In situ and *Ex situ* Conservation of Commercial Tropical Trees

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Foreword

Forests create vertically stratified habitats upon which virtually all other forest-dwelling organisms – microorganisms, smaller plants, insects, reptiles, birds, and mammals -depend. Because forests exert such a major influence upon all that lives above, amidst, and beneath them, and on local and regional climates and soils as well, the loss of biological diversity in the species of trees composing various forest ecosystems is especially significant in terms of its potential for triggering subsequent changes in biodiversity within and among associated organisms. In the tropics, more so than elsewhere, subtle changes in dominant forest cover may lead to major changes in plant species composition – and thus, food chain dynamics – that may in turn initiate much greater and more obvious losses in local and regional biological diversity.

Due to their value for a great variety of wood products, many species of tropical forest trees have been severely overexploited over the past two centuries, and especially during the latter half of the 20th Century. To that add the continuing practices of shifting cultivation, the wholesale landscape-level clearing of forests for agriculture and urbanization, and the catastrophic effects of fire and erosion often attendant to those practices, and it becomes obvious that, in tropical forest ecosystems, the key to maintaining biological diversity is to maintain the diversity of the dominant organisms - that is, the trees themselves.

Tropical forests typify, to most people, the ultimate source of biological diversity on this planet. Indeed, of the more than 50,000 species of trees, worldwide, greater than 70% of them are tropical endemics. Moreover, the typical species mixtures of tropical forests are remarkably diverse – often, more than 250 tree species may be found growing on a single hectare in a young tropical rainforest. Conversely, in the more-or-less pristine, late-seral or old-growth tropical forests composed of lesser numbers of very large trees, it is not unusual to find only one or two individuals of the same species over an area of ten hectares. And, of course, it is in those very areas where most of the harvesting of trees occurs in the tropics.

If there is inadequate advance reproduction and/or reproduction is impeded by fire, erosion, grazing, or cropping, it is highly likely that a sought after species might be eliminated from that local or regional flora. Even in the case of the widely practiced selection harvesting, where only one or a few species are removed, the sparse spatial distribution of these contributes to local elimination. In fact, this selective practice often leads to so-called “high-grading” - i.e., the removal of the largest and best-formed individuals from the canopy - and this represents dysgenic or negative genetic selection as well as species removal. At this point, there are many forest tree species in SE Asian forests known to be rare and endangered (sandalwood, ebony, and several dipterocarps to name but a few) and probably as many others in that category as yet unknown.

It can be argued that, ultimately, the sustainability of biological diversity depends upon the maintenance of diverse and healthy gene pools of all of the many organisms constituting the particular ecosystem under consideration. And as discussed earlier, dominant forest tree species are, literally, often the "keystone species" for maintaining the "biodiversity" of tropical forest ecosystems. Without adequate **genetic** diversity within all of the species comprising that ecosystem, sustaining its **biological** diversity over both time and space is questionable.

On a more pragmatic level, geneticists and plant breeders, and evolutionary biologists as well, are interested in conserving genetic resources for many obvious reasons. The sustainability of species and species groups in nature has already been mentioned. Equally important and obvious are the needs to preserve wild-type gene pools for purposes of selection and breeding for domestication and/or other purposes such as natural resistance to pests, pathogens, and environmental pollutants. The several forms of *ex situ* conservation – i.e., genetic conservation **off-site** - practiced by geneticists and breeders certainly play a major role here. These include botanical gardens, arboreta, seed orchards and banks, clonal banks, common gardens or provenance tests and, more recently, DNA libraries. However, all but the most pragmatic of breeders will readily acknowledge that, in the final analysis, these *ex situ* techniques best serve as backup or emergency measures or as vehicles for research convenience, and that to successfully conserve wild gene pools of tree species, *in situ* conservation areas are also needed.

In situ or **on-site** genetic conservation depends basically upon establishing protected reserves of a species or groups of species in natural locations appropriate to ranges and patterns of distribution. In theory, *in situ* is the preferable long-term genetic conservation solution for most species and especially those that are rare and endangered. This is because by dedicating the sites containing the populations to be conserved, one is also preserving, in effect, the set of **ecosystems** in which the selected species populations are growing. This then allows for the continuation of genotype x environment interactions, adaptations, and evolution of the conserved populations. Thus, the *in situ* option provides for a long-term dynamic situation wherein the populations continue to evolve in nature, as opposed to the static, one-dimensional or artificial environments afforded by all of the various *ex situ* conservation options.

To the evolutionary biologist, geneticist, or breeder *in situ* gene conservation areas of adequate size and distribution provide the needed elements of intra- and interspecific competition and natural selection to drive the evolutionary process. At the same time, these areas represent to the ecologist and conservation biologist sources of biological diversity both for study and demonstration, and also for assistance with designing and implementing reintroductions and ecological restoration projects of various sorts. On the other hand, *ex situ* conservation often serves to facilitate breeding and regeneration for genetic improvement and reforestation programs. It is generally agreed that the two systems are not mutually exclusive, and in fact may serve to complement each other in many ways.

This volume represents the Proceedings of the "International Conference on ***Ex situ*** and ***In situ*** Conservation of Commercial Tropical Trees" held 11-13 June 2001 on the campus of Gadjah Mada University (GMU) in Yogyakarta, Indonesia. This conference was co-sponsored by the International Tropical Timber Organization (ITTO), the Indonesian Ministry of Forestry, PT. INHUTANI I-V, PERUM PERHUTANI, and the Faculty of Forestry, GMU. The conference was planned and organized as an authorized activity of the ITTO-funded Project PD 16/96 Rev.4 (F), "*Ex situ* Conservation of *Shorea leprosula* and *Lophopetalum multinervium* and Their Use For Future Breeding and Biotechnology".

More than 110 persons from ten nations convened to share information and to learn about conserving genetic resources of tropical trees throughout the tropics, with a particular focus on forests of Southeast Asia. Papers and posters covered a wide variety of topics and species, and representatives from several ASEAN nations reviewed the status of genetic conservation in their countries. There is obviously an appreciation of the importance of such programs in the region as well as a significant level of current activities aimed not only at conserving rare and endangered indigenous species, but also at supporting the selection and breeding programs necessary for sustainable commercial plantation forestry.

During the conference, a new concept of genetic conservation was discussed, wherein the forest is not only considered as a biophysical ecosystem but also as a "soft system" which considers and accommodates interactions among all levels of society within a defined forest landscape. That concept greatly expands the community of institutions, persons, and activities that might influence or directly affect genetic conservation efforts, and especially those aimed at highly valued indigenous species. This concept highlights the importance of public education in science and natural resources as well as the opportunity to involve local communities, farmers, and smaller companies in conserving tropical tree genetic resources as well as producing plant materials to complement and extend the activities of forest industries and agencies.

One aim of the conference was to increase regional and pan-tropical cooperation in research and implementation of tropical forest trees. With continuing concern, interest, and support from government, industry, and the international forestry community, the work presented herein could form the nucleus of a center for the conservation of tropical tree genetic resources. That center could serve to coordinate and expand efforts to learn more about tropical species' biology, to develop strategies and systems for conservation that also consider social and economic issues and needs, and to significantly improve the sustainability of both natural and planted forests in the tropics.

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September 2001

Report of the International Conference on *ex situ* and *in situ* Conservation of Commercial Tropical Trees Yogyakarta, June 11 – 13, 2001

The International Conference on *ex situ* and *in situ* Conservation of Commercial Tropical Trees, held in Yogyakarta on 11-13 June 2001, was attended by 112 participants from 10 countries. Representatives from international organizations included: ITTO, JICA, World Bank, DANIDA, Forest Research Institute of Malaysia (FRIM), Gadjah Mada University (UGM), Bogor Agricultural University (IPB), University of Bengkulu (UNIB), University of Papua, University of Philippines Los Banos (UPLB), University Putra Malaysia (UPM), Oregon State University (OSU), The Australian National University (ANU), Ministry of Forestry of Indonesia, Thailand Royal Forest Department, USDA-Forest Service, Forest Companies, (Perum Perhutani, PTs INHUTANI I-V, Sari Bumi Kusuma, Sumalindo Lestari Jaya, Musi Hutan Persada, Menara Hutan Buana) The Indonesian Institute of Sciences (LIPI), the Indonesian Biodiversity Foundation (Yayasan Kehati) and the Indonesian Forest Concessions Association (APHI).

The conferees presented and discussed relevant aspects of *ex situ* and *in situ* conservation of genetic resources, with special emphasis on commercial tropical trees. The conference agenda included five keynote addresses, eight invited papers, and twenty three contributed papers. Six posters were displayed and four voluntary papers were submitted. The discussions focused on the importance of genetic conservation of commercial tropical trees, and on the relationships of such conservation to selection and breeding and biotechnology programs.

The Conference produced an awareness that a new vision for conservation is needed to understand the forest not only as an ecosystem, but also as a “soft system” wherein there are interactions between all levels of society and the forest landscape. The objective of selection and breeding programs is to produce high quality planting stock for forest rehabilitation and afforestation, and it was deemed important to involve communities and smallholder companies in producing and maintaining genetic materials for those purposes.

The conferees were also of the opinion that just establishment of high quality plantations in itself is insufficient - appropriate downstream industries that would process and add value to the timber and fiber produced are also needed. These secondary or downstream industries would produce high quality products that would bring increased returns from both domestic and international markets thereby benefiting not only the nation but also generating greater income and returns to communities. Besides maintaining genetic materials on local farms, some communities could grow trees on small landholdings to complement and extend the resources of forest industries.

Presenters noted that unlike "static" conservation methods such as seed or clonal banks, an evolutionary conservation approach would better facilitate continued natural selection in response to new or changing environments. The importance of continued basic genetic studies to support both development of appropriate selection and breeding techniques for commercial and non-commercial tree species and the creation of effective and sustainable conservation programs, was emphasized. It was recognized that *in situ* and *ex situ* conservation strategies for a species are complementary and not mutually exclusive entities, and that one or both may be necessary and appropriate depending upon circumstances dictating the extent and speed needed to conserve a particular species or population.

Significant efficiencies may be obtained by combining genetic improvement programs with appropriate conservation strategies to support them, where possible. Success in combining these activities depends greatly upon adequate short- and long-term planning based on sound genetic principles and knowledge of species biology and site requirements. In particular, gene frequencies and population size are critical for adequate sampling, while proper design and maintenance of conservation areas are needed to maximize genetic recombination and minimize contamination from outside sources. Maintaining separate breeding populations based on traits of interest is one effective way to maintain diversity and minimize loss of low-frequency alleles.

Breeding and supportive conservation are expensive programs, and cooperative approaches were encouraged to reduce costs and maximize efficiencies. Presentations of the results of such combined programs, for indigenous as well as for introduced species and from both the public and private sectors, illustrated both scale of threat to several species and a promising degree of success in alleviating those threats. Results also showed that properly-planned and conducted, combined programs of breeding and genetic conservation can have favorable cost benefit ratios and can serve to improve the public's perception of plantation forestry.

The conferees also recognized that ideal *ex situ* and *in situ* conservation programs carry significant cost implications. Considering the constraints of the various land tenure systems and the economic status of many of the producers, it would be a real challenge for them to carry out such programs without some level of support from national and local governments. And this situation is particularly true of those forest species and systems that currently are not utilized by forest industries but have great values for local communities and the nation-at-large, nonetheless.

The conferees also recommended that most of the genetic resources available within a particular region or province should be conserved not only *in situ* but also *ex situ* in gardens at appropriate locations. This to a great extent will ensure the sustenance of these valuable resources in protected areas. An example of such a garden established in Puspiptek Serpong near Jakarta, Indonesia, was highlighted.

Plantation forestry programs supporting *in situ* and *ex situ* genetic conservation should differentiate and learn from the successes and failures of general plantation programs. Plantation forestry should be encouraged only on degraded forest and agricultural lands and, where possible, appropriate agroforestry systems should be introduced to reduce shifting cultivation activities. To encourage private sector investment, including smallholders, it is necessary to make available attractive incentives and loans at reasonable interest rates.

The conferees agreed that plantation establishment will significantly reduce the pressure on natural forests and that this in itself will contribute to preserving valuable genetic resources. To ensure viable plantations, there is a need for a continuous supply of quality planting stock produced through conventional tree improvement programs and currently available biotechnological tools. Studies to estimate the effects of management on carbon sequestration in the soils of degraded forests and agricultural lands should be enhanced to elucidate carbon fixation and emission levels to further contribute to our scientific understanding and conservation efforts.

Conferees were also made aware that farmers showed great interest in tree planting and, while they have indigenous knowledge, they often lack of technical skills and tools. Increasing their awareness of the importance of using high quality planting materials and providing access to those materials and technical assistance was deemed essential for capacity building in rural communities. Such a program would enhance the success of genetic conservation efforts, in general and especially for species of higher value.

The potentials of the newly emerging biotechnological tools that could used to enhance genetic conservation and breeding programs were also highlighted. Various molecular techniques for enhancing breeding, and for understanding the phylogeny and ecology of various populations and species were highlighted and discussed.

The conferees noted that these are powerful techniques that can be useful to conservation strategies. With these techniques genetic diversity, population structure, gene flow, mating systems can be elucidated fairly accurately. They will greatly improve the efficiency of design and sampling. Research on such technologies needs to be further enhanced throughout the region.

Summary

- Both *in-situ* and *ex-situ* conservation activities are of prime importance and need to be enhanced. An evolutionary conservation approach is recommended
- Strategies for plantation development should be put in place to alleviate the pressure on natural forests.
- Development of appropriate downstream industries to give added value to the timber produced is needed.
- Where possible, local communities and small landholders should be involved in conservation activities
- There is a need to establish, as protected areas, regional or provincial gene conservation gardens to help conserve indigenous genetic resources.

Yogyakarta, June 13, 2001

In situ Conservation

In situ Forest Conservation: a Broader Vision for the 21st Century

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Abstract. A broader vision for *in situ* forest conservation began to emerge in the late 20th century. This broader vision recognises that, while traditional protected areas are the cornerstone of *in situ* conservation strategies, they are seldom - if ever - adequate to achieve conservation goals in isolation from the complementary management of other forests. Such a vision suggests that we need to develop integrated forest conservation strategies, which incorporate forests of all tenures and management regimes.

The broader vision for forest conservation envisages conservation goals being attained by cumulative contributions from many sources – a continuum of contributions from across the forest landscape. Protected areas can provide some of this contribution, but other important and sometimes irreplaceable contributions will come from forests conserved on private lands, from other forests such as community and sacred forests, and from forests managed under sustainable forest management regimes. Such an approach broadens the constituency for *in situ* forest conservation to include, for example, private forest owners, local authorities, indigenous and local communities, and commercial forest managers and workers – all of whom can contribute to conservation goals in complementary, mutually supportive ways.

The best geographic basis for conservation planning and implementation is a “bioregion”, which is defined by ecosystem rather than cadastral boundaries. A bioregional approach to conservation planning is consistent with the ecosystem approach endorsed by the Conference of Parties to the Convention on Biological Diversity. Conservation planning tools have been developed to facilitate this regional planning process, and maximise the achievement of forest conservation goals at minimum opportunity cost. These tools are widely applicable, and adaptable to different levels of data and technology.

The broader vision for *in situ* forest conservation recognises that achieving and sustaining forest conservation also requires the integration of social and economic goals into conservation planning processes. It therefore recognises the development of more collaborative participatory modes of conservation planning and management as essential to achieving and sustaining forest conservation goals. New forms of partnership between many of the actors with interests in forests, which recognise the diversity of their roles and contributions, are especially important in delivering conservation outcomes. A related policy challenge is enabling locally appropriate responses to emerge in a world in which both commerce and regulation are increasingly globalised.

Background - the world's forests

The world's forests cover more than a quarter of the land surface of the globe. They are very diverse ecosystems, occurring from sea level to the alpine tree line and from the equator to the Arctic Circle. Their diversity of form, structure and composition reflects the combined influences of their physical environment, of ecology and evolution, and of people throughout human history. The diversity of forest ecosystems is accentuated by their dynamic state, reflecting both the expression of ecosystem processes and responses to natural and human-induced change and disturbances.

(a) The values of forests

Forests have many values: some are intrinsic, and others are conferred by human use and preference. Forest values have environmental, material and cultural dimensions. Forests serve ecosystem functions at scales from the global to the local, and are the most biologically diverse terrestrial ecosystems. Forest products and services have a high economic value, only part of which is captured by the market. Forests have profound cultural values, both material and spiritual. Forest values are now often summarised in the terms identified in Box 1.

Box 1. Forest values

Forest values can be expressed and summarised in many ways. International processes to define criteria and indicators of sustainable forest management are based around a common set of values (Wijewardana et al 1997):

- biological diversity
- forest health and vitality
- productive functions - including wood & non-wood products
- protective functions - including ecosystem services such as watershed values and biogeochemical cycles
- social benefits - including the aesthetic, cultural, and spiritual values of forests, and
- economic benefits

Societies, and groups and individuals within societies, value and use forests in different ways. Throughout history, societies have developed conservation and management regimes, which reflected the values they accorded forests. At one extreme, this value might be in the conversion of forest to recover minerals or to realise the agricultural potential of the soil; at the other, it might be in the reservation of forest from human exploitation to protect its intrinsic values.

Between these extremes lie a myriad of forest management regimes which variously integrate conservation and production – for example, the land use practices of forest dwelling and forest dependent peoples, traditional and modern agroforestry systems, or production forestry. Each represents and delivers a different mix of forest values.

(b) Forests under pressure

The world's forests are subject to many pressures. As human population increases, so do the demands on forests of both poor and affluent people. As societies change, so do their needs and uses of forests. As technologies advance so does our capacity to impact on forests, both adversely and favourably. As economies and trade globalise, so these forces influence land allocation and forest management on an unprecedented scale. Under these pressures, forest loss is occurring on a scale and at a rate unparalleled in human history; the remaining forests are becoming more fragmented in the landscape, and their integrity and quality frequently diminished; many forests are being exploited for a variety of products at a rate greater than they can sustain; global and local environmental changes challenge the resilience of forest ecosystems and of ecosystem processes (FAO 1997).

Many forest values are threatened as a consequence. The scale and immediacy of these threats have focused the world community's attention on the imperatives of forest conservation and sustainable forest management to protect forest – for example, through the Intergovernmental Panel and Forum on Forests, or the Convention on Biological Diversity.

(c) Contemporary responses to forest loss and degradation

The world community and individual nations have responded to the pressures on forests in a variety of ways. The responses share a common emphasis on forest conservation and sustainable forest management. The terms "forest conservation" and "sustainable forest management" are sometimes used interchangeably, and sometimes in ways that imply that one is subsumed by the other[1] . This paper reflects contemporary usage of the terms and considers forest conservation and sustainable forest management to be complementary,

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[1] for example: Chapter 11 of Agenda 21, Programme area B: "Enhancing the protection, sustainable management and conservation of all forests …";
the CBD S10a;

interdependent and inseparable themes. The concept of forest conservation describes the goal of maintaining the extent of forests and the full range of forest values, implying their sustainable management. The concept of sustainable forest management – that which respects the environmental, economic and social dimensions of forest values, and which is integrated with forest conservation - describes the common framework which has emerged for maintaining forest values in those forests managed for production as well as for conservation.

All forest management regimes can maintain some forest values - both those at which management practices are directed, and those maintained by association rather than intention. For example, many traditional agro-ecosystems are effective in conserving key elements of biodiversity as well as in producing food (Halladay & Gilmour 1995); forests managed for wood production can also protect catchment values, amongst others. The maintenance of various suites of forest values is frequently mutually supportive and interdependent: in the two preceding examples, traditional agro-ecosystems may only sustain food production because they conserve biodiversity, and the protection of catchment values allows sustained wood production.

Consequently, different strategies are possible to maintain forest values. Each approach delivers different conservation and development benefits, confers different costs, and has its own strengths and limitations in achieving forest conservation and development goals. The international community has recognised that different nations will adopt different strategies in working towards the shared goals of forest conservation and sustainable forest management[2]. Strategies may include a variety of mechanisms including the establishment of protected areas, management agreements such as conservation covenants with forest owners, and forest management regulations such as codes of forest practice.

The Australian National Forest Policy Statement states the goal of forest conservation as: "The goals are to maintain an extensive and permanent native forest estate and to manage that estate in an ecologically sustainable manner so as to conserve the full suite of values that forests can provide for current and future generations…";
The first Australian Approximation Report for the Montreal Process (1997) defines sustainable forest management as: "integrating the commercial and non-commercial values of forests so that the welfare of society (both material and non-material) is improved, whilst ensuring that the values of forests, both as a resource for commercial use and for conservation, are not lost of degraded for future generations."
The Santagio Declaration of Montreal Process refers to the "development of criteria and indicators for conservation and sustainable management of temperate and boreal forests …"

[2] for example, as in the signing of the International Tropical Timber Agreement, at the G8 Meeting in Birmingham 1998.

The variety of mechanisms allows different forms and degrees of protection to be afforded to different forests. The conservation of forests therefore can be pursued to varying degrees through the establishment of areas protected from exploitation and those in which both conservation and development goals are pursued jointly, through the development of community- and landowner-based conservation programmes, and through sustainable forest management regimes in forests managed for industrial production.

Protected areas

The deliberate reservation of specific areas of forest from particular uses, to protect the features for which they are valued, is an ancient and widespread human practice (Colchester 1994, Gomez-Pompa & Kaus 1992). Historical examples of protected forests include the sacred groves of Asia and Africa and the royal forests of Europe. In some cases protection was imposed by a ruling elite; in others it was conferred by more general agreement; in all cases various sanctions were applied to those who failed to respect the protection regime.

Since the world's first National Park, Yellowstone in the USA, was declared in 1872, the concept of 'protected areas' has emerged as a key element of national and international strategies to protect forest values. Protected areas now make a fundamental contribution to the conservation of the world's natural and cultural resources; the values they protect include landscape features, representative ecosystems, biological diversity, environmental services, and cultural heritage. The purpose of protected areas has also evolved from a focus on the protection of landscape features to an emphasis on the protection of biological diversity.

The internationally agreed definition of a protected area is:
An area of land and/or sea especially dedicated to the protection and maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means (IUCN 1994).

The World Conservation Union (IUCN) recognises six categories of protected area, summarised in Box 2. These categories represent different degrees of emphasis on conservation and development, and acknowledge the complementarity of different approaches to protecting forest values. All categories are intended to be permanent designations that provide long-term protection to biodiversity and other values. IUCN's World Commission on Protected Areas does not consider large-scale industrial activities as compatible with any category of protected area (Phillips 1998).

Box 2. IUCN Protected Area Categories
IUCN Category & Description

I (a) *Strict Nature Reserve*
I (b) *Wilderness Area*
Protected areas managed for science or wilderness protection.

II *National Park*
Protected areas managed mainly for ecosystem conservation and recreation.

III *Natural Monument/Natural Landmark*
Protected areas managed mainly for conservation of specific natural features.

IV *Habitat/Species Management Area*
Protected areas managed mainly for landscape/seascape conservation and recreation.

V *Protected Landscape/Seascape*
Protected areas managed mainly for landscape/seascape conservation and recreation.

VI *Managed Resource Protected Area*
Areas managed mainly for the sustainable use of natural ecosystems

Source: IUCN 1994, in WWF/IUCN 1998

There are currently some 30,000 protected areas in 150 nations worldwide, with a total area of around 13.2 million km^2. Terrestrial protected areas occupy around 9 per cent of the world's land surface (IUCN WCPA 1997) The size of individual protected areas varies enormously, from less than one hectare to hundreds of thousands of square kilometres. Protected areas incorporate a diversity of terrestrial and marine ecosystems and cultural sites, exist on a range of tenures, and function within a great diversity of social contexts - from some of the least-developed and –populated parts of the world to densely-populated cultural landscapes. About half of the protected areas world wide, and up to 80 per cent in some regions such as Latin America, have significant human populations; in India alone, for example, it is estimated that about 4 million people live within protected areas (Borrini-Feyerabend 1996).

Protected areas are managed through a variety of institutional arrangements - by govenments, non-government organizations, private enterprise, community groups, indigenous peoples, individual landowners and in various forms of partnership between these actors. The management of protected areas is largely focused on conserving areas for their own intrinsic values. Increasingly, management is attempting to achieve a mix of social, economic and cultural, as well as environmental, objectives.

Some protected areas are of principally local significance; others have international status such as UNESCO's Biosphere Reserves and World Heritage Areas.

Forests outside protected areas

Conservation in forests outside dedicated protected areas is often described as "off-reserve conservation" (eg Binning 1998, Hale & Lamb 1997). Off-reserve conservation provides a mechanism for contributing to the conservation of those values that cannot be fully protected in conservation reserves. Off-reserve forest conservation can make a significant contribution to regional biodiversity conservation, provided appropriate management systems and processes are in place.

The emerging emphasis on such mechanisms (eg , Binning & Young 1997, Hale & Lamb 1997) reflects growing recognition that many forest values were not well conserved in existing protected areas, principally because of land tenure and land use patterns.

Existing protected areas and forest conservation

Although some estimates are lower (WWF/IUCN 1996), the World Conservation Monitoring Centre estimates that around eight per cent of the world's forests are designated as IUCN Category I-VI protected areas (WCMC 1998). Indicative locations, areas and summary statistics for forest protected areas are have been summarised by the UNEP World Conservation Monitoring Centre (2000). As their data illustrate, the coverage of existing protected areas varies considerably between forest ecosystems and regions.

The mere designation of protected forest areas, however, does not necessarily result in the achievement of forest conservation objectives. For a variety of reasons, many protected areas are not achieving the conservation objectives for which they were established.

The effectiveness of protected areas

The effectiveness of protected areas can be assessed in terms of protection of biodiversity, institutional capacity, social impacts and legal status (Dudley & Stolton 1998). Effectiveness of biodiversity protection can be broadly interpreted to cover the degree to which the design and management of the protected area meets goals for conservation of genetic diversity, species, populations and ecosystems, as well as ecosystem processes. Institutional capacity refers to the ability of the managing agency to effectively manage the area and is affected by skill levels, degree of control and resource availability. Social impacts relate to the cultural and social appropriateness of protected area systems, how protected areas are integrated into the broader social context of the region, and the level of support from and involvement of local people in the protected areas. Legal status refers to the degree of protection afforded the protected area through gazettal under appropriate legislation and implementation of that legal status on the ground.

Most assessments to date have focused on environmental effectiveness, and there has been limited analysis of social, institutional or legal situations. The WCPA Working Group on Management Effectiveness recently released a draft discussion paper suggesting a framework for evaluating effectiveness of protected area management (Hockings 1997). The paper identifies the need to evaluate the design of protected areas, the management and resourcing of inputs, the management demands, and management systems and processes. However, considerable work is still required in developing methodologies for such assessments. A number of organizations - such as IUCN, the World Commission on Protected Areas (WCPA) and WWF - and individual nations are currently developing systems for undertaking rapid assessment of management effectiveness of protected areas (Dudley and Stolton 1998).

The development of a consistent global approach to the assessment of protected area effectiveness has been proposed by numerous actors, including the WCPA, IUCN and the World Bank/WWF Forest Alliance. Initial steps towards such an approach have begun, with field testing of the WCPA Framework proposed for 1999 (Dudley and Stolton 1998).

Effectiveness in relation to biodiversity conservation goals

Unfortunately, existing protected areas have a number of significant deficiencies in relation to the achievement of forest biodiversity conservation goals. Common deficiencies relate to reserve network principles, and to the effectiveness of management in achieving conservation goals.

Reserve network principles

The biodiversity conservation principles, which apply to any conservation reserve network, are summarised in Box 3.

Box 3
Reserve network principles for biodiversity conservation

A reserve system for the conservation of biodiversity should follow the principles of:

* comprehensiveness: the network includes the full range of forest ecosystems across a landscape;
* adequacy: the network maintains the ecological viability and integrity of populations, species and communities, and acknowledges the issue of risk by replicating areas across the landscape;
* representativeness: the network reflects the biotic diversity within ecosystems by sampling different areas of the same ecosystem across the landscape;
* reserve design and connectivity: ideal reserve models suggest that protected areas should be large and contiguous, with spatial configurations that maximise the area to perimeter ratio, and with connectivity to other protected areas.

Sources: Commonwealth of Australia 1997, Frankel *et al.* 1995

Few existing protected area networks were established according to these principles. Some protected areas have been chosen for their outstanding natural beauty, or because they protect rare species or wilderness areas. Many, perhaps the majority, appear to have been chosen because the land was of little value for alternative uses or human habitation (Runte 1987, Kirkpatrick 1987, Pressey 1994). With few notable exceptions (MacKinnon & MacKinnon 1986), protected area selection has tended to be opportunistic or *ad hoc*. As a result, much of the biological diversity most in need of protection has not been protected (Pressey & Tully 1994). There is now a very biased representation of biological diversity in existing protected areas, favouring that associated with areas with least potential for extractive uses (Leader-Williams *et al.* 1990). Consequently, if inadvertently, limited conservation resources have been used inefficiently.

Similarly, the long-term conservation of many forest vertebrate species require areas in the order of hundreds of thousands of hectares (Frankel *et al* 1995). Because it is very difficult to achieve idealised protected area size and design in most situations, there has been considerable debate about the best alternatives (eg Frankel *et al* 1995, Hawkes *et al* 1997). It is clear that many existing protected areas are of inadequate area or inappropriate configuration to conserve forest biodiversity in the long term. For example, as a result of the

Regional Forest Assessment process recently undertaken in Australia, between 40 and 50 per cent of remaining forest in some regions has been included in protected areas. However, the conservation of many rare and threatened species continues to depend on the management of production forests or on private land outside the protected area system, exemplifying the need to have forest conservation strategies, which extend beyond protected areas if biodiversity conservation goals are to be achieved.

Protected areas cannot function as islands disconnected from their surrounding landscape. Their effectiveness in conserving biodiversity will be greatly diminished unless they are very large and contiguous, or they are functionally connected by intervening forests.

Effectiveness of management – "paper parks"

The term "paper parks" (eg Phillips 1997, World Bank/WWF Alliance 1998) describes those protected areas, which are legally established but not achieving the conservation objectives for which they were established. Many of the world's protected areas can be described in these terms; it is widely agreed that improving the management of existing protected areas would greatly assist the achievement of forest conservation goals. This task has been identified by the international community as a high priority for forest conservation (World Bank/WWF Alliance 1998).

Even protected area systems that fulfil the ecological criteria discussed above will still fail to deliver conservation objectives if their management is ineffective. The reasons for and manifestations of this lack of effectiveness are as many and varied as are paper parks themselves. Common themes include unsupportive policy and institutional structures, a lack of political and community commitment to protection, lack of capacity within the management agency, the alienation of communities from their traditional lands, and insufficient resource provision for effective management.

More generally, the effectiveness of protected areas in contributing to forest conservation objectives is constrained by those factors, which also constrain sustainable forest management: population growth, poverty, commercialism and market and institutional failures. The difficulties of managing protected areas are exacerbated where land tenure and access rights are poorly defined or disputed (Western & Wright 1994).

Targets - quantifying conservation goals

In recent years, the international community and individual nations have been engaged in discussions about the quantification of conservation goals. Debate around these issues of quantification has focused on the nomination of arbitrary targets, principally the proportion of forest ecosystems, which should be represented in protected areas. For example, WWF and IUCN (1996) are campaigning for a minimum of 10 per cent of all forest types to be represented in protected area networks. To date, some 20 countries[3] have committed to this goal and some, for example Australia, are committed to exceeding it[4] .

The specification of targets has both advantages and disadvantages. On the one hand, specification of targets identifies a clear goal against which achievement can be assessed; it is probably necessary if national and international communities are to agree on objectives and make progress towards them. On the other hand, any specific target for the area or proportion of forest to be protected is an essentially arbitrary choice, guided rather than defined by science, and usually reflecting political compromise. In addition, a focus on protected area targets may diminish the necessary focus on other, complementary, means of achieving forest conservation goals. Once arbitrary targets have been met the incentive to address values not adequately protected may be diminished.

Setting targets for conservation planning should therefore be seen in the same light as target setting in other areas of human endeavour. It is a means to an end rather than the end in itself. As knowledge of forest biological diversity and other forest values accumulates and as social, economic and environmental conditions change, conservation planning goals should be revisited to ensure they remain appropriate.

Conclusions – protected areas and forest conservation

It is clear that existing protected areas make important contributions to forest conservation, that they do protect many forest values, and that they represent very considerable effort and achievement on the part of all concerned in their establishment and management. It is also clear, however, that existing protected areas are not, in themselves, sufficient to achieve or sustain forest conservation goals. Many are in the wrong place, of inadequate size or inappropriate configuration, too disconnected from their surrounding environment, and inadequately protected from pressures that impact adversely on their conservation values. They seldom comprise more than 10 per cent of any forest

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[3] Argentina, Armenia, Australia, Austria, Bolivia, Canada, Chile, People's Republic of China, Colombia, Greece, Lithuania, Malawi, Mozambique, New Zealand, Nicaragua, Romania, Russian Republic of Sakhs, Slovak Republic, Tunisia, Uzbekistan.

[4] The Australian target is 15 per cent of pre-1750 forest ecosystems (Commonwealth of Australia 1997).

ecosystem, seldom protect forests on tenures other than public lands, and are often culturally inappropriate. They are subject to a range of social and economic pressures which may not be compatible with the protection of their conservation values, and which many cannot sustain.

As Paine (1997) recognises, the principal policy implication for biodiversity conservation is that effective strategies must be based on scientific criteria rather than political expediency. However, contemporary human pressures on forests are such that forest conservation has to be achieved in the context of competing demands for forest use; it cannot be based solely on the establishment of protected areas. As many analysts and interested parties have argued vigorously (Bridgewater *et al.* 1995, Kramer *et al.* 1997, IUCN WCPA 1997, McNeely in press, Expo 2000 1998), a broader vision for forest conservation is needed if forest conservation goals are to be achieved and sustained.

Broadening our vision of forest conservation

This broader vision for forest conservation has been evolving in many parts of the world. This builds on the achievements and experiences of the past, and on contemporary understanding of science and societies. It is one in which forest conservation strategies are more diverse but also more integrated, more broadly based and more inclusive, and consequently more effective in achieving forest conservation goals.

The broader vision for forest conservation seeks to conserve the full range of forest values. It recognises the conservation of biological diversity and the maintenance of ecosystem services as fundamental to the conservation of other forest values – for example, the sustained harvest of forest products, or the protective functions of forests. It also recognises the importance of conserving other forest values – for example, their productive wealth, their roles as water catchments and as carbon sinks, and their cultural and spiritual values. The broader vision for forest conservation recognises the ecological and economic interdependencies between forest values, and seeks to develop relationships that enhance rather than diminish the synergies between conservation and development.

This evolving vision for forest conservation recognises that protected areas are the cornerstone of conservation strategies[5]. It also recognises that protected areas are seldom, if ever, adequate to achieve conservation objectives in isolation from the complementary management of other forests, and that we need to make protected areas a component of integrated conservation systems. It recognises that simply setting targets for the number or extent of protected areas is insufficient to achieve conservation goals, and that effective management for conservation is critical to the success of forest conservation efforts. The broader vision for forest conservation recognises that achieving and sustaining forest conservation goals also requires the integration of social and economic goals into conservation planning processes. It therefore recognises the development of more participatory modes of conservation planning and management as essential to achieving and sustaining forest conservation goals.

The broader vision for forest conservation reflects an evolution in our thinking about how conservation should be achieved. It envisages conservation goals being attained by cumulative contributions from many sources – a continuum[6] of contributions from across the forest landscape. Protected areas could provide much of this contribution, but other important and sometimes irreplaceable contributions will come from forests on private lands, from forests managed under sustainable forest management regimes, and from other forests such as community and sacred forests. As a consequence, the constituency for forest conservation is broadened – to include, for example, private forest owners, local authorities, indigenous communities, forest workers and commercial forest managers – all of whom can contribute to conservation goals in complementary, mutually supportive ways.

Strategies to deliver the broader vision for forest conservation share a number of common features, recognising:

- the diversity of forest values and benefits, and the complex relationships between achievement of conservation and sustainable development goals
- that different situations will require different strategies to achieve conservation goals
- that protected areas have a key role in achieving conservation goals, but are not the only means of achieving forest conservation goals
- that forests outside protected areas also have a key role in contributing to the achievement of conservation goals
- the power of partnerships between interested parties to achieve and sustain conservation goals
- the need to develop integrated conservation strategies, informed by bioregional conservation planning processes which recognise and accommodate the issues identified in the preceding points, and
- the importance of policies and institutional arrangements that support the achievement of forest conservation goals.

The development and implementation of integrated forest conservation strategies, within the context if a supportive policy framework and with adequate

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[5] as stated, for example, in the ITTO Guidelines for Biodiversity Conservation in Tropical Production Forests

[6] also described as "spectrum" (Pressey & Logan 1997)

resources, are the key actions necessary for forest conservation. The remainder of this paper outlines the features common to the development and implementation of integrated forest conservation strategies.

Integrated strategies for forest genetic conservation: issues and elements

Forest genetic diversity is complex, heterogeneous and dynamic; it is shaped by interactions between the physical environment, the biology of forest systems and populations, and the influences of people and societies (Kanowski *et al.* 1997, Ledig 1992). Strategies for its conservation must recognise these forces and their interdependencies.

This context suggests some key issues and elements that these strategies should address if they are to achieve genetic conservation objectives:

- recognition and accommodation of the diversity of interests and rights;
- policy integration, coordination and innovation;
- institutional capacity and cooperation;
- the respective roles of *in* and *ex situ* conservation;
- the development of integrated conservation strategies;
- the respective roles of both protected areas and off-reserve forests.

Each of these is discussed below.

Recognition and accommodation of the diversity of interests and rights

Discussions around the IU on Plant Genetic Resources, between the Conference of the Parties to the CBD, and in the context of the WTA, have been severely hampered by the difficulties of defining broadly acceptable means of recognising and accommodating the diversity of interests in and rights to genetic diversity. The spectrum of immediate interests encompasses, amongst others, indigenous peoples and local communities, farmers and forest owners, governments and their agencies, enterprises seeking to commercialise or otherwise profit from forest genetic diversity; there are also, as the CBD recognises, the broader interests of the global community and of future generations. These issues have been reviewed in the context of forest genetic diversity by, amongst others, Ten Kate (1995) and Kanowski *et al.* (1997).

As with other arenas of public policy, establishing a policy framework which achieves this objective, and thus acts as an enduring foundation for more specific policy development, is particularly challenging - the negotiations which preceded and have followed the international agreements relevant to genetic diversity, or associated with corresponding national initiatives, amply demonstrate the difficulties of reconciling diverse and often competing interests. However, these processes can be instructive, and we now have access to a wealth of experience and body of literature specifically concerned with how we might address these challenges for forests: examples range from the international (eg Thomson 1996) and national (eg Carew-Reid *et al.* 1994) levels to those negotiated with and between local communities in recognition of their rights (*eg* Hughes 1998, Posey & Dutfield 1996, Reid *et al.* 1994, Western *et al.* 1994). Flexibility, diversity and negotiation are important elements of these approaches, and co-management and partnership are important common themes that emerge from them.

Policy integration, coordination and innovation

Competing or inconsistent policies frequently arise as a means of attempting to satisfy different interest groups, and emphasize the importance of developing the coherent and consistent policy framework discussed above. Notwithstanding nor diminishing its limitations, the CBD - as a generally-agreed legally-binding framework, with objectives broadly consistent with those declared by national governments for forests under their jurisdiction - offers one framework for the coordination of disparate policies relevant to the conservation of forest genetic diversity. The development of national biodiversity strategies consistent with the objectives of the CBD illustrates how it might play such a coordinating role. Other fora, such as those provided by other intergovernmental processes and by FAO, can also play an important complementary role to the CBD.

The separation of authority and responsibilities between government ministries and agencies concerned with management of forests for conservation and for production is a particular challenge to policy integration and coordination; experience with agencies integrating these functions suggests, unsurprisingly, that agency restructuring alone is not a solution, and generates its own set of challenges. Another common limitation of major consequence for achievement of conservation objectives is the vexed relationship between public policy and forests under private ownership or control. A range of incentives and partnerships have been employed to promote conservation objectives on private land; examples were described by Kanowski *et al.* 1999. However, the effectiveness of these measures varies widely (Binning & Young 1997, Tasmanian Public Land Use Commission 1997); indeed, some well intentioned but poorly-conceived policies have acted as perverse incentives for the conversion or unsustainable management of forests (*eg* Mayers & Bass 1999). National strategies that engaged rather than avoided the complexities of achieving conservation goals on private land would advance considerably the cause of the conservation of forest genetic diversity.

Engagement between public and private sectors, and with non-government and community organizations, is also an imperative because of the prevailing political ideology of the late twentieth century, in which the traditional role of the State is diminished. The relative decline of State authority over forests has been paralleled by an increasing role for the private sector and for the community at large. In many countries, "civil society" has asserted its interests in public forest management; those same interests, particularly in respect of environmental values, are slowly being extended across tenure boundaries to forest in private ownership. One of the principal manifestations of these expressions of interest in forest management is the development of consultative or collaborative processes through which stakeholders exercise influence over forest management decisions. Corporations are also finding themselves subject to pressure, internally from shareholder associations, and externally from community expectation, and many are responding with the establishment of environmental management systems and reporting mechanisms. All of these developments should assist the realisation of genetic conservation objectives.

There are already encouraging examples of initiatives within the private sector, and of partnerships between the private, government and non-governmental sectors, which illustrate the potential of private ownership and enterprise to contribute to genetic conservation objectives. Recent work exploring the role of the private sector in forestry generally (*eg* Bass & Hearne 1997), and in achieving conservation objectives in particular (*eg* Tasmanian Public Land Use Commission 1997), offer good foundations for developing successful partnerships between the public, private and community sectors. In recognition of these changes, the traditional emphasis in conservation-directed policies on regulatory frameworks and sharply defined institutional roles is likely to evolve to encompass more flexible and innovative institutional arrangements, and stronger cross-sectoral partnerships supported by incentive structures and market-mediated mechanisms.

Institutional capacity and cooperation

Issues of institutional capacity and cooperation also emerge from the discussion above. Institutional capacity has been limited by the low status historically accorded the environment and the conservation of forest genetic diversity relative to that of other priorities, such as agricultural expansion; this is reflected both within national governments and in their positions at intergovernmental fora. Consequently, much of the onus for strategic development, coordination and action for the conservation of forest genetic diversity has fallen to relatively informal multilateral mechanisms, such as FAO's Panel of Experts or IUFRO'

Working Parties, to those national institutions which have been able to assume international responsibilities, and more recently to non-governmental organizations and the few international or regional centres with a focus on forests. A major constraint is that few of these are politically influential, and well- or securely-resourced. It is less a lack of institutional structures or collaborative frameworks, than the adequacy of support for those which already exist, which most limits effective cooperative action - including that in research, education, and the exchange of information - to promote the conservation of forest genetic diversity.

Funding mechanisms emerging from new collaborative partnerships with the private sector (eg Nature 1998) offer one way forward; these include innovative use of the capital markets (*eg* Mansley 1996), as evidenced in debt-for-nature swaps (WWF/IUCN 1996), or in the emergence of carbon trading regimes (eg Moura-Costa and Stuart 1998). New or renewed multilateral mechanisms, such as the role being developed for the Global Environmental Facility in supporting implementation of the provisions of the CBD (IISD 1998), represent another important source of funds to support achievement of genetic conservation objectives.

The respective roles of in and ex situ conservation

The complexity of forest ecosystems, the dominant role of tree species in them, the environmental and economic value of forests and trees, and the poor conservation status of most tree populations *ex situ*, has led to the characterisation of forest trees as a paradigm of *in situ* conservation (Frankel *et al* 1995). Effective *in situ* conservation demands that both ecosystem function and process, and intra-specific population genetic processes, are maintained in a network of sites, which are comprehensive and representative in terms of all levels of genetic organization.

The *ex situ* conservation status of forest species is generally correlated with the extent of their domestication, and is therefore either poor or non-existent for most. Only a trivial proportion of forest species (*eg* around 100 tree species) are conserved adequately *ex situ*. These species are almost exclusively those whose genetic resources have been assembled for domestication programmes, with which almost all substantive *ex situ* forest conservation activities are associated (NRC 1991). For forest species, the value of *ex situ* seed storage is further limited by the relatively large number of species, many of economic importance, whose seed is not amenable to storage, and by the impracticalities of periodic regeneration. Although some progress has been made with other storage technologies, few are currently operationally feasible for trees (Haines 1994). Whilst research to develop these technologies has merit, their technical limitations and cost will continue to preclude their use, other than when exceptional circumstances or strong economic imperatives prevail.

The situation of trees in terms of *ex situ* conservation is also true for most other forest species - the majority of which are not yet described by science. Consequently, *in situ* conservation strategies - in the broad sense discussed below - will remain of fundamental importance for achieving the conservation of forest genetic diversity.

In situ conservation strategies

Most *in situ* conservation strategies have been developed around the foundation of a reserve system of protected areas - the ultimate expression and focus of *in situ* conservation (eg Western & Wright 1995, World Commission for Protected Areas/ IUCN 1997). Acknowledgment that protected areas do not function as islands isolated from their broader environments – ecological, economic or social - has fostered the development of a parallel suite of policies, which seek to reinforce the effectiveness of the reserve system through complementary off-reserve management. These policies are now focused on the concepts of sustainable forest management, and its assessment against criteria and indicators (Braatz 1997, Wijewardana *et al.* 1997).

Perceptions of the purpose of both protected areas and forests outside reserves, and of their roles in achieving conservation objectives, continue to evolve – and are the subject of considerable debate (eg Brandon 1995, Hale & Lamb 1997, Kramer *et al* 1997, Western and Wright 1994, Wood 1995, WWF/ IUCN 1998). Experience at both policy and operational levels has led, for example, to a reassessment of assumptions about protected area function and management (eg Western & Wright 1995, WCPA/IUCN 1997), and the reinterpretation of IUCN's Protected Area categories (WWF/IUCN 1998). A common theme is that achieving conservation objectives requires more than the declaration of conservation reserves, and demands the integration of on- and off- reserve management through the development and implementation of integrated conservation strategies.

In response both to the limitations of the traditional model of protected areas, and as a consequence of the changing role of governments, there has also been increasing diversification in the means by which protected areas are established, financed and managed; examples include the involvement of NGOs such as The Nature Conservancy or WWF, the establishment of community-based partnerships such as those described by Western *et al.* (1994), and the emergence of various forms of voluntary conservation agreements between private forest owners and governments or NGOs (Binning & Young 1997). A major challenge to contemporary conservation efforts is to develop policies that build on the role and contributions of protected areas, whilst recognising their limits.

Identifying priority areas for in situ conservation

Although the means by which conservation objectives should be achieved is the subject of considerable debate, there is broad agreement that efforts should be focused on areas of highest priority. Recognition of the need to identify priorities for the conservation of biological diversity has generated considerable methodological development in planning for *in situ* conservation. Contemporary conservation planning methodologies seek to maximise the achievement of forest conservation goals, through the identification of priority areas for conservation, whilst minimising the opportunity costs associated with realising the goals. In practice, this requires comparison of the values of different forest areas, so that the benefits and costs of different forest conservation options can be assessed and used to inform decisions. Suites of conservation planning tools (*eg* BioRap – Margules & Redhead 1995, C-Plan – Pressey and Logan 1997) are now available for this purpose; they are based on a number of common principles (Kanowski *et al.* 1999):

- making the best use of both biological and environmental data, which will inevitably be incomplete;
- incorporating the social and cultural values of stakeholders by including them in the planning process;
- minimising bias and maximising efficiency in the achievement of conservation goals;
- incorporating economic costs into the process for achieving conservation goals;
- providing the information necessary for informed negotiation about the best way to achieve conservation goals, and to strike a balance with other goals.

The best geographic basis for conservation planning is a "bioregion", which is defined by ecosystem rather than cadastral boundaries (eg Breckwoldt 1996, Saunier and Maganck 1995). A bioregion can be defined as (Bridgewater *et al* 1996):

a land and water territory whose limits are defined not by political boundaries, but by the geographical limits of human communities and ecological systems.

The scale of a bioregion will reflect the level at which planning is being conducted and the pattern of ecological variation. Australia, for example, has been partitioned into 80 bioregions for conservation planning (Thackway & Cresswell 1995). Within each bioregion, planning seeks to identify a set of priority areas, which are (ANZECC/ MCFFA 1997):

- comprehensive – all forest ecosystems are represented;
- adequate – the viability and integrity of populations, species and communities are maintained, and risks of loss minimised by replication across the landscape;
- representative – diversity within ecosystems is sampled by replication within the same ecosystem across the landscape;
- efficient – the network of priority areas meets the three criteria above according to any efficiency criteria specified. Two such criteria might be the minimisation of area and of opportunity cost.

This approach to conservation planning begins with stakeholders agreeing on a set of regional goals for forest conservation – for example, that 10% of each ecosystem should be protected, by whatever mechanisms are most appropriate. The region is then divided into smaller spatial units, for example, grid cells, catchments or some other mapped unit. The contribution each of these units makes to the various goals is then measured or estimated, and - in consultation with stakeholders - priorities for the use of each are assigned according to these contributions. Kanowski *et al.* (1999) summarized a conservation planning process conducted on this basis in Papua New Guinea.

These priority areas for *in situ* conservation will occur across the landscape and, most likely, on a range of tenures: some may be in existing protected areas, whereas others will be on forests outside protected areas - under public, communal, private or other ownership or control - subject to various forest management regimes. Their identification in the conservation planning process allows informed consideration and negotiation about how their genetic diversity might best be conserved – for example, through the establishment of reserves, or the modification or maintenance of particular forest management regimes.

Four common elements are evident from experience with these conservation planning processes (Kanowski *et al.* 1999):

- they work best when they make use of all sources of data, including traditional and local knowledge;
- recent technological advances – especially in Geographic Information Systems – are revolutionising our capacity to capture data describing forest values and represent it in easily-interpretable ways;
- they provide a powerful means of informing all interested parties about priorities and options for achieving conservation goals;

- supportive policy frameworks and institutional processes are essential if the values held by all interested parties are to be recognised and incorporated in the conservation planning process.

The integration of on- and off-reserve management

Recognition of the importance of forests and trees outside reserves in contributing to conservation objectives has focused attention both on the sustainable management of off-reserve forests and the integration of on- and off- reserve management. The concept of a spectrum or continuum of contributions from different parts of the forest landscape has been used by, amongst others, Pressey and Logan (1997) and Kanowski *et al.* (1999) to describe the process of achieving conservation objectives on a bioregional scale.

Consequently, Kanowski *et al* (1997, 1998) have argued that the principles of landscape ecology and of adaptive management (*eg* Margules and Lindenmayer 1996), which recognise the importance of the biogeographical context and the limits to knowledge, define essential elements of off-reserve forest management. These principles include:

- the maintenance or restoration of connectivity between protected areas;
- the maintenance of heterogeneity across the forest landscape;
- the maintenance of structural complexity and floristic diversity within forest stands;
- the use of an array of management strategies implemented at different spatial scales;
- the state of processes that generate and maintain genetic structure and diversity (*eg* Stork *et al* 1997).

The translation of these principles to operational forest management regimes poses many challenges, but one to which researchers and forest managers are beginning to respond (eg Bachmann *et al.* 1998, Boyle & Boontawee 1995, Hale & Lamb 1997, UBC-UPM 1996). For example, Pressey and Logan (1997) demonstrated how conservation planning tools could be adapted to assist in the development of off-reserve forest management regimes; Halladay and Gilmour (1995) reviewed the role of traditional agro-ecosystems in contributing to conservation objectives; suites of indicators (eg Montreal Implementation Group 1998, Namkoong *et al.* 1996, Saunders *et al.* 1998, Stork *et al.* 1997) are being proposed and tested, under the auspices of a number of international processes (Wijewardana *et al.* 1997), to assess the impacts of forest management on forest genetic diversity.

Conclusions

Recent advances in our understanding of ecology and genetics are dramatically improving our capacity to identify the key scientific elements of effective genetic conservation strategies - helping to resolve issues such as options for reserve location and design, the most appropriate silvicultural regimes for managed forests, and the respective and complementary contributions of various *in* and *ex situ* measures. Whilst these advances have highlighted the limitations of our knowledge, they are also informing us about the most effective use of human and financial resources to achieve genetic conservation objectives.

Scientific advances have been paralleled by the evolution of our thinking about how best to achieve these conservation objectives in the context of diverse social, economic and political circumstances. Locally-appropriate responses, consistent with the international regimes defined by the CBD and World Trade Organization, are emerging as a result. New forms of partnership between many of the actors with interests in forests, which recognise the diversity of roles and contributions, are especially important in delivering conservation outcomes.

This broader vision for forest conservation suggests that we need to develop integrated forest conservation strategies which incorporate forests of all tenures and management regimes. The best geographic basis for conservation planning and implementation is a “bioregion”, which is defined by ecosystem rather than cadastral boundaries. A bioregional approach to conservation planning is consistent with the ecosystem approach endorsed by the Conference of Parties to the Convention on Biological Diversity. Conservation planning tools have been developed to facilitate this regional planning process, and maximise the achievement of forest conservation goals at minimum opportunity cost. These tools are widely applicable, and adaptable to different levels of data and technology.

The broader vision for *in situ* forest conservation recognises that achieving and sustaining forest conservation also requires the integration of social and economic goals into conservation planning processes. It therefore recognises the development of more collaborative participatory modes of conservation planning and management as essential to achieving and sustaining forest conservation goals. New forms of partnership between many of the actors with interests in forests, which recognise the diversity of their roles and contributions, are especially important in delivering conservation outcomes.

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The Role of *In Situ* Conservation In Sustainable Utilization of Timber Species

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Abstract. *In situ* conservation as an ideal way to protect species in their natural habitats has been known by conservationists long before the world community who gathered in Rio in 1992 considered the loss of biological diversity as an important subject to be included in environment and development issues. To ensure the availability of biological resources for now and for the future a Convention on Biological Diversity (CBD) was signed by more than 150 nations symbolizing their commitment to conserve the global biological diversity for human interests. Article 8 of the Convention deals with *in situ* conservation.

Being one of the countries which are rich in biological diversity and the fact that such a richness is used for national development, Indonesia has allocated 18.6 million hectares of its forests for protected areas. Of course not all genetic resources which are important for tree breeding are represented in the existing protected areas. To complement the *in situ* conservation of timber species, arboreta, botanic gardens, and collection gardens, etc., are becoming an important part of a conservation system. The concept of *in situ on farm* management of genetic resources of cultivated trees may be developed to complement species population which are conserved *in situ*.

Another way of conserving sample of timber species is in provincial gardens, an example of which is shown in Serpong Garden. In this way, each provincial garden only keeps native timber species of its own.

From the management point of view *in situ* and *ex situ* conservation is primarily the concern of the government while *in situ on farm* is in the hands of communities. For the management of *in situ* conservation the participation of those community living within and surrounding the protected areas is considered of prime important. Ways and means to linking conservation of trees genetic resources with their sustainable utilization should be enhanced so that the benefits of conservation could be felt by those who should participate in conservation activities.

In Indonesia, conservation and development should be regarded as two sides of the same coin. This paper discuss further the role of the scientific community in unraveling the potential of tree genetic resources for enhancing the income generation at the local and national level. Moreover, an enabling environment which is necessary to make the *in situ* conservation works is highlighted.

Introduction

For more than 30 years Indonesia, like many developing countries in Asia, has utilized its forest resources for national development. At the same time a number of conservation areas, such as National Parks, Biosphere Reserves, or Protected Forests have been established in many provinces to protect ecosystems and the biological resources they contain.

At the global level, in the early 1970s the world community began to notice a drastic decline in environmental quality due to the impacts of development. Ever since the Stockholm summit meeting on human environment (1972), various concepts of sustainable development have been elaborated at the global level. One such concept that is widely discussed is the socio-ecological system. In this concept, the inter-dependency among sub-systems, i.e., environment, society, and economy is stressed (Gallopin *et al.* 1997). In many developing countries, forests are one of the natural resources utilized for national development. Hence, forests have become a favorite subject of international discussions.

Twenty years after the Stockholm Conference the world community gathered in Rio de Janeiro (1992) to express concerns about the loss of biological diversity. Their commitment to conserve biological diversity for sustainable utilization led to the signing of the Convention on Biological Diversity. At the same time, the world community agreed to handle matters on forests through the Statement of Principles on Forest Management, Conservation and Sustainable Development (Watson *et al.* 1998).

Indonesia was among the first countries to sign the Convention on Biological Diversity. However, only in late 1994 did Indonesia ratify the Convention through Act no 5, 1994. This Act signifies the political commitment of Indonesia to protect the biological diversity within its territory not only for the sake of Indonesia itself, but also for the world community. Soon it was realized that a political commitment at the global level alone is not sufficient to implement obligations as a party to the Convention. Not only the complexity of institutional arrangements contributed to delays in implementing the Convention, but also the fact that there is so much socio-cultural diversity throughout Indonesia.

To support its national development Indonesia relies heavily on its natural resources. Even before its independence, estate crops such as tea, coffee, cacao, and rubber were prime export commodities. In the late 1960s, timber assumed the prime export role. And, unlike estate crops that have been cultivated in plantation with a system of monoculture, most timber species are extracted directly from mixed natural populations. Because of the complexity of these mixed forests, even though a selective timber extraction method was adopted, damage to neighboring, non-targeted species cannot be avoided. And, in addition, over harvesting has characterized forest exploitation in Indonesia.

The fact that forests in Indonesia are degraded has advanced fears of those who are aware of the need to conserve timber species in their natural habitat. Such *in situ* methods of conservation are necessary, especially when we consider that the cultivation of many timber species, and most non-timber species, has not been initiated. This paper presents facts about forests in Indonesia, including their richness in timber species; the opportunity and benefit for Indonesia to be a party to the Convention on Biological Diversity; the need to integrate efforts of *in situ*, *in situ-on farm*, and *ex situ* conservation with sustainable utilization of timber species; the analysis of the players in conservation; and, finally, the identification of the enabling environment to make these conservation efforts work.

Indonesia on the World Forestry Map

Tropical forests are considered as one of the areas richest in diverse species of plants, animals and microbes (Heywood & Watson 1995). Amongst the sub-regions in Asia-Pacific, South East Asia is considered the richest in plant diversity, and particularly that found in its tropical forests. Within S.E. Asia, Indonesia has the largest area of forests. In fact, Indonesia is listed amongst those seven countries (Russia, Brazil, Canada, the United States, China, Indonesia, and the Congo) wherein 60 percent of the remaining world's forests are found. Of those seven countries the forests of Brazil, Indonesia, and Congo are tropical in nature (Abramowitz 1998).

In discussing forests in Indonesia Collins *et al.* (1991) summarized key features of the original forest cover which once dominated its islands and island groups:

1. Sumatera: lowland evergreen rain forests dominated by dipterocarps, peat swamp forests and mangroves, montane rain forests, and natural pine forests.
2. Java: rain forests, natural monsoon forests, montane forests, temperate herbaceous formations, limestone karst, freshwater swamp forests, and mangroves.
3. Nusa Tenggara: monsoon forests and extensive grasslands, natural sandalwood forests, and a bit of montane rain forests.
4. Kalimantan: lowland evergreen forests, montane forests, extensive mangroves, peat and freshwater swamp forests, and large heath forests.
5. Sulawesi: montane rain forests, lowland rain forests, karst limestone, small inland swamp forests, and mangroves.

6. Maluku: lowland and montane forests, mangroves, and freshwater swamps.
7. Irian Jaya: monsoon forests and savanna, tropical rain forests, lower montane rain forests, upper montane forests, subalpine forests, alpine heath land, freshwater swamp forests, mangrove forests, peat swamp forests, monsoon forests, savanna woodlands, limestone, grassland, and beach forests

The different forest formations mentioned in these seven bio-geographical areas represent all the tropical rain forests in the Malesian region. The fact that Indonesia is a huge archipelago with more than 17,000 islands, of which the 5 biggest islands (Sumatera, Java, Kalimantan, Sulawesi, and Irian Jaya) encompass a wide range of altitudes, enables such a diversity of natural forest formations.

On the world map, the 17,000 islands of Indonesia are no more than dots between continental Asia and Australia and, in terms of land area, these islands occupy only 1.3 per cent of the global surface. Yet these islands support a diverse species of plants, animals, and microbes, many of which are unique and cannot be found in any other part of the world. In terms of animal species distribution, the famous "Wallace line" divided Indonesia into two parts; the western part - the Sunda shelf - is rich in large animals, and the eastern part - the Sahul shelf - is characterized by marsupials and is also rich in bird diversity. A famous example of one of those unique birds is the "bird of paradise". The giant lizard (Komodo Dragon) *Varanus komodoensis* on the islands of Padar and Rinca, and the anoa (*Bubalus sp.*) on Sulawesi are among the many unique species of Indonesian animals.

Floristically, Indonesia is also one of the richest countries. A study in Jambi, Sumatra, has shown that a square kilometer there is richer than Brazil in plant species. According to FAO (1982) there are no less than 25.000 species of Malesian flowering plants, of which 10,000 are tree species. The largest flower in the world, *Raflessia arnoldi,* and also the most gigantic inflorescence - *Amorphophalus titanum* - are found only in Indonesia. Of the many useful species, Indonesia is known as the center for tropical fruit species such as durian (*Durio zibethinus*), rambutan (*Nephellium lappaceum*), and salak (*Zalacca edulis*) (Zeven & Zhukovsky 1963). Concerning endemism, it is estimated that out of the 37,000 diverse higher plants in Indonesia, between 39 and 49 percent are endemic (Conservation International 1998).

For centuries, people in Indonesia have utilized plants for food, energy, building materials, and medicines. Most of these species have not been cultivated and people generally collect what they need directly from the forests. Some useful species are commonly grown in villages around the forests signifying the beginning of plant domestication. Some species of rattan, for example, have been under village domestication in Kalimantan for decades. Rattan needs trees to support its climbing growth habit, and the villagers have selected appropriate species for that purpose. Another multi-purpose species in the process of domestication is bamboo and the same holds true with fruit species such as durian and mango.

Indonesia, together with Malaysia, is at the top of the list of countries exporting tropical timber. Although Indonesia has a larger forest area than Malaysia, export earning from forest products of the two countries is almost the same. In 1995, Indonesia exported US$4,728 million in forest products, while Malaysia exported US$4,232 mill. (Abramowitz 1998). Compared to 1970 figures, Indonesia increased its forest products exports by 1267 percent, an amazing jump that has most conservationists worried.

No less than 18.6 million hectares of forests have been set aside for conservation areas. There were more than 326 locations of gazetted reserves as of August 1990 (IUCN 1990) that are scattered all over Indonesia. Of this number, there are 164 Nature Reserves, 44 Wildlife Sanctuaries, 54 Recreation Parks, 13 Marine Recreation Parks, 4 Grand Forest Parks, 13 Hunting Reserves, 10 Marine Nature Reserves, and 24 National Parks. There are protected areas which are small (less than 5 ha) and those that cover more than 300,000 hectares belonging to several categories of protected areas, i.e., strict protected areas, National Parks, Biosphere Reserves, Game Reserves, etc.

Indonesia has experienced several great forest fires since 1982 that made the world news. East Kalimantan was the most afflicted area during the 1982 fires that destroyed approximately 8,000 sq km of forestland. El Nino was identified as the main cause of the 18-month long drought preceding those fires. It was reported that the fires started in farmers' fields while clearing logged-over areas for agriculture (Malingreau *et al*. 1985). Great fires also struck Indonesian forests in 1996. This time the land clearing activities of the big plantations was identified as the cause. The impacts of these fires were felt not only in Indonesia but also in Singapore and Malaysia. Since then the Ministry of Forestry has actively monitored the growth of the "hot spots" not only in many parts of Indonesia but also in neighboring countries. Prior to the 2000 planting season, the numbers of hotspots increased, reaching a peak in July, as reported by the Ministry of Forestry.

Timber: Keystone Economic Species

Forest valuation is based primarily on the value of the standing timber trees. Such a valuation is, of course, questionable if the other functions of the forests in national development are considered. Commercially, there is no doubt that natural forests are the primary source of cheap timber. The trees are readily available without capital or planting and maintenance efforts. In Indonesia there are more than 4000 tree species that are potentially useful for timber. Out of these 4000 species, only 400 are considered economically valuable, although a mere 200 species are listed as commercial timbers (Soerianegara & Lemmens 1993). The Malesian region, of which Indonesia forms the largest part, is well known for dipterocarp species belonging to the genera of *Dipterocarpus, Shorea, and Dryobalanops*. However, in natural forests these species grow in a mixture with other species, including the many non-timber species.

Commercial logging is identified as the major threat to sustaining natural forests stands. According to FAO figures (GBA 1995), since 1970 no less than 300,000 ha have been logged annually in Indonesia. The actual acreage is hard to determine due to illegal logging. In addition, village communities follow suit by felling trees that are left by loggers and, in this way, forest degradation proceeds faster and further.

Conversion from natural forest to other land uses is very common in Indonesia. Some areas are converted into estate plantations (oil palm) or forest plantations (*Acacia mangium, Eucalyptus urophylla, Pinus merkusii*), a trend all over the world. According to FAO (Mattoon 1998) acreage of tree plantations doubled in 15 years (1980-1995). Compared to the regions of Africa, and Latin America/Caribbean, Asia/Oceania ranked high in the expansion of tree plantations with 32.6 million hectares in 1980 increasing to 66.9 million in 1995. It is generally believed that tree plantations have the potential to protect natural forests by substituting as wood producers. In Indonesia, significant tree plantations can be found in North Sumatra, East Kalimantan, and on Jawa.

Another land use system introduced to deal with degraded forests is agroforestry. Much research on this subject has been done in Indonesia. One of the Consultative Groups on International Agricultural Research (CGIAR) is the International Centre for Research in Agroforestry (ICRAF), which has an office in Indonesia. Through the Global Environment Facility (GEF), ICRAF together with the Central Research Institute for Food Crops, Indonesia conducted a study on “Alternatives to Slash-and-Burn” (ASB, 1998). This study identified 30 species of trees that are valuable for agroforestry. Based on their origin and uses they can be classified into three types: exotic species (e.g. rubber, teak, mahogany), indigenous multipurpose species (damar, kemiri, kelapa), and the pioneer timber species (sungkai, sengon, and terap). Policy reform in timber production is needed to promote these species for planting on small farms.

If it is true that forest products industries are looking more toward tree farms (Mattoon, 1998) then it is only logical for Indonesia to consider enhancing her tree plantation on degraded lands that are characterized by alang-alang species (*Imperata cylindrica*). For such purposes, both introduced and native timber species are available. From the large number of species that have potential to be developed into timber resources, one can select and evaluate the best species for various industries. Many fast growing species, for example, are good for papermaking, boxes, furniture frames, etc. while hardwood species are sought for building materials and high quality furniture. Currently, scientific information on the silviculture of minor timber species is insufficient to be used as the basis for their cultivation. Moreover, the monoculture systems employed in most timber plantations may experience the same problems with pests and diseases as do crop monocultures.

The CBD and *In situ* Conservation

As stated in Article 1 of the Convention of Biological Diversity (CBD), the Convention has three objectives which should be looked on as one entity; i.e., the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources. The inclusion of sustainable uses and the sharing of benefits are the reasons that many countries, and especially the developing countries, joined the Convention. The two last objectives have promoted public and private sector awareness of the need to conserve biodiversity.

For conservationists, the most attractive article among the 42 articles of the CBD is Article 8, which deals with *in situ* conservation. This Article highlights the needs for the establishment of a system of protected areas; the protection of ecosystems, habitats and populations; the promotion of environmentally sound and sustainable development surrounding the protected areas; the degraded ecosystems; and compatibility between conservation and development. Also related to *in situ* conservation is Article 7 on Identification and Monitoring, particularly point 7a, the identification of biological diversity important for its conservation and sustainable use. The indicative list of categories of such components is given in Annex 1, which emphasizes ecosystems and habitats containing high diversity as well as species and communities that are threatened.

Long before the CBD era where *in situ* conservation of biological diversity is attempted at the intergovernmental level, many international organizations, such as the International Union for Conservation of Nature (IUCN), the World Wildlife Fund for Nature (WWF), and UNESCO-Man and the Biosphere were already engaged in biological conservation. The famous Red Data Book of the IUCN has a long list of species that are on the brink of extinction. It should be noted that earlier activities in conservation dealt especially with charismatic species. Therefore panda, lion, rhino, and the like were the focus of these conservation efforts. With the CBD, an ecosystem approach to conservation is further emphasized allowing the protection of various species together with their habitat.

The holistic approach to conservation in the CBD includes human beings as part of the living system. In this way, conservation may also be considered as part of national development. Moreover, never before in convention efforts has attention been paid to the rights of the traditional or local communities who live within and adjacent to protected areas. The needs to respect, preserve, and maintain traditional knowledge, innovations, and practices of indigenous and local communities are outlined in Article 8(j).

In most developing countries where poverty still prevails, a conservation agenda is at the bottom of the list of their national programs. Therefore, in Article 8(i) of the CBD it is stated that conditions needed for compatibility between present uses and the conservation of biological diversity should be established. Moreover, a call for financial and other support for *in situ* conservation, particularly aid to developing countries, is presented in Article 8(m).

As a party to the CBD, Indonesia has received financial support for *in situ* conservation activities either through bilateral arrangements or through the Global Environment Facility (GEF), which at the moment is the financial mechanism for the CBD. The implementing agencies for the GEF are the World Bank, the United National Environment Programme (UNEP), and the United Nations Development Program (UNDP). Although there is no specific provision in the GEF mechanism to deal directly with *in situ* conservation of timber species, the fact is that in many protected areas in Indonesia timber species are growing naturally. Thus, *in situ* conservation of timber species should not necessarily be regarded as a separate activity from forest ecosystem conservation, in general. The immediate question that needs to be answered is “how may we best know what species we have within each protected area?” This knowledge is important for deciding whether or not a species should be protected by *ex situ* condition to back up the *in situ* conservation efforts.

The Need to Expand Conservation of Timber Species

Several timber species, especially introduced commercial species, have been cultivated in Indonesia. Jati (*Tectona grandis*), for example, has been in cultivation since the Dutch time. The same holds true for mahogany (*Swietenia mahagoni*) and pine (*Pinus sp*). In some parts of Indonesia, the fast growing kayu Africa (*Maesopsis emenii*) has been introduced for further cultivation. In recent years there is a tendency to utilize the rubber tree (*Hevea brasiliensis*), the cultivation of which is well practiced, as source of timber.

Plantations of fast growing indigenous tree species have been attempted either for pulp or for wood. Among those that have been tried on large areas are mangium (*Acacia mangium*), sungkai (*Peronema canescense*), and ampupu (*Eucalyptus urophylla*). The more slow growth native species that have been identified as having potential for timber are, among others, sonokeling (*Dalbergia latifolia*), surian (*Toona sureni*), kayu hitam (*Diospyrus ebenii*), and bayur (*Pterospermum javanicum*).

A few tree species have attracted the attention of villagers based on usefulness for village life. Among these species are jeungjing (*Paraserianthes falcataria*), terap (*Artocarpus elasticus*), nyamplung (*Callophyllum inophyllum*) and jabon (*Anthocephalus cadamba*). These are planted in *ladang* or in backyard gardens. The number of individual trees planted per family is of course small, but at the village level the total number can be substantial.

In the Biodiversity National Plan for Indonesia there is a list of animal species, including birds and reptiles, that have top priority for conservation (BAPPENAS 1993). Unfortunately, plant species, including timber trees, are absent from that list. The reason is quite obvious - animals are more attractive to humans than plant species, so plant conservation is somewhat lagging behind that for animals. It seems that development of a list of timber species to be conserved is in the hands of the Ministry of Forestry.

The future of many native timber species of Indonesia is uncertain. This is due to the recent uncontrolled extraction of trees from the forests following the change of the government in 1997. Moreover, the slow process of decentralization of governance (autonomy) from the central government to provinces has caused uncertainty as to who is responsible for, or who has the right to exploit protected areas that have been declared for *in situ* conservation. The uncertainty has placed several National Parks such as Tanjung Puting (Central Kalimantan), Kutai (East Kalimantan), Kerinci Seblat (in 4 provinces in Sumatera), and Gunung Leuser (North Sumatra) in jeopardy. Timber species are the most sought after objects in those forests..

To promote *in situ* conservation of forest tree genetic resources in areas where concessions have been granted, the National Committee on Genetic Resources works together with the Association of Forest Concessionaires to design conservation fields within the concession areas. It was agreed that around 200 hectares of forests should be left uncut in each concession area. In this way there is a remnant of original forest in each locality, which will serve as a reference for future studies, as well as a place where seeds of native trees can be collected. The Committee has developed guidance for the concessionaires to set aside the conservation areas. It would be useful to know how many such areas are successfully developed and managed at the moment.

Anticipating a worsening condition for *in situ* conservation in Indonesia, *ex situ* conservation may be an alternative way to conserve genetic resources of timber species. However, it should be borne in mind that most timber species produce seeds that are recalcitrant in character - these seeds cannot be kept in standard storage conditions of low temperature and relative humidity. In addition to seed storage, a new method of *ex situ* conservation is by tissue or cell culture. These techniques, however, still need further development to be used in the conservation of timber species in general.

Botanic gardens, arboreta, or collection gardens may be the best solution for the time being for the conservation of some timber species. In such gardens the number of individuals per species to be maintained is limited. Soekotjo (2001) has attempted to improve the *ex situ* conservation of populations of a species by growing samples of species populations in several collection gardens. At the same time selection and evaluation are conducted to improve the genetic makeup of future planting materials. The financial consequences of managing such collections are undoubtedly significant. The existing collection gardens, be it botanic garden, experimental garden, or arboreta are insufficiently budgeted. The newly established Serpong Garden, under the State Ministry of Research and Technology, has tried to accommodate collections of timber species using a representation of at least 50 individuals per species to allow a gene pool to be maintained. Again, the lack of minimum financial support prevents the garden to operate as planned. To be effective, this method of *ex situ* conservation should be combined with other objectives of national development such as education, research, and recreation.

In agricultural crops, *in situ-on-farm* management systems have been introduced. In this way farmers are actively selecting and evaluating crops on their own farms. Research institutes provide technical and scientific backup. This arrangement allows farmers to keep their traditional crops but at the same time improve the genetic makeup as well as the farming practices. By so doing, conservation and sustainable utilization activities are combined to get optimum benefits. This system of management can be employed for tree species that have been cultivated in farmers field for decades, such as *Paraserianthes falcataria* and *Anthocephalus cadamba*. However, a question remains to be answered; i.e., whether the scientific community is ready to provide technical and scientific backup for managing tree species *on farm*. The long life cycle of tree species and the small number of individuals on each farm might not be attractive to those who might engage in *on farm* research. Moreover, it appears that in *situ on farm* management of tree species may not be economically viable as well, unless a series of farms are aggregated into a “cooperative” system of management.

In Indonesia, each method of conservation for timber species cannot survive in its own right. Therefore, an integration of *in situ*, *ex situ* and *on farm* conservation into a system of management may be the best way of conserving tree species in the long run. Within this system sustainable utilization is part of the exercise. In this way, conservation becomes an integral part of national development. From a technical point of view it is feasible to integrate conservation efforts into development activities. However, the biggest challenges and obstacles to doing so are institutional, rather than technical.

Considering that *ex situ* conservation will play a more important role for saving timber species in the future, the idea of establishing provincial gardens, which was expressed again and again in Indonesia, can now be revitalized. Each garden will be unique in its own right since such a garden is expected to feature the local species. The acreage of the famous Bogor Botanic Garden, which was established in 1817, is only about 84 hectares. The collection of trees maintained in the garden came from all over the tropical world. In this way the representation of native trees is limited. The same holds true for Cibodas Garden, which is dedicated to the collection of subtropical trees of high elevation origins. The gardens in Purwodadi and Bali contain more native species of Indonesia then the other two gardens. Thus, the establishment of provincial gardens would complement the existing botanic gardens. If each province in Indonesia was able to establish around 100 - 200 hectares of land to save its native timber species, then it could be expected that most of the timber species of Indonesia would be saved. A provincial garden can serve as a place for education, research, and recreation. In the long run, a provincial garden can be self-sustaining when the private sector is invited to join in the management.

Beyond the Government Authority

Forests in Indonesia are under the jurisdiction of the Ministry of Forestry - thus, all the protected forest areas, as well as the production forests, are under the same ministry. Under this system if there was a conflict of interest between the conservation and the utilization of forest areas it was settled within the ministry without difficulty. Nowadays, however, there is a movement aimed at letting other parties provide suggestions to the Ministry of Forestry.

The most significant and intense logging activities in Indonesia began in the late 1960s. As far as logging permits are concerned, no institution outside the Ministry of Forestry has authority to grant them. Thus, when the concessionaires began their operations, people who lived in the forests were the ones who were most affected. The same holds true when a type of forest was designated as a protected area. Local communities within such a forest became marginalized. They either moved further inside the forest where no

Forestry official could reach them, or they stayed but had to face all sorts of legal complications from time to time. Social conflicts colored the process of logging or gazetting protected areas.

The concern of the CBD is for putting human within the system of conservation. In this way conservation efforts can accompany development. More than seven years after Indonesia ratified the Convention, public awareness of the importance of biological diversity has been enhanced significantly. This is due to the fact that not only is the government actively informing the public, but also the Non Government Organizations (NGOs) are complementing these efforts. External aid allows those who are concerned with biological diversity to act according to the specifications of those providing the aid. Yet implementation of Article 8 and the closely related articles (Art. 9 on *ex situ* conservation, 10 on sustainable utilization, 15 on access to genetic resources, and 16 on access to and transfer of technology) of the Convention is still far from ideal. Therefore, if *in situ* conservation is to work in Indonesia, complementary conservation activities between institutions should be organized and attempted as one system through periodic communication.

A new development - involving stakeholders at the grass roots level in conservation activities - has been promoted within the era of the CBD. Almost all projects supported by external agencies have required a participatory approach from the beginning of the planning process to the end of the project cycle. In this way activities can be sustained beyond the project's life. The change of political atmosphere in Indonesia in 1997 has promoted further the role of NGOs in public advocacy at the grass roots level. Unfortunately, however, the involvement of the scientific community in providing scientific and technical know-how at that level is still limited.

From experience we have learned that government alone, particularly the Ministry of Forestry, is unable to cope with overall management of the *in situ* conservation areas. Pressures from other sectors of government to use these conservation areas for other purposes are mounting. Resettlement programs require large areas to be developed into housing facilities, tourism programs must find attractive places to assure a flow of visitors, estate plantations need to expand their business onto less contested lands, i.e., conservation areas, and local governments are eyeing these areas to increase local development budgets. At the same time, communities within and surrounding the protected areas demand to use conservation areas for their needs. Meanwhile, the number of field personnel in protected areas is very small compared to the large areas that need to be protected. Thus, at the grass roots level conflicting interests between stakeholders poses problems for the managers of the protected areas.

Therefore, an adaptive management system of conservation areas involving as many stakeholders as possible at the grass roots level may be an option to be explored.

Enabling Environment for Sustaining Timber Resources

Conservation of renewable natural resources in Indonesia, including living organisms, was initiated in the Dutch era. After proclaiming her independence (1945), Indonesia joined various global movements in nature conservation. Unfortunately, the public at large has different perceptions concerning conservation issues. When national development began, conservation was viewed as a government affair only. To them, a conservation area was a place where plants and animals were protected and the public was not allowed. Such a perception fostered a growing feeling of resentment toward conservation.

The most recent global trend in conservation is to invite and encourage public participation. This change in philosophy has enlarged the constituency of the conservation movement. Not only the public at large is invited to participate but also the private sector, which before was often considered the enemy of conservation. Thus, public awareness of the needs to conserve biological diversity is considered as the spearhead of the movement. All channels of communications have been used to raise such an awareness. If at the global and national levels the number of the people who are affected measures the program's success, at the grass roots level a different measure should be applied. At this level, probably the number of actions to implement conservation is a much more valuable indicator.

People may be aware of the needs to conserve biological diversity for present or future uses, but in order to act in accordance with their understanding they must acquire benefits by doing so. At the grass roots level such benefits from conservation are hard to deliver, especially if the benefits are perceived to be monetary. At the moment, in terms of biological diversity conservation, the benefits to local food security and income generation are considered as attractive goals. If these two objectives can be achieved then poverty issues are dealt at the same time. The problem is not easy to solve because each locality has its own unique socio-cultural institutions, infrastructure, and biological resources. So there is no uniform formula for solutions that will work in every locality. Such diversity offers a great challenge to all concerned with conservation.

Competence of government officials at all levels is required to deal with the diverse background of the stakeholders if conservation of biological diversity is to work. Social issues are more prominent in Indonesia than technical or scientific questions. Moreover, conservation actions should be on the agenda of all sectors in the government, not just that of the Ministry of Forestry. At the moment, Indonesia is preparing a Revised Biodiversity Strategy and Action Plan with financial support from the World Bank. The First Plan had a comprehensive range of coverage from conservation *in situ*, *ex situ,* and also *in situ* outside protected areas. The targets and the actions needed are also focused and thus are easy to follow. However, some believe that adequate monitoring and evaluation activities are lacking. In this area it is hard to tell just how far Indonesia has gone to implement her obligation to the CBD. Therefore, it is hoped that within the Revised Plan the sector that should monitor and evaluate the implementation of the Plan is identified and specified.

Political will is well expressed by the Indonesian government to participate in the Convention on Biological Diversity. This commitment was shown by the mode of ratification of the CBD through Law no 5/94 and its Biodiversity Action Plan (1993). However, political actions are needed to implement the activities that have been listed in the Plan. Without political action to allocate appropriate budgets, that Action Plan is only good to look at. It should also be kept in mind that decentralization of governance is in the process of implementation. Unless every level of governance is aware of the need to conserve our biological wealth for our development, there is a danger that the wealth will slip from our hands.

One of the assets that Indonesia can depend on for her sustainable development is her timber resource. Timber, like other biological resources, is renewable. In theory, then, timber resources will remain available as long as water and sunshine are available. Sunshine will be in Indonesia forever until the day the globe stops revolving. Water, however, is beginning to be a problem because of the loss of forests that can hold it in storage. If we can manage the flow of rainwater through good management of forests, including reforestation and forest rehabilitation activities, even if there is substantial climate change, we will be better off in maintaining our timber resources. It is up to all of us to select the best options for financing our development. If we believe that timber is our choice, then the conservation of timber species through good management of forests, protected areas, various collection gardens, and farms is a must.

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Status of *In Situ* Conservation of Commercial Tree Species In Malaysia

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Abstract. The tropical rainforest of Malaysia is a unique natural heritage containing a vast biodiversity of natural resources. These forests provide many significant contributions such as resources for breeding programmes and socio economic benefits, while at the same time they are required for ensuring environmental quality and ecological stability. Currently, the issues of rising demand for wood and wood products, which have resulted in the depletion of forest resources, are also no exception in Malaysia. Thus, effective conservation measures of these forest resources form logical strategies to alleviate such problems. In the present era of increasing environmental awareness, any conservation effort for forest resources should be conducted in tandem with sustainable development. This paper reviews the status of *in situ* conservation efforts for commercial tree species being carried out in Malaysia. Some issues pertaining to these efforts are highlighted and discussed.

Introduction

It is well known and virtually recognized that the wet tropical moist forests of Southeast Asia possess the highest biodiversity amongst the terrestrial ecosystems and habitats ever recorded on the earth. Recently, these forests were immensely threatened and several factors that have contributed to the rapid decline of forested areas of the region at large were identified so that they can be dealt with in the most appropriate manner (ITTO 2000). Nonetheless, awareness of this exceptional diversity and its uniqueness, coupled with vigilance to the rapid loss of this heritage, is highly conceived by all concerned and has been translated into policies and management plans and operational measures to safeguard its continued availability for sustainable use.

In situ conservation measures adopted by Malaysia are similar to those conducted in many countries in the Asia Pacific region. The forests in Malaysia are generally classified into Permanent Reserved Forests (PRFs) and state lands. The former refers to areas kept permanently or as permanent as possible, to be managed on a sustainable basis for maximum economic, social, and ecological benefits, while the latter can be earmarked for non-forest development such as agriculture, infrastructure, industry, and residential purposes. However, in the context of conservation, only PRFs are applicable and they can be further classified according to their functional objectives i.e., production and protection.

Production forests refer to forest areas zoned for harvesting of timber and other produce that are managed sustainably according to criteria and indicators (C&I). Protection forests consist of forest areas purposely set aside for fulfilling many ecological services including maintenance of climatic conditions, water catchments, and flood mitigation. These include National Parks, Virgin Jungle Reserves (VJRs), Wildlife Reserves, Big and Heritage trees. The above two classification viz. land use classifications and functional objectives are interrelated. Therefore, this paper intends to review the state of the art of *in situ* conservation activities in Malaysia

Permanent Reserved Forest Estate (PRF)

PRF refers to forest reserves maintained and managed on a sustainable basis for their maximum economic, social and ecological benefits. A total of about 4.78 million hectares out of 13.15 million hectares of the forested land in Malaysia have been gazetted as PRF in accordance with the National Forestry Policy (1978).

Peninsular Malaysia, Sabah and Sarawak have their own individual forest classifications of PRFs. There are 11 types in Peninsular Malaysia, 7 in Sabah, and 3 in Sarawak. Peninsular Malaysia classifies the PRFs according to their ecological functions, while protection of a particular forest ecosystem forms the basis of classifications for Sabah and Sarawak, as shown in Table 1. The distribution of PRFs across the states in Peninsular Malaysia is given in Table 2.

Table 1. Classifications of PRFs in Malaysia

No	Peninsular Malaysia	Sabah	Sarawak
1.	Timber Production Forest Under Sustained Yield	Protection Forest Reserve	Forest Reserves
2.	Soil Protection Forest	Commercial Forest Reserve	Protected Forest
3.	Soil Reclamation Forest	Domestic Forest Reserve	Communal Forest
4.	Flood Control Forest	Amenity Forest Reserve	
5.	Water Catchment Forest	Mangrove Forest Reserve	
6.	Forest Sanctuary for Wildlife	Virgin Jungle Reserve	
7.	Virgin Jungle Reserves (VJRs)	Wildlife Reserve	
8.	Amenity Forest		
9.	Education Forest		
10.	Research Forest		
11.	Forest for Federal Purposes		

Table 2. PRFs by forest types in Peninsular Malaysia as at 31.12.99

State	Permanent Reserved Forest (PRF)			
	Inland Forest	Peat swamp forest	Mangrove forest	Total
Johore	321,154	5,099	17,029	343,282
Kedah	332,057	0	7,949	340,006
Kelantan	625,843	0	0	625,843
Malacca	4,859	0	77	4,936
Negri Sembilan	162,885	0	454	163,339
Pahang	1,396,787	97,406	2,786	1,496,979
Perak	956,065	0	41,302	997,367
Perlis	10,409	0	0	10,409
Penang	5,955	0	451	6,406
Selangor	145,515	76,134	15,090	236,739
Terengganu	538,730	13,819	1,295	553,844
Federal Territory	61	0	0	61
Total	4,500,320	192,458	86,433	4,779,211

Source: Forestry Department, 1999

To date, Malaysia has no official national list of target species for the purpose of conservation for production. However, efforts have been made to list some potential target species in three regions of Malaysia. They are selected on the basis of (i) commercially important, (ii) potentially important but somehow threatened, (iii) rare but of high value and (iv) species with low regeneration potential. Table 3 presents the list of these species in the three regions of Malaysia.

There are several classifications under Protected Areas (PAs) including National and/or State Parks, Wildlife Reserves and/or Sanctuaries, and Nature Reserves depending on the legal instruments used to govern their gazettement. The PA systems operating in the three regions of Malaysia viz. Peninsular Malaysia, Sabah, and Sarawak are different. A similar designation normally has different interpretation under different legislations in these regions.

Table 3. Potential target species for the three regions of Malaysia

Peninsular Malaysia	Sarawak	Sabah
Dyera costulata	Shorea macrophylla	Dtyobalanops lanceolata
Endospermum malaccense	Shorea hemsleya	Eusideroxylon zwageri
Shorea leprosula	Shorea beccariana	Shorea beccariana
Hopea odorata	Shorea splendida	Shorea glaucescens
Dyobalanops aromatica	Dipterocarpus sarawakensis	Shorea mecistoptery
Azadirachta excelsa	Dtyobalanops beccarii	Shorea leprosula
Pterocarpus indicus	Eusideroxylon zwageri	Shorea superba
Anisoptera laevis	Palaquim	Shorea gysbertsiana
Dryobalanops oblongifolia	Gonystylus bancanus	Shorea macrophylla
Neobalanocarpus heimii	Azadirachta excelsa	Shorea ovata
Koompasia malacensis		Octomeless sumatrana
Shorea curtisii		
Shorea singkawang		
Shorea parvifolia		
Shorea platyclados		
Khaya ivorensis		
Hevea sp		
Acacia hybrid		
Tectona grandis		
Maesopsis eminii		

National and State Parks

Malaysia has established a network of protected areas for the conservation of biological diversity in the form of National and State Parks. These form the best-protected areas under the existing laws and regulations in Malaysia. For instance, the National Park Enactment of 1938 and 1939 has governed the National Parks for decades. As of 1999, Malaysia had 1.43 million hectares of protected areas; 0.78 million hectares in Peninsular Malaysia, 0.39 million hectares in Sabah and 0.26 million hectares in Sarawak as depicted in Table 4.

Table 4. Areas under National Parks, Wildlife and Bird Sanctuaries in Malaysia (Million hectares)

Region	National Park	Wildlife & Bird Sanctuary	Total
Peninsular Malaysia	0.43	0.35	0.78
Sabah	0.25	0.14	0.39
Sarawak	0.08	0.18	0.26
Total	0.76	0.67	1.43

Source: Forestry Department, 1999

In Peninsular Malaysia, there are about 0.14 million hectares of areas overlapped with PRFs and 0.64 million hectares of areas not overlapped with PRFs. In certain instances, legislative conflicts might arise when dealing with the management of Wildlife Reserves and Sanctuaries in Malaysia. This is because there are different implementing agencies involved; for instance, the Forest Department for Sarawak, the Department of Wildlife and National Parks (PERHILITAN) for Peninsular Malaysia, and the Sabah Wildlife Department. Different ministries manage these in turn. Conflicts in land use may also arise when wildlife reserves and sanctuaries are overlapped with those of the production forest in the PRFs. One unique example of PAs is the Taman Negara, Malaysia's largest national park, gazetted in 1939 and covering a tri-state area of 434,351 hectares. It is virtually a virgin forest comprising various forest types according to altitude and soils. It has also been estimated to contain 5000 species of plants per hectare (Soepadmo 1971, Berger 1990). Examples of common tree species, especially the Dipterocarps, at different ecological zones are highlighted in Table 5.

Table 5. Common Trees and Shrubs of Taman Negara at Different Ecological Zones

Undulating Lowland Dipterocarp		Hill or Ridge Dipterocarp	Riparian Vegetation
Anisoptera spp	Erismanthus obliquus	Dipterocarpus grandiflorus	Tristania whiteana
Dipterocarpus spp	Annonaceae (F)	Dipterocarpus baudii	Lithocarpus wallichianus
Koompassia spp	Rubiaceae (F)	Garcinia spp	Dipterocarpus oblongifolia
Shorea spp	Memycelon spp	Eugenia spp	Casnatopsis oblongifolia
Dispyros spp	Helicia spp	Lithocarpus wallichianus	Eugenia spp
Lithocarpus wallichianus	Garcinia spp	Castanopsis inermis	Aglaia salicifolia
Lithocarpus Cantleyanus	Gironneira spp	Castanopsis lucida	Calophyllum rupicolum
Lithocarpus Emcleiaccarpus	Aracaceae (F)	Agrostistachys longifolia	Nauclea spp
Castanopsis inermis	Zingiberaceae (F)	Anisoptera laevis	Ixora stenophylla
Castanopsis lucida	Maranthaceae (F)	Shorea leprosula	Dysoxylum angustifolia
Paranephelium spp	Gesneriaceae (F)	Calophyllum spp	Dipteris lobbiana
Canarium littroole	Myristica spp	Koompassia malaccencis	
Donax grandis	Cycas rumphii	Parkia spp	
Xerospermum spp	Gigantocholoa wrayi	Agathis dammara	
Eugenia spp	Payena spp	Gironniera parvifolia	
Phrynium capitatum	Ochanostachys amentacea		
	Knema spp		

Source: Soepadmo (1971)
Note: (F): Family name

In addition, this reserve contains 60% of the local mammals, 211 of 241 lowland forest birds, and 60 out of 76 known mountainous species (Davidson, 1982). Other examples of these national parks include the Kinabalu National Park (75,370 hectares) in Sabah, and Mulu National Park (52,887 hectares) in Sarawak. Recent efforts to safeguard transboundary or bilateral biological diversity between Malaysia and Indonesia include the establishment of a protection area of 800,000 hectares between the Lanjak Entimau Wildlife Sanctuary in Sarawak, Malaysia and the Bentuang Karimun Nature Reserve in Kalimantan, Indonesia.

