200, as many were found to be impractical in the field. One of the major deficiencies in the set of C&I they had worked with were those of relevance to the assessment of sustainability of biodiversity in managed forests. This problem was addressed through workshops looking specifically at genetic C&I (Namkoong et al. 1996) and biodiversity C&I (Stork et al. 1997). Field trials have since been carried out on both genetic and biodiversity C&I, and revisions are likely in the near future.

One of the practical considerations in designing C&I for biodiversity in forests around the world is the fact that the resources that are likely to be available for such assessments in most countries are extremely limited. There is, therefore, no point in designing an assessment package that requires a team of four or five world-class specialists on plants, insects, vertebrates, or forest ecology to carry out the assessment. Rather, the package should be relatively straightforward to use by a team of one or two unskilled laborers with local knowledge of the forest in question under the supervision and training of someone with more technical and scientific training.

The team designing the biodiversity C&I (Stork et al. 1997) took the fundamental view that if the processes that create and maintain biodiversity are functioning well then we can use assessments of the health of these processes to provide an indication of the health or sustainability of biodiversity in the forest in question. From this viewpoint, a series of process-based indicators and verifiers have been identified and are being tested in the field in terms of their utility and practicality (Box 3). This differs from other more skill and labor-intensive approaches based on rapid biodiversity assessment techniques, where a variety of indicator taxa are assessed, and these are used as surrogates for biodiversity as a whole. As discussed above, Lawton et al. (1998) found that many of the taxa often used as indicators for others are poor surrogates. The biodiversity package of C&I was field tested in Kalimantan in late 1997 and the results of this will be published by the Centre for International Forest Research.

In summary, the C&I initiative will provide an exciting package of tools to rapidly assess sustainable management, in its broadest sense, of forests across the world.

Box 3. Examples of indicators and verifiers (from Stork et al. 1997)

Indicator	Verifiers
Landscape pattern is maintained	V1.1.1: Real extent of each vegetation type
	V 1.2.1: Number of patches per unit area
	V 1.2.2: Largest patch size of each vegetation type
	V 1.2.3: Area weighted patch size
	V 1.2.4: Contagion
	V 1.2.5: Dominance
	V 1.2.6: Fractal dimension
	V 1.3.1: Average distance between two patches of the same cover type
	V 1.3.2: Percolation index
	V1.4.1: Total amount of edge for each vegetation type
	V1.4.2: Edge round largest patch
Changes in habitat diversity within critical limits	V 2.1.1: Vertical structure
	V 2.1.2: Size class distributions
	V 2.1.3: Relative abundance of leaf sizes
	V 2.1.4: Gap frequency/forest regeneration phase
	V 2.1.5: Canopy openness
	V 2.2.1: Standing and fallen dead wood.
	V 2.2.2: Other structural elements
Community guild structures do not show significant changes	V 3.1.1: Relative abundances of tree species in different guilds
	V 3.1.2: The abundances of avian guilds
	V 3.2.1: Abundance of nests of social bees
The richness/diversity show no significant changes	V 4.1.1 Species richness reported by local people
	V 4.1.2: Number of different bird calls
	V 4.1.3: Numbers of large butterfly species
	V 4.1.4: Number of species in local markets
	V 4.1.5: Number of leaf types in litter
Population sizes/structure do not show significant changes	V 5.1.1: Measures of the population size of selected species
	V 5.2.1: Age or size structure
Decomposition and nutrient cycling show no significant change	V 6.1.1: Diameter and height/length of all standing and lying dead wood
	V 6.1.2: State of decay of all dead wood
	V 6.1.3: Abundance of small debris
	V 6.1.4: Depth of litter/gradient of decomposers
	V 6.1.5: Abundance of important decomposers
	V 6.1.6: Leaf bags
	V 6.2.1: Frequency of N-fixing plants
	V 6.2.2: Soil conductivity and pH
	V 6.2.3: Soil nutrient levels
No significant change in water quality/quantity from the catchment	V 7.1.1: Abundance/diversity of aquatic organisms
	V 7.1.2: Chemical composition of stream water organisms
	V 7.1.3: Leaf bags
	V 7.2.1: Stream flow