Virgin Jungle Reserves (VJRs)

Virgin Jungle Reserves (VJRs) are gazetted by the State Forest Departments. In Malaysia, within every large production forest area or forest reserve, an area of 80 – 200 ha of undisturbed vegetation has been protected as VJRs, commonly termed as ‘pockets’. The purpose of these pockets is to establish permanent nature reserves and natural arboreta as controls for comparing harvested and silviculturally treated forests, and as undisturbed natural forests for ecological and botanical studies.

Eighty-seven VJRs, covering 23,002 ha, have been established throughout Peninsular Malaysia. (Pers comm. Azahar, Forestry Department

Annual Report 1999). The VJRs represent the many forest types of Malaysia including the lowland (LD), hill (HD), and upper Dipterocarp (UD) forest. All the VJRs in Peninsular Malaysia are currently being inventoried to assess their flora (including tree species) and their distribution for more effective conservation of these resources. The sizes of these VJRs vary from areas as small as 10.9 to 2,744 ha in Peninsular Malaysia to as large as 6,735 ha in the reserve of Sabah. Examples of Dipterocarp-rich VJRs compartments in selected forest reserves in Peninsular Malaysia are given in Table 6.

Table 6. Some Dipterocarp-rich VJRs compartments in Peninsular Malaysia

State	Forest Reserve	Compartment Number	Forest Type	Year of Establishment	Area (ha)
Johore	Gunung Ledang	62-69 and 77	LD, HD, UP, HM	1969	1,059
Kedah	Gunung Jerai	20, 25, 26 and 27	HD	1960	1,480
Malacca	Tanjung Tuan	All	HD	1953	65
Kelantan	Relai	15	LD, HD, UP	1960	523
Negeri Sembilan	Berembun	21 and 32	LD, HD, UP	1990	1,619
	Berembun	33	LD, HD, UP	1959	1,595
Penang	Telok Bahang	2	LD	1976	98
Selangor	Ulu Gombak	22 and 23	HD, UP, HM	1959	275
Terengganu	Jambo Bongkok	2	LM	1960	123
	Sungai Kijal	1	LD, HD	1986	361
Pahang	Lesong	371 and 372	LD	1990	291
Perak	Bukit Larut	41-46	HD, UP, LM	1962	2,747
Perlis	Mata Air	24	LD	1953	55

Source: Forestry Department, 1999
Key: LD: Lowland Dipterocarp Forest; HD: Hill Dipterocarp Forest
UP: Upper Dipterocarp Forest; LM: Lower Mountain Forest
HM: Upper Mountain Forest

Big and Heritage Tree Conservation

The production forests of Malaysia were almost exclusively managed under the Malaysian Uniform System (MUS) or the Selective Management System (SMS). The two aforementioned systems selectively remove the biggest trees of the selected species. However, the consequences of this practice pose a strong possibility for genetic erosion and degradation.

Shade and ornamental tree planting is relatively recent in Malaysia as compared to Europe and the United States of America. To counteract the various environmental problems brought about by the rapid urban and industrial development in the country, recently wayside trees and shrubs are being planted and gardens developed to create a more aesthetic landscape.

Selected Big Trees

These comprise exceptionally big-size trees found in PRFs or elsewhere. Efforts have been made to collect information about these biggest trees in all PRFs in Peninsular Malaysia to preserve them as heritage trees. The initiative succeeded in the registration of the nine biggest trees in Peninsular Malaysia as shown in Table 7.

Table 7. Registry of the nine biggest trees in Peninsular Malaysia

Tree No.	Family	Local Name	Scientific Name	Location	Height (m)	DBH (cm)
1.	Diptrocarpaceae	Chengal	Neobalanocarpus heimii	Pasir Raja FR, Dungun, Terenganu	65	533
2.	Diptrocarpaceae	Chengal	Neobalanocarpus heimii	Pasir Raja FR, Dungun, Terenganu	45	342
3.	Leguminosae	Batai	Paraserianthes falcataria	Bukit Lagong FR, Rawang, Selangor	-	253
4.	Diptrocarpaceae	Mersawa	Aniosptera spp.	Chabang besar FR, Padang Terap, Kedah.	16	246
5.	Diptrocarpaceae	Mersawa	Anisoptera spp.	Chabang besar FR, Padang Terap, Kedah.	18	228
6.	Diptrocarpaceae	Chengal	Neobalanocarpus heimii	Senaling FR, Kuala Pilah, N. Sembilan	45	202
7.	Apocynaceae	Jelutong	Dyera costulata	Chebar Besar FR PadangTerap, Kedah.	25	196
8.	Apocynaceae	Jelutong	Dyera costulata	Sg. Lalang FR, Selangor	-	174
9.	Diptrocarpaceae	Keruing Gumbang	Dipterocarpus cornutus	Moakil FR, Lenga, Johor.	28	136

Source: Forestry Department, 1999.
DBH: Diameter at Breast Height FR: Forest Reserve

Heritage Trees

Extensive roadside planting together with the increasing appreciation of the role of trees in the amelioration of urban environment and the shaping of the landscape to encompass more aesthetic values to urban and rural landscapes have contributed to the conservation of some valuable indigenous tree species. The popular and magnificent native angsana tree *Pterocarpus indicus* that had been planted extensively as a shade and ornamental tree along major promenades during the 18th century in Malacca and Penang is a good example. This tree species has become one of most popular roadside trees in Peninsular Malaysia today.

Seed Production Areas

Natural forests offer opportunities for selecting candidate plus trees as seed sources. Seeds are collected from them for immediate and long-term usage. Initiatives of the Forest Department and FRIM have identified both Dipterocarp and non- Dipterocarp plus trees of the target list. The list of seed production areas (SPAs) established by states of Peninsular Malaysia is given in Table 8 and Fig. 1.

Table 8. List of Seed Production Area (SPAs) in Peninsular Malaysia

States	Forest Reserve (FR)	Comp. No.	No. of Plus Tree
Johore	1) Gunung Banang F.R.		94
Kedah	1) Gunung Enggang F.R.		30
Negri Sembilan	1) Sungai Menyala F.R. 2) Pasir Panjang F.R. 3) Teriang F.R.		68
Kelantan	1) Hulu Sat F.R.	Comp.12	68
Pahang	1) Lentang F.R. 2) Lepar F.R. 3) Balok F.R. 4) Mergastua Taman Negeri Endau-Rompin F.R. 5) Kemasul F.R. 6) Som F.R. 7) Jengka F.R. 8) Terengganu F.R.	Blk.I,Comp.8 Blk,II,Comp-.4 Blk.I Blk.II Blk I Blk II Comp.1	347
Perak	1) Bukit Tapah F.R. 2) Keledang Saiong F.R. 3) BubuF.R.	Comp.24 Comp.4	 53
Perlis	1) Mata Ayer F.R..	Blk.1953 Blk.1962	69
Penang	1) Bukit Pancor F.R. 2) Pantai Acheh F.R.		32
Selangor	1) Sungai Buluh F.R. 2) Bukit Cerakah F.R.		31
Terengganu	1) Bukit Bauk F.R. 2) Kersing F.R. 3) Besut F.R. 4) Sungai Paka F.R. 5) Bukit Jemalang F.R. 6) Bukit Bandi F.R.	Comp.21 Comp.9 Comp.10 Comp.16 Comp.7 Comp.1 Comp.6	93

Source: Personal communication; Rahman Jalil (FDHQ).
Note: Most of the plus trees in the respective states are of similar species belonging to the Dipterocarpaceae e.g. *Shorea leprosula, S. parvifolia, S. macroptera, S. acuminata, S. foxworthy, S. multiflora, S. pauciflora, Neobalanocarpus heimii*, and *Dryobalanops aromatica*.

Some examples of species composition in selected SPAs are also given in Table 9. Because seed production is closely related to the overall phenological behavior of trees and their interaction with the environment, a monitoring system of phenological data was devised on these SPAs to enable selection of appropriate method and time for seed harvesting of the target tree species. Phenological monitoring will also contribute to our knowledge of periodicity of seed production. In addition, management of information systems utilizing these data served as an impetus for the development of the Forest Genetic Data Base (FGRID) that was later upgraded to the Forest Genetic Resources Information system (FORGRIS). This system has been used by the Forest Department in all the three regions of Malaysia to store important information necessary for seed production, storage, background information on mother trees, and the like. Predictions of seed production trends can be made using this system, which provides updated data that are readily accessible. One example is the case study of *Neobalancarpus heimii* for which 20 years (1980-1999) of phenological data have resulted in a means of predicting the trend of its flowering behavior at different sites. Using these data systems, it was found that flowering behavior could be either annually or biannually and flushing periods were found to be March to May or September to November. The flowering pattern in the FRIM locality was found to be twice annually and twice biannually. On the contrary, it was found that *Neobalancarpus heimii* flowered annually followed by twice biannually in Ulu Gombak and Ampang, Selangor, Peninsular Malaysia. Therefore, the prediction made for the year 2000 was twice for FRIM and once for Ulu Gombak and Ampang. In addition, this useful system revealed the following:

1. Mass flowering seasons to fruiting last 4-5 months.
2. Two distinct categories of flowering trees were identified:
    a) Species restricted to mass flowering syndrome.
    b) Species that flower regularly, either annually or biannually, but skip a year or two, or irregular intervals.
3. The Dipterocarps are confined to the mass flowering category (50-70% of the spp. flowered).
4. *Shorea* species flower annually with different intensities.

Table 9. A subset of data from Table 8 showing details of species composition in some representative compartments in FRs in Peninsular Malaysia

State	Forest Reserve	Compartment	Species
Pahang	Balok FR	1	Dryobbalanops aromatica Shorea macroptera Palaquiumrostrastum Madhuca utilis
	Jengka FR		Dipterocarpus cornutus Shorea leprosula Dyera costulata Scaphium macoropodum Heritiera javanica
Terengganu	Bauk FR	21	Shorea macroptera Shorea parvifolia Endospermum malaccense Neobalanocarpus heimii Dipterocarpus costulata Dryobbalanops aromatica
Perak	Kledang Saiong FR	-	Shorea leprosula Shorea lumutensis Shorea gluca Shorea coriacea Koompassia malacceneis Aquilaria malaccenseis
Kedah	Gunung Enggang FR	-	Dipterocarus grandiflorus Shorea paviflora Shorea macroptera Mesua ferrea Shorea coriacea Shorea dolichocarpa
Negeri Sembilan	Teriang FR	-	Shorea parviflora Shorea acuminata Shorea macroptera Shorea ovalis
Penang	Bukit Pancor FR	-	Drybalanops aromatica Shorea leprosula
Selangor	Bukit Cerakah FR	-	Shorea leprosula Dyera costulata Palaquium gutta Endospermum malaccense Koompassia malaccensis

Genetic Resource Area (GRA)

As part of a long term strategy, an area of about 5517 ha within the Ulu Sedili Forest Reserve, Johor and 19 ha in the Semengoh Forest Reserve, Sarawak have been set aside for seed production and conservation of genetic diversity. This is a joint project between the Federal and State Forestry Departments and the ASEAN Forest Tree Seed Center (AFTSC) for Johore and the ASEAN-Canada Forest Tree Seed Center for Sarawak, which provides the technical expertise and training to undertake the project. The GRA in Johore comprises 30 compartments, 19 of which have been logged, while 11 are scheduled for the future (Lim & Chin 1995).

The Ulu Sedili Forest Reserve Genetic Resource Area can be described as a Lowland Dipterocarp Forest, dominated by trees from the family Dipterocarpaceae including species belonging to the genera *Dipterocarpus, Shorea, Dryobalanops, Neobalanocarpus, Hopea* and *Anisoptera.* Other important timber producing species include trees from the families of Burseraceae, Guttiferae, Myristicacae, Myrtaceae, Sapotaceae, Annonaceae, Euphorbiaceae, Flacourtiaceae and Rubiaceae. Since one of the objectives of this project was to identify and manage a set of target species intended to be monitored and managed as seed production stands, the GRA in Ulu Sedili Forest Reserve therefore involves 8 target species as depicted in Table 10, and the one in Semengoh utilizes 12 target species as in Table 11.

Table 10. Target species of Dipterocarps and non-Dipterocarps in Ulu Sedili Forest Reserve for GRA (*in situ* conservation)

Scientific Name	Local Name	Family
Shorea singkawang	Meranti Sengkawang Merah	Dipterocarpaceae
Shorea laevis	Balau Kumus	Dipterocarpaceae
Shorea curtisii	Meranti Seraya	Dipterocarpaceae
Anisoptera laevis	Merawan Durian	Dipterocarpaceae
Dryobalanops aromatica	Kapur	Dipterocarpaceae
Neobalocarpus heimii	Chengal	Dipterocarpaceae
Dyera costulata	Jelutong	Apocynaceae
Koompasia malacensis	Kempas	Fabaceae

Note: Based on one compartment out of 30 compartments.
Source: Lim & Chin 1995.

Table 11. Selected target species in Semengoh Forest Reserve for GRA

Scientific Name	Local Name	Family
Shorea macrophylla	Engkabang jantung	Dipterocarpaceae
S. hemsleyana	Engkabang gading	Dipterocarpaceae
S. splendida	Engkabang bintang	Dipterocarpaceae
S. beccariana	Engkabang langgai	Dipterocarpaceae
Dipterocarpus sarawakensis	Keruing layang-layang	Dipterocarpaceae
Shorea sp.	Selangan batu	Dipterocarpaceae
Dryobalanops beccarii	Kapur bukit	Dipterocarpaceae
Eusideroxylon zwageri	Belian	Lauraceae
Palaguim spp.	Nyatoh	Sapotaceae
Dyera costulata	Jelutong bukit	Apocynaceae
Gonystylus bancanus	Ramin telur	Gonystylaceae
Azadirachta excelsa	Sentang	Meliaceae

Source: Joseph Jawa Kendawang, pers. Comm.

Research and Education Forests

In addition, some examples of forests allocated for research and education purposes include the Air Hitam Forest Reserve belonging to Universiti Putra Malaysia, FRIM's research stations in Kepong, Jengka, Ulu Tekam Forest Reserve, and Pasoh Forest Reserve; Setiu in Terengganu, research plots in Bukit Hari, Bukit Lagong, and Sg. Menyala, and Gombak Field Study Centre of the University of Malaya.

The research findings and other issues of indigenous tree species pertaining to their use in *in situ* plantation programmes in Malaysia have been documented in the proceedings of a national seminar held at UPM in 1992 (Sajap *et al.* 1992). Lim and Faridah (1992) stressed that the use of indigenous species has often been overlooked. Indeed, there are problems associated with the use of indigenous species such as the lack of ecological understanding of requirements for growth, the inadequate collection of seed, the relatively slow growth rate, and economic feasibility. However, most of these problems can be overcome by research and, indeed, some are already being studied and some solutions were suggested. Thang and Mokhtar (1992) reviewed the status on the usage of indigenous tree species in Peninsular Malaysia. They concluded that several indigenous species have the potential to become plantation species. However, appropriate research and development in all aspects of plantation establishment is needed.

Kendawang (1992) reported that research on indigenous species for plantation establishment dated back to the 1930's in Semengoh Forest Reserve using *Shorea* spp. Recently, research on indigenous species for use in forest plantation in Sarawak was intensified. Major indigenous species intended for plantation use include *Shorea macrophylla, Dryobalanops beccarrii, Durio zibenthinus, Anthocephalus cadamba, Alstonia* spp and *Artocarpus integer.*

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Genetic Resource Conservation Strategies for Timber Trees in the Philippines

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Abstract. Timber production forests in the Philippines are now less 2 million ha. These are under various kinds of agreements between the government and private sector. Natural forests under license agreements for timber production represent the majority of these remaining areas. About 600,000 ha are tree plantations established mainly with four species for pulp and paper. The extent of the genetic diversity within and between populations of the commercial timber species in the plantations, and much less, in the natural forests, remains largely unknown. A few companies are involved in limited tree improvement programs for plantation species. Some tree plantations and natural forest stands have been designated by the government as seed production areas to serve as primary sources of quality seeds for tree plantations. The network of protected areas serve as *in situ* conservation areas for many forest trees; however, these usually do not contain genetic resources of the commercial timber trees (e.g. dipterocarps) characteristic of the lowland rain forests. Some specific measures are being developed for conservation of genetic resources of timber trees in areas subjected to commercial logging. Despite great natural diversity of species and habitats, resources for conservation in the Philippines remain limited.

Introduction

The Philippines ranks 8th on the world's list of 25 megadiversity countries (Mittermeier *et al.* 1999) owing to its impressive record of species diversity and endemism. However, it is also considered a "hotspot" (Myers1988, Myers *et al.* 2000). As such, its species and habitats are among the most endangered in the world and face imminent threat of destruction.

In 1997, the actual forest cover in the Philippines was a mere 5.4 million ha or just 18% of the total land area, despite the fact that 15.8 million ha were still classified by the government as forestlands (FMB 1997). Only 25 years before, at least 15.6 million ha of the archipelago were still covered with forests (BFD 1972). Hence, during the last 2½ decades alone, the Philippines lost more than 10 million ha of its forests.

Of the remaining 5.4 million ha under forest cover, 66% are lowland dipterocarp forests with about 805,000 ha classified as old-growth forests and 2.7 million ha as residual forests. The remaining forest types are categorized by the Forest Management Bureau (1997) as follows: pine forest, 227,900 ha;

sub-marginal forest (includes the limestone and ultramafic forests), 475,100 ha; mossy forests (includes the upper montane and subalpine rain forests), 1.04 million ha; and mangrove forest, 112, 400 ha (Table 1).

Table 1. Land-use and area of forest types in the Philippines (after DENR UNEP 1997).

Land use / Forest type	Area (in hectares)	Percentage of total area
Forest	5,391,717	17.9
Dipterocarp	3,536,017	11.7
Old-growth	804,000	2.6
Residual	2,731,117	9.1
Pine	227,900	0.7
Submarginal (includes the limestone and ultramafic forests)	475,100	1.5
Mossy	1,040,300	3.4
Mangrove	112,400	0.3
Brushland	2,232,300	7.4
Other land use	22,375,983	74.5
Total	30,000,000	100.0

The Philippine lowland dipterocarp forests have been regarded, at least in the early part of this century, as the richest of their kind in the world, especially those on Negros and Mindanao (Wyatt-Smith 1954). Those in Surigao in Mindanao, in particular, have been considered ‘amongst the grandest in the world’ (Cox 1990).

Timber production in the Philippines

Some 1.9 million ha of production forests have been declared by the Forest Management Bureau (FMB) as of 1997. These include natural forests (mainly residual forests) under timber license agreements (TLAs) with private corporations, and forest plantations under industrial forest plantation management agreements (IFMA) or industrial tree plantation lease agreements (ITPLA), and tree farms. The annual log production by TLAs often does not exceed the annual allowable cut granted to them. The overall volume of log production for the entire Philippines has generally dropped from about 6.5 million m^3 in 1980 to 0.5 million m^3 in 1997.

Natural forests

About 1.3 million ha or 50 % of the residual dipterocarp forests have been leased under timber license agreements to 26 private logging companies with a total annual allowable cut of 854,252 m^3. About 72% of this area is on the island of Mindanao. At least 360,670 ha (or 27% of country total) are in the province of Surigao del Sur in eastern Mindanao. In 1997, this province alone contributed 43% of the total volume (555,917 m^3) of timber production for the entire country.

Forest plantations and tree farms

Nearly 600,000 ha of the production forests are industrial forest plantations and tree farms currently established mainly for pulp and paper. The species commonly cultivated in the plantations are *Paraserianthes falcataria, Gmelina arborea, Endospermum peltatum,* and *Eucalyptus deglupta*. The last two are indigenous species that occur naturally in the lowland dipterocarp forests. Some plantations and tree farms include other exotic species such as *Acacia mangium, Pinus caribaea,* and *Eucalyptus urophylla.* About 75% of the forest plantations and tree farms are in Mindanao. Three of the large companies with forest plantations and tree farms are discussed further below.

Timber tree species in the Philippines

There are an estimated 3500 species of trees indigenous to the Philippines (Salvosa 1963); about 10% of these have economic importance as a timber source (Meniado *et al.* 1974). The bulk of the commercial timber extracted from Philippine forests, however, belongs to species of *Dipterocarpaceae*. The *Dipterocarpaceae* is the pre-eminent timber family of the Asian tropics (Newman *et al.* 1996) and about 45 species of this family occur in the Philippines (Ashton 1982, Newman *et al.* 1996, Table 2).

Table 2. Summary of dipterocarp species indigenous to the Philippines and their trade names (after Newman *et al.* 1996). Only species known to attain timber size (45-50 cm diameter) are included in this list. Species marked with an asterisk (*) are endemic to the Philippines.

Philippines	Indonesia and Malaysia (nearest equivalent)
Light hardwoods (air dry density c. 450-720 kg./m3) a. Red lauan (Philippine red mahogany * Shorea negrosensis Foxw. Shorea ovata Dyer ex Brandis * Shorea polysperma (Blanco) Merr.	Seraya/Dark red meranti
b. White lauan (Philippine light red mahogany) Shorea almon Foxw. * Shorea contorta Vidal * Shorea palosapis (Blanco) Merr. Parashorea malaanonan (Blanco) Merr.	Seraya/Light red meranti White seraya (Sabah)
c. Manggasinoro Shorea assamica Dyer ssp. koordersii (Brandis) Symington Shorea assamica Dyer ssp. philippinensis (Brandis) Symington * Shorea polita Vidal Shorea virescens Parijs	Seraya/Yellow meranti Melapi/White meranti
d. Palosapis * Anisoptera aurea Foxw. Anisoptera costata Korth. * Anisoptera thurifera (Blanco) Blume ssp. thurifera	Pengiran/Mersawa
Medium hardwoods (air dry density c. 720-880 kg./m3) a. Apitong Dipterocarpus alatus Roxb. ex G. Don * Dipterocarpus caudatus Foxw. ssp. caudatus Dipterocarpus eurhynchus Miq. Dipterocarpus gracilis Blume Dipterocarpus grandiflorus (Blanco) Blanco Dipterocarpus hasseltii Blume Dipterocarpus kerrii King Dipterocarpus kunstleri King * Dipterocarpus orbicularis Foxw. Dipterocarpus validus Blume	Keruing
b. Manggachapu * Hopea acuminata Merr.	Merawan
Heavy hardwoods (air dry density c. 880-1120 kg./m3) a. Yakal * Hopea basilanica Foxw. Hopea plagata (Blanco) Vidal * Shorea astylosa Foxw. * Shorea falciferoides Foxw. ssp. falciferoides * Shorea malibato Foxw. Shorea seminis (de Vriese) Slooten	Giam Balau
b. Guijo Shorea guiso (Blanco) Blume	Red balau
c. Narig * Vatica pachyphylla Merr.	Resak

Among other families that include commercially important timber species are *Sapotaceae, Burseraceae, Anacardiaceae, Leguminosae, Meliaceae, Combretaceae, Meliaceae*, *Myrtaceae* and the conifer families *Pinaceae* and *Podocarpaceae*.

Several species have been protected by law from cutting for some time, *viz*., *Sindora supa*, *Albizia akle, Afzelia rhomboidea*, *Intsia bijuga*, *Vitex parviflora*, and *Agathis dammara.* The cutting of *Pterocarpus indicus* is also strictly regulated. More recently, some species have been considered as endangered or potentially threatened (see Table 3).

Table 3. Endangered and potentially threatened timber tree species in the Philippines (after Tan *et al.* 1986, Florido 1993)

Species	Family
Afzelia rhomboidea (Blanco) Vidal	Leguminosae
Agathis philippinensis Warb.	Araucariaceae
Dacrycarpus imbricatus (Blume) de Laub.	Podocarpaceae
Glenniea philippinensis (Radlk.) Leenh.	Sapindaceae
Heritiera sylvatica Vidal	Malvaceae
Hopea malibato Foxw.	Dipterocarpaceae
Intsia bijuga (Colebr.) O. Ktze.	Leguminosae
Litsea leytensis Merr.	Lauraceae
Pinus merkussii Jungh. & De Vr.	Pinaceae
Podocarpus costalis Presl.	Podocarpaceae
Sindora supa Merr.	Leguminosae
Tectona philippinensis Benth. & Hook. f.	Lamiaceae
Toona calantas Merr. & Rolfe	Meliaceae
Xanthostemon verdugonianus Naves	Myrtaceae

Government policy initiatives and programs for forest tree genetic resource conservation

Ex situ and *in situ* conservation are generally regarded as complementary, rather than mutually exclusive strategies. Both strategies have since been applied in the Philippines. Except perhaps for the protected areas, there has not really been a sustainable genetic resource conservation program for timber trees in the Philippines. Garcia (2000) even noted that 'conscious efforts on genetic conservation have been nil'. Many of the efforts were implemented as *ad hoc* programs or projects, and any research was often largely fragmented. Some of these *ad hoc* projects formed the core of current *ex situ* conservation projects, but many have simply been abandoned for lack of government support.

Ex situ conservation

Ex situ conservation strategy refers to the collection and maintenance of species germplasm outside their natural habitats. For timber trees in the Philippines this generally involves field gene banks or plantations for provenance and species trials.

Species provenance trials and seed orchard establishment have historically been conducted by the Department of Environment and Natural Resources (DENR) for species of *Pinus, Acacia, Eucalyptus*, *Casuarina, Gmelina,* and other multi-purpose trees species (Garcia 2000). Many of these projects faltered with changes in leadership and institutional reorganizations and the government's lack of sustained support (Ordinario 1992). Some of the provenance trial plots later served as the seed sources for the younger plantations by DENR and private planters. PICOP Resources Inc., (PICOP), Provident Tree Farms Inc. (PTFI), and Bukidnon Forests Incorporated (BFI) have been practicing *ex situ* conservation activities through provenance introduction and multiplication of phenotypically superior industrial forest plantation species such as *Paraserianthes falcataria*, *Gmelina arborea*, *Endospermum peltatum* and *Eucalyptus deglupta*.

PICOP Resources, Inc.

PICOP Resources, Inc. of Mindanao is one of the first few organizations that established, early in the 1970s, large-scale forest plantations in the Philippines to support its pulp and paper mill. There are six species in PICOP's plantations, including *Swietenia macrophylla*, *Pinus caribaea, Gmelina arborea, Acacia mangium, Paraserianthes falcataria,* and *Eucalyptus deglupta.* The last two species are the most commonly planted. In 1987, PICOP's industrial tree plantations totaled more than 46,000 ha of mainly *Paraserianthes falcataria* and *Eucalyptus deglupta* (Reyes 1987). PICOP used to have a decent and active forest research and tree improvement program for its plantations that included species provenance trials, progeny testing, and parent tree selection. However, several changes in company ownership during the last decade have put all these at a stand still.

Provident Tree Farms, Inc.

Provident Tree Farms, Inc. (PTFI) began in 1966 as a collaborative project with the Philippine Match Company (Phimco) to establish plantations of *Endospermum peltatum* for matchwood production. By 1992 PTFI had an industrial forest plantation agreement with the government covering some 20,770 ha in Agusan del Sur, Mindanao (Anonymous 1992). PTFI's plantations currently include *Endospermum peltatum, Acacia mangium, Anthocephalus chinensis, Gmelina arborea, Eucalyptus deglupta* and *Xylopia* sp. Its focus is mainly for matchwood, and other special wood uses such as toothpicks and chopsticks. PTFI has an active tree improvement program that includes species provenance trials, progeny testing, and parent tree selection.

Bukidnon Forests Incorporated

Bukidnon Forests Incorporated (BFI), also in Mindanao, started in 1989 as a tree plantation project between the Philippines' DENR and the Government of New Zealand. BFI now covers a total land area of 39,000 ha under a government scheme through an Integrated Forest Management Agreement (IFMA). Its net plantable area is about 21,000 ha. BFI has been regarded as a model project in the domestication of exotic tropical acacias (Arnold *et al.* 1998), eucalypts, and pines, and in site-species matching and practical plantation silviculture in the Philippines (Wilcox 1996). The species planted in BFI are mainly exotics such as *Acacia mangium*, *Pinus caribaea,* and *Eucalyptus urophylla*. *Eucalyptus deglupta,* an indigenous species, is also among the most commonly planted species by BFI. By 1998, BFI had successfully established about 6,300 ha of forest plantations of various ages. To date BFI has been undertaking provenance trials of 58 species/provenances mainly from Australia, Papua New Guinea, and Indonesia and supplied by the Australian Tree Seed Centre. BFI also started, in 1997, trial plantings of Philippine indigenous species such as *Shorea contorta, Anisoptera thurifera,* and *Vitex parviflora.*

Seed orchards

The Ecosystems Research and Development Bureau of DENR has recently implemented the UNDP/FAO Regional Project on Improved Productivity of Man-made Forests through the Application of Technological Advances in Tree Breeding and Propagation (FORTIP). This project, in cooperation with the Australia Tree Seed Centre, has established about 10 ha of seed orchards of *Acacia mangium* and *Eucalyptus urophylla* in Cavite Province (Luzon Island) and Mindoro, and *Acacia crassicarpa* in Bukidnon (Mindanao Island). *Gmelina arborea* seed provenances and improved clones were also brought from Thailand and Sabah, Malaysia.

Seed production areas

In 1995, the DENR, having finally realized the value of establishing a good and sustained forest genetic resources for the country's reforestation efforts, initiated through Administrative Order No. 9 the identification, establishment, maintenance, and protection of Seed Production Areas (SPAs) throughout the Philippines. The objective was to make the SPAs the primary sources of seeds for forest plantations. As embodied in the order, SPAs may be established in government tree plantations, industrial tree plantations, private forest tree plantations, and in natural forest stands. These identified plantations and natural stands should meet the following criteria: (1) for established plantation of similar species, the minimum area should not be less than 10 ha; (2) for contiguous natural stands of mixed species, the area should not be less than 10 ha; and (3) all identified mother trees shall be marked on the site, recorded in the inventory form, and plotted on a map. All established SPAs, except those on private lands, were to be delineated and proclaimed as permanent SPAs, subject to re-evaluation every 5 years. Commercial timber harvesting within the proclaimed SPA inside public forest was also to be strictly prohibited. Private landowners with at least 2 ha of forest plantation may also apply for accreditation with the DENR as seed production area (SPA).

The National Forest Tree Seed Committee

For the proper implementation of the SPAs, the DENR created the National Forest Tree Seed Committee (NFTSC) under the Chairmanship of the Director of the Forest Management Bureau (FMB), and the Regional Forest Tree Seed Committees (RFTSC) in each DENR Regional Office in the country.

The RFTC, together with PENRO / CENRO is responsible for the identification, evaluation and documentation of SPAs and potential SPAs. This information was to be processed by the FMB and stored in the Forest Genetic Resources Information Database System (FGRID) of the DENR for policy formulation and other relevant purposes, such as the development of an integrated management plan for SPAs.

The DENR requires that seeds designated for forest plantation purposes shall be collected only from selected trees within established SPAs by trained and authorized DENR personnel. If seed collection is undertaken in private plantations, it shall be done under the supervision of DENR authorized personnel, until such time that owners shall have been trained by DENR experts in seed collection and handling.

For this purpose, the DENR was set to establish at least eight seed storage and testing centers in strategic regions of the country. This is to ensure that collected seeds would be tested promptly and stored properly until their distribution. These centers were also asked to conduct periodic seed testing and provide an updated list of seeds sourced from certified SPAs. Once these storage and testing centers are established, according to DENR plan, all forest tree seeds shall only be sourced from said centers to eliminate the risk of using inferior planting materials, which may cause the failure of a forest plantation.

However, like many typical government projects, the implementation of this seed orchards project and National Forest Tree Seed Center has moved very slowly and is likely to be abandoned again. To date, only one seed storage and testing center at the DENR Central office has been established.

Botanic Gardens and Arboreta

The Makiling Botanic Gardens (MBG) in Los Baños maintains an arboretum of *Dipterocarpaceae* representing more than half of all the species known from the Philippines (see also Table 2). It also has plantations of *Swietenia macrophylla* representing probably the earliest seed lot of this species, first introduced in the Philippines in June 1913 from the Royal Botanic Gardens in Calcutta, India (Ponce 1933). MBG's collections of commercial timber trees in its approximately 5-ha site also include, among others, *Vitex parviflora, Pterocarpus indicus, Afzelia rhomboidea, Intsia bijuga, Sindora supa, Madhuca betis, Petersianthus quadrialatus, Agathis philippinensis, Tectona grandis, Tectona philippinensis, Cedrela odorata,* and *Endospermum peltatum.*

In situ conservation

This conservation strategy involves the maintenance of genetic diversity of species in their original or natural habitats. In the Philippines, this remains the principal strategy for genetic resource conservation of timber trees, despite the rapid deforestation rate in the country.

Network of protected areas for in situ conservation

The focal response of the Philippines government to conserving its biodiversity – and forest tree genetic resources – is the establishment of a network of protected areas – the national integrated protected areas system - or NIPAS - which was formally established in 1992. This network of protected areas includes national parks, watershed forest reserves, wilderness areas, game refuges and bird sanctuaries, and mangrove swamp forest reserves (see Table 4). The NIPAS is the major legal instrument requiring *in situ* conservation of forest tree genetic resources and biodiversity in general (Catibog-Sinha 1994) and forms a significant component of the Philippine National Biodiversity Strategy and Action Plan (PAWB-DENR 1998). Like many protected areas in the S.E. Asian region, the establishment of NIPAS in the Philippines was not determined on forest tree genetic resource considerations. But these, nonetheless, serve as *de facto* genetic resource areas or genetic reserves for many commercial timber species. Protected areas provide the best means for conserving genetic resources of forest trees (Ledig 1988).

The Philippines has one of the oldest national park systems in S.E. Asia, established in the early 1900s. The 4,244 ha Makiling Forest Reserve on Luzon Island was one of the earliest, having been established since 1910. This and other national parks established before 1992 became the initial components of NIPAS. Currently, there are 294 of these in the NIPAS with a total area of more than 4 million ha, including some marine parks and reserves and mangrove swamps (Table 4). The great majority, are however, terrestrial ecosystems representing different types of forest formations ranging from a small 1-ha forest park to the large 72,000-ha national park.

Table 4. Categories of protected areas in the Philippines (after DENR-UNEP 1997)

Category	Number	Area (in hectares)
National parks, national marine parks and reserves	67	455,314
Game refuges and bird sanctuaries	8	924,150
Wilderness areas	16	3,297
Watershed forest reserves	85	1,200,129
Mangrove swamp forest reserves	27	undetermined
Tourist zones and marine reserves	56	undetermined
Protected areas declared through administrative and memorandum orders	14	127,749
Newly proclaimed protected areas under NIPAS category	21	1,410,261
TOTAL	294	4,120,900

For nearly a decade now, the government of the Philippines has banned timber harvesting in old-growth forests, mossy forests, and forests above 1000 m elevation and with 50% slope. Many of these areas now form part of the national integrated protected areas system (NIPAS).

In situ **conservation in logging areas**

Despite the expansion of the network of protected areas in the Philippines, these remain very limited in their coverage, especially for many commercial timber trees of the lowland dipterocarp forests. A consensus has been growing that protected areas alone will not be sufficient to effectively conserve biodiversity and forest tree genetic resources in the Philippines. For example, the great bulk of the genetic resources of commercial timber trees (*e.g.* dipterocarps) are not found in currently declared forest reserves or protected areas. These timber species are usually restricted to the lowland rain forests where much of the large-scale commercial logging in the Philippines has been undertaken for many decades. The challenge, therefore, has been to include biodiversity conservation measures, even as timber is harvested from natural forests. Many around the world have begun to develop measures to maintain biodiversity within the practice of forestry (see for example Aplet *et al.* 1993).

In the Philippines, the Surigao Development Corporation (SUDECOR), a private logging company in eastern Mindanao, in cooperation with the government's Department of Environment and Natural Resources (DENR), has been undertaking a research project implemented by the Sustainable Ecosystems International Corporation (SUSTEC) and funded by the International Tropical Timber Organization (ITTO). The overall objectives of the research project are to collect information useful in assessing the biodiversity present in the area and to develop conservation measures for integration in a sustainable forest management (SFM) plan for the logging company. Can forests managed for timber production contribute to the overall effort of conserving biodiversity in general, and forest tree genetic resources in particular, in the Philippines, without impairing their capacity to yield commercial timber?

The sustainable forest management plan and the guidelines that integrate biodiversity and genetic resource conservation measures with timber production, are currently being developed. The plan includes, among others, very specific management strategies for the timber production zone within the logging concession. There are proposed strict standards in pre-logging inventory and tree marking (of trees to be cut) and felling.

Among the specific guidelines being designed are the following:

(a) Tree marking will be governed not only by the volume, number, class-size of the trees, and replacement growth rates, but also by the species and its biodiversity and genetic resource conservation value. For instance, tree species characterized by small populations or restricted distribution (e.g. rare species) will be marked as trees to be left.

(b) Adherence to the minimum 60 cm diameter breast height requirement for trees to be cut. The Philippines follows a selective logging limiting the diameter of trees to be cut to 60 cm and above.

(c) Deviation from high grading or cutting the largest or best trees all the time. This is to ensure that the phenotypically superior trees in the site are not completely depleted; and

(d) Exclusion from marking for cutting both individual flowering and fruiting trees and those in the priority list for conservation.

(e) Directional felling to minimize damage to saplings, especially those of timber species with high commercial value or in priority list for conservation.

(f) At least one mother tree of not less than 40 cm dbh per timber tree species per hectare will be marked as residual. This is to guarantee that pre-logging timber tree species will continue to exist in the area and at the same time promoting a better distribution of tree species across all cutting areas. This will be on top of the tree-marking goal estimated for the area.

This concept of sustainable forest management plans that also integrate biodiversity and genetic resource conservation measures with timber production is similar to that being developed for the Berau Forest Management Project in East Kalimantan (Tyrie & Natadiwirya 2000). The Kalimantan SFM plan allows forestry practices to continue while at the same time maintaining environmental quality.

Summary and Conclusions

The genetic resource conservation strategies for timber trees in the Philippines include both *ex situ* and *in situ* methods. The timber production forests currently total less than 2 million ha, including about 600,000 ha of forest plantations and tree farms. Some private companies are involved in limited tree improvement programs for plantation species. Although tree plantations and natural forest stands have been identified by the government as seed production areas to serve as primary sources of quality seeds for tree plantations, these remain largely undeveloped and undocumented.

Despite the Philippines' rapid decline of its forest habitats, *in situ* conservation through the protected areas system remains its best hope for conserving genetic resources of timber trees. The protected areas, however, are still limited in their scope, often excluding lowland dipterocarp forests that harbor the majority of the commercial timber trees. Sustainable forest management systems involving integrated and careful planning of timber harvesting operations that incorporate genetic resource conservation measures offer a promising strategy.

The extent of the genetic diversity within and between populations of the commercial timber species in the plantations, and much less, in the natural forests, remains largely unknown. Although there is a high diversity of species and habitats in the Philippines, the financial resources for genetic conservation remain limited.

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In Situ Conservation of Forest Genetic Resources in Thailand

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Abstract. Alarmed by the dramatic decline of forest areas, from 54% to 27.9%, during 1960-1989, prompted the Thai government to declare one of the most drastic forms of environmental protection of the forest resources – a complete ban on logging in the natural forest. As of 1999, *in situ* conservation of forest resources, as protected areas, in Thailand accounted for 96 national parks, 66 forest parks, 48 wildlife conservation areas, 49 non- hunting areas, 15 botanical gardens, and 53 arboretums and covered a total of 86,611.8 sq.km., about 16.9% of the country's area. This figure is one of the highest ratios of protected area to total country area in the world. Aside from the protected areas, national forest reserves have also been proclaimed throughout the country.

Long-term ecological studies of viable populations in their natural habitats should lead to the development of prime and precise management practices for conservation and sustainability of the forest resources. In this regard, an initiative on "*In situ* conservation of forest genetic resources in Thailand" was established in 1999. The aim of this study is to continually obtain comprehensive data from dynamic plant communities including growth, regeneration, mortality, and behavior. These research activities are being conducted in 15 forest-reserve areas throughout Thailand. The fifteen study sites were chosen inside national parks, wildlife sanctuaries, and national forest reserves that represent various types of forests including Tropical evergreen forest, Dry evergreen forest, Coniferous forest, Mangrove forest, Swamp forest, Mixed deciduous forest, and Dry Dipterocarp forest. At each site, a species area curve index was constructed using the "Nested plot" method. A permanent sample plot of 400x400 m (16 ha) was set up and subdivided into 16:100x100-m (1 ha) compartments. Data of species diversity, growth, height, density, frequency and abundance of trees, saplings, and seedlings were collected from diagonal 4:100x100-m plots, and from 25 systematic 4x4-m and 1x1-m plots, respectively. Tree profiles were drawn from a 10x100-m plot. An intensive survey of all selected areas could be performed only at 2 sites due to financial constraints. Research activities in 2001 will emphasize soil analysis, litter fall, C/N ratio, and forest dynamics.

Introduction

Forests are sources of timbers, wood products, foods, fuels and other non-wood forest products including bamboos, rattans, and medicinal plants. These products were adequately available in the past for domestic consumption but supplies are now diminishing due to overexploitation forest areas, mismanagement, and uncontrollable expansion of urban development. Major losses of forestlands in Thailand have occurred during the past 30 years. A forested area of about 53.33% of the total area of Thailand (approximately 27.36 million hectares) in 1961 had been reduced rapidly to only 27.95% (approximately 14.34 million hectares) by 1989 (Forestry Statistics of Thailand, 1998). In response to this situation, the Thai Government declared, in 1989, one of the most drastic forms of environmental protection of the forests - a complete ban on logging in the natural forests. However, illegal logging, shifting cultivation, and agricultural encroachment have continued in some areas and consequently, the remaining forest lands as a percent of total land in 1998 was estimated at only 25.28%, or approximately 12.97 million hectares.

At present, programs for reforestation, management, and development of both the remaining forest resources and the degraded lands are being promoted, with the aim of attaining 40% forest lands, which would then consist of 15% conservation forests and 25% commercial forests. Thailand's protected areas currently account for 96 national parks, 66 forest parks, 48 wildlife conservation areas, 49 non- hunting areas, 15 botanical gardens, and 53 arboretums covering 8,661,180 hectares or about 16.9% of the country's area (Forestry Statistics of Thailand 1999). This figure is one of the highest ratios of protected area to total country area in the world. In accordance with the National Park Act of 1961, management responsibilities of all protected areas are administered and regulated by the Royal Forest Department (RFD). Aside from the protected areas, national forest reserves have also been proclaimed throughout the country. The government has now reached and exceeded its target goal by having more than 15% of the forestland in conservation forests. The next question would be "How can Thailand conserve these diverse forest resources?" To achieve that goal, the country needs participation at all levels, including government enterprises, non-governmental organizations, other institutions, and its people. Recently, a wide range of research activities were instituted, such as long-term ecological studies of the forest resources and diversity of the species, especially those species that possess high commercial values, including timber trees, medicinal plants, bamboos and rattans. The next approach is to promote the utilization of these potentially valuable species. Direct extraction of the species from their natural habitats should be absolutely prohibited while *ex situ* conservation of the forest resources should be emphasized. At the *ex situ* scale, various studies can be done in the areas of genetic improvement, silviculture, and management practices. The improved genetic materials and appropriate technologies can be subsequently transferred to the commercial sectors. This comprehensive study programme will lead to the sustainable management and utilization of the tropical forests, the major source of global biodiversity.

Vegetation types

Forests in Thailand can be classified into two major types, evergreen and deciduous forests, based on plant community: dominant and co-dominant vegetation (Kutintara 1998). The evergreen forests include Tropical evergreen forest, Hill evergreen forest, Dry evergreen forest, Coniferous forest, Mangrove forest, Scrub forest, Beach forest and Swamp forest. The deciduous forests consist of Mixed deciduous forest and Dry Dipterocarp forest, Savanna, Tropical grassland and Bamboo forest. Areas of major forest types in 1998 are shown in Table 1.

Table 1 Areas of major forest types in Thailand in 1998

	Forest type	1998		
		Area (ha)	%1	%2
Evergreen forest	1. Tropical evergreen forests	5,219,818	10.17	40.24
	2. Hill evergreen forest	-	-	-
	3. Dry evergreen forest	-	-	-
	4. Coniferous forests	164,011	0.32	1.26
	5. Mangrove forests	167,559	0.33	1.29
	6. Scrub forests	236	0.0005	0.002
	7. Swamp forests	73,430	0.14	0.57
	8. Beach forest	-	-	-
Deciduous forest	1. Mixed deciduous forests	4,405,696	8.59	33.96
	2. Dry Dipterocarp forests	2,680,527	5.22	20.66
	3. Bamboo forests	260,952	0.51	2.01
	4. Savanna	-	-	-
	5. Tropical grassland	-	-	-
	Total area of forest	12,972,229	25.28	100

Source: Forest Resources Assessment Division, Forest Research Office, RFD
Note: $\%^1$ = % of country area
$\%^2$ = % of forest area

In Situ Conservation of Forest Genetic Resources in Thailand

Study sites and methods:

Long-term ecological studies of plant communities, including their growth and dynamic behavior, have been studied at 15 study sites of 7 major forest types (Table 2). Forest structure and dynamics are studied to understand stand characteristics and changes in tree populations as well as some aspects of stand and individual tree growth. Tree censuses including species compositions and dynamic changes in growth and viability of trees, saplings, and seedlings are planned at an interval of 2 years.

Table 2. Type of forests and study sites for long-term ecological studies on forest genetic resources in Thailand

	Type of Forest	Study Site	Area (ha)
1.	Tropical Evergreen Forest (TEF)		
	- TEF - Hopea [Site 1] - TEF Intsia [Site 2] - TEF - Shorea [Site 3]	- Vieng Ko Sai NP, Phrae - Khao Luang NP, Nakhon Si Thammarat - Hala Bala WS, Narathiwat	160 48 400
2.	Dry Evergreen Forest (DEF)		
	- DEF Dipterocarpus [Site 4] - DEF [Site 5] - DEF [Site 6]	- Pa Klang Ao RF, PRachuab Khiri Khan - Mae Salid - Pong Daeng RF, Tak - Khao Phu Luang RF, Nakhon Ratchasima	192 480 160
3.	Coniferous Forest		
	- Pine and Oak Mixed Forest [Site 7]	- Nam Nao NP, Phetchabun	480
4.	Mangrove Forest [Site 8]	- Ao Khung Kra Ben, Chanthaburi	128
5.	Swamp Forest [Site 9]	- Bang Nara Swamp Forest, Narathiwat	160
6.	Mixed Deciduous Forest (MDF)		
	- MDF with Teak [Site 10] - MDF with Teak [Site 11] - MDF with Teak [Site 12]	- Mae Yom Np, Phrae - Um Phang WS, Tak - Mae Yuam RF, Mae Hong Son	96 560 760
7.	Dry Dipterocarp Forest (DDF)		
	- DDF [Site 13] - DDF with Pine [Site 14] - DDF [Site 15]	- Huay Kha Khaeng WS, Uthai Thani - Phu Khao Kaew & Dong Pak Chom RFs, Loei - Phu Phan Np, Sakhonnakhon	104 160 160

General information of the study sites:

Site 1: TEF – *Hopea*: Vieng Ko Sai: National Park, Phrae
- located at Wang Chin district, 218 km. southwest of Pare
- 600-700 m. above sea level
- average annual rainfall (at Phrae)= 1,227.7 mm.
- average temperature = 26.8 °C
- the lowest temperature = 4.6 °C (during January, 1961-1990)
- the highest temperature = 39.7 °C (during April, 1961-1990)
- relative humidity = 73.6 %

Site 2: TEF – *Intsia*: Khao Luang National Park, Nakhon Si Thammarat
- located at Lan Saka district, 36 km. west of Nakhon Si Thammarat
- lies at the latitudes of 8°23/ N and the longitudes of 99° 45/ E
- 300-400 m. above sea level
- average annual rainfall (at Lan Saka) = 2,683.9 mm.
- average temperature = 26.6 °C
- the lowest temperature = 21.4 °C
- the highest temperature = 31.8 °C
- relative humidity = 92.8 %

Site 3: TEF – *Shorea*: Hala Bala Wildlife Sanctuary, Narathiwat
- located at Sukhirin district, 90 km. south of Narathiwat
- lies at the latitudes of 5°49/ N and the longitudes of 101° 48/ E
- 400-700 m. above sea level

Site 4: DEF – *Dipterocarpus*: Pa Klang Ao Reserved Forest, Prachuab Khiri Khan
- located at Bang Saphan district, 85 km. south of Prachuab Khiri Khan
- lies at the latitudes of 11°15/ N and the longitudes of 99° 30/ E
- 2 m. above sea level
- average annual rainfall (at Bang Saphan) = 1,300 mm.
- average temperature = 27 °C
- relative humidity = 70-80 %

Site 5: Dry Evergreen Forest: Mae Salid – Pong Daeng Reserved Forests, Tak
- located at Sam Ngao district, 60 km. north of Tak
- lies at the latitudes of 17°19/ N and the longitudes of 99° 15/ E
- 600-700 m. above sea level
- average annual rainfall (at Tak) = 1,037.6 mm. (1999)
- average temperature = 27 °C (1999)
- the lowest average temperature = 22.8 °C (1999)
- the highest average temperature = 33 °C (1999)

- relative humidity = 85.8 %

Site 6: Dry Evergreen Forest: Khao Phu Luang Reserved Forest, Nakhon Ratchasima

- located at Wang Namkheaw district, 70 km. south of Nakhon Ratchasima
- lies at the latitudes of 14°26/ N and the longitudes of 101° 51/ E
- 460-520 m. above sea level
- average annual rainfall (at Wang Namkheaw) = 1,200 mm.
- average temperature = 24.8 °C
- the lowest temperature = 8 °C
- the highest temperature = 37 °C
- relative humidity = 78 %

Site 7: Pine and Oak Mixed Forests: Nam Nao National Park, Phetchabun

- located at Nam Nao district, 110 km. northeast of Phetchabun
- lies at the latitudes of 16°30/ N and the longitudes of 101° 23/ E
- 800 m. above sea level
- average annual rainfall (at Lom Sak district) = 1,050-1,300 mm.
- average temperature = 26.4 °C
- the lowest temperature = 19.3 °C
- the highest temperature = 31.6 °C
- relative humidity = 92.8 %

Site 8: Mangrove Forest: Ao Khung Kra Ben, Chanthaburi

- located at Tha Mai district, 30 km. west of Chantaburi
- lies at the latitudes of 12°37/ N and the longitudes of 101° 94/ E
- 0-1 m. above sea level

Site 9: Swamp Forest: Bang Nara Swamp Forest, Narathiwat

- located at Sungai Kolok district, 75 km. west of Narathiwat
- lies at the latitudes of 6°4/ N and the longitudes of 101° 58/
- 8 m. above sea level

Site 10: MDF with Teak: Mae Yom National Park, Phrae

- located at Song district, 90 km. north of Phrae
- lies at the latitudes of 18°35/ N and the longitudes of 100° 10/ E
- 160-180 m. above sea level
- average annual rainfall (at Phrae)= 1,227.7 mm.
- average temperature = 26.8 °C
- the lowest temperature = 4.6 °C (during January, 1961-1990)
- the highest temperature = 39.7 °C (during April, 1961-1990)
- relative humidity = 73.6 %

Site 11: MDF with Teak: Um Phang Wildlife Sanctuary, Tak

- located at Um Phang district, 230 km. southwest of Tak

- lies at the latitudes of 15°51.8/ N and the longitudes of 98° 82.5/ E
- 300-500 m. above sea level
- average annual rainfall = 1,338 mm. (1996-1999)
- average temperature = 18.15-30.86 °C
- relative humidity = 94.7 %

Site 12: MDF with Teak: Mae Yuam Reserved Forest, Mae Hong Son

- located at Mae Sariang district, 165 km. south of Mae Hong Son
- lies at the latitudes of 19°11/ N and the longitudes of 97° 55/ E
- 300-800 m. above sea level
- average annual rainfall = 1,237.5 mm.
- average temperature = 20.6-29.8 °C
- relative humidity = 73.8 %

Site 13: DDF: Huay Kha Khaeng Wildlife Sanctuary, Uthai Thani

- located at Huay Mae Dee sub-station, Ban Rai district, 135 km. southwest of Uthai Thani
- 200-300 m. above sea level

Site 14: DDF with Pine: Phu Khao Kaew & Dong Pak Chom Reserved Forests, Loei

- located at Pak Chom district, 95 km. northeast of Loei
- lies at the latitudes of 18° N and the longitudes of 102° E
- 500-745 m. above sea level

Plot 15: DDF: Phu Parn National Park, Sakhonnakhon

- located at Muang district, 15 km. west of Sakhonnakhon
- lies at the latitudes of 17°12/ N and the longitudes of 104° 5/ E
- 250-310 m. above sea level

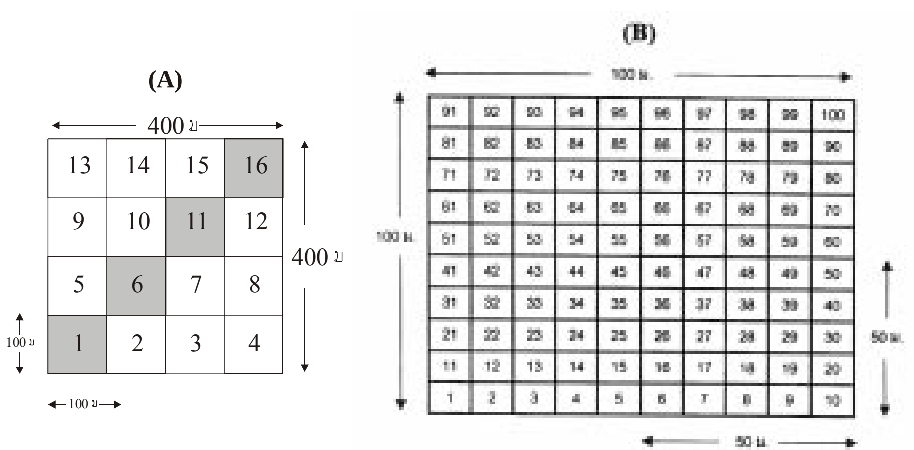
A common methodology has been set up for the research work at all study sites. There might be slight adjustments to fit the topography and other constraints at some sites. As a standard methodology, a permanent sample plot of 400x400 m (16 ha) was established and divided into 16:100x100-m (1 ha) compartments (Figure 1A). Data on species diversity and the growth of trees, saplings, and seedlings were collected from diagonal 4:100x100-m plots, 25:4x4-m plots and 25:1x1-m plots, respectively. Each 100x100 m plot was divided into 100:10x10-m quadrats (Figure 1B) and all trees with diameter at breast height (DBH) equal to or greater than 4.5 cm were marked with aluminum numbered tags. Tags were placed 15-20 cm below a painted ring marking DBH for accuracy when repeating that measurement. Data on tree species, DBH, and height of all tagged trees were recorded, and then the densities, frequencies, and basal areas for each species were calculated. Stem positions and crown projections were mapped, and species composition was checked.

A species area curve index was constructed using a "Nested plot" method. Stand profiles were drawn from a selected transect belt of 10x100 m. Structural characteristics of the trees inside the four 100x100-m plots were investigated to obtain their basic stand information. The Importance Value Index (IVI) - the sum of the percentages of relative density, relative frequency, and relative dominance (Curtis and McIntosh, 1951) - was calculated for each component species of the stands. The relative density and relative frequency of every tree species were determined for this purpose. The relative dominance was computed from the total basal area at breast height, relative to the sum of the basal area of all species. Information on species diversity was computed using the Shanon-Wiener index:H^* (MacArthur and MacArthur, 1961):

$$H^* = -\sum_{i=1}^{n} \boldsymbol{p}_i \log_2 \boldsymbol{p}_i$$

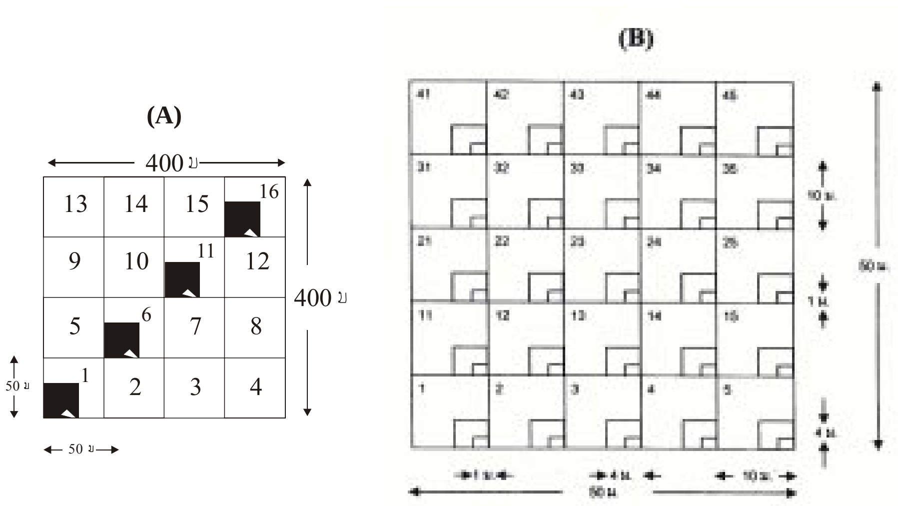
wherein n is the total number of species, and P_i is the proportion of species i to the total species. In this study, all the species that could or could not be identified were counted as one species.

Figure 1 (A) Setting of a permanent 400x400-m sample plot (16 ha) and systematic selection of 4:100x100-m (1 ha) plots.
(B) Setting of 100:10x10-m quadrats inside each 100x100-m plot



Structural characteristics of those saplings having a height equal to or more than 1.30 m but DBH less than 4.5 cm were done inside the 25:4x4-m plots. The 4x4-m plots were systematically sampled inside the 25:10x10-m plots (Figure 2A and B). For seedlings, data on species and covering area were recorded from the 25:1x1-m plots (Figure2B).

Figure 2 (A) Systematic selection of 25:10x10-m plots inside the 4: 100x100-m plots
(B) Setting of 25:4x4-m and 25:1x1-m plots.



Results and discussion

This study was established in late 1999. The first two years of this study should provide information on stand structure, species composition and its importance, species diversity index, species area curve index, and stand profiles at all 15 study sites which represent 7 major forest types throughout Thailand. This paper, however, reports only on the IVI values of the first 5 species in 4 study sites representing 4 different forest types (Tables 3-6) and the species diversity indices of 12 study sites (Table 7). The IVI values and the species diversity indices of some study sites are absent because data collection and calculation have not been completed.

The species composition of the Tropical evergreen forest at Hala Bala Wildlife Sanctuary, Narathiwat province is dominated by *Shorea leprosula*, Lun Ton (scientific name being investigated), and *Hopea sangal* (Table 3).

The ecologically- important tree species judged by the IVI values are *Shorea leprosula*, *Polyalthia viridis*, *Hopea sangal* and *Barrington* spp. The associated species differ from plot to plot. All four 100x100-m plots have different association types of the important tree species: *Polyalthia viridis-Shorea leprosula*, *Shorea leprosula*-Lun Ton (scientific name being investigated), *Shorea leprosula- Hopea sangal* and *Shorea leprosula- Hopea sangal*. The completely closed canopy of this forest type is at least 30 m high. Abundant epiphytes and lianas are found on the large trees and the undergrowth is mostly herbaceous.

The species composition of the Pine and Oak Mixed forest at Nam Nao National Park, Phetchabun province has been studied at 2 sites nearby. One site (Plots 1 and 2) was selected for studying the species composition of *Pinus kesiya*, and the other site (Plot 3) for studying on *P. merkusii*. The locations of these 2 sites are at almost the same altitude (800 m above mean sea level). The dominant and ecologically important tree species of Plots 1 and 2 are *Dipterocarpus obtusifolius, Pinus kesiya*, and Kor Talap (an Oak species, scientific name being investigated) (Table 4). The dominant and ecologically important tree species of Plot 3 are *Pinus merkusii*, and *Aporosa villosa*. These 3 plots have different association types of important tree species: *Dipterocarpus obtusifolius-Pinus kesiya*, Kor Talap (an Oak species, scientific name being investigated)-*Dipterocarpus obtusifolius-Pinus merkusii,* and *Pinus merkusii-Aporosa villosa*, respectively. A suitable description of this forest type might be “a transition zone between the deciduous forests at lower elevations and the temperate evergreen forests at higher elevations” (Anderson, 1993). There are only 2 native Pine species in Thailand- *Pinus kesiya* and *P. merkusii* - and both of them are the indicator species for this forest type. Natural regeneration of *P. kesiya* and *P. merkusii* is rare, since pine forests have been chronically disturbed. Local people habitually burn or tap the pine trees for resin, as well as removing the pitch-filled wood for sale as kindling to start charcoal fires. These activities have degraded the reproductive capability of the pine trees.

The species composition of Mixed Deciduous Forest with teak: at Um Phang Wildlife Sanctuary, Tak province is highly dominated by *Tectona grandis* (Table 5). The Mixed deciduous forests that are widespread throughout northern Thailand are best characterized by the presence of teak. The ecologically important tree species are *Tectona grandis*, *Schleichera oleosa*, *Terminalia alata*, *Croton* sp., and *Dillenia parviflora*. he associated species differ from plot to plot. All four 100x100-m plots have different association types of important tree species: *Tectona grandis-Schleichera oleosa*, *Tectona grandis-Terminalia alata*, *Tectona grandis-Croton* sp., and *Tectona grandis-Dillenia parviflora*. Teak is a highly important tree species in this study site with IVI values of about 87-155, while the IVI values of other top 5 species ranges from about 8-32. The upper-canopy trees (23-30 m. or more), mainly teak, are more-or less touching, but are not densely packed. Many tree species shed leaves during dry season. Bamboos commonly associated with this forest type are *Dendrocalamus membranaceus*, *Bambusa bambos*, and *B. longispatha*.

The Mixed deciduous forest without teak, with a more-or -less open canopy, can be found at the lower north southwards to the western region. Common tree species in the Mixed deciduous forest without teak are *Afzelia xylocarpa, Bombax insigne, Gmelina arborea, Terminalia bellerica, Lagerstroemia calyculata*, and *L. tomentosa* (Kutintara 1998).

The species composition of Dry Dipterocarp Forest with Pine: at Phu Khao Kaew – Dong Pak Chom Reserved Forests, Loei province is dominated by *Shorea siamensis*, *Shorea obtusa*, and *Pinus merkusii* (Table 6). The ecologically important tree species are *Shorea siamensis*, *Dipterocarpus obtusifolius*, *Shorea obtusa* and *Pinus merkusii*. The associated species differ from plot to plot. All four 100x100-m plots have different association types of important tree species: *Shorea siamensis-Dipterocarpus obtusifolius*, *Shorea siamensi-Shorea obtusa* and *Shorea siamensis-Pinus merkusii* and *Shorea obtusa-Pinus merkusii.* A better name for this forest type at this particular area may be " the Pine deciduous dipterocarp forest" as described by Greijmans (2001).

The total number of tree species and species diversity indices of 12 study sites representing 6 forest types are presented in Table 7. The Tropical evergreen forest undoubtedly has the highest values of species diversity and tree density. The species diversity indices of the 3 study sites in the Tropical evergreen forest (Sites 1-3) are 4.721, 5.923, and 5.738 whereas those in the Dry evergreen forest (Sites 4-6) are 3.770, 5.380, and 4.014, respectively. Coniferous forests at Nam Nao National Park, Petchabun, and Swamp forest at Bang Nara, Narathiwat have species diversity indices of 2.93 and 3.55, Dry evergreen forest (Sites 4-6) are 3.770, 5.380, and 4.014, respectively. Coniferous forests at Nam Nao National Park, Petchabun, and Swamp forest at Bang Nara, Narathiwat have species diversity indices of 2.93 and 3.55, respectively. The species diversity indices of the 2 study sites in the Mixed deciduous forest (Sites 10-11) are 4.508 and 3.831, whereas those in the Dry Dipterocarp forest (Sites 14-15) are 2.907 and 3.161, respectively. The Mixed deciduous forests at Mae Yom National Park (Site 10) and Um Phang Wildlife Sanctuary (Site 11) contain greater numbers of tree species than those of the Dry Dipterocarp forests at Phu Khao Kaew – Dong Pak Chom Reserved Forests (Site 14) and Phu Parn National Park (Site 15). The Dry Dipterocarp forests, both at Sites 14 and 15, on the contrary contain greater numbers of plant densities than the Mixed deciduous forests at Sites 10 and 11.

Table 3 Dominant tree species in 4:100x100 m plots: Tropical Evergreen Forest – *Shorea*: Hala Bala Wildlife Sanctuary, Narathiwat

Rank	Species	Density (tree/ha)	Relative Density (%)	Frequency (plot/ha)	Relative Frequency (%)	Dominance (m2/ha)	Rela Domina
Plot 1:							
1	*Polyalthia viridis*	115	11.70	63	8.89	2.04	6.8
2	*Shorea leprosula*	37	3.76	18	2.54	3.77	12.
3	*Barringtonia* sp. (Chik Nommeaw)	77	7.83	39	5.50	1.20	4.0
4	(Faad Khao)	56	5.70	35	4.94	1.46	4.9
5	*Syzygium sp.* (Waa Hin)	57	5.80	39	5.50	1.01	3.3
Plot 2:							
1	*Shorea leprosula*	24	2.22	19	2.18	13.04	27.
2	(Lun Ton)	1	0.09	1	0.12	6.84	14.
3	*Barringtonia* sp. (Chik Khao)	94	8.68	37	4.24	0.40	0.8
4	*Barringtonia* sp. (Chik Nommeaw)	57	5.26	41	4.70	1.14	2.3
5	(Daeng Khao)	42	3.88	37	4.24	0.86	1.7
Plot 3:							
1	*Shorea leprosula*	78	8.97	35	5.08	14.68	32.
2	*Hopea sangal*	78	8.97	50	7.26	3.35	7.3
3	*Syzygium* sp. (Faad Kao)	36	4.14	29	4.21	0.57	1.2
4	(Lun Ton)	1	0.12	1	0.15	3.83	8.4
5	*Syzygium sp.* (Waa Hin)	31	3.56	26	3.77	0.54	1.1
Plot 4:							
1	*Shorea leprosula*	44	6.56	33	6.08	20.95	46.
2	*Hopea sangal*	97	14.46	53	9.76	2.40	5.3
3	*Barringtonia* sp. (Chik Nommeaw)	53	7.90	33	6.08	1.35	3.0
4	*Syzygium* sp. (Faad Kao)	34	5.07	26	4.79	1.99	4.4
5	*Litsea monopetala*	22	3.28	15	2.76	1.37	3.0

Note: ? = scientific name still under investigation
(name in brackets) = Local name

Table 4 Dominant tree species in 1:100x100-m and 2:50x50-m plots: Pine and Oak Mixed Forests: Nam Nao National Park, Phetchabun

Rank	Species	Density (tree/ha)	Relative Density (%)	Frequency (plot/ha)	Relative Frequency (%)	Dominance (m2/ha)	Rela Dominan
Plot 1: 50x50 m: *P. kesiya*							
1	*Dipterocarpus obtusifolius*	95	37.11	38	26.76	2.60	18.
2	*Pinus kesiya*	32	12.50	23	16.20	7.35	53.
3	*Quercua/Lithoscarpus/Castanopsis ?* (Kor Talap)	73	28.52	33	23.24	7.35	22.
4	*Querkus kerrii*	15	5.86	10	7.04	0.64	0.4
5	*Lithocarpus fenestratus* ?	6	2.34	6	4.23	0.14	1.0
Plot 2: 50x50 m: *P. kesiya*							
1	*Quercua/Lithoscarpus/Castanopsis* ? (Kor Talap)	201	29.65	81	23.41	39.90	21.
2	*Dipterocarpus obtusifolius*	109	16.08	57	16.47	3.77	20.
3	*Pinus kesiya*	61	9.00	34	9.83	5.83	31.
4	*Lithocarpus polystachyus*	81	11.95	42	12.14	10.02	5.5
5	*Quercua/Lithoscarpus/Castanopsis ?* (Kor Hin)	59	8.70	28	8.09	0.75	4.1
Plot 3: 100x100 m: *P. merkusii*							
1	*Pinus merkusii*	131	27.35	72	27.80	48.25	89.
2	*Aporosa villosa*	189	39.46	68	26.25	1.96	3.6
3	*Dipterocarpus obtusifolius*	19	3.97	18	6.95	0.68	1.2
4	*Lithocarpus polystachyus*	24	5.01	14	5.41	0.41	0.7
5	*Quercua/Lithoscarpus/Castanopsis* ? (Kor Baileai)	24	5.01	11	4.25	0.89	1.6

Note: ? = scientific name still under investigation
(name in brackets) = Local name

Table 6 Dominant tree species in 4:100x100-m plots: Dry Dipterocarp Forest: Phu Khao Kaew & Dong Pak Chom Reserved Forests, Loei

Rank	**Species**	**Density (tree/ha)**	**Relative Density (%)**	**Frequency (plot/ha)**	**Relative Frequency (%)**	**Dominance (m2/ha)**	**Dom**
Plot 1:							
1	*Shorea siamensis*	683	53.40	97	23.21	10.51	
2	*Dipterocarpus obtusifolius*	167	13.06	74	17.70	3.43	
3	*Quercua/Lithoscarpus/Castanopsis ?* (Kor Kao)	117	9.15	53	12.68	2.21	
4	*Shorea obtusa*	91	7.11	36	8.61	1.41	
5	*Quercua/Lithoscarpus/Castanopsis ?* (Kor Nam)	59	4.61	35	8.37	1.27	
Plot 2:							
1	*Shorea siamensis*	277	17.40	86	18.66	3.66	
2	*Shorea obtusa*	179	16.69	66	14.32	3.51	
3	*Quercua/Lithoscarpus/Castanopsis ?* (Kor Kao)	154	14.87	63	13.67	3.13	
4	*Shorea roxburghii*	81	14.01	50	10.85	2.95	
5	*Dipterocarpus obtusifolius*	174	11.23	69	14.97	2.36	
Plot 3: with Pine							
1	*Shorea siamensis*	242	29.30	66	17.84	3.48	
2	*Pinus merkusii*	77	9.32	43	11.62	7.86	
3	*Dipterocarpus obtusifolius*	164	19.85	74	20.00	2.95	
4	*Shorea obtusa*	89	10.77	42	11.35	1.85	
5	*Quercua/Lithoscarpus/Castanopsis* ? (Kor Nao)	83	10.05	30	8.11	1.76	
Plot 4: with Pine							
1	*Shorea obtusa*	484	38.20	83	18.28	7.56	
2	*Pinus merkusii*	74	5.84	36	7.93	6.34	
3	*Dipterocarpus obtusifolius*	134	10.58	71	15.64	1.80	
4	*Quercua/Lithoscarpus/Castanopsis ?* (Kor Kao)	106	8.37	38	8.37	1.87	
5	(Kaokao) = Unknown 1	101	7.97	49	10.79	1.28	

Note: ? = scientific name still under investigation
(name in brackets) = Local name

Table 7 Total number of tree species, total density, total frequency, total basal area and species diversity index of 12 study sites within 6 forest types

Conclusion

Long-term ecological studies of plant communities, including their growth and dynamic behavior, have been conducted at 15 study sites representative of 7 major forest types including the Tropical evergreen forest, Dry evergreen forest, Coniferous forest, Mangrove forest, Swamp forest, Mixed deciduous forest and Dry Dipterocarp forest. The study was established in late 1999. Data on tree species, DBH, and height of all trees were collected and the density, frequency, and basal area of each species were calculated. The IVI values of the first 5 ecologically important tree species in 4 study sites and the species diversity indices of 12 study sites are reported in this paper.

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Conserving Tropical Forests: Brazil's Pilot Program

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Abstract. In situ and ex situ conservation of commercial trees are not purely scientific or technical endeavors; they take place within a broader social, economic, institutional, and political framework. Within this broader framework, the Pilot Program to Conserve the Brazilian Rain Forest offers a range of relevant experience for tropical forest management in Southeast Asia. This paper presents: a) background on Brazil's tropical forest ecosystems; b) an overview of the Pilot Program (brief history, objectives, highlights); c) summaries of relevant projects; and d) programmatic lessons learned and next steps. It is hoped that the paper will assist with the second half of the Conference goal, "to identify fruitful areas for improving the tropical forests."

Background on Brazil's Tropical Forest Ecosystems

The Pilot Program operates in Brazil's two tropical forest ecosystems – the Amazon and the Atlantic Rain Forest. The Amazon Forest is characterized by:

- Size – the Brazilian portion of the forest covers 4.8 million km.^{2}, or 58% of the country's total land mass;
- Biodiversity – the Amazon contains more than half of the world's biodiversity and includes 20 million examples of the species *Homo sapiens*;
- Water resources – the aquatic systems of the forest yield 15% of world's freshwater river discharge;
- Deforestation – through the year 2000, about 580,000 km.^{2} of the Amazon has been deforested, representing 14% of the total forest area. Of this, 150,000 km.^{2} has been abandoned after use for farming and pasture; and
- Timber – The Amazon currently produces about US$1 billion in timber annually. Unlike Southeast Asia, over 85% of this production is consumed domestically. Brazil is also the world's largest consumer of tropical hardwood, using 34 million m^3/yr. (as a reference point, Indonesia consumes 19 million m^3/yr.).

The Atlantic Rain Forest presents a much different picture. It has been intensively exploited for 500 years and only fragments remain today. However, it provides valuable services and is one of the world's most critical ecosystems, as indicated by the following points:

- Size – The original dominion (ca. 1500 AD) covered an area of 1.48 million $km.^2$. Today, however, only 95,000 $km.^2$ (or 7.3%) remains in a series of coastal and inland fragments;
- Biodiversity – there is a high level of biodiversity and endemism throughout the ecosystem. It has been identified as one of the world's top four biodiversity "hotspots" and one of Conservation International's priority ecosystems for the year 2002;
- Water resources – the dominion encompasses the watersheds that provide water supply for more than 120 million people; and
- Human value – more than 80% of Brazil's population lives within the dominion. Importantly, it provides shelter for various traditional populations (indigenous as well as descendants of Afro-Brazilian slaves).

Overview of the Pilot Program

Objectives. The Pilot Program to Conserve the Brazilian Rain Forest was created to protect the biodiversity of the Amazon and Atlantic Forests, reduce carbon emissions, promote the improved quality of life of local populations, and provide experience in international cooperation on global environmental issues. It is designed to develop, test and learn from opportunities to maximize rainforest environmental benefits while being consistent with Brazil's development objectives. Goals and objectives were revised in June 2001 as reflected in Box 1.

Box 1: Pilot Program Goals and Objectives

To contribute to policies that promote conservation and sustainable development of Brazil's Amazon and Atlantic rain forests, by pursuing the following objectives:

* generating and disseminating relevant and reliable knowledge within Brazil and in the Amazon region
* catalyzing the adjustment of policies and mobilizing political support for such policies
* promoting the mainstreaming of successful experiences and models
* building capacity in public, private and civil society institutions to implement such policies and apply new knowledge

History. The Program was first proposed by the German Government in 1990 at a G-7 meeting in Houston, USA. Formulated in 1991 and established in 1992, it is a joint effort of the Brazilian Government, the G-7 and Dutch Governments, and the Commission of European Communities, broadly supported by their civil societies.

With approximately US$340 million in resources and pledges, the Pilot Program is now managed by the Brazilian Government. The World Bank administers the Rain Forest Trust Fund, which provides 22% of total resources. The remainder is provided by bilateral and multilateral donors through co-financing and technical cooperation, and by Brazilian funds and the project beneficiaries themselves.

The Program began implementation in 1995. Its projects are grouped in five complementary areas: 1) experimentation and demonstration, 2) conservation, 3) institutional strengthening, 4) conducting scientific research, and 5) learning and disseminating lessons. Overall, Program implementation should serve as a model for supporting sustainable development in rain forest regions.

Achievements. Highlights of Program accomplishments by area include:

Experimentation and Demonstration

- Funding of demonstration projects in some 190 communities and organizations that experiment with new approaches to using and conserving natural resources in the rain forests;
- Training of more than 12,000 people from 322 municipalities in fire prevention through a partnership between civil society organizations and the government;

Conservation

- Establishing and consolidating four extractive reserves**,** totaling 2 million hectares (ha.) in the Amazon;
- Demarcating and legalizing 29 million ha. of lands inhabited by indigenous people, in part with the active participation of the indigenous people themselves and of specialized NGOs;
- Introducing and gaining wide acceptance for the concept of rain forest corridors, (networks that link protected areas and the buffer zones around them);

Institutional Strengthening

- Building capacity at the state and municipal level for natural resource management in the nine states of the legal Amazon;
- Supporting the creation of strong NGO networks in the Amazon and Atlantic rain forests that link 650 NGOs;
- Encouraging public-private partnerships around specific products to be produced and marketed under innovative, sustainable conditions in the Amazon and the Atlantic rain forests; and

Scientific Research

- Investments in infrastructure to modernize two important scientific centers in the Amazon (the Emilio Goeldi Museum in Belém and the National Institute for Amazon Research - INPA) in Manaus, and US$14 million in funding for 23 "directed research" projects.

Relevant Projects

The Program has strategically organized its projects in five complementary lines of action: 1) experimentation and demonstration, 2) conservation, 3) institutional strengthening, 4) conducting scientific research, and 5) learning and disseminating lessons. In addition, there are plans for new crosscutting initiatives.

Program implementation should serve as a model for supporting sustainable development in rain forest regions. Each line of action has projects in various stages of implementation and preparation, as indicated in footnotes to the figure. Overall, the Program currently has three projects/subprograms completed which are being renewed (Extractive Reserves, Science and Proteger), six ongoing projects (Natural Resources Policy, Demonstration Projects, Indigenous Lands, Forest Resources Management, Forest Fire Prevention, and Monitoring and Analysis), one ready to begin (Floodplain Resources Management), and several in latter stages of preparation (especially the cross-cutting, business-oriented Sustainable Production Project, the Indigenous People's component of Demonstration Projects, Rain Forest Corridors, and Deforestation and Fire Control). Objectives and recent accomplishments are described below by line of action.

Experimentation and Demonstration

This line of action seeks to generate and transfer knowledge on sustainable management of natural resources through local communities and civil society organizations. Two projects are being implemented (Demonstration Projects and Forest Resources Management) and one has met conditions precedent and will start up very shortly (Floodplain Resources Management). Several additional components are being prepared for Demonstration Projects, especially the Indigenous People's Component. The latter two have built on the successes of the Demonstration Projects, especially its agile implementation procedures and development of monitoring techniques.

Demonstration Projects (PD/A)

Objectives and Subprojects

The PD/A has the following objectives:

a) Generate knowledge on the conservation and sustainable management of natural resources through demonstration activities and involvement of local communities;
b) Transfer knowledge resulting from experiences to other communities, NGOs, decision-makers, government technicians and other representative groups
c) Strengthen organization and articulation capacity of local populations, as well as their capability to prepare and implement projects.

The project supports subprojects in four theme areas: environmental conservation projects (5%), forest management systems (31%), agroforestry systems and recuperation of degraded areas (53%) and aquatic resource management systems (11%).

By end-May 2001, 188 subprojects were approved, including 11 with conditions. Of these, 147 are either under implementation or concluded. The project monitoring and evaluation system is now fully tested and refined by the technical team, and monitoring activities are currently underway. In addition, the Technical Secretariat is now delivering capacity-building courses to help executors of subprojects beginning develop self-monitoring systems.

Based on the geographic concentration of subprojects in the same thematic areas, the Project is developing a sustainable development pole concept which will help unify and package subproject results for dissemination, and also facilitate the development of partnerships with state governments, and key institutions like BNDES, BASA, universities and the DED (German NGO which provides technical cooperation services).

Project review

A sample of 29 subprojects was selected among the 46, which concluded or are about to conclude implementation this semester. According to the reviewers who consolidated the conclusions and recommendations of the evaluation process, the project has contributed to changes in the behavior of local implementers and neighboring communities, reduced use of fire and deforestation in and around subproject areas, thus enhancing biodiversity conservation; it has also strengthened producer organizations, thereby increasing their chances to access additional grant funds and/or commercial credit lines. However, there is insufficient data on their economic and financial feasibility.

The Project review process was completed at the end of May 2000 with a very participatory workshop. Key follow-up recommendations of workshop participants are:

1. Project continuity:
   - Continue to meet demands, learning from consolidated lessons to improve new projects;
   - Maintain and expand the government-civil society partnership in management beyond the Program;
   - Support changes in the formal rural school system to build local capacity for sustainable development;
   - Strengthen the small project line as an incubator of medium and large projects;
   - Request budgetary funds to support Project activities;
   - Stimulate proposals which take an integrated view of the family or micro-basin production unit, taking into account issues such as food security, increased respect for traditional practices, environmental characteristics and legislation;
   - Integrate subprojects into the context of regional development policies, without losing sight of the role in promoting projects which support conservation and sustainable natural resource use;
   - Strengthen technical assistance to prepare project proposals and implement them;
   - Study the possibility of redirecting PDA resources to seek partnerships with other environmental financial mechanisms to design and implement a training program for technical assistance, implementers and technical staff;

2. Strengthening ongoing experiences
   - Intensify and implement strategies of exchange and dissemination, including mechanisms to exchange technological and management information within the Pilot Program;
   - Strengthen PDA staff and partnerships for continuous technical monitoring, evaluation and feedback of subproject activities;
   - Invest in articulating and monitoring partnerships seeking new financing, improved administrative structure, research, coordination with the public sector, based on a regional pole approach and theme-based networks;
   - The SCA should interact with state governments to incorporate lessons from PDA subprojects into public policies;
   - Intensify studies of lessons learned, interacting with other projects to avoid duplication;

- Stimulate subprojects to analyze and organize their own lessons for dissemination;

The environment education component is still not effective.

Indigenous People's Component

After some delays in the definition of scope and funding, the Indigenous People's Component (PDPI) of the Demonstration Projects has been moving forward. Its objective is to contribute to the protection of Brazilian rain forests by improving the economic, social and cultural sustainability of indigenous people, promoting conservation on their lands and supporting planning and implementation for local initiatives that could serve as demonstration projects. Appraisal was completed in February 2000 under KfW and GTZ leadership, the first application of the "lead donor" concept where the Rain Forest Trust Fund and thus the World Bank do not participate in project funding and supervision. Preparation is being completed with a US$494,800 grant from Japan, including a number of participatory activities with indigenous organizations.

The component will include four subcomponents: (i) small grants for surveillance and protection of indigenous lands, economically sustainable activities and cultural revitalization, enhancing self-esteem and improving capacity to manage natural resources, (ii) subproject management, (iii) capacity-building and dissemination of subproject results to other communities and specialists, and (v) institutional strengthening of indigenous organizations, empowering them to negotiate with other actors.

The component of the Demonstration Projects will be implemented over a five-year period with an estimated funding of DM 20 million from Germany, £1.5 million from UK and US$2.5 million from the Brazilian Government. As requested by the Council of Indigenous Organizations in the Amazon (COIAB), the coordinating unit will be located in Manaus and headed by a selected indigenous leader with management experience.

Forest Resources Management Project

Objectives and Initial Accomplishments

Started in January 1999, the US$20 million Promanejo project is well underway. The objective is to promote sustainable forest management through:

- Strategic studies to revise forest policy and incentives. The Forest Sector Study Group was created and met in February and April 2000 to prepare a work plan and define terms of reference for the first five studies. Results are feeding directly into the forest management component of the National

Forest Program, launched in April 2000. This is an example of cooperation between agencies within the Ministry of Environment towards a common goal.

- Support for promising sustainable forest management initiatives. The Project's Executive Committee selected four of eight initial proposals, totaling approximately $2 million including: a) sustainable forest management by the University of Amazonas – Gelthal Amazonas partnership, b) the Socio-Environmental Institute's partnership with the Xicrin indigenous people, c) a forest management training film with subsequent training to be prepared by the Tropical Forest Foundation, and d) community forest management in the Mamiaua Reserve. A second call for proposals went out in February 2000 with a deadline in June 2000. The Project has benefited from PD/A's experience with evaluation and selection procedures due to the close collaboration between the two projects.
- Development and testing of monitoring and control systems of forest activities. Signed agreements with SECTAM and IPAAM (Pará and Amazonas state environment agencies respectively) to help carry out this component. Prepared first phase terms of reference and planned workshop.
- Support for community forestry conservation and management in the Tapajós National Forest. The 2000 annual work plan was reviewed and training of community volunteers both took place in December 1999. A workshop was held in Santarém in February 2000 to review a regional ecotourism plan. An environmental education plan was prepared. Meetings with communities were held to promote the component. Four community forestry proposals have been approved by the Management Group for a total of over $100,000. A total of 300 people organized for vegetable oil production and a management plan being prepared. A surveillance plan is being prepared for the Tapajós National Forest. A consultant was hired to facilitate exchanges among various community initiatives. Assistance was provided to National Forest management in establishing its management group and in surveying resident families.
- The project has also promoted two major events: In September 1999, a workshop was held in Marabá on community forest management that brought together representatives of communities and NGOs to share experiences. In October 1999, a workshop was held in Manaus on "Sustainable Wood Production" attended by 140 participants from the wood industry, banks, buyers, government and NGOs. Forest certification was seen as a key instrument to promote sustainable management. A buyer group is setting up a certification standard in Brazil while a task force is defining forestry management and financing criteria. Meetings were held with lumber firms to promote the component. Both workshops were done

in close partnership with several institutions (PD/A, GTZ, WWF, USAID/ SUNY, Friends of the Earth).

As a result of the sustainable production workshop:

- Six lumber firms have sought BNDES funding to certify their forestry management plans, including one that is ready to obtain the first loan of its kind.
- Twelve lumber firms are seeking risk capital from the Banco Axial to adapt to new market requirements (management and certification). One has received a loan and six of the requests are in advanced stages.
- BNDES and BASA are redesigning their credit lines to favor certification.
- The German investment bank DEG received two Amazon lumber firm proposals to certify and export to Europe.
- IMAFLORA, the FSC certification entity in Brazil has been swamped with Amazon visitors interested in certification, multiplying demand by 5 since the workshop.
- A certified wood buyers group established at the workshop now has 40 members, coordinated by the NGO Amigos da Terra.

- The Project Coordinating Units in Manaus and Santarém are fully staffed and functioning. The GTZ has provided technical assistance for overall project management and consultants are being hired to help implement the Tapajós component. The professional project staff, Bank of Brazil and beneficiaries were trained in financial, monitoring and reporting procedures with PD/A assistance.

Floodplain Resources Management Project (PROVÁRZEA)

This US$15.5 million project aims to promote the conservation and rational use of floodplain ecosystems, with an emphasis on fisheries and other aquatic life, through technical and scientific knowledge needed to formulate policies. It has three components:

- Studies to provide the knowledge base needed;
- promising initiatives to test the feasibility of sustainable practices; and
- an integrated system for control and monitoring fisheries activities.

Funding from DfID and the Rain Forest Trust Fund is in place. Effectiveness conditions for the RFT grant should be fulfilled in June of this year, with the Bank's waiver of one condition of effectiveness, relating to the signing of KfW grant agreements.

Three senior project coordination staff are being recruited via a national open call. Once effectiveness is reached and staff are in place, the launch seminar will be held and the already-nominated Project Advisory Commission (PAC) will be convened. The MMA is giving this project high priority and visibility.

According to the Mid-Term Review, the following lessons were learned during project preparation:

- Broad participatory discussions lead to stakeholder integration
- Joint actions by IBAMA, MMA and other actors facilitated project preparation and start-up.
- Exchange with other Program projects avoided duplicated efforts.

Reducing delays and bureaucracy in preparing and approving projects limits beneficiary and participant complaints and loss of Program credibility.

Conservation

This line of action is key to reducing deforestation and protecting biodiversity, by supporting efforts of the forest's peoples or connecting protected areas into corridors. Two projects have been recognized as having a significant impact on reducing deforestation: Extractive Reserves and Indigenous Lands. The third project, Rain Forest Corridors, the first to apply the principle of establishing corridors by connecting various types of protected areas, thus enhancing biodiversity, is in the process of being redesigned.

Extractive Reserves Project (RESEX I)

Results

The Program Mid-Term Review concluded that RESEX brought the following results:

- Extractivists have reduced deforestation within the reserves and also led to encouraging results in surrounding areas.
- Amazonian and Brazilian organized civil society and extractivists are more aware of the reserves' importance.
- Partnerships with NGOs, universities and research institutions increased.
- An efficient participatory monitoring system was developed which is being copied on other projects.
- Reserve development planning procedures have become consolidated instruments for enhancing resource use and income.

Current Status

The completion of first phase of the project was extended from April to December 1999. RESEX II, concentrating on achieving long-term sustainability, was appraised in October 1998, but has not become effective due to differences between the World Bank and the European Community on provisions of a Financing (Trust Fund) Agreement between them. The lack of additional funds has resulted in discontinuity of the project and demobilization of its actors.

Accomplishments

Major achievements during this period include:

- The use concessions for the Chico Mendes and Alto Juruá Reserves are being negotiated with the Federal Property Secretariat since March 1999. Chico Mendes data were updated by researching local registry offices. Regular surveillance campaigns with tapper organizations have been carried out to maintain reserve integrity from acts of degradation by outsiders.
- The Rio Cajari Reserve Brazil nut and Porto Velho rubber processing plants were completed and marketing plans continued. The CNPT is cooperating with the Amazônia Exchange in strengthening cooperatives and promoting business. IBAMA funds were provided for marketing rubber and Brazil nuts to the Chico Mendes and Rio Ouro Preto reserves. Studies for Rio Cajari ecotourism facilities and vegetable oil potential were completed.
- Training provided to 150: 15 teachers, 30 cooperating inspectors and 54 statistical agents, 50 families on production and marketing of líquid smoked leaves (FDL).

In order to maintain extractivist self-confidence in their management capacity while faced with reduced resources, the CNPT sought other funding sources. IBAMA allocated an additional R$804,000, Amazon Bank (BASA) financing was obtained for 239 families in three reserves, IBAMA worked with extractivist collaborating inspectors on environmental control campaigns and by constantly expressing confidence in the continuation of the project.

Indigenous Lands Project (PPTAL)

Objectives

The project's objectives are to improve the conservation of natural resources in indigenous areas and increase the well being of indigenous people by i) regularizing indigenous lands in the Legal Amazon; and ii) improving protection of indigenous populations and areas. It develops and applies approaches that are compatible with both indigenous forest management and environmentally appropriate technologies.

Performance and Plans

Under the PPTAL, 29 million hectares of indigenous lands have already been demarcated. However, during 1999, a supervision mission expressed concern about slower project performance, noting that only seven of the fifteen working groups to conduct identifications were formed. None of the expected demarcation activities were completed due to delays in contracting firms to carry them out. While the pace picked up in the second half of 1999, administrative bottlenecks are still a concern. The recent resignation of FUNAI's president heightened concerns regarding administrative instability and project implementation. A new president, Mr. Glenio da Costa Alvarez, was appointed May 2000.

The 2000 Annual Operating Plan (POA), approved in March 2000, succeeded in intensifying land regularization efforts throughout the year to bring PPTAL back on track in terms of meeting project goals. It includes plans to identify 20 and demarcate 32 indigenous lands and implement six participatory surveillance and protection plans.

Achievements

The Project continues to make important inroads in the enhancement of technical and participatory aspects of indigenous land regularization, improved identification procedures and environmental evaluations, and in socio-economic assessment methods of non-Indian residents (regarding tenure, compensation and resettlement procedures).

Enhanced indigenous participation in demarcation is being achieved in Vale do Javari, Munduruku and Poyanawa lands and in ethno-ecological studies, underway in eleven lands. The latter will provide a comprehensive view of the biophysical, land use and socio-economic characteristics of each area, assisting management planning and sustainable use of natural resources.

Monitoring

While the priority list of indigenous lands continues to function as a powerful monitoring tool, the monitoring and evaluation system is not yet fully operational, pending a final list of impact indicators (Also see indigenous people's component).

Rain Forest Corridors Project

The Project is designed to conserve biodiversity by implementing “corridors” in the Amazon and Atlantic rain forests, strengthening, creating and, where possible, linking protected areas, indigenous lands and private reserves. It currently consists of four components, concerning the two priority corridors in the Central Amazon and Northern Atlantic Forest: (i) planning and monitoring, (ii) management and establishment of conservation units, (iii) management of critical connecting areas, and (iv) biodiversity protection on indigenous lands. A fifth component of strategic coordination and studies, including plans for further corridors, may be carried out by MMA based on further consultations.