26.3 Socioeconomic Limitations to the Development of Farm-Forestry in North Queensland

26.3.1 Community Rainforest Reforestation Program

The Wet Tropics World Heritage Area (WTWHA) covers almost 900,000 ha of tropical rainforest in North Queensland. As a result of world heritage listing, timber production ceased within the nominated area. The timber industry has subsequently struggled to continue, with current supplies of rainforest cabinet timbers being sourced almost exclusively from freehold rainforest areas that were not included as part of the listing. In order to redevelop this industry, the government has supported a Community Rainforest Reforestation Program by heavily subsidizing the establishment of plantations on private land through the use of unemployed persons and by the provision of free trees and extension services. This program has largely targeted forestry on farms with mixed endemic hardwood species, rather than exotics, being planted. In this way, the scheme has helped to reforest areas with native species of farms that were formerly rainforest. Although this scheme has generally been hailed as a success, the uptake of the opportunity by most farmers has been poor. It would seem at face value that the lack of uptake is caused by the hesitancy of farmers to invest resources (land, time, and money) into a venture that might take up to 40 years to produce real economic returns. However, a series of detailed studies (Broome 1993; Eono and Harrison 1996; Eono 1997a, b; Harrison et al. 1997a; Qureshi et al. 1997) indicate that this a simplistic view and that in practice the situation is much more complex. These and other studies indicate that there are potentially a large number of factors limiting the growth of farm-forestry in the region (Box 4). Some of the more critical factors are discussed here.

26.3.2 Timber Supply

Within Australia, the debate prior to the declaration of the WTWHA became extremely fierce, and as part of the package to fund the World Heritage Area, compensation was provided for those sawmills that ceased operations, or for retooling others to accept smaller diameter plantation timber. There is still much concern among the environmental movement that there is no protection from timber harvesting for the few areas of forest outside the WTWHA. Therefore, those sawmills that have survived are constrained by reduced timber supplies and the controversy that might be caused if they promote timber harvesting too visibly. This latter problem has meant that many of the traditional users of their products, the cabinet makers in Queensland, have experienced difficulty in obtaining regular supplies of rainforest timbers or information on the availability of timber (see, for example Fig. 1). This appears to be a major factor affecting the demand for these timbers, especially in North Queensland where there has traditionally been a high rate of utilization (see

Box 4. Some of the socioeconomic problems limiting the development of farm-forestry in North Queensland

- lack of certainty on economic returns to farmers
- high cost of establishing and maintaining forestry plots
- lack of economies of scale in small area plantings
- lack of information on how and when to thin and prune
- poor return to farmers from cut timber — large differential between value of timber before going to saw mill and after being dried, milled, and marketed
- low demand for good quality timber due to poor marketing and information flow to cabinet makers and intermittent supplies of particular species
- improved tax incentives required and removal of existing impediments
- concern that future conservation orders may restrict the use of plantations (harvest rights)
- losses incurred during drying and storage— improved timber handling methods required
- historical acceptance of low prices in the timber industry in order to promote social goals
- possible rent capture by timber mills and timber merchants at the expense of landholders
- lack of demand of products to be made from rainforest timbers
- uncertainty of timber supplies (lack of resource security) leading to a lack of investment in industry infrastructure and obsolete milling equipment
- input substitution from other products
- trends towards high-volume, low-cost production using exotic pine and composite timber, particularly in furniture and kitchen production
- possible lack of product recognition, i.e., cabinet makers do not recognize, highly regard, or extensively use native cabinet timbers
- price competitiveness of cabinet timber imports resulting in a price ceiling
- continued timber removal from private land as a result of land-clearing operations, resulting in reduced supplies in the future
- reduced community demand for products made from native timbers because of
  - concerns about the perceived damage that logging operations cause to native forests
  - a belief that these timbers are no longer available because of World Heritage listing, leading to a reduction in demand
- lack of direct nexus between reforestation and increased land values, particularly in the early years of plantations

Herbohn et al. 1997a; Herbohn et al. 2001). In short, in the absence of an explicit demand for the use of these timber from customers, cabinet makers will prefer other timber inputs that are more easily available. Similar patterns also exist in the Brisbane market, but other factors, such as poor price competitiveness with substitutes, appear to be more important (Herbohn et al. 2001). Despite the recent difficulties experienced by cabinet makers in obtaining rainforest cabinet timbers, these timbers are still highly regarded for use in furniture and kitchens. Indeed, in recent surveys of cabinet makers (Herbohn et al. 1997a; Herbohn et al. 2001), rainforest cabinet timbers were consistently ranked the highest of all available timber products in Townsville, Cairns, and Brisbane (for example see Table 2). There is also evidence