The project was pre-appraised in June 1998, leading to a consensus on the future vision for these corridors, yet leaving questions concerning project management and implementation mechanisms. Government had provided a project proposal in December 1998. It was presented to donors and received comments in February 1999. Several issues remained unresolved until discussions on the project resumed in 2000 and will start implementation during the fourth quarter of 2001. Total donor support is currently estimated at about US$ 30 million.

Institutional Strengthening

This line of action has made significant inroads in decentralizing environmental issues by strengthening organizations of state and municipal governments as well as supporting civil society organizations to spread new ideas and practices. It consists of three projects: Natural Resources Policy, Forest Fire Prevention and Deforestation and Forest Fire Control. The first, providing state governments with control and enforcement capacity, is in latter half of its implementation. The second has been successfully implemented by the Amazon Working Group, an NGO network established with Program support; a second phase is being designed. The latter project is in the process of being redesigned.

Natural Resources Policy Project (NRPP)

Key design strengths and results

This Project’s design strengths and main achievements are:
- greater coordination among the agencies involved in fire-deforestation control campaigns (state secretariats, the federal environmental agency - IBAMA, public attorney offices, environmental police);

- new partnerships among government, civil society and the private sector to analyze environmental problems and seek solutions;
- integrated environmental management instruments, including ecological-economic zoning, for evaluation, monitoring and control actions; although zoning work has produced valuable information, there has been only limited public discussion and negotiation of a land use policy at state or municipal level;
- decentralized environmental issues, introducing them in State political agendas, while establishing the legitimacy and leadership of state environment organizations and strengthening state and municipal governments to progressively take on these functions;
- advances in the development and implementation of public policies, including sustainable funding mechanisms;
- building consensus as part of the Project's management strategy, gradually enhancing mutual understanding among participants. Planning procedures have been improved but continue to require further adjustments. Complex decision-making processes and relationships among actors have improved with expanded dialogue and increased mutual confidence. This process, however, limited implementation.
- identifying project management issues and make several conceptual and management improvements; and
- training numerous groups, contributing significantly to strengthen environmental management institutions, especially with technical cooperation assistance.

Project Concept and Design Issues

However, among the limitations identified during the mid-term review were that:

- The project defined environmental management strategies based command and control instruments. For several historical reasons, it had not, until recently, addressed federal and state coordination with other, largely development-oriented public policies, This has limited the application of sustainable development principles to natural resource management and the capacity to prevent and solve problems. The MMA recognizes this need to expand the scope of its actions and is asserting itself with other federal agencies, whether or not they included as part of this project.
- The Integrated Environmental Management Plans (IEMPs or PGAIs) are effective tools for participatory planning, contrary to the first phase of institutional strengthening without clear objectives and geographic limits.

However, they should be more strategic and less detailed. The Working Groups were effective in bringing together many of the stakeholders but not all. Broader participation in project design, planning and implementation is needed to achieve legitimacy and ownership.

- While the project has helped several states to enhance their ownership of environmental management, in many states, state government ownership was lacking. This was partly due to a lack of effective measures to promote sustainable practices by government constituencies, including stakeholders involved in degrading the environment (loggers, ranchers, etc.). Recognizing this failing, the MMA has been meeting with all actors, thus building mutual trust, understanding and commitment to change among previously reluctant participants. The decentralization of MMA representatives supports these changes, with state representatives to help articulate stakeholders, coordinate policies and facilitate project implementation.
- The objective of decentralized environmental management was constrained by delays in the sharing of control responsibilities with the states and limited participation of municipal government, civil society organizations, NGOs, private sector, etc. Improved Federal-State Government cooperation is demonstrated in the Get Legal, Amazon! Project (see Box 2).
- Financial implementation are currently limited by donor, federal and state budgeting and purchasing systems resulting in numerous delays. Proposals for alternative procedures are being considered.

Box 2: "GET LEGAL, AMAZON!" Component

Designed to improve the prevention and control of illegal forest fires and deforestation and thus revert deforestation trends, this component of the NRPP is being carried out by the MMA, in cooperation with IBAMA and Amazonian states. It is made up of five action areas, broken into detailed state plans (each with preventive and enforcement elements): intensive surveillance (with remote sensing and GPS), audits of forest management plans and deforestation authorizations, forest product control, mobile user service and environmental education and consciousness raising (including training). The component established 22 control stations of various types, planning for the field participation of 442 agents for continuous 30-day periods, 400 audits of sustainable forest management plans, exploration plans and deforestation authorizations.

The key accomplishments during the past year include:

- Implementation of joint federal-state enforcement campaigns during the year 2000 cutting and burning seasons throughout the Amazon;
- Federal and state situation rooms worked in coordination with the fire prevention courses given to 10,000 small farmer and indigenous families in Roraima state by the PROTEGER project; and
- Basic surveillance and control courses given to 152 IBAMA staff .

Recent NRPP accomplishments

In synthesis, due to some limitations in the Project's original concept and design, its ability to support alternative sustainable practices and obtain state political support was reduced. The removal of these constraints will soon release the project's considerable potential to promote environmental management and sustainable development.

Capacity-Building Initiatives

The Program is supporting the strengthening of Brazilian organizations to fully take on environmental policy and management responsibilities. In addition to building state and municipal capacities through the NRPP project, the RFT is funding the deepening of MMA's policy and coordination capabilities as well as consolidating the NGO Networks' capacity to assure civil society participation in implementing and monitoring programs and policies.

Ministry of Environment (MMA)

The MMA, through the Secretariat for the Coordination of the Amazon – SCA, is in charge of coordination of the Program, with new additional responsibilities, in addition to other programs, with the double challenge of achieving environmental management and sustainable development of the Amazon. The SCA has mobilized a staff of qualified and experienced professionals. However, it still lacks the resources and some skills to better coordinate and articulate actions to face these challenges. Additional resources to those already mobilized from multilateral, bilateral and government budget sources, will significantly enhance its coordination capacity.

The Government presented a proposal for Program coordination, temporarily increasing total program management costs from US$2.6 to US$4 million. The Government and the Bank agreed to analyze needs function-by-function. Meanwhile, the JSC approved initial funding of US$ 300,000 from the

RFT for MMA to cover a six-month period beginning April 2000, during which plans for the Coordination Project would be finalized. Such project would also have the support of the Netherlands.

Amazon Working Group (GTA)

The GTA, a network of 450 NGOs working in the Amazon, has been receiving institutional support already under the Demonstration Projects and under a special grant from the RFT. It continues to be in need of institutional and financial support. The objective of a GTA Institutional Action project for 2000-2002 is to assure civil society participation in the monitoring and implementation of the Program and of relevant policies. This will be accomplished by consolidating management structure, planning, institutional coordination and strategic communications of the GTA. The total budget proposed by GTA is about US$3 million. The JSC has so far approved US$500.000 for a period of one year or more.

Atlantic Rainforest Network (RMA)

The RMA, a network of 200 NGOs working in the Atlantic Rainforest dominion, has also received support under the Demonstration Projects, and in 1999 established an additional office in Brasilia with program support. The objective of the RMA is to promote the conservation of the Atlantic Rainforest through coordinated political action and NGO mutual support and exchange to promote effective public sector conservation programs. Specific objectives include monitoring and influencing public policies, strengthening national actions and articulating and training affiliated NGOs, promoting the exchange of experiences, dissemination of the Program and maintaining an RMA offices. The JSC has temporarily approved funding of US$163,000 for a non-specified period.

Fire Prevention Training and Mobilization Campaign (PROTEGER)

Mobilization and training in the prevention of forest fires was carried out by the GTA Network from June 1998 to October 1999, in coordination with state and federal situation rooms, divided into three phases:

- Mobilize and raise consciousness of 12,000 rural worker families in the prevention and control of fire in the Amazon
- Study and exchange ideas on existing sustainable alternatives to the use of fire and
- Mount a campaign in the state of Roraima with farm workers and indigenous

populations, reaching 9000 families. During the critical period, no accident occurred with the use of fire for agriculture.

According to local reports, the project was highly satisfactory and effective, despite potential for managerial improvements.

The Mid Term Review concluded that:

- Participation and social control are effective means to supervise development projects with high community involvement.
- Alliances among local institutions are vital to the success and allow a large number of interested out-of-the-way parties to be reached in a short time.
- Alliances are more effective when carried out through institutional agreements, relieving implementation of bureaucracy.
- Realism, rather than exaggerated optimism, in planning and implementation, avoids or reduces frustrated expectations.

Proteger II

The GTA prepared a draft proposal to continue this excellent program. It is being revised with the participation of the MMA and the Federation of Agricultural Workers (FETAGRI).

Fire and Deforestation Control Project

The Fire and Deforestation Control Project (PRODESQUE) had been designed to reduce deforestation and burning rates by developing a unified system of monitoring and surveillance of forest clearing, burning and degradation. While it was being prepared, accidental fires raged out of control in Roraima in March 1998. In response, the Emergency Fire Prevention and Control Project (PROARCO) was designed and implemented (see Issues Section above). In October 1998, a project proposal was submitted by Government, which received Bank and donor comments in December 1999. Meanwhile, other related projects were implemented that contained elements of the original PRODESQUE project (PROARCO, PROTEGER and "Get legal, Amazon!"). It is likely that PRODESQUE will not be implemented in the form proposed, and that certain of its components will be integrated into the Natural Resources Policy Project.

Scientific Research

This line of action seeks to promote the generation and dissemination of scientific knowledge relevant to conservation and sustainable development activities. It is carried out through the Science and Technology Subprogram.

Projects and Components

The subprogram consists of two coordinated projects, the Science Centers and Directed Research Project - Phase I, and the Emergency Assistance Project.

The Phase I Project – consisting of the science centers and directed research components – closed on December 31, 1999. It aimed to Amazon region by supporting competitive grants for scientific research and strengthening two established research institutions, the National Institute for Research in the Amazon (INPA) and the Emílio Goeldi Museum (MPEG).

- The Directed Research Component under the Phase I project provided resources for 23 research grants to institutions, selected by a 1995 competitive process (call) in three areas: (i) ecosystem studies; (ii) sustainable management and technology development; and (iii) socioeconomic and cultural studies. Subsequently, donors supported funding of a second 1998 call for another 30 grants to institutions and individual researchers. The total cost was US$5.11 million.

  The IAG made specific suggestions at its 13th meeting, considering that the current point-based selection system places insufficient importance on neither Pilot Program objectives nor research staff qualifications. Priority research topics should include: causes of deforestation, valuation of environmental services, public policy impacts, production and marketing of forest products, sustainability of agrarian reform settlements and demarcated indigenous areas. The MTR preliminary report indicates that the Component improved its performance considerably. The 1998 call and the resulting contracts include several innovative and commendable aspects: They speed up and simplified the processing and administration of individual grants and gave more degrees of freedom to research teams to optimize their work.
- The Science Centers Component supported activities aimed at institutional strengthening and facilities improvement of the two regional science centers. Spending totaled US$20.86 million, of which US$9.2 million from the Brazilian Government and US$11.7 million from donors. As a result, despite some implementation problems and differences in priorities, the centers strengthened institutional management and planning, rehabilitated and expanded infrastructure and equipment, increased human resource capacity for research and education and improved science and dissemination management. The Emergency Assistance Project was a supplementary project designed to support infrastructure renovations at the two science centers. The project closed on June 30, 1998.

Reviews and Continuing Project

Discussions on the design of a possible follow up operation are currently underway. A project preparation visit by the World Bank, the U.S. Agency for International Development (USAID) and the European Commission (EC) took place in June 1999. The team concluded that a final rigorous evaluation of the Phase I and Emergency Assistance Projects should be completed prior to making decisions about the design of the next project. It was agreed that the implementation completion report (ICR) would be done by the Bank for both projects, based on the government's own evaluation report, which is expected to be completed by the end of June 2000.

The June 1999 mission also included a workshop to discuss lessons learned from the two initial science projects, as well as the design of the next project. The participants, including representatives of the scientific community, NGOs and technical secretariats of the Pilot Program, made many positive observations and recommendations for the projects.

On the other hand, the mission emphasized the need for: (i) better integration of the science subprogram into the overall objectives of the Pilot Program (including emphasis of the MTR team on improved linkages and feedback mechanisms between this Project and other PPG-7 programs); (ii) more consideration of regional and local research needs; (iii) greater emphasis on collaborative, inter-institutional and interdisciplinary research; (iv) consideration of research fellowships, stipends and other support, and (v) better dissemination of research results to a broad spectrum of users.

A final wrap-up workshop took place in December 1999. Organized by the Ministry of Science and Technology, the workshop presented the preliminary results of the 23 Directed Research sub-projects supported under the Phase I project, and reviewed the results of investment under the two projects into the Science Centers. A new Science and Technology project is being prepared with financing from USAID and possibly the RFT with an indicative budget of US$12 million.

Learning & Disseminating Lessons (AMA Project)

The Rain Forest Trust Resolution specified the careful monitoring of the Pilot Program's activities and results as essential to the Program's objective of learning and broadly disseminating lessons. This is being carried out in all projects to varying degrees.

As a result, the Monitoring and Analysis Project (AMA), the Program's first crosscutting project, became effective in 2000 after delays in meeting established pre-conditions (selection and appointment of key staff). It consists of three components: (i) monitoring the achievement of the four broad objectives by the Program and its projects; (ii) cross-cutting strategic analyses and (iii) development and implementation of Program-wide dissemination strategies. With resources from the RFT a small project team was maintained prior to start-up to develop indicators and plan monitoring in conjunction with other projects. The project is still in its start-up phase. A draft set of Program-level indicators has been prepared, for discussion by the IAG at its next meeting. Several projects have requested technical assistance in monitoring.

New Cross-Cutting Initiatives

The Program has initiated two new cross-cutting initiatives: a) a new sustainable production project to provide business know-how to existing projects, with start up planned for this year, and b) a new Atlantic Rain Forest Subprogram, which has received RFT pre-investment funding.

Sustainable Production Project

Based on the experience to date with productive and commercial activities under the Demonstration Projects, the MMA and the World Bank have agreed on the concept of a project to strengthen "sustainable businesses", with Dutch funding, which would bring essential business know-how to small producer communities or individual enterprises. It would help innovative producers obtain the skills and information needed to create and/or maintain viable businesses. The MMA sees this as the Program's second crosscutting project.

One of the premises underlying existing promising initiatives (Demonstration Projects, Extractive Reserves, Forest Resources and Floodplain Resources, as well as a number of initiatives external to the Program), is that forest conservation and sustainable development can be pursued together. While enthusiastically undertaken, beneficiaries mostly lack essential managerial skills, technological know-how, business acumen, marketing knowledge and access to appropriate financing mechanisms. Without these, they may fail in the medium or long run.

A workshop took place in June 2000 to discuss project design and stimulate partnerships. Appraisal will take place in 2001. The total cost is estimated at US$5 million, of which US$3.8 million from the Government of the Netherlands.

The project is likely to consist of: (i) screening of promising initiatives, including an analysis of existing efforts to select at least 100 initiatives for assistance, (ii) establishment of a data bank on selected initiatives, potential investors, business partners and consultants, (iii) promoting sustainable partnerships, with this information, among producers, commercial firms and investors (iv) technical support to subprojects with management and accounting training, business planning, market research and legal support and (v) adaptation of official credit lines, training of bank staff and rural extension staff.

Atlantic Forest Subprogram

Beginning in 1995, the Pilot Program began working to preserve the Atlantic Forest through several Demonstration Projects, budgeting up to 5% of the PD/A program's resources, representing US$7 million for 44 subprojects. Subsequently, about 30% of the Rain Forest Corridor Project, still to be implemented, was also assigned to the Atlantic Forest.

Recognizing the biome's additional needs, the Secretariat of Biodiversity and Forests of the MMA prepared an action plan establishing priorities. A concept paper was presented at the 1999 Participants Meeting, and the meeting approved, in principle, funding for the preparation of a sub-program. In April 2000, a Pilot Program Atlantic Forest advisory nucleus was established within the Secretariat. A US$114 million Atlantic Forest subprogram was designed during 2000 and is now seeking funding as well as beginning a pilot phase of implementation. An Atlantic Forest Working Group, created by order of the Minister, discusses and monitors the Nucleus' work, including the opinions of civil society, state governments and the private sector.

Lessons Learned and Next Steps

Lessons. At the programmatic level, a number of key lessons have been learned. Overall, the Program has demonstrated that a wide range of activities are needed to achieve conservation objectives. In addition to the scientific work that is the focus of this conference, it is necessary to undertake actions that touch on the economic, institutional, social, and policy dimensions of forest management. More specifically, the Program has learned about:

- **Partnerships** – a major initial limitation has been partnering with the weakest federal ministry through a donor-driven program with only 10% initial counterpart funding. This weakness was overcome by diversifying the base of partners through involvement of the Ministries of Science and Technology and Justice, the federal environmental protection agency (IBAMA), state and local governments, NGOs, and the private sector;
- **Civil Society** – the projects in the Program with the best implementation record have been those executed by civil society (PD/A, Resex I, Science

Sub-program), indicating the advantages of working outside of the public sector framework;

- **Tailoring** – several projects attempted to fit a single model to a diverse range of situations, without much success. For example, the NRPP has gone through several revisions in order to adjust its capacity building work to the levels of political will and technical ability found in the nine states of the legal Amazon;
- **Targeting** – the initial design of projects was generally opportunistic, working where there was demand and interest, but not necessarily the greatest need. The Program has since grown more sophisticated in its geographic targeting. For example, the Ecological Corridors project will work in the two top priority corridors as identified by a rigorous scientific and participatory exercise. The Fire and Deforestation project, now to be a component of the NRPP, will focus on the 43 municipalities in three states that accounted for 70% of Amazonian deforestation in 2000;
- **Balance** – there is a need for balancing approaches to forest management by a) ensuring a better blend of command-and-control with economic instruments and b) working in partnership between the public, private and civil society sectors; and
- **Political cycle** – the federal and state electoral cycle has important consequences for Program implementation. There are initial delays when a new administration comes to power. Then, there is keen interest in the Program as it is seen as a source of money and capacity to implement the new Government's political agenda. This is followed by a period of disillusionment and disinterest as it becomes clear that the Program cannot be easily manipulated. Finally, as the next election nears, there is heightened interest as politicians seek to demonstrate results to the electorate.

Next Steps. The Program should enter into a Second Phase early in 2003, after a phase of transition period of 18–24 months, with activities and results as described further below. The Second Phase should run for about four years (through 2006). During the transition phase lessons learned, replicable models, policy proposals, and mainstreaming recommendations should be available, e.g. by mid-2002.

The Second Phase will be characterized as follows:

- Guidance by a shared Brazilian vision (macro-zoning) of the long-term future of the Amazon;
- Overall concentration on the support to mainstreaming of new or improved policies, instruments, and programs;

- More of a strong program rather than a collection of projects character;
- Concentration on generation and application of knowledge gained, lessons learnt, and models validated;
- Implementation of thematic lines, possibly with a modified funding structure;
- Inclusion of themes that need greater attention in the future (Land settlement; land titling; cattle ranching; transmission of know-how to end-users through technical assistance and extension services; regional policies and plans for infrastructure);
- Striving for specific results in terms of policy impact for each line of action;
- Stronger integration of policy development actions with other federal ministries and state governments;
- Stronger focus on the areas with the highest risk of deforestation;
- Emphasis on financial sustainability in the public sector and economic sustainability in the private sector;
- Strengthened integration with other projects and programs in the rain forest;
- Continued strengthening of civil society organizations;
- A wider array of relevant institutional actors;
- Stronger private sector involvement;
- Continued monitoring and analysis, learning and dissemination;
- Larger domestic funding (at least 30% of the overall Pilot Program funds available during the second phase); and
- A more diversified group of foreign donors (official and non-official).

For information on the Program in English, please visit www.worldbank.org/rfpp. For the Brazilian Government's official Web site in Portuguese, please visit www.ppg7.mma.gov.br

Ex situ Conservation

Ex situ Conservation of Commercial Tropical Trees: Strategies, Options and Constraints

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Abstract. The role of *ex situ* conservation in a given conservation programme depends on the objectives of the gene conservation effort. In the first part of this presentation we therefore focus on the basic question: 'What should be conserved and with what purpose?' We separate *ex situ* conservation efforts into two categories: (1) efforts designed to minimise genetic changes in order to maintain the original genotypes to the extent possible (static conservation), versus (2) efforts that actively support continued natural selection in response to new or changing environments and growth conditions (evolutionary conservation). Both types of conservation efforts have a role to play in strategies for sustainable use and conservation of genetic resources. Still, we find type (2) activities to be of most general value for traditional conservation, while type (1) activities often have an important role to play in connection with breeding programmes.

In the second section of this presentation we focus specifically on the role of combined conservation and utilisation. This is an obvious option to consider when dealing with commercial species, and we argue that better management and utilisation can often contribute substantially to conservation of the genetic resources of many such species. Intense utilisation of commercial species is often a major threat against their genetic resources, but utilisation may at the same time be a key to their conservation. We argue that increased utilisation in terms of planting and domestication of commercial species can be a case of true integrated conservation and development (ICD). Bringing the hundreds of precious tree species into planting programmes is a major challenge: not only as part of a required conservation effort, but first of all as part of long term sustainable management of the valuable genetic resources. Nobody would like to lose the options for growing these precious species in the future. This is a challenge for timber producers, but certainly also for programmes involved with development of non-wood products and on-farm plantings. Products from trees are of substantial importance to the livelihood of millions of farmers in almost any tropical country. Support to conservation through domestication and commercialisation of species that can provide fruits and medicine for people, and fodder for livestock, is therefore a means to poverty alleviation, rural development, and conservation.

In the last part of our presentation we discuss choice of different *ex situ* strategies in given situations. We recommend a holistic approach, where threats against given genetic resources are analysed together with available options for effective conservation. The best conservation effort is often found in a combination of changed management practices and additional conservation efforts.

Introduction

Ex situ conservation deals with protecting components of biological diversity outside of their natural habitats (Glowka *et al*. 1994). It covers widely applied conservation techniques such as establishment of clonal archives, seed banks, *in vitro* conservation, and conservation plantings. In general, *ex situ* conservation is applied as an additional measure to supplement *in situ* conservation, which refers to conservation of biological diversity in its natural habitats. The present paper focuses only on the role of *ex situ* conservation, because *in situ* conservation is the topic of other presentations in the present proceedings. Still, it is important to emphasise that the approaches should be seen in combination as *e.g.* discussed in more details in Graudal *et al.* (1997).

The objective of the present paper is to provide input to a discussion on lessons learned and ideas for further development of *ex situ* conservation of commercial tropical tree species. The paper rests on our experience with conservation of genetic resources of tropical tree species at Danida Forest Seed Centre (DFSC), gained through field work and discussions with colleagues from many organisations including: the Royal Forest Department, the Forest Genetic Resources Conservation and Management Project, and the Chiang Mai University in Thailand; the Forest Department in Zambia; Centre National Semences Forestières in Burkina Faso; Institute Forestral and University of Sao Paulo in Brazil; the Natural Resource Management Sector Assistance Programme (NARMSAP) in Nepal; the National Tree Seed Project in Tanzania; the Indochina Tree Seed Project in Laos, Vietnam and Cambodia; and the Medicinal Plant Conservation and Revitalisation of Local Health (MPC-RLHT) project in India. A second paper (Theilade *et al.* these proceedings) presents one of the specific *ex situ* conservation projects we have been working with at DFSC, while the present paper provides an overview and presents some general ideas. There are many technical and institutional aspects of *ex situ* conservation, and we will not review all these important details. In Table 1 we have listed some general advantages and disadvantages of the various *ex situ* techniques, and we will elaborate these below. Comprehensive accounts of technical and institutional aspects can be found in references such as NCR 1991, Wang *et al.* 1993, Given 1994, Erikson 1994, Guarino *et al.* 1995 & Frankel *et al.*1995, Miller & Lanou 1995, Bowes 1999, Young *et al.* 2000.

*Table 1.*General advantages and disadvantages of various *exsitu* technique

	ADVANTAGES	DISADVANTAGES
Evolutionary conservation		
In plantings and domestication programmes	⇨ Can conserve genetic resources in the habitats of expected use ⇨ Conservation of intra-population variation can be combined with conservation of inter-population variation through a network of spatially separated areas ⇨ Can develop into multiple population conservation programmes where new intra-population variation is developed as response to different conditions of growth or selection criteria ⇨ Can be combined with utilisation ⇨ Can function as seed sources allowing rapid procurement of seed in commercial scale in early domestication	⇨ Many areas required ⇨ Spatial isolation to conserve population identity required ⇨ Lack of pollinators may cause problems ⇨ Relatively expensive if not combined with utilisation
Static conservation		
Seed banks:	1) Propagules ready for use (although the amount of seed typically is too limited to serve as input to commercial use) 2) Little space required (at least for species with small seeds) 3) Intra- and inter-population can be easily conserved provided species range adequately sampled 4) Seed can be conserved far away from the *in situ* environment if requested	**5) FACILITIES REQUIRED** **6) NOT APPLICABLE TO SPECIES WITH RECALCITRANT SEEDS** 7) Regular regeneration of seed lots pose severe practical problems even for many species with orthodox seed. 8) 'Short term storage rather than conservation' for the majority of species
Tissue culture banks	• Minimum space required • Aseptic conservation (minimises disease risk); • Time required to produce propagules for use is short. • Germplasm can be conserved far away from the *in situ* environment if requested	• Expensive facilities required • Sampling problems (representative individuals and within individual) • Difficult to conserve adequate number of genotypes • Protocols are specific for species and often even for genotypes • Problems of soma-clonal variation and early maturation

Clonal archives	⇨ Intra- and inter-population variation can be conserved provided species range adequately sampled but this will require a large number of genotypes ⇨ Useful method for unique phenotypes/ genotypes ⇨ Can readily provide scions for grafting, if requested ⇨ Clones can be conserved away from the *in situ* environment if requested.	1) Suitable site(s) required 2) Relatively expensive 3) Risk of confusion with rootstock (if grafted).
Botanical gardens and arboreta	• Can be combined with demonstration and education • Botanical gardens are often part of very stable institutions and likely to be continuously maintained by trained staff	• Suitable site(s) required • Difficult to collect seed due to hybridisation • In general not apt for conservation of inter- and intra- population variation (requires a larger number of individuals than usually planted in botanical gardens/arboreta).

What to conserve?

The tropical forests harbour enormous biological diversity. Here we use the term *genetic resource* to describe "genetic variation of potential or present benefit to human beings" (cf. e.g. FAO 1988), i.e. a perception where:

- *Commercial trees* refers to all kinds of woody plants capable of providing valuable wood or non-wood products
- *Genetic* refers to variation of genetic origin. DNA polymorphisms as such (genes), but also genetic patterns of variation at different levels: 1) variation between species, 2) variation between populations within species, and 3) variation between individual trees within populations. The largest variation is between species, and loss of whole species is therefore also the most dramatic loss of future options. Variation between populations (level 2) often reflects adaptation to different conditions of growth, and loss of variation at this level may reflect a dramatic loss of options for optimal utilisation of a species over a wide range of environments. The patterns of variation are therefore important in addition to the variation itself (genetic polymorphisms).
- *Resources* refer to genetic variation - in the broad sense stated above - considered to be of potential value for mankind at present or in the future.

It is a resource because it possesses the possibility of improving the livelihood of present and future generations.

Conservation of the genetic diversity (at all three levels) as such is not the sole objective of gene conservation. Protection of evolutionary processes of adaptive significance (e.g. natural selection in favour of healthy trees) is also of major importance. This may sound somewhat theoretical, but merely reflects that natural selection continuously works within and between populations, favouring the healthiest trees. Thereby, it contributes to a continued adaptation to changing climatic conditions or new pests (see e.g. Eriksson *et al.* 1993). We consider this continuous "selection of the fittest" to be important, because populations need to respond to changing environments and new competitors in order to maintain their relative fitness in the long run. Most, if not all, tree species are constantly challenged by competition from other species and changing environments. This perception is sometimes referred to as the Red Queen theory after Van Valen (1973). The name comes from L. Carroll's 'Through the looking glass' where the Red Queen tells Alice in Wonderland that 'she has to run as fast as she can just to stay in the same place'. In our context, this means that a population must be able to respond and adapt to changes in the environment as fast as it can - otherwise the population may disappear in the long run. Again, it may sound theoretical but it does have practical implications for choice of practical conservation technique. The point is that conservation techniques roughly can be separated into two categories depending on whether or not evolutionary processes are targets of the conservation effort, or at least *de facto* are included (Guldager 1975). Static conservation (preservation), where genetic processes are avoided to the extent possible, versus evolutionary (sometimes called dynamic) conservation, where genetic processes are maintained.

Clonal archives, seed banks and in vitro cultures: static approaches

Static conservation activities try to avoid genetic changes to the extent possible and are therefore characterised by:

- *Genotypes* as the targets for conservation and vegetative propagation,
- The effects of natural selection and other genetic processes being limited to the extent possible, preferably by maintaining all initially sampled genotypes ('nothing should change'),
- Human intervention almost always is required to avoid genetic processes taking place during conservation.

Static conservation is typically applied in connection with relatively intensive breeding programmes, where identified and characterised genotypes (clones) are grafted and kept in clonal archives. *Ex situ* conservation in seed banks is also a static conservation measure. Selective effects may take place during storage, but this will in general not be related to fitness of the genotypes, and will therefore not support continued adaptation. Seed of some species can maintain their initial high germination capacity for many years. However, the vast majority of species can only maintain high germination for relatively few years. This means that a seed lot must be regenerated in frequent intervals. Seed must be germinated, seedlings produced, the trees grown on protected sites isolated from other trees that may hybridise, and new seed must eventually be harvested once the trees starts flowering. Age of first flowering differs much between species, from only a few years for some species to many years for other species. Seen over a longer time perspective, the sampled gene pools may therefore easily "spend more time in the field than in the cold store". For this reason, we think seed banks in general should be seen as a short-term conservation (storage) activity.

Many tropical tree species, including some very important commercial species, have recalcitrant seeds that rapidly lose their capacity to germinate (Schmidt 2000). Dipterocarpaceae, which include many commercial tree species in the South East Asian tropical rain forests, is an example of a family where seeds maintain germination from only a few weeks (e.g. *Shorea dasyphylla*) up to a maximum of one year (e.g. *Hopea hainanensis*), here cf. FAO/IPGRI/DFSC (2001). For this genus and many others, seed banks cannot be used even for short-term conservation because most seed will die within a single year. For some of these species, *in vitro* conservation (including cryopreservation in liquid nitrogen) has proven to be technically possible, but from a conservation perspective, cryopreservation is rarely a cost-effective option. Depending on species and techniques, fairly strong genetic modification may take place during *in vitro* growth and storage, sometimes observed as so-called somaclonal variation (Fourré *et al.* 1997). Further, major disadvantages of the *in vitro* approach are probably constraints such as (i) the associated costs, (ii) the requirement for stable supply of electricity, and (iii) the fact that in general only few genotypes can be conserved. *In vitro* conservation techniques therefore seem to be of limited general interest when it comes to conservation of forest genetic resources, whereas seed banks can be important for short-term storage of some species in an interim phase until more suitable techniques can be applied.

Conservation in plantings: evolutionary approaches

Evolutionary conservation is the opposite of static conservation in the sense that it aims at supporting genetic changes to the extent that they contribute to continued adaptation. Evolutionary conservation is characterised by:

- The trees reproduce themselves by seed in successive generations. *Genes* are conserved - but specific *genotypes* are not, because recombination takes place during each generation turn-over
- Human interventions (if any) are designed to facilitate moderate genetic processes rather than to avoid them ('things should be allowed to change'). Natural selection takes place in favour of well-adapted trees (disfavouring genotypes that carry genes coding for low fitness in the given environment). Gene frequencies will change, in general increasing for alleles with positive influence on fitness, and decreasing for alleles associated with low fitness. Neutral genes should in general be maintained through sufficiently large population sizes, but some genes will inevitably be lost along the way, while new genetic variation will be generated through polygenic mutations (Lande & Barrowclough 1987)
- Genetic variation between populations from different environments is, in general, maintained and expected to increase over time (Namkoong 1984).

Ex situ conservation in a planted conservation stand is an example of evolutionary conservation, at least if natural selection is allowed to work (favouring healthy and vigorous trees). If thinned systematically (for example every second tree), a planting can to some extent serve as a static conservation measure, but selection will always take place during propagation and establishment. Continued response through selection is desirable in an evolutionary approach, and thinnings that mimic and support natural selection are therefore preferred in an evolutionary approach. Cases where trees are grown in one environment, but expected to be (re) introduced in a completely different environment at a later stage, can represent a different situation. Here systematic thinning may be preferred in order to avoid adaptation to the temporary conservation site (i.e. a static conservation strategy). In such cases it will often be important to re-introduce the genetic resource as soon as possible and thereby return to an evolutionary approach (work with natural selection rather than trying to work against it).

Pure conservation stands established as traditional plantation or mixed "pseudo- in situ" stands

Plantings are sometimes established with the sole purpose of conservation. Such plantings have been established for many commercial tropical plantation species including eucalypts and pines. Although technically quite easy for well-known plantation species, the concept may cause practical problems after some years (Theilade *et al*. 2001). Plantations are normally associated with intensive and expensive tending and fire protection. Also, thinning is required after some years and the plantings develop into mature stands and reach rotation age relatively soon. The short rotation age is important for the profitability of plantation forestry, but actually increases the cost of the conservation. After relatively few years, the conservation officer needs to consider how to make a generation turnover, i.e., collect seed and establish a new plantation. If not combined with utilisation of the trees, this can turn into a costly business. This especially if the conservation stand is not adequately isolated from other hybridising pollen sources, and the seed collection therefore has to be based on controlled crosses. Further, plantations are often relatively exposed to severe damage by occasional hurricanes or fires. This may be an acceptable, calculated, risk in intense plantation forestry, but from the point of view of conservation, it leads to requirements for replicated conservation stands and thereby increases the required conservation efforts. The objectives of conservation stands are very different from the objectives of wood producing plantations, and it may therefore be worth reconsidering how conservation stands can be established in ways that meet conservation requirements, rather than just applying standard plantation routines.

Some species are not easy to grow in plantings for a number of reasons: lack of appropriate seed sources, very specific ecological requirements of the species, exposure to pests and diseases when grown in monoculture, and/or because only very limited experience exists on how to plant them. However, most species can probably be successfully propagated and established if care is given, e.g., by establishing nurse crops to provide shade and suitable microclimate for 'climax' species. Skilled foresters should be able to develop such techniques for cultivation of species either in pure or mixed stands; for example, based on mimicking key conditions in natural stands. We believe that such stands under many conditions (but not always) will be capable of developing into "pseudo *in situ*" stands (*in situ*-like, cf. Prance 1997) and thus be far more stable than traditional monoculture plantations and often capable of regenerating naturally. Such a "pseudo-*in situ*" strategy may further be based on increased diversity from naturally associated species migrating into the plantings (FORRU 1998). Use of suitable nurse species is likely to be essential, and we are quite optimistic about foresters being able to handle the technical aspects of establishing successful plantings of the commercial species based on a "close to nature" approach.

The majority of tropical tree species depend on pollination by animals (Bawa 1990). Insects, birds and bats are important vectors, and several species are known to have very specialised pollination ecology based on a single or very few pollinators (Bawa 1992). Many kinds of intervention in forest ecosystems may have an unintended negative impact on the pollinators (Owens 1994, Lillesoe 1996), which can seriously reduce reproductive fitness of target species. This is obviously a serious problem in planted conservation stands. These may grow well, but will not produce viable seed unless pollinators are available once flowering starts. This is a problem of gene resource conservation activities in general, but will probably be first realised in *ex situ* plantings.

Pollinator management is not an easy issue. An important first step is to identify the prevailing pollinator. Lillesoe *et al.* (2001) listed a number of considerations to make (depending on the order of pollinators) with reference to management of seed sources in natural forests. These considerations will, to a large extent, also apply to pollinator management in planted stands.

Plantings with multiple purposes including conservation

Pure conservation plantings are discussed above. However, many other types of plantings can serve conservation purposes (cf. above) as long as they are managed in a way that supports: (i) continued adaptation and (ii) maintenance of genetic diversity at the targeted levels. But what kinds of plantings meet these requirements in practice?

Continued adaptation refers to selective processes as they take place during the lifetime of the trees and during regeneration. But, do we want to support adaptation of the species in conditions as natural as possible, i.e. their ability to grow and regenerate without any human influence? This could, e.g. be targeted by establishing mixed plantings, which mimic natural conditions and include interaction with other species (discussed above). Or do we want to focus on maintaining fitness in domesticated systems, where ease of artificial propagation, adaptation to limited shade conditions, etc. will be components of fitness? This could take place if *ex situ* genetic conservation is combined with use in planting programmes, including combining gene conservation with tree improvement programmes (discussed in section 2 below). For conservation of commercial species for future use, the latter is often the case - or at least partly the case.

However, there are three potentially 'inherited' conflicts to consider, mainly related to situations with rather intensive domestication programmes. The first is *correlated responses*. Selection for one trait influences other traits if these are genetically correlated to the desired trait. Such correlation can be positive or negative, and should be taken into account in managed domestication programmes. However, the correlation may remain unknown for a long time, because it may not be expressed until a fairly late stage, or because it is not easily observed. The effects of such correlated response can, in principle, be reversed – once it has been identified - by changing the selection criteria in favour of the correlated trait. However, this may be costly and may therefore reduce the potential value of a species for (economically) marginal areas or uses. This leads to a potential need for maintaining the unimproved base population. This can be as *in situ* populations, 'pseudo *in situ*' plantings or in utilised (commercial) plantings as long as they are based on unimproved stock, records are kept, and care is taken for regeneration as discussed above. Programmes based on multiple populations (Namkoong *et al.* 1980) may be especially protective as different populations can develop in different directions allowing for a range of alternative seed sources to evolve (Namkoong, 1984; 1989).

A second potentially 'inherited' conflict is *maintenance of the patterns of variation between populations* (inter-population variation). Comprehensive provenance studies rarely exist to an extent where one can say that the inter population variation has been fully mapped, not even for tested species. Not all provenances are represented by the included seed sources in existing provenance trials, far from all important planting sites are typically represented by the test sites, and/or not all important traits have been evaluated. For the majority of species no, or only very limited, knowledge is available from tests. Domestication is always based on limited knowledge, and it can therefore not be ruled out that unique seed sources are lost if the genetic resources are only conserved in plantings based on fairly few seed sources. Again, the problem is less relevant in domestication that is based on more extensive approaches in multiple populations. As a rule, the more multiple populations, the less conflict.

A third consideration is *rare genes*. Rare genes (alleles) have in several cases proven valuable for resistance breeding of agricultural crops. However, they are difficult to maintain – especially in intensely bred populations – due to the large number of required genotypes. Large *in situ* conservation areas will be required, or alternatively large *ex situ* conservation plantings based on seed collected from a large number of trees (FAO/IPGRI/DFSC 2001). Concerns for rare genes are often not given high priority in programmes for conservation of trees (cf. discussion in Graudal *et al.* 1995), in which case it is easier to integrate conservation in commercial plantings. A multiple population approach will provide relatively good protection to genes with low frequency compared to breeding in a single population (Eriksson *et al.* 1993).

Besides the above more-or-less 'inherited' conflicts, domestication and utilisation based on unsound principles can cause severe genetic degradation. Loss of genetic diversity and random selection can take place if plantings are based on seed from too few trees. Negative selections can happen during seed collection (e.g., if only collected from a few poor-performing but easily accessible trees) or during tending and harvesting (e.g. if straight trees are cut leaving the inferior for future seed harvest). Other problems can be due to hybridisation between species. Loss of genetic diversity can also take place if e.g., seed are distributed by exchange between farmers based on seed collected from few trees (Simons 1996). Also, seed may be moved around between ecologically very different zones with no record of its origin (in centralised seed distribution systems). However, these problems are basically a result of genetically unsustainable practices that are in conflict with good utilisation as much as they are in conflict with good conservation. The point is that using the plant is *not a guarantee* of protection of the genetic resources. The use of the plant has to be based on genetic sound principles. Otherwise, domestication can actually deplete the genetic resource and lead to a situation where conservation measures are needed simply to avoid the negative effects from domestication. However, if genetic considerations are taken into account, genetic diversity can, in general, be effectively protected within domesticated plantings (cf. Namkoong *et al.* 1980, Namkoong 1989).

Conservation through increased utilisation

Many commercial tree species have become rare and endangered because of their valuable products. This may be because they provide precious timber or high value non-wood products such as medicine or aromatic compounds. In such cases, conservation and utilisation should not be regarded separately (Burley 1994, Dvorak 1996) and *increased* utilisation of the endangered tree species may actually be the most effective conservation measure (Kjaer & Nathan 2000).

Increased utilisation of genetic resources can be through plantings in forest areas, watershed areas, degraded areas and, last but not least, on-farm (Fig. 1). Cultivation of a valuable but endangered tree species can result in multiplication and distribution of its germplasm, and may thereby be a very efficient way to protect valuable genetic resources. Moreover, when rare species become common due to planting and their products can be harvested, the pressure on natural populations will often decrease (Fig. 2). At the same time, cultivation of threatened valuable species can help to meet local people's needs for tree products and services, or for cash incomes from sale of tree products.

The increased utilisation approach can also be effective for tree species with less valuable products suitable for use in planting programmes connected to land rehabilitation or watershed management programmes. Utilisation of threatened local species may be suitable for such purposes, because they have adapted to local conditions for a long time and therefore carry less risk of dieback due to biotic or abiotic events. Moreover, they will often be suitable for mixed species plantings where future management can be reduced to a minimum (pseudo-in situ). As such they may indeed in many situations prove to be much better suited for land rehabilitation and protection than species used at present for these purposes.

Increased utilisation through planting may be limited by constraints of mainly technical nature. Sometimes valuable trees are not planted because of lack of access to viable propagation material. In such cases, development of practical solutions can be decisive. One solution could be mobilisation of the gene pool (e.g. establishment of seed sources). Other solutions can be related to improved methods for collecting and handling of seed locally (cf. IPGRI/DFSC, 2000). It is also important to support the organisation of programmes that can ensure rural people's access to high quality plant material of the targeted species suitable for on-farm planting. This practice improves the farmers' benefits from growing the trees, and reduces the chances that the distribution of poor seed between farmers will lead to degradation of the genetic resources of the species at the landscape level (cf. Simons 1996). For domestication programmes, it is important that these rest on genetically sound principles (cf. above) based on utilising existing knowledge. Resources will be required, but for many species the required 'investment' will be small compared to the value that can be generated through future utilisation.

Program that aim at distributing plant material to rural people must ensure that rural people are given the opportunity to identify their own needs and that the planting program respond to these needs. This is important both for conservation and for development objectives. Second, introducing new crops should not result in rural people running an economic risk, which would put their livelihood at stake. Experiments with domestication may be costly and not all species will turn out to be suitable for plantings. The market also may be uncertain for new types of products, and for some species it may take 25-30 years before valuable timber can be harvested. Different problems can be encountered when dealing with commercialisation of non-wood products. Support for storage, transport, and/or trade may be useful (Hansen & Kjaer 1999), but other factors need also to be considered (cf. a detailed discussion in Neumann & Hirsch 2000). For precious timber species, a well-developed market often exists, or has existed recently, because they have been traded internationally for decades before the supply slowly vanished as logging of these very valuable species in the natural forests gradually has come to an end.

Figure 1. Conceptual presentation of the few planted species, compared to the many native, under-utilised ones

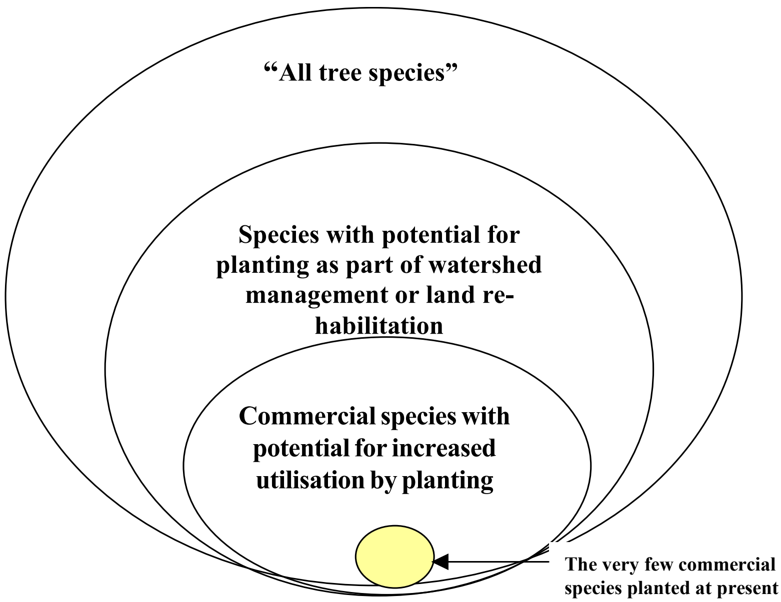

Figure 2. Conservation through increased utilisation

Endangered natural gene pool of valuable species

↓

Increased utilisation through planting

↓

Germplasm widely distributed at the landscape level

↓

Protection status increased + Harvest potential increased +
Reduced pressure on the remaining trees in the natural forests.

Source: Kjaer & Nathan (2000)

Two examples on conservation through increased utilisation

An example of a species that is well suited for an increased utilisation strategy is Prunus africana, a tree species native to the African highlands. Its bark is utilised to extract a medicament used for treatment of benign prostatic hyperplasia. The bark is usually harvested by felling trees in natural stands, and this has led to extreme overexploitation of the species. The species is listed under CITES appendix II regulating trade with endangered species, and all international trade must therefore be under licence. World Conservation Centre has listed the species as vulnerable at the species level. Genetic diversity within the species is certainly being strongly eroded.

ICRAF has analysed options for conservation of the genetic resources of Prunus africana (see e.g. Dawson & Were 1999). They find that the species is fairly easy to cultivate on-farm and that the bark from the planted trees can be extracted in a non-destructive way. Prunus africana offers the potential of generating income for farmers cultivating the tree. The value of the global trade of Prunus africana is approximately 220 mill US$/year (Dawson & Were 1999). At the same time, on-farm cultivation of this species will be an effective way to protect its genetic diversity, as well as to release the pressure on the few remaining natural populations.

Dalbergia cochinchinensis is one example of a valuable timber species found in the Indochina region (Soerianegara & Lemmens 1994) that has – like other *Dalbergia* species - been subject to heavy logging. In Vietnam, *Dalbergia cochinchinensis* has been exposed to high rates of exploitation of the prime timber, and it is now considered a threatened species. In Thailand the pressure on the species has caused concern too. The species has been appointed top priority by the FAO Panel of Experts on Forest Genetic Resources (FAO 1999), and is classified as vulnerable by the World Conservation Monitoring Centre.

For a long time, it has been a general assumption that *Dalbergia cochinchinensis* trees grow slowly when cultivated. Also, that the seed was not easy to handle. The species has therefore not been included in planting programmes. However, a demonstration plot in Laos has shown that it can grow quite fast if cultivated under suitable conditions (STRAP 1995). The wood is extremely valuable, and the value produced per ha far exceeds the value produced by the fast growing eucalypts or *Acacia mangium*. Planting *Dalbergia cochinchinensis* as alternative to the often-planted eucalypts can thus both provide superior income and protect the genetic resource of the species. Efforts have been made to support identification of - and seed collection from - good seed sources to be used for plantings. Such plantings can serve as seed sources for commercial seed procurement later on (LTSP 2000), and thus form the basis for future domestication of the species in large parts of Laos. It is therefore very important that they are carefully planned, documented and most important, *not* based on collection from a few random trees.

The two above cases are merely examples, and many other species could have been mentioned. Butterfield (1995) reports on the encouraging growth of several native species from Costa Rica, of which only very few at present are planted. Dvorak (1996) mentions several examples of Central American pines with large potential, Bierwagen *et al.* (1996) report the promising growth of the valuable Brazilian *Zeyheria tuberculosa* including options for tree improvement. FSIV (1996) lists more than 200 valuable, commercial species native to Vietnam of which many are endangered and only very few planted. Lillesoe & Jafarsidak (1996) suggest increased plantings of Kayu Kuku (*Pericopsis mooniana*) as an important way to protect the Indonesian base populations of this very valuable species against strong genetic erosion. Many other species can surely be listed given the large number of commercial tree species (cf. e.g. Verheij & Coronel 1991, Soerianegara & Lemmens 1994, Lemmens *et al.* 1995, Sosef *et al.* 1998). Immediate action will often be important, because many species at present are subject to strong genetic erosion, and because there will be a lapse of time from initiation of domestication activities (e.g. in terms of establishment of combined pilot test-plantings and simple, genetically diverse, multiple seed sources covering genecological zones) until knowledge and seed will be available for large scale plantings.

Increased utilisation as part of sustainable rural development

Summing up, the increased utilisation approach can be applied to target species with a high value in terms of trade or utility. We see a huge potential for growing such locally valuable species. If based on sound genetic principles (the activity should be designed to capture and maintain genetic diversity of the target species), the increased utilisation of these species can contribute substantially to their protection. It cannot ensure maximum conservation status, but probably much better conservation status than what in practice can be obtained from pure *ex situ* conservation program, overly dependent on continued external funding. Although, there are technical and institutional problems related to using the approach, it is often possible to find solutions to such problems. The largest amount of genetic variation is located between species, and loss of valuable commercial species is something that we simply cannot afford on behalf of our children.

However, if the strategy of growing threatened species on-farm is to be successful, it must be ensured that the users have been given the opportunity to identify their own needs, and that the selected species meet these needs (i.e., based on partnership). Moreover, it must be ensured that rural people's livelihood is not put at stake through experiments with new species. This is important both to the objective of conservation and to the objective of development for people. An interdisciplinary approach for combining genetic and social concerns is often required.

Development of a suitable ex situ conservation strategy

Efforts to conserve forest genetic resources are initiated when it is realised that the genetic resource is at risk, and/or valuable options for improving benefits from the species would otherwise be lost. The objective of conservation will vary depending on the nature of these threats and options, and the appropriate techniques must therefore work accordingly. A search for the best solution should therefore begin by analysing the problem (Kjaer & Graudal 2001):

- If the threat is caused by destructive extraction (e.g. logging), a straightforward answer can be to change management practices. Based on increased awareness of the values of the trees, management practices may be changed so that use and conservation can be combined: for species of great current value, probably over their entire area; for other species, maybe only in selected genetic resource conservation areas. In situ conservation in protected areas is likely to be an important additional option in many cases. Ex situ conservation may be relevant as part of planting program.
- If the threat is caused by overexploitation of the resource that cannot be changed (in an acceptable time perspective), it may be necessary to move or 'multiply' the resource, in which case ex situ techniques will be required. For some species, use may be promoted through domestication in plantings, as the product is valuable (cf. section 2 above). This will require that potential problems of propagation and cultivation can be solved.
- If the threat is caused by conversion of forestlands to other uses, solutions may be found in solving conflicts related to land tenure-ship. In situ conservation based on local partnership, involving, e.g., buffer-zone management, may be the solution and should be tried. However, in some cases, ex situ conservation may be required. The use of local species for land rehabilitation could be an option combining conservation and use. Specific ex situ conservation stands for long term conservation can also be considered e.g. in terms of 'pseudo in situ' plantings (cf. Section 1 above). A good match between the ecological conditions at the site of origin and the site of planting will usually be a prerequisite. The need for pollinators

must also be considered.

- If the problems are caused by the way seed is collected and distributed in the landscape, e.g. more or less erratic distribution of seed from "non-adapted' seed sources, the solution would be to support better practices. This can include improved use of locally well known (well adapted) seed sources that should be made available (physically and economically) – sometimes this will require initial propagation and distribution.
- If improvement programmes have identified superior seed sources (or clones), but excessive use of these over a huge area causes concern, then development and support of genetically sound deployment strategies would be the place to start.

There are many factors to consider in any conservation strategy. Focus on the local problem seems essential, but many biological factors will also influence the implementation of available options. Institutional aspects, availability of trained staff and economic resources are often the most critical factors when it comes to implementation of the conservation plans in the field.

To summarise, in very general terms we find that evolutionary conservation usually is more attractive than static conservation, and it is important to consider which properties (fitness) are targets for conservation and improvement. Further, we find that *in vitro* conservation seems to be of limited use for conservation of most tree species. Seed banks are a valuable tool for short-term storage, but in most cases, the seed must sooner or later be transferred to other types of conservation (often within a year). We believe that combining conservation and utilisation is important, especially when dealing with commercial species. Mobilising germplasm that at present is difficult to access sometimes seems to be the most straightforward and effective way to conserve a species *ex situ*. If done carefully, domestication can often provide good protection of the genetic resource at both the between- and within- population levels. Land rehabilitation and buffer zone management may also provide good opportunities to bring 'new' local species into cultivation, and thereby provide high levels of protection. Still, it will not remove the need for *in situ* conservation and protection of ecosystems, and *ex situ* conservation should never be seen as an exclusive alternative to *in situ* conservation in networks of protected areas.

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The Status of *Ex Situ* Conservation of Commercial Tree Species In Indonesia

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Abstract. There are two basic strategies for plant genetic resources conservation: *in situ* and *ex situ* conservation. In situ conservation is the conservation of entire ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their surroundings (protection of populations in nature). *Ex situ* conservation refers to the conservation of components of biological diversity outside of their natural habitats or conservation of samples/ target species in gene banks.

Both strategies can be considered together as Indonesia's national response to the UN Conference on Environment and Development 1992 and, as such, they represent a continuum of interactive activities focusing on development of new approaches combining conservation and utilization of forest resources. The aim is to alleviate the decline of genetic resources and the degradation of tropical forests, as well as to improve the quality of future forest plantations.

Ex situ conservation is essential for those who need ready access to tree germplasm. A majority of tropical rain forest trees produce recalcitrant seeds. Although cryopreservation and other conventional preservation systems offer technical possibilities for conservation of some valuable germplasm, conservation in live collection is better for long-lived, out-crossing and recalcitrant tropical forest tree species.

There are three eras of *ex situ* of tree genetic resources conservation identified in Indonesia. These are; (1) era of tree introduction, (2) era of breeding programs, and (3) era of conservation and more efficient breeding and biotechnologies programs. These three eras track the evolution of *ex situ* conservation activities over the past several decades.

Activities in the first era consisted of establishing three botanical gardens, three arboreta, and eleven test (trial) plantations. Samples were poorly characterized, too small to allow adequate evaluation and of limited geographic distribution. During the second era, a relatively wide spectrum of genetic diversity was sampled, with the primary focus on two to three specific traits. The third era is considered the era of more efficient utilization. Now the major activities consist of (1) more intensive and representative sampling of the populations to be conserved, and (2) establishment of the collected genetic materials in ex situ plantations, protected from potentially hybridizing between populations and managed to serve future breeding and biotechnology programs.

This paper traces the evolution of ex situ conservation efforts in Indonesia, and uses our current ITTO-supported project with Shorea leprosula and Lophopetalum multinervium to illustrate contemporary activity in genetic conservation of Indonesian tropical rainforest species.

Introduction

Indonesia's species-rich forests harbor the world's greatest diversity of both flora and fauna. An estimated 25,000 species of flowering plants (van Steenis 1971) including more than 380 species of dipterocarps (Ashton 1982), the most valuable commercial timber trees, are indigenous to Indonesian forests. Numerous wild plants and animals are harvested for domestic or commercial consumption as food, medicines, building materials, handicrafts and other uses. Several plant species of global and national importance originated from Indonesia. Therefore, the conservation of forest plants is vital to Indonesia both biologically and in a socioeconomic sense, as well.

There are two basic strategies for conserving plant diversity (Maxted *et al.* 1997), and these are: (1) the entire plants are conserved and protected in their natural populations within their native ecosystems and habitats, known as *in situ* conservation; and (2) the conservation of samples of plants outside of their natural ecosystems, considered as *ex situ* conservation. Each strategy is composed of various techniques that conservationist can adopt to conserve genetic diversity. The two strategies are complementary (Cohen *et al.* 1991), and one strategy serves to back-up or reinforce the other. Although *ex situ* conservation is certainly considered the most directly relevant to users (via breeding and biotechnology programs), this technique is insufficient to maintain the many physical and biological processes that the species interacts with as it evolves through the processes of natural selection.

The Indonesian Ministry of National Development Planning/National Development Planning Agency issued the *Biological Diversity Action Plan for Indonesia* in 1993. In this action plan the objectives of *ex situ* conservation are (1) to strengthen *ex situ* collections in botanical gardens, gene banks, germplasm collections and plant breeding centers; and (2) to implement the national policy for germplasm conservation. This action plan promotes both the collection of genetic resources and the development of appropriate techniques for improving food production, pharmaceutical products, industrial crops and wood products.

In this paper, *ex situ* conservation of tree species in Indonesia is grouped into three time eras, whereas Cohen *et al.* (1991) introduced the concept of four time eras, particularly for agriculture/crops. The eras indicate the evolution of *ex situ* conservation. The first era is characterized by introducing and testing indigenous and exotic tree species with limited genetic bases. During the second era, a relatively wide spectrum of genetic diversity is used in tree breeding/ improvement, with a specific focus on the 2 to 3 best traits of the selected trees. In the third era, the long-term viability of genetic resources is ensured;

therefore, conservation of some target species and development of more efficient methods is called for. These three eras are illustrated in Table 1.

Table 1. Three eras of tree genetic resource conservation in Indonesia

Three Eras and Elements	Timeline	Major Activitie
First Era: - Tree introduction of indigenous and exotic species with limited genetic based - Species (indigenous and exotic) trials - Samples are poorly characterized, too small to allow evaluation and limited geographic distribution	1817 - 1959	Establishment of Bogor, Purwodadi and Eka Karya Botanical Garden, 3 Arboreta (Bogor, Kaliurang and Watusipat) and Species Trials at 11 test plantation site
Second Era: - Era of tree breeding programs - Samples are relatively a wide spectrum of genetic diversity - Focus on certain traits (2 to 3 best traits	1976 - 2020	Establishment of: - Provenance testsProgeny test - Seedling Seed Orchards - Clonal Seed Orchards of some tree species
Third Era: - Era of more efficient utilization - More close linkages with breeders and biotechnologists - Maintain population differentiation - The stands must be able to produce reproductive materials in the new environmental conditions it represent	1998 - 2020 and beyond	- sampling target populations - establishment of ex situ plantations, kept separate from hybridizing among populations

The first era consisted of three activities: (1) establishment of botanical gardens, (2) establishment of arboreta, and (3) establishment of species trials. The presence of Bogor Botanical Garden, which was founded in 1817, facilitated the development of the agricultural, estate crop and forestry sectors of Indonesia and other countries in the world. Plants from many locations throughout the world have been cultivated in this botanical garden, and these activities have generated significant sources of information relating to introducing and testing exotic species for further research in such areas as silviculture and tree breeding.

The Indonesian Forest Research and Development Agency (FORDA-formerly Indonesian Forest Research Institute) established arboreta such as the Bogor Arboretum, established in1922. This Arboretum contains 50 families, 136 genera, and 167 indigenous and 67 exotic species. In addition to the Bogor

Arboretum there are two other arboreta located in Yogyakarta; the Kaliurang Arboretum (18 species, 10 hectares), and the Watusipat Arboretum (36 species, 10 hectares) both established in 1958.

FORDA also established indigenous and exotic species trials, such as those conducted at Pasirhantap (1937, 35 hectares, 78 species), Cikampek (1937, 45 hectares, 61 species), Sumberwaringin (1937, 21 hectares, 64 species), Pasirawi (1938, 14.25 hectares, 9 species), Haurbentes (1940, 100 hectares, 70 species), Pandekanmalang (1952, 21 hectares, 25 species), Yanlapa (1953, 46 hectares, 44 species), Arcamanik (1954, 16.27 hectares, 15 species), Cikole (1954, 39.8 hectares, 45 species), Carita (1955, 50 hectares, 54 species), and Darmaga (1956, 60 hectares, 123 species).

The second era consists of breeding activities such as provenance and progeny trials, establishment of seedling seed orchards and clonal seed orchards. We recognized that utilization of the materials collected in the first era were constrained by a number of factors, such as sample sizes too small for statistical evaluation, populations poorly characterized, and distribution of samples too restricted and we expanded our efforts to more and better collections in the 1970s. At the time the second era was introduced with these improved plantation programs, it also marked the establishment of genetic tree improvement work in Indonesia.

A tree improvement program was initiated in 1976 at Gadjah Mada University in cooperation with the Ministry of Forestry, GOI and was supervised by Dr. J.W. Wright and Dr. James Hanover, both from Michigan State University, USA. The program was started to genetically improve the stem form and growth rate of *Pinus merkusii*. Over the next ten years many other breeding programs and genetics research activities were initiated.

The third era may be considered as a genetic conservation effort within the context of current environmental conditions, which includes the continuous depletion of the tropical rain forests of Indonesia through overexploitation, illegal cutting, and forest fire. These issues represent potentially damaging losses of forest resources (e.g., entire tree and other species, genetic resources of target species, and other "non-tree" forest resources, as well), and therefore pose serious biological, ecological, and socioeconomic consequences. To generate research information and help alleviate these potential losses, the Faculty of Forestry, Gadjah Mada University in collaboration with Oregon State University, USA, submitted a proposal on: *Ex Situ* Conservation of *Shorea leprosula* and *Lophopetalum multinervium* and their use in future breeding and biotechnology programs. This proposal aimed to establish models of *ex situ* conservation for both well-drained and swampy forest ecosystems. This project proposal was submitted by the Government of Indonesia to the International Tropical Timber Organization (ITTO), and it was funded by ITTO in 1998.

The specific objectives of the project are to initiate research activities that will (1) avert a decline in genetic variability of target species, and (2) allow the use of these genetic collections for more efficient future breeding and biotechnology programs in which the output could be used to meet policy by developing effective *ex situ* conservation methodology. A third objective was to sponsor this Conference to provide a forum for genetic conservation activities in this region and to establish a network of scientists and practitioners who are active in this field of research and development.

The First Era: Tree Introduction

The era of tree introduction consists of the establishment of botanical gardens, arboreta and species trials. According to Radford *et al.* (1974) a botanical garden is "an institution organized to maintain plant collections, usually representing a large number of genera and species …. to serve educational, aesthetic, scientific and economic purposes". While an arboretum, on the other hand, is a representative and effective display of all the different kinds of woody tree species maintained for the purpose of reference and as a convenient source of seed for trial plantations.

This era promoted tree introduction and stimulated tropical plantations in such species as rubber, *Swietenia macrophylla*, *Swietenia microphylla*, *Styrax sp* and others. It was characterized by: (1) access to germplasm, and (2) establishment of the national quarantine system.

Indonesia has established three botanical gardens: (1) Bogor Botanical Garden, (2) Purwodadi Botanical Garden and (3) Eka Karya Botanical Garden

Bogor Botanical Garden was founded on May 18, 1817 in Bogor, West Java by Dr. C.G.C. Reindwart. This botanical garden is currently about 87 hectares and it represents the most suitable institution set up to rescue and conserve tropical rain forest species collections.

Purwodadi Botanical Garden is located in Purwodadi, Malang, East Java. This botanical garden was founded on January 30, 1941, as a branch of the Bogor Botanical Garden. The collections represent deciduous forest species of the Tropical Summer Rain Region.

The Eka Karya Botanical Garden is located in Bali. This botanical garden was founded July 15, 1959 as a branch of the Bogor Botanical Garden. It is located at Candikuning, on the eastern slopes of Tapak Hill, about 1,200 to 1,450 m above sea level. The collections consist of 156 families, 535 genera and 937 species.

These three botanical gardens demonstrate not only the opportunities for esthetic landscape design, but they also facilitate various taxonomic or other botanical research based on living materials. The functions of these three botanical gardens are:

Education

These botanical gardens are adjunct facilities to universities. They provide facilities in plant-related subjects for (a) graduate and undergraduate level botany and horticulture, (b) nature study programs for school groups, (c) environmental awareness programs for school, university and community, (d) workshops for teachers of natural sciences, and (e) workshops on many phases of plant study for the general public and professionals from other disciplines.

Conservation

With the increased pressure of population and development programs, the damage to natural forests presents potential loss of forest resources. This loss presents serious biological, ecological and economic consequences. Therefore, botanical gardens and other *ex situ* conservation areas are in a position of public and political interest and influence which will help the country to develop the scientific information and technology to relieve pressure and conserve the remaining natural forests.

Research

The botanical garden operation can continue to serve in the development and distribution of suitable strains of economic plants. Conservation should be a major responsibility of botanical gardens, whether it is the preservation of single species or a large-scale operation of administering the protection of an extensive natural area containing an entire ecosystem as a biological garden. The garden could support an extensive range of research in areas such as; genetics (hybridization, cytogenetics), physiology (development, water relations, stress physiology), taxonomy, pathology (disease control, host resistance) and others.

Arboreta (of both indigenous and exotic species), which have been established by the Indonesian Forest Research and Development Agency, are a means to attain the first step in reaching the ideal goal in tree planting. To select the right tree for the right place with just the right amount of growing space at each stage of development will help meet the human objective of effective stand management.

As a rule, differences among species are ordinarily very large compared with differences among populations within species. Thus, an arboretum is the most important step in tree introduction. However, in the past, most species planted in arboreta have involved seed of unknown origin (such as superiority in certain traits) and are not necessarily well adapted for that region. Due consideration should be given this when interpreting results in established arboreta.

The Indonesian Forest Research and Development Agency has also established indigenous and exotic species trials. The objectives of these trials are:

1. To assess growth characteristics of each species tested in these trials;
2. To identify, estimate, and develop the stand related silvicultural techniques required to achieve management objectives in future commercial plantation establishment;
3. To develop and transfer techniques and methods to enhance understanding of silvical characteristics of the species tested;
4. To be used as *ex situ* conservation areas.

These species trials are presented in Table 2.

Table 2. Plantation trials established by the Forest Research and Development Agency

No	Location	Distance from Bogor in KM	Year of establishment	size in HA	Number of species tested
1	Pasirhantap	50	1937	35	78
2	Cikampek	140	1937	45	61
3	Sumberwaringin	1,062	1937	23,6	64
4	Pasirawi	50	1938	14,25	47
5	Cirendeng	285	1939	7,65	9
6	Haurbentes	60	1940	100	70
7	Pandekanmalang	1,076	1952	21	25
8	Yanlapa	56	1953	46	44
9	Arcamanik	140	1954	16,27	15
10	Cikole	150	1954	39,8	45
11	Carita	200	1955	50	54

Selected species will not only survive and thrive on the site, but will also yield the wood or other benefits at some optimum rate. The nature of botanical gardens and arboreta as institutions and collections of living plants remains essentially unchanged. On the other hand, developments in genetics, cytology, *ex situ* conservation, breeding, and biotechnology have been largely responsible for the changes that have occurred. But, unfortunately, there has been some resistance to this change. Most silviculturists and tree breeders now use numerous approaches to problem solving, such as in introducing and testing new species.

Wright (1976) observed that the procedures and designs used to test exotics are the same as those used in the study of individual tree inheritance and racial variation. In this regard, exotic testing should be done in two or three stages. The first preliminary test should include several scattered plantations on different soil types and with different climates, with a few blocks per plantation. These first tests may well include a few hundred seed lots of several different species. The second stage tests should concentrate on those races or species that grow best in the first-stage tests. There should be more replication, perhaps at each test site, and observations should be concentrated on individual tree variation. The third-stage trials consist of pilot-scale commercial plantations designed primarily to assess production potential.

To connect the first era to the second, under the requirements as described earlier, Oemi Hani'in Suseno and Jonathan W. Wright initiated a tree improvement program for Indonesia. This activity will be discussed under the Second Era, and more specific information will be provided by Oemi Hani' in Suseno in the Section on Tree Improvement.

The Second Era: Initial Tree Breeding/Tree Improvement

The Indonesian plantation programs during the 1970's offered one of the major strategies to halt the process of tropical deforestation and to meet future demands for wood. Forest plantation development activities may be speeded up when successful genetic improvement is conducted. It is necessary to use quality seedlings with high growth potential and adequate adaptation to plantation sites. For example, Kjaer and Suangtho (1997) estimated that seedlings from classified seed stands will yield at least 8 % higher value production than seedlings from unclassified seed sources.

Teak provenance trials in Indonesia were introduced in 1932 (Coster & Eidman 1934). Activities basically comprised comparison of trees derived from seed of different origins throughout the whole range of teak (8 provenances of India, Myanmar, Thailand, Viet Nam, and Laos; 4 provenances of Java, and one provenance of Muna). But while these provenance trials were introduced in 1932, the large-scale tree improvement programs in Indonesia began in 1976. A collaborative tree improvement program between Gadjah Mada University and the Ministry of Forestry (GOI) began with the establishment of a seedling seed orchard program to improve growth rate and stem form of *Pinus merkusii*.

That program employed plus tree selection of more than 1,000 parent trees. Six series of open pollinated progeny tests were conducted in three provinces (East, Central and West Java). Each series contains 200 families, field-tested using randomized complete block designs with five-tree plots replicated 10 times. These progeny tests were progressively converted into open pollinated *seedling seed orchards* by selective thinning.

Following 10 years of successful collaborative work with *Pinus merkusii*, tree improvement programs were then conducted in Indonesia for several other target species (teak, *Acacia spp, Eucalyptus spp,* and others).

The Third Era: Conservation and More Efficient Use

This era was initiated by Gadjah Mada University in collaboration with State Owned Forest Companies, PT INHUTANI I, II, III, IV, V, Perum Perhutani, Government of Indonesia, and Oregon State University, USA, and supported by the International Tropical Timber Organization (ITTO). These conservation activities are conducted under ITTO Project PD 16/96 Rev. 4(F), on *ex situ conservation of Shorea leprosula and Lophopetalum multinervium and their use for future breeding and biotechnology.* This project initiated fundamental research activities aimed at: (1) averting a decline in the genetic variability within *Shorea leprosula* and *Lophopetalum multinervium,* and (2) use these genetic collections in the future to initiate more efficient breeding and biotechnology programs.

This ITTO project uniquely addresses both genetic conservation and utilization activities. The *ex situ* conservation areas developed herein are designed to also meet future requirements for improving those two species through breeding and biological technology, thus ensuring their productive conservation.

The reasons for selecting *Shorea leprosula* and *Lophopetalum multinervium* relate to the characteristics of these two species to serve as models of *ex situ* conservation techniques for tropical rain forest tree species. Basically, we proposed to consider two representative and wide-spread habitats: (1) well-drained sites and (2) swampy sites. In this context, *Shorea leprosula* will serve as a model for species on well-drained sites, and *Lophopetalum multinervium* for those on more swampy sites. It should be noted that these two model species are also of considerable commercial value to Indonesia.

To implement ITTO Project PD 16/96 Rev. 4 (F), the activities consisted of the following phases: (1) identification of populations of both species, (2) determination of type of reproductive materials to be collected, (3) collection and transport of materials, (4) nursery establishment, and (5) *ex situ* conservation site planting, monitoring and management.

Identification of populations of target species (*Shorea leprosula*)

A genetic population, according to Wright (1976), is a group of individuals related by common descent and treated as unit for convenience. There is no definite limit to the size or amount of variability contained within a population, nor is there necessarily a connotation that populations differ by any set amount.

The sampling strategy to achieve the target of minimum genetic material to be collected should be adjusted and increased depending on the availability of material and the desirability of sampling from a wide range of environments. In this regard, a collector is faced with a wide range of questions.

The preparatory elements to locate the actual populations consists of: (1) the ecological and geographic information and (2) field exploration. The ecological and geographic information will rarely be sufficiently comprehensive to precisely locate the actual population. Ideally, the objective is to conserve the target species to contain 95 % of all alleles at a random locus occurring in the identified population with a frequency greater than 0.05 (Marshall & Brown 1975). However, during the project implementation, the depletion of the tropical rain forest of Indonesia continued. This depletion is primarily due to forest fire, illegal cutting, deforestation for agriculture, and over exploitation of timber resources. This continued depletion represents loss of target species and, therefore, represents genetic loss, which will in the future pose serious biological, ecological and economic consequences.

Based on this urgency, we decided to immediately collect genetic materials rather than to wait until information on biochemical markers of target populations was available. We identified 5 to 6 populations of *Shorea leprosula* from Sumatera and 9 to 10 populations of *Shorea leprosula* from Kalimantan. So, the total "target" for populations of *Shorea leprosula* was set at 15.

Type of genetic materials to be collected

Brown and Brigg (1991) introduced the range of types of genetic materials and the amount of material be collected. The genetic materials range from pollen, seeds, and vegetative cuttings or propagules (such as tubers, bulbs or corms) to whole individuals (plant). The genotypes of vegetative cuttings or propagules represent those of the parent materials, whereas in wildling or seed collections, the samples depend on the breeding systems. In self-fertilized species, wildings or seeds will genetically resemble the parent tree. But for out-crossing species, wildings or seeds will differ to a greater or lesser degree from the parent, and exhibit within-family diversity.

In general, Dipterocarps produce fruits only at irregular intervals, and have recalcitrant seed. During the duration of our project, no fruit of *Shorea leprosula* was available. Therefore, it was decided to collect wildlings, *in situ*.

For *Lophopetalum multinervium,* we were able to collect seeds, however.

Collection of genetic materials of Shorea leprosula

Since preserving genetic diversity is essential, the project needed to give careful consideration to developing and conducting a sampling strategy in each population. Conservation of genetic resources of trees requires an understanding of the biological dynamics of the population in which they exist. Especially important is knowledge of the diversity and distribution of genes in a tree population.

Basic sampling strategy

In collecting genetic materials we decided to use two-stage sampling (Brown & Marshall 1995, Brown & Brigg 1991). The population was divided into 5 to 10 sub-populations, then each sub-population was divided into 5 to 10 blocks or compartments. The size of each block was about 100 hectares. In each population we planned to have about 50 blocks or compartments. Sampling points were chosen randomly within each block and there are 10 points in each block. At each point we selected 5 “best” and 5 “average” trees, so that in each population we will have 2,500 best trees and 2,500 average trees. Wildings were collected under these selected trees.

Number and location of sampling points (sites) per population

In each population we selected 50 sampling units. These sampling units were located systematically in each sub-population. Each sampling unit encompasses 100 hectares of forest area and sampling points were chosen randomly within each sampling unit. Ten sampling points per sampling unit were chosen so that there are 500 sampling points per population.

Number of individual trees sampled at each population.

From the basic sampling strategy developed above the number of individual trees was determined as follows:

1. Identify about 15 populations, 9 populations from Kalimantan and 6 populations from Sumatera
2. Select about 5 sub-populations per population
3. Sample systematically 10 unit areas per sub-population
4. Sample randomly 10 points, consisting of 5 plus trees and 5 average trees per point area

Based on the above sampling strategy the project has selected 250 plus trees and 250 average trees per population.

Number of wildings collected.

Wildings identified from sampled parent trees (both plus and average tree) were collected. Each parent tree is represented by 10 wildings. The total wildings collected in this scheme are thus 2500 from average trees and 2500 from plus trees per population.

Nursery establishment

Permanent and temporary nurseries were established in 6 State Owned Forest Companies. Wildings obtained from target population were raised in each nursery, identified by sampling point and sub-population, and maintained until they were ready to be planted at identified sites. Materials destined for *ex situ* genetic conservation should remain representative of the source population from which they were derived. Thus, nursery technique and maintenance are more expensive than routine nursery practices to maintain this genetic identity.

***Ex situ* plantation establishment**

Establishment and maintenance of *ex situ* conservation plantations is expensive. However, *ex situ* plantations can be used (1) to safeguard genetic resources of target species, (2) as identifiable genetic resources for future breeding and biotechnology. The criteria for *ex situ* plantation establishment consists of: (1) site selection, (2) design and size of stand, and (3) isolation between populations.

Site selection

In selecting sites, the project decided that adaptability of the species and populations to the planting sites is of paramount importance. In this regard, the stands must be able to produce reproductive materials, and will eventually become the center of future commercial plantation development. In addition, the security of the sites selected for establishing *ex situ* plantations is also important, to minimize loss. To decrease the possibility for loss of materials, we decided to replicate the *ex situ* plantations at a minimum of two sites,

Size of planted stands

The size of each population stand is based on: (1) reducing possibility of genetic drift, (2) protection from contamination by outside pollen, and (3) maintaining each population separately (i.e., population differentiation). We realized that the populations established must be designed to maintain their genetic variation over many generations while subject to open pollination. Based on those assumptions, the number of individual trees planted in each population stand should be about 4,000. No thinning is required, so as to maintain the diversity of each original gene pool in each population throughout the life of the stand.

Based on the above, the spacing employed is 5 m x 5 m, so that the minimum size of a population plantation is 10 hectares. Attention was paid to promote the development of well adapted, healthy stands in which maximum cross fertilization between individuals in each population is assured, and screened to minimize contamination with outside pollen.

Isolation between populations

Establishment of *ex situ* genetic population plantations must be done to adequately isolate each from contaminating pollen to maximize maintenance of population differentiation. The minimum isolation strips are thus about 300 m wide. To facilitate future management of the plantations, continued ready access to them must also be assured.

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The Status of *ex situ* Conservation of Dipterocarp in Malaysia in Serving Breeding Programs

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Abstract. Despite the rich natural forests present in Malaysia, it has always been felt that a long-term management plan to manage these rich forests in a sustainable manner is essential. As the natural forest resources are dwindling, increased attention has been given to forest management, forest plantation and conservation of genetic resources with specific concentration on indigenous species. This paper describes some of the conservation strategies taken by the Malaysian government to address biodiversity issues. In the past, most of the effort has been concentrated on *in situ* rather than *ex situ* conservation. The biggest living arboretum - about 600 ha - which also serves as an *ex situ* conservation area is the Forest Research Institute Malaysia (FRIM) campus itself in Kepong, Kuala Lumpur. It consists of more than hundred species that are mostly dipterocarps. As the interest in forest plantation has become greater in the country recently, more research efforts have been taken to conserve some of the selected species out of their natural habitats and also to serve as progeny trial *cum* seedling seed orchards.

Introduction

The Malaysian tropical rainforest is generally synonymous with the species rich lowland and hill dipterocarp forest. The other forest types occurring are the mangrove and peat swamp forest, montane oak forest and montane ericaceous forest. The dipterocarp forests extend over large areas in the inland and uphill regions and are of vital ecological and economic importance to the country. These forests supply a variety of forest products besides timber, which contribute significantly towards the overall economic development of the country. As a naturally renewable resource, the forest will continue to provide vital economic returns to the country in terms of foreign exchange earnings, government revenue, income and employment. The forest-based industries generate economic activities providing added income and employment opportunities through downstream processing and the development of value-added products for the domestic and export markets. The sector has great potential for further development in the future. In addition, they also play a vital role in the protection and conservation of ecosystems and environmental quality.

Although the forest resources in Malaysia have been systematically managed since the beginning of the century, it has always been felt that a long-term management plan to manage these rich forests in a sustainable manner is essential. In general terms, sustainable development has two major components - improvement of the quality of human life and conservation of the earth's vitality and diversity. It is therefore very important to minimize forest depletion and remain within its carrying capacity while ensuring the sustainable use of its resources. The decade of the 1990's was characterized by a broader awareness of the Malaysian people, both politicians and the public, of the role of forests to human well-being, environmental quality, and the conservation of biological diversity. Increased attention has been given to forest management, forest plantation, and conservation of genetic resources with specific concentration on planting and management of indigenous species. Since then *in situ* and *ex situ* conservation strategies have received greater emphasis both in research and in management activities. *In situ* and *ex situ* conservation are complementary approaches that attempt to maintain, and in some instances, create genetic diversity. There seems to be a consensus that *in situ* conservation efforts will focus on the protection of entire ecosystems and not individual species. Conversely, *ex situ* conservation efforts strive to accumulate useful genes of individual species across a wide range of environments to supplement the *in situ* conservation efforts in the country.

Forest *in situ* conservation in Malaysia

Malaysians are keenly aware of the need for forest conservation not only to ensure a sustained supply of timber but also to maintain environmental stability, to provide sanctuary for wildlife and to serve as an invaluable storehouse of genetic resources useful for the improvement of our indigenous tree species, agricultural crops and livestock. Active conservation strategies go back as early as the Third Malaysia Plan (1976 - 1980) when environmental considerations in development were taken into consideration. A call was made for mitigating measures to the disruption of natural forest cover as a consequence of development. The National Environmental Policy outlined the requirement to ensure that in the process of development, the capability of the environment to support human need is conserved, if not enhanced, and that undesirable changes that occur are contained at reasonable costs relative to benefits. As such, the Third Malaysia Plan called for the incorporation of a larger variety of species in regenerated forest to be promoted for the purposes of genetic conservation. To be able to conserve the wide range of differences of forest ecosystems in the country, so that the full diversity of ecosystems is effectively conserved, a survey was carried out during this Plan period to provide the basis for the establishment of a system of national parks, nature reserves, wildlife sanctuaries and virgin jungle reserves. As a result, various multiple-use national parks with a total area of 2.22 million acres were proposed.

The Fifth Malaysia Plan, (1986 - 1990) called for the setting aside of forest areas for nature conservation, and preventive measures against the deterioration of the environment. Efforts were made by the government to coordinate policy- and program- implementing agencies throughout the country. The Plan specifically recognized that efforts should be directed towards the promotion of greater involvement of the public, citizen groups and the private sector in environmental awareness campaigns, education and training. The enforcement of environmental impact assessment (EIA) for future development plans, project implementation and operational maintenance was also reiterated in the Plan. In its effort to effectively manage the forest, the government established a National Forestry Policy in 1978. To enhance the implementation of this policy, the National Forestry Act of 1984 was adopted which provides for uniformity of laws of the different States with respect to the administration, management and conservation of forest and forestry development. The National Forestry Act contains ample provisions for the conservation, protection and rational utilization of forest resources. It also provides for the conservation of soil, water and wildlife resources.

Within the ambit of the National Forestry Policy, a total of 12.7 million ha of forested land has been set aside as Permanent Forest Estate out of which 9.0 million ha will be managed on a sustained yield basis as productive forest and the remaining as protective and amenity forest where no exploitation will take place. The establishment of this Permanent Forest Estate allows for proper planning and better management of the valuable renewable resource to enhance commercial returns while ensuring environmental conservation. Economic production in the Permanent Forest Reserves through logging is strictly on a sustained yield management regime.

Besides the 12.7 million ha of natural forest land maintained as the Permanent Forest Estate, there are many other natural forest areas outside the PFE that have been protected. The National Parks Act and the Protection of Wildlife Act provide the legal backing for the conservation of national parks, wildlife and protection of endangered species.

In Sabah, the State Ministry of Manpower and Environment manages a total of 265,794 ha of National Parks. Another 141,200 ha of wildlife reserves in the PFE is managed by the State Forestry Department. With the aim of conserving the plant and animal genetic resources in perpetuity and preserving of samples of ecosystem as reservoirs of species diversity, the Sabah government has reserved almost 20% of the forest areas by way of protective reserves, wildlife reserves, etc. All commercial activity in these areas is prohibited.

In Sarawak, the national parks and wildlife reserves are managed by the forestry department. With the constitution of the Gunung Gading National Park in 1983, the total area of national parks and wildlife reserves in Sarawak now stands at 252,870 ha. The established national parks include Bako, Gunung Mulu and Niah National Parks. The wildlife sanctuaries are the Samunsam Wildlife Sanctuary established to protect the Proboscis monkey, and the sanctuaries for Orang Utan covering 168,600 ha in the Gunung Lesong and the Lanjak-Entimau areas.

In its efforts to conserve various forest and ecological types in their original condition (*in situ*) the Forestry Department has set aside pockets of virgin forest known as Virgin Jungle Reserves (VJR) that are representative of all major forest types. These serve as permanent nature reserves. The VJRs also serve as research areas for ecological, botanical and phenological studies, seed orchards, and as gene pools. There are 81such VJRs covering an area of 91,000 ha. These are distributed in various forest types such as Mangroves, Beach Strand, Heath, Peat Swamps, Lowland Dipterocarp, Hill Dipterocarp, Upper Hill Dipterocarp and Montane forest types.

Ex situ conservation through forest plantation

As with the management of natural forests, plantation trials were first begun in Penninsular Malaysia, and then subsequently in Sabah and Sarawak. Records of plantings of forest species date back as far as 1880, when concern for loss of desired species was expressed (Hill 1900). There was concern over the rapid destruction of the taban forests and it was also becoming increasingly difficult to obtain railway sleepers. This was the gutta percha era when the nyatoh taban (*Palaquium gutta*) trees were heavily felled for gutta percha, which fetched a very high price. Interest in plantations of gutta taban caught on. Wildings were collected and planted in regular plantations. At Ayer Kroh, Malacca, 500 gutta percha plants were planted (Hill 1900). Similarly, the accessible forests. A scheme for planting hardwood trees in Sungai Buloh Forest Reserve was also initiated. They removed all mature timber, and line planted the hardwood species.

Besides planting of heavy hardwoods, afforesting mining land and BRIS soils and reforesting forest lands were also of high priority. In 1898, the areas around Pekeliling (Circular Road), Kuala Lumpur, were planted with species such as *Casuarina equisetifolia*, *Eugenia grandis*, *Dryobalanops aromatic*, *Swietenia macrophylla*, *Hevea brasiliensis* and *Fagraea fragrans*. Forest planting was limited to trials of extremely valuable exotics such as *Eusideroxylon zwageri* and *Hevea brasiliensis*, and local timber species such as *Casuarina equisetifolia, Fagraea fragrans, Intsia palembanica* and *Palaquium gutta.*

After a while, all these plantings fell out of favor. Plastics replaced gutta percha. Rubber planting was taken up increasingly by private planters. The reforestation work, experimental in nature, did not perform well and the results were considered not commensurate with the expenditure. Nevertheless, some planting trials persisted here and there. Plantings of *Intsia palembanica* and *Neobalonocarpus hemeii* were continued. These were the main developments , and by the end of 1912, there were 922 acres of regular plantations and 4,828 acres were line planted under shade. By that stage, results from improvement fellings in natural forests were available. In the 1912 Annula Report it was suggested that it is still better to improve the crop in the natural forest rather than planting in regular plantations. As a result, no further increase in plantations occurred, and in the 1920 Annual Report, it was noted that the area of regular plantations stood at 869 acres.

The Forest Department did most of the plantings but the records were meager. Initially exotics were tried, but subsequently local species were tested in the belief that they gave better growth. Foxworthy (1930) records that some 130 species were tested in all, but a high proportion of them yielded poor results. Overall, the plantings were haphazard, initiated by individuals, and scattered throughout the country. Many of the trials were lost when the officers got transferred. This was dubbed the "plant and forget" era (Oliphant 1932).

These disappointing results led to a major development in forestry in Malaya. It was decided that a central permanent experimental station should be set up, in Kepong. It was recommended that further planting should be initiated only after the species and methods had been tested in Kepong. The experimental plantations were started in Kepong in 1927. Between 1929 and 1941 experimental plantations of dipterocarps were started at the Forest Research Institute Malaysia. Main dipterocarp species planted were *Anisoptera scaphula, A. laevis, Dipterocarpus baudii, Dryobalanops aromatica, D. oblongifolia, Shorea acuminata, S. curtisii, S. leprosula, S. macroptera, S. macrophylla, S. ovalis, S. parvifolia, S. platyclados* and *S. sumatrana*. While more species were added to the trials in Kepong, much of it remained experimental. The emphasis was mainly on high quality timber species that have a long rotation. In the early 1930s, the position on planting did not shift as the researchers were not in favor of planting. The area which is about 600 ha and now known as Forest Research Institute Malaysia (FRIM) has become one of national heritage and a gene pool of more than hundred dipterocarp and non-dipterocarp species, and can also serve as seed stand or seed production areas.

Despite the reservation on planting, occasional experimental trials were conducted. In 1936 large scale planting experiments were carried out in Rantau Panjang and Bukit Sungai Puteh Forest Reserves, Selangor. In 1937, trials were begun to establish commercial pole crops on denuded areas in Selangor and on the poor BRIS land in Kelantan. The latter were totally unsuccessful. During the Japanese Occupation, (1942-1945) many areas in Forest Reserves were cleared for farming. After the war, there were some efforts to line-plant these areas and the Taungya system was also tried. Progress was made, but the survival rate was very poor. In the early 1950s, extensive plantations were established in devastated areas in Malacca, Selangor and Perak.

In the early 1960s, following sweeping changes to land-use policy, forestry became confined to poorer soils and in the hills. With these poorer forests, the old management systems were revised and planting was taken as an option to remedy logged sites that were poor in regeneration. The Forestry Departments embarked on Enrichment Planting under the Intensive Forest Management Scheme (Ismail 1964). Planting with potted seedlings and wildings of mainly indigenous species was carried out in several states, especially Selangor, Perak and Kedah. Such plantings dominated the Departments' activities for most of the 1970s. The results were however dismal, despite the high costs (Tang & Wadley 1976). Survival was moderate to low. Growth was good provided the canopy was kept open for a relatively long period. The species used were not necessarily the best for the purpose. Thereafter, enthusiasm for expensive enrichment planting faded, and is now employed to a minor degree only, to improve highly degraded sites.

Sarawak embarked on plantations of dipterocarps in the 1920s by planting *Shorea macrophylla*. While such plantings were pursued on a small scale until 1975 (Kendawang 1995), the state commenced large-scale plantings of dipterocarps in 1979 after disappointing results were obtained from research on exotic fast-growing species (Mok 1994). Dipterocarp plantations were established within the Reforestation Programme for Permanent Forest Estates on areas degraded by shifting cultivation (Kendawang 1995). About 4940 ha have been planted on an operational scale with *Shorea* species of the pinanga group and based on a species-site matching procedure.(Butt & Sia 1982, Ting 1986, Kendawang 1995).

In Sabah, dipterocarp plantations have been planted under the lnnoprise-Face Foundation Rainforest Rehabilitation Project through the enrichment plantings (Moura-Costa 1993, Moura-Costa & Lundoh 1993, 1994). Until 1994 about 700 ha had been planted within the Face Foundation Project. Dipterocarp species used were: *Dipterocarpus spp., Dryobalanops lanceolata, Hopea nervosa, Parashorea malaanonan, Shorea argentifolia, S. johorensis, S. leprosula, S. macrophylia, S. ovalis* and *S. parvifolia*.

Of all the dipterocarp species the *Shorea* spp. and *Dryobalanops* spp. have been given greater emphasis in past basically because of their relatively abundant seed supply as well as good growth and survival. Although all these planting activities were not initially meant for *ex situ* conservation, most of them are now preserved as Forest Reserve and some have been converted into seed production areas as well as gene banks as tree breeding activities in the country have become more important now and in the future.

Current developments

The decade of the 1990s saw increased attention given to the planting and management of indigenous species after fast-growing exotics yielded disappointing results. The increase in demand for indigenous planting materials has highlighted the importance of having tree breeding programmes and conserving the genetic resources. The Government of Malaysia has made a commitment to this by forming the National Forestry Planting Stock Procurement Programme to undertake and enhance the production of quality planting material of our indigenous species. This programme was initiated in 1994 as a joint project with assistance from GTZ of Germany.

For the plantation programme, there are about 18 indigenous species that have been selected for their potential for plantation purposes. Among the dipterocarps that have been selected suitable for plantation programme are *Dryobalanops aromatica*, *Hopea odorata*, *Shorea leprosula*, *Shorea parvifolia*, *Shorea macrophylla*, and *Shorea platyclados*. Plus tree selection has been carried out for all the species mentioned. Amongst the species, for a start, *Shorea leprosula* has been selected for establishment of seed orchards. Three progeny trials *cum* seedling seed orchards of *Shorea leprosula* have been established throughout Peninsular Malaysia in 1997 and 1998.

Besides the conventional ways of *ex situ* conservation mentioned earlier FRIM, as the research institution, has looked into other approaches especially through biotechnology methods. Knowing that dipterocarp seeds are recalcitrant, storage of the seed for conservation purposes is a problem. FRIM has come up with the protocol of storing the whole seed or the excised embryo through cryopreservation. This could offer an alternative method for future *ex situ* germplasm conservation of other dipterocarp species, beside the establishment of seed orchards for selected dipterocarps species.

Constraints

Planting dipterocarp is less popular

Although interest in man-made forests is growing in the country, planting dipterocarp species for industrial plantation purposes is less popular. This is due to the long rotation cycles, which result in a long gestation period before there are any cash inflows to the investment. Therefore, less emphasis was given to the improvement of these species. This is also reflected in the project priority and budget allocations, which directly affect the smooth progress of the research work.

Irregular fruiting

The dipterocarp species are well known for their irregular fruiting. Generally, flowering and fruiting are notoriously unpredictable. Infrequent flowering, together with sporadic fruiting seasons causes difficulty in collecting sufficient numbers of families for progeny testing. This has also become one of the crucial factors in establishing the progeny trial *cum* seedling seed orchard.

Conclusion

Conservation and environmental management in Malaysia are national issues not entirely new to its people. Malaysians believe that there is a need for careful planning, for conservative management, for preserving the gracious living heritage that is the forest. While there is no denying that there are still some outstanding issues unresolved, nevertheless, given the constraints, especially the lack of ascertained methodology and technology, the country has certainly not been side-stepping conservation and environmental issues. The present strategies to retain natural forest, to create man-made forests in strategic locations and to rehabilitate logged forests have been formulated because there is genuine concern for the continued existence of the tropical forest heritage.

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The Status of *In situ* and *Ex situ* Conservation of Dipterocarpus alatus Roxb. in Thailand

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Abstract. The dipterocarps, family *Dipterocarpaceae*, found in Thailand is composed of 65 species within 8 genera including *Anisoptera* (3 species), *Cotylelobium* (1 species), *Dipterocarpus* (16 species), *Hopea* (13 species), *Neobalanocarpus* (1 species), *Parashorea* (1 species), *Shorea* (22 species) and *Vatica* (8 species). Their natural habitats are normally confined to the tropical evergreen, semi-evergreen and mixed deciduous forests, except for 6 species, *Dipterocarpus intricatus*, *D. obtusifolius*, *D. tuberculatus*, *Shorea obtusa*, *S. roxburghii* and *S. siamensis*, that are found in dry dipterocarp forests. As a consequence of forest destruction, natural habitats of the dipterocarps have shrunk correspondingly. The 25% loss of forestland during the past decade resulted in a decline in the genetic resources of some important dipterocarp species. At present, an official estimate of the forest areas in Thailand is approximately 13 million hectares or about 25.6 % of the total country's area. Fortunately, about 18% of the forestlands are now declared as national parks, forest parks, wildlife sanctuaries and strict nature reserves. The habitats of dipterocarps have now been protected under the National Park and Wildlife Acts. A project on the *in situ* conservation of the forest genetic resources in Thailand, established in 1999 by the Royal Forest Department, has emphasized the conservation of forest tree species, including dipterocarps, inside their habitats. The project has also emphasized ecosystem biology including distribution, associated species, growth, litter, as well as pattern of regeneration of the forest trees.

Among the 65 dipterocarp species, *Dipterocarpus alatus* has long been recognized as one of the most essential economic trees in Thailand. The species provides not only good quality wood for plywood and construction but also resin and oil for multiple uses. One can say that it is the most prominent species of dipterocarps planted in Thailand. The first plantation of *D. alatus* (8 ha) was established in 1962 at Tumbol Tritreung, Muang district, Kamphaeng Phet province. It has presently been maintained as an efficient seed production area. Aside from the plantation at Tritreung, there have been approximately 4 small *ex situ* gene conservation plots at Prachuab Khiri Khan, Nakhon Ratchasima, Kanchanaburi and Chiang Rai. Other plantations were established for commercial purposes by semi-governmental organizations such as the Thai Plywood Company (Limited) and the Forest Industry Organization. The *in situ* conservation of *D. alatus* is also far ahead from of the other dipterocarps. There have been approximately 15 *in situ* conservation areas designated as important gene resources of the species.

Introduction

The forest area in Thailand has diminished quite rapidly between 1960 and1989 - from 54% to 27.9% of its total area - mainly caused by population pressure and compounded by wide spread log-poaching activities. Through the loss of forest area, natural habitats of many valuable and economic indigenous species have been destroyed resulting in decreased forest genetic resources in our country. This alarming situation prompted the Thai government to declare a complete ban on logging in the natural forest in 1989, as an initial effort aimed at complete protection of the remaining natural forest system.

In 1999, the government of Thailand established *in situ* conservation of forest resources in the country by announcing 86,611.8 sq. km or 16.9% of the country's forested area as protected areas, followed by initiation of the "*In situ* conservation of forest genetic resources in Thailand" project. The research activities aim to continually collect comprehensive data on dynamic forest ecological systems, including biodiversity, in 15 forest-reserve areas throughout the country. The study of genetic information such as gene markers of the species is going to be one of the main research focuses in the near future.

The "*Ex situ* conservation of forest genetic resources in Thailand" project was initiated in the following year, i.e. 2000. It is hoped that both *in situ* and *ex situ* conservation of forest genetic resources in Thailand will help provide useful and valuable forest genetic resources for the future.

This paper describes some important information on the *in situ* and *ex situ* conservation of *D. alatus* Roxb. in Thailand. It is one of the economic tree species that has historical values associated with the history of our country. It is hoped that the information presented here, although not yet completed, will be a useful contribution to the efforts toward *in situ* and *ex situ* conservation of forest genetic resources in this region.

Ex situ conservation of *D. alatus* in Thailand

An alternative approach to conserving genetic resource of *D. alatus*, where *in situ* gene conservation is not possible, is *ex situ* conservation. Although the planted trees may possess a narrower genetic base when compared to those in natural habitats, it is still a valuable tool when the natural stands are in a high-risk area. Other considerations necessary to implement *ex situ* gene conservation programs may include the availability of propagation techniques and the silvicultural aspects of *D. alatus* when plantations are established in designated areas. Fortunately, besides using seeds as reproductive materials for seedling production, many studies on vegetative propagation of *D. alatus* have been conducted, particularly on rooted cuttings. Growth performance has also been investigated under different silvicultural treatments.

Presently, ongoing activities towards *ex situ* gene conservation of *D. alatus* in Thailand are still in initial stages when compared to those of other tree species. Most of them are in form of establishment of plantations made by both state and private sectors, which have different purposes of establishment. Although much further information of *D. alatus,* such as that on some genetic aspects, is required to support the *ex situ* program in Thailand, existing plantations can be considered as an important initial genetic resource. So far, there are only four *ex situ* gene conservation plots of *D. alatus* established with the cooperation of the Danish International Development Agency through Danida Forest Seed Centre, and using the international standard for establishment. The experimental plots were established in 1992 and cover an area of 10 ha at each site in the provinces of Kamphaeng Phet, Pitsanulok, Nakhon Ratchasima and Ubon Ratchasima. A few other plantations established for commercial purposes are owned by the private sector and state enterprises, as listed below.

Plantation *D.alatus* Roxb. at the Royal House

In spite of extensive use of *Dipterocarpus alatus* wood in the past, there is now less attention to the establishment of large-scale plantations neither by both the private sector and the state. The first *D. alatus* planting for experimental purposes was undertaken on the campus of Suanjitlada Royal House at Dusit Royal Palace, using the seeds collected from the Tha Yang district, Phetchaburi province. His Majesty King Bhumipol later kindly allocated an area within the Royal Palace grounds, and the Faculty of Forestry, Kasetsart University established a plantation of 1,096 *D. alatus* trees in 1961, which is now the only *D. alatus* plantation situated in the City of Bangkok.

Plantation of *D. alatus* by the Royal Forest Department (RFD)

Under responsibility of the RFD, establishment of *D. alatus* plantations were first made in 1962 at the Tritreung Plantation Station under the supervision of Mr. Prasert Pakdee, station chief (as shown in Table 1). Tritreung Plantation Station is located adjacent to Paholyothin Road, between kilometer 376 and 377, at Pakdong village, Tritreung sub-district, Muang district, Kampaeng Phet province. The station is located at an elevation of 80 m above sea level. The area is mostly flat with a medium-fertile soil type of loam to sandy loam. The average annual rainfall is 1,252 mm, relative humidity 77.5%, and the mean maximum and minimum temperatures are 33.5°C and 22.7 °C, respectively.

D. alatus plantation area covers approximately 8 hectares, located along the highway Nakorn Sawan – Kampaeng Phet. During the early stages, *Cassia* sp. seedlings were also planted to provide shade for the *D. alatus* seedlings. For preparation of *D. alatus* seedlings, instead of using polythene bags, bamboo containers were used and filled with a planting media of soil – sand – horse manure (1:1:1). The seeds were collected from plus trees naturally grown in Kamphaeng Phet province using the method of ground collection during March through mid-April. Normally, seeds germinated within 11 – 15 days and seedlings reached an average of 25 cm in height after being tended in the nursery for 4 months. At this stage, the *D. alatus* seedlings totaled 2,937 and they were ready to be planted out in the designated plantation area. In 1966, an investigation of seedling survival rate was made, showing that only 1,801 seedlings (61%) remained in the area. In 1972, the Forest Protection Unit undertook the first selective thinning operation No. KP. 3 (Tritreung) to promote better growth. A method of crown thinning was applied, in which up to 101 trees (7.27 m^3) were removed as were all *Cassia* trees (214 trees) in the supplementary planting.

In 1973, 13 *D. alatus* trees (2.37 m^3) were also removed. However, no research such as the establishment of permanent plots was made to study growth performance. This was initiated in 1986, when tree girths and tree heights of 1,155 *D. alatus* trees were measured. Based on calculations using an average height of 12.4 m and an average GBH of 120 cm (average volume per tree 1.1 m^3), the total wood volume was 1,282.6 m^3 at the age of 24 years. The GBHs of the smallest and largest trees were 32 and 200 cm, respectively. The *D. alatus* plantation activity at Tritreung Plantation Station was continued until 1967. However, since 1963, local people had expressed their displeasure with the *D. alatus* plantation program, and this perhaps was the cause of the almost annual fires in the plantation. Therefore, some empty areas where fire-damaged trees were removed were additionally planted with teak (*Tectona grandis*). The data of plantation established in this area is presented in Table 1. The preparation of teak cuttings by coppicing is easier compared with *D. alatus*. In conclusion, it appears that the oldest *D. alatus* plantation, established in 1962, exists today. This plantation is demarcated, undisturbed, and well protected.

Table 1. D. alatus plantation area during 1962-1967, Tritreung Plantation Station

Planting Year	Area (ha)	Budget (Thai Baht)	Supervisor	Remarks
1962	8	34,000	Mr. Prasert Pakdee	1. *D. alatus* trees planted during 1963-1967 were damaged by fire and the area was substituted with teak. 2. Data from Nakorn Sawan Regional Forest Office was transferred to Silviculture Research Center No. 5 in Kampaeng Phet, in 1996.
1963	16	44,000	Mr. Prasert Pakdee	
1964	19	49,000	Mr. Thammasak Suwapat	
1965	19	41,923	Mr. Thammasak Suwapat	
1966	4.5	49,872	Mr. Prasert Pakdee	
1967	18	17,000	Mr. Prasert Pakdee	
Total	**84.5**	**235,795**		
remaining area today	8			

Source: Nakhon Sawan Regional Forest Office, 1986

Apart from the plantation at Tritreung, there are four other small plantations and two roadside plantings of *D. alatus* that have been used for gene conservation purposes and were mainly used as additional seed sources by RFD (Table 2)

Table 2. Major *ex situ* gene conservation sources of *D.alatus* by the RFD

No.	Site	Location	Status	Area (ha)	Remarks
1.	Bang Saphan Noi	Bang Saphan Noi District Prachuab Khiri Khan Province	Experimental plot (Undisturbed)	0.16	
2.	Reforestation Research and Training Station	Wang Naam Khaew District Nakhon Ratchasima Province	Gene bank	1.6	
3.	Paa Huay Khayaeng	Tong Phaaphum District Kanchanaburi Province	Plantation (Undisturbed)	0.16	
4.	Taa Yaang Petchaburi	Taa Yaang District Petchaburi Province		-	Planting along roadside
5.	Sarapee Forest Check Point	Nhong Phueng district Chiang Mai province		-	Planting along roadside
6.	Kew Tubyang	Mae Chan district Chiang Rai province	Plantation		
	Total			**1.92**	

Plantation of D. alatus by Forest Industry Organization (FIO)

FIO, a semi-government organization, has established plantations for industrial purposes. Although *D. alatus* was not a main species for plantation by FIO, *D. alatus* was planted both as pure stands and in mixed stands in an agroforestry system. The plantation of *D. alatus* was established at Lang Suan district, Chumphon province in 1977 on an area of 22 rai (3.52 ha) and at Somdet

Reforestation Station, Somdet district, Kalasin province during 1976-1977 in various planting systems such as pure stand, mixed stand with other tree species and mixed with cash crops (cassava) with 2mX8m spacing. The growth data of some *D. alatus* plantation is given in Table 3.

Table 3. Growth of *D. alatus* plantation of FIO at Somdet Reforestation Station, Somdet district, Kalasin province

Planting year	**Age**	**Spacing (mxm)**	**Planting area (ha)**	**DBH(cm)**	**Remarks**
1977	14	2x8	144	82.84	Mixed planting with *Pterocarpus marcrocarpus, Eucalyptus camaldulensis*
1978	13	2x8	1.6	51.42	Pure stand
1979	12	2x8	168	52.78	Mixed planting with *Pterocarpus marcrocarpus, Eucalyptus camaldulensis*

Source: Forest Industry Organization, 1999

Plantation of Plywood D. alatus by The Thai Plywood Company (Limited)

The Thai Plywood Company (Limited) or TPC (Ltd.) is one of the semi-governmental organizations under Ministry of Agriculture and Cooperatives which utilizes a massive amount of *D. alatus* and some other dipterocarp timbers as raw materials for producing plywood and fiber board. In the past, these timbers were mainly from natural stands. The TPC (Ltd.) later established the *D. alatus* plantation as reserved stocks for future supply. The plantations established by the TPC (Ltd.) were not large in scale but were for experimentation at Huay Rabum Plantation, Lansak district, Uthai Thani province and at Ladkrating Plantation, Sanamchaikhet district, Chachoengsao province. The investigation of growth at both sites was carried out in 1997 and 1998 respectively and the results are shown in Table 4.

Table 4. Growth of *D. alatus* in experimental plantation by Thai Plywood Company (Limited)

Plantation	**Planting year**	**Age**	**Spacing (mxm)**	**Planting area (ha)**	**Ht (m)**	**DBH(cm)**	**Remarks**
Huay Rabum, Uthai Thani province	1980	17	2x4	0.32	11.61	13.57	Planted in partly thinned *Melia azedarach* plantation
	1996	1	2x2	3.52	-	-	Pure stand
Ladkrating, Chachoengsao province	1973	36	4x4	0.72	18.72	28.34	Pure stand
	1992	6	2x4	4.84	5.20	6.92	Planted in partly thinned *Melia azedarach* plantation
Total				**9.40**			

Source: Thai Plywood Company (Limited) 1999

Plantation of D. alatus by private sectors

D. alatus is a somewhat slow growing species compared with several indigenous species and some exotic fast growing species such as *E. camaldulensis*, *Acacia mangium*, *A. auriculiformis,* or even teak. It is not the first choice of species for commercial planting, and often inter-planted with fast growing species. However, *D. alatus* is one of the promotional species in the RFD's Private Reforestation Extension Project and seedlings are readily distributed to other governmental organizations, the private sector, and the public at almost every RFD's Nursery Center, particularly those located in or near natural stands of *D. alatus*.

A number of demonstration plantations of *D. alatus* were also established at RFD Experimental Stations either in pure stands (in Krabi, Chumphon and Nakhon Ratchasima province) or in mixed stands with other tree species (Agroforestry system) which has led to much interest by the public to plant *D. alatus*.

In the past 5-7 years, more than 1,280 ha of *D. alatus* plantations were established by the private sector through RFD's Private Reforestation Extension Projects (Table 5). The majority of these were established within the native range of *D. alatus*, mainly in northeastern, central, and lower northern Thailand.

Table 5. *D. alatus* plantation by private sectors under RFD' Private Reforestation Extension Project

Planting year	No. of planters	Planting area (ha)	Remarks
1994	150	288.48	*D. alatus* plantations were mainly in mixed stands due to technical guidance by RFD
1995	195	328.64	
1996	404	612.28	
1997	2 7	52.24	
Total	**776**	**1,281.64**	

Source: Private Reforestation Division, 1999

Most of the *D. alatus* plantations owned by private industry were established through various forms of governmental extension program because it is difficult for the private sector to obtain the technical knowledge for production of seedlings.

Nowadays, there are increasing numbers of *D. alatus* plantations in Thailand, particularly those owned by private sectors, although the registered plantation areas are still limited. The areas of *D. alatus* plantation in Thailand by various organizations are summarized in Table 6. The major *D. alatus* plantation area belongs to the private sectors (1,394.72 ha).

Table 6. D. alatus plantation in Thailand

Organization	Age (year)	Planting area (ha)	Remarks
RFD FIO TPC (Ltd.) Private sectors	2-40 20-23 19-27 3-4	348.15 327.52 9.40 1,394.72	*D. alatus* planted by RFD as *Ex situ* gene conservation area and as experimental plantation with total area of approximately 48 ha was excluded.
T o t a l		**2,079.79**	

Some constraints to establishing *D. alatus* plantation in Thailand

Limited seed source

Overexploitation of *D. alatus* in the past has resulted in insufficient production of healthy seeds now. *D. alatus* seeds were normally collected from trees in natural stands that were mostly self-pollinated. That practice was reflected by poorer growth and less tolerance to diseases and insects. Supply of *D. alatus* seeds fluctuates, and seeds are attacked by insects that evidently cause more than 20% of immature seeds to fall to the ground prematurely (Boontawee 1999). Even mature insect-attacked seeds will not germinate if the seeds are seriously damaged.

Nursery techniques

Generally *D. alatus* seedlings are obtained from seed collected from the ground. Germination rate of *D. alatus* seeds is an important factor controlling the number of seedlings produced because the viability period of *D. alatus* is very short (1 - 2 weeks). Within these 2 weeks, germination of healthy mature *D. alatus* seeds will exceed 90%.

Storage of *D. alatus* seeds is possible after drying them in air-conditioned room (10-15°C) to slowly reduce moisture content of seeds to the level of approximately 10% or less, and storing them in sealed polythene bags in the freezer at -10°C for up to 2 years (Pukittiyacamee *et al.* 1994). Where cold storage facilities are absent, an alternative is to use aged seedlings. These can be maintained by placing potted seedlings on a wooden seeding bed 30-50 cm above the ground, and allowing the roots that come out of the pots to be air-pruned. These seedlings can be maintained over a one-year period for later use.

Many attempts have been made to produce *D. alatus* seedlings through tissue culture but with no success inducing roots of the seedlings (Boontawee *et al.* 1993). Various cutting techniques offer another satisfactory alternative for producing *D. alatus* plants (Soonhuae & Limpiyaprapant 1996).

***In situ* conservation of *D. alatus* in Thailand**

Due to extensive use of *D. alatus* wood in the past decades, natural stands have declined continuously, resulting in decreased genetic resources. At present, most *D. alatus* stands are limited to protected areas such as national parks, wildlife sanctuaries, or forest parks where they have strict laws and regulations. However, many attempts have been made to maintain *D. alatus* by also establishing an *in situ* conservation program in the Royal Forest Department in 1999. The program, basically, emphasizes ecosystem biology of existing *D. alatus* populations in the protected area, which possess high potential as genetic resources. Investigations focus on the natural distribution, growth performance, natural regeneration, and protection of the species.

Another possibility for expanding *in situ* gene conservation programs for *D. alatus* may be to include other existing natural stands found scattered about in the reserve forests and in public areas such as temples or schools. These stands are normally in high-risk areas and are reserved as seed production areas for the RFD's Nursery Division. When compared to the RFD *in situ* program in the protected areas, these stands, even though are of less potential for gene conservation, still have significant importance and genetic value, and may contribute to providing a broader genetic base for *D. alatus* in Thailand. Some of these promising *D. alatus* stands are listed in Table 7. However, only the most basic information has been received at this time, particularly related to natural distribution and disturbance status. Further information on detailed genetic aspects may help to determine whether or not all of these stands of *D. alatus* represent a supplemental genetic resource, aside from being used as seed production areas.

Table 7. Gene resources of *D. alatus* Roxb. in Thailand

No.	Site	Location	Status	Area (ha*)
1.	Paa Khao Phra Leu Si-Khao Bo Rae	Tong Pha Phum District Kanchanaburi Province	Natural forest (Disturbed)	200
2.	Forest Protection Unit No. ST 15 (Chaiya)	Chaiya district Surat Thani province	Natural forest	0.8
3.	Paa Klang Ao	Bang Saphan District Prachuab Khiri Khan Province	Natural forest (Disturbed)	192
4.	Wat Koo Phra Konaa	Suwannaphum District Roi Et Province	Natural forest (Undisturbed)	32
5.	Kosampee Forest Park	Kosumpisai District Maha Sarakham Province	Natural forest (Undisturbed)	8
6.	Tri Treung plantation	Muang District Kamphaeng Phet Province	Plantation (Undisturbed)	8
7.	Paa Wang Chao-Wang Chompoo	Kosampee Nakhon Branch-district, Kamphaeng Phet Province	Natural forest (Undisturbed)	8
8.	Baan Wang Yaang	Muang District Kanchanaburi Province	Natural forest (Undisturbed)	64
9.	Paa Khao Phuuluang	Wang Naam Khaew District Nakhon Ratchasima Province	Natural forest (Undisturbed)	8
10.	Nakhon Chai Borworn Forest Park	Phoe Talae District Phichit Province	Natural forest (Undisturbed)	172.8
11.	Dong Faa Huan	Muang District Ubon Ratchatani Province	Natural forest (Slightly Disturbed)	352
12.	Nhong Sanom	Muang District, Rayong Province	Natural forest (Disturbed)	35.68
13.	Khao Yai National Park	Pakplee district Nakhon Nayok province	Natural forest	100
14.	Paa Wang Naam Kheaw	Paktongchai District Nakhon Ratchasima Province	Natural forest (Undisturbed)	2.4
15.	Tub Larn National Park	Kornburi District Nakhon Ratchasima Province	Natural forest (Disturbed)	12.8
16.	Mae Salad-Pong Daeng National Reserved Forest	Ban Tak district Tak province	Natural forest	>480
17.	Paa Huay Khayaeng	Tong Phaaphum District Kanchanaburi Province	Natural forest (Disturbed)	90.4
18.	Ban Nhong Chiang Kaa Public Area	Sam Ngao district Tak province	Natural forest	8
19.	Paa Phangai Huay Tubtan-Kumphode (Nhong Koo)	Sangkhla District Surin Province	Natural forest (Disturbed)	3.2
20.	Ram Kamhaeng/Paa Khao Laung National Park	Khirimas district Sukhothai province	Natural forest	0.64
21.	Forest Protection Unit No. PL6 (Panchalee)	Wang Thong district Phitsanulok province	Natural forest	8
22.	Nursery Center Pattani province	Koke Phoe district Pattani province	Natural forest	1.6
23.	Paa Mae Jun Nation Reserved Forest	Jun district Phayao province	Natural forest	30.2
24.	Forest Management Unit Klong Tron Paa Klong Tron National Reserved Forest Phang Khaaw	Naam Paad district Uttaradit province	Natural forest	80
25.	Forest Protection Unit	Saena Nikom district	Natural forest	5.6
26.	Paa Chiang Rai. Experimental Station Mae Lao National Reserved Forest – Phang Sai	Mae Saruay district Chiang Rai province	Natural forest	16
	Total			**1,920.12**

source: Boontawee, 1999

Conclusion

The total area of *D. alatus* plantation in Thailand is approximately 2,080 ha, of which approximately 1,280 ha are used as *ex situ* conservation areas by the RFD. The *in situ* conservation of *D. alatus* occupies approximately 1,920 ha. Although most natural stands of *D. alatus* are well protected, it is the responsibility of the Thai people to maintain all these naturally distributed stands. *D. alatus* has been a prominent, high economic value species along with teak, and has played an important part in the history of Thailand. It is the RFD's main objective to increase the forested area of Thailand to 40% of the total area of the country. One pathway to this target of increased forest area is through the RFD-promoted Private Reforestation Extension Project. It is hoped that the private sector will also play a major part in increasing *ex situ* conservation areas of *D. alatus* in the country. Several studies on *D. alatus* are still needed to provide useful information to enable more successful establishment of *D. alatus* plantations in Thailand, and this research is an essential part of strengthening the future *ex situ* conservation program for *D. alatus*.

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Ex situ Conservation of Dipterocarp Species in West Java and Banten

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Abstract. Since 1937 the Forest Research Institute, now the Forest and Nature Conservation Research and Development Center (FNCRDC), has established 11 demonstration forests in West Java and Banten, eight of which – namely, Darmaga, Cikampek, Pasirhantap, Haurbentes, Yanlapa, Pasir Awi, Cigerendeng and Carita –have collections of dipterocarp species. The collection of dipterocarps in these demonstration forests consists of 41 species in 5 genera. The origins of the dipterocarp species are from the islands of Sumatera, Bangka, Java, Kalimantan, and Maluku. Various studies have been conducted on these demonstration forests including growth and yield, pest and disease resistance, and flowering patterns. Among the potentially fast growing dipterocarp are *Shorea leprosula, S. macrophylla, S. selanica,* and *S. guisso*. Currently the dipterocarp stands at these demonstration forests have become important seed sources for planting programs of several dipterocarp species.

Introduction

Over 20 million ha of tropical forest in Indonesia has been deforested since the early 1970's and has now become unproductive degraded forest (Badan Planologi Kehutanan 2000). Dipterocarps are the major commercial trees of the tropics and the deforestation is threatening the existence of some dipterocarp species. The family Dipterocarpaceae, which is naturally spread throughout the islands of Indonesia, consists of at least 8 genera and 155 species (Symington 1974).

Natural regeneration of dipterocarp species in natural forests has also been disturbed due to removal of mother trees by illegal logging. To make the problem worse, forest fires have destroyed millions of hectares of dipterocarp forests. Finally, the irregularity of flowering and fruiting makes it difficult to obtain seeds. Since the existence of many dipterocarp populations are now in danger, an awareness to save the last scattered dipterocarp populations must be generated nationwide, and quick action, such as establishing conservation areas must be taken before the species disappear.

Genetic conservation can be accomplished either *in situ* or *ex situ*. Currently there is an increasing trend of encroachment and illegal logging even in conservation areas such as National Parks. *In situ* conservation areas have also been under threat of illegal logging or encroachment, so under these present conditions, the quickest and safest way to conserve genetic material of dipterocarp species is through *ex situ* conservation. *Ex situ* conservation methods include field gene banks, seed banks, slow-growth seedlings, pollen storage, and *in vitro* gene banks (Chin 1993). Because dipterocarp seeds cannot be stored for long periods, field gene banks such as botanical gardens or demonstration forests, are appropriate methods for *ex situ* conservation of the dipterocarps. The goal of *ex situ* conservation of commercial tropical trees is to keep genetic resources in secure and accessible areas for future utilization, especially in tree improvement programs.

Since 1937 the Forest and Nature Conservation Research and Development Center (FNCRDC) has established 11 demonstration forests in West Java and Banten; namely, Carita, Haurbentes, Yanlapa, Pasir Awi, Darmaga, Cikampek, Pasirhantap, Cikole, Arcamanik and Cigerendeng. In addition, another 6 demonstration forests have also been established in Lampung, Central Java, and East Java. The aims of the establishment of demonstration forests are to test promising exotic and indigenous tree species and to conserve Indonesian native tree species, including dipterocarps, in secure areas.

This paper presents the descriptions of dipterocarp stands at eight demonstration forests in West Java and Banten, and the utilization of these stands.

Locations and Site Description

Locations of the dipterocarp demonstration plots in West Java and Banten are shown in Figure 1. There are 8 demonstration forests in West Java and Banten belonging to the FNCRDC, which has dipterocarp plots at Carita, Haurbentes, Yanlapa, Pasir Awi, Darmaga, Pasirhantap and Cigerendeng. All of the demonstration forests are accessible by car. The nearest demonstration forest to the FNCRDC main office in Bogor is Darmaga (about 7 km West of Bogor) and the farthest is Cigerendeng (about 300 km Southeast of Bogor).

Figure 1. Location of FNCRDC demonstration forests (1. Carita; 2. Haurbentes; 3. Yanlapa; 4. Pasirawi; 5. Darmaga; 6. Cikampek; 7. Pasirhantap; 8. Cigerendeng)

Site descriptions of those demonstration forests are presented in Table 1. Environmental conditions at most demonstration forests are suitable for the dipterocarps. Rainfall is relatively high, except at Cikampek with annual rainfall of only about 1891 mm. The elevations range between 50 m above sea level (lowland) to 650 m above sea level (upland).

According to the several *ex situ* conservation eras described by Cohen *et al.* (1991), the demonstration forests of the FNCRDC were established during the era of *plant exploration and introduction* between 1850 and 1950. The oldest demonstration forests are at Cikampek and Pasirhantap (established in 1937), and the youngest is at Carita (established in 1956). Most of the dipterocarp trees on the demonstration forests have reached maturity. Natural regeneration of some dipterocarp trees has grown to a diameter breast height of about 20 cm, and many have already borne fruit.

Most of the demonstration forests are located in rural areas adjacent to forest concession areas belonging to Perum Perhutani. But the Darmaga demonstration forest is located in an urban area surrounded by villages and the CIFOR campus.

Figure 2. FNCRDC demonstration forests in Carita (upper-left), Darmaga (upper-right), 60 years old *S. leprosula* at Haurbentes (lower-left) and 60 years old *S. macrophylla* at Haurbentes (lower-right)

Table 1. Site description of demonstration forest in West Java and Banten

No	Site name	Soil type	Rainfall (mm/year)	Elevation (m a.s.l)	Total area (Ha)	Establish-ment year
1.	Carita (Banten)	Grey alluvial	3959	50	50	1956
2.	Haurbentes (West Java)	Red-yellow podzolic	3348	200	100	1940
3.	Yanlapa (West Java)	Grey alluvial	2712	100	46	1953
4.	Pasir Awi (West Java)	Brown-reddish latosol	4016	150	14	1938
5.	Darmaga (West Java)	Brown-reddish latosol	3552	250	47	1954
6.	Cikampek (West Java)	Brown-reddish latosol	1891	50	45	1937
7.	Pasirhantap (West Java)	Brown latosol	3163	650	35	1937
8.	Cigerendeng (West Java)	Grey & brown alluvial	2429	50	8	1939

A total of 41 species of dipterocarps have been planted at the FNCRDC demonstration forests in West Java and Banten. The list of dipterocarp species is presented in Appendix 1. Only about 30 % out of 155 dipterocarp species have been planted so far. These dipterocarp collections were made primarily as species collections (whole genotypes). Unfortunately, information about mother trees and populations (genetic diversity) at the collection sites was not recorded during collections. The only information available is the name of the major islands of the seed origins and the years of collection.

Stand Description

Most of the dipterocarp stands in West Java and Banten contain mature trees. Although few original trees (mother trees) remain on the plots, the natural regeneration has been prolific and the progeny make these dipterocarp stands dense. The oldest dipterocarp stand now is the 64 year-old *Shorea macrophylla* planting in Pasirhantap (planted in 1937). The dipterocarp species plots were planted at 50 m x 50 m (0.25 ha). When it was planted, each plot normally contained a single dipterocarp species but now some plots have mixed species because of natural regeneration from adjacent plots.

Forest guards supervise the FNCRDC demonstration forests, and at least one is stationed at each site. Most of the demonstration forests are relatively safe from human disturbance, but two demonstration forests, namely Pasir Awi and Carita, are currently under pressure from illegal cutting and land encroachment.

Among the 41 species of dipterocarps in the eight forests, some species such as *S. leprosula*, *S. selanica, S. macrophylla* and *S. guisso* have performed well under plantation conditions at Haurbentes. Their growth rates are presented in Figures 3 and 4. The data indicate that if targeted stem diameter of the trees is 50 cm, then those four species can be managed at a 30-year cutting cycle at that site. Dipterocarp stem form is normally straight and produces long, clear boles up to 15 meters. The largest tree at Haurbentes is a 60 year-old *S. macrophylla* with a diameter of 127 cm and wood volume of 18 m^3, and the second largest tree at Haurbentes is a 60 year-old *S. leprosula* with a diameter of 120 cm and wood volume of 16 m^3 (Figure 2).

Apparently, there are no serious pests and diseases of the dipterocarps at the FNCRDC demonstration forests, except that gall diseases reportedly attacked *S. javanica* at Pasir Awi and Cigerendeng (Ardikoesoema 1954). Although gall diseases may reduce growth, most of the plants will recover. Fruits of tengkawang (*S. macrophylla* and *S. pinanga*) are reportedly infested by the Pyralidae worm (Intari 1993), but the seeds usually still germinate normally with only some damage to the cotyledons. The Pyralidae worm also infests smaller-seeded dipterocarps, such as *S. selanica*, and most of these infested seeds lose their germination capability (Natawiria *et al*. 1980).

Figure 3. Diameter increment of meranti at Haurbentes, West Java

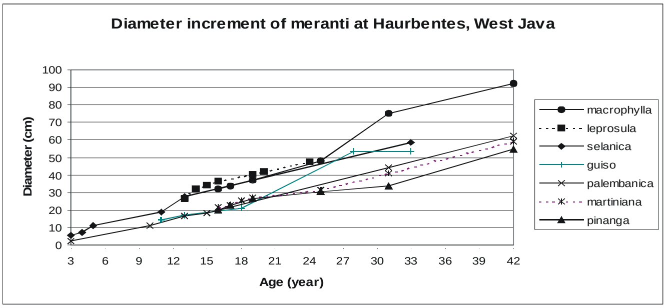
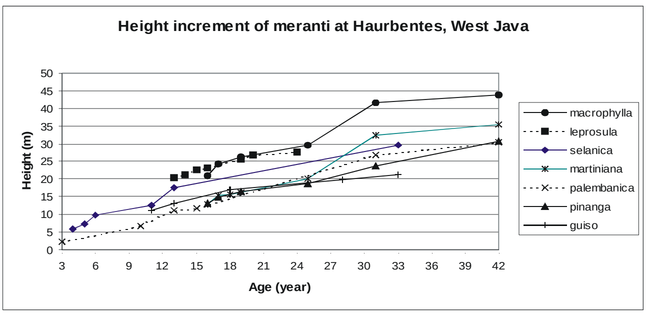


Figure 4. Height increment of meranti at Haurbentes, West Java



Utilization of The Dipterocarp Collections

The dipterocarp collection in the FNCRDC demonstration forests has been used for various purposes such as (1) research plots, (2) educational forest, and (3) seed source for planting programs of some dipterocarp species.

The FNCRDC demonstration forests are suitable places to conduct research in areas such as phenology (flowering observation and promoting flowering), growth and yield, pest and disease resistance, mycorrhizal relationships, and others. Several research institutions have conducted research at the FNCRDC demonstration forests, including BIOTROP, LIPI, Faculty of Forestry IPB and UGM, Forest Tree Seed Technology, and Forest Biotechnology and Tree Improvement Research and Development Center, Yogyakarta.

The FNCRDC demonstration forests are also good sites for educational purposes. Some short training courses have been taught in these forests including: tree identification (jointly held by Forestry Training Center and

FNCRDC); forest protection (held by BIOTROP); early detection of woody plant diseases (held by BIOTROP); biotechnology of forest trees (held by Biotechnology Research and Development Center of LIPI); seed biology (jointly held by Indonesian Forest Seed Project and Forestry Seed Technology Institute); and mycorrhizal technology (jointly held by Inter University Center for Biotechnology, IPB, and FNCRDC.)

As most of the dipterocarp stands of the demonstration forests consist of mature trees, the stands have produced abundant seeds at times. For example, almost two tons of seeds of *S. macrophylla* and *S. pinanga* were harvested at Haurbentes demonstration forest from January until April 2001. These seeds were used to produce planting stock for operational planting programs, especially for the establishment of meranti commercial plantations by Perum Perhutani and PT. Inhutani. It is generally recognized that flowering and fruiting in dipterocarps is irregular and, as an example, the last flowering season for *S. leprosula* was in early 1998 and it is expected that this species may flower again in early 2002, as it is known that its average flowering interval is 4 years. Stimulation of the dipterocarps by hormones has not successfully induced flowering (Samsuwida & Owen 1997). Table 2 presents the most probable occurrences of flowering and fruiting, by month, for some dipterocarps at Haurbentes.

Table 2. Commonly observed of flowering and fruiting months of dipterocarps in Haurbentes

Species	MONTH												Fruiting interval
	1	2	3	4	5	6	7	8	9	10	11	12	
Dipterocarpus grandiflorus	●	●				❀	❀	❀	●	●	●	●	Almost yearly
Hopea sangal	❀	❀	❀	●	●	●	●	●	●				Almost yearly
Hopea mengarawan	❀	❀	❀	●	●	●	●	●	●				Almost yearly
Hopea odorata	❀	❀	❀	●	●	●	●	●	●				Almost yearly
Shorea chrysophylla	●	●	●				❀	❀	❀	●	●	●	Every 3 years
Shorea compressa	●	●	●				❀	❀	❀	●	●	●	Every 3 years
Shorea guiso	●							❀	❀	●	●	●	Every 2 – 3 years
Shorea leprosula	●	●	●				❀	❀	❀	●	●	●	Every 2 – 4 years
Shorea macrophylla	●	●	●				❀	❀	❀	●	●	●	Every 3 – 4 years
Shorea martiniana	●	●	●				❀	❀	❀	●	●	●	Every 2 – 3 years
Shorea mecistopterix	●	●	●				❀	❀	❀	●	●	●	Every 2 – 3 years
Shorea multiflora	●	●	●				❀	❀	❀	●	●	●	Every 2 – 3 years
Shorea palembanica	●	●	●				❀	❀	❀	●	●	●	Every 3 years
Shorea pinanga	●	●	●				❀	❀	❀	●	●	●	Every 2 – 3 years
Shorea selanica	●	●	●				❀	❀	❀	●	●	●	Every 2 - 3 years
Shorea stenoptera	●	●	●				❀	❀	❀	●	●	●	Almost yearly
Shorea virescens										❀	❀		Every 4 years
Vatica sumatrana	❀	●	●	●	●	●	●				❀	❀	Almost yearly
Vatica wallichii	●	●				❀	❀	❀	●	●	●	●	Almost yearly

❀ Flowering months

● Fruiting months

Concluding Remarks

Only about 30% of 155 native dipterocarp species have so far been planted and domesticated either as demonstration plots or commercial plantations. As the natural habitats of the dipterocarps become degraded at an alarming rate, exploration and collection of planting material of these other dipterocarp species from their natural habitats for conservation purposes is an urgent task.

The FNCRDC demonstration forests have thus played an important role in conserving these 41 dipterocarp species and for planting programs. The plots are also suitable sites for forest scientists to conduct various studies. Unfortunately, the demonstration forests cannot be properly managed for these many purposes due to the limited funds currently allocated to maintain the stands.

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Appendix 1. Dipterocarps collection at FNCRDC demonstration forest

Species	**Planting site**	**Plot number**	**Planting year**	**Origin**	**No. of tree left**	**Natural regeneration**
Anisoptera costata	Haurbentes	64	1986	Kalimantan	38	None
Dipterocarpus gracilis	Darmaga	74	1957	Sumatera	16	Few saplings
		181	n.a	Kalimantan		
Dipterocarpus gracilis	Carita	I/9.a	1956	Bangka	110	Few
Dipterocarpus gracilis	Cigerendeng	4	1939	Sumatera	n/a	n/a
Dipterocarpus grandiflorus	Haurbentes	33	1950	Kalimantan	9	None
Dipterocarpus haselthii	Darmaga	101.a	1958	Sumatera	3	None
Dipterocarpus haselthii	Carita	I/8	1957	Java	8	Few
		II/19.a	1977	Java	n/a	n/a
Dipterocarpus haselthii	Yanlapa	41.b	1953	Java	n/a	n/a
Dipterocarpus imbricatus	Darmaga	103.a	1963	Sumatera	24	None
Dipterocarpus retusa	Darmaga	23	1987	Kalimantan	16	Few
		28.a	n.a	Kalimantan	20	Few
		29	1986	Kalimantan	55	Few
		38	1957	Sumatera	66	Few
		45	1983	Kalimantan	16	Few
		84	1987	Kalimantan	8	Few
		89	1957	Sumatera	41	Few
Dipterocarpus retusa	Yanlapa	9	1953	Java	n/a	n/a
		41.a	1953	Java	n/a	n/a
Dipterocarpus tempehes	Haurbentes	36	1940	Kalimantan Barat	21	None
Dryobalanops lanceolata	Darmaga	144.a	1987	Kalimantan	0	None
Dryobalanops lanceolata	Haurbentes	36	1954	Kalimantan	2	No fruit, flowering only
Dryobalanops lanceolata	Pasir Hantap	23	1973	Kalimantan	6	Do not flowering yet
Hopea bancana	Darmaga	68	1958	Bangka	66	Few
Hopea bancana	Carita	II/16.a	1974	Bangka	31	Few
		II/19.b	?	Bangka	0	None
		II/30.a	1958	Sumatera	21	None
		II/30.b	1958	Sumatera	15	None
		II/32.a	1958	Bangka	37	Few
		II/46	1963	Sumatera	27	Few
Hopea bancana	Haurbentes	44	1954	Sumatera	57	Few

Practical Experience with *Ex situ* Conservation of Tropical Pines

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Abstract. *Ex situ* conservation stands of *P. caribaea, P. oocarpa* and *P. tecunumanii* in 8 countries were assessed in the field in 1996-1999 by national institutions in cooperation with FAO and DFSC. Most stands were established within the FAO/UNEP project on forest genetic resources in 1975-85. Of 135 stands established 20 were lost. The remaining stands generally showed satisfactory survival rate and growth. The average stand size was well above the 500 individuals recommended for *ex situ* populations. However, a number of factors restricted the conservation value of the stands. Many stands were poorly isolated from contaminating pollen sources. Flowering and cone production was poor or non-existent and so far no seed has been harvested on a commercial scale. The countries involved experienced little benefits from maintaining the stands and at present it is doubtful whether the necessary regeneration will take place.

Based on our results, it is recommended that *ex situ* conservation as a conservation strategy for tropical trees be linked to domestication and use, preferably as part of active breeding and/or research programs, to ensure long-term management and regeneration. *Ex situ* conservation of trees is expensive and, on a long term, only applicable to a number of commercially important species. It may be the "last option" for certain important or unique provenances as an integrated supplement to *in situ* programmes. However, knowledge is wanting on practical management, regeneration and long-term stability of *ex situ* stands. So far, efforts have been restricted to pioneer species, which are relatively easy to propagate and grow. Establishment and management of *ex situ* conservation stands of climax species will often require more focus on reproductive ecology, seed collection and handling, use of nurse species and mixture of species in general.

Introduction

Ex situ conservation is often cited as a useful complement to *in situ* conservation of important forest genetic resources (FAO 1993, IPGRI 2000). The present study analysed *ex situ* conservation stands of valuable provenances of Central American pines, established under the framework of a FAO/UNEP project on conservation of forest genetic resources. The study showed that it was possible to establish an international network of conservation stands and that all provenances initially included are still represented by existing stands. The study also revealed a series of difficulties with management and flowering and cone production, which threaten the long-term viability of the stands. At present, regeneration of the *ex situ* stands at the end of their rotations seems unlikely.

So far, most *ex situ* conservation of trees has been on species of early succession, for which many species possess seed dormancy and readily adapt to open land. However, many tropical tree species, especially in rain forests, often and even predominantly possess seeds that lack dormancy. Seed handling may therefore represent a problem and typically, these species are not easily established on open land. In the same habitats species often depend on intricate pollinator relationships for their continuous regeneration. If *ex situ* strategy is to play a role in the conservation of these often highly threatened species, future efforts have to pay more attention to moving species back into *in situ-like* situations (Prance 1997). It is suggested herein that a mixture of species over larger areas, possibly in restored suitable habitats simulating the original ecosystem, may help sustain natural regeneration and long-term stability of *ex situ* populations.

Table 1. Number of existing *ex situ* conservation stands (N) included in the present study, by country and provenance. Stands of highest international importance indicated by light shading

		Thailand	**India**	**Australia**	**Côte d'Ivoire**	**Kenya**	**Tanzania**	**Brazil**	**Zambia**	**Total**
Species	**Provenance**	N	N	N	N	N	N	N	N	N
P. caribaea	Alamicamba	5	1	3	1		1	3		14
	Culmi	3	3					3		9
	Guanaja			1				1		2
	Karawala						1	3		4
	La Brea Colon	1		1						2
	La Mosquitia	2		1						3
	Los Limones	6	8			3	1			18
	Poptun	2	5		1					8
	San Carlos	1								1
	Santa Clara	1						1		2
	Unknown	3	1							4
	SUBTOTAL	**24**	**18**	**6**	**2**	**3**	**3**	**11**	**0**	**67**
P. oocarpa	Bonete		1						2	3
	Bucaral (El Castano)	1								1
	Dipilto	1								1
	Jocotan	1								1
	Las Crusitas		1							1
	Mal Paso	4								4
	Unknown	2								2
	SUBTOTAL	**9**	**2**	**0**	**0**	**0**	**0**	**0**	**2**	**13**
P. tecunumanii	Chanal							1		
	Mt. Pine Ridge	1		2	1	1		2	3	10
	Napite							1		
	Pachoc							1		
	Rancho Nuevo							1		
	San Rafael	2	3					4		9
	Yucul	4	1		1		1	4	1	12
	SUBTOTAL	**7**	**4**	**2**	**2**	**1**	**1**	**14**	**4**	**35**
	TOTAL	**40**	**24**	**8**	**4**	**4**	**4**	**25**	**6**	**115**

The *Ex situ* Programme on Tropical Pines

In the mid 1970s there was a major interest in the establishment of fast growing plantations of forest trees in many tropical countries. As suitable species and provenances were being identified it became increasingly difficult to procure seed from the identified sources and it was realised that many of the native stands of these species and provenances, not being of high priority in their country of origin, were vulnerable. On this background a FAO/UNEP project on *ex situ* conservation of forest genetic resources was developed. The objectives of the FAO/UNEP project were threefold: (i) *ex situ* conservation of species and provenances of proven value for plantations in a range of interested, developing countries (ii) establishment of seed sources for use at national levels (iii) gaining of experience regarding the feasibility and efficiency of *ex situ* conservation in living stands. Stands were established in Australia, Brazil, Côte d'Ivoire, India, Kenya, Tanzania, Thailand and Zambia in the late 1970's and early 1980's. The study population consisted of 135 *ex situ* conservation stands of *P. caribaea* var. *hondurensis, P. oocarpa,* and *P. tecunumanii* representing 28 different provenances. Thirty-six stands were established as part of the FAO/UNEP project on conservation of forest genetic resources (FAO 1985), while 99 were established through national efforts. The number of existing *ex situ* conservation stands by country and provenance is shown in table 1. In total, the *ex situ* stands covered almost 1000 hectares.

In 1996 countries hosting the stands, the Food and Agriculture Organization of the United Nations (FAO), and Danida Forest Seed Centre (DFSC) agreed to make a common evaluation of the established stands. The evaluation was carried out during 1996-1999 and was undertaken as a common effort among the countries involved, DFSC, and FAO. It is probably one of the most extensive *ex situ* conservation efforts on tropical trees to be documented.

Establishment and Management of *Ex situ* Stands

The FAO/UNEP project gave recommendations for stand establishment and management, and provided financial support for the initial phase of establishment (FAO 1985). The establishment and management of the national stands were financed locally in many cases with support from external donors. For these stands establishment and management followed local practices.

Planting sites

The 135 stands were established at 39 planting sites in Australia, Brazil, Côte d'Ivoire, India, Kenya, Tanzania, Thailand and Zambia. Some planting sites differed considerably from conditions found in the natural range of the species/ provenances, i.e. highland provenances were planted in the lowland and vice versa. Presumably, this was due to local and national constraints in identifying matching sites, rather than a conscious choice. The poor match did not influence survival and growth but seriously reduced the reproductive potential of many stands. Coastal provenances of *P. caribaea* var. *hondurensis* did not flower when planted above 800 m alt. and overall were most affected by the poor environmental matching. Inland *P. caribaea* var. *hondurensis*, *P. oocarpa,* and *P. tecunumanii* provenances generally were established at sites more similar to their origin.

Stand size

A minimum stand size of 10 ha was recommended at the project start in order to secure a viable conservation stand in the long run (FAO 1985). This was based on a number of biological/genetic considerations such as calculations on pollen flight to ensure sufficient pollination and, above all, the ability of the overall pollen cloud to dilute pollen arriving from outside sources and, thus, reduced risk of pollen contamination (FAO 1992).

A stand of 10 ha with a spacing of 3 x 3 m would initially consist of approximately 11,000 trees. If the recommended FAO/UNEP thinning regime is applied as an example, reducing the number of trees by 50% at each of two thinnings reduces the number of individuals 2,800 trees. This would still provide a buffer against loss due to natural and human disturbances and is well above the 500 individuals estimated as minimum viable population size (Franklin 1980).

Actual stand size in the programme after establishment varied considerably from less than 1 ha to 37 ha. The majority of stands were smaller than the recommended 10 ha. For stands established under the FAO/UNEP programme average size was 5.8 ha. Average size for national stands established through national effort was 7.5 ha. Nonetheless, most stands were considered to have a sufficient number of individuals to secure genetic variation and decrease genetic drift to an acceptable level even over a number of generations.

Isolation from contaminating pollen sources

Appropriate isolation of stands from possible, contaminating pollen sources is an important requirement for gene conservation and for providing an appropriate seed source for future conservation use. The survey revealed that about half of the stands fulfilled the recommended isolation of 300 m distance to nearest contaminating pollen source. In most cases more stands were found at one site, typically close to research or gene conservation and seed production stations. Many stands at one site provided advantages in terms of protection and management of the stands, but made it difficult to secure appropriate isolation. Moreover, stands were, as mentioned before, established with a range of objectives and *ex situ* conservation was not always the prime one (e.g., initial testing of performance of a seedlot/provenance in a given environmental condition, and seed production). Therefore, proper isolation was not always regarded as critical at the time of establishment, by the national institutes concerned.

Demarcation

Various means of field demarcation were used, i.e. forest roads, fire lines, border rows of different species, paint, or poles. Most of the assessed stands were well demarcated. However, for about one quarter of the FAO/UNEP stands the borders were difficult or impossible to identify. For example, three stands in Kenya were not demarcated or mapped and could not be located in the field. National stands were generally better demarcated. At sites with a single *ex situ* conservation stand demarcation was clear. Difficulties usually arose where more conservation stands of pines were clustered at one site, typically around a research station.

Lack of thinning

Although the *ex situ* stands were well established and managed in the initial stages during which external funding was available, active management in later stages was generally lacking. In particular, the large majority of stands were never thinned. Of 24 existing FAO/UNEP stands only one had been thinned, and only once. Of the 99 stands established through national efforts only 19 had been thinned. As a result, the *ex situ* stands were extremely dense and the trees had small, narrow crowns - conditions which usually negatively impact flowering in pines.

Conservation Status of the Stands

Do the stands still exist?

Of the 36 stands established within the framework of the FAO/UNEP project 8 were lost and a further three stands could not be properly identified in the field. For the stands established through national efforts, the number of stands initially established is unknown and hence the survival rate cannot be calculated. However, 12 out of the 99 stands included were lost. In addition, information on seed lot origin was lost for 6 stands why they have little or no value conservation wise. Fire originating from shifting cultivation and other human activity in nearby areas was the main reason for the loss of stands damaging 1/3 of the remaining stands as well.

Disturbance in stands

Of the 28 FAO/UNEP stands, 15 were undisturbed, while fire, illegal cutting or wildlife damaged 13 stands to some extent. Fire and illegal cutting accounted for approximately 2/3 of these damages. In India, domestic animals damaged 13 out of 16 stands in early stages of development. This resulted in high seedling mortality in the establishment phase, whereas the present health status of surviving trees is generally excellent. Encroachment, domestic animals, and termites caused damage locally.

Among the 87 existing national stands, 47 were undisturbed. Fire, illegal cutting, encroachment or domestic animals affected the remaining 40 stands to varying degrees. Fire was the dominant threat in Thailand, Tanzania and Australia and accounted for 24 of the disturbed stands. In Brazil, wind and hail constituted a serious threat at a number of sites causing stem break and wind throw.

In summary, fire was the all-important cause of damage to the conservation stands. Besides accounting for the loss of 14 of 20 stands, fire was the primary cause of damage followed by illegal cutting. Disturbances were in most cases slight to moderate, but 13 stands were so seriously disturbed, that their overall survival is unlikely.

Survival rate in existing stands

Survival rate was measured as the ratio between numbers of trees remaining over the number of trees originally planted. Measuring survival rate in these 20-25 years-old stands was difficult in the unthinned stands, as it was not always clear where dead trees were located. Therefore, survival rate was determined from sample plots in each stand. For stands with large gaps, plots were laid out in the denser parts of stand.

The average survival rate for FAO/UNEP stands was 57%, varying from 7 % to 100 %. The average survival rate for national stands was 68%, with a range from 13 % to 100 %. The higher survival rate for the national stands is believed to be partly a result of their younger age.

Growth

Height, diameter, and stems per hectare were measured. In addition dominant, co-dominant, or suppressed status and crown development was scored. Large variation between stands was observed reflecting different site conditions, different management schemes and differences in the growth potential between species and provenances at a given site. In general, the three pine species showed a remarkable adaptation to many different conditions and overall a satisfactory growth, although their full potential was probably hampered by the lack of thinning.

Flowering and cone production

Flowering and fruiting is of crucial importance for *ex situ* conservation stands. It is a prerequisite for maintaining the genetic resource over time and to allow adaptation to the prevailing site conditions. A good flowering is of course also important for seed production necessary for regeneration of the stands at the end of their rotation.

Flowering and cone production were quantified for all stands. Five developmental stages were registered: male and female strobili, conelets, closed cones, and old open cones. The flower and cone setting was assessed on single trees using a log score scale developed by Wellendorf (1989). The score scale is described in more details in the assessment manual (DFSC 1996). Occurrence of flowers or cones on the ground was also noted.

Studies of flowering and fruiting generally require re-occurring data collection throughout the year, and over several years. The present assessment is a ‘snapshot’ assessment and the results should be interpreted with care. The five stages are believed to give a good representation of how the flowering develops over the whole reproductive cycle, however the assessment of closed cones is probably the single best measurement of the ability of a stand to flower and produce cones.

The overall flower and cone crop was poor for all five development stages. None or very light flowering and fruiting were recorded for almost all stands. For 75% of the stands no male flowers were recorded and in 96% of the stands no female flowers were observed. More conelets and closed cones were observed though the majority of the stands had none. Old open cones were seen in 83% of the stands of which most showed a light fruiting. Light fruiting corresponds to an average crop rate of 2-8 cones per tree. None of the stands showed heavy flowering or cone production and all stands set far less cones than required for commercial seed collection.

The low level is believed or explained primarily by lack of thinning (see section 3.5). In general stands were dense with narrow crowns restricting flowering and fruiting. Thinning is an established practise in the management of seed production areas and seed orchards to secure a good seed crop (Granhof 1993, Lauridsen & Olesen 1994, Faulkner 1975).

Poor matching of provenance to planting site was also considered to be a likely cause for the poor flowering and cone production. For example lowland provenances of *P. caribaea* did not set seed when planted in the highlands. Thus the low levels of flowers and cones were for some stands a combined effect of lack of thinning and climatic factors. However, cone crops in *P. tecunumanii* are usually extremely light with fewer than 50 cones on trees as large as 45 m. An average of six filled seeds per cone was found for trees in high elevation populations, yielding a seed efficiency rate of 7% (Dvorak & Lambeth 1993). More detailed investigations are required to describe and explain the flowering and seed set of the three species under different environmental conditions.

The small number of cones is likely to be a serious constraint to the use and regeneration of the *ex situ* stands. It is questionable whether thinning at this late stage will facilitate cone setting, as mature stands may not be able to respond to thinning. For lowland provenances of *P. caribaea* planted at high altitude sites sufficient cone setting may not be possible at all.

The flowering and cone production data stress the importance of a close match of seed source to planting site. Furthermore, continued management to enhance seed production is necessary. Only then the full potential of *ex situ* conservation stands of trees can be achieved.

How to Assess the Conservation Value of the Stands?

An attempt was made to categorise the stands according to their conservation value. The conservation value was assessed, based on the following factors: appropriate documentation of seed source; genetic variation within the stand (number of mother trees); size of stand (number of individuals in the conservation population); reproductive potential (flowering and seed production); isolation from contaminating pollen sources; and threats.

What is the Conservation Value of the Ex situ *Stands?*

None of the stands in the study are ideal candidates for successful *ex situ* conservation. Poor environmental matches of seed source to planting site, poor isolation from contaminating pollen sources and lack of thinning were the most important factors limiting the *ex situ* conservation value of the stands. None of the stands complied with all requirements for *ex situ* conservation. Few stands fulfilled most of the basic requirements to size, proper isolation, and flowering and cone production. However, most stands went some way towards *ex situ* conservation but individual stand contribution may be quite variable. Thirteen stands had virtually no conservation value; for example stands could not be documented or identified, -or management was given up at earlier stages for a number of reasons. The assessed conservation value of the stands in the study is shown in figure 1.

Figure 1. Conservation value of *ex situ* stands included in the study. None of the stands fulfilled all requirements of documentation, demarcation, and isolation from nearest contaminating pollen sources, seed production, and survival rate. Most stands had a sufficient number of individuals to maintain genetic variation and decrease drift to an acceptable level provided regeneration takes place. 15% of the stands were lost.

The conservation value of some of the stands with 'restricted conservation value' may be improved by management intervention (thinning, isolation, and improved protection). However, the financial resources available for management intervention are limited in most countries in the study. Decisions on further management activities should therefore be based on a careful evaluation of the potential value and use of the stands.

Discussion

The *ex situ* conservation stands of tropical pines were generally well established and managed in the initial stages. Sufficient financial resources were allocated to early stand management. However, it was much more difficult to ensure long term management of the stands. Thinning and fire protection were often neglected, which substantially reduced the conservation value and success of the project. As a result, the stands have been characterised as the ‘forgotten stands’ - they were established but largely set aside and not managed or used.

At the time of establishment there was national interest in using the species and provenances in question for local plantation purposes. Hence, the purpose of the stands was both conservation and seed supply. The demand for seed for these species was much lower than anticipated seriously hampering the incentive to actively manage the stands. Most methods of *ex situ* preservation of live plant material require periodic regeneration and sexual reproduction of the stock. At present, regeneration of the *ex situ* stands included in this study seems unlikely. The costs involved and the poor prospects for recovering part of the expenses by seed sales probably hamper the interest of institutions in charge to regenerate the stands at the end of their rotation. As a basis for deciding how, -and, which of the stands to manage in the future, it is recommended that institutions in charge carefully evaluate the potential use and seed requirements of the species/provenances, define national conservation priorities and take action in accordance with these priorities.

Although a particular stand may have limited conservation or economic value from a national perspective it may still be valuable from an international conservation perspective. Hence, assessment of the international conservation value, identifying how stands may fit into an overall conservation network and thereby contribute to the conservation of genetic resources of the species, is important. Information on the present conservation status of the natural populations *in situ* complemented with information on other *ex situ* initiatives has been compiled by CAMCORE (2000). Based on this information it appears the stands with the highest international value are the *P. caribaea,* lowland provenances of Guanaja and Karawala together with *P. tecunumanii* provenances of San Rafael and Yucul. Stands of high priority internationally are indicated in Table 1.

For stands recognised for their national or international conservation value future management steps should be considered. Thinning measures to attempt to develop seed production from the better phenotypes is recommended. At the end of their rotation it would be desirable to collect seed and replant the block to the same number of individuals or larger. It is likely, however, that the effective population size will be substantially reduced, perhaps in the order of

1/2 to 1/3 of the original number of trees. So seed would need to be collected from as many trees as possible in order to avoid effects of poor genetic representation of the original populations. In most cases co-ordination and funding would need to be established to support national institutions in the implementation of the above general steps.

Some Considerations on Future *Ex situ* Conservation of Tropical Trees

The *ex situ* conservation program on three tropical pines illustrates some of the major problems in the establishment and management of *ex situ* conservation stands. Firstly, there is a need for informed and timely management in all stages of development from establishment to generation turn-over. This is often quite costly. Secondly, viable *ex situ* conservation should to a large extent be driven by interest in utilising the species and provenances in question. Otherwise the management costs is an expense without corresponding short-term benefits to local forestry authorities. This is especially compounded when the long-term conservation value is considered unknown as well. There may or may not be a demand for seed and other reproductive materials that will pay – fully or in part – for the costs of management, protection and regeneration of the *ex situ* stands. If not, the stands are likely to be seen as conservation for its own sake, with little local value. In the absence of appropriate external funding, it is most likely that these conservation stands will be entirely lost.

Based on our results, it is recommended that *ex situ* conservation as conservation strategy for tropical trees be linked to a program for domestication and use, preferably as part of active breeding and/or research programs, to ensure long-term management and regeneration. *Ex situ* conservation of trees in living stands is expensive and, on a long-term basis, is likely applicable for a small number of commercially important species. It may be, and should be used as, "last option" for certain important or unique provenances as an integrated supplement to *in situ* program.

So far, conservation of forest trees *ex situ* has mainly been restricted to pioneer species, for which seed are readily available, stored, and which are easy to propagate and grow. The design of *ex situ* stands has followed plantation practices for these pioneer species, using monocultures and even-aged stand structure. The study shows that for such species this is a feasible design provided that resources are available for intensive management and timely regeneration. Using this design, the conservation officer will, after relatively few years, have to initiate thinning and start considering how to make the generation turnover, i.e. collect seed and establish a new stand. If not combined with utilisation of the trees, this is a costly procedure (Kjær *et al.* 2001).

This same design will, however, frequently not be suitable in the case of many tropical forest tree species of subsequent successional stages. These species often occur in mixed or uneven-aged stands; special attention will thus be needed to issues such as nurse crops, mixtures of trees and shcrub species in the various strata of the stands, and reproductive ecology including maintenance of pollinators and seed dispersing animals and collection and handling of recalcitrant seed. If conservation of trees *ex situ* is to play a role in the conservation of the numerous threatened tropical tree species, and not just for a few pioneer species, it may be necessary to think in larger areas with a mixture of species. This will favour natural regeneration and long term stability of established *ex situ* populations.

We firmly believe that it is preferable to conserve both species and ecosystems *in situ*. However, it is obvious that this will not be an option for many species or provenances where habitat destruction is total. Where *in situ* and *ex situ* techniques for conservation come together most closely is in reintroductions and recreation of habitat for rare and endangered species (Prance 1997). As more habitat is lost and some tree species are conserved only *ex situ*, it will be necessary to restore suitable habitats. We recommend that future *ex situ* conservation efforts on tropical trees should focus more on man-made habitats that have been created to move species back into *in situ* situations. By mimicking *in situ* conditions it may be possible to facilitate natural regeneration. Such strategy will broaden the range of species that can be considered for ex situ conservation in living stands, and may very well be a regular component of large-scale reforestation and rehabilitation programmes already undertaken in many countries today.

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Genetical Studies for Conservation of Tropical Timber Species in Indonesia

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Abstract. As deforestation in Indonesia continues at an alarming rate, sustainable forest management and conservation of forest genetic resources have become very important issues. Most conservation programs in Indonesia, however, are usually based on ecological information, and rarely consider genetic aspects. Population genetic studies of some commercial timber species, consisting two dipterocarp species and one non-dipterocarp species in Central Kalimantan, showed that the species are one large panmictic population. Some disturbance such as logging would not immediately reduce the genetic diversity of the species but has, however, induced the disturbed populations to differentiate from the original ones, and reduced the outcrossing rates. Reduced genetic diversity has been observed in a completely isolated population of a mangrove species in Jawa, which was formed due to extensive development of the mangrove area leading to fragmented populations. Efforts to conserve those species in situ should be directed toward restoring the disturbed populations to resemble the original, panmictic ones, whereas ex situ conservation should attempt to collect as large as possible gene pools in the populations to maintain the outcrossing nature of most tropical timber species. Other important factors to be considered in designing an ex situ conservation program or a seed orchard, as shown by a study on pine, are a balanced ratio among alleles, and presence of assortative or preferential matings among individuals collected. Those factors would ensure that most individuals sampled will produce seeds for subsequent generations.

Introduction

Every year the world loses about 15 – 17 million hectares of forests and thousands of species, and most modern deforestation is happening in tropical areas (Blaser & Douglas 2000, Contreras-Hermosilla 2000). The World Bank estimated rates of deforestation in Indonesia between 263,000 to 1,315,000 ha per year (Sunderlin & Resosudarmo 1996). The primary causes of deforestation are human activities, which exploit forests in unsustainable ways in search of profits and means of subsistence. Although forest products are recognized as the biggest source of government income besides oil and gas, the cost to society from deforestation and forest degradation is often considered to exceed the benefits (Contreras-Hermosilla 2000).

Acute deforestation and forest decline have generated a strong call for conservation of forest ecosystems and genetic resources. In theory, forest genetic resources would include all species in the forest; however, emphasis has been given to forest trees, especially those of economic importance. The reasons for this are the important role of trees in the ecosystem and that loggers harvest trees, both legally and illegally, and are charged with being the main agents of forest decline. Tropical forest ecosystems, with their well-known high levels of species diversity, now have many tree species occurring at lower than average adult densities or in dispersed clumps, conditions that, under heavy exploitation, could lead to local extinction.

Most conservation programs in Indonesia, such as establishments of parks and reserve forests, are based solely on ecological information. However, their locations on marginal areas often do not consider distribution ranges of forest species. They may capture many species, but perhaps only a limited and biased proportion of tree species' gene pools may be conserved (Ledig 1988). Even more recent conservation measures performed by forest managers within forest production areas (Riggs 1990) do not consider the processes that maintain the diversity either. Ecosystem conservation may therefore serve as a basis for developing *in situ* genetic conservation programs, but one cannot be considered to be sufficient for the other (Namkoong1997). Conservation of forest tree genetic resources involves both preventing extinction as well as ensuring the availability of the resource for future use and adaptability to changing environments (Namkoong 1997).

According to Bawa (1997) conservation of forest genetic resources poses a number of difficulties as follows:

- the number of species that require attention is very large.
- ecosystem integrity must be maintained because a majority of tropical plant species are involved in mutualistic interactions for their growth and reproduction.
- many tropical forest trees occur in extremely low densities but their populations are expected to cover large areas.
- because of their long life, trees can survive as adults without regeneration, conveying the illusion of persistence when, actually, the population is on its way to extinction.
- progress in accumulating biological knowledge is often slow and requires considerable investment of time and resources.

To be effective, forest conservation programs should consider demographic, ecological, and genetic factors (Boshier & Young 2000). The science of genetics has made an important contribution to understanding threats facing natural populations. Perhaps the most important contribution of genetic information to conservation has been its general utility in the identification of species, understanding the structure and differentiation of populations, describing the mating or reproductive system, or determining other evolutionary, ecological, or behavioral information unrelated to selective differences (Hedrick *et al.* 1996).

This paper will review results of genetic studies on several commercial timber species in Indonesia using molecular markers such as isozymes, RAPD, or AFLP and will propose some implications from those findings to both *in situ* and *ex situ* conservation programs of commercial timber species in Indonesia. Although molecular marker gene loci may be considered as a neutral and nonspecific measure of adaptability to environmental change (Hattemer & Ziehe 1997, Hedrick 1999, Namkoong 2000) employment of markers has helped elucidate many genetic processes in populations.

Genetic diversity and population structure of several timber species

Several economically important timber species in Indonesia have been subjected to genetic investigation for two main purposes; i.e., tree improvement and conservation. Two Dipterocarp species were selected as representatives of species which are commercially logged, and because they have different distribution patterns in the studied area. One species, *Shorea parvifolia,* represents an abundant species in Central Kalimantan, whereas *Shorea laevis* occurs at low density. Locally known as ulin, *Eusideroxylon zwagerii* was chosen because it is of common occurrence and is very important to a forest community. All data were taken from Lowe *et al.* (1999), Siregar *et al.* (1999), and Sudarmonowati, *et al.* (1998).

As shown in Table 1, all species chosen have relatively high genetic diversity estimates. High levels of variation were also found in seedling families, with low genetic differentiation between seedlings occurring at different areas and indicating that those species are predominantly outcrossers forming a large, panmictic population. Gene flow can occur over long distance (about 6 km) within the forest. For all species significant levels of population sub-structuring were found, indicating that more genetically similar individuals are clustered, and thus that genetic variation is not randomly partitioned.

Pinus merkusii is another important commercial species in Indonesia. Plantations have been extensively established in Java and this species ranks second only to teak in terms of plantation area. Data on this species was taken from Siregar (2000). Both in its natural habitats; i.e., Aceh, and in plantations, the species also exhibits high genetic diversity (Table 1) with high outcrossing rates.

Studies on important mangrove species, i.e., *Rhizophora mucronata*, also revealed high genetic diversity found in natural habitats (Hamzah 1999). Unfortunately data on outcrossing rate was not available for this species.

Effects of disturbance and small population size

Disturbance to natural populations, such as selective logging of dipterocarp species and ulin in Central Kalimantan, does not immediately reduce genetic diversity of the species investigated. It does, however, reduce the outcrossing rates, which means that inbreeding will increase. Population sub-structuring was also enhanced, as it was found that logged-over populations had differentiated from original populations.

Small population size, formed due to fragmentation, was characteristic of the mangrove species populations on the north coast of Jawa island, and of the isolated population of *P. merkusii* at Kerinci National Park. In those small populations of mangrove species, reduced genetic diversity was observed. As an extreme case, in the isolated population of *P. merkusii,* no variability was detected on any of the isozyme loci investigated, indicating that homozygosity has been fixed. Another important finding from the *P. merkusii* study was the existence of mating preferences between genotypes in a seed orchard. More specifically, trees with high outcrossing rates preferred male gametes of low frequencies or very rare alleles. This phenomenon could lead to changes in allelic proportions in subsequent generations.

Implications for conservation programs

Considering the fact that almost all species studied are outcrossers, *in situ* conservation programs for most tropical species should be directed at maintaining high genetic diversity by allowing gene flow to take place among local or sub-populations. This means that severely disturbed forests should be restored to form a panmictic population. Gene flow would encounter inbreeding and/or genetic drift. To avoid negative effects of fragmentation, in forestry operations it is advisable to leave several mature individual trees, which will serve as "stepping stones" between local populations.

The results of population genetic studies will help to design sampling strategies for *ex situ* conservation programs. Sub-structuring of populations and different levels of inbreeding will affect sampling processes, because sampling depends on the amount of genetic diversity in each populations and on the extent of genetic divergence between them. Detection of assortative mating has the consequence that as many as possible alleles should be collected.

The provenance concept provides a link between *in situ* and *ex situ* conservation of forest tree genetic resources. Sampling should recognize provenances as units of genetic resources. As shown by a study in plantations of *Paraserianthes falcataria,* any plantations that are established from collections of several natural populations could have higher genetic diversity than the original natural populations.

Table 1. Genetic diversity of several timber species studied from various sources.

Species names	Genetic diversity	Outcrossing rate
Shorea parvifolia	0.304	0.750 - 0.465*
Shorea laevis	0.307	0.508 - 0.206*
Eusideroxylon zwagerii	0.247	0.464 - 0.284*
Pinus merkusii	0.361	0.870 - na
Rhizophora mucronaia	0.160	na
Paraserianthes falcataria	0.187	na

Note:
*) outcrossing rate after logging
na: not available

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Genetic Conservation to Serve Breeding Programs

Genetic Conservation in Applied Tree Breeding Programs

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Abstract. This paper reviews how population size and structure impacts the maintenance of genetic variation in breeding and gene resource populations. We discuss appropriate population sizes for low frequency alleles and point out some examples of low frequency alleles in the literature. Development of appropriate breeding populations and gene resource populations are discussed.

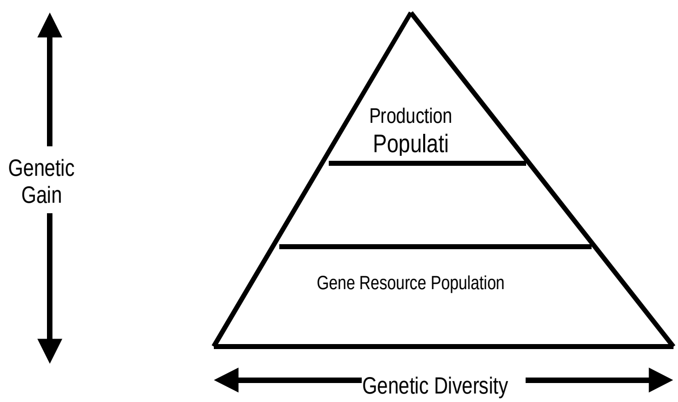
Introduction

The primary objective of a breeding program is to increase the frequency of desirable alleles found in the breeding population. While breeders know the traits they wish to improve, they do not know which alleles (genes) favorably impact the traits or their distribution in the native population. Breeding programs must maintain sufficient genetic variation to allow for continued genetic gains over multiple generations. Complicating matters is the fact that traits of interest change over time in response to new pests or changes in markets. Population sizes needed to maintain gains in polygenic traits of current interest are much smaller than population sizes needed to find potentially rare traits that may be desired in the future. This paper considers the impact of breeding population size and structure on the maintenance of genetic variation and on continued genetic gain.

The breeder needs to consider both short- and long-term objectives when structuring a breeding program. Short-term objectives usually include obtaining substantial gains in current traits of interest in the first few generations of breeding while maintaining well-adapted trees. Long-term objectives include the maintenance of low frequency alleles and control of inbreeding. A major conflict arises between short- and long-term objectives. Selection intensity must be high to obtain substantial genetic gains, yet maintaining rare alleles requires keeping a large breeding population in subsequent generations. There are ways to structure the breeding population and make selections to reduce this conflict.

Fortunately, the breeding population is only one aspect of gene resource management. Rowland Burdon developed a pyramid that conceptualizes the role of the breeding population in gene resource management (Figure 1). The horizontal axis represents genetic diversity and the vertical axis represents genetic gain. The gene resource population represents all of the available genetic variation that could contribute to the breeding population. This includes native stands, provenance trials, seed orchard parents, progeny in progeny tests, and operational plantations. The next level is the breeding population, which must have sufficient genetic variation to maintain genetic gain for multiple generations. It tends to be more improved than the gene resource population. At the top is the production population, consisting of seed orchard candidates or clones used for operational deployment. These selections are the best selections from the breeding population and provide diversity and genetic gain to operational plantations.

Figure 1. Conceptualization of gain versus genetic variation for a gene resource management program



While it would be nice to maintain all genetic diversity in the breeding population to account for unforeseen contingencies, this is not possible. Thousands of parents are required to maintain low frequency alleles for many generations (Millar & Libby 1991, Lynch 1995, Lande 1995, Yanchuk 2001), and breeding populations of this size are not financially or practically feasible. Breeders can make intelligent, or at least informed decisions by understanding which alleles are being affected by selection and by understanding the genetic variation within a species.

Population size and the conservation of genetic variation (alleles)

Unless population size is infinite, alleles are lost; it happens in nature all the time. The probability of a neutral allele being maintained in a population is primarily a function of the initial allele frequency and the effective population size. Effective population size (Ne) is an estimate of the number of individuals that would give rise to the sampling variance or the rate of inbreeding for the appropriate number of random mating parents with equal contribution of every parent (Falconer & Mackay 1996, p. 65). Ne is determined by the number of selections and their relatedness.

When deciding on a population size for a breeding or gene resource population, it is important to consider the probability of allele loss for varying allele frequencies and the associated risks. The probability of allele loss can be calculated using appropriate formula. Gregorius (1980) examined this probability for a population in Hardy-Weinberg equilibrium. Namkoong (1988) presented tables examining the minimum number of genotypes required to allow for the loss of only one allele when considering differing numbers of loci and alleles. Kang (1979a) modified a formula from Kimura and Ohta (1969) to determine the population size needed to maintain a neutral allele with specific initial allele frequencies. A general formulae (found in Frankel *et al.* 1995, p 36) for the sample size needed to be 95% certain of obtaining one copy of an allele with population frequency of *p* with an inbreeding coefficient of *F* is $\approx 3/\{(F\text{-}2)\log_e(1\text{-}p)\}$. Thus, to maintain at least one copy of an allele with a frequency of 0.05 for multiple generations, a maximum population size of 117 appears to be necessary (Table 1, Kang=79, Gregorius=117, Namkoong=117, Frankel *et al.*=59). For alleles at frequencies of ≈ 0.20, the number is reduced to 31 (Table 1, Kang=31, Gregorius=21, Frankel *et al.*=14).

Table 1. Population size recommended for maintaining neutral alleles in a population.

Allele frequency	Kang (1979) [1]	Gregorius (1980) [2]	Namkoong (1981) [3]	Frankel et al. (1995) [4]
0.5	18	6		5
0.2	31	21		14
0.1	49	51		29
0.05	79	117	117	59
0.01	269	754	597	299

1 - Population size necessary to maintain neutral alleles for 50 generations
2 - Minimum sample size required to ensure all alleles at a locus are detected with 95% probability
3 - Minimum number of genotypes required for average loss of one allele at any of 100 loci with 4 rare alleles per locus
4 - Sample size needed to be 95% certain of obtaining one copy of a gene for a population

with an inbreeding coefficient F=1

Practically, these numbers must be increased for several reasons. They assume neutral alleles, but specific alleles may be linked to loci being selected against. Also, “population size” refers to the effective population size (Ne), which is always less than the census number. To account for this, Namkoong and Roberds (1982) suggest doubling the calculated effective population sizes. Additionally, Yanchuk (2001) points out that one copy of an allele would not be useful to most breeding programs. Instead, breeding programs would want closer to 20 usable copies of a gene so that inbreeding can be controlled; this requires that the allele be evident in 20 phenotypes. Very large population sizes are needed to find 20 phenotypes for recessive alleles. A population size of 2,784 is needed if the recessive allele is at a frequency of 0.1, while 278,788 is needed if the frequency is 0.01 (Yanchuk 2001).

Maintaining low frequency alleles is important to the very long-term maintenance of populations and the genetic conservation literature has many suggestions as to how many individuals (Ne) are needed to maintain a population and its inherent genetic variation for hundreds of generations. Franklin (1980) and Soulé (1980) suggested that 500 individuals (Ne) provide the needed genetic variation. Lynch (1995) suggested an Ne of 1,000, whereas Lande (1995) calculated an Ne of 5,000. Millar and Libby (1991) suggested that effective population sizes (Ne) of 2,230 to 9,110 are needed to maintain heterozygosity levels (H_e) for *Pinus ponderosa* and *Pseudotsuga menziesii.*

It is unrealistic to think we will be able to preserve alleles with extremely low frequencies in the breeding population. These alleles are probably at low frequency because they are not presently beneficial; although, in the future they may be of use. Low frequency alleles are much better conserved in the gene resource population.

The breeding population: Maintaining alleles that contribute to gain in the short term

The most efficient way to achieve gain in early generations of a tree-breeding program is to start with the most appropriate population or land race. Most species have substantial among-provenance variation, so that beginning with the best provenance or land race is important. This requires that information from provenance trials be available. Eldridge *et al.* (1993) provide an excellent discussion of the value of provenances and land races.

The appropriate number of initial selections and families needed to obtain short-term gain has been addressed recently by Lindgren *et al.* (1997). Because many traits have low to moderate narrow sense heritabilities, we depend upon among-family selection to provide a substantial part of the gain.

One needs to start with large numbers of initial selections and families to allow for intense family selection to ensure early gain. Lindgren *et al*. (1997) suggest that 200 unrelated parents is a reasonable number to achieve gain when considering the costs and benefits of breeding.

Selection has the greatest impact on allele frequencies in the intermediate range (0.25 to 0.75) (Namkoong 1979, Falconer & Mackay 1996). These alleles are of primary importance for genetic gain in the first 5 to 10 generations of breeding, assuming that selection is for polygenic traits like growth. Based on the discussion above, 30 to 50 individuals in a breeding program is sufficient to ensure that the genes most influenced by selection will be maintained in the breeding population. In later generations, favorable alleles that were initially at low frequencies will be in the intermediate range and contribute the most to genetic gains. The genes that were initially at intermediate frequencies will be closer to fixation and not as important to achieving gains.

Baker and Curnow (1969) demonstrated that an initial Ne of 16 enables nearly as much gain as an Ne of 256 in the first five generations of breeding; very few favorable alleles are lost to random drift (random sampling) (Table 2). An Ne of 32 is almost as good as an Ne of 256 for 10 generations. Namkoong *et al.* (1988) also suggested that population sizes as small as 20 are adequate when dealing with limited generations. Kang (1979a) showed that an Ne of 17 would probably suffice to fix genes at a frequency of 0.25 and above.

Table 2 Expected progress (gain) from selection after 1, 5, 10, and infinite (∞) generations of selection for different values of Ne in a model population (Baker & Curnow 1969).

	Generation			
Ne	1	5	10	∞
4	3.3	12.4	19.5	28.4
16	3.3	16.0	31.4	114.5
32	3.3	16.8	34.6	177.5
64	3.3	17.2	36.4	220.9
256	3.3	17.5	37.8	240.0
∞	3.3	17.6	38.0	240.0

It would appear that if a breeder was only concerned about gain for 10 generations that an Ne of 30 to 50 would suffice for a single trait, provided breeding objectives did not change. However, most programs breed for multiple traits and traits of interest do change over time. Furthermore, the variance of the response must be considered. Smaller populations may have high expected gains, but the variability of those predicted gains can be high (reviewed by White 1992).

Gene resource populations: maintaining variation so that new traits can be incorporated in the future

The above discussion assumes that the traits of interest to tree breeders will remain constant. We do not foresee breeding programs losing interest in improving growth rate, but history shows that new traits are often desired. Examples of traits added to breeding programs include wood density, tree form, pulping characteristics, and disease and insect resistance. Most new traits will probably be polygenic and have genetic variation in the breeding population. The typical impact of selecting for additional traits is that gain in current traits is reduced. However, major difficulties arise when a new trait is controlled by alleles at very low frequencies or when the alleles can be found only in a few localized populations.

Examples of low frequency alleles

One example of a low frequency desirable allele is the MGR gene for blister rust resistance in *Pinus lambertiana* (Kinloch 1992). Over the range of the species, the frequency of this allele is 0.022; however, within individual seed zones the frequency varies between 0 and 0.087. Using the population wide frequency of 0.022, the population size needed to maintain and observe at least 20 phenotypes of this dominant allele is approximately 600 (Yanchuk 2001). Had the allele been recessive, over 55,755 individuals would be needed to observe 20 phenotypes with 95% probability. Fortunately, at least in crop breeding, disease resistance genes are usually dominant, although they are recessive around 10% of the time (Burdon 1987, in Burdon 2001).

Another example of a low frequency allele that is valuable to tree breeders is the allele that alters lignin properties in *Pinus taeda* (Ralph *et al.* 1997). This allele has only been demonstrated in one first-generation parent.

Examples of traits found in localized populations

Finding desirable genes or genotypes is further complicated when they occur only in isolated populations. Examples of pest resistance found only in specific populations include the MGR gene for blister rust resistance in *Pinus monticola* (Kinloch *et al.* 1999) and white pine weevil resistance in *Picea sitchensis* (Ying 1991, 1997). In the case of *Picea sitchensis*, resistance occurs predominantly in two British Columbia populations. These populations would not usually be considered for breeding in Oregon because growth rates of the local selections are much faster than those from British Columbia. Because very little resistance is evident in Oregon, resistant selections from British Columbia will be incorporated into Oregon breeding populations. An example of a wood property trait is wood density in the Guadalupe Island population of *Pinus radiata*. This island population has considerably higher core wood density than the New Zealand land race or the three mainland populations (Burdon & Low 1992, Low & Smith 1997).

Implications for breeding programs

The above discussions point out that, although 200 initial selections in a breeding program may maintain gains when breeding objectives do not change, many more individuals may be necessary to maintain genetic diversity for novel traits, particularly when those traits are rare. Most breeding populations have between 200 and 400 selections, although some have as many as 1,000 selections (Table 3, updated from White 1992). Most programs cannot afford to double their population sizes in order to maintain alleles like the MGR gene in *Pinus lambertiana*. Likewise, keeping poorly adapted provenances in a breeding program is a high price to pay to maintain genetic diversity. It becomes obvious that low frequency alleles must be maintained in gene resource populations. Levels of gain for current traits of interest in a gene resource population will be lower than in a breeding population. Therefore, one should expect a reduction in gain when genotypes from the gene resource population are incorporated into the breeding and production populations.

Table 3. Approximate census number (N) for breeding populations of some advanced-generation tree improvement programs. N is on a "per breeding unit" basis for programs with multiple breeding units (updated from White 1992)

Species	Program	N	Citation
Eucalyptus globules	CELBI - Portugal	300	Cotterill et al. 1989
	APM - Australia	300	Cameron et al. 1989
Eucalyptus grandis	ARACRUZ - Brazil	400	Campinhos and Ikemori 1989
Eucalyptus nitens	APM - Australia	300	Cameron et al. 1989
	New Zealand	270	Gea et al. 1997
Eucalyptus regnans	APM - Australia	300	Cameron et al. 1989
	New Zealand	300	Cannon and Shelbourne 1991

Eucalyptus urophylla	ARACRUZ - Brazil	400	Campinhos and Ikemori 1989
Picea glauca	Nova Scotia – CAN	450	Fowler 1986
Picea mariana	New Brunswick - CAN	400	Fowler 1987
Picea abies	Sweden	> 1000	Rosvall et al. 1998
Pinus banksiana	Lake States – USA	400	Kang 1979
	Manitoba – CAN	116	Klein 1995
Pinus caribaea	QFS – Australia	200-300	Kanowski and Nikles 1989
Pinus elliottii	CFGRP – USA	900	Hodge et al. 1989
	WGFTIP – USA	800	Lowe and van Buijitenen 1986
Pinus radiata	STBA – Australia	300	White et al. 1999
	FRI – New Zealand	550	Jayawickrama and Carson 2000
Pinus taeda	NCSU – USA	160	McKeand and Bridgwater 1998
	WGGTIP – USA	800	Lowe and van Buijitenen 1986
Pseudotsuga menziesii	BC – CAN	450	Woods 1993
	NWTIC - USA	70 - 404	Anon. 2001
Tsuga heterophylla	HEMTIC CAN-USA	150	King and Cartwright 1995

A risk analysis should be carried out by an organization to weigh the costs and potential benefits of enlarging a breeding program to maintain genetic diversity. For example, in a simple simulation examining a number of different breeding options, Johnson (1998) showed that strategies with the highest Ne were not always optimal for achieving gain after needing a low frequency allele.

Maintenance of genetic diversity in gene resource populations

There are several types of gene resource populations, traditionally categorized as either *in situ* or *ex situ*. *In situ* techniques involve conserving genetic resources in native habitats, while *ex situ* techniques involve storing genetic resources in special collections such as seed banks, progeny or provenance tests, and seed orchards. Both *in situ* and *ex situ* management are important in maintaining genetic diversity for a breeding program. They vary in effectiveness depending on objective, species, origin, management intensity, population size, etc. One important measure of effectiveness is whether an organization has control of the particular gene resource population. Because these are long-term populations, one needs some control over these populations to ensure they will be available in the future.

In situ reserves tend to be the less costly option for maintaining genetic diversity, but the difficulty with this approach is that most organizations have little control over the *in situ* reserves throughout a species' range. However, it is usually possible to monitor the *in situ* reserves in order to decide when *ex situ* measures are needed. Such efforts are underway in the Pacific Northwest of North America. In British Columbia, the BC Ministry of Forests has inventoried *in situ* populations in an effort to find populations that may be at risk (Lester & Yanchuk 1996). In Oregon and Washington, numerous organizations have come together to do a "gap" analysis of eight important conifer species (St. Clair & Lipow 2000). The cooperative approach minimizes the costs to any one organization.

Ex situ genetic conservation programs may also be carried out by multiple organizations working cooperatively. Examples include the provenance studies organized by IUFRO in the past and the current efforts of the Central America and Mexico Coniferous Resources Cooperative (CAMCORE). CAMCORE is a cooperative organization that is working to establish *ex situ* gene resource populations of tropical species, many of which are threatened. Presently, 24 organizations are members of the cooperative. *Ex situ* populations have been established for 22 conifer and 13 hardwood species (Dvorak *et al.* 1996, http://www2.ncsu.edu/camcore/index.htm).

Provenance studies are important to breeding programs, but most provenance studies are not suitable as "stand alone" gene resource populations. If a desired allele is identified in only one provenance, there will probably be fewer than the 20 unrelated copies recommended by Yanchuk (2001), because any one provenance is usually represented by a limited number of parents. Although provenance trials can only provide limited genetic variation to a breeding program, they are important because they can show the geographic distribution of a trait. For example, it was in provenance trials where the weevil resistance populations of *Picea sitchensis* were first identified (Ying 1991).

Ex situ conservation is extremely important when an organization is breeding exotics and the species is in jeopardy within its native range. One example is the collections and plantings made by Australia and New Zealand of *Pinus radiata* (Libby *et al.* 1966, Eldridge 1979, see also Matheson *et al.* 1999). The natural distribution of *Pinus radiata* is limited to five relatively small populations. Collections of these populations are planted in large blocks in both countries with management plans in place.

Many breeding programs are using their first generation selections as gene resource populations. For example, the Western Gulf Forest Tree Improvement Program has grafted all of its first generation *Pinus taeda* selections into scion banks (Byram *et al.* 1999). A similar conservation program was established with *Bombacopis quinta* by Monterrey Forsestal (Vallejo 1999). The Northwest Tree Improvement Cooperative (NWTIC) in Oregon and Washington USA is using their first generation progeny tests as gene resources populations (Lipow *et al.* in mans.). Because progeny tests will not survive indefinitely, methods are being discussed to regenerate stands to maintain these populations in the long term as multiple populations, as suggested for *Pinus taeda* by Namkoong (1997).

In Europe, gene conservation programs have been proposed that use both *in situ* and *ex situ* populations in different multiple populations (Eriksson 2001). Use of multiple populations conserves genetic variation better than a single population of the same size as the sum of the multiple populations (Namkoong 1984).

Suggestions in developing ex situ gene conservation populations

Before constructing *ex situ* gene resource populations, it is informative to evaluate the status of *in situ* reserves; are they in danger? Yanchuk and Lester (1996) considered a "population" adequately protected if it was represented by more than 5,000 m^3 of wood. They assumed 0.5 m^3 per tree, resulting in at least 5,000 trees. The definition of a "population" used by Yanchuk and Lester (1996) was a biogeoclimatic zone, which are used as seed zones in British Columbia. An organization should also be aware of *ex situ* populations that may be available, in cases like *Pinus radiata*, the species is probably best conserved in *ex situ* populations as long as they are appropriately regenerated.

If an organization decides that a valuable population is in danger, then establishment of *ex situ* gene resource populations may be warranted. Burdon (1987, 1995) discusses issues involved in developing *ex situ* populations. We would suggest that a minimum of 50 unrelated selections per population be used to establish a gene resource population. This would ensure the capture of genes with frequencies of 0.1 and greater. These populations should be established in stands of 1,500 stems or more. Multiple stands should be established to spread the risk of losing a population during a natural disaster or mistaken harvest. They should also be established in such a way that they grow for many years. The longer the life of the stand, the longer it will be before one needs to collect seed and reestablish new stands.

By planting and identifying family rows one can control the female genetic component of seed collections in the future. This may lead to some level of inbreeding from sib pollination, but it would be impractical to map single-tree plots in these populations so that one could identify maternal parents in the future. A small amount of inbreeding would not necessarily be bad because the objective of these populations is to conserve genes and gene complexes, not necessarily to "improve" the population. Any inbreeding depression can be removed by outcrossing in one generation. If regeneration plans depend on wind pollination rather than control pollination, larger stands should be considered to ensure that the pollen component in the next generation is from the appropriate provenance or population.

Maintaining genetic variation in breeding populations

Programs can also manage their breeding populations to better hold on to genetic variation while still obtaining genetic gain. Making wise decisions in early generations is crucial in maintaining the genetic variation needed later.

Breeding population structure

One way to maintain genetic variation in the breeding population is to structure it in subpopulations. There have been two basic methods proposed to structure breeding populations; either selections are stratified by their genetic value or they are stratified by their selection goals or geographic origins. These two methods are reviewed by Eriksson *et al.* (1993), Williams *et al.* (1995) and Williams and Hamrick (1996).

One method of structuring a population is to stratify the breeding population based on genetic merit. The very best selections are placed into the elite population and the remainder of the breeding population is placed in the "main population". The idea is to concentrate more effort on the elite population, where maximum gain is expected, and less on the main population. Ways to emphasize the elite population include making more crosses with parents, testing families on more sites and turning generations faster. The main population serves as both a breeding population and a gene resource population. This system was initially used in maize (Kannenberg 1981) and sheep breeding (James 1977), and later incorporated into forest trees by Cotterill *et al.* (1989). Such programs are referred to as nucleus breeding programs (James 1977 and Cotterill *et al.* 1989) or hierarchical open ended (HOPE) programs (Kannenberg 1981). Lindgren and Matheson (1989) proposed a strategy using a similar concept for seed orchards. They suggested that clones be used in proportion to their breeding value, with better clones having more ramets in the seed orchard. This idea was described for breeding programs by Kang (1989) and Kang and Namkoong (1988) where the better clones (parents) would be used to make more crosses than the poorer clones.