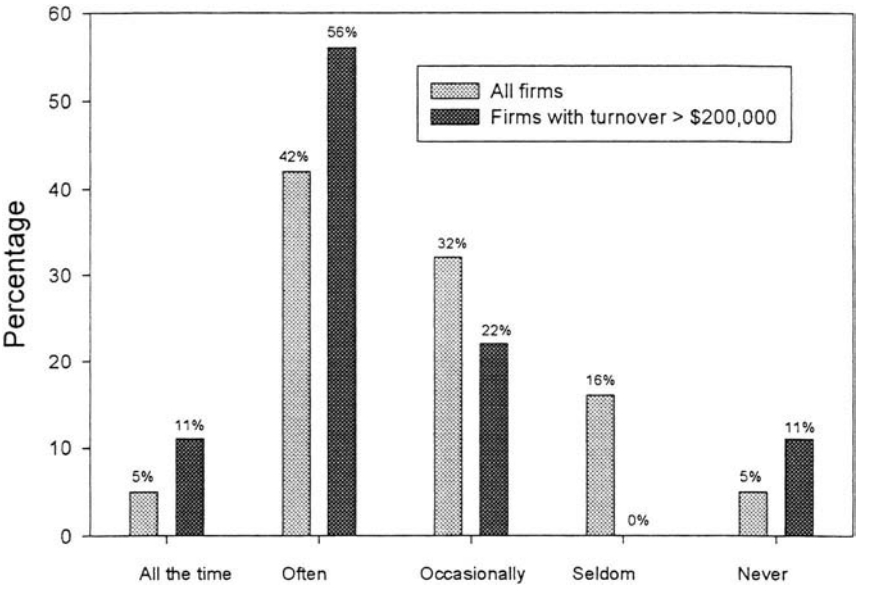

Fig. 1. Difficulties experienced by Townsville cabinet makers in obtaining rainforest cabinet timbers (n = 19). Reproduced from Herbohn et al. (1997a)

Table 2. The degree of influence that cost, quality, suitability, and customer requests have on managers choices of wood products for use in the manufacture of indoor furniture (n = 24). Ratings are on a scale of 1 (very little influence) to 5 (very strong influence). Reproduced from Herbohn et al. (1997a)

	Low cost	Quality (colour, grain)	Suitability for indoor furniture	Requested by customer	Aggre-gaterating
Australian rainforest timbers	2.4	4.4	4.2	4.5	15.5
Australian hardwoods	2.9	3.9	3.1	4.0	13.9
Australian softwoods	3.0	3.7	3.5	4.3	14.5
Imported tropical timbers	2.7	3.8	3.6	3.7	13.8
Imported softwoods	3.3	3.5	2.9	3.5	13.2
Other timbers	3.2	3.8	3.6	3.5	14.1
Composite products	3.2	2.6	3.1	3.4	12.2

that many consumers believe that rainforest cabinet timbers are no longer available, or at least are uncertain about availability (Smorfitt et al. 2001). Endemic hardwood timbers are losing out to imported timber and to non-timber products, and in the short term a marketing campaign is clearly needed to promote their use and availability to consumers. However, some caution needs to be expressed about such a strategy. It may well be an easy task to convince consumers that rainforest cabinet timbers are a high quality material, superior in many respects to other materials. It may also be possible to improve the availability of supply of these timbers, although

this will be less easy. The real difficulty will be to persuade consumers that it is worth paying a premium for this quality, when substitute materials are available at a lower price. This task will be made even more difficult because these substitute products have already gained market acceptance. Suppliers of these substitute materials are unlikely to give up market share without resistance. In the longer term, the problems associated with the lack of ready availability will be overcome as the industry grows in size.

26.3.3 Predicting Timber Values for Rainforest Species

One major impediment has been the apparent unwillingness of many farmers to invest in farm-forestry rather than cattle, sugar cane, fruit, or other crops where the turnover and profit are more visible. This problem has been addressed by the development of a simple EXCEL based spreadsheet to predict returns from small-scale plantations designed for use by farm-forestry extension officers and farmers themselves (Harrison and Herbohn 1997; Herbohn et al. 1999, 2001) (Fig. 2). This spreadsheet allows the farmer to estimate yield from a mixture of up to five species of tree in a given area. For example, a farmer can choose up to five of 32 species of endemic rainforest trees to plant, and can vary the proportion of land given to each species. The program provides the farmer with an estimated return for the timber over the 20–40-year period. Integrated into the program are data on the estimated growth rates of the 32 tree species and their current and predicted future stumping values. More work is needed to improve the accuracy of the growth rate and pricing data and, hence, the accuracy of the revenue estimates, but this system should provide the much-needed support for farmers in decision making. Technical reports advising farmers on when it is best to thin and prune trees are currently in preparation (D. Doley, D. Lamb and D. Yates unpublished), and these reports again should enhance the industry.