The use of "multiple populations" has been proposed as a way of better maintaining genetic variation in a breeding program. The multiple population breeding strategy refers to having many subpopulations of relatively small size (20-50) designed to maintain genetic diversity in the breeding population. The concept was introduced to forestry by Namkoong (1976, 1984) to account for uncertainty in the future value of selected traits. The idea is that each multiple population is selected for different traits (or different weightings), thus providing more options (genetic variation) in the future. Multiple populations, each selecting for different traits, will conserve genetic diversity better than one single breeding population (Namkoong *et al.* 1988, Kang & Nienstaedt 1987). While any one population may loose specific alleles to random drift (the effect of sampling) or selection, each population will lose different alleles. As a result, each population may end up with a different set of genes that correspond to different "adaptive peaks" as defined by Wright (1977).

Examples of breeding programs using the nucleus breeding strategy include the *Pinus taeda* program of the NSCU-Industry Tree Breeding Cooperative (McKeand & Bridgwater 1998), the Southern Tree Breeding Association's *Pinus radiata* program (White *et al.* 1999) and the New Zealand Radiata Pine Breeding Cooperative (Jayawickrama & Carson 2000). The New Zealand program has integrated aspects of multiple population breeding by having multiple nucleus populations, each emphasizing different combinations of traits. A number of *Pinus patula* breeding programs in southern Africa are using multiple breeding population strategies (Dvorak 1997). The second-generation Douglas-fir breeding programs of the Northwest Tree Improvement Cooperative are also utilizing aspects of the multiple population breeding strategy (Johnson 1998). In each breeding zone, selections are only mated with individuals from their local area. Thus, while the same traits are being selected upon, different gene combinations may be selected in each breeding group, since each population comes from different areas and there is clinal variation in adaptive traits.

Selection and mating procedures

Genetic gain is increased in the short term by increasing selection intensity, i.e. choosing only the very best. The drawback to this is that it ultimately decreases the maximum gain in the long term because some favorable alleles are lost to random drift. It also leads to inbreeding depression. However, for a given level of genetic gain, there can be a number of different selection choices, some of which maintain genetic variation better than others. Lindgren and Mullin (1997) and Zheng *et al.* (1997) present algorithms to maximize genetic gain when inbreeding (reduction in Ne) is given a negative weight. Kerr *et al.* (1998) expands the idea to maximize gain over many generations (instead of only one generation as done previously) and examines optimum mating algorithms. These methods are more complex than simply limiting the number of selections per family, but will lead to more gain for a given loss of genetic diversity.

King and Johnson (1993) used computer simulation to examine gains and effective populations sizes from a number of mating designs. They limited the number of selections per family as a means of controlling inbreeding. They found that by increasing the number of crosses per selection (i.e., increasing family number), they were able to maintain a higher Ne for a given amount of gain.

Summary

Most breeding programs have sufficiently large breeding populations to maintain rates of gain for up to ten generations for current traits of interest. As new traits of interest arise, additional populations may be needed, since many low frequency genes will not be in the breeding population. Organizations should monitor the existing *in situ* reserves and when necessary, develop *ex situ* populations, preferably in cooperation with other interested organizations.

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Current Status of Tree Improvement in Indonesia

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Abstract. Indonesia is part of the Malesian botanical region. The Malesian Rain Forests are among the richest in the world in terms of number of tree species, and one of their most important features is the abundance of valuable dipterocarp species. The total forestland in Indonesia is 144 million ha consisting of protection forests (30 million ha), conservation and recreation forests (19 million ha), production forests (64 million ha), and conversion forests (31 million ha). Most of the production forests are natural tropical forests located outside of Java, while all of the production forests on Java are plantations, with Tectona grandis and Pinus merkusii as the two main species.

In an effort to save the natural forest and increase forest productivity, the Government of Indonesia has launched a massive reforestation program - called HTI - through establishment of industrial plantations covering an area of 6.2 million ha. Zobel (1994) stated that the biological potential for plantation forestry in Indonesia is huge and this program aims at capitalizing on that potential.

There is abundant evidence from around the world indicating that availability of good quality seed and/or planting materials of the best-adapted species is one of the most critical factors in ensuring healthy, productive, and profitable plantations. Genetically improved materials can only be obtained through a series of tree improvement activities. Indonesia realized early on that tree improvement is an important tool to meet these needs for good quality planting materials.

The first tree improvement work in Indonesia was carried out in 1932 for teak, initiated by establishing provenance/varieties trials on several sites in Java. However, due to the Japanese occupation and the subsequent independence war, these studies were neglected. No serious effort in tree improvement was made until 1976, when the Directorate of Forestry, in cooperation with Gadjah Mada University, started a tree improvement program with tusam (*Pinus merkusii*). Since the development of the industrial plantation forest program (HTI) in the late 1980's, various tree improvement programs have been underway, mainly for short-rotation species. A significant teak tree improvement program initiated on Java by Perum Perhutani (the State-owned Forest Enterprise) included establishment of extensive clonal seed orchards. Since 1996, Gadjah Mada University in cooperation with Perum Perhutani has been involved in various tree improvement activities for teak including establishment of the Teak Center at Cepu. In the last few years a number of additional tree improvement activities for tropical rain forest species such as *Lophopetalum multinervium*, *Shorea polyandra*, *Shorea leprosula*, and *Dyospiros celebica* have been initiated.

A few other universities and some other research institutions have also been involved in various tree improvement programs. A number of forestry companies with industrial plantation forests handle their own research and development and they are very active in tree breeding programs. Although there are many problems facing forest tree improvement activities in Indonesia, current progress is good and the prospects for further success seems promising.

Introduction

Because of its geographic location, size, and broad range of physiographic conditions, the natural resources of Indonesia have an unusually high level of biodiversity and Indonesia is one of the world's Mega–Biodiversity countries. Indonesian species biodiversity encompasses a large variety of ecosystems and it is very rich in genetic variation. Many types of forests cover the country from coastal plain to the mountain top more than 3000 m above sea level.

The total number of production forests is 64 million ha and most of these are natural tropical forests located outside of Java. All production forests on Java are plantations managed by Perum Perhutani (the state-owned forest company). Plantation forests in Java cover 2 million ha. In order to both save the natural forest and to increase wood production to meet rising demand, Indonesia has planned a reforestation program through establishing the HTI (Industrial Plantation Forest) program covering an area about 6.2 million ha. It is planned to establish 400,000 ha of plantations under HTI, annually. For the success of the HTI program, Indonesia must use genetically improved seed and planting materials obtained through properly planned and executed tree improvement activities.

Tree improvement can be defined as the application of genetic principles in silviculture to produce high quality forest products with increased economic value. According to Zobel and Talbert (1984) all beginning tree improvement programs rely on, and consist, of the following:

1. A determination of the species, or geographic sources within a species, that should be used in a given area.
2. A determination of the amount, kind and causes of variability within the species.
3. A packaging of the desired qualities into improved individual trees to develop lines with combinations of desired characteristics.
4. Mass-producing improved individuals for reforestation purposes.
5. Developing and maintaining a genetic base population broad enough to cover needs in advanced generations of selection, breeding, and genetic transformation.

Since 1976, Indonesia has had significant tree improvement programs for some of its important commercial forest species, beginning with *Pinus merkusii*. A large number of genetic plantations have been established for it and other species. The objectives of these studies are:

(1) determine genetic variation for some important forest tree species in Indonesia;
(2) conduct species, provenance and progeny trials for guidance in seed collection and advanced generation breeding;
(3) serve as seed orchards which produce genetically improved seed

A number of tree improvement activities for various purposes have been initiated, including: exploration, plus tree selection, genetic test plantation establishment, clone bank, hybridization, vegetative propagation, seed stand & seed orchard establishment, tissue culture, multiplication garden, isozyme analysis, DNA analysis, conservation area establishment, and intensive silviculture. The forest tree species studied can be divided into six groups: (1) teak species, (2) pine species, (3) exotic, fast-growing species, (4) tropical rain forest species, (5) non-teak species for Java, (6) community forest species.

Geography and Forest Resources

Indonesia is the largest archipelago lying on the equator, and it consists of about 17,000 islands of which Kalimantan, Sumatra, Sulawesi, Irian Jaya and Java are the largest. Its geographical boundaries stretch from 6 degrees North Latitude to 11 degrees South Latitude, and from 95 to 141 degrees East Longitude comprising a total territory of about 780 million ha located between the continents of Asia and Australia and between the Pacific and Indian Oceans. Of that total territory, about 193 million ha are land, and approximately 74% of that is forested (144 million ha). That forest area represents about 50% of the tropical forest area of Southeast Asia, and about 10% of the tropical forests on earth.

Indonesia is a mountainous country. An active volcanic range runs through Sumatra, Java, Nusa Tenggara, Moluccas and Sulawesi. There are 128 active volcanoes, more than half of which have erupted throughout history. The most famous volcano is Krakatau, whose eruption in 1883 threw copious ash into the air that was believed to have circled the earth for two years.

The country is entirely within the tropics, but does not experience a uniform climate. Continuously wet and semi-wet climates are found primarily in Western Indonesia where most of the natural forest resources are found. All of other areas are monsoon or semi-arid climatic types, with the driest areas found in Nusa Tenggara. Average annual rainfall ranges from 700 to 4,000 mm with a mean day temperature of 32^0 C, mean night temperature of 22^0 C, daily average humidity of 90%, and monsoon winds outside the typhoon belt.

Because of its strategic location, the natural resources of Indonesia have very high biodiversity. Many types of tropical forests cover the country from the coastal plain to mountaintops more than 3,000 m above sea level. Major forest types in Indonesia are the following:

- lowland rain forest
- heath forest, associated with sandy soils of low quality
- sub-mountain forest, mainly between 1000 m and 2000 m above sea level
- mountain rain forests mainly over 2000m above sea level
- deciduous forest
- dry land forest, savanna forest in semi arid areas
- tidal forest
- swamp forest
- peat forest, on organic soils with peat layers of at least 50 cm.
- coastal forest on the beaches and dunes
- mangrove forest

Based upon their utilization, the Indonesian Ministry of Forestry classified our forests as follows:

a. Protection Forest	30 million ha
b. Conservation and Recreational Forest	19 million ha
c. Production Forest	
permanent	34 million ha
limited	30 million ha
d. Conversion Forest	31 million ha
Grand total	144 million ha.

Indonesia is part of the Malesian Region and the Malesian Rain Forests are among the richest in the world in term of the number of tree species. One of their most important features is the abundance of valuable dipterocarp trees, particularly in the western region of the country (Sumatra and Kalimantan). Seasonal monsoon forests are found in Central and East Java and in Nusa Tenggara, with savanna grassland in Nusa Tenggara and southern Irian Jaya and non-dipterocarp lowland forests and alpine vegetation types in Irian Jaya. Indonesia's 144 million ha of natural forests are the world's most biologically diverse (500 species of mammals, 1500 species of birds, and 10,000 species of trees) and represent 10% of the world's dwindling tropical rain forest. They are vital for Indonesia's economic development, providing for most of our domestic wood demand as well as US$3.8 – 5.95 billion annually in wood product exports. The forests are also important for sustaining agriculture, and for soil and water conservation. Due to various factors such as logging operation, conversion to other land uses, fires, forest encroachment, and shifting cultivation, the forest resources in this country have suffered from degradation and deforestation at an unprecedented rate (Hardiyanto & Na'iem 2001).

Production Forests, Large-Scale Planting

As mentioned earlier, the total number of production forests is 64 million ha and most of them are natural production forests located outside Java such as in Kalimantan, Sumatera, Sulawesi, Irian Jaya, and Maluku. In 1970, the government issued a concession right for state and private companies to utilize forest resources in the outer islands. Before 1990 more than 421 timber concessions were operational and that was the beginning of the exploitation of Indonesia's tropical rain forests on a large scale. The extracted wood, mainly from dipterocarps, is for export and accounts for 20.47% of the world's plywood market. Wood products exports contribute 54-85% of the total export earnings of Indonesia. The silvicultural system being used in natural forest management is mostly the Indonesian Selective Felling System. The tropical rain forests of Indonesia also are rich in non-wood forest resources such as rattan, fruits, nuts, tannins, resins, and other natural products.

All production forests on Java are plantation forests managed by Perum Perhutani (State Owned Forest Enterprise) except for the Yogyakarta Special Territory. Indonesia has very large-scale plantation forests. Plantation forests in Java cover a total area of about 2 million ha, consisting of teak (*Tectona grandis*), pine (*Pinus merkusii*), mahagoni (*Swietenia macrophylla* and *S. mahagoni*), rosewood (*Dalbergia latifolia*), Agathis (*Agathis loranthifolia*), Sengon (*Paraserianthes falcataria*), kayu putih (*Meulaleuca cajuputi*), and other, more minor species.

The largest teak plantation forest in the world is located on Java and is managed by Perum Perhutani. Teak forests in Java have been planted since Dutch Colonial times (1874) and now represent one of the best examples of intensive forest management systems in the world. Teak plantations on Java amount to over one million ha, and the annual cut is about 8,000 – 10,000 ha. There are also some teak forests outside Java such as those on the islands of Muna, Buton, Kangean, Sepinggan, Sumbawa, Timor, Buru, Saparua, and South East Sulawesi. Representative of the great success of the greening movement on private land, there are now plenty of teak plantations in places such as Gunung Kidul, Yogyakarta, Wonogiri, and at many sites in South Sulawesi (Suseno and Na'iem, 1999).

The second largest plantation area on Java is represented by pine forests of *Pinus merkusii*, or tusam, covering around of 800,000 ha mostly at higher elevations. There is also an impressively large area of planted pine forest in South Sulawesi as a result of the greening and reforestation movement conducted in the 1970's.

A fast-growing worldwide demand for wood has resulted in the increasing rate of depletion of our natural tropical forests, and this crisis in the tropical forest has become a global environmental issue. In an effort to save natural tropical forest, increase forest productivity, and enhance the size of the export market in wood products the Government of Indonesia has begun a reforestation program promoting establishment of industrial forest plantations called the HTI (Hutan Tanaman Industri) Program, covering 6.2 million ha. The Government aims at establishing 400,000 ha of forest plantation annually. Since late 1980's plantation forests have been developed on other islands other than Java, mainly short rotation plantations with *Acacia mangium*, *Acacia spp*, *Eucalyptus spp*, and *Gmelina arborea*. As of 2000 approximately 2.5 million ha of plantation forests have been established on the outer islands. Almost all of HTI holders prefer to grow fast growing species (exotics) for pulp and paper products.

Most of the valuable commercial woods such as meranti, kapur, kruing, and ramin have been extracted from the natural forests and at present, Indonesia is facing an extremely serious threat to its natural forests. The latest data indicate that about 40.26 million ha of forests have been degraded and fragmented and the annual rate of deforestation has reached approximately 1.46 million ha. On Sulawesi alone the rate of deforestation is around 1% per year, while the figure for Irian Jaya is 0.7 % per year (Harsono, 2000).

There will be many problems with sustainability of our wood products if we do not include tropical rain forest species for planting in the HTI establishment program. One way to overcome those problems is to establish meranti plantation forests in the tropical rain forest areas and applying intensive silviculture to their management. At the present time the Ministry of Forestry encourages HTI as well as the Forest Concession holders to grow tropical rain forest species - mainly fast growing meranti - on unproductive areas. Meanwhile, the government also has launched a greening campaign to reforest and rehabilitate marginal land with the main focus on private land.

A movement aimed at one million trees under cultivation has been launched by the government and has become popular now. There are many stakeholders and officials participating in growing thousands of various tree species in the open areas including the Minister of Forestry. Even the President and Vice President of R.I. have several times presided at the tree planting ceremonies, by cultivating "memory trees".

All of these activities indicate that Indonesia has decided to grow trees on a very large scale, and particularly as plantation forests. Zobel (1994) stated that the biological potential for forest plantations in Indonesia is very great. And these large-scale plantations are certainly very conducive to developing genetic improvement programs.

Genetic Tree Improvement

Activities

Since 1976, Indonesia has conducted a number of tree improvement activities with various species. There is abundant evidence from around the world to show that the availability of good quality seed or planting materials of the best species is one of most critical factors in ensuring healthy, productive, and profitable plantations. Genetically improved materials can be obtained through a series of tree improvement activities.

Tree improvement in the USA is generally recognized as the most cost effective and profitable forest management technique available. Therefore it is a widely acceptable practice in most regions of that country (Hanover, 1990). Tree improvement can be defined as the application of genetic principles to silviculture to produce high quality forest products.

Zobel and Talbert (1984) distinguished between tree breeding, which is aimed at solving specific problems, and tree improvement aimed at increasing the production and quality of products by combining genetics, silviculture, and forest management activities.

Indonesia early realized that tree improvement is an important tool to meet the need for good quality planting materials and genetic improvement is now an accepted activity in Indonesia. According to Zobel & Talbert (1984), a forest tree improvement program consists of the following major activities:

1. Determination of species to be improved and the geographic sources to be used.
2. Determination of the amount, kind, and causes of variability within a species.
3. Packaging of the desired qualities into the improved individuals to develop lines of trees combining those desired characters.
4. Mass production of improved individuals for reforestation purposes.
5. Developing and maintaining populations with a broad genetic base.

Using those activities as a guide, Indonesia has carried out the following:

1. Exploration to identify natural distribution of a particular species
2. Species trial plantation establishment
3. Provenance test plantation establishment
4. Progeny test plantation establishment
5. Plus tree selection followed by seed & vegetative materials collection
6. Clone bank establishment
7. Controlled pollination
8. Hybridization

9. Seed stand designation
10. Seed orchard establishment
11. Vegetative propagation
12. Tissue culture
13. Hedge/multiplication garden
14. Isozyme analysis
15. DNA analysis
16. Conservation
17. Intensive silviculture

There are now many Indonesian institutions involved in forest tree improvement activities and they have achieved much since the government encouraged the establishment of HTI. Several universities and forest research institutes have especially provided major contributions. Also, some of the HTI holders have their own tree improvement programs.

Generally, the major reason to start tree improvement activities is to meet the needs for adequate quantities of genetically improved seed (superior seed) for forest plantations. In many of the early tree improvement programs, gains in volume or value of 20–38% are being obtained in the first stages of simple mass selection (Hardiyanto & Na'iem 2001).

Field trials (genetic test plantations) for a number of species in Indonesia are presented in Table 1. Table 1 describes some of the species in current tree improvement programs in Indonesia.

Table 1. List of Field Trials for Various Species in Indonesia

Species	**Field Trials / Genetic test**		
	Provenances	**Families**	**Clones**
Acacia mangium	18	757	
Acacia crassicarpa	9	261	
Acacia auriculiformis	13	236	
Dyospiros celebica	11	158	
Eucalyptus pellita	4	223	
Gliricidia sepium	4		
Gmelina arborea	4	80	72
Meulaleuca cajuputi	17	412	
Lophopetalum multinervium	5	100	
Paraserianthes falcataria	10	300	
Pinus merkusii	3	1372	
Santalum album	8	421	
Shorea johorensis		25	
Shorea macrophyla	10 population	200	
Shorea pinanga		97	
Shorea polyandra		25	
Swietenia macrophylla		100	
Tectona grandis		600	200

Species

Based on species/group of species, forest tree improvement activities in Indonesia up to the present time, can be grouped into six groups as follows: **1.** teak (*Tectona grandis)*; **2**. pine/tusam (*Pinus merkusii*), **3**. *Acacia mangium* and other fast growing species for pulp & paper; **4**. non-teak species on Java, **5**. meranti (*Shorea spp*) and other tropical rain forest species; and **6**. community forest species. Only some of those species or group of species are described in Table 2.

Table 2. Partial List of Species In Current Tree Improvement Programs

Species	Provenance Test	Progeny Test	Clonal Test	Seed stand	Seed Orchard	Hybrid
Acacia mangium	x	x	x	x	x	x
Acacia crassicarpa	x	x			x	
Acacia auriculiformis	x	x			x	
A.mangium x A.auriculiformis						x
Artocarpus heteropyllus		x		x	x	
Eucalyptus deglupta	x	x				
Eucalyptus urophylla	x	x				
E. urophylla x E. alba						x
E. urophylla x E. pellita						x
E. urophylla x E. brassiana						x
E. urophyla x E. grandis						x
E. brassiana		x				
E. pellita		x				
Gmelina arborea	x	x				
Meulaleuca cajuputi		x				
Morus spp			x			
Paraserianthes falcataria	x	x			x	x
Pinus merkusii	x	x			x	x
Santalum album		x				
Shorea johorensis		x				
Shorea macropyhlla		x				
Shorea parvifolia		x				
Shorea pinanga		x				
Shorea stenoptera		x				
Swietenia macrophylla		x		x	x	
Swietenia mahagoni				x	x	
Tectona grandis	x	x	x	x	x	
Armelaria sp				x		
S. selanica				x		
Gliricidia sp.			x	x		
Aleuritas moluccana					x	
Armelaria campaca					x	

There are also species trials for:
1. Fire wood
2. New Eucalyptus introductions
3. Acacia
4. MPTS (Multi Purpose Tree Species)

Teak (Tectona grandis)

The first tree improvement work in Indonesia was carried out in 1932 for teak, initiated by establishing provenance/varieties trial on several sites in Java. However, due to the Japanese occupation and the later independence war, these earliest studies were neglected until 1980. Recognizing that the largest forest plantation is teak on Java (around one million ha), a significant program of tree improvement for teak was started by Perum Perhutani in 1981. The main objectives of this program were to produce genetically superior seed and also to establish seed production areas and Clonal Seed Orchards (CSOs). Perum Perhutani now has 3,700 ha of teak seed production areas and 1,200 ha of Clonal Seed Orchards. The first step was to select plus trees from East and Central Java teak forests for clonal seed orchards, clone banks, and progeny tests. The first CSO was established in 1983. In the period 1983-1993, Perum Perhutani selected 136 plus trees from East and Central Java teak forests. The clone banks are located in the forest districts of Randublatung, Cepu, Kebunharjo, Bojonegoro, Parengan and Saradan. Progeny testing was started in 1987 in the forest districts of Kendal and Saradan. Since 1985, Pehutani has produced tissue culture plantlets and, in 1988, they established experimental plantations with tissue culture plantlets in the Forest Districts of Cepu and Kendal (Perum Perhutani 1995).

In 1996 Perum Perhutani asked UGM to help design a tree improvement strategy for all of the commercially important species they manage, such as *Swietenia spp*, *Paraserianthes falcataria*, and *Meulaleuca cajuputi*. At this time, Perum Perhutani also began to plan the construction of a " TEAK CENTER" emphasizing teak genetic improvement activities and was asked to revise the teak tree improvement program. Its feasibility study for this Teak Center was conducted by UGM, and they also did a subsequent Action Program. In the implementation of the Teak Center, UGM provided some significant contributions, such as: plans for an Information Center, a Teak Arboretum, a Hedge Orchard, and establishing a research program on vegetative propagation with rooted cuttings. The Minister of Forestry opened this Center in February 1998. Since 1997, UGM has also been involved in several cooperative tree improvement activities on teak with Perum Perhutani, taking part in various activities such as exploration and plus tree selection, design and establishment of progeny tests, clone banks, clonal tests, *ex situ* conservation plantations, hedge gardens (multiplication gardens), as well as various studies on rooted cuttings and intensive silviculture. More specific information on the Teak Center was presented by Poedjorahardjo (1998) and Sadhardjo (2001), among others.

Exploration

Exploration in several teak forests on Java as well as outside Java to select more plus trees (phenotypically-superior trees) has been completed. About 600 plus trees were selected from the natural teak distribution areas in Indonesia, such as the islands of Kangean, Sepinggan, Muna, Buton, Sumbawa, Timor, Buru, Saparua and Southeast Sulawesi in addition to Java. The main purpose of exploration and selection was to increase the number of plus trees from 164 to 600.

Progeny tests

Progenies from all parent trees (600 families) were tested from 1996 – 2000 by establishing progeny test plantations at several sites. About 200 families were planted annually. Teak progeny tests were established at four sites: the Forest Districts of Cepu, Bojonegoro, Ngawi, and Ciamis. In each location the progenies are tested on two site qualities, good and poor; i.e., 200 families were progeny tested in eight site replications, annually. The design of all progeny tests is RCBD, 4-tree line plot, 10 blocks, and spacing 3 x 3 m.

Rooted cuttings, hedge / multiplication garden, clonal test

Rooted cuttings of teak have been studied by the Faculty of Forestry UGM as well as at the Teak Center in Cepu. The Faculty of Forestry fortunately found a way to easily propagate teak on a large scale using rooted cuttings. To produce rooted cuttings as planting material on a large scale, a hedge garden or multiplication garden of teak should be established. At the same time seed is collected from the selected trees, collection of vegetative materials in the form of dormant buds is also done. These scions are then bud grafted onto seedlings which were previously prepared as rootstocks for the hedge garden/multiplication garden. The grafted trees have to be coppiced several times each year to form a compact hedge which can produce as much juvenile material for rooted cuttings as possible in a small area. Rooted cuttings were prepared for establishing clonal test plantations in Cepu, Ngawi, Bojonegoro, and Ciamis. The design of the clonal test plantations is the same as for progeny tests.

Ex situ Conservation

In a two-year period, 20 ha of *ex-situ* conservation plantations have been established in Ngawi-East Java, including sixteen populations collected from Java as well as others outside Java. As generally recognized, there are various lines or varieties of teak that can be easily distinguished through wood properties or morphological performance. These include jati lengo, jati kapur, jati sungu, jati keling, jati benjol, jati batang halus, jati doreng, jati muare, etc. To conserve the genetic diversity of teak, before it disappears due to natural disasters or logging operations, *ex situ* conservation plantations of teak need to be established.

The main objectives of the new improvement program on teak managed by Perum Perhutani, according Danarto *et al.* (1999) are: (1) to study genetically controlled variation in some characteristics of teak such as growth, wood quality, branching habit; (2) to provide basic information from progeny tests for CSO evaluation; (3) to identify superior clones based on clonal test data; (4) to preserve a broad enough genetic base for advanced improvement through *ex situ* genetic conservation; (5) to find a proper intensive silviculture method for teak to shorten its cutting cycle.

Pine/tusam (Pinus merkusii)

Historical Background

Pinus merkusii Jungh et de Vriese is the only pine species native to Indonesia, occurring on the island of Sumatera in disjunct populations, namely Aceh, Tapanuli and Kerinci. It is also the only pine species in the world that extends naturally south of the Equator. It also occurs naturally in Thailand, Cambodia, Laos, Vietnam, and the islands of Luzon and Mindoro in the Philippines (Critchfield, 1966; Mirov, 1967). The species was introduced to Java in the early 1920s from one of its natural population in Sumatra, presumably Blangkejeren in Aceh provenance. Since then it has become the second most extensively planted species (after teak) by Perum Perhutani in Java, covering a total area of more than a half-million hectares. There are also now about 73,000 hectares of *Pinus merkusii* plantations in South Sulawesi as one of the results of major reforestation and land rehabilitations in the 1970s.

Pinus merkusii wood is of considerable value, and it is excellent for furniture, sawn timber, boxes and paper. The wood is also extracted for its high quality oleoresin. A program for genetically improving stem form and growth was initiated in 1976 by the Faculty of Forestry, Gadjah Mada University in cooperation with the Directorate of Reforestation and Land Rehabilitation and Perum Perhutani, under the supervision of Prof. Dr. Jonathan Wright from Michigan State University, USA.

First Generation Breeding Strategy

The objectives of the experiment (Suseno, 1988) were: (1) to study variation in stem straightness and other characters; (2) to ascertain the possibility of obtaining genetic improvement for stem straightness; and (3) to study the possibility of obtaining genetically improved seed by converting the progeny test into a seedling seed orchard.

The breeding program for *P. merkusii* began by plus tree selection. The plus trees were selected for stem form and growth from unimproved *P. merkusii* plantations in Java. Stem form was evaluated using the classification system for stem defect in use at the East African Agriculture and Forest Research Organization. The procedure of plus tree selection followed the comparison trees method introduced by Morgenstern, et al. (1975). More than 1000 parent trees were selected and 6 series of open pollinated progeny tests were then established at 3 sites: Sumedang (West Java), Baturaden (Central Java) and Jember (East Java) from 1978 to 1983. In every series, 200 families were tested using randomized complete block designs, 5-tree line plots with 10 replications, planted at a spacing of 4 x 4 m for Baturaden and Jember, and 3 x 3 m for Sumedang The procedure for nursery establishment followed the method described by Wright (1970a; 1970b; 1975; 1976).

Suseno (1988) reported on a number of traits, as summarized in Table 3. Narrow sense family heritability estimates and predicted genetic gains for a number of traits are presented in Table 4.

Results indicated that genetic improvement for *Pinus merkusii* is feasible. The heritability of stem crookedness was moderately high, and the predicted gain in stem straightness was also high.

Table 3. Analysis of variance for a number of traits for *P. merkusii* progeny test at six years of age

Traits	Plant	Fam.	Fam. X Plant
Degree of crookedness	**	**	**
No. of crooked trees	ns	**	**
Height	**	**	**
Diameter	**	**	**
No. of forking trees	**	**	**
No. of foxtailing trees	**	**	**
No. of trees with cones	**	**	**
No. of internodes/tree	**	**	**

ns = not significant ; ** = significant at the 0.01 level

Table 4. Narrow-sense family heritability estimates and predicted Genetic gains for a number traits for *P. merkusii* progeny test

Traits	Heritability	Genetic gain (% of plantation mean
No. of crooked trees	0.79	24.57
Degree of crookedness	0.72	38.54
Height	0.43	2.7
Diameter	0.68	4.11
No. of forking trees	0.37	20.80
No. of foxtailing trees	0.27	22.25
No. of foxtailing trees	0.67	18.60
No. internodes/tree	0.57	4.90

The progeny test plantations were progressively converted into open-pollinated seedling seed orchards (SSOs) by selective thinning, leaving only the best trees within the best families. At present all of the progeny tests have functioned as SSOs and the improved seeds from the orchards have been used for commercial plantations since 1991. This year (2001) we expect to be able to collect 2,000 kg of pine seed from those SSOs. This strategy of developing SSOs as the production population has been adopted due to the facts that *P. merkusii* commences flowering and seed production at early ages and that grafting has been found to be relatively difficult. These seed orchards were the first half-sib progeny test seed orchards or SSOs in Indonesia. But at the present time, almost all seed orchards in Indonesia are SSOs, using the same design as the *P. merkusii* SSOs.

Many theses and dissertation on forest tree improvement were produced from data collected in these half-sib progeny test plantations. The results of *Pinus merkusii* progeny tests have been reported very often by, among others, members of the Faculty of Forestry, GMU (1993), Hardiyanto (1996), Suseno & Nai'em (1999), Danarto *et al*., (1999). Some genetic information is presented in Tables 5 and 6. Genotype–site information was found to be important for the following characters: stem form, stem diameter, tree height, forking, and foxtailing.

Table 5. Individual and Family Heritabilities for tusam

Trait	Individual heritability	Family heritability
Stem form	0.20 - 0.37	0.64 – 0.72
Diameter growth	0.26 – 0.38	0.39 – 0.68
Height	0.19	0.26 – 0.43
Branching type	0.21	0.34
Oleoresin yield	0.52	0.69
Wood density	0.40	0.49
Forking	-	0.37
Foxtailing	-	0.27
Internode	-	0.57
Fruiting	-	0.38 – 0.67

Table 6. Genetic Correlations

Stem form	Diameter	Height	Oleoresin yield	Wood density
Stem form	0.11 to -0.53	0.56	0.12	0.06
Diameter	-	0.56 – 0.71	-0.37	0.08
Branching type	-	-	-0.23	-

Forward Selection

The existence of very low or adverse genetic correlations between stem form and diameter growth in the current breeding populations make the simultaneous improvement of these traits more difficult. Trees to be included in SSOs were determined based on their rank in a combined index, which combines both the individual value and family performance. Family mean was estimated using BLUP (Best Linear Unbiased Prediction). Trees having stem form less than 4 (Stem form was scored from 1-very poor to 6-excellent) were dropped from the population and the combined indices for diameter were then developed for the remaining trees.

Forward selection of materials in the second-generation test was also based on the highest rank of combined indices. These trees are basically correlation breakers - they have a favorable genetic correlation between stem form and growth.

Broadening the Genetic Base

It is believed that the existing breeding population of *P. merkusii* has a narrow genetic base and therefore needs to be broadened to sustain long term genetic gains. Infusion of genetic materials has been carried out by collecting seed from the natural population of Aceh since 1995. As of December 1997 the following numbers of families have been included in the current breeding population: Janto (41), Takengon (67), and Blangkejeren (386). These materials have been progeny tested in the field at Sumedang and Jember. Provenance resource stands were also established at Jember.

Second Generation Breeding Strategy

It was decided that a nucleus breeding strategy would be applied. In this strategy, the first generation breeding population is split into a two–tier structure involving: small nucleus (or elite) and large main populations. The short-term genetic gain is expected to be maximized in the nucleus population, which consists of selected individuals with the highest breeding values, and crossing these in many combinations to produce outstanding families and individuals. For the long term, a more gradual gain is achieved through the main population that has less intensively selected individuals and maintains a large effective population size.

The main population is divided into 6 sub lines, each sub line representing the year of establishment. The elite population contains 60 individuals taken from the 10 best trees of every sub line. Each sub line consist of 48 individual trees and it is further divided into 3 strata, each stratum containing 16 trees. In total, the number of individuals in the main population is 288, which is in the minimal range recommended for a sound improvement program. New infusions of genetic materials from seed collection in the natural population will certainly increase the size of the breeding population of *P. merkusii* in Java. In this new collection, the division of breeding populations follows the natural origin of seed collections.

The method of deployment of improved plants will remain as seedlings grown from seed collected from open pollinated SSOs. Separate SSOs will be established using families in the elite population and thinned based on progeny test evaluation. Composite SSOs will also be established using the best families from each sub line according to the assessment that will be done in progeny tests. Multiplication of superior full-sib families is still being planned and a clonal forestry program will be based on testing of juvenile materials in field trials.

Mating design

A complementary mating design is being adopted. Polymix is used for assessing the breeding value (GCA) in randomized and replicated progeny tests, while full-sib mating (single or double pair) is used to make forward selection from the best families (based on the GCA tests) in unreplicated full-sib selection plots to form the third generation breeding population.

Future Improvement

Following improvement in form and growth, likely traits to be included in the future breeding plan for *Pinus merkusii* will be oleoresin yield and wood density.

Acacia mangium and other Fast Growing Hardwood Species

The main objective of the Government's HTI Program is to save and protect our natural tropical forest, which is dominated by *Dipterocarp* species, from wood exploitation pressures. Unfortunately, in fact almost all of the HTI concession holders have established HTI with fast growing species for pulp and paper products. Hardiyanto (1996) reported that since the program of HTI was launched in the mid-1980s, the Government of Indonesia has approved 14 projects for HTI, mainly for pulp and paper production. At the beginning of the plantation forest development initiated by the government in the late 1980s, particularly with exotic species such as *Acacia mangium* and *A. crassicarpa*, the seed demand could not be fulfilled locally and the major portion had to be imported, mainly from Australia, Malaysia, and Papua New Guinea.

Nowadays, good quality seeds and other planting materials for industrial plantation forests are becoming available locally as a result of tree improvement programs. Some of the HTI holders have their own tree improvement programs such as PT Musi Hutan Persada (MHP), PT. Surya Hutani Jaya, PT. Arara Abadi, PT. Inti Indorayon, PT. ITCI Manunggal Hutani, and PT Riau Andalan Pulp & Paper.

In Indonesia, only a few species are used for short rotation plantations (mainly for pulp and paper) and most of them are exotic species such as: *Acacia mangium*; *Eucalyptus urophylla*; *Eucalyptus pellita*; *E. deglupta; E. tereticornis; E. grandis A. crassicarpa; A. aullacocarpa, Gmelina arborea; Paraserianthes falcataria*. A list of species used for pulp plantations by several companies in Indonesia is presented in Table 7. The limited number of species is due to problems such as seed availability, poor adaptation, and unsuitability for end products, unknown silvicultural aspects, and unknown growth potential and wood properties.

Table 7. Species used for pulp plantations in several companies in Indonesia

Company Name	Species planted
PT. Indonusa Indrapuri	- *Mangium, E.*
PT. Tusam Hutani Lestari	*urophylla*
PT. Inti Indo Rayon	*Pinus merkusii*
PT. Riau Andalan Pulp & Paper	2. *Urophylla, E. grandis, A. saligna*
PT. Surya Damai	- *Mangium*
PT. Arara Abadi (Indah Kiat)	• *Mangium, E. pellita*
PT. Wirakarya Sakti	*C. mangium, E. urophylla, E. pellita*
PT. Pakerin	• *mangium*
PT. Musi Hutan Persada	1. *mangium*
	⇨ *mangium, P. falcataria, E. urophylla,*
PT. Inhutani V	❑ *pellita, P. merkusii*
PT. Fintara Intiga	❑ *mangium, P. falcataria*
PT. Inhutani III	❑ *mangium*
	A. mangium, A. crassicarpa, A. aullacocarpa,
PT. Menara Hutan Buana	❑ *pellita, E. tereticornis*
PT. Inhutani II	❑ *mangium*
PT. Surya Hutani Jaya	❑ *mangium, P. merkusii, P. falcataria*
	❑ *mangium, G. arborea, E. deglupta,*
PT. ITCI Manunggal Hutani	❑ *urophylla, P. falcataria*
PT. Tanjung Redep Hutani	❑ *deglupta, A. mangium*
PT. Adindo Hutani Lestari	❑ *mangium*
	A. mangium

Table 7 indicates that the species most planted in HTI is *Acacia mangium*. Barito Pacific Group (PT. Musi Hutan Persada) has had experience in planting 50,000 ha/year of *Acacia mangium*. Various tree improvement activities have been carried out by this company including species trials, provenance trials, seed orchard establishment, and hybridization. Tree improvement programs for *Acacia mangium* by PT MHP have resulted in genetically improved seed. *A. mangium* for plantation establishment on a large scale and now can be supplied internally. A second generation breeding strategy for *A. mangium* has also been set up. Table 8 shows the growth performance of various species in South Sumatra. Growth performance of various provenances of *Acacia mangium* in South Sumatra is presented in Table 9.

Table 8. Tree Growth Performance of various species in South Sumatera

No	Species	Height(m/year)	Diameter
1	*Acacia mangium*	6	5
2	*Acacia crassicarpa*	4	4
3	*Acacia hybrid*	4	4
4	*Eucalyptus pellita*	5	3
5	*Acacia leptocarpa*	4	3.5
6	*Ochroma bicolor*	4	6
7	*Paraserianthes falcataria (var. solomonensis)*	3	5
8	*Acacia auriculiformis*	3.5	3
9	*Acacia aulacocarpa*	3.5	3
10	*Eucalyptus brassiana*	4	3
11	*Eucalyptus urophylla*	3	2
12	*Eucalyptus deglupta*	2	2
13	*Tectona grandis*	2	2
14	*Eucalyptus urograndis*	2	2
15	*Gmelina arborea*	2	2
16	*Swietenia macrophylla*	1.5	2
17	*Peronema canescens*	1.5	1.5
18	*Maesopsis spp*	1.5	1.5
19	*Acacia melanoxylon*	1.5	1
20	*Eucalyptus camaldulensis*	1	1
21	*Alstonia scholaris*	1	1
22	*Pinus merkusii*	1	1
23	*Pinus oocarpa*	1	1
24	*Eucalyptus globulus*	1	0.5
25	*Shorea spp*	0.5	0.5
26	*Shorea javanica*	0.5	0.5
27	*Palaquium sp*	0.5	0.5

Source : Arisman (2001)

Table 9. Growth performance of various provenances of *Acacia mangium* in South Sumatra

Provenance	Increment (m^3/ year)	Volume (m^3)	% Control
Oriomo River, PNG	65	355	146
Olive River, Qld	64	348	142
Wipim, PNG	63	347	142
Kini, PNG	59	323	132
Kiriwo, PNG	57	316	130
Bimadebun, PNG	57	315	129
Bensbanch, PNG	57	314	129
Muting, Irja, Ind	57	314	129
Malam, PNG	57	312	129
Deri-deri, PNG	57	310	128
Aurifi, PNG	55	300	124
Kuru, PNG	55	300	124
Keru, PNG	55	299	123
Mata, PNG	54	296	122
Claudi River, Qld	54	275	121
Tully Mission, Qld	50	257	113
Subanjeriji, Pohon Plus	47	257	105
Subanjeriji, biasa (kontrol)	44	244	100

Source : Arisman (2001)

PT. Arara Abadi has a significant staff for tree improvement research. They have a large area of seed orchards, a hedge (multiplication) garden, and clonal forests. Artificial hybrids between *A. mangium* and *A. auriculiformis* have been produced. They were able to shorten the rotation of *A. mangium* from 7-8 years to 5-6 years with Mean Annual Increment (MAI) yield class 40-60.

Eucalyptus urophylla is planted on a large scale by PT. Inti Indo Rayon. The Directorate General of Reforestation and Land Rehabilitation, in cooperation with UGM, conducted a series of tree improvement activities: exploration in natural forests, seed collection, progeny testing, and provenance test plantation establishment. PT. ITCI prefers to grow *E. deglupta* rather than *E. urophylla*.

PT. Surya Hutani Jaya, in cooperation with UGM, has conducted exploration for *E. deglupta* and *Paraserianthes falcataria* plus trees. Progeny test and provenance test plantations of both species have been established, and PT. Surya Hutani Jaya also conducted a significant tree improvement program for *Gmelina arborea*. Some HTI holders are interested on *Acacia crassicarpa* and *Acacia aollacocarpa* due to their ability to grow on swampy sites.

Eucalyptus urophylla (Ampupu)

Ampupu is one of the fastest growing species in Eastern Indonesia. Natural distribution of ampupu is on the islands of Timor, Flores, Adonara, Lomblen, Pantar, Alor, and Wetar. Ampupu is considered as a select species for industrial plantation.

A tree improvement program for ampupu was started in 1980 by exploration, parent tree selection, and by collecting seed from parent trees in natural stands over its geographic range. Twenty-four provenances were visited and seeds were collected from each parent tree and kept separated.

The objectives of the ampupu improvement program were: (1) to obtain superior races/provenances and individual genotypes for important characters on industrial and commercial plantations; (2) to determine the amount and mode of inheritance of specific traits; (3) to develop methods for creating additional genetic variation to add to the ampupu gene pool; and (4) to establish seed orchards. A progeny test was conducted and followed methods as described in progeny test above with 100 families. Twenty-two provenances were tested at Wanagama in 1983.

Non Teak Species in Java

As mentioned earlier, all production forests in Java are managed by Perum Perhutani; not only teak and pine species, as discussed, but also non-teak species such as *Swietenia spp, Meulaleuca leucodendron, Paraseriathes falcataria, Dalbergia latifolia,* and *Schleichera oleosa* have been planted by Perum Perhutani. The tree improvement strategy of these species has been designated, but not all have been initiated. Tree improvement activities on mahagoni and *Paraserianthes falcataria* have been conducted by Perum Perhutani. Plus tree selection and progeny test of both species has been accomplished. A collaborative research project between Perum Perutani and The Center for Research and Development of Forest Biotechnology and Tree Improvement, sponsored by JICA, has been conducted.

Meranti and other Tropical Rain Forest Species

The data show us that wood production from natural forests has declined significantly. Most of the valuable tropical wood such as meranti, kapur, kruing, ramin, and ebony has been harvested from natural forests. On the other hand, almost all HTI holders cultivate exotic, fast-growing species for pulp and paper products. There will thus be plenty of problems with sustainability of local wood products if we do not conserve our tropical rain forest species. The only way to sustain our tropical rain forest wood products is through establishing meranti plantation forests on appropriate sites and applying intensive silviculture. Several tree improvement programs for a few tropical rain forest species were started in 1995. There was cooperative research on tengkawang between Biotrop and the Faculty of Forestry, UGM. Population test plantations were established in 1995 in both West and South Kalimantan. The first two years, the plantations grew well. Unfortunately, fire destroyed our plantation trials in both locations. In cooperation with PT. Inhutani II, the Faculty of Forestry, UGM has selected 50 plus trees each of 3 local species from the Pulau Laut forest. Progeny tests were established and growth was evaluated at the age 2. However, all the plus trees as well as the progeny test plantations disappeared due to illegal cutting.

In the 1998, ITTO Project PD 16/96 Rev. 4(F) entitled "*Ex situ* Conservation of *Shorea leprosula* and *Lophopetalum mulitinervium* and their Use for Future Breeding and Biotechnology" was initiated. At present, most *ex situ* conservation activities for forest trees in Indonesia are limited to collecting and preserving genetic resources in Botanical gardens, Arboreta, and Taman Hutan Raya. It will not be possible to use these collections for breeding purposes. This ITTO project on *ex situ* conservation is being conducted to provide a model for meeting the requirements of future breeding and biotechnology programs to improve productivity and quality of tropical forest products. A development objective of this project is to create a Center of Excellence for *ex situ* conservation, which will serve Indonesia and the neighboring countries in research, technology development, training, and education in the genetic conservation and improvement of selected tropical species. The project focuses on determining appropriate methods of conserving two selected indigenous species of the tropical rain forest to provide a "reservoir of genetic materials" for future improvement and biotechnology efforts to improve those species.

The other specific objective is to establish *ex situ* conservation methodologies for *Shorea leprosula* and *Lophopetalum multinervium* and to develop these as models for general *ex situ* conservation techniques for tropical forest trees.

Shorea leprosula

S. leprosula is a common source of light-red meranti timber, a valuable export commodity. The wood density is 300-865 kg/m3 at 15% moisture content. *S. leprosula* is a large to very large tree, up to 60 m tall and up to 175 cm in diameter, with a clear bole up to 30 m and buttresses up to 2 m high. *S. leprosula* is commonly found growing on well-drained ultisols below 700 m in altitude. A resin called "Damar daging" is extracted from the roots and is used in traditional medicine. The bark is used for tanning.

Like most *Shorea* species, the genetic variability of *S. leprosula* has declined due to abuses of the Indonesian Selective Cutting practices by some loggers. Often, the best trees with diameters of 50 cm and above were cut and the poorest trees were left to reproduce, despite regulations that required leaving at least 25 of the best trees with diameters of 20 cm and above per hectare. Because of this practice, while there are still many *Shorea leprosula* seedlings growing on logged over areas, the genetic quality of these is not as high as it was prior to logging. Therefore, the sustainability potential of *S. leprosula,* especially in terms of its quality, has been reduced. Thus, in addition to its use as a model for species growing on well-drained sites, there is a real need to conserve, restore, and improve the genetic variation of *S. leprosula* and to regain this lost potential for sustainability.

Lophopetalum multinervium

The wood of *L. multinervium,* sold as perupuk, is a valuable export commodity. Perupuk timber is suitable for high quality general construction, interior finishing, paneling, and furniture manufacture. This odorless and tasteless wood is also used to make food containers and tea chests.

Perupuk is medium-to–large-sized tree with a clear cylindrical bole, growing to 45 m tall and 90 cm in diameter with buttresses up to 2 m tall spreading as far as 8m from the base. Perupuk grows in flooded forests and on peat soil from sea level to about 1,500 m altitude.

Perupuk timber is currently in great demand because it is considered very decorative. The increased effort to meet this demand might easily result in significant genetic erosion. The need to conserve, restore, and improve the genetic variation is thus a major reason the species was chosen for this study.

Shorea polyandra.

A cooperative research project in tree improvement between the Faculty of Forestry and PT. Inhutani II has been conducted on three species of indigenous tropical rain forest trees to Pulau Laut. One of these three species is *Shorea polyandra*. This species has good quality wood and is a valuable export commodity.

The study started with selecting 50 plus trees from the natural forest in Stagen Pulau Laut, South Kalimantan. Fruit was collected from all 50 plus trees for establishing a half-sib progeny test, which was established at 3 sites on Pulau Laut, South Kalimantan: Stagen, Mekarpura, and Teluk Kepayang. At age 1.5 years, measurements and other evaluations were made by

Nurprabowo. Results indicated that:

1. The mean survival percentage at all sites is high,
2. The heritability estimation value is relatively low for tree height and stem diameter at all sites,
3. Genetic correlation between tree height and stem diameter in Stagen, Mekarpura, Kepayang and all sites combined was 0.57; 0.79; 0.85 and 0.76, respectively.

Meranti Plantation Forest Establishment (PHTM)

The Ministry of Forestry, in collaboration with the Faculty of Forestry, has a program to develop meranti plantation forests on a forest management unit scale.

Each unit needs around 15, 000 ha of forest land of which 9,000 ha should be planted with meranti and the other 6,000 ha would be used for activities supporting the implementation of appropriate meranti plantations, such as: conservation, tree improvement, research related to intensive silviculture and buffer zones for the local people. Each unit must be cultivated with 300 ha of meranti per year with 30 years rotation. This program, which was planned by Prof. Soekotjo and approved by the Ministry of Forestry, has been socialized to some Local Governments (Pemda). All State-owned Forest Companies (Perum Perhutani, Inhutani I–V) always participate in discussing the possibilities of meranti forest plantation establishment. Unfortunately, due to lack of funding at the present time, they are still waiting for the opportunity to speed the program's implementation. However, they are prepared to start working at any time.

Seed Sources For Community Forests

All the earliest tree improvement activities were conducted to meet the needs of the state forests. There was no any effort to meet the needs for good quality seed on private lands or community forests until 1999. In that year, the Directorate General of Forest Community and Land Rehabilitation, in cooperation with the Faculty of Forestry, GMU established several seed stands/seed orchards in Java, North Sulawesi, South Sulawesi, and South Kalimantan.

Seed sources of *Artocarpus heterophylla* (nangka), *Paraserianthes falcataria* (sengon), *Armelaria campaca* (cempaka), *Aleuritas moluccana* (kemiri), and some dipterocarp species have been established in Java, North Sulawesi, South Sulawesi, and South Kalimantan, respectively. The maintenance looks good and performance is generally promising.

Education, Training

Training is a key element for the development of forest tree improvement in Indonesia. In Indonesia, there are 8 (eight) state universities offering forestry courses, four of which have an independent Faculty of Forestry, and the other four have a forestry curriculum within their Faculty of Agriculture (Hardiyanto, 2001). In the Faculty of Agriculture, Forestry is designated as one of its Divisions or Departments. Not all Faculties of Forestry or Departments of Forestry offer a specialized course in tree improvement. There are also some private Universities with a Faculty of Forestry or a Department of Forestry, and some of these offer a Tree Improvement Course.

Gadjah Mada University now has 18 Faculties and one of these is the Faculty of Forestry. Before 1964, the Faculty of Forestry at GMU was a Department of Forestry within the Faculty of Agriculture and Forestry. Before 1972, forest tree improvement science was covered in the general Silviculture course. Since 1972, students from the Department of Silviculture must take the Tree Improvement course in addition to the Silviculture course. Since that time, many students have taken forest tree improvement as their study program and done research on tree improvement for their thesis. From the cooperative research between GMU and various institutions, we have established many genetic test plantations and seed orchards on several sites. Students are thus able to take part in forest tree improvement activities in the field (such as exploration, plus tree selection, seed or pollen collection, nursery work, genetic testing, seed orchard establishment, controlled pollination, hybridization, and vegetative propagation) and use the experience and data for their thesis. GMU has produced more than 100 under graduates from the S1 program, more than 20 breeders from S2 program, and one current student in a doctorate or S3 program.

With regard to cooperative work with other institutions, GMU has experience with holding short course training in forest tree improvement. Almost all of the trainings were conducted at Wanagama I due to availability of various tests and facilities. Not only dormitory, dining hall, and classrooms are available, but also various genetic test plantations for tree improvement studies. The Indonesian Forest Seed Project (IFSP), in cooperation with Wanagama I, conducted some short courses on genetically good seed. The short courses related to forest tree improvement conducted by the Faculty of Forestry are as follows:

Table 10. Training on Tree improvement held by GMU

No	IN COOPERATION WITH	THEME OF TRAINING
1	University of Bengkulu (1990)	Forest Tree Improvement
2	PT. Sumalindo (13-15 Sept 1993)	Forest Tree Improvement
3	APHI I (25/8 – 3/9 1994)	Forest Tree Improvement
4	APHI II (1995)	Forest Tree Improvement
5	INHUTANI I (1999)	Forest Tree Improvement (*Pinus merkusii*)
6	IFSP (3 – 7 Mei 1999)	Basic Forest Genetics
7	IFSP (19 – 27 Oct 1999)	Seed sources
8	IFSP (12 - 17 June 2000)	Basic Forest Genetics
9	ITTO (2000)	Conservation, Tree Breeding & Biotechnology
10	ITTO (2000)	Conservation & Genetic Improvement
11	ITTO (2000)	Introduction to Molecular Genetic and Biotechnology
12	ITTO (2000)	Genetic Resource Conservation and Wise Use on the Global Market. (Workshop)
13	ITTO (2000)	Genetic Conservation & Tree Breeding

Generally, there are adequate facilities in Indonesia for degree courses in Forestry. However, there are still insufficient facilities, scientists, and budgets in forestry education for specialization to pursue postgraduate studies related to forest genetic resources.

It is necessary to correct this education and technology transfer focuses on the training of personnel who will then be able to produce genetically-improved seed and vigorous planting stock to ensure successful reforestation of logged-over forests, and establish new plantations.

Institutional Frame Work

The agencies within the Ministry of Forestry dealing with forest tree improvement and forest genetic resources include: **(1)** The Research and Development Center for Forest Biotechnology and Tree Improvement under the Agency for Forest Research and Development has adequate laboratory facilities and scientific resources to conduct research and development in tree improvement and forest biotechnology. The center has a number of tree improvement projects. **(2)** the Directorate General for Forest Protection and Nature Conservation is responsible for the protection and management of protected areas and protection forests. **(3)** the Directorate for Forest Tree Seed under the Directorate General for Land Rehabilitation and Social Forestry has responsibility for controlling, supervising, and facilitating the use of good quality seed for agroforestry and plantations. Other institutions with responsibility for managing biological resources include the Indonesian Institute of Sciences (LIPI); the National Planning Board (BAPPENAS), and the Provincial Planning Agency (BAPEDA).

The Faculty of Forestry, Gadjah Mada University, Yogyakarta Indonesia is one of the strongest Indonesian Institutions in the area of tree improvement. Many genetic test plantations of pine such as provenance and progeny tests and seedling seed orchards have been established since 1976. These test plantations were supported through collaboration with the Ministry of Forestry (Perum Perhutani). Since 1981, GMU has established progeny and provenance tests of other commercial species. This work is also supported by the Government of Indonesia, as well as by Perum Perhutani, PT Inhutani, and some concession holders. Since 1998, the Faculty of Forestry, GMU has had cooperative work with the Ministry of Forestry (including Perum Perhutani and PT Inhutani I–V) and ITTO on e*x situ* conservation.

Based on previous experiences in collaborative activities mentioned above, the Government of Indonesia and PT INHUTANI I to V held a meeting to discuss the proposal for the ITTO Project submitted by GMU. In this meeting, PT Inhutani I-V (as users) asked to participate in this project, and expressed their willingness to support exploration, seed collection, and the nursery and plantation establishment and maintenance activities. This support was used by the GOI as their matching funds. Participants at the meeting further suggested that ITTO should finance the cost of consultants (both in-country and abroad), and that ITTO include the technology transfer.

The cooperating institutions for this project are the Directorate General of Forest Protection and Nature Conservation (Ministry of Forestry), the Bureau of International Cooperation and Investment (Ministry of Forestry), Inhutani I to V, GMU, OSU (Oregon State University), and ITTO.

The role of OSU is basically to provide project support in education and technology transfer. This has included consultation and collaboration in the area of research project planning, proposal writing, and development of education and training plans. OSU will also provide basic scientific backup to this project through the design and conduct of any supplemental studies that may be appropriate to the success of the project.

Over the long term, the research and educational activities conducted under this project should conserve genetic materials and contribute to significant increases in the sustainability of forest plantations and logged-over natural forests. A unique feature of this project is its focus on facilitating the incorporation of the conserved genetic materials into future tree improvement programs by designing the *ex situ* conservation areas to accommodate future research in conventional selection and breeding as well as in biotechnology.

Problems

Reforestation is a high priority for Indonesia and the government is actively working to replant unproductive forestlands and to alleviate the accompanying problems of low soil fertility, erodability, and susceptibility to fire on those lands. In addition to site-related problems, the available genetic resources of many species have declined alarmingly.

According to Hardiyanto (1990) there are several factors that might be problematic to progress in tree improvement in Indonesia. **(1)** A great diversity of sites that might cause genotype x environment interactions and thus complicate breeding programs. **(2)** A large number of tree species, with the basic biology of many not adequately known. **(3)** A lack of adequate funding. **(4)** A lack of qualified scientists and support staff. A successful breeding program is dependent upon adequate and continuous funding, as well as continuous staff support. A short-term program, say 2-3 years, will not have a significant impact (Zobel and Talbert, 1984)

According Zobel and Talbert (1984), a major problem of many tree improvement programs is the impermanence of organizations and movement of the scientific staff. Tree improvement is a long-term process and, as such, it must have good permanent records that are maintained and handed on from scientist to scientist. This often has not been the case. Records are sometimes poorly kept or not kept at all. And valuable information moves out with the personnel who leave. Often, the new scientist is a highly trained specialist who wants to do her or his "own things" and therefore the old studies that were inherited suffer because they are only of passing interest to that person. The lost time and wastage caused by poor records and constant personnel changes cannot be overemphasized. Although not based on any credible data (none are available), the senior author estimated that at least 50% of the forest research that was initiated never comes to fruition because of a lack of care, lost of records, and/or the movement of key persons.

Difficulties associated with working with large, long-lived plants such as forest trees are obvious. The size of the trees creates problems with measurements, crossing, seed collection, laboratory and field-testing. Age to flowering is relatively long and that also complicates breeding and testing.

Conditions in the field in tropical forest areas commonly are not as good as in temperate forests. Generally, tree breeders in Indonesia and other SE Asian countries have to work harder than any other breeders, due to working at isolated sites, mountainous or swampy topography, high temperature and humidity, and heavy rainfall. All of these conditions make genetic improvement work with trees, laborious, time-consuming, dangerous, and expensive.

Prospect and Progress

Forest tree improvement is now a widely practiced forest management activity in over 40 countries, worldwide. Forest tree improvement is generally recognized as the most effective and profitable management technique available. In many of the early tree improvement programs, predicted gain in volume or value could reach 20-30%; indeed, in some cases these are being obtained at the first stage of simple, mass selection (Hardiyanto, 1990). Talbert reported that from one generation's selection in loblolly pine the predicted gains achieved were 17, 12, and 32% for height, volume, and harvest value, respectively.

The predicted gain in stem straightness for *Pinus merkusii* was high and was comparable to those for other *Pinus* species. Based on the results from our half-sib progeny tests, significant genetic improvement for *Pinus merkusii* is feasible (Suseno, 1988).

Tree improvement programs with ampupu and teak seem promising as well. In ampupu, differences among provenances were found to be very large. For example, the best provenances grew 27% faster than the mean (Andjalmo, 1987).

Tree improvement programs in the tropics are potentially more attractive than those with temperate zone trees, since tropical tree species generally have faster growth and shorter reproductive rotations. The potential genetic gains could be considerable in some species. So even though forest tree improvement is relatively new in Indonesia, there have been some significant positive results, to date, and both progress and prospects seem satisfactory. The number of foresters now interested in forest tree improvement has increased significantly. The Universities have produced some tree breeders who have found good, well-paying positions.

It is of interest to also note that the Ministry of Forestry has maintained a strong interest in tree improvement programs. In 1994, the Ministry of Forestry opened the "Research and Development Center for Forest Biotechnology and Tree Improvement", supported by JICA and located at Purwobinangun, Yogyakarta. In 1998, the Ministry of Forestry also opened the "Teak Center" located at Cepu and many if not most of the activities there relate to genetic improvement of that species.

Most of the Indonesian tree improvement work has thus far been initiated and funded by the Ministry of Forestry and/or the State Owned Forest Companies. Recently however, several private companies engaged in industrial forest plantation management have started their own tree improvement programs. We can learn much from the experiences of other countries such as the USA, where cooperative forest tree improvement programs such as those in Florida, Michigan, North Carolina, Oregon, and Texas bring together scientists and managers from the academic, industry, and government to address common issues and opportunities. Such cooperative organizations may be worth considering at national or even regional levels in the tropics to promote teamwork and sharing of scientific knowledge, and to help solve problems of logistics and funding.

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Ex situ Conservation of *Pinus merkusii* in Java, Indonesia

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Abstract. *Pinus merkusii* is one of the important forest tree species planted in Indonesia due to the considerable value of its wood and oleoresin. In its natural distribution in Sumatra (Aceh, Tapanuli and Kerinci) the species has constantly suffered from a variety of threats. Despite current efforts at *in situ* conservation, *ex situ* conservation of the species is deemed necessary to better safeguard it from unwanted loss. The selected strategy is to integrate *ex situ* conservation into the existing breeding program. During 1996-1997 an attempt was made to collect *P. merkusii* seeds from the natural populations and the new genetic materials were then incorporated into the existing breeding program. The infusion of new genetic materials from natural populations is one of the key elements in the breeding programs of *P. merkusii* in Java. The subpopulation and number of families from Aceh included in the breeding program are as follows: Jantho (64), Takengon (72), Blangkejeren (295). The new genetic materials were established as breeding populations at two sites in Java in 1997 and 1998, and structured as a multiple population breeding system. In 1997 seed collection was made from the Tapanuli and Kerinci populations; however, due to poor germination the establishment of *ex situ* conservation stands of the latter two populations has not been successful.

Introduction

Pinus merkusii is an important forest tree species planted in Indonesia due to the considerable value of its wood, which is excellent for sawn-timber, furniture, boxes and paper. The paper derived from its fiber has qualities comparable to those of other pine species. The species has also been recognized for producing a high quantity and quality of oleoresin.

P. merkusii is the only pine native to Indonesia, namely in Sumatra. On this island the species is found in three disjunct populations; namely Aceh, Tapanuli, and Kerinci. The species also occurs naturally in northeastern India, Thailand, Laos, Cambodia, Vietnam, and the islands of Luzon and Mindoro in the Philippines (Cooling 1968).

The species was introduced to Java in the early 1920s from an unknown source of its natural population in Sumatra, presumably from Blangkejeren of the Aceh provenance. The species was also introduced to South Sulawesi at the end of the 1930s. Since then the species has been planted quite extensively in Java (500 000 ha) and South Sulawesi (76 000 ha). On a smaller scale, it has also been planted in other parts of Indonesia.

A program for genetically improving *P. merkusii* was started in 1977 in Java and it is now entering into the second generation breeding program. One of the key elements in this breeding program is to infuse new genetic materials from natural populations. The newly introduced genetic materials will also function as *ex situ* conservation measures for *P. merkusii* (Hardiyanto and Danarto 2000).

Genetic Variation of *P. merkusii* Population in Sumatra

Three populations of *P. merkusii* differ morphologically and differences in the following characteristics are noted: stem form, foliage, branching habit, internode, and bark. In general, the *P. merkusii* trees of the Aceh population are characterized by broader and coarser crowns, heavier and more horizontal branches, and thicker and darker colored bark in comparison with *P. merkusii* of Tapanuli and Kerinci populations. The Kerinci population is similar to that of Tapanuli, except it has smoother bark.

Genetic variation in growth and stem form based on the open-pollinated progeny tests using materials of *P. merkusii* plantations in Java was reported to be intermediate (Hardiyanto 1996). Genetic variation in oleoresin production is considerably high (Leksono and Hardiyanto 1996). Differences within and between subpopulations of Aceh provenance were also recorded (Hardiyanto *et al.* 2000). Data on the growth differences between Aceh, Tapanuli and Kerinci populations based on field trials, however, are not yet available. However, it is suspected that the growth of Tapanuli and Kerinci populations is slower than that of Aceh provenance.

Results of molecular marker work using allozyme technology show that within Aceh population, *P. merkusii* has relatively high genetic diversity; the expected heterozygosity (He) varies from 0.271 to 0.322, with mean heterozygosity of 0.304. The *P. merkusii* plantation in Java has slightly lower genetic diversity than that of natural population; the expected heterozygosity ranges from 0.274 to 0.285 with mean expected heterozygosity of 0.276 (Kartikawati 1988, Na'iem 2000). Using allozyme markers in his study, Siregar (2000) reported similar results in which the Aceh population showed considerable genetic variation (He=0.361); however, he recorded that the plantation of *P. merkusii* in Java had slightly higher genetic variability (He=0.395). As expected, the level of genetic variation of the Tapanuli and Kerinci populations is much smaller than that of the Aceh population. Siregar detected no genetic variation within Kerinci population (He=0.00), while expected heterozygosities of Tapanuli and Kerinci populations were 0.219 and 0.042, respectively (Na'iem & Munawar 2001, pers. comm.). Tapanuli and particularly Kerinci populations are relatively small and scattered and consequently, the inbreeding rate is expected to be high which gives rise to low genetic variation.

Threats to the Natural Populations of *Pinus merkusii*

Among three natural populations of *P. merkusii* in Sumatra, the Aceh population is the largest in terms of size. The population is divided further into three disjunct subpopulations, namely Jantho, Takengon and Blangkejeren. At the end of the 1960s, the Aceh population occupied around 200 000 ha (Cooling 1968); however, its size is steadily declining due to logging and conversion of its habitat into agricultural land. Since 1989 the subpopulation of Takengon has been logged to supply wood fiber for a kraft paper mill nearby and many stands in this subpopulation have been cleared. The threat to this population comes also from genetic contamination, in which some of the logged-over areas are planted not using available genetic materials from the local seed source, but seed imported from Java.

Realizing the serious threat to the subpopulation of Takengon, an effort is being made to conserve some of the populations in this area. In this program, 13 stands varying from 20 to 425 ha in size were designated as conservation stands. The stands were selected based upon stand condition, representative nature of the site, and accessibility. Details of the *in situ* conservation of *P. merkusii* in Aceh were previously described by Hardiyanto (1993).

P. merkusii in Aceh is most prominent at elevations from 500 to 1700 m. However, many stands of the subpopulation of Jantho are found at lower elevations (200 - 300 m) and considered as outliers. It is fortunate that the subpopulation is still in good condition due to its poor accessibility. Illegal cutting is only minor there.

Despite the absence of extensive logging operations in the Blangkejeren subpopulation, this subpopulation has long been suffering from genetic impoverishment due to the practice of negative selection. Local people have selectively felled the best trees in the stand and left the poor ones (Hardiyanto 1993).

P. merkusii in Tapanuli occurs at an elevation of around 950 m and it is fragmented into a number of subpopulations. Previously, the extent of the Tapanuli population was estimated to be around 3500 ha (Hardiyanto 1993) but its size has been steadily declining in recent years, mainly due to logging. It is true that many logging activities have occurred in the stands growing on the local community land, but illegal logging has also taken place in the state forest land, some of which has been designated as conservation areas. Genetic contamination has also occurred in this population as reforestation often uses seed of *P. merkusii* imported from Java.

Among three populations of *P. merkusii* in Sumatra, the Kerinci population is the most seriously threatened. The population has been severely fragmented, and some of the stands now occupy an area less than 1 ha and consist only few trees,which are often mixed with other tree species. *P. merkusii* in this population occurs at elevations between 1250 and 1400 m. While one subpopulation is relatively secure as it grows on the protected area of Kerinci-Seblat National Park, continued tree felling and conversion of the forest into *Cinnamomum* plantations likely caused some significant loss of valuable genes of this population. As found in Aceh and Tapanuli, genetic contamination has also occurred in the Kerinci population.

Management of *Ex situ* Conservation

Continuing threats to the natural populations of *P. merkusii* in Sumatra and the need to broaden the genetic basis of the current breeding program of this species have led to the effort of securing genetic materials from natural populations. For species under intensive domestication and for which breeding programs are in place, such as *P. merkusii*, conservation efforts are better integrated with the existing breeding program (Namkoong 1986, Palmberg-Lerche 1999).

Seed collection from Aceh was carried out during 1996 and 1997. Since information on the pattern of genetic variation was not available at this time, the strategy for seed collection was to sample as many subpopulations as possible. These new genetic materials were then planted as progeny tests on two sites in Java (West and East Java) in 1997 and 1998. These breeding populations also function as *ex situ* conservation stands. The infusion of genetic material from the natural population is structured according to a multiple population breeding system (MPBS). In this regard, populations are subdivided into subpopulations or sublines. MPBS was reported to be very effective in maintaining genetic diversity in *Pinus taeda,* and the MPBS aggregate had genetic diversity similar to that of natural stands (Williams and Hamrick 1996).

The grouping of the newly introduced genetic materials was based upon the place of origin of seed collection. Table 1 presents the subpopulations and number of families successfully progeny tested. It is worth noting that the original number of families in the seed collection was larger than those presented in Table 1. Due to poor viability, not all families were included in the progeny tests but the number of families in each subpopulation is considered adequate to maintain the current level of diversity. Taking seeds from at least 15 trees per subpopulation has been recommended as the minimum sample size for use in *ex situ* conservation (Brown and Briggs 1991, Brown and Hardner 2000). For maintaining genetic variability in the breeding program, the family size in each subpopulation presented in Table 1 is also considered sufficient; the minimum size of a breeding population is recommended to be 50 genetic entries (Eriksson 1997).

These newly added genetic materials should certainly broaden the genetic basis of the existing breeding program for *P. merkusii* in Java. It is worth mentioning that the plantation of *P. merkusii* in Java has been known to have a narrow genetic basis; its genetic material was initially introduced in the 1920s from an unknown source, presumably from Blangkejeren of Aceh provenance.

Table 1. Subline and number of families of the new genetic materials collected from Aceh

Subpopulation	Year of seed collection	Number of families
Jantho	1996	64
Takengon	1996	
North Takengon	1996	40
South Takengon	1996	32
Blangkejeren	1996	70
Arul Rengit	1997	50
Pagedungan-Uning	1997	50
Rikit	1997	60
Kendawi-Uring	1997	65

In 1997 seed collection was carried out in Kerinci and Tapanuli populations. However, due to poor germination, conservation stands of these two populations were not successfully established. An effort to make another seed collection from these two populations is still in progress. The Tapanuli population is suspected to have high oleoresin yield and it is certainly worth exploring this valuable genetic resource, considering oleoresin extraction represents considerable financial return for *P. merkusii* growers.

Siregar (2000) argued the insignificant importance of conserving the Kerinci population as a gene resource due to its considerably low genetic variation. This is probably true if the population is developed and maintained as a pure population. However, the use of the Kerinci population in interpopulation hybridization might be worth exploring.

Seed collection has also been made from open-pollinated parent trees selected for high oleoresin yield in *P. merkusii* plantations in South Sulawesi, which are known for high oleoresin production. It is suspected that the early *P. merkusii* plantation in South Sulawesi was developed from a seed source different from that used in Java. Seeds have been successfully collected from 280 parent trees and will be used to establish progeny tests. This subline will increase further the number of breeding populations in the current breeding program for *P. merkusii* in Java. .

It is believed that jointly managing *ex situ* populations for both gene conservation and breeding purposes is advantageous. Both are designed so that diverse germplasm can be maintained and readily available for tree improvement program. Broad sampling to establish multiple populations and continued development of interpopulation variation assure the presence of diversity to meet changing environmental conditions and market forces (Eriksson *et al.* 1993, Palmberg-Lerche 1999).

Concluding Remarks

The genetic resources of *P. merkusii* natural populations are continuously decreasing due to various causes, particularly logging and conversion of habitat into agricultural land. *Ex situ* conservation, integrated into the existing breeding program, is considered one of the more effective efforts to conserve this valuable genetic resource.

Acknowledgements

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The Benefits of Tree Improvement Cooperatives to Serve Breeding and *Ex situ* Conservation Programs of *Gmelina Arborea*, Roxb.

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Abstract. Provenance/ progeny tests of Gmelina arborea in Colombia, Costa Rica, Guatemala, and Indonesia were analyzed to estimate both potential genetic gain and financial benefit of tree improvement cooperative. The tests consist of 60 open-pollinated families from 3 provenances in Thailand that were collected by the International Gene Conservation and Tree Improvement Cooperative (CAMCORE). Only data from test locations with large trees (mean height > 10 m) were used in the study. The yield model and cost from a typical forest plantation in Indonesia were used to generate financial information. The objectives of the study were to determine potential genetic gains using the selection index and to estimate the financial benefits of tree improvement cooperative under a seed orchard strategy.

The predicted genetic gains and production gain of the best 30 trees using family and within family selection were 39% and 31% above the overall test mean, respectively. The estimates of Net Present Value (NPV) and Internal Rate of Return (IRR) were calculated for both single and multiple sites. These results were generated assuming a 3,000 ha annual planting program and a 6% interest rate. Even with a conservative assumption about yield and gain, tree improvement cooperative programs were shown to be beneficial, both financially and for gene conservation.

Introduction

Gmelina arborea is a fast growing species that has the potential to become a major international timber species over wide range of sites in the tropics. It grows naturally in semi-deciduous forests in Bangladesh, Cambodia, southern China, India, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam (Lauridsen *et al.* 1987, Palmer *et al.* 1984, Tewari 1995). It remains to be verified whether its reported occurrence in the Philippines and Malaysia is natural. The geographic range of *G. arborea* encompasses an area of nearly 12 million km^2 from latitudes 5° to 30°N, and longitudes 70° to 110°E. Its altitudinal range is approximately 50 m to 1,300 m above sea level (Lauridsen *et al.* 1995). *G. arborea* often occurs in moist fertile valleys with sandy loam soils but occasionally is found on clay loams (Tewari 1995). *G. arborea* is found in association with *Tectona grandis, Melia azedarach,* *Lagerstromia spp., Pterocarpus macrocarpus, Bombax spp., Dipterocarpus alatus, Hopea odorata* and some other species depending on the forests sub-types (Champion & Seth 1968). The tree is medium to large in size and a member of *Verbenaceae* family (Chudnoff 1979). It produces well-formed boles that are well suited for various wood products. The works done by Palmer et al. (1984) in the Solomon Islands, Twimasi (1991) in Ghana, and Laurila (1995) in Indonesia confirmed its suitability for sulfate kraft pulp. It had superior tear strength in the kraft-anthraquinone process in a study in Nigeria (Sobanjo et al., 1994-95). Interestingly, Su et al. (1995) found that younger trees at 2.5 years of age gave higher pulp yield and better properties than older trees at 9.5 years of age. *G. arborea* was also found suitable for laminated veneer lumber (Dolores *et al.* 1990), Phenol-formaldehyde particle board (Fuwape 1996), fuel wood or even charcoal (Salazar 1988). Recently, PT Sumalindo Lestari Jaya in Indonesia successfully used the wood in a mixture with *Acacia mangium* Willd. to produce a high quality medium density fiberboard (Sumalindo 1997).

In the last 20 years, *G. arborea* has been the subject of moderate to intensive tree improvement activities. International provenance trials sponsored by DANIDA (Danida Forest Seed Centre) were established from 1977 to 1981 on 27 sites in tropical Africa, India, Malaysia, the Solomon Islands, South America, and Vanuatu. The seeds came from 30 provenances and sources collected in Africa, India, Latin America, Malaysia, Oceania, and Thailand (Lauridsen *et al.* 1987). Several national provenance trials have been established in Cameroon (Fonwehan *et al.* 1997), Colombia (Ladrach 1986), India (Tewari 1995), the Solomon Islands (Sandiford 1989), Venezuela (Jurado-Blanco & Kane 1992), and in other regions such as Costa Rica, Malawi, Nigeria, and Tanzania. The findings from these provenance trials indicated the importance of seed source related to differences in survival, growth, form, and susceptibility to diseases and insects. In 1994, CAMCORE (International Gene Conservation and Tree Breeding Cooperative), North Carolina State University, in collaboration with the Royal Forest Department of Thailand, initiated ex *situ* gene conservations and seed collection programs of *Gmelina arborea* in native forest of Thailand (Donahue 1994).

Time is one of the most important considerations when assessing the financial returns of an active tree improvement program (Zobel 1978). A desired increase in productivity takes years to obtain through extensive selection, breeding, and testing. In a large planting program, the use of unimproved rather than improved seeds is considered a loss in future revenues. There is an urgency to obtain gains as quickly as possible. Gain is a function per unit time (Zobel & Talbert 1991). The same financial consideration was one of the driving forces for members of CAMCORE to collect and test *G. arborea* from the natural forest of Thailand in 1994 (Donahue 1994). Most of CAMCORE members represent private industry that together with universities and host-government organizations have combined technical and financial resources for both *ex situ* forest gene conservation and tree breeding (CAMCORE 1997). A careful and accurate assessment of financial returns is important for evaluating the benefit of testing and improvement. Analysis of tree improvement programs from an economic stand point can indicate what improvement is important to profitability, what costs are reducing profitability, and what research is most likely to pay off (Friedman 1992). It is essential that genetic and economic information are combined and presented in a realistic and meaningful manner. Zobel and Talbert (1991) suggested calculating the added value that genetically improved materials will contribute to an organization.

A series of international provenance/progeny tests of *Gmelina arborea* was established by CAMCORE members in Colombia, Costa Rica, Guatemala, Indonesia, and Venezuela during 1994 and 1995. The trials consist of samples of seeds from Thailand planted in a common genetic test design and measured annually. The measurement and cost related data provide information needed to evaluate both genetic and economic information. The main objective of the study is to assess potential genetic gain using a selection index. The second objective is to use the information on potential genetic gain to estimate the financial benefits of tree improvement cooperative under a seed orchard strategy.

Materials and Methods

Provenance/Progeny Tests

Five provenance/progeny tests of *G.arborea* established in Colombia, Costa Rica, Guatemala and Indonesia with common tree size (mean height > 10 m) were chosen for the study. Test location information is given in Table 1. The seeds for the test came from the CAMCORE collections made in Thailand in 1994. The tests were established using a randomized complete block design with 9 replications. Provenance plots were randomly located within blocks and families were randomized within provenance. The tests included 3 provenances and 30 to 60 families. Six trees per family were planted in a row plot in each provenance block. The trees were planted at spacing 3 x 3 m.

Table 1. Location of provenance/progeny tests of *Gmelina arborea* included in the analyses

Company name	Date of establishment	Latitude Longitude	Elevation (m)	Annual precipitation (mm)	Number of family tested	Data observed (age)
Smurfit Cartón de Colombia-Colombia	Nov' 94	3° 34' N 76°30' W	950	994	30	3 years
Ston Forestal-Costa Rica						
- Site 1	Sep'94	8°44'N 83°32'W	40	3,000	60	3 years
- Site 2	Sep'94	8°54'N 83°10'W	20	3,800	57	3 years
Simpson Forestal-Guatemala	Feb'95	15°37'N 88°55'W	50	1,975	50	2 years
PT Surya Hutani Jaya-Indonesia	Nov'94	0°7'N 117°3'E	100	1,796	58	3 years

Individual tree volume (over bark) from second and third year measurements were calculated using the following formula:

$$\mathbf{V = 0.00003d^2h} \qquad \textbf{[1]}$$

where ***v*** is individual tree volume in m^3, ***d*** is diameter at breast height in cm, and ***h*** is tree height in m.

In order to minimize effects of heterogeneous variances ("scale effect") the individual tree volume was standardized by dividing the data from each test using the square root of phenotypic variance as suggested by Falconer and Mackay (1996). The phenotypic variance of each test was obtained by adding variance components of family within provenance ($\sigma^2_{F(P)}$), plot (σ^2_{plot}) and error (σ^2_{E}).

Genetic Gain Predictions

Estimates of variance components were obtained through the Mixed Procedure of SAS (1997). Family ($\mathbf{h^2_f}$) and within family heritabilities ($\mathbf{h^2_w}$) for volume were calculated at ages 2 or 3 using standard formula presented by Falconer and Mackay (1996). Since the material was from open-pollinated collection with a possibility of some full sibs and/ or inbreeding, it was assumed that the coefficient of relationship was 0.33 (Squillace, 1974). Calculation for both family and within family heritabilities are given as follows:

$$\text{Family heritability } (\mathbf{h^2_f}) = \frac{\sigma^2_{F(P)}}{\sigma^2_{F(P)} + \sigma^2_{TF(P)}/\mathbf{i} + \sigma^2_{Plot}/\mathbf{ij} + \sigma^2_{E}/\mathbf{ijm}} \quad [2]$$

$$\text{Within-family heritability } (\mathbf{h^2_w}) = \frac{2\sigma^2_{F(P)}}{\sigma^2_{Plot} + \sigma^2_{E}} \quad [3]$$

where $\sigma^2_{F(P)}$ is family within provenance variance, $\sigma^2_{TF(P)}$ is variance of interaction between family within provenance with test, σ^2_{Plot} is plot variance, σ^2_{E} is error variance, and **i**, **j** and **m** are the number of test, block and tree respectively.

Family and within family selections were derived using a modified index developed by the CAMCORE Cooperative (Balocchi 1990). The formula for the index is as follows:

$$\mathbf{I = D_{(prov)} + h^2_f\, D_{(f)} + h^2_w\, D_{(w)}} \quad [4]$$

where I = index value

$D_{(prov)}$ = deviation of the provenance mean from overall mean

h^2_f = family heritability

$D_{(f)}$ = deviation of a family from a provenance mean

h^2_W = within family heritability

$D_{(w)}$ = deviation of an individual value from the family mean within a test

The index had been multiplied by the average square root of the phenotypic variance in order to convert it back to absolute value since the data were analyzed in standardized format. All trees that did not meet threshold values for stem straightness, forks, broken tops, pests and diseases were rejected using an independent culling method. Therefore, calculations were based only on the remaining good phenotypes. The index used volume as the selection criterion and the family and within family heritabilities as the genetic weights.

Individual tree was ranked based on percent gain. The percent gain was calculated using the following formula:

$$\textbf{Percent gain} = (\hat{g}_i / m_x) \times 100 \quad [5]$$

where $\hat{g}_i$ = expected genetic gain for volume from index selection

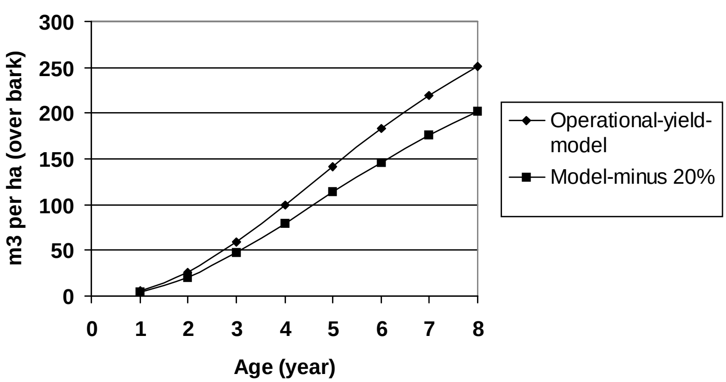
m_x = overall test mean

The percent gain was derived using the index to make projections for growth and yield of improved material. It was assumed that the best 30 trees were selected across test locations to form an elite population. The orchard would be a clonal seed orchard for obtaining short-term gain. In order to maximize genetic gain, the number of selected trees per family was limited to 5 trees without minimum number of trees per provenance. Operational yields were assumed to be 20% less than the research plot yield. This adjustment is necessary because the calculation was based only on a small plot in the tests. This discounted gain is called the production gain. The formula is given below:

Production Gain = 80 % x Percent Gain **[6]**

A yield model for the unimproved plantation was developed for *G. arborea* plantations at PT Surya Hutani Jaya, Indonesia. The model was constructed by Jääkko Poyrii (1990). Since the model was derived from a relatively young plantation, two different levels of yield were estimated as given in Figure 1. The first is the Operational-yield-model that assumed the harvested yield would follow the yield model of unimproved plantation. The second approach uses the Operational-yield-model minus 20% (Model-minus 20%). It suggests that the actual yield will be 20% lower than that predicted by the Operational-yield-model. These two different models were used as the baselines, and the two different levels of projected gains from single and multiple-sites were applied to them to evaluate the marginal benefits of tree improvement cooperative.

Figure 1. The 2 different yield models for the baseline of projected gain in *G.arborea*



Financial Parameters

Planting objectives

The main objective of the base scenario is to establish a *G. arborea* timber plantation in Indonesia. The plantation program is projected to supply high quality pulpwood at 8 years rotation. The program consists of two different activities that are performed simultaneously. The first activity is development of an operational plantation program and the second is application of a tree improvement program.

The operational plantation includes several activities such as growing containerized seedling in a permanent nursery, site preparation by using chainsaw and bulldozer, planting by hand, and maintenance by using either hand clearing or herbicides. It is also assumed that *G. arborea* will be planted at a rate of 3,000 ha annually. Spacing is 3 m x 3 m or approximately 1,100 trees per ha.

Tree improvement begins with the selection of trees and collecting seeds from the best mother trees of *G. arborea* in natural stand in Thailand. This activity is a part of the privilege of membership in the CAMCORE Cooperative. The following year, at year 0, provenance/progeny tests are established by several members all using the same field design. The test is maintained and measured annually. At the same time, an *ex situ* conservation bank is also established. At 3 years of age, an across site analysis is performed on the data and the best trees across test locations are selected and scion material collected and grafted into a clonal seed orchard. The orchard is assumed to start producing seed at 2 years after the establishment. Based on the results in Costa Rica, the seed production is projected to be around 100 kg per ha during the first production at 2 years of age and reach the full production of 300 kg per ha at 4 years of age (Zeaser 1998). Since the number of seeds per kilogram varies between 1,130 to 1,750 seeds and the germination capacity ranges from 75% to 90% (Tewari 1995), the orchard would need to be as large as 10 ha in Indonesia. It also assumed that the orchard has a life cycle of 10 years before new improved material takes its place.

The pulp wood production begins at 8 years from unimproved seed and gradually would be replaced by the trees from an improved orchard at 14 years. The harvesting of trees generated from improved seeds in the first generation seed orchard would continue through age 24 years. The time schedule of activities is given in Table 2.

Table 2. The schedule of activities for *Gmelina arborea* plantations in Indonesia

Company name	Date of establishment	Latitude Longitude	Elevation (m)	Annual precipitation (mm)	Number of family tested	Data observed (age)
Smurfit Cartón de Colombia-Colombia	Nov' 94	3° 34' N 76°30' W	950	994	30	3 years
Ston Forestal-Costa Rica						
- Site 1	Sep'94	8°44'N 83°32'W	40	3,000	60	3 years
- Site 2	Sep'94	8°54'N 83°10'W	20	3,800	57	3 years
Simpson Forestal-Guatemala	Feb'95	15°37'N 88°55'W	50	1,975	50	2 years
PT Surya Hutani Jaya-Indonesia	Nov'94	0°7'N 117°3'E	100	1,796	58	3 years

Cost and Revenue

Plantation costs were estimated from typical operations in Indonesia (S.K. MenHutBun No. 126/Kpts-IV/1999) as given in Table 3. It was adjusted to reflect the average currency exchange in US dollars in 1999 i.e. US$ 1.00 equals to IDR 8,000.00. For the ease of calculation, these costs have been transferred to cost per unit including overhead to support an annual planting of 3,000 ha per year.

Table 3. Present value cost per ha of operational plantations of *G.arborea* in Indonesia.

Description of activity	Unit	Present Value ($US)
1. Plantation establishment		
a. Nursery	ha	36.60
b. Site preparation	ha	167.40
c. Planting		40.60
d. Maintenance		
- 1st year	ha	60.00
- 2nd year	ha	41.80
- 3rd year	ha	32.50
- 4th year	ha	13.90
- 5th year	ha	7.60
2. Overhead	ha	225.20
Total Planting cost	ha	625.60

Tree improvement costs include all activities from establishing provenance/progeny tests to create a production orchard. It was estimated that the cost of tree improvement activities such as provenance/progeny test or orchard establishment was about double normal operational costs. The overhead cost of tree improvement includes an annual CAMCORE membership fee, the direct expenditure to send 2 delegates to annual CAMCORE meetings, and the overhead cost to personnel and land allocated to the test and orchard. Table 4 gives the present cost of the tree improvement program.

Table 4. Present value cost per ha for tree improvement activities in *G. arborea* plantations in Indonesia

Description of activity	Unit	Present Value ($US)
1. Clonal seed orchard		
a. Provenance/ progeny test		
- Establishment	ha	955.60
- Test maintenance	ha	188.80
- Evaluation		2,500.00
b. Tree selection and grafting		
c. Seed production		
- Orchard establishment	ha	955.60
- Seed harvesting and handling	ha	2,000.00
- Orchard maintenance	ha	472.00
2. Overhead and Annual Fee		50,000

Revenues come from the total clearing of the plantation at the end of the 8 years rotation. The standing stock value is US$ 7.00 per m^3 estimated from the current FOB log sale at US$ 20.80 per m^3 minus the harvesting cost and transportation at US$ 13.70 per m^3. The standing stock value is preferred because the main product of the plantation is the standing log and the harvesting is likely to be done by a different department. A real interest rate of 6% was used throughout (Bank Indonesia 1999). No adjustment was made for either price due to value increase or cost due to inflation.

Financial analysis

The present value calculation was divided into calculations of costs and revenues of the plantation. Net present value (NPV) is the excess of the revenue over the cost. In general term, as given in Formula 7, NPV is the sum of revenue in each year, **y**, discounted to year 0 minus the sum of costs in each year discounted to year 0 as shown below.

$$\textbf{Net Present Value (NPV)} = \sum_{Y=0}^{n} \left[\frac{R_y}{(1+i)^y} - \frac{C_y}{(1+i)^y} \right] \quad [7]$$

where ***R*** and ***C*** are revenues and costs in the subscripted years, ***y*** is the years and ***i*** is the interest rate.

Another decision criterion frequently recommended and used in businesses is the calculation of the internal rate of return (IRR). IRR of an investment is defined as the interest rate that results in an NPV equal to zero dollars when all cash flows associated with the investment are discounted to the present (Klemperer 1996). It is derived by solving NPV for ***i***, which would be the interest rate or the average annual rate of return on an investment that paid $\mathbf{V_0}$ and received $\mathbf{V_n}$, ***n*** years later. The formula for IRR is as follows:

$$\textbf{Internal rate of return (IRR)} = \sqrt[n]{\frac{V_n}{V_0}} - 1 \quad [8]$$

Results and Discussion

The best 30 trees selected from the multiple tests for the clonal seed orchard gave an average estimated genetic gain of 39% over the general mean as given in Table 5. This gain is 10% higher than the results from a single-site selection in Indonesia (Table 6). This difference reflects the multiple-site selection had higher intensity. It also indicates that the natural variations of *G. arborea* are responsive to intensive selection. The influence of the number of trees per family allowed to be selected also played an important role in the magnitude of genetic gains. The choice of using a limit of 5 trees per family is a compromise between gain and variability. Namkoong *et al.* (1980) suggested the importance of achieving rapid exploitation of existing genetic variability while at the same time providing for continued genetic gain and maintaining flexibility for possible future changes. This choice of 5 trees per family returned reasonable genetic gain while maintaining a reasonable number of families selected. A reasonable number of families in tree breeding is important to minimize the possibility of inbreeding. There is improved gain when trees in mildly inbred population are out-crossed.

Table 5. The best 30 trees selected from multiple-tests for clonal seed orchard of *G. arborea*.

Test ID	Block	Provenance	Family	Tree	Index	Gain Index	% Genetic gain	Ranking
472901B	6	2	23	5	1.0811	0.0414	50.07%	1
473301E1	1	2	22	1	1.0516	0.0403	48.70%	2
473301E3	2	2	23	1	1.0474	0.0401	48.51%	3
473101C	5	2	27	1	1.0041	0.0385	46.50%	4
470201G	4	2	23	6	0.9597	0.0368	44.44%	5
470201G	4	2	23	3	0.9581	0.0367	44.37%	6
472901B	6	2	23	3	0.9497	0.0364	43.98%	7
473101C	6	2	40	5	0.9244	0.0354	42.81%	8
473301E3	3	2	27	4	0.9237	0.0354	42.78%	9
473101C	2	1	5	6	0.8764	0.0336	40.59%	10
473101C	7	2	27	2	0.8547	0.0327	39.58%	11
473101C	1	2	27	6	0.8369	0.0321	38.76%	12
473101C	8	2	40	6	0.8247	0.0316	38.20%	13
473101C	3	2	27	6	0.8186	0.0314	37.91%	14
470201G	1	2	31	3	0.8105	0.031	37.53%	15
473101C	5	2	28	2	0.8088	0.031	37.46%	16
470201G	2	1	3	3	0.7974	0.0305	36.93%	17
473101C	3	2	40	2	0.7926	0.0304	36.71%	18
472901B	9	2	36	1	0.7909	0.0303	36.63%	19
472901B	2	2	28	6	0.7864	0.0301	36.42%	20
473301E3	6	1	4	2	0.7674	0.0294	35.54%	21
473101C	4	1	17	2	0.761	0.0291	35.24%	22
473101C	7	1	14	1	0.7534	0.0289	34.89%	23
473101C	2	2	28	2	0.7531	0.0288	34.88%	24
473101C	8	1	4	6	0.7442	0.0285	34.46%	25
473101C	9	1	5	5	0.7431	0.0285	34.42%	26
473101C	3	2	28	5	0.7378	0.0283	34.17%	27
472901B	3	2	28	2	0.724	0.0277	33.53%	28
473101C	7	1	10	5	0.7212	0.0276	33.40%	29
472901B	3	1	3	1	0.7071	0.0271	32.75%	30
					Average gain :		39.07%	

Table 6. The best 30 trees selected from single-test in Indonesia for clonal seed orchard of *G. arborea*

Test ID	Block	Provenance	Family	Tree	Index	Gain Index	% Genetic gain	Ranking
472901B	8	2	30	1	0.8488	0.0306	39.80%	1
472901B	6	2	23	5	0.8331	0.0301	39.06%	2
472901B	9	2	30	4	0.7456	0.0269	34.96%	3
472901B	1	2	30	4	0.7428	0.0268	34.83%	4
472901B	8	2	27	1	0.7418	0.0268	34.78%	5
472901B	8	2	27	6	0.7418	0.0268	34.78%	6
472901B	9	2	27	6	0.7212	0.0260	33.82%	7
472901B	7	2	30	4	0.7142	0.0258	33.49%	8
472901B	9	2	36	1	0.7075	0.0255	33.17%	9
472901B	6	2	23	3	0.7016	0.0253	32.90%	10
472901B	3	2	27	3	0.6853	0.0247	32.13%	11
472901B	8	2	27	3	0.6809	0.0246	31.93%	12
472901B	8	2	30	2	0.6728	0.0243	31.55%	13
472901B	2	2	28	6	0.6670	0.0241	31.28%	14
472901B	3	2	23	3	0.6335	0.0229	29.70%	15
472901B	6	2	32	4	0.6283	0.0227	29.46%	16
472901B	7	1	5	6	0.6257	0.0226	29.34%	17
472901B	4	2	32	6	0.6125	0.0221	28.72%	18
472901B	7	2	21	3	0.6118	0.0221	28.69%	19
472901B	3	1	3	1	0.6075	0.0219	28.49%	20
472901B	4	2	23	4	0.6068	0.0219	28.45%	21
472901B	7	1	17	4	0.6060	0.0219	28.42%	22
472901B	3	2	28	2	0.6046	0.0218	28.35%	23
472901B	9	2	23	3	0.6000	0.0217	28.13%	24
472901B	4	2	28	2	0.5971	0.0216	28.00%	25
472901B	7	1	18	5	0.5958	0.0215	27.94%	26
472901B	8	2	28	3	0.5920	0.0214	27.76%	27
472901B	8	3	57	5	0.2298	0.0083	10.78%	28
472901B	9	3	41	3	0.1392	0.0050	6.53%	29
472901B	7	3	57	2	0.1075	0.0039	5.04%	30
					Average genetic gain :		29.07%	

The production gain was used to calculate the yield at the end of the 8 years rotation. Using Formula 6, the production gain for multiple-sites and single-site assumptions were 31% and 23%, respectively. Figures 2 and 3 show the projected yield using the combination of multiple-sites and single-site projected gain models with 2 different baselines of unimproved plantations, i.e. Operational-yield-model and Model-minus 20%. These projected yields reflect the options that one is often faced in an applied tree improvement program. Returns from unimproved and improved plantations may be lower than expected. Various things may happen during the length of a rotation that affect growth such as periodic climatic change, changes in soil fertility, fire damage, and attack from pests and diseases. The two baselines were generated in order to show how sensitive the effect of unimproved and improved genetic materials were to the overall product. This is critical information because the introduction of new improved material will not immediately change the overall plantation productivity. In this scenario, the harvesting of improved material is started at year 14.

Figure 2. The projected yield of Operational-yield-model for *G. arborea* over a rotation of 8 years

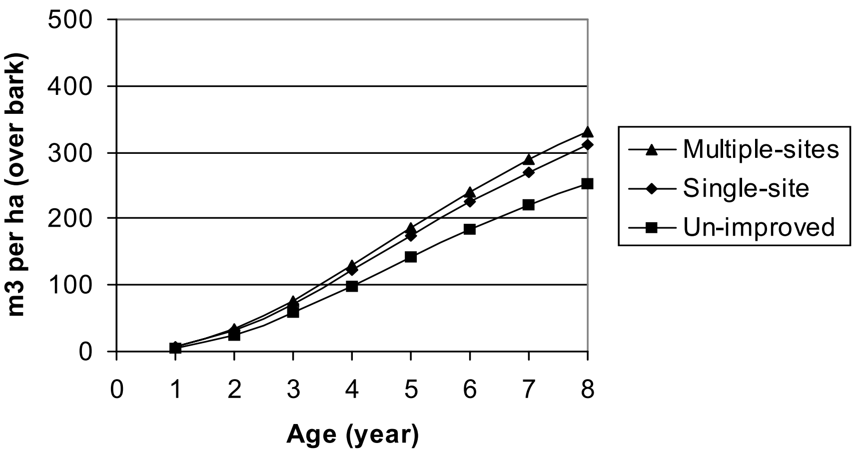
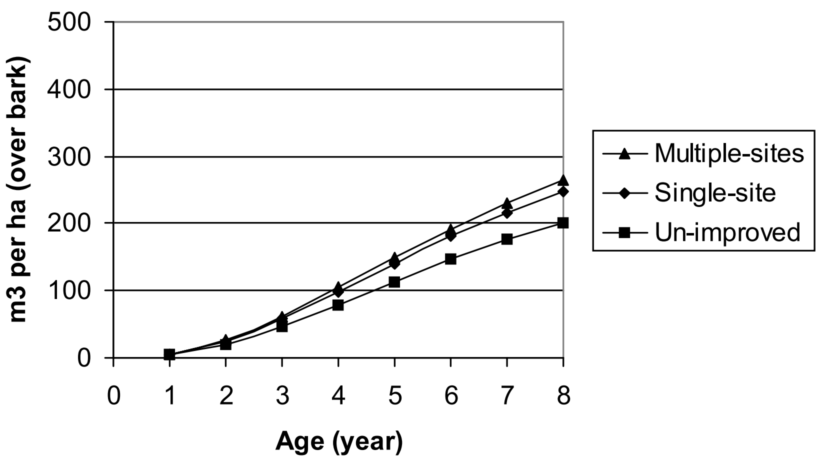


Figure 3. The projected yield of Model-minus 20% for *G. arborea* over a rotation of 8 years



Figures 4 and 5 show the NPV and IRR for various yield models. The results indicated that all combinations of yield model and projected gains had positive value of NPV. The projected gains for multiple-sites gave the best value of NPV. The NPV in the Multiple-sites projected gains were US$ 19,581,309.00 and US$ 11,143,432.00 for operational-yield-model and model-minus 20% respectively. The IRR for all combinations were also above the interest rate. The IRR ranged from 10.8% to 15.2%. In general, the results of multiple-sites were higher than the NPV from the single-site selection at PT Surya Hutani Jaya.

Figure 4. Net Present Value for combinations of yield model and projected gain in *G. arborea*

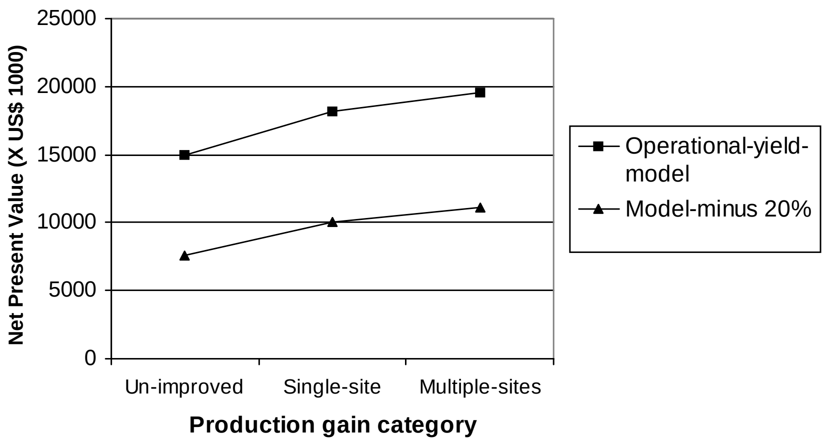
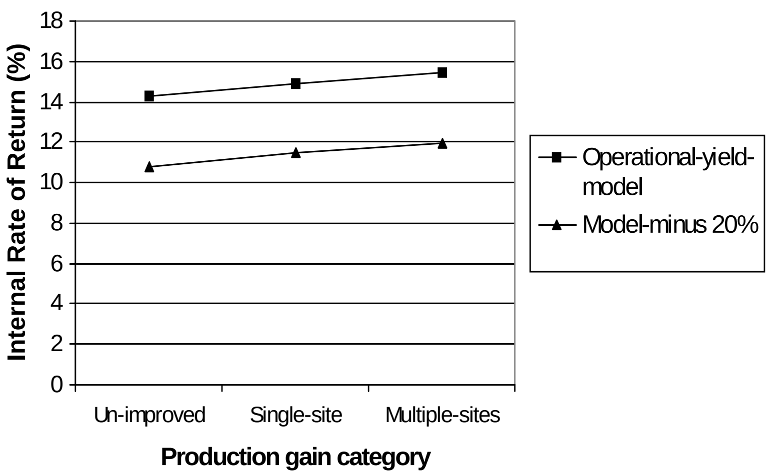


Figure 5. Internal Rate of Return for combinations of yield model and projected gain in *G. arborea*



In order to quantify the effectiveness of tree improvement program, a simulation was conducted by setting the projected gain to zero i.e. improved yield equals to either operational-yield-model or model-minus 20%. This simulation showed that tree improvement program reduced the NPV by only 5% and 10% for operational-yield-model and model-minus 20% respectively. On the other hand, the program was able to increase the NPV ranging from 22% to 47% for combination of single-site selection with operational-yield-model and multiple-sites selection with model-minus 20% respectively. These results shows that tree improvement program is very cost effective.

There are two important considerations that arise from the study. The first, the advantage of multiple-site selection is hardly achieved without joining a tree improvement cooperative. Other than providing the materials, the cooperative will ensure the use of uniform test design that allow to conduct a multiple-sites selection. On the other hand, taken across all sites, more than 13,500 half sib individuals and 5 conservation banks were included in the study. This number of trees sampled and the percentage of progeny that survive would be adequate to capture the genetic diversity in a certain population of *G. arborea*. More studies are needed to verify this effectiveness, but a study by Dvorak *et al.* (1999) in large and small population of *Pinus tecunumanii* that using a similar sampling approach was found to be effective.

The second is the fact that the financial analysis only included the benefit from volume increase. There are two other benefits inherent with improvement, i.e. quality improvement and greater resistance to insects and diseases. In developing the selection index, all inferior trees were independently culled. This would actually increase the wood value by having better straightness, smaller limbs, and better boles. Better resistance to insects and diseases might be expected since only healthy trees were included in the selection. As suggested by Zobel *et al.* (1978), tree improvement may be the only way to ensure wood production in the case of insects and diseases attack. These benefits may significantly increase the financial feasibility of tree improvement that were not quantified in this study.

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Ex Situ Genetic Conservation of *Acacia mangium* for Production Plantation Forests in South Sumatra

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Abstract. PT. Musi Hutan Persada (MHP) is a private forest plantation company with a 300,000 ha forest concession grant in South Sumatra. Through the end of the 1996/97 planting season, MHP has successfully established 193,000 ha of plantations, most of which are *Acacia mangium*. Since early 1999, MHP has supplied acacia wood as the raw material to a pulp mill with a maximum capacity of 2.2 million tonnes of wood per annum. A tree improvement program was established at the beginning of the project and it is aimed at improving growth, stem form, pest and disease resistance, and wood quality of *A. mangium*. A broad genetic base is maintained for this long-term tree-breeding program. MHP has also established its own seed sources which also function as *ex situ* conservation stands for *A. mangium* and consist of 16 provenances (of PNG, Queensland Australia, and Merauke Indonesia) and represent 531 half sib families of those provenances and also about 130 half sib families of the Subanjeriji materials that originally came from the Cairns Region of Queensland. The seed sources are either provenance seed stands or breeding seedling orchards. The breeding seedling orchards were established under a multiple breeding population system to restrict mating only within each provenance subpopulation. Composite seed orchards will be established should there be any sign of inbreeding depression within each subpopulation. Three provenance test stands consisting of 20 provenances will be kept as genetic conservation stands for future use. MHP has also established 8 groups of second-generation seed orchards (breeding seedling orchards) as well as retaining the individual second generation seedlots representing more than 350 half sib families.

Introduction

PT. Musi Hutan Persada (MHP) is a joint venture company between PT. Enim Musi Lestari and PT. Inhutani V. MHP has been granted a 300 000 hectare forest concession area for establishing a forest plantation. The previous condition of the area could be described as Alang-alang (*Imperata cylindrica*) grasslands, sparsely scattered old scrubs, and logged-over forests typical of traditional shifting cultivation with repeated burning and no cropping management. That condition caused prolonged soil erosion and serious depletion of soil nutrients. The natural forests were harvested for lumber production long before MHP came in, and only small remnants are left in the concession area. The remaining natural vegetation is now designated as conservation areas. The landform mostly ranges from flat to undulating and is dominated by red- yellow podzolic soils. These soils are characterised by low pH, low fertility, and high clay content.

Average annual precipitation is 2900 mm with a rainy season from January to April and October to December, and a dry season from May to September. Daily temperature average is 29 –30°C, and average humidity is 70%.

MHP started the plantation project in early 1990 and through the end of the 1996/97 planting season about 193,000 hectares of plantation had been established, mostly with *Acacia mangium*. The plantation will produce wood as raw material for a pulp mill with a maximum capacity of 2.2 million tonnes of wood per year to produce 450,000 tonnes of bleached kraft pulp per year. This is a huge undertaking for MHP and, since 1999 the plantation has been harvested to supply acacia wood to the mill. The mill's consumption is still lower than its maximum capacity at present, but this will steadily increase to maximum capacity.

Acacia mangium has been chosen as our major species for the following reasons:

- Fast growing, and suitable for a short rotation plantation forest
- Easy to handle both in the nursery and the field
- Early and prolific flowering and seed production enabling rapid generation turn-over in breeding programme
- Relatively free from serious pests and diseases
- Soil reclamation ability
- Suitable fibre for the pulp industry

At the beginning of the project, MHP used available seeds from what was then the only seed source of *A. mangium* in South Sumatra. This seed stand was established in the late 1970s as a part of the reforestation project conducted by the Government of Indonesia. The genetic origin of this seed stand was the Cairns Region (Julatten, Mossman, Cassowary, and Daintree) of Queensland, Australia and these seed sources were later proven to be sub optimal compared with other sources in several provenance trials. In addition, the first seed lot used to establish the stand in Subanjeriji, South Sumatra was, genetically, very narrowly based (from only a few parent trees) which is not suitable for a long term breeding programme.

To achieve long term sustainability of the plantation, MHP then initiated a tree improvement programme to broaden the genetic base and establish its own seed sources to produce more improved seeds for its planting programme. The targets for improvement are volume production, stem form, pest and disease resistance, and wood quality.

Ex Situ Genetic Conservation of *Acacia mangium*

To ensure continual genetic improvement, MHP must secure as much genetic material as possible for its future tree improvement programme. The company can neither rely solely upon seed supply from outside parties nor be sure about the future of the natural sources of *A. mangium*. Therefore, having as complete a seed collection of genetic materials as possible is an initial positive step taken by the company. More thorough provenance trials have been set up in the vicinity of the plantation area to find suitable and superior provenances to be included in the breeding programme, as reported by Hardiyanto *et al.* (2000). Based on the information from the provenance trial, we have established our own seed sources that now consist of 16 introduced provenances. Those provenances include; the PNG provenances of Oriomo R., Wipim, Kini, Kapal, Pongaki, Lake Murray, Deri-Deri, Gubam, Wando, Bensbach, Bimadebun, Boite; Queensland, Australia provenances of Claudie River, Pascoe R., and Cassowary; and one provenance from Merauke, Indonesia (Muting area). Those provenances are from South latitude 8° to 13° and from altitudes of less than 100 metres above sea level - which is more-or-less similar to our plantation area - to 3° to 4° South latitude and about 80 metres above sea level.

We have established provenance seed stands (from Wipim, Wando, Deri-Deri, Gubam, and Claudie R.) with a broader genetic base as one of our interim steps to produce improved seeds. The provenance seed stands were planted in block plantings of 3 to 5 hectares with obvious buffer strips for identification in the field and to also function as fire breaks to prevent wild fires from spreading into the seed stands. The seed stands were regularly thinned, by phenotype, about 120-130 trees per hectare. Selection was based upon height, diameter, stem form, and health. At present, we have established around 80 hectares of provenance seed stands, which have produced seed and further contributed to the genetic variation in the plantation. We intend to keep these provenance seed stands beyond their productive age as provenance conservation stands.

For its long term breeding programme, MHP has been establishing seedling seed orchards. These SSOs are breeding seed orchards (BSOs) that is, the progeny test and the seed orchard are one, corresponding to the scheme suggested by Barnes (1995). We established the BSOs in a multiple breeding population system where each provenance is planted as one group (a breeding subpopulation) to confine mating only to within that provenance. As mentioned by Eriksson *et al.* (1993), this multiple population breeding system emphasizes interpopulational diversity within an array of populations both in traits targeted for improvement and in environmental adaptabilities. This system is a dynamic process that can be coincidental or supported by a long term breeding program. Interpopulational variation ensures the presence of diversity to meet changing environments and/or market forces - lost diversity is loss of options for future use (Kjaer *et al.* 1999). These BSOs consist of more than 531 half-sib families from superior provenances, and an additional 130 half-sib families from the Subanjeriji material (originally from Queensland, Cairns Region).

These seed orchards are now of various ages and while some of them have just been thinned phenotypically, some others have undergone final selection using a combined selection index for growth and stem form (forking and straightness). Around 115-130 trees per hectare remain after the final rouging and, to reduce risk of inbreeding, the number of trees per family is restricted to three. So, to meet the target number of trees retained in the seed orchard, we need to include some lower-ranking families. By doing so, we ensure that we do not drastically narrow the genetic base in the production population - some lower-ranking families remain in the breeding population. At present, the MHP has established around 50 hectares of first generation SSOs, and seed collections have been made in some of these seed orchards for operational plantings.

Before proceeding to the final roguing, individual seedlots as well as cuttings from the best member tree of each family are procured for establishing second generation seed orchards and setting up a clone bank. Numbers of families tested in the second generation is the same as those of the first generation. Eight groups of second generation SSOs with a total area of 30 hectares have been in place since 2000. These second generation SSOs consist of more than 350 half-sib families.

Most of the individual seedlots are still kept in the refrigerator for future use. The cuttings from the best trees of each family were planted in our clonal garden and will be hedged to provide vegetative materials for further clonal tests. We are still trying to improve our vegetative propagation techniques and have used rooted cuttings and air layering. We are also planning to establish a small and simple tissue culture laboratory.

Composite seedling seed orchards will be established should there be any sign of inbreeding depression resulting from recurrent selection. Yeatman (1987) observed that recurrent breeding schemes could cause a narrowing of the genetic base. Loss of genetic diversity may cause inbreeding depression, which can result in reduced vigour and susceptibility to pests in future generations (Hansen & Kjaer 1999). Mating of unrelated individuals from several provenances in one population is one way to combat inbreeding depression. This composite seedling seed orchard will be established using seeds from the top-ranked members of each subpopulation. The seeds from each group will be of the same quantity and will be bulked before being sown at the nursery to obtain more-or-less equal representation of each provenance in the seed orchard. Phenological studies need to be conducted to ensure that flowering of each provenance is synchronised. The seed orchard will eventually be thinned by phenotype.

Infusions of fresh genetic material into the breeding population may also be needed to meet potential demands for special breeding programmes such as to improve resistance to pests and diseases or to increase wood density. Larger provenance resource stands that would serve as a genetic pool for future use must therefore be established to handle such demands. These provenance resource stands can be block plantings of each provenance that contain as much genetic variation as possible, and only very light thinnings are required for these stands. Broad sampling or random seed collections within each provenance will also be conducted to provide for the next generation of conservation stands.

Currently, MHP keeps the SPAs as well as the SSOs established from the former Subanjeriji material, and the total area for this material is around 40 hectares. When we are very sure that we have secured the best genetic materials both for seed production and gene conservation, and the total area of improved seed sources is adequate to support our annual planting programme, we will eliminate the Subanjeriji material. The provenance trial stands that contain 20 provenances will also be kept as provenance conservation stands for future studies.

Closing Remarks

MHP realises that prior selection practices that were conducted in the seed sources may have caused some traits to be eliminated from the breeding population. This will lead to a narrower genetic base for the population. Therefore, as mentioned before, as a long term strategy we need to establish more provenance resource stands of *A. mangium* to serve as future sources of fresh genetic material that will be included in the breeding population. Material exchange with research institutions as well as other private companies that have strong tree improvement program must be greatly enhanced to enable each party to continue improving the quality of *A. mangium* in the future.

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Potential for Combining a Tree Improvement Program with *Ex situ* Gene Conservation of *Duabanga moluccana*

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Abstract. *Duabanga moluccana* is an indigenous species in East Kalimantan with suitable properties for various wood products. This species usually grows in open areas following forest disturbance such as road construction and other forest opening activities. Currently, most native *Duabanga moluccana* stands have been cut, and the land converted to other usage.

In 1995 PT. Sumalindo Lestari Jaya Tbk began to collect seeds from 100 trees of *Duabanga moluccana* in East Kalimantan. The seeds were used to establish a progeny test and a trial plantation, and that practice may be amenable to combining both breeding and conservation aspects of *Duabanga moluccana*. The progeny test may serve as part of a breeding program while also being maintained as an *ex situ* gene conservation area.

Introduction

Duabanga moluccana is an indigenous species in East Kalimantan. This species usually grows in open areas after forest disturbance such as road construction and other forest opening activities.

Duabanga moluccana, in the local language of East Kalimantan, is called "Bulung–bulung" or "Benuang Laki", while in Java it is called "Takir". This species is part of the Sonneratiaceae family and it can achieve 25–45 meters of height, and diameters of 70 – 100 cms. *Duabanga moluccana* mainly grows in East Indonesia. At Besuki, East Java the tree grows well at 300 – 900 meters above sea level. This species is not found in west and central Java (K. Heyne 1987).

The production of genetically improved seed and appropriate silvicultural techniques are fundamental to increasing forest productivity. Genetic improvement through selection and breeding may yield increases of 10–25 percent in volume growth in the first generation, and 35–45 percent in a second-generation seed orchard (Nienstaedt 1978).

PT. Sumalindo Lestari Jaya Tbk is aiming at obtaining significant gains in productivity and quality. So it must establish a breeding program that would be realistic and suit its needs. This program is planned to both protect existing genetic resources, and to yield continuous benefits.

Developing a Strategy

The strategy for improvement and conservation of *Duabanga moluccana* is divided into two parts – a research program and an operational program. The research program involves both tree improvement and silvicultural research. Silvicultural studies must be conducted because of the lack of information and literature about *Duabanga moluccana*. Tree improvement research will provide new information on the utilization of this species and its genetic conservation.

The objective of the operational program is to establish trial plantings of this species in the plantation area. This will allow an evaluation of potential yields and comparisons of performance with other species.

Tree Improvement Program

Selected Species for Tree Improvement Program

There are many choices of species for large-scale industrial plantations, and generally, industry chooses the species that best fulfill their needs. However, one general criterion for commercial plantation is that the species chosen must yield a marketable commodity at a competitive price. *Duabanga moluccana* wood has suitable properties for various wood products, especially plywood. As an indigenous species, it also offers some biological advantages.

Mother Tree Selection

The first step in a tree improvement program is to survey and select mother trees from the natural forest. In 1995, 100 mother trees were selected in Berau, East Kalimantan. Mother trees were selected based on height, diameter, straightness, and health. Thirty of the best mother trees were selected using a query system and tested in a progeny test.

Seed Exploration

The exploration took place in East Kalimantan, especially in the forest concession Sumalindo following the steps below:

- Identify the limits of the regional population
- Keep a distance of about 500 meters between individual mother trees
- Collect seeds from each mother tree
- Keep the seed individually

Progeny Trial And Conservation Bank

Thirty of the best mother trees were selected from 100 mother trees after ranking. The seeds collected from the natural stands during the exploration were grown and planted directly. In 2000 we established one progeny test and conservation area for *Duabanga moluccana*. This project consisted of 30 families with RCBD design, with single tree plots in 30 blocks.

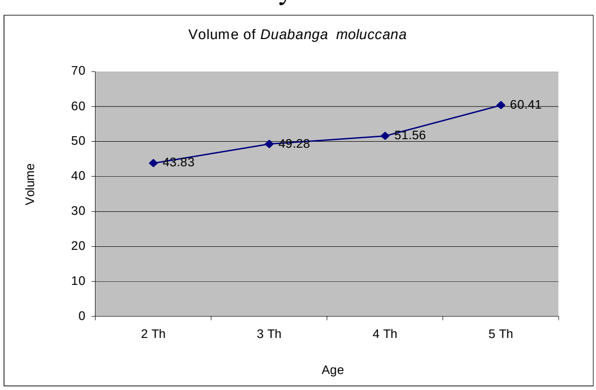
At 3 years, we will evaluate the progeny test and select the best trees from the best families and convert to a hedge orchard. A genetic conservation bank consisting of all families in the progeny test will be established with 10 blocks.

The research program, especially in tree improvement, must be expanded and also focused. From 2001 onward we will conduct new surveys and select additional mother trees from natural forests. The objective is to increase the genetic base for the next breeding cycle. We will rogue the initial hedge orchard after evaluating the progeny test and select the best trees from the best families. We will also establish clonal tests of the best trees of the progeny test and add the best mother trees newly-selected from the natural forest.

Operational Program

In the short-term operational program, we are using unselected material and silviculture methods appropriate to other fast-growing species. We planted with vegetative propagules because obtaining seedlings presented problems such as viability and irregularity of fruiting season. Now we have hedge orchard that we used to produce seedlings. Through December 2000 we have planted about 145 Ha.

Figure 1. Trend of Volume of *Duabanga moluccana* at Plantation PT. Sumalindo Lestari Jaya Tbk.



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Molecular Approaches to Conserving Tropical Forests for Sustainable Forestry

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Abstract. Tropical rain forests are becoming fragmented into small patches and simplified in structure and composition due to their exploitation for timber. Consequently, genetic variation may be decreased and the genetic differentiation between the patches may rise. Developing sustainable forestry regimes that retain genetic diversity is one of the major goals in tropical forestry today. Tropical tree species have predominantly allogamous mating systems, mediated by many kinds of pollinators, so the gene flow through pollen and seeds is one of the most important factors influencing genetic diversity in tropical forests. Recently, molecular markers have been used to determine the genetic diversity and differentiation of forest tree populations. In particular, microsatellite DNA markers, which are highly polymorphic, have been used to evaluate the gene flow through pollen and seeds within forests. We have also developed microsatellite DNA markers in dipterocarp species, and used these markers to analyze mating systems, genetic structures and gene flow in several dipterocarp species. I discuss, here, characteristics of the pollination and breeding systems in dipterocarps that have been revealed by microsatellite analysis, and the factors that influence them. Finally, the potential value and limitations of using molecular approaches to develop genetic criteria for gene conservation in sustainable forestry are discussed.

Introduction

Knowledge of gene flow and mating systems is essential for understanding the reproductive processes of outcrossing plants, as well as for developing methods to conserve plant populations. Theoretical studies suggest that restricted gene flow probably reduces effective population size, and causes inbreeding depression (Slatkin 1985). In general, restricted gene flow, due for instance to selfing or mating with relatives, causes serious inbreeding depression in outcrossing species (Wang *et al.* 1999). Thus, restricted gene flow presents a threat to the viability of populations of outcrossing plants.

The Asian tropical rain forests contain a great variety of dipterocarp species, which are predominantly allogamous species pollinated by insects (Bawa 1998). These commercially important timber species are some of the prime targets of selective logging in Southeast Asia. The selective logging has resulted in intense fragmentation of the forests, and low-density populations in the remaining forests. The restricted gene flow caused by selective logging may also reduce the population viability of tropical trees. Outcrossing rates in some tropical tree species were shown to be positively correlated with flowering tree density in self-compatible species (Murawski & Hamrick 1991). Thus, to conserve viable populations in tropical tree species, it is important to obtain good estimates of outcrossing rates and pollen flow. To maintain the sustainability of selectively logged forests, we have to know how much genetic variation is retained in the forest after logging.

Several types of DNA markers have been developed in recent years, including microsatellite DNA markers, which have been frequently used to study genetic variation and natural mating systems of plants and animals. This is because they are very sensitive to genetic polymorphism, and can be used to determine parental individuals with a high probability of correct assignation (Chase *et al.* 1993, Dow & Ashley 1996). I report here on recent progress in dipterocarp study using molecular markers such as microsatellite DNA markers and discuss the kind of information needed to promote sustainable forestry in Southeast Asia.

Genome and DNA Markers

Many types of DNA markers of different genomes have been developed and used to assess genetic diversity at different levels, both between and within species and populations. At the species level, DNA sequences of chloroplast genes such as *rbcL*, *matK* and spacer regions are used to analyze the phylogenetic positions of each species (Soltis & Soltis 1998). Organellar DNA, such as chloroplast and mitochondrial DNA, has been used to examine genetic differentiation between populations because organellar DNA is generally inherited via the maternal line in angiosperms. Thus, organellar DNA markers will detect more genetic differentiation between populations than nuclear DNA markers, since seeds are the sole means of dispersing organellar DNA. Organellar genomes may therefore be suitable for studying the effects of forest fragmentation (Hamilton 1999) and assessing methods of conserving genetic resources. Historically, allozymes encoded by the nuclear genome have often been used to evaluate how genetic diversity is divided between and within species and populations, and a large amount of important information regarding plant evolution has been obtained from them. Recently, however, highly polymorphic DNA markers such as microsatellites have been used for studies on gene flow, genetic structure and mating systems (Chase *et al.* 1996, Dow & Ashley 1996). Microsatellite markers are especially useful for determining the pollen donors of plants and thus analyzing the effective pollen flow and seed dispersal within populations. Microsatellite DNA markers of tropical species have been reported in several species, including *Pithecellobium elegans* (Chase *et al.* 1996), *Carapa guianensis* (Dayanandan *et al.* 1999), *Shorea curtisii* (Ujino *et al.* 1998), and *Neobalanocarpus heimii* (Iwata *et al.* 2000). Markers developed in one species can also generally be applied to other, closely related species (Ujino *et al.* 1998, Dayanandan *et al.* 1997).

Molecular Phylogeny of Dipterocarpaceae

The Dipterocarpaceae family of trees is distributed in Africa, South America and Asia. In Africa and South America the species are not numerous and are mostly shrubs. However, in Asia the family is remarkably diverse, comprising more than 400 species (Ashton 1982), most of which are emergent trees that dominate tropical rainforests. The taxonomy of dipterocarps has been studied quite intensively because of their great value in the timber market (Symington 1943, Ashton 1982). However, phylogenetic relationships among the taxa of Dipterocarpaceae have not been so closely studied.

Progress in molecular systematics in the last two decades has tremendously increased our knowledge of phylogenetic relationships among land plants (Chase *et al.* 1993, Soltis *et al.* 1997). Because of the simplicity of acquiring large amounts of molecular data, they are more reliable than morphological data for constructing phylogenetic trees. Thus, phylogenetic relationships of the Dipterocarpaceae have recently been studied at various taxonomic levels, as they have in many other families of land plants. At the sub-family level, Dayanandan *et al.* (1999) found that the members of the Dipterocarpaceae, including *Monotes* and *Pakaraimaea*, form a monophyletic group closely related to the family Sarcolaenaceae and are allied to the Malvales. The phylogenetic relationships between genera within the family have also been probed, using PCR-RFLP analysis of chloroplast genes (Tsumura *et al.* 1996) and sequence analysis of chloroplast DNA (Kajita *et al.* 1998). According to the cited studies, all of the Dipterocarpaceae, except Dipterocarpus can be separated into two distinct groups, one with seven pairs of chromosomes, and the other with 11 pairs. Kamiya *et al.* (1998) also reported phylogenetic relationships within the genus Shorea; the Selangan Batu, Yellow Meranti, and White Meranti groups appear to be monophyletic, while the Red Meranti group, which has the greatest nucleotide diversity in the genus, is divided into three clades.

Understanding phylogenetic relationships between genera and species is not only fundamentally important for evolution and conservation studies but molecular markers can also have important practical uses, for instance in breeding strategies.

Mating System and Genetic Variation

Using isozyme data, Murawski and Hamrick (1994) demonstrated that the outcrossing rate of *Stemonoporus oblongifolious*, another species of the Dipterocarpaceae, was 84 %, while Kitamura *et al.* (1994) estimated the outcrossing rate of the dipterocarp *Dryobalanops aromatica* to range from 79.4 to 85.6 % in primary and secondary forests. The average outcrossing rate of *Shorea curtisii* in undisturbed forest was found to be 96.3 % using microsatellite markers (Obayashi *et al.* unpublished data), which is within the range of estimates for tropical tree species reviewed by Loveless (1992) and those reported by other authors. However, in a selectively logged plot, the average outcrossing rate was found to be only 52.2 % (Table 1, Obayashi *et al.* unpublished data): about half the average value in the undisturbed plot and the values reported in previous studies. The high inbreeding rate at the selectively logged plot suggested that this species might not have a self-incompatibility mechanism, although some dipterocarp species do have such systems (Soepadmo 1989).

Table 1. Outcrossing rate and the number of alleles received from paternal trees by outcrossing in each mother tree of *S. curtisii* in undisturbed forest and a selectively logged plot (Obayashi *et al.* unpublished data)

Forest type	Investigated No.	No. of seeds	No. of detected alleles				Out crossing
	of mother tree	Analyzed	Shc09	Shc07	Shc11	Mean	rate (%)
Undisturbed plot	10	35. 1	4.5	5.9	2.5	4.3	96.3
Logged plot	5	37. 0	3.8	4.6	1.8	3.4	52.2

Outcrossing rates may be strongly influenced by flowering-tree density (Murawski & Hamrick 1992) and the types and behavior of pollinators governing the pollen movement (Ghazoul *et al.* 1998). Both of these factors may be partly responsible for the low outcrossing rate of *S. curtisii* observed at the selectively logged site, since tree density at the site was much lower than at the undisturbed site, and the principal pollinators for the genus Shorea are thought to be thrips and/or small beetles with low mobility (Appanah & Chan 1981, Ashton *et al.* 1988, Momose *et al* 1998). The outcrossing rate may not be influenced by tree density if the species has a wide-ranging pollinator such as Apis and/or Trigona (Konuma *et al.* 2000, Kitamura *et al.* 1994).

The outcrossing rate varied greatly among individual trees within both plots, especially in the selectively logged plot of *S. curtisii* (Obayashi *et al.* unpublished data). The outcrossing rate of individuals should depend on the neighborhood density of conspecific flowering trees and be lower in isolated individuals than in clusters of trees (Murawski *et al.* 1990). Nagamitsu *et al.* (2000) also suggested that *Shorea leprosula*, a tree species with a low outcrossing rate and a similar pollinator, might be isolated from reproductive neighbors. The degree of flowering synchrony with surrounding conspecific trees could also cause variations in outcrossing rate among these trees, since individual Shorea trees have relatively short flowering periods of 16 to 26 days (Ashton *et al.* 1988).

The number of alleles per locus (Na) and heterozygosity (He) of the adult *S. curtisii* population in the selectively logged plot, in which 30 % of the wood volume was removed and the density of mature trees was decreased by approximately three quarters about ten years ago, showed similar values to those from the undisturbed plot (Obayashi *et al.* unpublished data). This suggests that the disturbance caused by such selective logging events exerts little influence on the population genetic parameters Na and He, as evaluated by highly variable DNA markers like microsatellites. Expected heterozygosity, He, is less sensitive to population bottlenecks than the proportion of polymorphic loci or the number of alleles per locus (Barrett & Kohn 1991). In contrast, the mean number of alleles per locus per maternal tree derived from paternal trees by outcrossing was significantly lower in the selectively logged *S. curtisii* plot compared to the undisturbed plot (Table 1, Obayashi *et al.* unpublished data). Disturbances such as selective logging are thought to cause a strong bottleneck, and thus reduce the population size in the following generation. This suggests that some degree of genetic variation might be lost in the next generation of selectively logged forest trees that germinate from newly produced seeds, because the total number of alleles per unit area of the future generation will be decreased if new alleles are not supplied through pollen flow and/or seed dispersal from outside of the plot. Consistent with this hypothesis, Dayanandan *et al.* (1999) reported that the allelic richness was lower in the sapling cohort of an isolated fragment of *Carapa guianensis* forest than in a more extensive stand. In allogamous species such as dipterocarps, the low outcrossing rate may influence the genetic variation of the future forest. Thus, knowledge of pollinator behavior and flowering tree density for each species must be obtained. A more suitable parameter than heterozygosity to evaluate differences in the genetic diversity between forests would be also needed.

Gene Flow

Konuma *et al* (2000) observed long distance pollen flow among flowering *Neobalanocarpus heimii* at a 36 ha study plot using microsatellite DNA markers. Pollen from outside the study plot sired 35% of the offspring in their study, and the estimated average mating distance was 524 m. *N. heimii* is mainly pollinated by stingless bees or honey bees (Appanah 1985) and the honey bees are known to be long distance pollinators (Dayanandan *et al.* 1990). Thus, this long distance pollen flow most probably depends on the performance of the pollinator. Pollinator behavior is probably affected by inter-flowering-tree distance (Bawa 1998), thus, flowering tree density can also influence the mating distance of tropical trees. For example, Ghazoul *et al.* (1998) reported that a pollinator (bee) of *Shorea siamensis* flew much further at a site where there were long distances between flowering host trees than it did at sites where the distances between flowering host trees were shorter. However, there must always be limits on the inter-flowering-tree movements of pollinators.

Seed migrations of *N. heimii* have also been inferred, from the observation of offspring with haplotypes that were incompatible with those of neighboring reproductive trees (Konuma *et al.* 2000). Clearly, some dispersal vector, such as small mammals, had carried fruits or seeds of *N. heimii.* In tropical forests, squirrels disperse fruits of some tree species (Becker *et al.* 1985). Nagamitsu *et al.* (2001) reported similar phenomena in *S. leprosula* using microsatellite analysis. Thus, microsatellite analysis is a very sensitive method for observing gene flow within forests, and it can be used to determine parents of seeds, seedlings and saplings at the study site. Such analyses will give us understanding of the range and scope of gene flow, including pollen flow and seed dispersal parameters, for important forest tree species, and thus provide essential information to help design sustainable forestry regimes.

Inbreeding Depression

If seeds are produced by consanguineous matings in allogamous plants, inbreeding depression may be manifested at many subsequent developmental stages, such as fruit maturation (Nagamitsu *et al.* 2000), germination (Konuma *et al.* unpublished data), seedling growth (Konuma *et al.* 1999), or sapling development. Dipterocarps are also predominantly outcrossing species, so inbreeding depression may be expected in selfed seeds. Konuma *et al.* (2000) found selfed offspring among seedlings but not saplings of *Neobalanocarpus heimii*, and suggested that inbreeding depression may occur in surviving seedlings. Self-incompatibility is a system that effectively avoids inbreeding, and some dipterocarpous species are known to have this system (Soepadmo 1989). However, in low-density populations caused by selective logging, the probability of mating via foreign pollen might decline if the main pollinator does not fly far. Thus, we have to maintain a suitable flowering tree density for genetically sustainable forestry, according to the ecological characters of the trees concerned.

Genetic Criteria For Sustainable Forestry

To conserve genetic diversity, both within- and between-population diversity should be considered. For between-population diversity, the forest fragmentation caused by timber exploitation presents critical challenges for genetic conservation (Nason & Hamrick 1996). Microsatellite markers can be used to test the effects of fragmentation on genetic diversity, but organellar DNA markers such as chloroplast DNA might be much more suitable for this purpose (Hamilton 1999). To gauge within-population diversity, microsatellite analysis can be used to obtain detailed information on mating system, genetic structure and, especially, gene flow through pollen and seed. To understand fully the genetic features affecting the within-population diversity of species, their ecological characteristics must be thoroughly known, including characters such as flowering phenology and synchrony, pollinator behavior, self-incompatibility systems, and apomixis (Kaur *et al.* 1978). Finally, we have to combine these data in appropriate models to construct valid genetic criteria if we are to maintain genetic diversity for conservation and sustainable forestry.

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Genetic Structure of Natural Populations of *Dryobalanops aromatica* Gaertn. f. (Dipterocarpaceae) in Peninsular Malaysia Using Microsatellite DNA Markers

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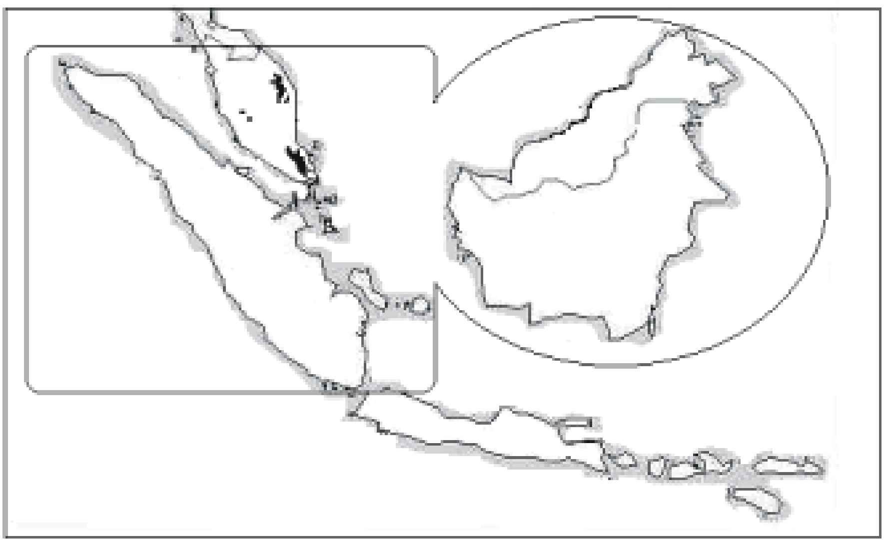
Abstract. Nine microsatellite DNA markers developed for *Shorea curtisii* (i.e. Shc01, Shc02, Shc03, Shc04, Shc07, Shc08, Shc09, Shc11 and Shc17), and one developed for *Dryobalanops lanceolata* [DL(GT)202] were tested for cross-species specificity on *D. aromatica*. The primer developed for *D. lanceolata* showed no amplification in *D. aromatica*. Although all the primers designed for *S. curtisii* gave amplifications, primer Shc01 and Shc08 yielded many non-specific bands. The other seven codominant microsatellite DNA loci were used to assess the genetic variation of *D. aromatica* from five populations in Peninsular Malaysia, namely Lenggor and Ulu Sedili (Johore), Lesong (Pahang), Kanching (Selangor) and Bukit Sai (Terengganu). The expected mean genetic diversity (H_e) was high (0.709) with values ranging from 0.684 (Lenggor) to 0.735 (Lesong). Most of the populations showed high and positive fixation indices, indicating an excess of homozygotes. This implies a high level of inbreeding that may be caused by selfing or/and mating between closely related individuals. A gene diversity analysis showed that 93.3% of the observed genetic diversity was contained within populations and 6.7% (G_{ST} = 0.067) was due to difference between populations. The smallest genetic distance (D) was between Bukit Sai and Lesong (0.068), and the largest between Ulu Sedili and Lesong (0.477). Mean gene flow (Nm) was high (2.94), with the highest value being between Lenggor and Bukit Sai (-6.98). The lowest gene flow occurred between Kanching and Ulu Sedili (1.95). The findings from this study imply that the four natural populations of *D. aromatica* on the east coast of Peninsular Malaysia may have been a single unfragmented population in the recent past. The results of this study also support an earlier alternative hypothesis that the Kanching population on the west coast of Peninsular Malaysia originated from the east coast populations. This is supported by the dendrogram derived from the UPGMA cluster analysis of genetic distances, in that the Kanching population is closely related to the Lesong and Bukit Sai populations from the east coast.

Introduction

Dryobalanops aromatica Gaertn. f., locally known as kapur, occurs naturally in Sumatera, Peninsular Malaysia, the Riau Archipelago, and Borneo (Symington 1943) (Figure 1). In Peninsular Malaysia, this tree is found naturally only on the east coast, south of latitude 5° N, except for small pockets in Rawang, Selangor (Wyatt-Smith 1963). In Terengganu, Pahang and Johore the tree is gregarious, representing nearly 90% of the total timber volume (Foxworthy 1927). The population history of *D. aromatica* on the west coast of Peninsular Malaysia is unclear. Burkill (1935) suggested that traders of crystalline camphor introduced the tree from the east coast. This substance is obtained from the kapur tree and was traded from the seventh century between Europe, the Malacca port and other ports on the west coast.

Figure 1. Distribution of *D. aromatica.* General distribution area in circle. Distribution in Peninsular Malaysia in black.



Source: Ashton (1982) and Symington (1943)

Kapur is an important timber that is of economic value. The medium hard wood of kapur is suitable for heavy construction, to make poles, rafts, flooring, furniture, window panels, doors, stairs and railway sleepers (Ser 1981). Kapur also produces crystallized camphor and oleoresin used in traditional medicine.

Kapur is one of several local species identified for planting of quality timber in Peninsular Malaysia (Appanah & Weinland 19?). However, before effective selection and breeding can be carried out, information on genetic parameters like mating system, genetic variation and genetic structure of the species is needed. Only a few studies have been made on the genetic aspects of kapur. Shiraishi *et al.* (1994) used isozyme markers to study the population genetics of this species in Brunei. Lee (2000) studied the mating system of this species in three different forest types and a seed production area using isozyme markers. Recently, genetic diversity of this gregarious species in Peninsular Malaysia was studied by Lee *et al.* (2000b) using isozyme markers. The objective of this study is to further estimate genetic diversity and genetic differentiation of *D. aromatica* in Peninsular Malaysia using highly polymorphic microsatellite DNA markers.

Materials And Methods

Leaf samples from 50 to 70 seedlings were collected from five natural populations of *D. aromatica* in Peninsular Malaysia i.e. Kanching (Selangor), Ulu Sedili and Lenggor (Johore), Bukit Sai (Terengganu) and Lesong (Pahang) (Figure 1). DNA was isolated with a modified method of hexadecyltrimethylammonium bromide (CTAB) (Murray and Thompson 1980). Extracted DNA templates were further purified with a High Pure PCR Template Preparation Kit® (Boehringer Mannheim).

Nine microsatellite DNA markers developed for *Shorea curtisii,* i.e. Shc01, Shc02, Shc03, Shc04, Shc07, Shc08, Shc09, Shc11 and Shc17 (Ujino *et al.* 1998), and one developed for *Dryobalanops lanceolata,* DL(GT)202 (Terauchi 1994), were tested on *D. aromatica.*

Polymerase chain reaction (PCR) was performed using GeneAmp® PCR System 9700 (Perkin Elmer) with 25 ml reaction volumes containing 10 ng genomic DNA. Annealing temperature was 50-56, as appropriate for each primer pair (Table 1). PCR reaction mixtures contained 1X PCR buffer, 1.8mM $MgCl_2$, 0.2mM of each dNTP, 5pmol of each primer, and 0.8 units of Platinum *Taq* DNA polymerase (Gibco BRL®). A PCR amplification was carried out for 3 min at 95°C, followed by 35 cycles of 45 s at 94°C, 30 s at 50-56°C and 45 s at 72°C, with a final 5 min incubation at 72°C.

Amplified fragments of DNA were separated using 3.5% high resolution agarose Metaphor® (FMC Corporation) in TBE buffer for about 3½ hours at 80 V. 50 bp and 100 bp (Gibco BRL®) markers were used. 10^{-5}X Gelstar Gelstain® (FMC Corporation) was used to stain the gel before it was checked under ultraviolet light with Gelstar Gelstain® (FMS) filter. Gels were documented using 667 Polaroid film.

Analysis of microsatellite DNA diversity in *D. aromatica*

Samples of 30 leaves from each population were collected randomly. PCR was performed using the seven primer pairs (Shc02, Shc03, Shc04, Shc07, Shc09, Shc11 and Shc17) that showed specific amplification in *D. aromatica*. Allele sizes were estimated for each locus and scored. The program BIOSYS-1 (Swofford and Selander 1989) was used to test for deviation from Hardy Weinberg equilibrium, and to calculate allele frequencies, mean sample size per locus, mean number of alleles per locus, observed and expected heterozygosities, fixation index, genetic differentiation (G_{ST}) and genetic distance (Nei 1978). Effective number of alleles per locus (A_e) was calculated according to Crow and Kimura (1970). A statistic derived for microsatellite DNA loci, R_{ST} (Goodman 1997), was used to estimate levels of genetic differentiation and gene flow between populations. As R_{ST} is based on a stepwise mutation model, those loci that do not follow this model, namely Shc07 and Shc11, were excluded from this estimation. A jackknife procedure (without Kanching) was made to determine the effect the west coast population had on the overall diversity.

Results

Allele frequency

Allele frequencies for the seven loci are presented in Table 1. The most frequent allele at each locus differed from population to population. Some alleles were present only in certain populations.

Table 1. Microsatellite loci allele frequency in the five populations of *D. aromatic*

Loci	Allele	Population				
		Kanching	Lenggor	Lesong	Bukit Sai	Ulu Sedili
Shc02	1	0.023	0.056	0.079	0.132	0.114
	2	0.000	0.028	0.000	0.026	0.023
	3	0.182	0.111	0.211	**0.237**	0.182
	4	0.136	**0.472**	0.132	**0.237**	0.182
	5	**0.227**	0.111	**0.316**	**0.237**	0.068
	6	0.068	0.000	0.079	0.026	0.114
	7	0.182	0.083	0.132	0.053	0.045
	8	0.182	0.139	0.053	0.053	**0.273**
Shc03	1	**0.441**	0.079	0.300	0.316	0.026
	2	0.324	**0.789**	**0.475**	**0.447**	0.368
	3	0.235	0.079	0.050	0.158	**0.526**
	4	0.000	0.053	0.175	0.079	0.079
Shc04	1	0.250	0.206	0.250	0.029	0.250
	2	0.250	0.382	0.250	0.294	**0.688**
	3	**0.500**	**0.412**	**0.500**	**0.676**	0.063
Shc07	1	0.050	**0.289**	0.083	0.059	**0.306**
	2	0.200	0.158	0.083	**0.353**	0.250
	3	0.025	0.237	**0.306**	0.235	0.000
	4	0.225	0.053	0.139	0.147	0.028
	5	0.175	0.184	0.083	0.088	0.194
	6	**0.275**	0.053	0.167	0.059	0.111
	7	0.025	0.000	0.000	0.059	0.083
	8	0.025	0.026	0.139	0.000	0.028
Shc09	1	0.000	0.029	0.000	0.133	0.000
	2	0.065	0.265	0.156	0.133	0.024
	3	**0.348**	0.059	**0.250**	0.067	0.071
	4	0.152	**0.324**	0.156	**0.333**	**0.357**
	5	0.043	0.118	0.125	0.000	0.143
	6	0.130	0.029	0.156	0.133	0.214
	7	0.261	0.176	0.156	0.200	0.190
Shc11	1	0.048	0.294	0.375	0.412	0.250
	2	0.214	**0.382**	0.125	0.059	**0.406**
	3	0.286	0.176	0.094	0.088	0.313
	4	**0.452**	0.147	**0.406**	**0.441**	0.031
Shc17	1	0.333	0.033	0100	0.300	0.079
	2	0.133	**0.500**	0.033	0.067	**0.500**
	3	0.000	0.267	**0.567**	0.200	0.237
	4	**0.533**	0.200	0.267	**0.433**	0.079
	5	0.000	0.000	0.000	0.000	0.053
	6	0.000	0.000	0.033	0.000	0.053

Most frequent allele at each locus in bold

On the whole, genotypic frequencies at most loci in the five populations did not adhere to Hardy-Weinberg proportions. Only Shc17 in the Kanching population and Shc02 in the Ulu Sedili population fit Hardy-Weinberg proportions. In the Lenggor population, three loci fit Hardy-Weinberg proportions, i.e. Shc02, Shc03 and Shc04. Lesong had three loci, which segregated according to the Hardy-Weinberg principle, namely Shc02, Shc03 and Shc17. Genotypes at loci Shc04, Shc09 and Shc17 also conformed to Hardy-Weinberg proportions in the Bukit Sai population.

Population structure

The genetic diversity parameters for the five populations of *D. aromatica* are summarized in Table 2. Mean number of alleles per locus ranged from 4.9 to 5.4 with a mean of 5.1 ± 0.2. Effective number of alleles per locus A_e was 3.3 to 3.9 with a mean of 3.6 ± 0.02. The mean observed heterozygosity was 0.491 ± 0.060 with the lowest value found in the Ulu Sedili population (0.407 ± 0.108) and the highest value in the Kanching population (0.555 ± 0.078). Expected heterozygosity, H_e ranged from 0.684 in the Lenggor population and 0.735 in the Lesong population with mean value 0.709 ± 0.020. Mean expected heterozygosity (H_e) was higher than mean observed heterozygosity (H_o) by 0.218 ± 0.154.

Table 2. Genetic diversity parameters in the five *D. aromatica* populations

Population	N	P	A_a	A_e	Mean heterozygosity	
					H_o	H_e
Kanching	19.1 (1.2)	100	4.86 (0.80)	3.69	0.555 (0.078)	0.721 (0.035)
Lenggor	17.4 (0.5)	100	5.14 (0.67)	3.36	0.454 (0.082)	0.684 (0.057)
Lesong	17.1 (0.7)	100	5.14 (0.59)	3.92	0.506 (0.104)	0.735 (0.039)
Bukit Sai	17.0 (0.6)	100	5.14 (0.70)	3.60	0.531 (0.087)	0.707 (0.048)
Ulu Sedili	18.7 (0.9)	100	5.43 (0.69)	3.60	0.407 (0.108)	0.700 (0.049)
Mean	17.9 (1.0)	100 (0)	5.14 (0.20)	3.63 (0.20)	0.491 (0.060)	0.709 (0.020)

Standard deviation given in parentheses

N - Mean number of samples per locus
P - Percent polymorphic loci
A_a - Mean number of alleles per locus
A_e - Effective number of alleles per locus
H_o - Observed heterozygosity
H_e - Expected heterozygosity

Fixation index

The overall fixation index was positive in each population for all loci, except for Shc02 (Table 3). Mean fixation index was high for all populations, ranging between 0.225 and 0.428. Negative values for Shc02 showed an excess of heterozygotes in all populations. Positive values at all other loci in all the other populations showed an excess of homozygous individuals.

Table 3. Fixation index (F) in the five *D. aromatica* populations

Loci	**Population**				
	Kanching	**Lenggor**	**Lesong**	**Bukit Sai**	**Ulu Sedili**
Shc02	-0.101	-0.154	-0.175	-0.043	-0.100
Shc03	0.362	0.126	0.002	0.292	0.547
Shc04	0.400	0.175	0.200	0.224	0.458
Shc07	0.249	0.537	0.254	0.397	0.151
Shc09	0.033	0.393	0.469	0.073	0.501
Shc11	0.428	0.259	0.907	0.717	0.907
Shc17	0.205	0.791	0.440	0.016	0.533
Mean	0.225	0.304	0.300	0.239	0.428

Genetic differentiation

From values of isozyme data, total gene diversity of *D. aromatica* was very high with the highest value at locus Shc07 (0.850) and the lowest value at locus Shc04 (0.637) (Table 4). Mean H_T for *D. aromatica* was 0.759. The degree of genetic differentiation among populations was low (0.067); that is, 93.3% of the observed genetic diversity was among individuals within populations, and 6.7% was among populations.

Table 4. Genetic differentiation measures among the five *D.aromatica* populations

Locus	**H_T**		**H_S**		**D_{ST}**		**G_{ST}**	
	+KC	**-KC**	**+KC**	**-KC**	**+KC**	**-KC**	**+KC**	**-KC**
Shc02	0.836	0.832	0.815	0.807	0.021	0.025	0.025	0.030
Shc03	0.665	0.646	0.597	0.580	0.068	0.066	0.102	0.102
Shc04	0.637	0.633	0.579	0.562	0.058	0.071	0.092	0.112
Shc07	0.850	0.843	0.816	0.816	0.034	0.027	0.039	0.032
Shc09	0.823	0.820	0.802	0.808	0.021	0.012	0.025	0.014
Shc11	0.744	0.736	0.689	0.688	0.055	0.048	0.073	0.065
Shc17	0.754	0.747	0.655	0.667	0.099	0.080	0.132	0.107
Mean	0.759	0.751	0.708	0.704	0.051	0.047	0.067	0.062
Mean (without Shc07, Shc11)	0.786		0.737		0.049		0.062	

+KC – analysis including Kanching population

-KC – analysis excluding Kanching population

Analysis without loci Shc07 and Shc11 did not show much difference, with the degree of genetic differentiation 0.062; that is, 93.8% diversity within populations and 6.2% among populations. Mean H_T and D_{ST} were not very different with an increase of only 2.7% and a decrease of 0.5% each.

Jackknife analysis without Kanching (Table 4) showed that the degree of genetic differentiation in Kanching was not much different from the differentiation in the other populations. Lowest gene diversity was at locus Shc09 (0.014), whereas highest gene diversity was seen at locus Shc04 (0.112).

Comparison of G_{ST} analysis (Table 4) and R_{ST} (Table 5) showed that genetic differentiation from R_{ST} analysis is higher by 2.8%.

Table 5 R_{ST} and Nm values of five D. aromatica populations (without Shc07 and Shc11)

	Kanching	**Lenggor**	**Lesong**	**Bukit Sai**	**Ulu Sedili**	**Mean Nm**
Kanching		9.66	5.71	-1.16	1.95	
Lenggor	0.06		21.00	-6.98	2.74	2.94
Lesong	0.06	0.04		13.48	2.19	**Mean R_{ST}**
Bukit Sai	0.09	0.03	0.04		1.96	
Ulu Sedili	0.15	0.11	0.10	0.15		0.09

Nm values given above diagonal line
R_{ST} values given below diagonal line

Gene flow

Mean R_{ST} for all populations was 0.09 and ranged from 0.03 to 0.15 (Table 5). The smallest mean R_{ST} value was between populations Bukit Sai and Lenggor (0.03). The largest values were between populations Kanching and Ulu Sedili and also Bukit Sai and Ulu Sedili (0.15).

Mean gene flow, Nm was high (2.94). Negative values of gene flow between Bukit Sai and Kanching populations (-1.16) and Lenggor and Ulu Sedili populations (-6.98) showed that gene flow was extremely high. Lowest gene flow was between Ulu Sedili and Kanching (1.95) and Ulu Sedili and Bukit Sai (1.96).

Genetic distance

Genetic distance and geographic distance of the five populations are given in Table 6. Bukit Sai and Lesong populations gave the lowest genetic distance (0.068). Genetic distance between Ulu Sedili and Lesong populations was the highest (0.477). Geographically, Lenggor is nearest to Ulu Sedili (5 km) whereas Kanching is furthest from Ulu Sedili (280 km). Genetic distance between Lenggor and Ulu Sedili was low, that is 0.172, whereas Kanching and Ulu Sedili genetic distance was high at 0.442.

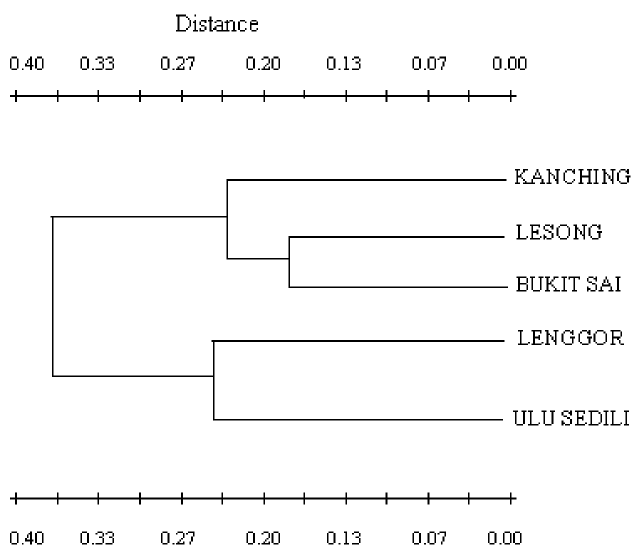
Table 6 Genetic distance and geographic distance among five D. aromatica populations

	Kanching	Lenggor	Lesong	Bukit Sai	Ulu Sedili
Kanching		270	190	210	280
Lenggor	0.352		80	240	5
Lesong	0.183	0.197		180	90
Bukit Sai	0.113	0.208	0.068		250
Ulu Sedili	0.442	0.172	0.477	0.474	

Geographic distance above diagonal (in km)
Genetic distance below diagonal

UPGMA dendrogram showed two main clusters with Kanching, Lesong and Bukit Sai as one cluster and Lenggor and Ulu Sedili as another (Figure 2). Kanching was separated from Lesong and Bukit Sai.

Figure 2 Dendrogram from UPGMA cluster analysis based on Nei's genetic identity (1978) among five D. aromatica populations



Discussion

Application of inter-specific microsatellite primers

Primer DL(GT)202 did not show any amplification, perhaps because there was no annealing site in *D. aromatica*. Primers Shc01 and Shc08 were non-specific for *D. aromatica.* Non-specific amplifications may have been due to the presence of more than one annealing site.

Other primers, i.e., Shc02, Shc03, Shc04, Shc07, Shc09, Shc11 and Shc17, revealed specific amplifications on agarose gel, showing that these primers can be used on *D. aromatica*. Downey and Iezzoni (2000) succeeded in amplifying black cherry (*Prunus serotina*) using primers developed for other species but in the same genus i.e. sweet cherry (*P. avium*), peach (*P. persica*) and sour cherry (*P. cerasus*).

Numbers of alleles detected at microsatellite loci, which had mixed repeats, i.e., Shc02 and Shc07, were high. Loci Shc03 and Shc04, which had simple CT repeats, had low numbers of alleles, i.e., only four and three respectively. However, locus Shc09, which also contained simple CT repeats, revealed a high number of alleles (7). This is because the application of microsatellite primers across species can cause a deviation in the expected number of alleles. Numbers of alleles were higher at loci that were developed specific for a particular primer compared to loci that are not specific (Bruford & Wayne 1993). This is because during sequence selection of the original primer, longer sequences (typically 12 repeats) are selected as primers. When used across-species, alelle variation at these loci may be shorter or contain other repeats in between, hence reducing the mutation rate and diversity without regard to population size. This deviation still needs to be studied. This was observed in amplifications among sea turtles (FitzSimmons *et al*. 1995), but not among wombats (Taylor *et al*. 1994).

Hardy-Weinberg equilibrium

Genotype frequencies of the microsatellite loci that deviated from Hardy-Weinberg expectations may be caused by inbreeding and small population sizes. Seedlings have been reported to have high homozygosity (Muona 1989). Samples that could not be amplified due to oxidation by phenolic compounds caused the sample size to drop. Therefore, estimation of homozygosity might not be accurate. However, sampling was done at random for this study. Thus there was no bias in selection of monomorphic or polymorphic loci. Edwards *et al*.'s (1992) study on populations of different human races in North America also gave genotypic ratios that deviated from Hardy-Weinberg estimation due to a lack of heterozygotes.

Fixation index

High homozygosity was observed from positive fixation indices in seedling samples that may have been produced through selfing or half-sib mating. This had also been shown through a study on *Pinus* sp. (Muona 1989). According to Muona (1989), positive fixation indices at the seedling level might be due to self-pollination. Seedlings produced through inbreeding usually die off before maturity as they are weaker. This follows the theory of natural selection, which states that selection is the dominant force in evolution (Hartl 1980). Excess of heterozygosity in locus Shc02 as shown by a negative value might mean that this locus responds to selection pressure and may be linked to adaptive traits.

The Wahlund Effect (1928) states that natural populations that are divided into sub-populations might have allele frequencies that deviate from populations due to natural selection, or random genetic drift in the case of small populations. The effect is that the homozygous genotype frequency of the whole population exceeds Hardy-Weinberg equilibrium, whereas heterozygotes of the whole population decrease from the Hardy-Weinberg ratio. In the case of *D. aromatica*, it could be said that all the populations in Peninsular Malaysia originated from one large population that had been sub-divided into a few sub-populations. This would explain the high fixation indices observed in all *D. aromatica* populations.

Genetic diversity in D. aromatica populations

On the whole, the five populations showed a high level of genetic diversity. Out of the seven microsatellite loci studied, 40 alleles were observed, which is a mean of 5.14 ± 0.20 alleles per locus. This value is higher than those obtained from isozyme markers for conifers (2.29) and tropical species (2.02) (Hamrick and Loveless 1989). *D. aromatica*, using isozyme markers, gave a value of 4.1 (Lee *et al.* 2000b). Generally, microsatellite markers yield a high number of alleles per locus due to length mutations that causes differences in repeat units. Amos et al. (1993) obtained 54 alleles from one whale microsatellite locus and Fornage *et al.* (1992) obtained 15 alleles in one locus located on the first intron of the C-II apoliprotein gene in a French human population.

Expected heterozygosity was more than the observed value by 0.218, showing an excess of homozygotes. This may be because leaf samples of seedlings were used in this study. Expected heterozygosity (H_e = 0.709) was higher than other tropical timber species ($H_e = 0.125$, Hamrick *et al.* 1992) and also higher compared to temperate tree species, especially conifers ($H_e = 0.145$, Hamrick *et al.* 1992), based on isozyme markers. The H_e value obtained in this study was also higher than that estimated by Lee *et al.* (2000b) for ten natural populations of *D. aromatica* using isozyme markers ($H_e = 0.459$).

According to Hamrick *et al.* (1979), long-lived trees have a higher level of heterozygosity. This is important for the survival of species in a changing environment, especially when natural selection occurs. This was proved by Nikolic and Tucic (1983) on *Pinus nigra*, a long-lived plant. Hiebert and Hamrick (1983) supported this theory with a study on *P. longaeva* that showed a high level of H_e. High levels of genetic diversity can be associated with population history, strategy and life history of the species like outcrossing, long life, distribution and high fecundity.

The highest effective number of alleles per locus (A_e) was found in the Lesong population whereas the lowest was found in the Lenggor population. Studies on these five populations with isozyme markers showed that the Ulu Sedili population had the highest effective number of alleles per locus (2.7), whereas the Kanching population had the lowest (2.4) (Lee et al. 2000b). The Lesong population showed the highest expected heterozygosity (0.735), whereas the Lenggor population revealed the lowest (0.684) from microsatellite markers. This differed from an isozyme study carried out by Lee, et al. (2000b) wherein out of these five *D. aromatica* populations, the Lenggor population showed the highest expected heterozygosity (0.475) and the Kanching population revealed the lowest (0.459). Isozymes are markers that are limited to coding regions of genes. Meanwhile microsatellite markers can be found in the whole eukaryote genome. Therefore, microsatellite markers may give a clearer picture of the genetic diversity of a species.

Allele frequency at each locus differed from population to population. This was also portrayed in the study of human populations of Negroes, Mexicans, whites, and Asians in America (Edwards *et al.* 1992). Fornage *et al* (1992) also reported a complex allele frequency distribution at the first intron of the apoliprotein gene C-II. High mutation rate at microsatellite loci, that is 10^{-4} to 10^{-6} (Dallas 1992, Edwards *et al.* 1992) may cause the presence of a high number of alleles. Therefore, a large sample size may be needed to represent all the alleles present in the population and for the all the alleles to be in Hardy-Weinberg equilibrium. The number of alleles at microsatellite loci can be very high. The long-finned whale exhibited 54 alleles at one locus (Amos *et al.* 1993). One human locus had 20 alleles (Edwards *et al.* 1992).

Genetic differentiation and gene flow

Tropical species have been reported to have low genetic differentiation (Hamrick & Loveless 1989). G_{ST} values of locally distributed tropical species, flowers of which are pollinated by animals and seeds dispersed by gravity, have values of 0.119, 0.092 and 0.131 each (Hamrick *et al.* 1992). Mean G_{ST} of conifers was 0.068 (Hamrick 1989). Tropical pine species showed low genetic differentiation among populations (Hamrick 1989). Lee *et al.* (2000a) reported a G_{ST} of 0.117 for *Shorea leprosula*, a dipterocarp in Peninsular Malaysia. G_{ST} for *D. aromatica* based on isozyme analysis also revealed low values (0.042, Lee *et al.* 2000b). From this study, genetic differentiation was higher (G_{ST} = 0.062, R_{ST} = 0.09) than that obtained using isozyme markers for *D. aromatica* in Peninsular Malaysia. This is because microsatellite loci are more polymorphic than isozyme loci. Gaggiotti *et al.* (1999) showed than in small sample sizes (n £10) and few loci (n £20), R_{ST} gave higher estimates. In this study R_{ST} estimates exceeded G_{ST} by 0.028.

High gene flow levels cause low values of genetic differentiation among populations (Mitton 1992). Lee *et al.* (2000b) obtained high gene flow among ten *D. aromatica* populations in Peninsular Malaysia with gene flow, Nm = 5.70 and genetic differentiation, G_{ST} = 0.042.

Negative Nm values showed that allele size variation within populations was higher than variation among populations. Therefore, most genetic variation was within populations and variation among populations was less. Negative Nm values are considered to be too high to be estimated using this approach (Goodman 1997), which can clearly be seen from microsatellite data of *D. aromatica* obtained from this study which has a low mean genetic differentiation (0.09) and a high mean gene flow (2.94).

Genetic differentiation analysis, R_{ST} that was designed especially for microsatellite data showed correlation between the level of genetic differentiation, R_{ST} and gene flow, Nm. Bukit Sai and Lenggor populations, which had the lowest genetic differentiation, showed the highest level of gene flow (-6.98). Whereas the Ulu Sedili and Kanching populations and the Ulu Sedili and Bukit Sai populations, which had the highest genetic differentiation, had the lowest level of gene flow.

Geographically, Kanching is the furthest from Ulu Sedili. Gene flow decreases with distance. This was clear from gene flow and genetic differentiation values that showed low gene flow and high genetic differentiation between these two populations.

G_{ST} analysis without the Kanching population showed that Kanching was not much different from the other populations in the east coast of Peninsular

Malaysia. This supports Burkill's hypotheses (1935) that the Kanching population was introduced from other populations in the east coast. The low genetic differentiation value supports the hypothesis that all the populations of *D. aromatica* in Peninsular Malaysia might have been one large population that had been divided into sub-populations due to fragmentation.

Genetic distance

Results from this study did not show any significant correlation between genetic distance (D) and geographic distance. Even though Ulu Sedili was nearest to Lenggor in terms of geographic distance, genetic distance between these two places was not the lowest. The same applied for the furthest genetic distance, that between Lesong and Ulu Sedili, which was not reflected geographically, as the furthest geographic distance is between Ulu Sedili and Kanching.

This may be because *D. aromatica* populations in Peninsular Malaysia are sub-populations as a consequence of fragmentation and limited migration of one original large population. Because of high gene flow among sub-populations on the east coast, genetic distance did not correlate positively with geographic distance. The lack of difference in genetic distance between Kanching and the other populations may be explained by Burkill's (1935) suggestion that the Kanching population was introduced from the east coast of Peninsular Malaysia.

In situ conservation of *D. aromatica* may require a few populations, as higher diversity within populations compared to among populations was detected. For *ex situ* conservation, it is suggested that focus be given to variation among individuals within populations so that all the variation is sampled. Population selection can be done based on the highest diversity in terms of alelle or genotype.

High diversity within populations also gives a wider base for improvement programs. The basis for selection in this process is similar to *ex situ* conservation but is more focused upon the highest level of heterozygosity. To carry out a selection program effectively, selection of more individuals within population needs to be done so that high genetic diversity can be maintained.

The samples for this study were from leaves of seedlings. It is suggested that further studies be carried out with samples from different age classes to give a better picture of the genetic differentiation between populations. As there is no study that relates microsatellite genotypes with morphological and physiological characteristics thus far, results from this study cannot be used as the sole criterion for sampling with the aim of conservation and breeding. Empirical studies on the level and distribution of genetic diversity for morphological and physiological characteristics are suggested. For breeding programs, selection criteria like wood quality, growth rate and adaptability to certain environments have to be taken into consideration.

Acknowledgement

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A Study of Genetic Variation Using AFLP Technique in Populations of *Kandelia candel* in Ryukyu Islands and Southern Japan

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Abstract. To examine the extent of genetic variation within and among populations of *Kandelia candel*, AFLP analysis was applied to 7 populations from the Ryukyu Islands and Southern Japan. A total of 553 bands were detected using 4 selective primer pairs. The proportion of polymorphic loci and the average heterozygosity for each population ranged from 29.8 to 58.2%, and from 0.067 to 0.152, respectively. Mean average heterozygosity (H) and nucleotide diversity (π) are 0.099 and 0.0098, respectively. The nucleotide diversity shown here is in the range of 52 to 80% of the values shown in that of the moderate temperate forests of Japanese beech (*Fagus crenata*) and Japanese oak (*Quercus mongolica* var. *grosseserrata*); however, the fixation index (G_{ST}) was shown to be 0.151, larger than the value shown in oak (0.083), and indicating considerable genetic differentiation among populations. The Iriomote-jima population showed the largest variation in both H and π. The UPGMA tree was constructed based on nucleotide diversities and showed no association with geographic distance. This result suggests that gene flow rate by seed migration is not simply correlated with the geographic distance between islands. The Kiire population is possibly a man-made population.

Introduction

Species are composed of genetically diverse individuals; that is to say, genetic variation has been accumulated in populations. It has been said that genetic variation is the source of adaptive evolution in a changing environment and it is thus important for a population to maintain its adaptive and evolutionary potential (Newton *et al.* 1999). The extent of genetic variation depends on the length of time the population has been established, the population size, and the mutation rate. Subdivided populations have been processed by both random genetic drift and natural selection to adapt to different environments. They have then accumulated genetic variation in different combinations of alleles and have become genetically diversified. These processes have been studied by using electrophoresis to detect protein polymorphism since the experiments of Lewontin and Hubby (1968) in *Drosophila* and Harris in humans (1966). Recently, however, a method called PCR has been more commonly used for these studies to detect genetic variation at the DNA level. Variations at the DNA level, such as nucleotide substitution, RFLP, SSCP, SSR, RAPD, and AFLP have been used as genetic markers and mapped on the chromosomes. Analysis of genetic variation can also lead to clarifying the genetic structure of populations and the mechanisms maintaining that variation. These studies of genetic variation are necessary to properly plan the conservation and rehabilitation of forests as ecosystems, and for developing strategies to preserve genetic resources.

In Japan, three species of Rhizophoraceae are found in the mangrove forests of the Ryukyu Islands; *Rhizophora stylosa*, *Kandelia candel* and *Bruguiera gymnorrhiza*. Among these, *K. candel* is most broadly distributed up in the north at Kiire in Kagoshima Prefecture. In this study we estimated the amount of genetic variation accumulated in the island populations of *K. candel,* and the extent of genetic differentiation among the populations to assess differential adaptation to environments. For detecting genetic variation we used a recently developed fingerprinting method, AFLP (Vos *et al.* 1995).

Materials and Methods

Sample collection

Leaf samples were collected from seven populations including Kiire in Satsuma Peninsula, Ooura River in Tanegashima, Gima in Kumejima, Kawamitsu in Miyakojima, Makiya in Okinawajima, Kabira in Ishigakijima, and Arapara River in Iriomotejima. Samples were collected from trees that are separated from each other by at least 10m, to avoid collecting half sibs. Four to five leaves were collected from individual trees and stored in a freezer until use.

DNA extraction

DNA was extracted using the modified CTAB method (Murray 1989). Two to three grams of leaf tissue was used for extraction and the final concentration of DNA was adjusted to 0.2?g/ml by TE.

AFLP analysis

Genomic DNA was digested with *Eco*RI and *Mse*I, and ligated with adapters that had complementary sequences for the restriction sites of these enzymes using the AFLP core kit (Gibco BRL). Primary amplification by PCR was done using primers specific to the adapters. Secondary amplification by PCR was done using selective primers that attached an additional three base pairs. Selective primers used were E-ACG/M-CTT, E-AAC/M-CAT, E-ACG/M-

CAG, E-AAC/M-CTG, where E and M indicate the specificity to *Eco*RI and *Mse*I adapters, respectively. *Eco*RI primers were labeled with NED, JOE and ROX (Applied Biosystems) in the secondary amplification. Amplified products were run on an auto sequencer (Applied Biosystems, Genetic Analyzer 310). Amplified bands (fragments) were detected by GeneScan software (Applied Biosystems) and analyzed by Genotyper (Applied Biosystems). Nucleotide diversity was calculated on the fragment data by Pai (Innan *et al.* 1999).

Results and Discussion

Fragments were identified and constructed on 0/1 data matrix where the locus is assigned as 1 if a fragment is present, and 0 if it is absent. Allele frequency was calculated assuming the Hardy-Weinberg equilibrium. Based on the 0/1 data matrix, proportion of polymorphic loci (l), average heterozygosity (H) and nucleotide diversity (π) were calculated for each population (summarized in Table 1.) The greatest genetic variation was shown in the Iriomotejima population for all three parameters above-mentioned. There is a tendency that populations located in the lower latitudes have more genetic variation although the trend is not statistically significant (see Table 1).

Table 1. Genetic variation in the island populations of *Kandelia candel*

Population	Latitude	No. Sample	m	l	k	H	π
Kiire	31° 20′	20	553	193	34.9	0.089	0.0084
Tanegashima	30° 25′	23	553	245	44.3	0.097	0.0097
Okinawajima	26° 40′	24	553	263	47.6	0.104	0.0091
Kumejima	26° 20′	25	553	165	29.8	0.067	0.0070
Miyakojima	24° 40′	26	553	257	46.5	0.107	0.0116
Ishigakijima	24° 25′	18	553	172	31.1	0.079	0.0083
Iriomotejima	24° 20′	19	553	322	58.2	0.152	0.0147

m: Total number of fragments. l: number of polymorphic fragments. k: proportion of polymorphic fragments. H: Average heterozygosity. p: Nucleotide diversity

This tendency may be hampered by the low degree of genetic variation in the Ishigakijima population, located about 20 km East of Iriomotejima. Lesser genetic variation in Ishigakijima may be caused by the small population size and the shorter history of the population. The generality of this tendency must be further examined based on data that include more island populations.

Fixation index (G_{ST}) was calculated by the following formula (Nei 1973).

$$G_{ST} = \frac{H_T - H_S}{H_T}$$

Here, H_S and H_T are the mean average heterozygosity for sub-divided populations and the heterozygosity for the total population, respectively. G_{ST} was calculated to be 0.151, indicating moderate divergence among subpopulations according to Hartl's criteria. These values, together with the nucleotide diversity (*?*), are compared with those of Japanese oak (*Quercus mongolica* var. *grosseserrata*) and Japanese beech (*Fagus crenata*) similarly obtained by AFLP analysis (Okaura and Harada, unpublished data) in Table 2. This shows that genetic variation is smaller in *K. candel* than in either Japanese oak or beech in both average heterozygosity (*H*) and nucleotide diversity; however, the fixation index is second to Japanese beech. This indicates that in *K. candel*, although genetic variation is low for each population, considerable differentiation has occurred among the populations.

A distance matrix was obtained based on the nucleotide diversity between populations, and a phylogenic tree was constructed using the UPGMA method (Fig. 1). Results suggest populations that are closely located geographically are not necessarily closely related, genetically. For example, the Ishigakijima population and the Iriomotejima population, and the Okinawa population and the Kumejima population are not genetically similar although they are geographically close. The seeds of *K. candel* in the Ryukyu Islands may be dispersed by the ocean current, Oyashio. It is known that the direction of Oyashio often changes during the seasons, and is quite complicated around these islands. This may cause a disassociation between geographic and genetic distances.

Pairwise nucleotide diversities were obtained for the whole population and a Neighbor Joining (NJ) tree was constructed (Fig. 2). Many individuals are grouped together for sub populations; however, individuals in the Kiire population are dispersed in different island populations. This may indicate that the Kiire population is an artificial population in which the original plants were collected from different islands in the Ryukyu chain. The relatively long branch length of individuals of Iriomotejima reflects the larger genetic variation in this population.

Figure 1.

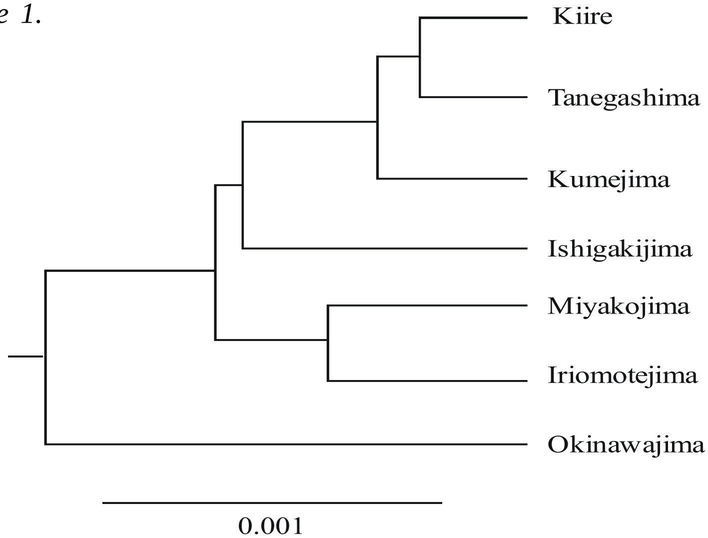
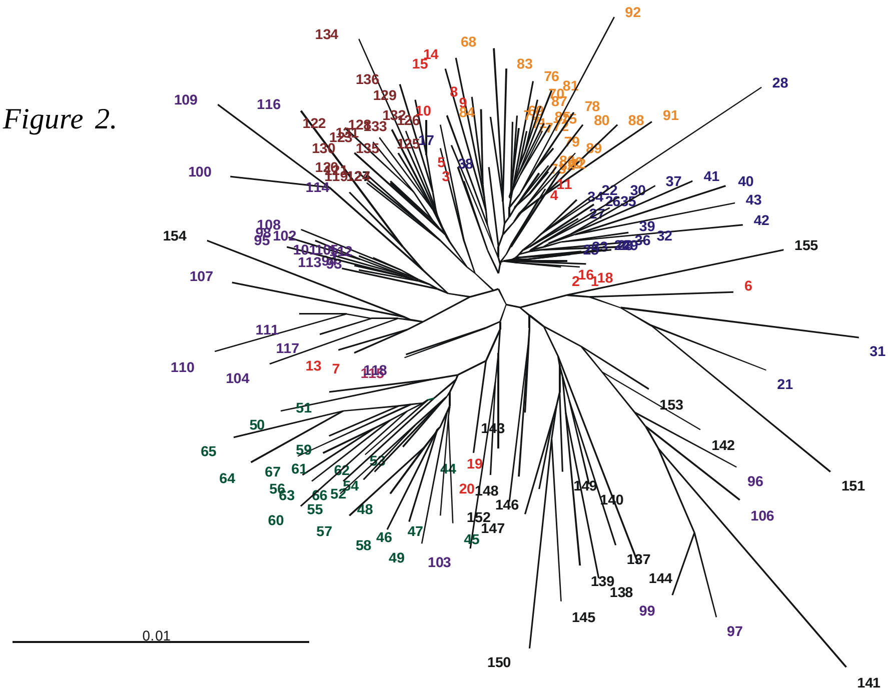


Figure 2.



Conclusions

Genetic variation of *Kandelia candel* in seven populations in Southern Japan and the Ryukyu Islands was examined using the AFLP method. It was found that the amount of genetic variation measured by both average heterozygosity and nucleotide diversity was smaller than in temperate climate forest tree populations in Japan such as beech and oak, but that the genetic differentiation as measured by fixation index is considerably larger. These findings may indicate that effective population size in *K. candel* is small or that the populations have adapted in different environments. The greatest genetic variation was found in the southernmost island, Iriomotejima. This suggests that some kind of balancing force has acted to maintain genetic variation such as was shown for southern populations in *Drosophila* (Takano *et al.* 1987). To address this possibility, further studies are required including more island populations.

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Genetic Structure of *Shorea leprosula* in a Single Population Revealed by Microsatellite Markers

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Abstracts. Microsatellite markers were applied to examine genetic divergence of *Shorea leprosula* from a population in Jambi (Sumatera). Three putative families, each with open pollinated progeny of 44, 46, and 19 trees were selected to investigate genetic characteristics, and 64 trees from 27 different putative families were used as a reference population. Three out of four loci (Shc01, Shc04, Shc09) were highly variable with a heterozygosity value of 0.913, 0.833 and 0.707 respectively. Mean expected heterozygosity across loci was 0.709. Genetic diversity within putative families did not differ with those of reference population. On the other hand, allele frequencies between putative families and the reference population differed greatly. The high genetic diversity with fewer numbers of alleles may be attributed to the existence of more than one major allele at each locus. Based on the characteristics of its natural population in Jambi, a sampling strategy for genetic conservation based on collection of a large number of wildlings from one or a few small areas is suggested.

Introduction

Shorea leprosula is an important tropical rain forest species. Because of its commercial value it has received utilisation tremendous pressure, which threatens the sustainability of the species. ITTO Project PD 16/96 Rev.4 (F) has initiated a comprehensive approach of conservation and utilisation through establishing *ex situ* conservation plots designed also for future breeding and genetic improvement of *Shorea leprosula* and *Lophopetalum multinervium*. Among the many positive features of *Shorea leprosula*, it is one of the fastest growing dipterocarp species, with an average annual increment of 1.2 cm (Appanah & Weinland 1993).

Broad genetic diversity is essentially important for genetic conservation as well as genetic improvement. Long-term survival of a species depends on its ability to adapt to environmental changes and the presence of genetic diversity in a population provides the basis for species adaptation. In a breeding program, genetic improvement depends upon the existence, nature, and extent of the genetic variability available for manipulation. Such a program will not only require access to this variability but also will depend upon the conservation and management of biodiversity.

Microsatellite DNAs, also known as simple sequence repeats (SSR), are abundant polymorphic elements in nuclear genomes consisting of tandemly repeated short DNA sequences (1 – 4 nucleotides) and they are highly informative genetic markers. These regions are interspersed throughout eukaryotic genomes, generally embedded in unique DNA sequences. Polymorphism among individuals arises through differences in the number of repeats present in the microsatellite sequence.

Microsatellites are ideal markers for population and conservation genetic studies (Powell *et al.* 1995, Rajora *et al.* 1999) because they are codominant, highly variable, amenable to screening large numbers of individuals, and require small amounts of DNA for analysis.

The objective of the work reported here was to examine the nature of genetic divergence of *S. leprosula*. The information would be used for designing the sampling strategy for a genetic conservation program.

Materials and Methods

Plant materials

A total of 162 wildlings were used for molecular analysis. Wildings were collected from natural populations in Jambi (south Sumatra). More than 5000 wildlings were collected for *ex situ* conservation, but only three putative families were used in the study since the family origins of most trees are unknown. The three families (38, 39, and 40) were selected to investigate the genetic characteristics. The numbers of trees for each putative family were 44, 46, and 19 respectively. Some 27 putative families were also selected, and one to five trees per family were used as representatives of the population/reference population.

DNA extraction

Total genomic DNA was extracted from 100 mg of leaves using a modified Murray and Thompson (1980) method (Shiraishi & Watanabe 1995). Grinding of leaves was done in liquid nitrogen and the powder was incubated in CTAB extraction buffer containing EDTA, Tris-HCl, NaCl, and ß-mercaptoethanol for 1 hour at 65^{o}C. After incubation, 800 uL chloroform was added to the mixture mixed by rotation for 20 minutes, centrifuged at 12,000 rpm for 10 minutes, and then removed as supernatant to new tubes. This step was repeated once with less chloroform (700 uL), removed as supernatant to new tubes then added 20 uL sodium acetate. The DNA was precipitated by adding 650 uL of isopropanol, centrifuging at 15,000 rpm for 5 minutes, and washing the precipitate with 70% ethanol. Remaining ethanol was removed by vacuum drying (PerSeptive Biosystems) for 2 minutes then adding 300 uL H_2O.

Microsatellite markers

Four microsatellite markers (Shc 01, Shc03, Shc04, Shc09) developed for *Shorea curtisii* (Ujino *et al.* 1998) were used in this study. These four loci were selected because they exhibited the highest levels of polymorphism in *S. curtisii.* Fluorescence labelled primer and non-labelled primers were synthesised for each marker according to the primer sequences. Details of the markers are presented in Table 1.

Table 1. Microsatellite loci and primer sequence.

Locus	**Primer sequence (5' to 3')**
Shc01	GCT ATT GGC AAG GAT GTT CA CTT ATG AGA TCA ATT TGA CAG
Shc03	TTG AAG GGA AGG CTA TG CTT CTC AAC TAC CTT ACC
Shc04	ATG AGT AAC AAG TGA TGA G TAT TGA CGT GGA ATC TG
Shc09	TTT CTG TAT CCG TGT GTT G GCG ATT AAG CGG ACC TCA G

Molecular analysis

PCR reaction mixtures (10µL final volume) contained 25 *ng* genomic DNA, 200 µM of each dNTP, 0.5 µM primer, 1x PCR buffer, 1.5 mM $MgCl_2$ and 0.5 unit Ampli*Taq* Gold DNA polymerase (Perkin Elmer).

The PCR conditions were set at 95°C for 10 minutes (pre-denaturation), followed by 35 cycles of denaturation at 95°C for 30s, annealing at 50 – 60°C for 30s, extension at 72°C for 60s, and final extension at 72°C for 60s using GeneAmp 9700 (PE Applied Biosystem). Annealing temperature of 60°C was decreased 1°C at each cycle for initial 10 cycles, and then fixed at 50°C for the remaining cycles.

PCR products were electrophoresed using ABI 310 Genetic Analyser (PE Applied Biosystem) and data were analysed using GeneScan and Genotyper programs (PE Applied Biosystem).

Data analysis

Microsatellite alleles were determined for each locus according to the length of PCR products. Total number of alleles (*N*a), effective number of alleles (*A*e), expected and observed heterozygosity (*h*e and *h*o), and measurement of deviation from Hardy-Weinberg expectation (*F*is) were calculated (Nei 1987).

Results

Sixty-four trees from 27 different putative families were considered to represent the natural population in Jambi, and these were used as the reference population. Statistics of 4 microsatellite loci for the reference population are summarised in Table 2. Three out of four loci (Shc01, Shc04 and Shc09) were highly variable with heterozygosity values of 0.913, 0.833, and 0.707 respectively. The remaining one locus (Shc03) showed much lower variability. Mean expected heterozygosity across loci was 0.709. The effective number of alleles (A_e) was much smaller than the total number of alleles at each locus. This is due to the existence of many private alleles. Positive values of F_{IS} were detected in three loci and one of them was significantly different from zero. This is an indication of the lack of heterozygosity.

Microsatellite genotyping was carried out for 44, 46, and 19 individuals from family 38, 39 and 40, respectively. The mean values of effective number of alleles, expected and observed heterozygosity, and F_{IS} over 4 loci are shown in Table 3. The genetic diversity parameters (H_e, H_o and A_e) within each putative family were slightly lower than those in the reference population. However, when comparing each locus, it was not always lower but was higher in some cases. Therefore, the differences in genetic diversity should be very small. On the other hand, total number of alleles per locus was always lower in the putative family.

Allele frequency of each locus was also compared among the putative families and the reference population. Unlike genetic diversity measurements, allele frequency was largely different in Shc01, Shc04, and Shc09. For example, the allele frequency of Shc01 within the putative families and the reference population is shown in Fig. 1. In this locus, some of the major alleles in the reference population were missing in one or more putative families. Instead of this, some of the rare alleles appeared to be major alleles. The changes of allele frequencies were particularly high in putative family 40 in three loci.

Allele frequencies were estimated from four loci. Frequency distributions show proportion of major alleles (Figure 1). Family 40 shows a significantly high frequency at three alleles.