26.3.4 Land Values and Financial Returns to Landowners

As many farmers and other private landowners in North Queensland invest in plantations of mixed rainforest trees, it has been difficult for land agents to estimate the added value of this reforestation. Valuers have been loath to place significant values on reforestation without authoritative information on which to base these values. There is evidence that reforestation increases land value, but the full cost of tree planting and maintenance is not fully factored into the land value (Harrison et al. 1997b).

Probably one of the greatest impediments to tree planting is the current low returns obtained by landholders for timber harvested from freehold rainforest areas. Typically, landholders have received between A$30 and A$50/m^{-3} for standing timber, whereas woodpack values for the same species from timber merchants are from A$2,000–3000. Before any prescriptions can be made on how to improve these returns to landowners, we need to gain an understanding of the cost levels and price

markups along the timber-production pipeline. In addition, to increase the returns to landowners it is important to determine the efficiency and cost effectiveness of fixed sites versus portable sawmills, particularly as the timber industry is small and dispersed. Portable mills appear to offer a cost-effective alternative means of milling high-value timber species.

26.3.5 Non-Market Benefits of Forestry

Recently, Costanza et al. (1997) estimated that the various environmental services (such as climate regulation, nutrient cycling, water supply, and soil formation) provided by the world's ecosystems (oceans, tropical and temperate forests, freshwater, etc.) are worth at least US$33 trillion a year. To put this value into perspective, the world's annual Gross National Product is US$18 trillion. These non-market benefits of such ecosystems are, therefore, not insignificant. Using the figures from Costanza et al. as a guide, Driml (1997) estimated the non-market benefits of the WTWHA as A$2.4m, with the major contributing ecosystem services as follows: nutrient cycling (46%), raw materials (16%), erosion control (12%), climate regulation (11%), and recreation (6%). This first analysis is extremely crude and assumes that the value for these services is the same as the values across the world. Eono (1997a) has looked more precisely at the non-market benefits from timber plantings by the Community Rainforest Reforestation Program (CRRP) and has estimated carbon sequestration values. The economic benefits from tree planting along rivers and streams, in terms of improved water quality and, hence, reduced damage to estuaries and the marine environment, may be extremely high. For example, Zorzetto (1994) estimated a saving in water treatment costs for the Johnstone River alone of $63,000 from revegetation of 4200 ha of streambanks (20 m width).

In that reforestation generates social benefits in excess of the private benefits to landholders (e.g., protection of water quality, landscape amenity, carbon sequestration), there is a case for government intervention to support tree planting. This suggests a need for reliable information about the extent of non-wood benefits. An understanding of the non-timber benefits of tropical forestry is also important for fulfilling the reporting requirements of agencies such as the CRRP (Herbohn et al. 1997a).

It is notable that experience elsewhere (e.g., New Zealand) suggests that tree planting does not really take off until landholders perceive it as a profitable investment. This suggests the need to remove impediments to profitability, such as restrictions on log exports, artificially low stumpage prices, uncertainties in harvest rights and taxation arrangements, and perhaps give more favorable local authority rates treatment on reforested land.

The Queensland Plantation Joint Venture Scheme (PJVS) is similar to schemes in other states, and is designed to increase the rate of planting trees on private land. The Department of Primary Industries Forestry shares the costs of tree establishment and maintenance and the timber revenue with landholders, each partner having at least 20% equity. The PJVS overcomes shortages capital and labor by landhold-

ers, shares the risk taking, and brings in the marketing ability of the forest service (Harrison et al. 1998). The minimum area requirement of 10 ha overcomes the lack of economies of scale in planting and marketing. While joint venture plantations involve mostly native species (particularly eucalypts), each block is typically a single species planting. The biodiversity benefits of such schemes are clearly less than those from mixed plantings of endemic rainforest species.

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