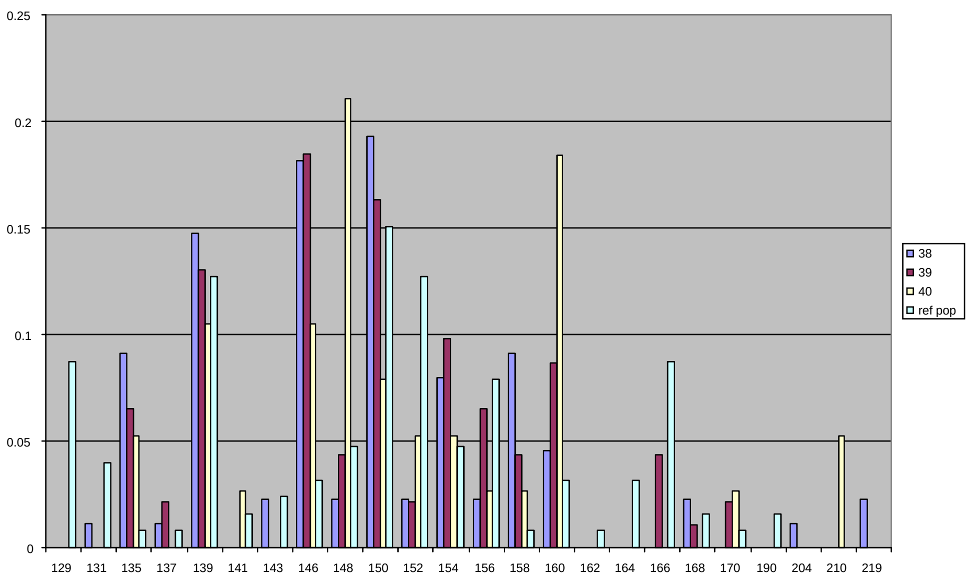
Table 2. Genetic diversity based on heterozygosity of the reference population.

Loci	N	Na	Ae	He	Ho	Fis
Shc 01	63	21.0	11.5	0.913	0.746	0.183
Shc 03	64	6.0	1.6	0.385	0.391	-0.016
Shc 04	64	16.0	6.0	0.833	0.594	0.287
Shc 11	64	14.0	3.4	0.707	0.672	0.050
ref pop	63.8	14.3	5.6	0.709	0.601	0.126

Table 3. Genetic diversity based on heterozygosity of individual family (means of 4 loci).

Sample	N	Na	Ae	He	Ho	Fis
Fam 38	44	12.0	4.3	0.675	0.563	0.147
Fam 39	46	11.8	4.7	0.685	0.576	0.126
Fam 40	19	10.0	4.6	0.691	0.566	0.168
Mix	63.8	14.3	5.6	0.709	0.601	0.126

Figure 1. Allele frequency distribution of Shc01.



Discussion

Genetic diversity

Heterozygosity of *S. leprosula* obtained in this study (He = 0.709) is relatively high. The high level of genetic diversity of *S. leprosula* conforms to several aspects of its biology, such as its breeding system and its geographic distribution. The breeding system of a species determines the genetic variability at species and population levels. *S. leprosula* is predominantly an outcrossing and insect pollinated species (Chan 1981). It is also a widely distributed species and, as such, would tend to maintain more variation than narrowly distributed species (Hamrick & Godt 1989).

Genetic diversity within the putative families did not show clear differences with those of the reference population. However, the lower number of alleles in the putative families indicates that the loss of some rare genes has occurred because only few number of plants were collected and these originated from a small area.

The frequency of alleles showed large differences between putative family and reference population. Although there are losses of some rare and/ or major genes, some of the rare and/or non-detected genes are major genes. The high genetic diversity with fewer numbers of alleles seems to be influenced by the existence of more than one major allele at each locus.

The four microsatellite loci used in this study were highly variable. Maximum value of expected heterozygosity was 0.913 (Shc01), and minimum was 0.385 (Shc03). If there is high variance of heterozygosity among loci, a large number of loci should be used to estimate mean heterozygosity (Nei 1987). Therefore, it should noted that the mean heterozygosity of these 4 loci might not reflect the true genetic diversity of this population.

Generally, but with exceptions, out-crossing species are assumed to follow the Hardy-Weinberg expectation. In this study, however, positive values of F_{IS} (measurement of deviation from the H-W expectation) were found in three loci, and in one locus it was significantly different from zero. A positive value indicates loss of heterozygosity and this normally happens when inbreeding has occurred. In this study, however, that is unlikely because the species is highly outcrossing (Nagamitsu *et al* 2001) and this population is a large natural population. And, if there was any inbreeding, all four loci should have lost heterozygosity. The possible explanation of this high F_{IS} value is the existence of null alleles. Although the homozygote of a null allele was not found in the sample of 173 individuals, they might exist in a frequency of only a few percent (if there is 5% of null alleles, they might not be detected).

Sampling strategy

The objective of sampling genetic materials for *ex situ* conservation is to capture as much genetic variation as possible within practical limits. Through this analysis of putative families (wildlings collected from a small area) and the reference population (regarded as a representative of the natural population in Jambi), genetic characteristics of natural populations were estimated and used as a guide in developing sampling strategy.

When collecting wildlings for conservation purposes, four different schemes of collection could be considered. First, very large number of wildlings from one or a few narrow areas. Second, a few wildlings/plot but from a wide area covering the whole population. Third, an intermediate number of wildlings from an intermediate number of plots, wherein the number of plots is more than the number of individuals per plot. Fourth, an intermediate number of wildlings from an intermediate number of plots wherein the number of individuals per plot is more than the number of plots.

For the first scheme, the chance of losing a certain amount of genes is high because the total number of alleles per locus was smaller in the putative families than in the reference population. Therefore, this scheme should be avoided. The second scheme could be the best if the same genetic composition as the natural population is to be conserved. This scheme, however, is time consuming and laborious. The third and fourth schemes are biologically similar but differ greatly in terms of time and labour. Both of them would capture high genetic diversity and the loss of genes would be minimum. Moreover, when these schemes are carried out, increasing heterozygosity due to the Wahlund's principle could be expected. Wahlund's effect occurs when two isolated populations that are differentiated are combined. The genetic characteristics of the wildling population of *S. leprosula* from a limited area were specific in this study, and this is the same effect as in isolated populations. Scheme four would be more efficient than scheme three and less laborious. If the other population possess similar genetic characteristics, a sampling strategy based on scheme four could be sufficient.

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Genetic Variation of *Lophopetalum multinervium* (Celastraceae) in the Sebuku Sub-Population

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Abstract. The genetic variation of *Lophopetalum multinervium*, an important swamp forest species, was investigated using eight enzyme systems – namely, ACP, DIA, EST, GDH, GOT, 6PG, POD and SOD enzymes. For that purpose, selected sprouting buds of three- month-old seedlings of 55 parent trees from the Sebuku, East Kalimantan sub-population were subjected to isozyme analysis. Of the eight enzyme systems used for isozyme study, genetic variations were detectable only in four enzymes and were controlled by 10 alleles in 4 loci. Results of the allele frequencies from investigated samples indicated that the level of genetic variation of *Lophopetalum multinervium* in the Sebuku sub-population was very low (0.128). However, further genetic studies of this species will be conducted using the four detected polymorphic loci as genetic markers.

Introduction

Tropical forests have very high species diversity and it is stated that about 65% of the total number plant species (250,000 or more) are found in tropical regions (Loveless 1992). The species richness of tropical forests has led to research on the evolutionary processes by which this diversity has arisen. The persistence of evolutionarily viable populations of tropical trees is crucial to the conservation of tropical forest habitats. Because of that, evolutionary processes can be used to understand tropical diversity at all levels.

Perupuk (*Lophophetalum multinervium* Rild.), is an important swamp forest tree species in Indonesia with limited natural distribution in some parts of the islands of Sumatra, Kalimantan, Sulawesi, and Irian Jaya between altitudes of sea level to 150 masl. (Abdurrahim & Iding 1977). In East Kalimantan for instance, prupuk growths in three disjunct sub-populations; Sebuku, Pimping, and Berau representing a total area about 15,000 ha.

Considering this species' great tolerance of various site conditions and environments, it might be expected that a large number of genetic variations would be found in its sub-populations. Genetic studies of common tropical plantation species such as teak, pines, eucalyptus, and rubber have been carried out using provenance trials and measuring quantitative traits under various growth conditions. However, these have not provided information on the population structure or evolutionary processes in natural populations (Loveless 1992).

In forest trees, allozyme analyses have been widely used in genetic studies, such as analysis of genetic variation within species, Chase *et al.* 1995, Lee *et al.* 2000), certification of parent trees and clones (Adam & Joly 1980, Cheliak & Pitel 1984) and estimation of mating system parameters (Doligez & Joly 1997).

Allozymes are expected to be inherited codominantly in accordance with simple Mendelian principles. However, in principle, allozymes may not show codominance due to modifiers or the absence of activity or 'null' alleles. Loci of related species often resemble one another in general isozyme characteristics, although species can differ in isozyme numbers, in phenotype of similar loci, or in interaction between loci and the expression of modifier loci (Adam & Joly 1980). It is therefore essential, when making comparisons of isozyme variation between populations, that the genetic control of the enzyme is understood. The absence of information on inheritance may lead to overestimation of heterozygosity and/or biased estimates of allele frequencies and mating system parameters (Lee *et al.* 2000).

The purpose of this study was to describe the level of genetic variation of perupuk (*Lophophetalum multinervium* Rild.) in the Sebuku sub-population using isozyme analysis. The elucidation of genetic variations in the natural population is essential as the basis for future genetic improvement and gene conservation activities for this species.

Materials and Methods

Sampling

Mature, open-pollinated seeds of *L. multinervium* collected from 55 parent trees of the Sebuku population, East Kalimantan were collected in April 1999. The distance between parent trees was about 100 meters. Collected seeds were germinated and grown in the nursery for about three months. Sprouting buds of those seedlings were subjected to isozyme analysis. Each parent tree was represented by 20 seedlings. Selected sprouting buds of each parent tree were stored separately and kept in fresh condition using ice boxes. They were then sent to the Laboratory of Biotechnology, Department of Silviculture, Faculty of Forestry, Gadjah Mada University in Yogyakarta.

Enzyme extraction

Polyacrylamide vertical slab gels for electrophoresis were prepared according to the methods of Davis (1964) and Ornstein (1964); 7.5 % running gel and 3.75 % spacer gel were used. Leaf tissues (100 mg of sprouting bud) of each parental sample were ground in a mortar after adding 1 ml. extract buffer containing 1 M Tris- HCl pH 7.5, 60% glycerol, 15% Tween 80, 0.4% mercaptoethanol, 200 mm Ditiothreitol, and 100 mg Polyvinyl polypyrrolidone. The homogenates were centrifuged at 10,000 rpm at 0^{o} C for 20 minutes. Twenty microliters supernatant of each extracted sprouting bud was used for electrophoresis.

Electrophoresis

Electrophoresis was carried out at 4^{o} C and 12.3 mA/cm^2 for three hours. Gels were stained using the formula of Tsumura *et al.* (1990). A total of 12 enzyme systems were analyzed in this study; namely Acid Phosphate (ACP). E.C. 3.1.3.2), Alcohol Dehydrogenase (ADH. E.C. 1.1.1.1), Diaphorase (DIA. E.C. 1.6.4.3), Esterase (EST. E.C. 3.1.1.1), Glycerate Hydrogenase (G2DH. E.C. 1.1.1.29), Glutamate Dehydrogenase (GDH, E.C. 1.4.1.2), Glutamate Oxaloacetat Transaminase (GOT. E.C. 2.6.1.1), Leucine Amino Peptidase (LAP. E.C. 3.4. 11.1), 6- Phosphogluconate Dehydrogenase (6-PG. E.C. 1.1.1.44), Peroxidase (POD. E.C. 1.11. 1.7), Shikimate Dehydrogenase (ShDH. E.C. 1.1.1.25.) and Sorbitol Dehydrogenase (SOD. E.C. 1.1.1.14) enzymes.

Genetic Analysis

Since material from controlled crosses was not available, verification of the genetic control and the respective allozyme variants was performed by observing zymograms detected from the 12 enzyme systems for all available samples. Genetic diversity was defined by four parameters; the proportion of polymorphic loci, the average numbers of alleles per locus, the mean observed (*Ho*) and expected (*He*) heterozygosity, respectively. The fixation index (*Fis*), $Fis = 1 - Ho/He$ was used to examine the deviation of genotypic frequencies from Hardy-Weinberg proportions (Nei 1973).

Results and Discussion

Among the 12 enzyme systems investigated, only 8 (ACP, DIA, EST, GDH, GOT, 6PG, POD and SOD) enzymes produced activity bands. No band patterns appeared in the other four enzymes (ADH, G2DH, LAP and ShDH). However, among the eight banding enzymes there were only four enzymes (ACP, EST, GOT and 6PG) that showed very clear band patterns that could be resolved and scored. The unsuccessfully developed enzyme systems exhibited very weak band activity and observations were difficult. As stated by Loomis (1974), inhibited enzyme activity in higher plant species is mostly due to endogenous phenols, tannins, phenoloxidases, and other cellular constituents such as terpenes, pectins, resins, coumarins, and carotenoids. Some losses in enzyme activity such as in Hexokinase (HK) and Aconitase (ACO) was also inevitable during freezing (Lee *et al*. 2000). A total of 11 loci could be detected in the four remaining enzyme systems.

1) Acid phosphatase (ACP)

Three zones consisting of one invariant band in zone 1, one segregation band in zone 2, and one unclear band in zone-3 were observed in all gels stained for ACP. We suppose that zone 1 was encoded by a single locus *Acp-1* and zone 2 seemed to be controlled by *Acp-2* loci with two alleles. In zone 3, however, due to having an inactive band, it was unobservable (Figure. 1)

2) Esterase (EST)

Many bands appeared on the EST gel. However, only three zones of band activity could be detected. Two single-banded phenotypes were detected in zone 1. There were segregation banded phenotypes detected in zone 2, while an invariant single band was observed in zone 3 (Figure 1). These results denoted that EST was encoded by three loci with one allele in *Est-1*, three alleles in *Est-2*, and one allele in *Est-3*.

3) Glutamate oxaloacetate transaminase (GOT)

There were three zones of band activity detected in all gells stained for GOT. There was one invariant-banded phenotype detected in zones 1 and 3. In zone 2, however, a segregation-banded phenotype was detectable. These results denoted that GOT was encoded by three loci with 1 allele in the *Got-1* locus, 2 alleles in the *Got-2* locus, and a single allele in the *Got-3* locus (Figure 1).

4) 6-phosphogluconate dehydrogenase (6-PG)

There were three zones of band activity detected on 6-PG gels (Figure 1). Three invariant single-bands were observed in zone 1. In zone 2, three segregation bands could be detected, while in zone 3 there was one invariant clear band observed. These results indicated that 6-PG enzymes were encoded by three loci, namely the *6Pg-1* locus with one allele, the *6 Pg-2* locus with 3 alleles, and the *6 Pg-3* locus with a single allele.

Allelic frequencies for eleven loci are shown in Table 1. Most loci observed in the Sebuku population were monomorphic, except for the *Acp-2, Est-2, Got-2* and *6Pg-2* loci. The number of alleles ranged from 1 to 3 with an average of 1.54. The average of expected heterozygosity was 0.128. (Table 1). If the values of genetic variations of *L. multinervium* were compared to those which were summarized by Hamrick and Godt (1989), where the expected heterozygosity (He) for tropical species = 0.211, then the results of this study yielded lower values (Table 1). However, the lower value for expected heterozygosity detected in this study (*He*=0.128) might be due to the following. First, there was a limited number of enzyme systems used in this study and a limited detectable polymorphic loci applied. Second, there may be a strong level of inbreeding depression within the Sebuku population. *Lophopetalum multinervium* is an endemic swamp forest tree species and is distributed naturally in the form of small populations. Because of that, long-term selection activities due to legal and illegal logging in this sub-population have had a serious impact on genetic diversity. Thus, many if not most of the remaining trees in this population may be closely-related to each other.

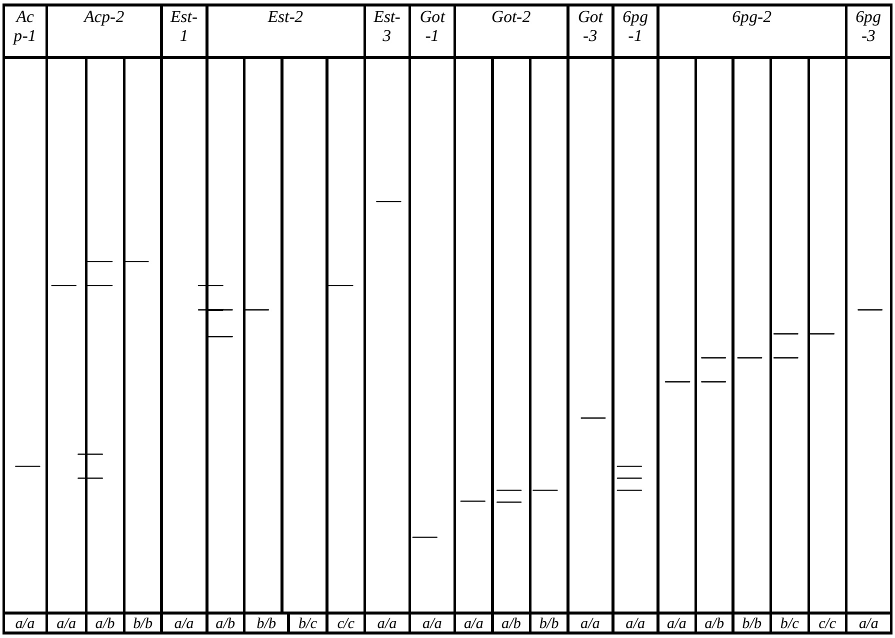
Table. 1. Allele Frequency, Heterozygosity and F- index for 11 isozyme loci of *Lopophetalum multinervium* in Sebuku population

No.	Locus	Allele	Allele Frequency	Observed Heterozygosity (*Ho*)	Expected Heterozygosty (*He*)	F- index (Fis)
1.	*Acp-1*	a	1.000	0.000	0.000	0.000
2.	*Acp-2*	a	0.645			
		b	0.355	0.389	0.458	0.151
3	*Est-1*	a	1.000	0.000	0.000	0.000
4.	*Est-2*	a	0.002			
		b	0.886	0.158	0.202	0.217
		c	0.112			
5.	*Est-3*	a	1.000	0.000	0.000	0.000
6.	*Got-1*	a	1.000	0.000	0.000	0.000
7.	*Got-2*	a	0.832			
		b	0.168	0.262	0.280	0.064
8	*Got-3*	a	1.000	0.000	0.000	0.000
9.	*6Pg-1*	a	1.000	0.000	0.000	0.000
10.	*6Pg-2*	a	0.003			
		b	0.600	0.413	0.482	0.143
		c	0.397			
11.	*6Pg-3*	a	1.000	0.000	0.000	0.000
	Mean			0.111	0.128	0.143 *⊗

* The mean of the *Fis* for polymorphic loci

⊗ significant at 5% level

Figure 1. Banding pattern for 12 detected loci in *Lophopetalum multinervium*



* The mean of the *Fis* for polymorphic loci

⊗ significant at 5% level

The genetic structure of the Sebuku population can be described in more detail by investigating the Fixation Index (Fis). Based on the calculation from the total available samples (around 800 seedlings) deviation from the Hardy-Weinberg equilibrium was found at the 5% significance level in this population for all loci studied (Table 1). This indicates that there was a deficiency of heterozygotic families in the Sebuku population.

However, at the level of individual samples, wherein each sample ranged from 15-20 seedlings, some of these fit the Hardy-Weinberg equilibrium for all loci observed while in others, significant deviations from the equilibrium were found. For instance, for the *Acp-2* locus a high departure from Hardy Weinberg equilibrium occurred in parent trees S 12 and S 62 (3.6 % of the total samples). For the *Est-2* locus, 10 of the 55 parent trees, namely S12, S 15, S 23, S 30, S31, S37, S 38, S 62, S 64, and S 92 or about 18.18% of the sample, were highly significant deviations from the Hardy-Weinberg equilibrium. The deficiency of heterozygote genotypes in the *Got-2* locus occurred in parent trees S 06, S15 and S62 (5.4 % of the samples) while for the *6Pg-2* locus the higher deviations from Hardy Weinberg equilibrium occurred in parent trees S06, S12, S17, S20, S29, S37, S62, S64, S71, S93 or about 18.18 % of the sample. It was also observed that 37 of 55 parents or 67.27% of the sample showed no deviation from Hardy-Weinberg equilibrium at the 1% significance level.

These results indicate that even though deviations from Hardy-Weinberg equilibrium was detected at some levels in the population, no deviation from the equilibrium was detected in more than half of the individual parent trees used (Table 2). That indicates a high inbreeding rate may have occurred within the sub-population while **bold** values denoting significance at the 1% level for individuals denotes that certain parent trees still have heterozygote genotypes. This information is very important from the viewpoint of genetic conservation, especially when devising a sampling strategy for seed collection from which *ex situ* conservation areas would be established. Samples taken from representative parent trees of *L. multinervium* from the entire area of distribution would maintain a higher level of genetic diversity. The results of this investigation would be more useful for supporting a genetic conservation strategy if additional populations were investigated. But these initial results will serve as valuable information for designing future research on genetic variability of this species.

Conclusions

The results of this experiment denoted that the level of genetic variation of *L. multinervium* was relatively low. Even though a deficiency of heterozygotes was observed at the sub-population level, for most of the individual parent samples there were not significant deviations from the Hardy-Weinberg equilibrium. These results will be useful for supporting the selection of a genetic conservation strategy and also for further breeding activities for this species.

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Table 2. Value of Fixation Indexes (Fis) based on group samplings evaluation

No. Of Fam.	Fixation Index (Fis)				No.	No. of Fam.	F-Statistics (Fis)			
	Acp-2	Est-2	Got-2	6Pg-2			Acp-2	Est-2	Got-2	6Pg-2
S 05	-0.216	-0.176	-0.176	-0.333	29	S 60	-0.133	0.375	-0.111	0.360
S 06	-0.111	0.314	-0.067	**0.400**	30	S 62	**0.467**	**1.000**	**-0.667**	**0.595**
S 07	-0.025	-0.081	0.134	-0.315	31	S 64	0.303	**0.634**	0.386	**0.446**
S 08	0.193	-0.086	**0.467**	0.333	32	S 65	0.000	0.124	-0.176	-0.133
S 09	0.000	-0.176	-0.111	0.214	33	S 66	-0.091	0.000	-0.143	-0.111
S 12	**-0.457**	**-0.600**	0.000	**0.607**	34	S 67	-0.086	0.000	-0.111	-0.083
S 15	-0.325	**0.438**	**1.000**	0.005	35	S 68	-0.152	0.000	-0.111	-0.333
S 16	0.232	0.375	-0.091	-0.025	36	S 71	0.000	0.000	-0.290	**-0.625**
S 17	-0.111	-0.143	-0.086	**0.600**	37	S 72	-0.004	0.000	-0.172	0.232
S 18	-0.175	0.081	-0.152	0.267	38	S 74	-0.111	0.000	0.218	-0.111
S 20	-0.111	0.139	0.000	**-0.600**	39	S 75	-0.057	0.000	-0.026	-0.175
S 23	-0.267	**0.686**	-0.004	0.066	40	S 76	0.000	-0.053	-0.111	-0.111
S 24	-0.211	-0.091	-0.111	0.000	41	S 79	-0.172	0.000	0.086	-0.267
S 27	0.066	-0.143	-0.057	0.000	42	S 80	-0.143	0.000	0.025	-0.303
S 28	0.000	0.048	-0.111	0.000	43	S 81	-0.091	0.000	0.000	-0.133
S 29	0.386	-0.052	-0.037	**-0.500**	44	S 82	0.000	0.000	-0.111	-0.176
S 30	0.005	**-0.504**	-0.141	-0.211	45	S 86	0.081	0.000	0.019	0.346
S 31	0.232	**0.444**	0.296	0.292	46	S 87	0.066	0.000	0.176	0.298
S 32	0.000	-0.028	-0.055	0.000	47	S 88	0.000	0.000	0.200	0.373
S 33	0.000	0.286	-0.003	0.067	48	S 92	-0.325	**1.000**	-0.143	0.059
S 36	0.124	0.134	0.063	0.047	49	S 93	0.333	0.001	0.062	**0.600**
S 37	0.000	**0.444**	-0.143	**-0.432**	50	S 94	-0.111	-0.027	-0.173	-0.179
S 38	0.200	**0.475**	-0.053	0.296	51	S 95	0.286	-0.026	0.271	**0.497**
S 40	-0.141	0.216	-0.111	-0.143	52	S 96	-0.086	0.000	-0.250	-0.049
S 56	-0.111	0.216	0.063	-0.176	53	S 97	-0.052	0.000	-0.250	0.059
S 57	0.025	-0.143	0.053	-0.250	54	S 98	0.000	0.000	-0.067	-0.010
S 58	0.066	0.000	-0.240	0.000	55	S 99	0.124	0.000	0.167	-0.200
S 59	0.025	0.000	0.122	-0.111						

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Evaluating Genetic Diversity of *Dipterocarpus alatus* Genetic Resources in Thailand Using Isozyme Gene Markers

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Abstract. Dipterocarpus alatus is a tropical tree species of Thailand and other areas of Asia. It is a source of highly valuable timber and it also has ecological value. However, genetic information about this species is scarce. This paper describes the genetic diversity of D. alatus from 16 natural forests of the North, Northeast, Center, and South of Thailand as revealed by isozyme gene markers. Thirteen isoenzyme systems were investigated in this species by horizontal starch gel electrophoresis. Thus far, eight putative isoenzyme gene loci were identified. The number of polymorphic loci varied from 12.5-75%. The average gene diversity and observed heterozygosity were 0.092 and 0.088, respectively.

Partitioning genetic variation into within- and among-population components revealed that 12.8% of the total variation was attributable to differences among populations. These results suggest that natural populations of D. alatus in Thailand show considerable levels of genetic divergence that is useful for future gene conservation and management of this species.

Introduction

The family Dipterocarpaceae is represented in Thailand by eight genera and 65 species, comprised of Anisoptera**,** three species; Cotylelobium, one species; Dipterocarpus**,** sixteen species; Hopea, thirteen species; Neobalanocarpus, one species; Parashorea, one species; Shorea, twenty two species; and Vatica, eight species. Most of these dipterocarps are confined to evergreen, semi-evergreen, and mixed deciduous forest. Exceptions are *Dipterocarpus intricatus, D. obtusifolius, D. tuberculatus, Shorea obtusa, S. roxburghii* and *S. siamensis,* all of which are found in dry, deciduous dipterocarp forests.

Dipterocarpus alatus is a very large tree reaching a height of 45-55 meters and a girth of 4-5 meters, with a cylindrical bole about 20-30 meters long. It is of high economic value and provides good timber and veneer for construction, railway sleepers, plywood, and furniture as well as oleoresin. It has a very wide distribution in Bangladesh (Chittagong), lower Myanmar, Thailand, Laos, Cambodia, South Vietnam, the Andaman Islands, and northern

Malaysia (Smitinand *et al.* 1980, FAO Regional Office for Asia and the Pacific 1985). In Thailand, it is distributed widely in the North, Northeast, East, Center, and South of the country. However, because of the demand for wood and land clearing for agriculture, forest area has been reduced to 25.28% of the total area of Thailand (Royal Forest Department, 1999). Consequently, the genetic resources of *D. alatus* in Thailand have been significantly disturbed.

One of the fundamental requirements for conserving and using forest tree genetic resources is to understand the dynamics of genetic variation within and between species. Considerable variation exists among tree species with respect to the extent of genetic diversity and the way such diversity is organised within and among populations. The extent and the pattern of this diversity are strongly dependent on the amount of genetic polymorphism, patterns of gene flow, and mating system (Changtragoon & Szmidt 1993). To understand and characterize the remaining gene pool of *D. alatus,* this genetic information is required. This paper reports a preliminary survey of genetic diversity of *D. alatus* in natural forests of Thailand using isoenzyme gene markers.

Materials and Methods

Seeds were collected randomly under 50 *D. alatus* trees separated by 100 meters in each of sixteen natural populations in the northern, northeastern, central and southern parts of Thailand as shown in Table 1.

Table 1. Investigated populations of *Dipterocarpus alatus*

Population name	Longitude and Latitude	Altitude
1. Khirimat, Sukhothai	E 99° 42’18” N 16° 52’24”	200
2. Long, Phrae	E 99° 50’ N 18° 3’	100
3. Samngao, Tak	E 99° 3’ N 17° 20’30”	120
4. Pho Thalae, Phichit	E 100° 36’ N 16° 48’	26
5. Kosumphisai, Mahasarakham	E 103° 2’ N 16° 15’	144
6. Kuphrakona, Roiet	E 103° 50’26” N 15° 34’	141
7. Dong Fahuan, Ubonratchathani	E 104° 52’ N 15° 14’	113
8. Sakaerat, Nakhonratchasima	E 101° 54’ N 14° 27’57”	224
9. Wat Chantarangsri, Angthong	E 100° 18’ N 14° 7’	6
10. Khao Phraresri, Kanchanaburi	E 98° 47’ N 14° 43’	100
11. Huai Khayeng, Kanchanaburi	E 98° 34’ N 14° 37’	100-200
12. Koh Samrong, Kanchanaburi	E 99° 31’ N 13° 57’	30
13. Muang, Ratchaburi	E 99° 48’ N 13° 32’	56
14. Bang Saphan Noi, Prachuapkirikhan	E 99° 33’ N 11° 12’	90
15. Chaiya, Suratthani	E 99° 16’ N 9° 23’	8
16. Hat Yai, Songkhla	E 100° 26’36” N 7° 28’	5

Isoenzyme Analysis

Embryos were extracted from each seed and ground in a homogenizing buffer. Crude extracts of each sample were applied to12% horizontal starch gel and separated for 4-5.5 hrs. Thirteen isoenzyme systems were analyzed (Table 2). The electrophoresis and staining procedure used were modified from those described in Conkle *et al.* (1982), Changtragoon and Finkeldey (1995a and 1995b), Changtragoon *et al.* (1996). Forty to ninety seeds per population were used in this analysis of genetic structure and diversity of D. *alatus*.

Table 2. List of the investigated isoenzyme systems

Isoenzyme system	**Abbreviation**	**Enzyme code**
Leucine aminopeptidase	LAP	3.4.11.1
Glutamate-oxaloacetate transaminase	GOT	2.6.1.1
Glutamate dehydrogenase	GDH	1.4.1.3
Isocitrate dehydrogenase	IDH	1.1.1.42
6-Phosphogluconate dehydrogenase	6-PGDH	1.1.1.44
Phosphoglucomutase	PGM	2.7.5.1
Malate dehydrogenase	MDH	1.1.1.37
Glucose 6-phosphate dehydrogenase	G-6PDH	1.1.1.49
Diaphorase	DIA	1.1.4.3
Formate dehydrogenase	FDH	1.6.99.3
Shikimate dehydrogenase	SKDH	1.1.1.25
NDH-dehydrogenase	NDH	1.6.99.1
Phosphoglucose-isomerase	PGI	5.3.1.9

Statistical Analysis

Measures of allelic frequency, genetic structure, diversity, and distance were performed using TFPGA computer programs (Miller 1997).

Results and Discussion

Eight putative gene loci were identified. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. The maximum number of alleles per locus was three. The average number of alleles per locus was 1.75. The percentage of polymorphic loci per population was 12.50-75%, with the average 31.25%. There were six populations that had percentages of polymorphic loci higher than average (Table 3). Dong Fahuan population in Ubonratchathani province had the highest percentage of polymorphic loci (75%) and Kao Phraruesri population in Kanchanaburi province had 50% of polymorphic loci. The other four populations had 37.5% of polymorphic loci. In the remaining populations, the percentage of polymorphic loci was lower than average (Table 3). Dong Fahaun population in Ubonratchatani also had the highest genetic diversity (0.1750). The mean observed and expected heterozygosity levels were 0.0884 and 0.0924, respectively (Table 3).

Table 3. Genetic variability of investigated *Dipterocarp alatus* populations

Population	Sample Size	Men no. alleles per locus	Percentage of polymorphic loci		Mean heterozygosity	
			at criteria 95%	99%	observed	Hywbg* expected
1. Khirimat,Sukhothai	90	1.57	25.00	62.50	0.0597	0.0588
2. Long, Phrae	40	1.50	25.00	50.00	0.1344	0.1045
3. Samngao, Tak	90	1.75	25.00	50.00	0.0417	0.0402
4. Pho Thalae, Phichit	45	1.88	25.00	62.50	0.1694	0.1169
5. Kosumphisai, Mahasarakham	85	1.75	37.50	62.50	0.0709	0.0918
6. Kuphrakona, Roiet	40	1.50	25.00	37.50	0.0513	0.0533
7. Dong Fahuan, Ubonratchathani	40	2.00	75.00	75.00	0.1750	0.1595
8. Sakaerat, Nakhonratchasima	40	1.63	25.00	50.00	0.1625	0.1044
9. Wat Chantantarangsri, Angthong	85	1.88	37.50	50.00	0.1483	0.1591
10. Khao Phraruesri, Kanchanaburi	68	1.88	50.00	62.50	0.0616	0.1255
11. Huai Khayeng, Kanchanaburi	85	2.00	37.50	62.50	0.0794	0.1333
12. Koh Samrong, Kanchanaburi	90	1.63	25.00	50.00	0.0319	0.0586
13. Muang, Ratchaburi	45	1.75	12.50	50.00	0.0514	0.0681
14. Bang Saphan Noi, Prachuapkirikhan	45	1.57	12.50	37.50	0.0056	0.0267
15. Chaiya, Suratthani	45	1.57	25.00	37.50	0.0250	0.0238
16. Hat Yai, Songkhla	90	2.00	37.50	75.00	0.1458	0.1542
Average	63.93	1.74	31.25	54.68	0.0884	0.0924

*Unbiased estimate (see Nei, 1978)

Genetic differentiation and diversity of the populations were also estimated. Results showed that the proportion of the total genetic variation due to differences among populations was 0.128 (theta P = 0.128). According to Jackknifing over loci, theta p was equal to 0.1351±0.0570 indicating that genetic differentiation among populations was about 13%. This result was lower than the differentiation among other populations of this species (18%) as determined by Changtragoon and Boontawee (1999) in two natural populations and two plantations. Genetic distances among populations are shown in Table 4. The present results suggest that natural populations of *D. alatus* in Thailand show considerable levels of genetic divergence, which is useful for future gene conservation and management. However, more allozyme loci and other polymorphic genetic markers such as SSR DNA markers, which have been developed by Ujino, *et al*. (1998) and successfully tested in *D. alatus* recently by Szmidt, A.E. (personal communication) should be used to further investigate genetic diversity, mating system, and gene flow in this species.

Table 4. Matrix of Nei (1978) unbiased genetic distance coefficients at isoenzyme gene loci

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*****															
2	0.0079	*****														
3	0.0047	0.0219	*****													
4	0.0065	0.0036	0.0336	*****												
5	0.0118	0.0222	0.0036	0.0368	*****											
6	0.0090	0.0239	0.0011	0.0390	0.0009	*****										
7	0.0068	0.0020	0.0314	0.0020	0.0294	0.0345	*****									
8	0.0063	-.0005	0.0304	0.0015	0.0313	0.0337	0.0008	*****								
9	0.0146	0.0216	0.0188	0.0182	0.0258	0.0234	0.0244	0.0252	*****							
10	0.0050	0.0294	0.0058	0.0438	0.0067	0.0050	0.0398	0.0392	0.0173	*****						
11	0.0012	0.0197	0.0072	0.0298	0.0098	0.0095	0.0275	0.0262	0.0164	0.0037	*****					
12	0.0078	0.0219	0.0010	0.0362	0.0030	0.0003	0.0339	0.0314	0.0198	0.0042	0.0076	*****				
13	0.0132	0.0207	0.0040	0.0391	0.0037	0.0014	0.0343	0.0305	0.0276	0.0061	0.0099	0.0012	*****			
14	0.0070	0.0221	0.0002	0.0349	0.0032	0.0005	0.0330	0.0311	0.0212	0.0069	0.0088	0.0004	0.0027	*****		
15	0.0005	0.0287	0.0005	0.0398	0.0059	0.0021	0.0402	0.0388	0.0175	0.0051	0.0046	0.0022	0.0065	0.0007	*****	
16	0.0064	0.0104	0.0109	0.0229	0.0104	0.0112	0.0183	0.0162	0.0179	0.0062	0.0030	0.0094	0.0082	0.0120	0.0136	*****

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Genetic Markers for Assessing Genetic Diversity and Improvement of Several Tropical Forest Tree Species to Support Conservation Program

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Abstract. Disturbances caused by human activities such as logging, natural catastrophes, or fragmentation might affect genetic diversity as they might lead to loss of populations, removal of superior genotypes, reduction in remnant population size, changes in density of reproductive individuals, and increased inbreeding and all of these might, in turn, influence a genetic conservation program. Thus, the techniques used to assess genetic diversity must be appropriate in order to be able to arrive at the correct conclusions and recommendations for supporting genetic conservation programs for commercial tropical forest tree species. Attempts have been made to assess genetic diversity of several tropical forest tree species, namely *Paraserianthes falcataria, Scaphium macropodum, Shorea parvifolia, S. laevis, and Eusideroxylon zwagerii* all of which are considered important species in Indonesia. Genetic markers of those species were screened using isozyme, RAPD (Random Amplified Polymorphism DNAs), or AFLP (Amplified Fragment Length Polymorphism) techniques. The AFLP, which is based on the selective amplification of restriction fragments from a total digest of genomic DNA, was applied to generate high-density genetic maps for marker-assisted selection (MAS) of desirable traits. Isozyme analysis of young leaves of *P. falcataria* collected from Parung Panjang (West Java) was conducted to investigate relationships between quantitative and qualitative traits, while leaves of *S. macropodum* collected from Jambi Province, Sumatra were evaluated for evidence of forest fragmentation effects on genetic diversity. RAPD markers were screened to assess genetic diversity of *S. laevis,* while both RAPD and AFLP markers of *S. parvifolia* and *E. zwagerii* from leaves collected in Central Kalimantan have been obtained in addition to the isozyme markers. Results obtained suggest that isozyme markers can be used to detect genetic diversity as well as relationships between quantitative and qualitative traits, and that both RAPD and AFLP markers are efficient for assessing genetic diversity and, eventually for, mapping desirable traits of tropical forest tree species. It was also observed that fragmentation has led to significant reduction of genetic diversity of species studied on certain sites, and that logging has the potential for reducing genetic diversity in species studied. Therefore, it is recommended to pay more attention to activities such as illegal logging and fragmentation when establishing strategies and areas for tropical forest conservation and sustainable utilization as these activities were proven to have a potential to affect genetic diversity.

Introduction

The rate of forest loss in Indonesia, caused mainly by illegal logging, land conversion and forest fire, has been increasing. With tropical forests estimated to contain more than 50% of all terrestrial biodiversity, the way these forests are used can have very significant implications for global biodiversity, and both the direct and indirect benefits resulting from thoughtful conservation and use. Many factors control the process of forest loss, but human activities are the agents of degradation and habitat fragmentation, which disturb habitats of flora, fauna and microbes. These disturbances affect indigenous populations and may cause reduction of genetic diversity and, eventually, loss of regeneration. Such changes to forests are not simply sequential. At a landscape level, different areas of forests may remain undisturbed, while others may have already become fragmented or reduced to residual trees in an agricultural landscape.

The potential impacts of selective logging are, among others: (1) preferential removal of superior genotypes, and (2) increased inbreeding. Genetic impacts may also be expected to vary by species, as related to reproductive ecology. Rare species are more likely to be affected then more common species. The impacts of logging are not necessarily limited to species that are harvested. Non-commercial species are also often affected via a “top down process” in which the largest trees are affected first and this proceeds downward until the targeted reduction in basal area has been achieved. Below the threshold diameter specified by the targeted basal reduction and diameter size distribution of the species, smaller trees are not affected.

Another condition that may potentially affect genetic diversity is forest fragmentation. Direct effects of ecosystem fragmentation on plants include loss of populations, reduction in remnant population size, changes in density of reproductive individuals and increased isolation of remnant populations. Based on this, the general prediction has been that fragmentation will be accompanied by an erosion of intraspecific genetic diversity through generation of genetic bottlenecks, reduction of interpopulation gene flow, increased random genetic drift, and elevated levels of inbreeding (Young 1996). In the short term, loss of individual genetic diversity (heterozygosity) may reduce individual fitness, and through this, lower the population viability. In the longer term, lowered population-level genetic diversity (population and allelic richness) will limit a species’ adaptive abilities.

Both biochemical and molecular markers have been used as powerful tools for assessing genetic diversity and for trait improvement of forest trees. Genetic markers have many potential applications. Uses of molecular markers in forest conservation genetics are for DNA finger printing, to identify hybrids, assessing seed purity (quality control in breeding), estimating gene flow, determining mating systems and paternity, evaluating disease resistant lines, measuring genetic diversity, determining phylogeny, and constructing genetic linkage maps. Some of these uses have been applied to study forest trees in Indonesia, both in primary forests and plantations. In crop plants, with shorter life cycles, the use of markers to genetically improve plants through Marker Assisted Selection (MAS), and to construct genetic maps has been widely applied. Each technique has advantages and disadvantages, and the choice of technique depends on the purpose and on economic considerations. Marker systems are mainly selected based on: number of available loci, degree of polymorphism, dominance, reliability/reproducibility, amount of sample required, automation, cost, ease of assay and multiplexing. Table 1 compares isozymes, RAPDs and AFLPs.

Table 1. Comparison of characters of genetic markers used in the studies

	Isozymes	RAPDs	AFLPs
Theoretical no. loci	30-50	Unlimited	Unlimited
Practical no. loci	30-50	100's	1000's
Degree of polymorphism	Low-moderate	Moderate-high	Moderate-high
Dominance	Co-dominant	Dominant	Dominant
Reliability/reproducibility	Very high	Low-medium	Medium-high
Amount of sample required	Not applicable	5-10 ng	25 ng
Ease of assay	Easy	Easy	Moderately difficult
Automation	Difficult	Yes	Yes

Unlike DNA markers, the expression of isozymes varies between plant tissues but isozymes have the advantage of being technically simpler and inexpensive compared to DNA markers. Although isozymes may yield lower absolute levels of genetic diversity, the patterns of population genetic structure revealed seem to be similar to that from nuclear DNA markers. Isozymes may be inadequate when markers are required to quantify the effects of domestication on genetic resources in some species with low isozyme variability such as *Acacia mangium* (Moran *et al*. 1989). In other situations, because isozymes have only medium levels of genetic diversity, they may not provide enough statistical power when testing hypotheses about changes in levels of genetic diversity.

All DNA markers other than RFLPs are based on Polymerase Chain Reaction (PCR). The RAPD reaction uses only a single, short primer of only 10 bases in length. By chance, sequences matching this primer will occur in many places throughout a large, eukaryotic genome. The technical ease of RAPD markers and the facility of their application to new species has led to their employment in large number of studies in forest trees, both in genetic linkage mapping and population genetic applications (Grattapaglia & Sederoff 1994, Nelson *et al*. 1994). Clearly, these markers have proven very useful, and their use has led to rapid advances in studying the molecular genetics of trees. However, the degree of reproducibility of these markers can sometimes be low, particularly between different laboratories. This is due to the sensitivity of RAPD banding patterns to reaction conditions, and the difficulty in exactly replicating reaction conditions across laboratories where different brands of thermocyclers may be used.

AFLPs are more recent and powerful genetic markers that can be thought of as a combination of RFLP and PCR technology. AFLPs have shown a high degree of reproducibility in contrast to RAPDs, likely due to the use of longer PCR primers, which allows for the use of more stringent conditions in the PCR step. AFLPs, like RAPDs, are dominant markers with only two alleles - the presence or absence of a given band, which can be a result of loss during the evolution of the species. Recently, however, many scientists have claimed that AFLPs are co-dominant markers like isozymes that are indicated by low and high intensity of the bands. Although AFLPs are a relatively recent development, there are already several examples of their application in forest trees (Beismann *et al*. 1997, Gaiotto *et al*. 1997).

Shorea parvifolia and *S. laevis,* which belong to the family Dipterocarpacea, are considered to be important timber species and the dipterocarps are one of the most dominant families in tropical rainforests of Indonesia. Timber from *Shorea spp* can be used for decorative work, furniture, paneling, flooring, and plywood manufacture. Research on *Shorea spp* has been hampered by their very high contents of phenolic compounds, which makes isozyme extraction and molecular biology studies relatively more difficult than with other forest tree species.

Scaphium macropodum Beumee, locally known as tempayang, belongs to the family Sterculiaceae and it is widely distributed in Sumatra. The timber of this species is particularly used for veneer and plywood as well as for interior finishing and furniture. It is sometimes used also as a substitute for oak (*Quercus spp*) and, in India, as a substitute for timber from *Salmalia spp*. It is also suitable for match splints, boxes, and crates (Soerianegara & Lemmens 1993).

Paraserianthes falcataria, which is locally known as sengon, belongs to the family Leguminosae. Because of its characteristics as a fast growing, multipurpose tree, this species, along with several others, has been recommended by the Government of Indonesia to be used in reforestation. The timber can be harvested at 15 years, while fiber for the pulp and paper industries can be obtained age 10.

Eusideroxylon zwagerii, locally known as ulin, is a timber species widely distributed in Kalimantan. This species was chosen to represent the least dense population at our study site in Central Kalimantan, as opposed to the most dominant species, *S. parvifolia*.

The aims of the studies were:

- To establish standard procedures for detecting genetic diversity of several tropical forest tree species and determine their use for tree improvement,
- To assess the impact of logging on the genetic diversity of *Shorea parvifolia*, *Eusideroxylon zwagerii*, and *S. laevis* in Central Kalimantan,
- To evaluate the effects of forest fragmentation on genetic diversity of *Scaphium macropodum* in the Province of Jambi,
- To investigate correlations between quantitative characters and isozyme markers of *Paraserianthes falcataria* in Parung Panjang, West Java.

Materials And Methods

Sample collection

Tissues of trees collected included young leaves or inner bark tissues of mature trees of *Shorea parvifolia*, *S. laevis*, *E. zwagerii*, *Scaphium macropodum,* and *P. falcataria*. The number of samples collected varied depending on the availability of samples in the field and the purpose of the study. Samples of the first three species were collected from both logged and unlogged areas in Wanariset Sangai in Central Kalimantan. Samples of *S. macropodum* were collected from the Silvagama area in Kuamang Kuning (managed by Faculty of Forestry, Gajah Mada University) and in the area of PT. IFA- Barito Pacific Timber Group, which is collaborating with SEAMEO-BIOTROP in Pasir Mayang, Province of Jambi. Samples of *P. falcataria* were collected from provenance/progeny trials in Parung Panjang, West Java, which were established in 1995.

Numbers of samples collected ranged from 600-700 depending on availability and purpose of the study.

Techniques used

The isozyme technique was used for detecting genetic diversity and paternal relationships of *S. parvifolia*, *E. zwagerii*, *P. falcataria* and *Scaphium macropodum,* while DNA-based techniques, both RAPD and AFLP, were used to assess genetic diversity of *S. laevis*, *S. parvifolia* and *E. zwagerii* and to assess that of *S. parvifolia* and *E. zwagerii*.

Extraction and electrophoresis

- Extraction: The extraction buffer used for the isozyme analysis was more complex than that used for DNA extraction (Sudarmonowati *et al.*1997). The extraction buffer for isozyme analysis was a modification of that described by Wickneswari and Norwati (1992), while that for DNA analysis was a modified mini preparation CTAB method described by Harris (1995). Certain compounds included in both extraction buffers that are critical for forest trees were PVP, EDTA and mercapthoethanol. Grinding samples for both isozyme and DNA extraction was conducted in liquid nitrogen to ease the grinding and to prevent oxidation.
- Electrophoresis: separation of isozymes was conducted in 13% potato starch gel in Histidin, MC or Tris citrate buffer depending on the species studied, and run for 5-6 hours while that of DNA was in 1% agarose with TBE electrophoresis buffer and run for 2 hours.
- Major procedures:
    - Isozyme: staining of enzyme systems was carried out for several hours to overnight, while ethidium bromide was added directly in agarose for DNA analysis. Three to seven enzyme systems were studied, and these differed for each species depending on results of preliminary studies. For example, PER, IDH, SDH, MDH, EST, PGI and ACP were used for analyzing *S. parvifolia*, and the first five of these enzyme systems were also used for analyzing *E. zwagerii*, while AAT, SDH and EST were used for analyzing *P. falcataria*.
    - RAPD: 10-mer of oligonucleotides were used as random primers and amplification of DNA fragments was conducted under PCR conditions as follows: 45 cycles of 3 minutes at 94^{o}C, 30 seconds at 94^{o}C, 45 seconds at 36^{o}C, 2 minutes at 72^{o}C and final extension for 4 minutes at 72^{o}C. Samples were maintained at 4^{o}C prior to electrophoresis. Primers used for analyzing *S. parvifolia* were OPB-15, OPE-3, OPF-10; those for *E. zwagerii* were OPB-15, OPB-20, OPE-7 and OPH-20; while those used for *S. laevis* were OPB-7 and OPE-3 of Operon Technologies Ltd., USA.
    - AFLP: steps involved in the analysis were digestion with restriction enzymes EcoRI and MseI, ligation with EcoRI/MseI adaptors, preamplification and selective amplification with pairs of primers (EACC-MCAA, EACA-MCTC, EACA-MCAG, EAGG-MCAC). Conditions for PCR pre-amplification were: 20 cycles of 30 seconds at 94^{o}C, 60 seconds at 56^{o}C, and extension for 60 seconds at 72^{o}C

and maintained at 4°C. Conditions for selective amplification involved three steps. Step I consisted of 1 cycle (30 seconds at 94°C, 30 seconds at 65°C, 1 minute at 72°C), step II consisted of 12 cycles with 0.7°C temperature reduction per cycle, and step III consisted of 23 cycles (30 seconds at 94°C, 30 seconds for 56°C and 1 minute at 72°C). After denaturation and electrophoresis in polyacrylamide gel, samples were stained using silver.

- Data analysis for all techniques: BYOSYS-1 software was used for assessing correlations between growth and isozyme markers, while POPGENE computer software, version 1.21, was used to analyze data obtained from all genetic marker techniques applied. Parameters of genetic diversity observed were number of alleles, effective number of alleles, number of polymorphic loci, percentage of polymorphic loci, Nei's gene diversity, and Shannon's index.

Results

Protocol establishment

Three protocols were established which seemed to be appropriate for a wide range of forest tree species. (A) isozyme analysis for *S. parvifolia*, *E. zwagerii*, *Scaphium macropodum* and *P. falcataria;* (B) RAPD analysis for *S. laevis*, *S. parvifolia,* and *E. zwagerii;* (C) AFLP analysis for *S. parvifolia* and *E. zwagerii*. It was observed that some major critical factors influenced results. For example, the addition of PVP to the extraction buffer and double centrifugation were necessary to obtain good resolution for isozyme analysis, while the composition of PCR reaction and PCR conditions were critical for both RAPD and AFLP analysis. All optimum conditions developed were appropriate for the species studied. For example, the same composition of extraction buffer could be applied for all species, the same number of cycles could be applied for RAPD analysis of *S. laevis*, *S. parvifolia*, and *E. zwagerii*, and the same number of cycles and PCR conditions for AFLP analysis of both *S. parvifolia* and *E. zwagerii* were adequate. It was also observed that certain RAPD primers gave high numbers of scorable bands and major bands in all three species studied (S. *laevis*, *S. parvifolia,* and *E. zwagerii*) and two of them, i.e. OPB-15 and OPE-3 indicated they could be used for analyzing a broad range of tropical forest tree species. Table 2 shows the number of scorable bands of two of the species studied as generated from isozyme, RAPD and AFLP techniques.

Table 2. Range of scorable bands generated from *S. parvifolia* and *E. zwagerii* using isozyme, RAPD and AFLP techniques.

Techniques	Scorable bands from *S. parvifolia*	Scorable bands from *E. zwagerii*
- Isozyme	3-4	3-6
- RAPD	10	9-10
- AFLP	101-182	10-81

The very low cost of isozymes, along with their ease of use and the speed with which data can be obtained, combine to make the system very attractive for obtaining markers for many conservation genetics applications. Hence it would seem that an efficient strategy would be to use isozymes wherever possible as the first tool for investigating the specific conservation genetic question at hand. DNA markers could then be used to supplement information obtained from isozymes whenever the number of loci and/or the degree of polymorphism are insufficient. A combination of techniques was reported to be useful in obtaining more valid data as reported by Ayres and Ryan (1997) in their work with *Wyethia reticulata,* which combined isozyme and RAPD markers.

The establishment of an AFLP protocol for assessing genetic diversity will benefit tropical tree genetic improvement in the future, as markers generated from this technique are comparably higher than those from isozyme, RAPD and RFLP (Restricted Fragment Length Polymorphism) systems. All techniques developed have potential value for assessing genetic diversity, which is highly important to developing an appropriate strategy for both *ex situ* and *in situ* conservation programs.

Correlation between morphological performance and isozyme markers of P. falcataria.

There were correlations between morphological characters and isozyme markers in several of the results obtained. Based on three enzyme systems studied (AAT, EST and SDH), there was a significant positive correlation for growth rate in locus EST-2, allele 22 in the Kediri subpopulation, and in locus EST-3 alleles 11, 12 and 22 in the Wamena subpopulation. Slow growth was correlated with locus EST-2 (alleles 11, 22) in the Jonggol subpopulation. This indicated that the distribution of loci was not uniform and significantly different in terms of growth for subpopulations, and that there was a close correlation between certain loci and the growth of *P. falcataria*. Similar research with *Calamus* *manan* indicated that there were five loci correlated with the growth of seedlings of that species (Salwana *et al*. 1996). By identifying the linkage between quantitative characters and isozyme markers, tree improvement programs could be initiated at very early ages, thus contributing to more efficient use of both human resources and finances. In addition to provenance considerations, information on genetic linkage would assist conservation programs and sustainable utilization of *P. falcataria* by guiding development of appropriate plantation programs in Indonesia.

Impact of logging on genetic diversity of *S. laevis, parvifolia* and *E. zwagerii*

Results based on isozyme, RAPD, and AFLP analyses of *S. parvifolia* and *E. zwagerii* indicated that logging has not had significant effects on reducing genetic diversity of either species. However, results suggested that it may effect future genetic diversity, which means that precautions must to be taken. This study also indicated that the three techniques used led to the same conclusion, although they gave slightly different results in terms of clustering or grouping. The differences found between the techniques for genetic relatedness between individuals and for variation patterns of individuals within the group, were presumably due to differences in evolution rates which affect the genetic variation pattern (Baruffi *et al*. 1995). According to Williams *et al*. (1990) the DNA sampling region for the RAPD assay is less responsive to selection, which leads to a higher degree of resistance to mutation than DNA coding for isozymes. The different results obtained from RAPD and from AFLP were probably due to the result of random sampling of total genome and the result of functional protein (Peakall *et al*., 1995). It is suggested that the use of these techniques can be applied complementary to each other. Initial analysis can be carried out using isozyme or RAPD, then AFLP may be used for further analysis to determine those groupings still not clear. Combinations of various markers will give a better estimate of genetic correlation between individuals and could produce a genetic structure hierarchy based on empirical data.

Results also suggest that, based on RAPD and AFLP analysis of S. *parvifolia* and *E. zwagerii*, there were no significant differences between logged and unlogged areas in all parameters of genetic diversity observed although all values for *E. zwagerii* were reduced in the logged area (Table 3). Logging practices are therefore an important factor in terms of decreasing genetic diversity in natural populations. Threshold values need to be determined for each species and used for implementation of appropriate conservation programs for Indonesian species.

Table 3. Values of genetic parameters of *E. zwagerii* based on RAPD analysis

Parameters	Unlogged	Logged
1. Observed number of alleles	1.9737	1.9434
2. Effective number of alleles	1.0900	1.0672
3. Gene diversity	0.0786	0.0595
4. Number of polymorphic loci	37	36
5. Percentage of polymorphic loci	97.37	1.1265

Results based on RAPD analysis using two primers screened from 109 random primers of *S. laevis* showed that a considerable amount of genetic variation can be found in natural populations. The results were reproducible, indicating that RAPD could serve as a good technique for relative comparisons as long as it could be standardized in one lab. This information could be utilized for *ex situ* and *in situ* conservation programs when the degree of genetic variation is known.

Impact of fragmentation on genetic diversity of Scaphium macropodum

Based on analysis of 6 enzyme systems, i.e. IDH, MDH, ME, PGD, PGI, and SDH there was a significant difference in genetic diversity between S. *macropodum* grown in Pasir Mayang, which represents a site linked with continuous forest cover, and those grown at one site, Silvagama in Kuamang Kuning, which represents a fragmented forest in Jambi Province (Table 4). The difference in genetic diversity between these two sites might be caused by the different ages of the trees from which samples were collected. Younger trees most probably grew in after the site was fragmented, while more mature trees (>60 cm in dbh) were growing before the fragmentation process. Data would probably be more reliable if only mature trees were studied, but there were a very limited number of mature trees available on the study sites. Work on *Acer saccharum* indicated that populations in mosaic fragments were more diverse than those under continuous forest cover although extreme spatial separation led to a reduction in genetic diversity (Young & Meeriam 1995). Other work on other species concluded that fragmentation placed species at great risk due to reduced gene flow (Ackerly *et al.* 1990). Some data provide evidence for the existence of a fragmentation threshold, with regard to remnant population size and isolation, below which reductions in genetic diversity are apparently not encountered. The relationship between population size and allelic richness for *Scabiosa columbaria* suggests that remnant populations larger than 200-300 individuals maintain high diversity, while those lower than this do not. If reductions in allelic richness are primarily attributable to initial sampling effects, then further losses due to genetic drift may still be expected, and current levels of diversity may provide little indication of the long term potential for remnant populations to maintain genetic diversity. At smaller scales, rather than resulting in increased genetic isolation, fragmentation leads to increased interpopulation gene flow, a breakdown of local genetic structure, and possibly even elevated population-level genetic diversity. Fragmentation may lead to reduced heterozygosity either as a function of reduced population level variation, and/or as a result of increased inbreeding. As with population-level variation, current empirical data suggest that reductions in population size due to ecosystem fragmentation are accompanied by reductions in average heterozygosity. Consequently, conservation programs need to be implemented in the near future in those areas wherein forests have been fragmented or are faced with anticipated fragmentation, as negative effects have been shown for certain species studied in Sumatra.

Further research is required to obtain more accurate data by employing more species, different histories of fragmentation, and varying physical conditions.

Table 4. Estimates of genetic diversity in three sites in Jambi Province for *Schapium macropodum*

Parameters	Pasir Mayang	Silvagama 1, Kuamang Kuning	Silvagama 2, Kuamang Kuning	Silvagama Poole (S1+S2)
-Number of allele per locus	2.1176	2.000	1.5000	2.1429
-Effective no. of alleles per locus	1.8792	1.6813	1.3507	1.8692
-Percentage of polymorphic loci	88.27	64.71	29.41	70.59
-Number of polymorphic loci	15	11	5	12
-Observed of heterozygosity	0.2409	0.1852	0.1071	0.1599
-Expected heterozygosity	0.4314	0.4007	0.2459	0.4610
-Shannon's information index	0.6261	0.5321	0.2819	0.6338

Conclusions and Recommendation

Isozyme, RAPD, and AFLP techniques were well suited for all species studied, which indicates that the techniques developed could be used, with or without slight modifications, for assessing genetic diversity in relation to conservation programs for a broader range of tropical forest tree species. Genetic diversity of S. *parvifolia*, *S. laevis*, *E. zwagerii* and *S. macropodum* and linkage between certain phenotypic characters with genetic marker in *P. falcataria* could be assessed using either isozyme, RAPD, or AFLP techniques. As an appropriate AFLP technique has been developed for two tree species, construction of a genetic map could be made, since the number of scorable bands was extremely high as compared to that obtained with isozyme and RAPD techniques. The superiority of this technique is beneficial for the development of commercial tropical forest tree breeding, as it will give strong fundamental information on important traits.

Comparison between the three techniques - isozyme, RAPD, and AFLP - when used for analyzing one species led to the same conclusion for assessing the impact of logging on genetic diversity. However, the grouping shown in the dendrogram was slightly different in that the genetic diversity values were reduced in the logged area compared with the un-logged area. For handling large numbers of samples when time is limited, RAPD may be applied first for assessing genetic diversity as long as the procedure is established in one lab. But if the goal is to determine outcrossing rate or mating system in regard to developing a conservation strategy based on the status of inbreeding in a species or population, then isozyme markers would be the first choice.

Isozyme techniques could be used to detect linkages between growth and quantitative characters of *P. falcataria*. The correlation was significant in certain loci and in certain subpopulations. Development of species should consider not only performance during provenance trials but also during genetic tests, as good growth correlates with certain isozyme patterns.

Although the impact of logging on genetic diversity of *S. parvifolia* and *E. zwagerii* in Central Kalimantan was not significantly different, appropriate conservation programs should be undertaken as it indicates that genetic diversity may be reduced in the near future. Strengthening existing forest conservation reserves may be a first step to combating illegal logging in Indonesia. Stronger recommendations could be proposed if there were further studies to obtain more valid data. Future research must consider different logging and fragmentation histories, a broader range of environmental and physical conditions, and inclusion of more species.

Fragmentation gave significantly different effects in terms of genetic diversity of *S. macropodum,* but only between that grown in Pasir Mayang and one site of Silvagama in Kuamang Kuning, in Jambi Province. Forest fragmentation in Jambi Province and on the entire island of Sumatra is mainly due to land clearing for settlement and transmigration, as well as to land conversion for agriculture, and it has to be controlled. Further research involving more species, various histories, and various physical conditions is needed to contribute in formulating conservation action plans in Sumatra and in Indonesia.

One factor to consider in decision making for genetic conservation is how effective the plan for management might be. The management options may involve only locking up the resource in some kind of reserve, or leaving it alone. The decision is to create a reserve or not, and the problem for the conservationist is to estimate the relative probability of sufficient survival of the resource with and without the reserve. For other conservation programs, more options may be available, but each has its own cost and probability for achieving the desired results and values. The benefit of each option may then be estimated for both present and future values to society.

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Mating System Parameters of *Dryobalanops Oblongifolia* Dyer. (Dipterocarpaceae) Planted in Peninsular Malaysia

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Abstract. An allozyme analysis of a progeny array of Dryobalanops oblongifolia planted in Peninsular Malaysia provided quantitative estimates of outcrossing rates for this species in a plantation environment. Under those conditions, D. oblongifolia has a mixed mating system, with approximately 26% of the seeds produced from selfing. The mean single outcrossing rate (t_s) for four loci was 0.630, while the multilocus outcrossing rate (t_m) was 0.741. Heterogeneity of individual multilocus outcrossing rates was prevalent, ranging from 0.48 to 0.99, with a mean of 0.77. As expected, violations from a mixed mating model were evident from the differences in pollen and ovule allele frequencies, and heterogeneity in pollen pools. The progeny inbreeding coefficient estimated with the assumption of inbreeding equilibrium was 0.149. A moderate outcrossing rate and a significant inbreeding coefficient might suggest that the species is self-compatible. Genetic substructuring, in terms of biparental inbreeding (detectable by the difference between multilocus and single locus outcrossing rates) was significant ($t_m - t_s = 0.111$). This may indicate that the plantation was established using closely related seed sources.

Introduction

Type of mating system is one of the most important life history traits that determine the genetic structure and evolutionary processes of a plant species. Generally, outcrossing species maintain more genetic diversity within populations and exhibit less genetic differentiation among populations than do selfing species (Brown 1979, Hamrick & Godt 1989). However, biparental inbreeding (mating among relatives) can occur even within outcrossing populations, particularly if seed dispersal is local. Many tropical tree species possess mechanisms that ensure or encourage outcrossing (e.g. Chan 1981, Bawa *et al.* 1985, Dayanandan *et al.* 1990, Murawski & Hamrick 1990, Murawski *et al.* 1994a, Lee 2000, Lee *et al.* 2000a).

Dryobalanops oblongifolia, locally known as Keladan, belongs to the Dipterocarpaceae, the main timber family in the tropical forests of Southeast Asia. This species occurs on the islands of Borneo and Sumatra and also in Peninsular Malaysia (Appanah & Weinland 1993). In Peninsular Malaysia, it is more common in the eastern states of Kelantan, Terengganu, Pahang, and Johor. It commonly occurs in low-lying, poorly drained, and frequently inundated sites (Appanah & Weinland 1993). The shaggy purple-brown bole, together with its oblong and aromatic leaves, easily distinguishes the species from other dipterocarps. The tree is big - often >100 cm in diameter at breast height (dbh), with a large buttress, and a tall but crooked bole. The bark sheds in long thin flakes. The flowers resemble those of *D. aromatica*, but are slightly smaller. Appanah (1981) reported that this species flowers gregariously, presenting large numbers of attractive flowers that are visited by swarms of bees (*Apis dorsata*). The fruit is subspheroid or obovate, enclosed at the base by the 5-lobed calyx tube. and dispersed by gravity. The regeneration is abundant. In plantations, keladan grows rapidly and attains a dbh of 30 cm in 15 years (Appanah & Weinland 1993). The timber is categorized as Medium Hardwood and can be used for construction, posts, beams, and joists. On the east coast of Peninsular Malaysia, it is used to make sampans.

Of 470 species of Dipterocarpaceae, multilocus outcrossing rates have been quantified for only seven: *Shorea congestiflora* and *S. trapezifolia* (Murawski *et al.* 1994a); *Stemonoporus oblongifolia* (Murawski & Bawa 1994); *Shorea megistophylla* (Murawski *et al.* 1994b); *Dryobalanops aromatica* (Kitamura et al. 1994; Lee 2000); *Shorea leprosula* (Lee e*t al.* 2000a) and *S. macrophylla* (Ng *et al.* 2001). Information on type of mating system is important to genetic studies in both natural and artificial populations. In natural populations, knowledge of the mating system is necessary to understand the distribution of genetic variation within and among individuals, gene flow within and among populations and the resultant substructuring of populations, all of which are crucial for the proper design of sampling strategies for tree improvement and genetic conservation programs. However, for artificial populations, such as seed orchards, knowing the mating system is important for assessing potential genetic improvement and probabilities of contamination from external sources (Moran *et al.* 1989, Lee 2000, Lee *et al.* 2000a).

To date, no quantitative study of the mating system in *Dryobalanops oblongifolia* has been reported, and no information is available on actual outcrossing rates under natural or artificial conditions, or on the extent of inbreeding occurring either through apomixis, selfing or biparental mating. The mass fruiting season of *Dryobalanops* species in June 1998 provided a good opportunity for a quantitative study on *D. oblongifolia*.

Materials and Methods

Sampling

This study was conducted in an artificial forest located in the Forest Research Institute Malaysia (FRIM). This 200 ha forest, which is surrounded by primary and secondary forests, was established in 1927 and it includes *D. oblongifolia* and various other dipterocarp and nondipterocarp timber tree species. Thirty-nine to 40 open-pollinated seeds were collected from each of eight single progeny families using the "shaking-catch" method as described by Lee *et al.* (2000b).

Electrophoresis

Enzyme extraction and starch gel electrophoresis of germinated embryo tissues was carried out following the procedure described by Lee *et al.* (2000b). Mating system parameters were estimated using four polymorphic allozyme loci (*Gpi, Pgm, Me* and *Ugp*) based on their Mendelian segregation patterns. Genotypes of maternal parents were determined directly *via* electrophoresis of adult inner bark tissue.

Genetic Analysis

Mating system parameters were estimated by using the MLTR computer program of Ritland (1994) for a mixed-mating model. From progeny array data and through maximum likelihood procedures, the program simultaneously estimated; (1) multilocus population outcrossing rate (t_m) by the Newton Raphson method; (2) the average single locus population outcrossing rate (t_s): (3) the average single locus inbreeding coefficient of maternal parents (F); (4) the pollen and ovule allele frequencies (p and o) by the expectation maximization method; and (5) variances of the above using the bootstrap method where the progeny array (within families) is the unit of resampling (250 bootstraps were used). Difference between t_m and t_s (biparental mating) was also tested using Student t-test as $t = (D - 0) / S_D$, where D is the mean difference between t_m and t_s, and S_D as the mean standard deviation. Homogeneity of the pollen pool over all the maternal trees was tested by chi-square distribution statistics as $\chi^2 = NG_{st} (A-1)$, where N is the total number of pollen gametes, G_{st} is the proportion of among-tree variance in pollen allele frequencies relative to the total variance in pollen allele frequencies, and A is number of alleles at a locus (James *et al.*

1998). Degrees of freedom were (M–1), where M is the number of maternal tree examined. Differences between pollen and ovule frequencies were tested using $\chi^2 = 2NF_{st}(A-1)$, where N is the sum of the number of pollen and ovule gametes, F_{st} is the genetic diversity between pollen and ovule pools, and $(A-1)$ are the degrees of freedom (Murawski & Hamrick 1992). The inbreeding coefficient of seedlings was calculated from the estimation of outcrossing rate (t_m) as $F_e = (1-t_m) / (1+t_m)$ (Ritland & Jain 1981, Ritland 1983).

Results and Discussion

The multilocus outcrossing rate (t_m) was 0.741 ± 0.068 (Table 1), suggesting that the species, under plantation conditions, exhibited a mixed mating system. This observed value is lower than the reports on other Dipterocarpaceae species in natural environments, such as *S. congestiflora* (t_m = 0.87, Murawski *et al.* 1994a), *D. aromatica* (mean t_m = 0.82, Kitamura *et al.* 1994; t_m = 0.92, Lee 2000), *S. megistophylla* (t_m = 0.81, Murawski *et al.* 1994b), *Stemonoporus oblongifolius* (t_m = 0.84, Murawski & Bawa 1994), and *S. leprosula* (t_m = 0.84, Lee *et al.* 2000a). However, for other forest types, this value is comparable; e.g., *S. megistophylla* (t_m = 0.71 for a selectively logged population, Murawski *et al.* 1994b), *D. aromatica* (t_m = 0.77 for a logged forest, 0.55 for a seed orchard, and 0.66 for an artificial forest, Lee 2000), and *S. macrophylla* (t_m = 0.61 for a planted population, Ng *et al.* 2001). Availability of pollinators might be the main factor that can influence outcrossing events. According to Lee (2000), density of pollinators might be higher in natural populations (where the breeding system evolved) than in plantation populations. In addition, the climatic and weather conditions in the plantation environment may affect the timing of floral development resulting in lack of flowering synchrony, which can serve to reduce the effective breeding population size.

Table 1. Outcrossing rates and inbreeding coefficients for *D. oblongifolia.*

No. of seeds examined	Mean t_s (SE)		t_m (SE)	$t_m - t_s$ (SE)	F_e*
	Locus	t_s			
302	*Gpi*	0.505 (0.170)	0.741 (0.068)	0.111 (0.026)	0.149
317	*Pgm*	0.212 (0.161)			
308	*Me*	0.968 (0.181)			
310	*Ugp*	0.718 (0.132)			
	Unweighted mean	**0.630 (0.074)**			

* Inbreeding coefficients of seedlings if FRIM population is in inbreeding equilibrium. $F_e = (1 - t_m) / (1 + t_m)$ where t_m is the multilocus outcrossing rates. Standard error is not calculated.

Allele frequencies in the pollen and ovule pools contributing to the sampled progeny differed significantly ($p < 0.05$) for *Gpi*, *Me*, and *Ugp* (Table 2). This could be due to pollen coming from outside, unequal male and female contributions among adult trees within the population, occurrence of selection after fertilization, nonrandom mating of genotypes during outcrossing events, and the existence of mating among relatives (Ritland & Jain 1981). Homogeneity of pollen pool gene frequencies over female parents was significant at $p < 0.05$ for *Gp*i, *Pgm*, *Me* and *Ugp* (Table 3). This may indicate that the maternal trees did not receive pollen at random from all synchronously flowering trees, but rather received a significant proportion of their pollen from relatively few individuals nearby.

Table 2. Maximum likelihood estimates of pollen and ovule allele frequencies and chi square statistic to test the differences between pollen and ovule frequencies in the progeny of *D. oblongifolia*.

Locus	*N*	*A*	Pollen	Ovule	F_{st}	DF	$\chi^{2(a)}$
Gpi	302	1	0.298	0.375			
		2	0.274	0.313			
		3	0.147	0.125			
		4	0.282	0.188	0.006	3	10.451*
Pgm	317	1	0.657	0.611			
		2	0.309	0.278			
		3	0.030	0.056			
		4	0.004	0.056	0.003	3	6.0302
Me	308	1	0.566	0.421			
		2	0.351	0.421			
		3	0.053	0.053			
		4	0.022	0.053			
		5	0.009	0.053	0.012	4	29.478*
Ugp	310	1	0.095	0.053			
		2	0.190	0.159			
		3	0.318	0.158			
		4	0.223	0.368			
		5	0.109	0.158			
		6	0.061	0.053			
		7	0.004	0.053	0.017	6	63.226*

(a) $\chi^2 = 2NG_{st}(A - 1)$, where N = number of seeds examined, G_{st} = genetic differences between pollen and ovule pool, A = number of alleles and $(A-1)$ = degree of freedom (DF).

*Significance levels are $p < 0.05$.

Table 3. Test of homogeneity of the pollen pool over all the maternal trees.

Locus	*N*	*A*	*Allele frequencies*								G_{st}	DF	$\chi^{2\,(a)}$
			fam1	fam2	fam3	Fam4	fam5	fam6	fam7	fam8			
Gpi	302	1	0.34	0.73	0.05	0.45	0.04	0.55	0.03	0.36			
		2	0.15	0.06	0.84	0.07	0.87	0.35	0.28	0.54			
		3	0.46	0.15	0.08	0.45	0.01	0.05	0.03	0.05			
		4	0.05	0.06	0.03	0.04	0.08	0.05	0.66	0.05	0.318	7	288.22*
Pgm	317	1	0.66	0.03	0.74	0.86	0.98	0.14	0.83	0.78			
		2	0.24	0.81	0.15	0.07	0.01	0.76	0.12	0.17			
		3	0.05	0.09	0.08	0.03	0.00	0.05	0.02	0.02			
		4	0.05	0.03	0.03	0.03	0.00	0.05	0.02	0.02	0.313	7	297.74*
Me	308	1	0.42	0.77	0.62	0.18	0.22	0.26	0.50	0.75			
		2	0.44	0.12	0.23	0.65	0.72	0.52	0.17	0.17			
		3	0.05	0.05	0.03	0.10	0.03	0.11	0.02	0.02			
		4	0.05	0.03	0.10	0.03	0.02	0.05	0.02	0.02			
		5	0.05	0.03	0.03	0.03	0.02	0.05	0.02	0.02	0.538	7	662.61*
Ugp	310	1	0.05	0.07	0.03	0.10	0.01	0.27	0.10	0.05			
		2	0.23	0.19	0.50	0.27	0.01	0.09	0.03	0.05			
		3	0.19	0.37	0.08	0.49	0.63	0.39	0.10	0.35			
		4	0.24	0.03	0.26	0.03	0.34	0.04	0.52	0.25			
		5	0.19	0.03	0.05	0.03	0.00	0.13	0.13	0.25			
		6	0.05	0.27	0.05	0.03	0.00	0.04	0.10	0.02			
		7	0.05	0.03	0.03	0.03	0.00	0.04	0.03	0.02	0.127	7	236.02*

[(a)] $\chi^2 = 2NG_{st}(A - 1)$, where N = number of seeds examined, G_{st} = genetic differences between pollen and ovule pool, A = number of alleles and $(A-1)$ = degree of freedom (DF).
*Significance levels are $p < 0.05$.

Single locus estimates of outcrossing value ranged from 0.212 to 0.968, with an unweighted mean (average single locus outcrossing rate) of 0.630 (Table 1). Estimation of population outcrossing rates suggested that there was a substantial level of inbreeding. This was further supported by the inbreeding coefficient based on the assumption of inbreeding equilibrium, which was estimated as 0.149 (Table 1). Inferences about inbreeding other than selfing (i.e., biparental mating) can be made from the comparison between multilocus and average single locus outcrossing rates. Comparison of these two values in Table 1 showed that the multilocus outcrossing estimated was significantly higher than the average single-locus estimates ($t_m - t_s = 0.111 \pm 0.026$, $p < 0.05$). This may indicate that the plantation was established using closely related seed sources.

In conclusion, *D. oblongifolia* planted at FRIM exhibited a moderate level of outcrossing rate and a significant level of inbreeding (either due to selfing or biparental mating). The significant level of selfing rate might suggest that the species is self-compatible. The moderate level of outcrossing rate may indicate the nature of this species, which exhibits mixed-mating system, or it may be due to an unsuitable environment for promoting wide-scale development of pollinator populations in this forest plantation compared to a natural forest environment. The significant level of biparental mating may indicate that there is mating among relatives, and strongly proved that the plantation might have been established using closely related seed sources. Future studies are needed to compare these results with other estimates based on natural populations.

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Estimation of Genetic Variation of *Shorea leprosula* in the Hedge-Orchard of the Inhutani I Dipterocarp Center East Kalimantan using DNA markers

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Abstract. Genetic variability of *Shorea leprosula* in one of the hedge-orchards in the Dipterocarp Center, East Kalimantan was investigated using RAPD (random amplified polymorphic DNA) and microsatellite markers. PT Inhutani I established the hedge-orchards to produce cutting materials for their operational plantation program. Eighty-four polymorphic RAPD markers obtained by 26 arbitrary 10-mer primers were used for analyzing the genetic relationship among 20 individual trees in the hedge-orchard and 4 in the ITTO gene conservation site in East Kalimantan. Genetic distances between individuals (d) calculated from RAPD data varied from 0.28 to 0.76 (mean d = 0.54). UPGMA cluster analysis using genetic distance indicated that there are some genetically similar individuals in the hedge-orchard. Microsatellite analysis was also carried out using 4 previously reported markers. Mean expected heterozygosity (H_e) was 0.686 within hedge-orchard. This value was slightly smaller than that of the Jambi population (0.710) in Sumatra (Rimbawanto and Isoda 2001). Allele frequency was largely differing in all loci. These results indicate that selective collection of wildlings in the field and selection in the nursery for better growth could result in changing the genetic characteristics of the population.

Introduction

Inhutani I has conducted plantation of meranti species widely throughout East Kalimantan. One of the main species for plantation is *Shorea leprosula* since this species is the dominant species in the area. Although some of the materials for plantation are from seedlings or wildlings, many are from cuttings because the amount and period of seed production of this species is not sufficient enough to provide seedlings for large-scale plantation. In order to produce a certain numbers of cuttings, hedge-orchards were established using wildlings collected from the natural population.

The current concern is the genetic variation in those source materials. As the initial step of the hedge-orchard's establishment, wildling plants were collected from the natural population and grown in the nursery. In the nursery, the plants were selected according to initial growth rate. This selection might have reduced their genetic variability. When considering operational plantations, it is preferred to have higher genetic gain and uniform quality. It is also important to keep genetic diversity as high as possible. It is, however, sometimes difficult to obtain both requirements. Therefore, estimating the genetic diversity of materials for the operational plantings is necessary for designing better schemes for sampling and selection.

In this study, DNA analysis using RAPDs (random amplified polymorphic DNAs, Williams *et al.* 1990) and microsatellites were conducted to evaluate the genetic diversity in one of the hedge-orchards in the Dipterocarp Center, East Kalimantan.

Materials and Methods

Plant materials and DNA extraction

Leaf materials were collected from 28 trees of *S. leprosula* planted in a hedge-orchard in the Dipterocarp Center, Inhutani I, Batuampar, East Kalimantan, Indonesia. They were dried and preserved in silica gel. Total genomic DNA was extracted from *ca.* 100mg of leaves using a modified protocol (Shiraishi & Watanabe 1995) of the CTAB method (Murray & Thompson 1980). For RAPD analysis, DNA was purified using Wizard Clean Up System (Promega).

Microsatellite analysis

Four microsatellites (Shc01, Shc03, Shc04, Shc09) developed for *S. curtisii* (Ujino *et al.* 1998) were applied to *S. leprosula*. For each microsatellite, fluorescence labeled forward primers and non-labeled reverse primers were synthesized according to the reported primer sequences (Ujino *et al.* 1998) and used for PCR amplification. Each 10 μL reaction contained 1x PCR buffer (supplied with AmpliTaq Gold DNA polymerase, Perkin Elmer), 2.0 mM $MgCl_2$, 200 μM each dNTP, 0.5 μM each Primer, 0.5 unit AmpliTaq Gold DNA polymerase (Perkin Elmer), and 25 ng template DNA. PCR amplification was performed at 94°C for 10 min, followed by 35 cycles at 94°C for 30 s, 50-60°C for 30 s, 72°C for 60 s, followed by 1 min at 72°C using a GeneAmp PCR System 9700 (Perkin Elmer). Annealing temperature at the initial cycle was 60°C, and it was decreased 1°C / cycle for the following 9 cycles. After the initial 10 cycles, annealing temperature was fixed at 50°C. PCR products were electrophoresed using ABI 310 Genetic Analyzer (PE Applied Biosystems). The lengths of PCR products were determined using GeneScan and Genotyper software (PE Applied Biosystems).

RAPD analysis

Twenty-six arbitrary decamer primers (Operon Technology) were used for RAPD analysis. Each 10 µL reaction contained 1x PCR buffer (supplied with AmpliTaq DNA polymerase stoffel fragment, Perkin Elmer), 3 mM $MgCl_2$, 200 mM each dNTP, 0.25 µM Primer, 0.5 unit AmpliTaq DNA polymerase Stoffel Fragment (Perkin Elmer), and 10 ng template DNA. PCR amplification was performed at 94°C for 1 min, followed by 45 cycles at 94°C for 30 s, 37°C for 30 s, 72°C for 90 s, followed by 7 min at 72°C using a GeneAmp PCR System 9600 (Perkin Elmer). PCR products were electrophoresed in 1.5% agarose gel in 0.5 x TBE and stained with ethidium bromide for visualization on a UV (302 nm) transilluminator.

Data analysis

Microsatellite alleles were determined for each locus according to the length of PCR products. Total number of alleles (N_a), effective number of alleles (A_e), expected and observed heterozygosity (h_e and h_o respectively), and measurement of deviation from Hardy-Weinberg expectation (F_{IS}) were calculated (Nei 1987).

RAPD bands were scored as present (1) or absent (0), and a (1/0) data matrix was made. Pair wise comparisons of genetic similarities were calculated using Nei and Li's similarity coefficient *F*, (Nei & Li 1979) and genetic distances (*d*) were estimated as $d = 1 - F$ using RAPDistance computer program (Armstrong *et al.* 1994). The UPGMA cluster analysis was carried out using PHYLIP ver. 3.57c (Felsenstein 1995).

Results

Microsatellite analysis

Twenty-eight individuals from the hedge-orchard were analyzed using 4 microsatellite loci and compared with data from the ex situ conservation site in Jambi, Sumatra (Rimbawanto & Isoda 2001) as a reference of the natural population. The number of alleles was 12, 6, 8, and 12 for Shc01, Shc03, Shc04, and Shc09 respectively. The comparison of allele frequency with the Jambi population showed quite a large difference (Figure 1). In Shc01, the second to sixth major alleles in the Jambi population were missing in the hedge-orchard material, but the frequency of the first major allele was largely different (0.679 and 0.151 in hedge-orchard and Jambi population respectively). Similarly in

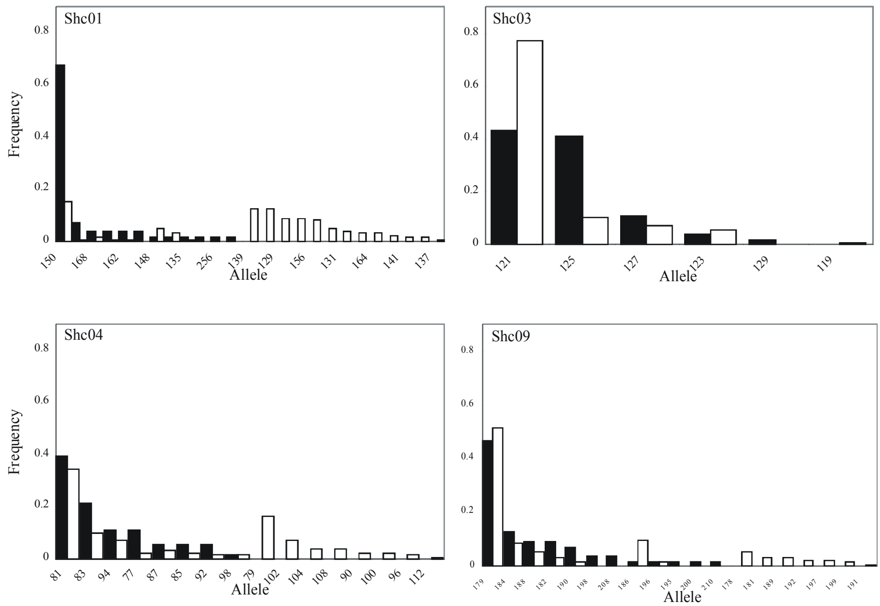
Shc04 and Shc09, second major allele and some other alleles were missing in the hedge-orchard. In Shc03, two major alleles were detected in the hedge-orchard, whereas only one major allele was detected in the Jambi population. Large differences between the two populations were also found in genetic diversity measurements. In the hedge-orchard, the effective number of alleles (A_e) and expected heterozygosity (h_e) was much lower in Shc01, but much higher in Shc03 (Table 1). The mean expected heterozygosity over 4 loci was somewhat smaller within the hedge-orchard (H_e = 0.686) than in the Jambi population (H_e = 0.710, Rimbawanto & Isoda 2001). Significant deviation from Hardy-Weinberg expectation (F_{IS}) was not detected in any of the 4 loci in the hedge-orchard.

Table 1. Summary statistics for 4 microsatellite loci, total number of allele (N_a), expected and observed heterozygosity (h_e and h_o, respectively), effective number of alleles (A_e) and measurement of deviation from H-W

		N	N_a	A_e	h_e	h_o	F_{IS}	Chi test
Shc01	Hedge-orchard	28	12	2.1	0.527	0.464	0.1197	NS
	Jambi[1]	63	24	11.5	0.913	0.746	0.1826	NS
Shc03	Hedge-orchard	28	6	2.7	0.635	0.571	0.0995	NS
	Jambi[1]	65	5	1.6	0.391	0.400	-0.0242	NS
Shc04	Hedge-orchard	28	8	4.3	0.768	0.536	0.3023	NS
	Jambi[1]	65	20	5.8	0.828	0.585	0.2937	*
Shc09	Hedge-orchard	28	12	3.9	0.744	0.821	-0.1046	NS
	Jambi[1]	64	19	3.4	0.707	0.672	0.0499	NS
Mean	Hedge-orchard	44	12.4	4.6	0.686	0.589	0.1417	NS
	Jambi[1]	64	17.0	5.6	0.710	0.601	0.1535	NS

[1] Rimbawanto et al. 2001

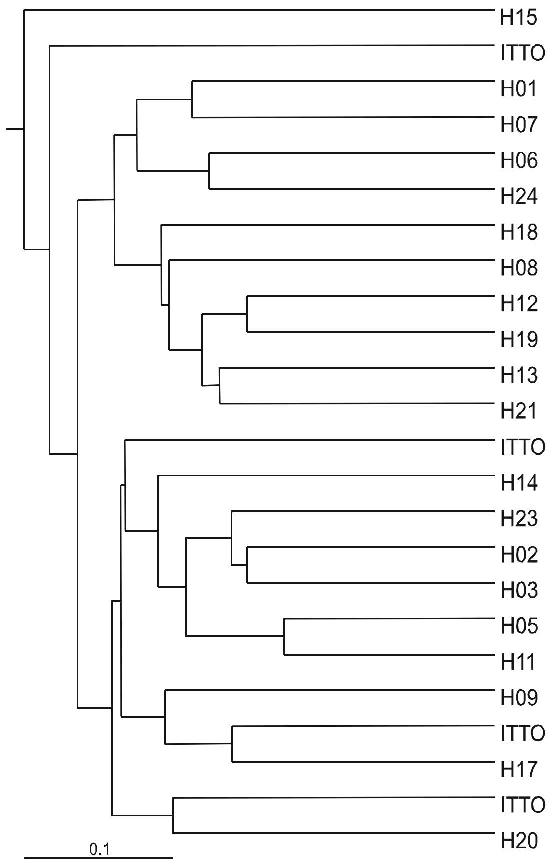
Figure 1. Allele frequency of four microsatellite loci. The black bars indicate the frequencies in hedge-orchard and the white bars indicate those in Jambi population. Alleles were arranged according to the frequency in hedge-orchard.



RAPD analysis

Twenty individuals from the hedge-orchard and 4 individuals from the ITTO's *ex situ* conservation site in Batuampar, East Kalimantan were used for RAPD analysis. Twenty-six RAPD primers produced 84 polymorphic bands. The mean genetic distance between individuals (mean *d*) was 0.54. The value varied from 0.28 to 0.76 in the hedge-orchard and from 0.5 to 0.59 in the *ex situ* conservation site. A dendrogram constructed by UPGMA cluster analysis showed several groups, but did not show any differentiation between the hedge-orchard and the ex-situ conservation site (Figure 2).

Figure 2. Dendrogram of each individual constructed using RAPD data. The samples from hedge-orchard were indicated with a letter H



Discussion

Genetic characteristics of one of the hedge-orchards established in the Dipterocarp Center, East Kalimantan largely differ from those of the Jambi, Sumatra population. The wildling materials used to establish the hedge-orchard were derived from the natural population in Berau, north of East Kalimantan. The differences may be caused by the differentiation of the populations. However, a population genetics study using RAPDs showed only slight differentiation between the Sumatra and Kalimantan populations (Prihatini *et al.* 2001). Differences in genetic characteristics could result from both the non-random sampling from the natural population and the following selection in nursery.

The genetic diversity (mean expected heterozygosity over the 4 loci) in the hedge-orchard was slightly lower than that in the Jambi population. However, it should be noted that only 4 microsatellite loci were used in this study, and they did not always indicate lower genetic diversity in the hedge-orchard (Table 1). One locus (Shc01) showed much lower heterozygosity, and one (Shc04) showed a slightly lower value in the hedge-orchard. On the contrary, Shc03 had a much higher value, and Shc09 also showed a slightly higher value. Considering these results, genetic diversity in the hedge-orchard may not be lower than that of the natural population. This should be confirmed with greater numbers of loci, using the natural population in the Berau region.

The dendrogram constructed using RAPD data indicates the existence of some groups consisting of closely related individuals within the hedge-orchard (Fig. 2). Trees in the same group may be derived from one and/or some related mother trees. RAPD data also suggested that not all the individuals in the hedge-orchard are related, since the genetic distances within the hedge-orchard were variable (0.28 to 0.76) compared to those within the *ex situ* conservation site (0.50 to 0.59). These results suggest that there are some genetically closely related tree groups, but that each group is unrelated.

DNA analyses of the hedge-orchard detected a different genetic composition, but did not detect any obvious loss of genetic diversity compared to the reference population. This could be due to effects of combining several genetically related groups, which are unrelated to each other. In the case of the present hedge-orchard, the sampling and selection scheme seems to be appropriate from the standpoint of genetic characteristics.

Greater genetic gain is one of the most important factors for operational plantation, thus selection according to performance is important. Therefore, genetic diversity should be maximized in the sampling strategy for collecting materials for the hedge-orchard. The microsatellite analysis of the natural population in Jambi, Sumatra (Rimbawanto & Isoda 2001, unpublished) indicated that combining several wildling populations would not cause the loss of genes but would retain the genetic diversity. In the Dipterocarp Center, there are several hedge-orchards that are currently producing cutting materials for plantation. It is essential to evaluate the genetic diversity of the plantations by investigating the genetic characteristics in other hedge-orchards, as well.

In this study, we demonstrated an application of DNA analysis to forestry, and achieved some useful information. This type of analysis should be done for many other cases including different schemes for sampling and selection, in order to obtain some general idea of appropriate sampling and selection methods.

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Forest Plantation

Commercial Plantation Strategy to Reduce Pressure on Tropical Forest Resources

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Abstract. Over the last decade, clear trends in the management of forest resources in Southeast Asia have emerged. Some countries have ceased timber production from natural forests and are now net importers. Others are perceiving incipient declines in production. While these trends have serious economic implications for many countries in the region, concern about the rapid degradation and loss of some of the world's greatest species-diverse forests has turned into an international issue. A number of international initiatives were instituted to improve management of these ecosystems and to ensure they are not totally degraded. Forest management has ceased to be mere pursuit of yield increments. It now must address an array of issues collectively described under the principles of sustainable forest management. These encompass socio-economic considerations, environmental issues, and biodiversity conservation matters. Currently, there are approaches to enforce such measures by introducing the concept of certified "green" timber as the only such material allowed to be traded in the international markets. There are calls to have independent assessment of the forest management as well.

With global awareness that remaining natural forests in the tropics must be conserved and managed with great care as a source of wood and for the many other known benefits, there is increasing recognition that greater development of plantation and agro-forests are essential for economic, environmental and aesthetic benefits to the community. Indeed, the rapidly expanding demand for wood to provide for the downstream wood-based industries throughout the world cannot be met without substantial development of plantation forestry. As a consequence, the private sector is beginning to participate in an industry that was once the domain of the public sector. This paper explores those aspects relating to the management of plantation forests. It also focuses on the potentials and constraints relating to the establishment of commercial forest plantations to make up for the expected future shortage of logs from natural forests, while at the same time relieving logging pressures on natural forests.

Introduction

A cursory glance at the timber trade worldwide would reveal some important clues. The area of closed forest in North America and Europe does not come close to that found in the Third World Countries of Africa, South America and Asia, yet the former have higher sector production and exports (Table 1) (FAO 1999). This could mean that the wealth of forests in the third world countries, which are mainly in the tropics, has not been truly realized as is the case with developed nations.

It is also apparent that among the third world countries, Asia and the Pacific region, while not in possession of the largest forest area, is yet enjoying a bigger production and export level. The Asia-Pacific region exports about 33,000 m^3 of industrial round wood, against about 17,000 m^3 from Africa and South America. Likewise, the export value of forest products from the Asia-Pacific region in 1988 was over US$10 billion while that from the other two regions was a dismal US$4 billion (FAO 1989).

What are the specific factors behind the strength of the Asia-Pacific region for this high productivity? A glance at the forest production and export values from Asian countries would provide the explanation (Table 2). Aside from the developed Asia-Pacific nations, the Southeast Asian countries are unusually productive, particularly Indonesia and Malaysia. The Asia-Pacific countries export over US$10 billion worth of forest products annually, of which half of it comes from those two Southeast Asian countries.

Table 1. Total Forest Cover and Exports, 1995 (FAO 1999).

Region	Total Forest Area (x 1000 ha)	Industrial Roundwood Export (mil. cu. m)	Sawnwood Export (m. cu. m)
Africa	530273	7439	1351
Asia	503000	14336	6666
Ocenia	90695	18347	1067
S. America	870594	10066	3493
Central & N. America	536529	23701	58185
Europe	933326	46074	41865

Forest Resource Base of Southeast Asia

The Southeast Asian countries can be subdivided into the continental and insular Asian countries (Table 3). With the exception of Thailand and Vietnam, all the countries have between 30 – 66% of the land covered with closed forests. In general, the insular region is still well covered with forests, whereas continental Southeast Asia appears to have lost some considerable areas. In terms of economic importance, forestry is critical for many of these countries. With the exception of Thailand, Brunei and the Philippines, all the rest appear to derive around 10% of their GDP from forest products. Of them, the biggest money earners are Indonesia and Malaysia.

The forests in the continental region are tropical seasonal forests, and those in the insular areas are the rainforests (Whitmore 1984). The rainforests are the major source of hardwoods from the region. For example, of the 25 million m^3 of tropical hardwood logs traded in the world market in 1986, 21 million m^3 came from Southeast Asia and the Pacific region (Nectoux *et al.* 1991). Of that, 19 million m^3 came from Malaysia. It is the same case with sawn hardwood – a total of 6.7 million m3, which is 70% of the world's tropical sawn wood trade, originated from Southeast Asia. This is repeated for plywood as well – Indonesia is now the world's biggest tropical plywood producer, with an export of 5.5 million m^3 in 1987. The strength of Indonesia and Malaysia in the hardwood export market is mainly due to the presence of dipterocarps, which dominate the rainforests of the region. These forests are particularly rich in timber as a result.

Table 2. Summary of Forestry Statistics for Asia-Pacific countries (Only countries with >5 mil. ha of closed forest included) (ca. 1988) (FAO 1989)

Countries	Closed Forest Area (x 1000 ha)	Forest Sector Production (mil. US$)	Forest Products Exports (mil. US$)	Round-wood Exports (1000 m3)	Mechanical wood (1000 m3)
Australia	41,658	2,549	352	8,497	4,416
China	97,847	17,007	772	10	29,908
India	51,841	10,691	16	76	17,902
Indonesia	113,895	8,450	2,873	1,131	16,807
Japan	24,158	17,566	1,031	16	39,507
Kampuchea	7,548	154	0	0	45
Laos, PDR	8,410	101	10	34	21
Malaysia	20,996	3,922	2,572	20,853	7,706
Mongolia	9,528	128	0	0	474
Myanmar	31,941	599	87	206	498
New Zealand	7,200	1,485	439	1,728	2,445
Papua New Guinea	34,230	265	109	1,383	136
Philippines	9,510	1,349	279	603	1,573
Thailand	9,235	1,403	130	152	1,295
Turkey	8,856	1,629	37	23	5,704
Vietnam	8,770	756	0	0	394

Table 3. Forest resources of Southeast Asian countries (000 ha) (ca. 1985)

Countries	Land area	All forests	Closed forests (% land covered)	Timber Plantations	% GDP
Continental:					
Kampuchea	17,550	12,648	7,548 (43.0)	6	14.0
Lao PDR	23,080	13,625	8,410 (36.4)	11	12.9
Myanmar	65,770	31,941	31,941 (48.5)	15	7.5
Thailand	51,180	15,675	9,235 (18.0)	129	2.4
Vietnam	32,540	10,110	8,770 (26.9)	204	8.1
Insular:					
Brunei	590	560	393 (66.6)	0.5	1.0
Indonesia	181,160	116,895	113,895(62.9)	1,918	7.4
Malaysia	32,850	20,996	20,996 (63.9)	120	10.0
Philippines	29,850	13,330	9,510 (31.9)	300	3.3

Deforestation

The Asia/Pacific region has, for more than three decades, experienced an exceptionally high rate of tropical hardwood removals (Table 4) (Grainger 1986). As noted earlier, most of it - over 60%, comes from just two countries. This appears to result from forest conversion to other land uses, mainly agriculture and logging activities.

A step back to the past would provide future scenarios. Not too long ago, countries like Thailand and the Philippines, well endowed with forests, were important exporters of hardwoods. Their rate of deforestation was in excess of 2%, and by the mid-1990s they ceased to be exporters, and are now net importers (Table 5) (WRI 1990)

Table 4. Trends in tropical hardwood removals (1965 – 1995)(in millions of m3)

	1965	**1975**	**1985**
All Tropics	77.746	113.747	134.418
Africa	11.666	14.296	17.370
Asia/Pacific	51.348	77.647	85.955
Latin America	14.732	21.804	31.084

Table 5. Area of natural forest and deforestation rates (ca. 1980) (area x 1000 ha)

Countries	Land area	% as cropland	% as forest land	Annual Deforestation rate
Continental:				
Kampuchea	17,550	17	76	0.3
Lao PDR	23,080	4	57	1.3
Myanmar	65,770	15	49	0.3
Thailand	51,180	39	29	2.8
Vietnam	32,540	20	40	0.6
Insular:				
Brunei	590	n.a.	79	0.3
Indonesia	181,160	12	67	0.9
Malaysia	32,850	13	60	1.2
Philippines	29,850	27	37	2.7

Indonesia and Malaysia, although still in possession of over 60% of their forests, are currently losing about 1% of their forests annually. If this trend is not arrested, these timber-rich countries would see the same fate of several other countries in the region.

Causes of Deforestation

Deforestation and the causes behind it are numerous and complex. The following are the leading factors behind deforestation (Panayotou & Ashton 1992):

a) Conversion to Other Economic Uses

Large tracts of easily accessible natural forests have been converted to other forms of land use such as agriculture, mining, timber plantations, pasture land, urban development, hydroelectric dams, etc. Conversions of natural forest to perennial tree-crop agriculture such as rubber, oil palm, cacao, fruit trees, spices, coffee, sugar cane, etc. have been important economic developments in the region. The pressure to convert more forested land for such development has not ceased, considering the apparently high profits from such activities. These activities result in definite loss of tree cover from the area.

In several countries, governments sponsored settlement programs whereby people were relocated. This has been done in Indonesia, Malaysia and Vietnam. They are called the FELDA scheme in Malaysia and the

Transmigration or resettlement scheme in Indonesia (Whitten *et al.* 1991). The schemes were a means to raise the economic standards of the settlers. Under the schemes, in excess of 10 million ha of natural forests were converted in the region to oil palm and other tree crops for the settlers to work on.

b) Uncontrolled Exploitation

Although statistics are not available, effects of over-harvesting, overgrazing and fire damage, all of which lead to forest degradation, must also be taken into account. The incidences of fire are increasing, with three major episodes of fires in the region in the last two decades. In each instance, about 1 million ha, mainly the peat swamps, were burned (e.g. Leighton & Wirawan 1986).

c) Shifting Agriculture

The problem of shifting agriculture has been highlighted extensively. Easily more than half of the deforestation in the region can be attributed to unsustainable shifting agricultural practices (Spencer 1966). The rapid growth of the populations and the shrinking of existing forested areas are the main reasons behind the failure of this age-old system of agriculture. Besides shifting agriculture, encroachment by landless populations into newly logged forests is also taking its toll.

d) Logging

Commercial extraction of timber in Southeast Asia has been shown to be largely destructive. An ITTO study concluded that a very small percentage (less than 1%) of the natural forests in the region are managed on a sustainable basis (Poore et al. 1989). In all cases, over-harvesting has been the usual practice. The growth figures obtained from more recent studies rarely support the rate of harvesting, and the cutting cycles of 25-40 years are believed to be on the short side. The harvesting using heavy skidder-tractor machinery usually results in damage of over 60% to the residual vegetation (Appanah & Weinland 1990). The loss of potential tree crops as a result of logging damage has not been clearly recognized, but it is showing up to be considerable, and extensively depletes the stock left behind for the second cut (Appanah & Harun 1999). Logging is carried out with maximum speed, and rarely are skidding tracks pre-planned and controlled, very little road maintenance is carried out, directional fellings are rarely employed, pre-felling climber cuttings are not conducted, and little silvicultural tendings are done to improve the commercial regeneration.

Besides damage to vegetation, the poor construction of roads, low maintenance, and the use of heavy machinery result in excessive soil erosion.

The overall conclusion is that, in the long term, there will be less and less commercially valuable timber left behind. While total destruction of forests is not likely in every country, many of the logged forests would be poorly stocked, and natural regeneration would be scarce.

Environmental and Economic Problems

While deforestation is required to develop sustained agriculture and viable cash crop plantations, beyond certain limits such forest openings would cease to be economically beneficial. Some of the countries are already beginning to reach such threshold levels. But agriculture on poor or ill-suited soils has proven to be disastrous and wasteful. The harvests have declined, and farmers have become impoverished as a consequence.

Deforestation, besides loss of valuable wood, which was often burned, has resulted in other serious problems. Heavy and unplanned encroachments have resulted in loss of major watersheds, which are facing severe devegetation and erosion (Hamilton & King 1983). This disrupts the water cycle - rivers and lakes are sedimented, and affect agricultural development and hydroelectric dams, and ports may also silt up. In many cases, the economic gains from logging are heavily offset by costs to society from the environmental damage ensuing. The cost of repairing flood damage has not been estimated in the region, but in the example of the Himalayas, it was estimated at US$250 million per year (Spears 1982).

Logging often comes at the cost of loss of environmental services whose values may even exceed the gains from timber (Repetto & Gillis 1988). Forests are the major source of potable water for large segments of the populations in the tropics. In recent times, many countries have experienced acute water shortages during unusual drought periods. The impact was most severe in areas that have lost their forests. It is indeed ironic that Southeast Asian countries, which are some of the wettest in the world, suffer from water shortages. A whole lot of other economic activities can be disrupted as a result of deforestation. They include river transport and ecotourism benefits. Climatic changes are also beginning to become apparent as a result of large scale logging activities (Sagan *et al.* 1979). Scientists speculate that large scale clearing of tropical forests may affect the reflectivity of the surface of the earth, which could alter global climatic patterns and shift rainfall distribution. Another deep concern is the release of carbon into the atmosphere as a result of burning tropical forests. This additional carbon dioxide in the atmosphere can cause global warming as a result of the green house effect.

Loss of forests has been considered to also having affected the livelihood of the indigenous and forest-dependent populations. Large-scale logging has resulted in loss of non-timber goods and environmental services, impoverishing the local people dependent on them. People who subsist on hunting, gathering fruits, nuts, cane, bamboo, medicinal plants, etc. have been affected (Caldecott 1987). Increasingly, non-governmental organizations have been vociferously campaigning against large-scale commercial loggings.

An additional facet to deforestation and forest degradation is the loss of biodiversity (Myers 1984). The rainforests of insular Southeast Asia fall among the richest zones for plant and animal biodiversity known in terrestrial ecosystems (Whitmore 1984). The loss has not been quantified, but considering some countries have already lost about 60% of their forest, the loss in biodiversity should have been substantial. Since not all the countries have done adequate surveys of the plants and animals, the losses may never be even recognized. The loss of biodiversity is not one of scientific curiosity. Biodiversity is necessary to: i) sustain and improve agriculture and animal husbandry; ii) provide opportunities for medical discoveries and industrial innovations; and iii) preserve the choices for future generations (OTA 1987). The rare discovery of an important drug can revolutionize medicine, and that option should not be lost to future generations, which may face new and unknown life-threatening diseases. Well-known drugs derived from tropical forests include the rosy periwinkle (*Cantharanthus roseus*), steroids from Mexican yams (*Dioscorea composita*), and antihypertensive drugs from serpent-wood (*Rauwolfia serpentina*). Next, the discovery of the rubber tree in the Amazon should trenchantly state the point. Within this century, the rubber crop has grown into a US$4 billion industry, and many countries are quite dependent on rubber exports for their foreign exchange. I am certain there still are economically important plants in tropical forests waiting to be discovered.

Plantation Forestry

In view of the rapid loss of natural forests, plantations have often been promoted as a better alternative to enhance timber production in the region. This has been considered the best option for countries like Malaysia, Thailand, Indonesia and the Philippines where the downstream industrial capacity has far exceeded the production from natural forests. The rationale is that natural forests at the most produce about 2.5 m^3/ha/yr of commercial timber, whereas plantations can produce annually from 10 m^3/ha of hardwoods to 30 m^3/ha of softwoods. If the plantations are well developed, they will indeed lift the pressure off the natural forests. Albeit, the benefit of natural forests, which includes the ecological services and biodiversity, may not be fully realized in plantations.

The success of eucalypts, pines and teak in the sub-tropics and dry tropics is used as evidence for their viability in the wetter regions of Southeast Asia. However, the history of plantations in the region does not appear easy and straightforward. There are numerous difficulties whether they are indigenous or exotics, and whether they are monocultures or mixed species. In Table 6, the plantation areas in the region are shown. It is obvious that hardly any of the countries have even reached 1% in area compared to the natural forest areas found in their countries. Indonesia is the only one that has so far gone aggressively into developing their plantations.

Table 6. Forest resources of Southeast Asian countries (000 ha) (ca. 1985)

Countries	Land area	Closed forests	Timber Plantations
Continental:			
Kampuchea	17,550	7,548	6
Lao PDR	23,080	8,410	11
Myanmar	65,770	31,941	15
Thailand	51,180 32,540	9,235	129
Vietnam		8,770	204
Insular:			
Brunei	590	393	0.5
Indonesia	181,160	113,895	1,918
Malaysia	32,850	20,996	120
Philippines	29,850	9,510	300

The majority of plantations are monocultures with exotic species originating from subtropical parts of the world. In the 1970s, fast growing hardwood species such as *Acacia, Eucalyptus, Paraserianthes, Gmelina*, and *Leucaena*, and softwood species such as *Pinus* and *Araucaria* were planted (Appanah & Weinland 1993). All of these have short rotations, simple stand structure, and uniform timber and can be harvested at one felling. Monocultures are not without problems either.

At present, more species are being tested, particularly the indigenous species and those with longer rotations. With appropriate silviculture and hygiene, the plantations are beginning to look more promising than the initial experiences indicated. With more research, particularly in production of planting material and tree selection and improvement, the plantations would meet their initial expectations and may produce more of the industrial timber by the end of the next two decades.

But most countries will still be dependent on natural forests until the plantations reach maturity. Big planting programs have to start now if that success is going to be realized. In the interim, other sources of timber have to be sought. Agroforestry programmes also offer some hope there. Agricultural waste wood, such as rubberwood and fibre from oil palm fruit bunches and trunks are important sources of wood material for countries with such extensive rubber and oil palm plantations. Already, a US$1 billion furniture industry using rubberwood exists in Malaysia.

Place of Forest Plantations in the ecosystem

Plantation development must take place within a holistic approach to land use and ecosystem management. In the humid tropics, there is evidence that well-planned ecosystem-based plantation forestry can play a role in improving the environment. In our climatic conditions, tree plantations are ecologically more in harmony with the ecosystem compared to the growing of annuals in agriculture. Tree plantations minimize soil erosion and do not disrupt the nutrient cycle.

Potential of Forest Plantations

Forest plantations are generally more efficient in producing commercial timber than natural forests. For example the increment from tropical plantations may be between 10-30m^3 per hectare per year compared with less than 3m^3 from managed natural forests. Furthermore, plantations are easier to manage due to the mono or double species mix only, in contrast to very diverse natural forest stands. From the logistics point of view, the location of plantations can be predetermined to reduce transportation costs. Currently available degraded and idle lands can be converted into productive forest plantations. Hence, plantation development will serve as a strategy for maintaining a sustainable supply of timber and, at the same time, relieve the natural forest for providing non-timber benefits such as water catchment, recreation, biodiversity, and germplasm conservation.

Agroforestry Approach

The term ‘agroforestry’ commonly refers to a dynamic system involving integration of agricultural crops and/or livestock with plantation tree crops for the purpose of increasing land productivity. It is a sustainable land use system and has the function of meeting the social and economic needs of both the forest and agriculture on the same piece of land. It can be introduced simultaneously or at different stages of tree growth.

The benefits of agroforestry in forest plantations are many. Generally, for the investors it means extra income, early cash flow and better return on investment. Besides, it maximizes land use and optimizes labour use over longer period from onset of establishment to final harvest of the tree plantation. For the nation it will encourage private sector involvement in forest plantation and thereby improve utilization of idle land as well as damaged forest. The overall effect will be a sustainable or even increased timber and food production.

Agroforestry has important roles to the forest environment and social activities of the forest dwellers. It improves biodiversity; increase biomass production and provides a better microclimate. Where agroforestry is developed on damaged forests or land areas resulting from shifting cultivation, it will lead to the development of permanent resettlement centres. This factor, among others, will discourage collection of items such as bamboo, herbal plants and rattan over wide areas and hence leave the natural forest undisturbed. In essence agroforestry has the potential to transform 'wasteland' or disturbed forest into an integrated productive-protective system.

Constraints of Forest Plantations

Generally, while forest plantations are a lucrative option to supply the ever increasing demand for wood on a sustainable basis, the planting of timber trees on a plantation scale is constrained by a number of other factors that are critical. These are:

a. Ecology

The establishment of a forest plantation involves extensive alteration of the ecosystem, particularly when heavy equipment is employed. The complex closed nutrient cycle in tropical rain forests is disrupted for a long time. This can lead to reduction of productivity unless ameliorative measures are undertaken. Furthermore, monocultures further destabilize the system, and require heavy use of fertilizers and pesticides. Next, with many slower-growing species, those grown faster under intensive plantation conditions may have poorer quality. Finally, there is the problem of matching species with site over the heterogeneous area of large plantations. The danger of fire may also increase in exotic species plantations.

b. Land

It is an established fact that land is the world's most valuable resource and public scrutiny of land is becoming more intense with each passing year. With increasing population pressure, the competition on land for agriculture and development are ever increasing.

For a forest plantation investment to be commercially viable, a large area is required. The size of the land required will vary with the objective of the plantation. If the timber were for saw milling and furniture manufacturing, than an area of around 15,000 to 20,000 hectares would suffice. On the other hand, if the objective is in establishing a chip or pulp and paper mill, than economic sized plantations should be in the range of 60,000 to 150,000 hectares. It would always be desirable to have a single contiguous piece of land area and if not, the required land area should just be in about two or three nearby parcels only. This is to ensure easy and efficient management of the activities. It is always preferable that the acquired land is close to basic amenities and near a relatively accessible road system and within an economic range to a processing mill or market. As an example, to operate efficiently a pulp or a chip mill, the plantation should be located within a 100 km radius. Otherwise, exorbitant cost for the transportation of logs would render the operations uneconomical.

In Malaysia, land is under the State's jurisdiction. This implies that in Peninsular Malaysia a large plantation project may stretch across state borders. Land being a state prerogative implies that commercial organizations may have to deal with different procedures adopted by individual State governments. Often, inquiries on information regarding land can become very difficult. Details for example on information regarding forest reserves is obtainable from the respective State Forest Departments while that on State land is obtainable from the Department of Land and Mines or Department of Agriculture. The setting up of a coordinating agency is desirable to overcome this and thus encourage the easier establishment of plantation forests.

Besides sufficient land size, the location with suitable infrastructure and the premium rate for leasing are also crucial factors. Considering the long period of forest plantations, many companies in Malaysia have requested that they be given the prerogative of allocating a fraction of the land leased for planting agricultural plantation crops, which can begin providing some revenue after three years of establishment. This is considered a necessary activity to cushion the long waiting period before final harvests. However, according to the Forest Department such a request is not permissible unless the land allocated is State land and lies outside the forest reserves. Forest reserve land is strictly to be planted with forest trees.

If forest reserves are ever to play a role in forest plantation establishment this issue needs to be reviewed.

The leasing period over land requested by commercial organizations varies. In order to attract their interest in forest plantations, land should be made available for leasing ranging from a period of at least four rotations to 99 years. The intention is to have tenureship long enough to assure that sufficient returns are obtained for the investments ploughed in.

Another concern that is slowing down the commercial sectors' participation in forest plantation investments, either as joint venture partners or outright investments, is the issue of claims for customary rights by natives residing in affected logged over forest lands where plantations are to be established. In Sabah for example, although these lands belong to the State and are untitled, under the provision of the Land Ordinance a native can claim customary rights on them as long as he or she has been living in it for at least 3 years. Such issues need to be resolved before investors would consider investing in forest plantations.

c. Species Selection

A review of about 45 reforestation projects in the Tropics revealed that about 95% of all projects utilized exotics in their reforestation program. Sixty percent of all projects carried out their species trials in parallel with their project activities. About 60% of the projects received additional information during their life span that resulted in new species selection (Abod 1995)

For plantations, although indigenous species are available, a greater preference is given for the selection of exotic species. The reasons for this are:

- there is generally a lack of adequate knowledge of the propagation and silvicultural management of indigenous species
- there is generally a plentiful supply of seeds of the exotic species
- the exotic species are handled more easily and
- the exotics are fast growing and high yielding

There is a great challenge ahead to carry out adequate studies on the indigenous species and to assess their viability for forest plantations.

In Malaysia, in 1982, under the Compensatory Plantation Project fast growing hardwood timber species such as *Acacia mangium, Gmelina arborea*, *Paraserianthes falcataria* and *Eucalyptus camaldulensis* were introduced. However, due to difficulty in procuring planting material, the majority of the areas were planted mainly with *Acacia mangium*, whose seeds were readily available. That species has not performed as initially expected. Growth has remained below expectation, and many of the trees appeared to be susceptible to heart rot damage in some sites (Hashim *et al.* 1991). Overall, its performance for sawlog production has remained dismal. As a consequence, additional planting of the species for sawlog production has been halted since 1992. But plans for planting the species for pulp production are being pursued on a big scale with the development of one pulp mill in Sabah and additional ones planned in Sarawak and Peninsular Malaysia.

Since the mid 1980s, rubber wood has become an important source of timber for the furniture industry. At present the main source of rubber wood for the industry is from replanting schemes and from large plantations. But the species has now been planted on trial basis exclusively for timber production. While the demand for rubber wood is high, supply is decreasing as fewer people are interested in planting it due to low price.

Besides rubberwood, other promising candidates are also being tried out on larger scales. This includes teak (*Tectona grandis*) and Sentang (*Azadirachta excelsa*). Teak was planted in the drier north-west of P. Malaysia before. But at present, it is being planted on a small scale in the wetter southern sites as well. The tree seems to grow just as well, the only drawback being the absence of distinct close growth rings and therefore veneer quality being less inferior. The other species that has caught the attention of the plantation industry is Sentang. This too has shown good growth rates in early years, and is mostly pest free. The plantings have to mature somewhat before their true value can be ascertained. A few other species that are also being considered for planting at the moment include *Khaya ivorensis*, *Dyera costulata* and to a lesser extend *Swietenia macrophylla*. Some of the dipterocarp species such as *Shorea leprosula, S. parvifolia,* and *Hopea odorata* also appear to be good and are being investigated as potential candidates

Table 3. Growth Measurements of Some Indigenous and Exotic Species

Species	**Age**	**MAI diam. (cm)**	**MAI ht (m)**
Dyera costulata (Jelutong)	48	0.9	0.6
Khaya ivorensis (Khaya)	26	1.7	1.0
Swietenia macrophylla (Mahagony)	25	2.4	0.8
Shorea leprosula (Meranti tembaga)	35	1.1	1.0
Hopea odorata (Merawan siput jantan)	32	1.2	1.0

d. Inadequate Supply of Quality Planting Material

In general, high levels of productivity are achieved when genetic and physiological potential of the species are well matched with management practices which promote rapid growth. Valuable improvements can be made in important properties such as stem form and wood density through selection and breeding. One major constraint that is currently perceived is the shortage of good planting material for the various plantation program. Quality seeds and plus trees that have been selected and reproduced vegetatively are inadequate to meet current and projected needs. While efforts are being stepped up to overcome this problem , middlemen and overnight nurseries are providing planting material whose genetic sources are unknown.

FRIM has now signed MOU's with four private nurseries to step-up production of quality planting materials of the required species while the Forest Department of Peninsular Malaysia is in the process of setting up the National Seed and Planting Material Procurement Centre at Lentang, Pahang to address this need also. In Sabah and Sarawak too, similar efforts are underway.

e. Labour and Mechanization

Labour supply is another issue of great concern. In Malaysia, the agricultural sector is experiencing a shortage of labour because of the rural to urban migration of youth to work in factories. Although the labour requirement in forest plantations is less then in agriculture, it still has to compete for labour in an expanding Malaysian economy, where the working conditions in other industries are usually more conducive.

A natural tendency is for the plantation sectors to engage foreign workers. The foreign labour recruitment process had never been efficient. The weaknesses have been attributed to inconsistent government policy on foreign workers' employment coupled with lack of dedication of the foreign workers resulting in extremely high turnover of manpower. One option to alleviate the labour shortage is increased mechanization. Machines developed in countries like Finland and Canada for example are environmentally friendly and highly flexible in their operation in forest plantations.

f. Finance and Private Involvement Issues

The planting of timbers on a plantation scale is constrained by a number of economic factors as well. These are:

1. the high initial capital investment to establish the forest plantations,
2. the long period between initial planting efforts and harvesting and thus the corresponding concern for the high capital cost or interest being carried until harvesting period,
3. the high biological and economic risk involved in forest plantations and
4. unattractive and inappropriate investment incentives provided by the government for forest plantation investments in the past.

Government Tax Incentives and Regulations

Considering the constraints to private venture in forest plantations, in order to attract such ventures, attractive tax incentives and regulation have to be put in place. In Malaysia, the Government has recently offered a monetary incentives package in the form of :

i) Pioneer Status

Tax exemption from corporate tax of 100% for 10 years on all statutory income

ii) Investment Tax Allowance

An investment tax allowance at the rate of 100% of the statutory income for 5 years

The above incentives, though in place, are not yet sufficient to attract private sector venture into plantation forestry. Perhaps the situation could be improved if the following could be put in place:

- Provision of 'group relief' that would encourage companies with other business ventures to undertake forest plantation activities since losses incurred in forest plantation in the initial years can be offset against income of other profitable ventures.
- Exemption at the state level on royalty payments and quit rent for at least the first rotation
- Setting up of a plantation forestry fund, which offers a soft loan or subsidy for establishing new forest plantations and management costs.

Privatization

Commercial ventures in forest plantation development are relatively few and new in the ASEAN countries, when compared to USA, Chile, Brazil, New Zealand or Japan and China in Asia. In view of the rapidly declining resources, the Government should formulate new policy directions and incentives to promote privatization of the existing forest plantations or even establish new forest plantations. Only then can long term security of wood and the growth of an expanding wood-based industry in this country can be assured.

Conclusion - The Way Forward

Given the scenario of declining timber production from natural forests and the need of wood based industries for supplementary timber supplies, forest plantations will continue to be an important part of the national forest resource development strategy for the years ahead. These plantations are best seen as tree farms with multiple values designed to provide one or more very specific services to society. Whether managed by the private sector or the government, timber from these plantations will play an important role in relieving the pressure on natural forests by supplementing wood supply in the future.

To ensure a greater potential of wood production from plantations, the immediate strategies to be adopted are:

- to review present forest plantation management objectives in line with timber utilization and marketing prospects
- to encourage private sector participation in forest establishment and development through attractive incentives for investments
- to strengthen and intensify current research on the production of high quality planting materials
- to formulate more effective silvicultural regimes for the plantation based on R & D findings
- to continue planting trials with other fast growing indigenous species that have potential

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Dipterocarp Plantation:
the Strategy and the Approaches of PT Inhutani I

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Abstract. This paper discusses the experiences of PT Inhutani I in plantation establishment. Many factors may influence the successful establishment of Dipterocarp plantations. The major constraint is how to continuously supply planting stock for large-scale plantation programs. The complementary use of seedlings, wildlings, and cuttings was implemented to overcome that problem.

Rooting of cuttings is an important method used by PT Inhutani I to produce planting stock in the nursery. A tree improvement program employing selection of superior material in the hedge orchard is included in the plantation program for Dipterocarps. These techniques, developed by PT Inhutani I, are discussed in this paper.

Ex situ and *in situ* conservation activities for the purpose of conserving genetic resources are considered to have an important role in the forestry program. Species that are not available in the planting region are introduced by *ex situ* conservation in the plantation area, while the conservation of the original forests within a plantation area represents *in situ* conservation.

Introduction

The Forest Plantation Establishment program is one of the most encouraging government forest policies to offset anticipated raw materials deficits. The dependence upon natural forests for raw materials could not meet future demands of the wood and paper industries due to declines in both the quantity and quality of natural forests. It is certain that timber from forest plantations will play an important role in the future of the wood industry in Indonesia.

As a state-owned forestry enterprise, Inhutani I has had experience in timber estate establishment since the early 1970s when the reforestation program commenced. At that time, the government introduced pine plantations all over Indonesia. Since 1980, Inhutani I also became actively involved in the Timber Estate Program for fast growing species like *Acacia mangium* and *Eucalyptus spp*, and this was followed in 1987 by Dipterocarp plantation establishment with *Shorea spp.* at Long Nah and Batuampar in East Kalimantan.

In line with the government policy to rehabilitate the degraded forest with promising indigenous species, the program of Dipterocarp plantation continues with a new perspective - how to establish plantations on degraded forest areas using indigenous species, especially in Sumatera and Kalimantan. This new program needed an integrated tree improvement and conservation effort to support the program in relation to options for future breeding of Dipterocarps for plantation. The strategy and the approach of Inhutani I to this issue are described below.

Selection of Priority Species

There are more than 500 species within the family Dipterocarpaceae (Ashton 1982) and not all of them are commercially interesting to the wood industry. Furthermore, among the commercial species, not all are suitable for commercial plantation. Before decisions can be made to establish commercial Dipterocarp plantations, the selection of the priority species must be done to assure the plantations are economically and technically feasible.

The major aim of this selection program is to match the correct species with the technical and environmental requirements. Selections are implemented under several criteria as follows:

1. The selected tree species must have high value timber
2. The selected tree species must have fast growth
3. The selected tree species originally existed in the planting region and thus, it is adapted to the local environment
4. There is sufficient supply of planting stock through use of wildlings, cuttings, and seedlings
5. There is adequate information on the silviculture of the species

Using these criteria, six priority species have been selected for commercial plantation by PT Inhutani I. These species are *Shorea leprosula, S. johorensis, S. parvifolia, S. smithiana, S. pauciflora, and Dryobalanops lanceolata.*

Planting Stock Production

The main constraint to obtaining dependable dipterocarp planting stock is the very irregular fruiting season, which occurs only once every 4 -5 years. So instead of relying on a single production method, which might encounter problems, planting stock production is derived from 3 complementary sources: seed (seedlings), wildlings, and/or cutting. PT Inhutani I uses the following approaches to produce planting stock for dipterocarp plantations.

Seed (whenever available, mostly for 2 - 3 months during a fruiting season)

Although the mast flowering season occurs once every 4-5 years, 10% of dipterocarps may not follow that pattern, and these produce seeds during the interval years (Smits 1990). The seed supply is usually collected and sown before germination.

Wildlings (when seeds have already germinated in the forest floor)

Another way to supply planting stock is by taking young, individual seedlings from the forest floor. This method can be relied on for at least 2 years after a fruiting season. The best wildlings are those with 2 – 5 leaves, including the first shoot, a maximum height not more than 50 cm, and collected from beneath the crown of the mother trees. From one mother tree more than 15,000 wildlings can be collected. Best collecting time is right after rain or whenever soil conditions are completely wet. The young wildlings should be pulled carefully to avoid root damage. Wildlings are the main planting stock resource for plantations because the method is cheap and technically easy to implement by most field workers.

Cuttings (when seed and wildlings are not available)

When the two previous methods cannot provide sufficient planting stock, vegetative propagation through rooting cuttings is often used to fill the shortage. Inhutani I has used this technique for a long time, and it has been used on operational scale. There are two kinds of cuttings; shoot-cuttings and stem-cuttings. Cutting production can be maximized if supported by a productive hedge orchard where from the cutting material can be continuously taken. And cuttings can be obtained all year when seeds and wildlings may not be available.
Related to the tree improvement program, this technique is highly useful to propagate selected superior material. This “clonal forestry” approach needs genetic resources from both *ex situ* and *in situ* conservation areas that must be established in the plantation areas as described below.

Planting techniques

Successful plantations depend upon the quality of the planting material, the planting method, and maintenance and management of the plantation. The experience of Inhutani I has been that environmental factors, such as light intensity manipulation in the field, play an important role in successful plantation establishment. Several different techniques have been tried to find an appropriate method:

· Clear cutting followed by plantation of fast growing species as "shadow trees" for Dipterocarps. The first step is planting the fast growing trees at 3 x 3 m then the dipterocarps at 5 x 5 m in between. After they reach an appropriate height, Meranti is planted under the shade to avoid exposure.
· Secondary Forest Areas
  This area can be planted with meranti using a line-planting system of 5 x 5 m distance and 2 m line width. Inhutani I employs this system, which is suitable for Dipterocarp plantations, in logged-over areas or heavily damaged forests.

Maintenance of the plantation starts after planting and continues until the plantation is 5-years-old. The first three years is the most critical period wherein the plantation needs intensive maintenance. In the next period, light manipulation is necessary, with vertical openings providing sufficient light for the trees to grow better. The light conditions must be monitored to maintain the soil temperature below 33 degrees Celsius, which is the critical soil temperature for development of ectomycorrhizae of Dipterocarps (Smits 1994, Yasman 1995). This temperature regime requires about 50 to 70 percent of full light intensity.

Genetic Resources and Conservation

Improving productivity through tree improvement of Meranti (Dipterocarpaceae) can only be guaranteed by conserving superior genetic resources to maintain the program. Rapid deforestation that might have the effect of eliminating superior genetic material from lowland Dipterocarp forests is proceeding at alarming rates, not only in production forests but also in conservation forest areas. Obviously, these conservation areas are needed to support plantations as the genetic source for species development in subsequent rotations. This includes both *ex situ* and *in situ* conservation activities within the plantation areas.

Ex situ conservation as well as *in situ* conservation were designed to provide genetic resources that can be directly used for future breeding and biotechnology programs. In the plantation programs, *ex situ* conservation activities are mostly to introduce and support the non-endemic species and programs are designed to plant mixed species to improve the species diversity in the area. In East Kalimantan, we introduced species like *Shorea selanica* (endemic to Maluku) and *Shorea javanica* (endemic to Sumatera), and some other non-Dipterocarps like *Khaya anthotheca, Swietenia mahagoni*, and

Gmelina arborea. Current activities were specifically designed for breeding purposes as recommended in the ITTO Project PD 16/96 Rev. 4(F), '*Ex situ* Conservation of *Shorea leprosula* and *Lophopetalum multinervium* and Their Use for Future Breeding and Biotechnology.

In situ conservation in the plantation area is designed to provide a genetically diverse resource that still has evolutionary potential to improve the breeding program. Most of the *in situ* conservation areas are natural forests that are excluded from the plantation area within a region. These areas may take the form of a compact, contiguous forest or be scattered along the riverbanks within the plantation concession. From these *in situ* conservation areas the good phenotypes of selected priority species were identified and their seeds were collected for breeding programs.

To improve genetic diversity in the selected priority species, the seeds and seedlings of identified plus trees from outside the region are collected for planting material, as well as for establishing hedge orchards which will be used in the tree improvement programs. Although this approach may change the genetic characteristic of the population (Isoda *et al.* 2001, this conference) this system is still considered to be the most appropriate for a Dipterocarp breeding program, since the conventional methods utilizing sexual reproduction would have limitations like floral phenology and lengthy reproductive cycles.

Conclusion

Dipterocarp plantation is one of the most important programs in forest plantation and rehabilitation in Indonesia. Despite some limitations in planting stock production and the variability in growth rate of the Dipterocarpaceae, tree improvement through selection and vegetative propagation are considered to be appropriate methods for these species.

Ex situ and *in situ* conservation areas within the plantation concession areas play an important role in providing genetic resources for these improvement programs. Therefore, these conservation activities must be included in the dipterocarp plantation establishment strategy in order to have a sustainable, highly productive forest plantation.

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Planting *Meranti* (*Shorea sp.*) Trees: an Experience of PT Sari Bumi Kusuma in Forest Concessionaire

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Abstract. The logged over areas under control of PT. Sari Bumi Kusuma that needed to be planted consisted of 4.71% of post harvesting rehabilitation replanting in open areas, and 2.06% of enrichment planting in areas where natural regeneration was lacking.

It was confirmed that in the logged over natural forest, post-selective cutting did not provide adequate space for strip planting of Meranti. When Meranti plants reached 13 years of age, only 32.5% of the planted trees had survived. The majority of the mortality was caused by competition with existing natural regeneration. Yet only 2.3% of the surviving trees received full sunlight. Therefore, to conserve logged over natural forests under selective cutting systems, efforts should be focused on maintenance and protection of the forests, and not on replanting activities.

Meranti trees (*Shorea* sp.) grew best when their upper canopies received full sunlight at a perpendicular angle. In general, these trees would produce more branches when they received additional horizontal light. At age 13, Meranti plants with open canopies had mean annual increment (MAI) of 1.65 cm per year, and mean annual height growth of 1.48 m per year.

Under natural regeneration, the highest rate of height growth, 1.85 cm/year, occurred in the diameter class of 40.0 to 49.5 cm for Meranti Merah (S. *leprosula*), and 1.34 cm/year in the diameter class of 30.0 to 39.5 cm for Meranti Putih (S. *hopeifolia*).

A Selective Cutting and Strip Planting System (TPTJ) could be adopted as one promising alternative for natural forest management, and it offers the following advantages:
(a) increases logging productivity,
(b) prepares enough room for optimal plant growth,
(c) absorbs more labor, and
(d) boosts participants motivation to conserve and protect the logged over forest to secure investments.

Finally, it is suggested that the TPTJ should not be implemented indiscriminately on all logged over areas in a Forest Concession as its proper execution depends on the status of the forest and some other factors such as economics, social conditions, and the surrounding environment.

The Condition of the Logged Over Areas

In 1978, PT Sari Bumi Kusuma (PT SBK) was granted the right to manage natural forest production in Central Kalimantan under a selective cutting system.

Under this system, the need for replanting, as well as the extent of the logged over areas that need to be planted, depend on the condition of the pertaining areas. One year after logging activities, a post harvesting inventory (ITT) was conducted on every logged over area. Based on that ITT, the logged over areas under PT SBK are as follows:

Table 1. Forest Condition of PT SBK at One Year Post-logging (Et + 1) under a Selective Cutting System

No.	Logging		Diameter (20 cm and up)		Open Areas				Less Natural Regeneration Areas ***	
	Year	Area (ha)	Tree/ ha	m3/ ha	Permanent (ha) *	%	Temporary **	%	(ha)	(%)
1.	1984/85	4,250	30.95	64.81	103.13	2.42	136.01	3.20	105.84	2.49
2.	1985/86	4.300	25.75	41.93	183.08	4.25	133.94	3.11	118.85	2.76
3.	1986/87	3,600	23.19	47.48	114.92	3.19	104.11	2.89	82.24	2.28
4.	1987/88	2,600	26.92	62.55	67.60	2.60	89.70	3.45	87.36	3,36
5.	1988/89	2,800	26.22	50.69	78.40	2.50	115.32	4.12	66.43	2.37
6.	1989/90	5,000	26.22	50.69	131.13	2.62	255.68	5.11	140.44	2.81
7.	1990/91	4,300	25.22	48.08	157.76	3.66	215.00	5.00	109.22	2.54
8.	1991/92	4,300	25.35	49.81	130.13	3.02	250.90	5.83	124.08	2.89
9.	1992/93	3,125	25.04	29.62	109.28	3.49	261.66	8.34	79.06	2.53
10.	1993/94	4,150	31.98	61.78	218.18	5.25	346.04	3.34	104.99	2.53
11.	1994/95	4,125	28.34	34.98	254.70	6.17	148,83	3.61	7.76	0.19
12.	1995/96	3,950	46.54	36.53	302.35	7.65	168.24	4.26	27.55	0.70
13.	1996/97	4,290	60.06	48.37	562.66	13.12	171.17	3.99	35,67	0.83
14.	1997/98	3,984	56.06	59.84	212.80	5.54	184,00	4.62	40.50	1.01
	Total	54,774	459,84	667.13	2,626.12	65.58	2,580.60	60.87	1,129.79	29.29
	Average	3,912	32.85	47.65	187.58	4.79	184.33	4.71	80.70	2.06

* infrastructure (logging road, etc) ** needs to be replanted (skid trails, etc) *** needs enrichment planting.
Note: In 1984/85 to 1992/93, observations were concluded when 25 main trees had been found.

Meranti Planting in Logged Over Areas

One year after logging under the selective cutting system, only a small portion of the forest areas needed to be replanted; that is, 4.71% + 2.06% or 6.77%. In subsequent years, the logged over forest showed an ability to restore itself and the only actions necessary for such areas were good maintenance and protection of the forest.

Public opinion commonly prevails in our society and, unfortunately, it is often contradictory to the facts. There have been some public inquiries on why the replanting areas are significantly lower than those of the logged over areas. Often, when no replanting activity is performed, an enterprise would be condemned as irresponsible. Therefore, in 1987, PT SBK initiated a test case for Meranti planting:

Replanting areas	: forest of the 1983/84 selective cuttings
Coverage	: ca. 100 hectares
Row width	: 3 by 5 m or 660 trees/hectares
Width of cleared alley	: 1 m
Kinds of plants	
Main	: *Shorea leprosula* (mostly)
Others	: *Shorea johorensis*, *S. parvifolia*, *S. stenoptera*, and *Shorea* sp.

It turned out that the majority of the plants either could not survive or had inhibited growth due to competition with natural regeneration. In a 3-hectare monitored area, routine measurements on 1,980 plants yielded the following data:

- Observations made from 1992 (5 years of age) until 1997 (10 years of age) revealed that the numbers of surviving plants in each year were 1,377 plants (69.55%) in 1992, 1,189 plants (60.05%) in 1993, 1,036 plants (52.32%) in 1994, 918 plants (46.36%) in 1995, 850 plants (42.93%) in 1996, and 736 plants (37.17%) in 1997.
- On the other hand, in observations made in the year of 2000 (at 13 years of age), there were only 643 plants remaining, a survival rate of 32.5%. Details on plant status in 2000 are as follows:

Table 2. Meranti Plant Condition in 2000 (13 Years of Plant Age)

No.	**Plant Condition**	**Plant**		**Diameter (cm)**		**Plant Height (m)**		**Main Stem Height (m)**	
		Numbers	**%**	**Mean**	**MAI**	**Mean**	**MAI**	**Mean**	**MAI**
1.	Group I: Plant canopy was covered by natural regeneration	371	57.7	11.52	0.89	13.21	1.02	8.06	0.62
2.	Group II: Plant canopy was half-covered by natural regeneration	257	40	17.76	1.36	17.90	1.37	9.46	0.73
3.	Group III: Plant canopy was fully exposed	15	2.3	21.45	1.65	19.28	1.48	7.44	0.57
Total / Average		643	32.5	16.91	1.30	16.80	1.29	8.32	0.64

Based on observations, it can be concluded that the growth of Meranti was best when the top canopy received sufficient sunlight at a perpendicular angle. In contrast, when plants also received sunlight from a horizontal direction, there was a tendency for the plants to produce more branches (or, in other words, the height growth of the main stem was lower).

It is worth mentioning, though, that during this trial test, no Meranti plants were fertilized or pruned.

The Growth of Natural Regeneration in Logged Over Areas

To determine the mean annual increment (MAI) of natural forest in logged over areas, a permanent sample plot (PUP) of 100 hectares was made in areas logged over in 1993/94. Observations on the growth of natural regeneration produced the following information:

- There were 51 kinds of plants existing inside the PUP of PT SBK. Measurements were made on all plants within the PUP, starting from plant diameter of 10 cm and up.
- The MAI of diameter and height of 6 dominant plants are shown in Table 3.

Table 3. The Growth of dominant plants from natural regeneration within the PUP of PT SBK

No.	Species	Diameter MAI (cm/year)	MAI of Total Plant Height (m/year)	MAI of Main Stem Height (m/year)
1.	Meranti Merah (*Shorea leprosula*)	1.11	1.08	0.84
2.	Meranti Putih (*Shorea hopeifolia*)	0.95	1.14	0.96
3.	Bangkirai (*Shorea levifolia*)	0.82	0.86	0.73
4.	Keruing (*Dipterocarpus* spp.)	0.65	0.74	0.80
5.	Medang (*Litsea firma*)	0.65	0.98	0.96
6.	Kulim (*Scorodocarpus borneensis*)	0.49	1.13	0.69
Note: Measurements were taken from 1994 till 1998				

- Out of six kinds of plants observed, the Meranti Merah had the highest diameter MAI.
- Plant conditions after reaching maximum growth varied among species. For example, the Meranti Merah had its highest diameter MAI (1.85 cm/year) in the diameter class of 40 to 49.5 cm. The highest diameter MAI of the Meranti Putih (1.34 cm/year) occurred in the diameter class of 30 to 39.5 cm.

As a comparison, the following data on MAI of some Meranti were obtained from Forest Research Institute in Bogor (Table 4).

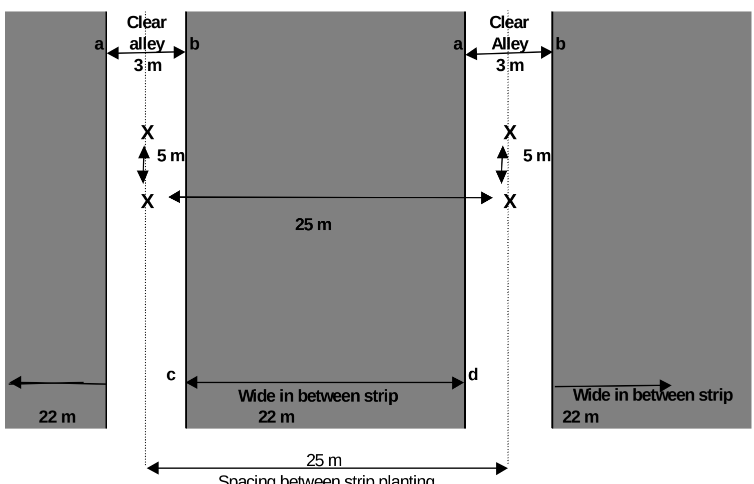
Table 4. Mean Annual Increment (MAI) of Meranti in Haurbentes, Bogor

No.	Species	Age	Diameter		Height		% survival
			Mean (cm)	MAI (cm)	Mean (m)	MAI (m)	
1.	*Shorea leprosula*	2 years	-	-	1.92	-	75.0
		35 years	77.2	2.21	44.60	1.27	-
2.	*Shorea selanica*	6 months	-	-	0.69	-	97.3
		32 years	54.2	1.69	28.20	0.88	-
3.	*Shorea compressa*	6 months	-	-	0.33	-	78.0
		42 years	55.5	1.32	31.90	0.76	-
4.	*Shorea pinanga*	6 months	-	-	0.29	-	79.0
		42 years	54.8	1.30	30.7	0.73	-
5.	*Shorea seminis*	12 months	-	-	0.96	-	86.3
		42 years	31.4	0.75	25.70	0.61	-

Growth of *Meranti* on Selective Cutting and Strip Planting

In 1998, PT SBK was granted a concession to develop a Selective Cutting and Strip Planting System (TPTJ) in logged over areas, with the following pattern:

The TPTJ System of PT Sari Bumi Kusuma



Notes:

X Plant with a spacing of 5 m within the strip and 25 m between strips

a - b A-3 m clear alley, free from non-Diterocarpaceae plants

c - d A-22 m wide in between strips of natural forest on the logged over areas

The limit diameter for cutting was 40 cm (to allow larger growing space as well as increase logging productivity)

The preferred plant was *Shorea leprosula*, and the other tree species grown were *Shorea johorensis*, *S. parvifolia*, *S. fallax*, and *Shorea* sp. Planting activities were initiated in 1999, and currently the logged over area that has been planted is 5.861 hectares. The adopted plant spacing was 5 m by 25 m, with a within-the-strip plant spacing of 5 m, a clear alley of 3 m, and width between the strips of 25 m. Therefore, in one hectare of TPTJ in the logged over area, there were approximately 80 Meranti plants within the strip and approximately 0.8 hectare of natural forest trees in between the strip.

The ITT data showed that approximately 28 commercial trees per hectare with a diameter of 20 cm or more still existed between the strips. These figures excluded numerous smaller poles, saplings, and seedlings.

The growth of Meranti plants up to age 2 years in the TPTJ system was promising. Plants showed excellent growth, negligible levels of pest and disease incidence, and plant vigor of 90.12% (replanting was done when seedlings died, however). The average plant height was 3.83 meter with an average diameter of 4.89 cm. These figures can be translated into average annual growth of 2.45 cm for tree diameter and 1.92 m for plant height.

On the other hand, the growth parameters of *Shorea leprosula* up to 2 years of age were the best of all species tested. The average plant height was 4.04 m and average plant diameter was 5.32 cm. The detailed measurements are presented in Table 5.

Table 5. Growth of Plants in the TPTJ System at 2 years (1999 planting date)

No.	Plant species	Diameter		Total Height		Mean % of growth
		Mean (m)	MAI (cm)	Mean (cm)	MAI (cm)	
1.	*Shorea leprosula*	5.32	2.66	4.05	2.03	90.12
2.	*Shorea johorensis*	4.50	2.25	3.55	1.78	
3.	*Shorea parvifolia*	4.99	2.50	4.37	2.19	
	Shorea fallax	4.72	2.36	2.53	1.27	
5.	*Shorea* sp.	4.91	2.46	4.67	2.34	
	Average	4.89	2.45	3.83	1.92	

Conclusions

1. The natural forest selective cutting areas under PT Sari Bumi Kusuma that needs to be replanted are 4.71% for rehabilitation planting and 2.06% for enrichment planting.
2. It is necessary that replanting not exceed these suggested figures to prevent unsuccessful plant growth. It was found age 13 years, only 32.5% of the Meranti plants still existed in the field, and the majority died due to competition

with natural replanting. Also, only 2.3% of the remaining plants received adequate sunlight. Therefore, to conserve the forest at this stage of post-selective cutting activities, priority should be addressed to the maintenance and protection of the natural forest itself, not to replanting activities.

3. Selective cutting and strip planting (TPTJ) is one of the alternatives that could be adapted to the management of logged over areas since it offers the following advantages:
   a. increasing logging productivity,
   b. preparing the land for optimal plant growth,
   c. utilizing more labor, thus, opening more job opportunities,
   d. boosting the motivation of the people to conserve and protect the logged over areas in order to secure investment.

The TPTJ, however, should not be carried out indiscriminately on all logged over areas in any Forest Concessionaire. Several factors, such as forest condition, economics, social situation, and the surrounding environment need also to be taken into a consideration before its implementation.

Establishment of Meranti Trial Plantations in Indonesia

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Abstract. Establishment of meranti trial plantations was studied at three Indonesian sites: Dramaga and Leuwiliang in West Java, and Riau in Sumatra. Red meranti (*Shorea leprosula* and *S. selanica*) derived from cuttings and seedlings were used for planting trials at the three sites. White meranti (*Shorea javanica*) was used only at the Dramaga site.

The average height and maximum height of *S. selanica* cuttings at five years in Dramaga was about 10m and 12m, respectively. However the growth of *S. javanica* was inferior to that of *S. selanica* due to a gall disease.

Preliminary data for the first three years at the Leuwiliang site indicated that *S. selanica* grow slightly faster than *S. leprosula*, and a closer planting density (2m by 2m) resulted in better survival and growth than the wider planting.

The growth of meranti on mined peat land in Riau was quite poor, whereas performance on undisturbed peat land was better. This data indicates that *S. leprosula* and *S. selanica* are prospective meranti species for planting programs such as commercial plantations, rehabilitation of degraded forests, and reforestation of conservation areas. Rehabilitation of logged over peat forest with meranti is also possible if the peat is undisturbed.

Introduction

Indonesian tropical forests have been unsustainably exploited for more than 20 years, resulting in more than 20 million ha of degraded, unproductive secondary forest (Badan Planologi Kehutanan 2000). Meranti, a major commercial tree of the tropical forest, has been the main target of logging activity. To protect the remaining tropical forest from total destruction, two important strategies (1) rehabilitation of secondary forests by enrichment planting using native trees such as meranti, and (2) establishment of commercial meranti plantations to meet increasing demand for meranti timber must be implemented quickly.

Rehabilitation of degraded tropical forests and establishment of meranti plantations requires continuity of planting stock supply. Unfortunately, there is a problem for producing meranti planting stock from seed. Fruiting of meranti is irregular (Chan & Appanah 1980, Ashton 1982), seed viability is of short duration (Sasaki 1980), and we lack adequate seed storage techniques (Otsamo *et al*. 1998). Vegetative propagation technologies offer important solutions to providing a continuous supply of meranti planting stock.

The Forest Research and Development Agency (FORDA) developed a technique of mass vegetative propagation for meranti, the KOFFCO system (Komatsu – FORDA Fog-Cooling system), in cooperation with Komatsu (Subiakto *et al*. 1999). Basically, the system controls humidity, temperature, and light intensity at levels most suitable for transpiration and photosynthesis. A model large-scale cutting production nursery was developed in Bogor, with an annual production of about 100,000 cuttings.

Establishment of commercial meranti plantations requires appropriate silvicultural techniques. A model for plantation management of meranti has yet to be established. The Komatsu – FORDA collaboration project is currently studying silviculture practices for the establishment of meranti plantations using vegetative propagules and several meranti trial plots have been established in West Java, South Sumatera, and Riau.

This paper represents a preliminary report on meranti planting trials using vegetative propagules in West Java and Riau.

Materials and Methods

Planting stock of *Shorea leprosula* and *S. selanica,* derived from both cuttings and seedlings, was tested at three locations: Dramaga and Leuwiliang in West Java, and Riau, Sumatra. *Shorea javanica* was tested only at Dramaga.

Experimental treatments were planting stock origin, spacing regimes, and site conditions. The spacings used in this experiment were 3 x 3 m in Dramaga, 2 x 2, 3 x 3, 4 x 4, and 5 x 5 m at Leuwiliang, and 3 x 2.5 m and 3 x 5 meter at Riau. Site conditions were represented by mined peat and un-mined peat at the Riau site. Trees in each treatment were planted in square plots of 100 x 100 m. The parameters assessed in these trials were survival and height.

Results And Discussions

Performance of planting stock from seedlings vs. cuttings

Growth of vegetative propagules of *S. selanica* in Dramaga was quite good but that of *S. javanica* was constrained due to gall disease on stems and branches (Figure 1).

Growth rates of vegetative propagules and seedlings of *S. leprosula* and *S. selanica* in Leuwiliang in the first experiment are shown in Figures 2 and 3 (seedlings only for 4 x 4 m), and those at Riau on the un-mined peat land are shown in Figure 4 (only for *S. selanica*).

Figure 1

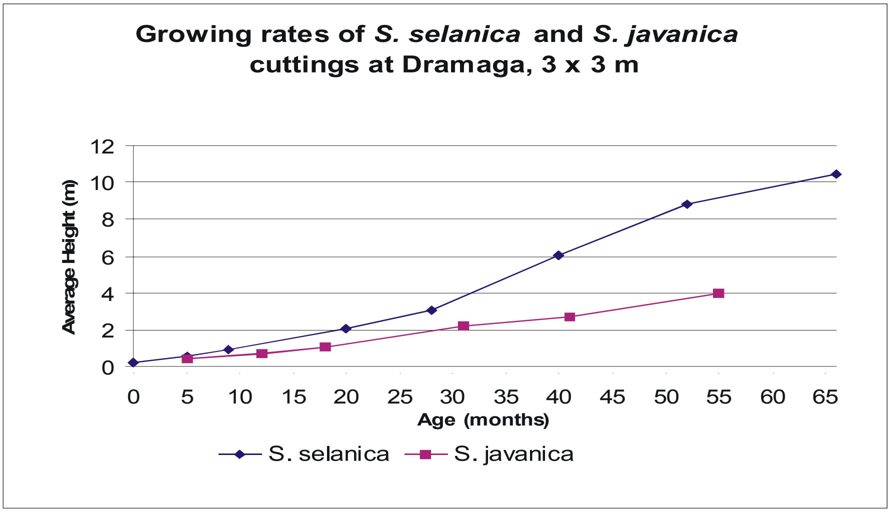


Figure 2

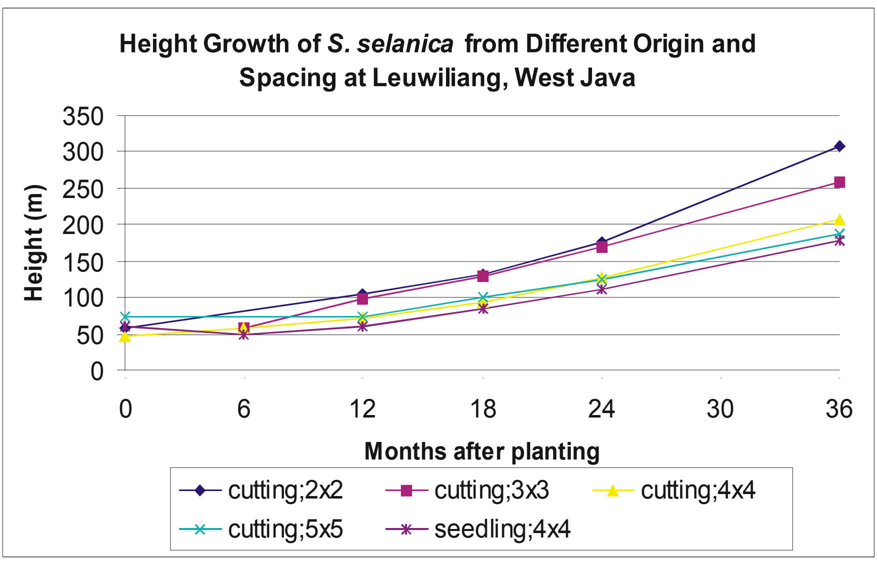


Figure 3

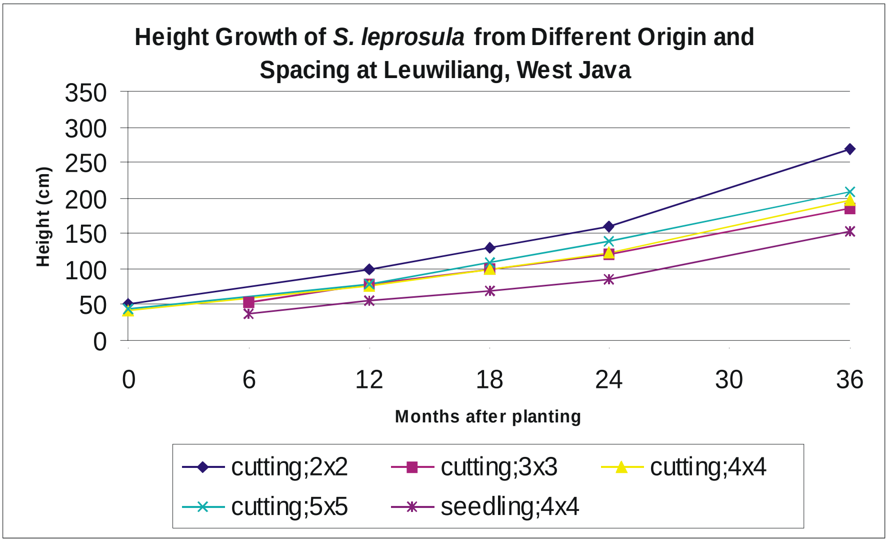
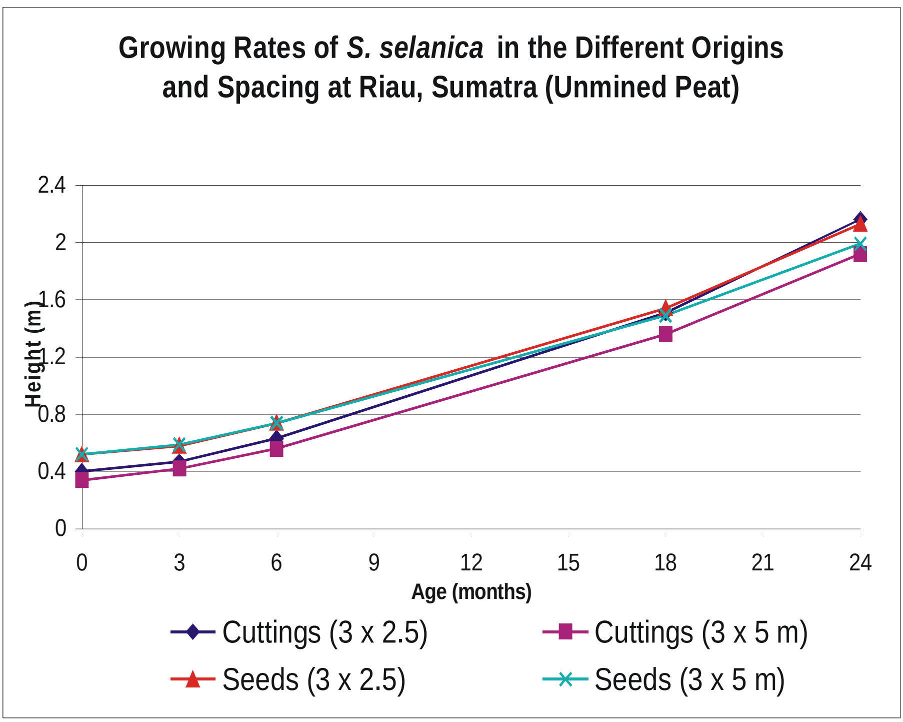


Figure 4



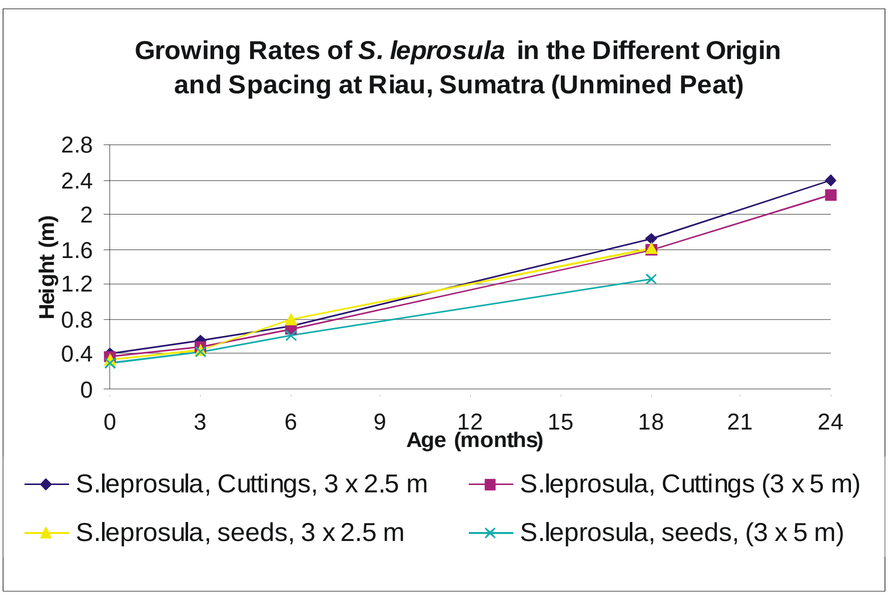
Overall, growth and survival of meranti trees planted as cuttings were better than for those planted as seedlings on the Leuwiliang site. However, survival and growth for the meranti seedling and cutting propagules at the Riau site were almost identical. The exact reason for this phenomenon is not known yet. One explanation may relate to differences of root systems between both planting stocks, so root systems should be investigated at both the nursery stage and the mature stage. Although these data were obtained from early stages of meranti growth, they provide an indication that planting stock from cuttings can be used for operational planting programs.

Effect of spacing

Growth rate of *S. leprosula* and *S. selanica* grown at different spacings at Leuwiliang are shown in Figures 2 and 3, and those at Riau are shown in Figures 4 and 5.

Both growth and survival of *S. leprosula* and *S. selanica* were better at the closer spacing of 2 x 2 m than at any of the wider ones at Leuwiliang. This may be partly due to less competition from surrounding vegetation than in the wider spacings, since planting with wider spacing may stimulate growth of surrounding vegetation (Forestry Department, Peninsular Malaysia 1996).

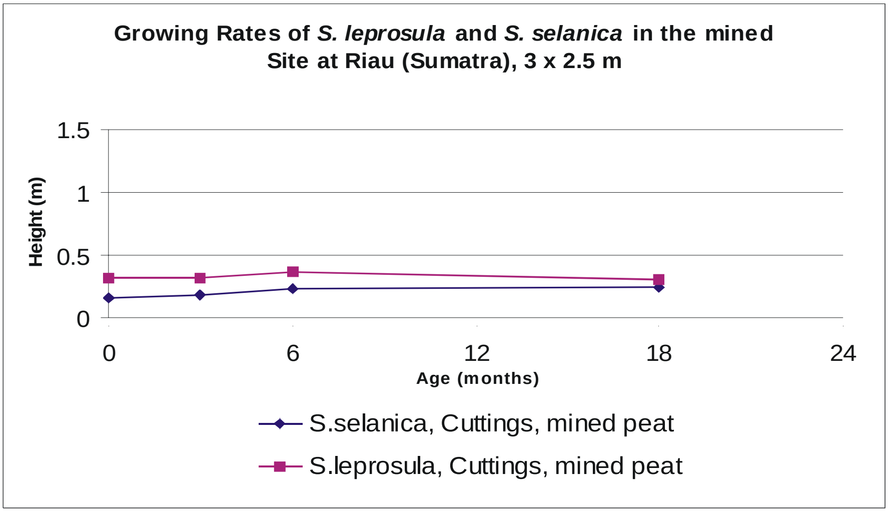
Figure 5



Effect of mined peat

This experiment was carried out only at Riau. Growth rate of *S. leprosula* and *S. selanica* cuttings on mined peat is shown in Figure 6.

Figure 6



Planting *S. leprosula* and *S. selanica* on mined-peat land suppressed the overall growth and survival of both species. Peat surface of the study area was removed to a depth of about 1 meter. The mined peat area was successfully planted with *Acacia mangium* and *A. crassicarpa*. Unfortunately, indigenous vegetation such as grasses and ferns do not grow on this site, and introduced meranti showed hardly any growth, perhaps due to insufficient nutrients when the surface soil was removed. This finding indicates that meranti is not suitable for rehabilitation of mined peat land.

Concluding Remarks

Performance of vegetative propagules on the three sites was quite promising. However, further observations are required before one can make any conclusions about prospects of using cuttings to establish meranti plantations.

Closer spacing resulted in better growth and survival of meranti planting stock; however, the choice of spacing for operational plantings must also consider the cost and availability of planting stock.

Shorea leprosula and *S. selanica* are suitable for reforesting logged-over peat swamp areas; however, planting should be done before disturbing surface soil layer.

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Potential of Carbon Sequestration After Reforestation and Grass Establishment on Tropical Degraded Soils

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Abstract. Increased attention is being given to the accumulation of CO_2, a potential 'greenhouse gas', in the earth's atmosphere, and to the potential for global warming caused by the consequent 'greenhouse effect.' Carbon dioxide is increasing in the atmosphere, partly from the combustion of fossil fuels and also from changes in land use from forest and range ecosystems to agriculture. Recent concerns about global warming have led to attempts at estimating the effects of land management on carbon sequestration in the soil. The research described herein was initiated to help assess the potential of C sequestration in forest soils, agriculture soils, and grasslands in tropical regions to ameliorate the buildup of atmospheric CO_2. The objectives of this study were to determine the amount of soil organic C (SOC), the rate of CO_2 emission, and the potential rate of C-sequestration in soils after restoration of forests and grasslands at a certain time (year), and to predict the economic valuation of soil C-storage assets. The C pool storage of previously cultivated clay soils (Typic Palehumult and Typic Paleudult) in Bengkulu, Sumatra returned to forest and to grasslands of *Imperata cylindrica* and *Chromolaena odorata* were compared with those of cultivated soils in three areas. Both field and laboratory studies measured carbon dioxide emissions from soils. Estimation of C sequestration utilized the method of Rosenzweig & Hillel (2000). Potential carbon dioxide emissions were reduced by about 31-40% when the soils were returned to forest cover, and about 14-36% when the soils were under grass. Soil carbon storage increased from 39-54% and 16-35% when cultivated fields were re-established to forest and grassland, respectively. Returning the previously cultivated soils resulted in an increase in soil organic carbon in the surface 60 cm at a mean rate 0.59-1.22 g C kg^{-1} yr^{-1} in the forest and 0.27-1.04 g C kg^{-1} yr^{-1} in grassland. In general, the relative rate of carbon sequestration during reforestation was two times higher compared to grass establishment following cultivation. This implies that an aggrading forest ecosystem has the highest capability to sequester C in the soil, thereby helping to offset carbon emissions. The SOC mass in the surface 60 cm of the agricultural soils was 25% to 32% less than that of forest soils and 5% to 11% than that of grassland sites. In conclusion, the forest and grass sites can be said to be providing a global service function worth US$ 95 billion and $47 billion on the unit of the global cropland area (1.5 x 10^{7} km^2), respectively. However, this study also suggests that the values of C accumulation/sequestered vary from site-to-site according to the extent and value of agricultural activity associated with each.

Introduction

Increasing attention is being given to the accumulation of carbon dioxide, a potential 'greenhouse gas,' in the earth's atmosphere and to the potential of global warming caused by the consequent 'greenhouse effect'. Carbon dioxide is increasing in the atmosphere, partly from the combustion of fossil fuels and also from land use changes (Mann 1986). Recent concerns about global warming have led to attempts to estimate the effects of land management on carbon sequestration in soils and to determine the total worldwide carbon storage available (Handayani 1996).

Estimates of sources and sinks for carbon dioxide have consistently provided evidence of an imbalance, indicating the existence of a substantial terrestrial sink located in tropical regions. An understanding of the processes associated with the terrestrial C sink is crucial to predicting environmental responses to continued atmospheric loading of carbon dioxide. Little is known about whether this C sink is currently increasing or decreasing, or how the flux of C associated with it may change in the next several decades. The stability of the sink is of particular concern because any decrease in the rate of C sequestration would result in an increase in the concentration of atmospheric carbon dioxide.

Among all of the many studies focusing on the global carbon cycle, few are concerned with the soil reservoir, and especially with the fluxes of carbon dioxide respired by soils. Recent studies have shown that soils contain the largest active terrestrial carbon pool on Earth and, through soil respiration, contribute an annual flux of carbon dioxide to the atmosphere that is 10 times greater than that from fossil fuel combustion (Andrews *et al.* 1999). Because of the size of this flux, even small changes in the rate of soil respiration could have large effects on the atmospheric carbon dioxide concentration and recent studies demonstrate that increased rates of soil respiration may result from increases in atmospheric carbon dioxide (Ball & Drake 1998, Cheng 1996).

Soil respiration (*heterotrophic respiration*) is probably one of the largest inputs of carbon dioxide to the atmosphere with an annual flux estimated to about 68 to 76 10^{15} g C yr-1 (Raich & Schlesinger 1992, Raich & Potter 1995). Therefore, soil respiration must play an important role in the regulation of the concentration of carbon dioxide in the atmosphere, i.e., at least as important as the vegetation-atmosphere exchanges (net flux 50 to 60 Gt. C. yr^{-1}) and the ocean-atmosphere exchanges (net flux 1 to 2 Gt.C.yr^{-1}).

The upper meter of the world's mineral soils represents a large reservoir of C - about 1500 Pg C (Batjes 1996) – or about twice that of the atmospheric reservoir. Moreover, recent studies have suggested that forest soils contain a significant portion of the world's C reserves (Eswaran *et al.* 1993). Human-induced changes of land cover - such as the replacement of natural ecosystems by agrosystems – drastically alters C dynamics in soils. Therefore, the estimation of C pools and their turnover rates in natural and human-influenced systems is fundamental to our ability to estimate fluxes in C between the ground and atmosphere. About 7 Pg CO_2-C are released to the atmosphere each year from deforestation and the burning of fossil fuels. Over the period 1980 to 1989 the average annual carbon dioxide emission to the atmosphere from tropical deforestation was 1.6 ± 1.0 Pg C (IPCC 1994).

Carbon dioxide fluxes from the soil surface consist of contributions from the decomposition of plant litter and root detritus, plus the respiration of living plant roots and microorganisms. As areas originally covered with natural vegetation are transformed into cultivated fields, much of the vegetative biomass originally present is converted to carbon dioxide. Land-use change and biomass burning cause the release of carbon - in the form of carbon dioxide - that had previously been contained in plant biomass and soil organic matter through cumulative prior photosynthesis. The aboveground material is either burned or decomposes rapidly. The decline of soil organic matter after deforestation is caused largely by losses from the labile pool, also known as the light fraction (Handayani *et al.* 2000 a, b). In contrast, the resistance pool or heavy fraction of organic matter in the forest soil, while not entirely stable, decomposes at a much slower rate (Feller & Beare 1997, Handayani *et al.* 2001).

Deforestation, biomass burning, drainage, plowing, cultivation, and overgrazing all promote the decomposition of organic matter and the release of carbon dioxide into the atmosphere, thus lowering soil organic carbon content (SOC). The decrease in SOC content depends on the land use established after forest clearing. Lal *et al.* (1998) estimated that cropping tropical forest soils reduces their C content by 40% while the use of these soils to support pastures reduces C by about 20%. Soil degrading processes (such as erosion, crusting and compaction, acidification, and salinization) further exacerbate loss of soil carbon. As crop production continues over time, soil organic matter declines still further (albeit at a slowing rate), resulting in more carbon dioxide release, until a steady state is reached or until the field is abandoned.

Appropriate management strategies may be selected to store carbon that would otherwise be released to the atmosphere. Tillage effects on soil organic carbon (SOC) contents have been studied for a substantial period of time (Handayani 1991, 1996, Handayani *et al.*1998, 1999, 2000a). An estimated 5×10^9 metric tons of C have been lost from United States soils as a result of cultivation (Lal *et al.* 1998). More recently, studies have been directed toward potential increase of SOC with improved management practices such as returning agricultural soils or cultivated fields to grassland or forest management. After conversion of long-term arable land to forest, more than twice as much SOC was present in the surface 23 cm than at time of conversion (Martins *et al.* 1991). However, little difference was found in C content or in C:N ratios in the surface 23 cm of soils under continuous grass cover for 120 years.

Indonesian soils, because of extent (approximately 5,000,000 km^2), high carbon density, and high deforestation rates, play an important role in the global C cycle. The capacity of these soils to sequester C through the establishment of plants (trees or grass) in deforested areas is thus considerable. However, the time required for organic C to obtain equilibrium, especially under tropical conditions, is uncertain as there is little information concerning carbon sequestration.

The research described in this paper was initiated to assess the potential for carbon sequestration after reforestation and grass establishment on degraded soils in Bengkulu Province, Sumatra. The objectives of this study were to develop reliable preliminary data concerning the amount of soil organic C, the rate of carbon dioxide emissions, and the rate of carbon sequestration in soils previously degraded by shifting cultivation practices after restoration of grass and forest cover, and the economic valuation of soil C-storage assets in forest and grass ecosystems. Our study was divided into three phases: i) constructing a preliminary database for soil carbon dioxide emissions; ii) providing comparable data on organic C content of soils returned to grass and forest, and iii) estimating the economic value of soil C-storage in forest and grass ecosystems.

Site Description

Three areas in Bengkulu Province, Sumatra were identified as having sites with a unique combination of management histories; grassland (*Imperata cylindrica*), shrubland (*Chromolaena odorata*), forest sites, cultivated fields with a history of inversion tillage, and a previously tilled site that had been returned grass and forest. Soils at these sites are Ultisols, with large kaolinitic clay content in the soil profile.

Methods

Soils were sampled in the areas of Kabupaten Rejang Lebong (Bukit Daun) in 2000, and Kabupaten Bengkulu Utara (TAHURA Rajolelo and Bukit Ilalang) in 1999. These sites were selected based on previously published information (Handayani & Prawito 1998, Handayani 2000 a, b). Soils were analyzed at Soil and Climate Laboratorium Research in Bogor, using prescribed methods

(Page *et al.* 1982). Soil organic carbon was determined with the wet digestion method. Bulk density was measured on saran-coated clods. Carbon dioxide emissions from soils were determined by the method of Page *et al.* (1982) and Schinner *et al.* (1996).

Results And Discussion

Carbon Sequestration

Potential CO_2 emissions

Carbon dioxide emissions for the three locations and surface conditions are presented in Table 1. On a volumetric basis, potential carbon dioxide emissions rates varied from 11.05 to 90.89 ton ha^{-1} yr^{-1}, depending on land use and study sites. In general, potential carbon dioxide emissions were highest for the cultivated field, intermediate for *Imperata cylindrica* grassland and *Chromolaena odorata* shrubland, and lowest for forest soils. The greatest differences in carbon dioxide emissions occurred between forest and cultivated fields in Transos. When degraded soils were returned to forest and grass, the carbon dioxide emissions were lower. The values of carbon dioxide emissions at all fields were comparable to those measured at other forested sites in other tropical countries (Moraes *et al.* 1996, Uhl *et al.* 1988). There is a large potential for further carbon dioxide emissions in the soils of cultivated fields and grasslands based on differences in measured carbon dioxide at forest, cultivated, and grassland sites.

The rate of decomposition was lowest at the forest sites, intermediate at grassland sites, and highest at cultivated sites. The largest difference - ± 52% - was between forests and cultivated fields, which is closely related to the amount of fine litter in each (Table 2). This implies that more stable pools of C were accumulated in the reforestation sites compared to the grassland sites. Therefore, these data confirm that reforestation sites are aggrading, and suggest that there is a substantial potential for decreasing carbon dioxide emissions through increasing forest cover.

Table 1. Potential CO_2 emissions under different land uses in Bengkulu

Study sites	Land use	CO_2 emissions (ton ha^{-1} yr^{-1})
Bukit Ilalang*	Grass (5 yrs) Grass (10 yrs) Forest (35 yrs)	39.11 35.96 11.05
TAHURA Rajolelo	Forest (40 yrs) Forest (15 yrs) Cultivated field (6 yrs) Grass (15 yrs) Grass (2 yrs)	62.78 66.80 90.89 77.75 87.60
Transos*	Forest (25 yrs) Cultivated field (3 yrs) Grass (1 yr) Grass-Schrubland	23.48 39.14 41.93 26.97

* indicated that the CO_2 emissions were measured in the field

Table 2. The rates of decomposition and percentage of fine litter in forest and deforested areas

Land use	Rate of decomposition (% yr^{-1})	Fine litter (%)
Forest (40 yrs)	1.56	19
Cultivated field (6 yrs)	3.24	39
Grass (15 yrs)	2.16	36
Grass (2 yrs)	2.52	-

Soil organic carbon content

Soil organic carbon content for the three locations and surface conditions are presented in Table 3. Differences in soil organic C concentration by surface conditions were generally limited to the surface of 60 cm. The forest sites had the greatest soil organic C concentration, ranging from 3.53 - 6.41% in the surface 20 cm. The cultivated fields had the lowest soil organic carbon, with values from 2.12 - 2.98%. The surface soil organic carbon contents of cultivated fields are typical of those previously reported (Handayani 1991, 1996, 1998, 1999, 2000 a). Soil organic carbon declined with the lower age of *Imperata cylindrica* grasslands in all cases, but it was still generally higher than in cultivated fields. Carbon concentrations at grassland sites were intermediate to those reported for the forest and cultivated fields.

Table 3. Mean soil organic carbon concentration values (in %)

Kedalaman (cm)	Land use			Soil Type
Bukit Daun	***Forest (40 yrs)***	***Cultivated fields (7 yrs)***	***Grass (15 yrs)***	***Typic Paleudult***
0-20 20-60 60-120	6.41 1.03 0.48	2.98 1.55 0.90	4.55 0.97 0.31	
Bukit Ilalang	***Grass (1yr)***	***Grass (5yrs)***	***Grass (10yrs)***	***Typic Palehumult***
0-20 20-60 60-120	2.37 0.88 0.55	2.68 0.70 1.19	2.86 1.14 1.03	
TAHURA Rajolelo	***Forest (30 yrs)***	***Cultivated fields (6 yrs)***	***Grass (15 yr)***	***Typic Palehumult***
0-20 20-60 60-120	3.53 1.20 -	2.12 0.73 0.52	2.53 0.74 0.56	

Carbon concentrations in the soil profile may indicate C fluxes or soil C accumulation rates during a certain time (year). In general, there was an increase of 39-54% of carbon when cultivated fields were returned to forest and 16-35% when established as grassland. Increases of 12% and 6% occurred when the soils returned to grass for one and ten years, respectively. Carbon accumulation rates during reforestation following agricultural abandonment ranged from 0.59 g kg^{-1} to 1.22 g kg^{-1} but when the soil returned to grass cover, C accumulation rates ranged from 0.27 g kg^{-1} to 1.04 g kg^{-1}, clearing tropical forests has reduced the mass of carbon by about 2.55×10^{10} tons, but when agricultural sites were returned to forest, the soil will accumulate about 2.70×10^{10} tons of carbon.

Our results thus indicate that depleted soil carbon may be restored by re-establishing forest and grass. Under such practices where litter fall is left in the field and erosion is controlled, organic carbon can be replenished in a soil where it has been depleted by cropping. Appreciable carbon dioxide is sequestered in the process.

It was demonstrated in this study that carbon sequestration rates were two times higher during reforestation than carbon sequestration rates for grass establishment. A question of great concern is how long soils can serve as a sink for carbon. Estimates vary upward from as little as 25 years (Lal *et al.* 1998).

Economic valuation of C-fixation assets

There is a growing recognition that the values of environmental goods and services should be factored into national accounts to obtain realistic assessments of whether economic development is proceeding sustainably or not (Dixon 1990). The principle here is that traditional means for measuring Gross National or Domestic Product (GNP or GDP) do not adequately reflect depreciation of renewable natural resources through unsustainable use. This has led to suggestions that national accounts be reformulated to generate figures on *Sustainable Net National Product* (SNNP) rather than GNP. This approach stresses the importance of categories of value, which represent *the total economic value* of a set of natural resources.

Carbon fixation properties of certain ecosystems (i.e. forests, grasslands, agricultural fields, prairies, arable land) are values that have indirect use derived from regulatory environmental fractions. The C sequestered or contained in ecosystems may have an important role in maintaining regulatory functions, e.g., nutrient cycling and global climate change. This indirect use value for environmental functions is also related to the change in the value of production or consumption of the activity or property that it is protecting or supporting. Thus, the C-fixation asset values suggest strongly that maintaining/increasing the ecosystem's capacity to sequester C is fundamental to the continued integrity of our environment, especially the forest environment, and hence the continuation of national economic output and future prosperity.

*Economic value of soil C-storage under forest and grass ecosystem***s**

According to McNeely *et al.* (1990) the destruction of one hectare of tropical forest releases at least 75 tons of C into the atmosphere, and the marginal cost of sequestration of this carbon has been estimated in the range US$ 2-5 per ton, or an average of US$ 3.5 per ton. Based on our data, it can be estimated that clearing tropical forests in Bengkulu has reduced the mass of C per global cropland area about 2.55 x 10^{10} tons, which is equal to US$ 8.93 x 10^{10} or 89 billion, but when cultivated sites were returned to forest, the soil will accumulate C about 2.7 x 10^{10} tons or about US$ 9.5 x 10^{10} (95 billion), and when returned to grassland will sequester C about 1.35 x 10^{10} tons or about US$ 4.7 x 10^{10} (47 billion).

Thus, the forest and grassland sites in Bengkulu, Sumatra can be said to be providing a global service function worth US$ 95 billion and 47 billion on the unit of the global cropland area (1.5 x 10^{7} km^2), respectively. However, this study suggests that the values of C accumulated/sequestered varied from site-to-site according to the extent and value of agricultural activity associated with each.

Finally, it is clear that ecosystem diversity, which is associated with capability for C-sequestration, species richness, and intraspecific genetic variability, altogether must be worth many billions of dollars to the Indonesian economy. This implies that there is a strong reason for any organization or institution whose activities impact C-fixation assets to consider very carefully the true economic benefits of its activities relative to the true economic benefits of avoiding or reducing those impacts.

Conclusion

Potential carbon dioxide emissions were reduced about 31-40% when the soils were returned to forest cover and by 14-36% when the soils were returned to grassland. Soil carbon storage increased from 39-54% and 16-35% when cultivated fields were established to forest and grass, respectively. Returning the previously cultivated soils resulted in an increase in soil organic carbon in the surface 60 cm at a mean rate 0.59-1.22 g C kg^{-1} yr^{-1} in forest and 0.27-1.04 g C kg^{-1} yr^{-1} in grassland. In general, the relative rate of carbon sequestration during reforestation was two times higher compared to grassland establishment following cultivation. In conclusion, the forest and grass sites in Bengkulu, Sumatra can be said to be providing a global service function worth US$ 95 billion and 47 billion on the unit of the global cropland area (1.5×10^{7} km^2), respectively. However, this study also suggested that the values of C accumulated/sequestered varied from site-to-site according to the extent and value of agricultural activity associated with each.

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Possibility of Timber Estate Development on Degraded Coal-Mined Lands in Sumatra Region

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Abstract. Surface mining activities can change drastically the ecosystem of the outer-layer of the earth. The remaining disturbed soils (mine spoils) are bare, susceptible to erosion, and unfavorable for plant growth. Based on findings from 5 years of research on mine-soil reclamation in Bengkulu, the possibility for timber estate development was assessed from the performance of plant growth.

Results showed that four species (*Paraserianthes falcataria*, *Sesbania glandiflora*, *Acacia mangium* and *Pneronema canescens*) grew relatively well in the degraded ecosystem. Field experiments also indicated that inoculation with endomycorrhizae induced better growth, especially for *Acacia mangium*. Within the first 16 months of the studies, *Paraserianthes falcataria*, *Sesbania grandiflora* and *Acacia mangium* grew better than *Peronema canescens*. Development of undergrowth species was better in the plots that had been prepared by ripping.

Favorable conditions for plant growth were indicated by soil development under the four stands, and this is promising for further ecosystem development. For example, forest plantations would be feasible in some mined land areas. Even in this harsh environment, forestry activities could generate some sustainable forest resources.

Introduction

As one of the major export commodities of Bengkulu, coal is important to the Province's economic development. After South Sumatra (Bukit Asam), Bengkulu is the most important coal-producing area in Sumatra, and Sumatra's coal deposits represent approximately 68 % of total Indonesian coal deposits. The Provincial Office of the Department of Mining and Energy (1999) reported that measured coal deposits in Bengkulu are approximately 123 million metric tons while total Indonesian coal deposits are about 750 to 1050 million metric tons (Umar 1986). In the last four years, the average annual coal production in Bengkulu was around 800 thousand metric tons. Most of the yields are for export, particularly to Asian countries such as Japan, India, Malaysia, Philippines, and Taiwan.

Coal mining in Bengkulu is accomplished primarily by surface mining and mining activities can drastically alter ecosystems. Mining removes above ground cover vegetation and fertile topsoil, exposes rock materials of the lithosphere, and depletes biologically active nutrient pools. Heavy machines, such as scrapers, loaders, draglines, shovels, and excavators greatly increase levels of soil compaction (Porterfield 1981) and reduce soil water permeability and water holding capacity (Grandt 1988). Consequently, the leftover soils, which are usually called mine spoils, have highly unfavorable properties for supporting plant growth.

To support plant growth, therefore, successful reclamation of the mine spoil is necessary. Not only is this required to increase the productivity of the soil, but also to reduce negative impacts to the environment. Reclamation may also be considered as the first stage of restoration, and the re-establishment of active biological nutrient cycles and microorganism populations are important keys to reclaiming disturbed lands for productive uses. Mineral nutrients are chemically bound in the organic structures of leaves on the forest floor, fine roots, and soil humus. The quantity of nutrients released depends solely on the rate of decomposition of organic matter, which is determined by many factors involving activity of soil flora and fauna and chemical quality of decomposing materials. Therefore, establishment of vegetative cover is a critical initial aspect in reclaiming or rehabilitating disturbed land to achieve post-mining land uses goals.

This paper discusses the possibility of timber estate development on lands degraded due to coal mining activities, based on findings from reclamation research in North Bengkulu and surveys from South Sumatra

Methods

The experiment was designed in two levels; species selection (green house), and field experiments with selected species.

Green house experiments

The green house studies were done at the Faculty of Agriculture, University Bengkulu and involved testing 13 woody species and two grasses (Suhartoyo & Munawar 1998).

A Completely Randomized Design was used to test the performance of thirteen timber species and two grasses species, namely *Acacia mangium, Delonix regia, Gliricidia sepium, Hurea sp, Phitecellobium jiringa, Cassia siamea, Calliandra callothyrus, Leucaena leucocephala, Mimosa sp., Ceiba petrandra, Paraserianthes falcataria, Peronema canescens* (timber species) and *Pennisetum purpureum* and *Setaria spacellata* (grass species). Plant materials were prepared by growing seedlings or rooted cuttings for about three weeks prior to transplanting them into 10 kg containers of mine soil or forest soil. Growth measurements were made every two weeks.

Field Experiment

The field experiment was located at a six-year-old dumping site in the mining area of PT. Bukit Sunur, North Bengkulu. The experiment was designed as a split plot with ripped and non-ripped treatments as main plots and endomycorrhizal inoculation as sub-plots. Four legume timber species were tested, namely *Acacia mangium* (mangium), *Paraserianthes falcataria* (sengon), *Sesbania grandiflora* (turi), and *Pneronema canescens* (sungkai) (Suhartoyo & Munawar 1998).

Results and Discussion

Green House Experiment

In general, observations showed that all plants were able to grow relatively well on both mine and forest soils. Mortality was only found when trees were derived from cuttings, e.g. gliricidea and in the grasses (Suhartoyo & Munawar 1998).

Four species were determined to be the best for field-testing - Sengon, Turi, Mangium, and Sungkai.

Field Experiment

In general, all legume plants except Sungkai grew relatively well on mine soil. However, data on plant height and stem diameter (Tables 1 to 4) during the first months of the growing period (10 months for mangium) showed that all species grew better on ripped mine soil than on non-ripped soil. As indicated by Munawar (1998) and Suhartoyo and Munawar (1998), soil compaction was identified as the main constraint to plant growth on coal mined soils in Bengkulu. Very compacted soils with strengths of more than 4.5 kg/cm2 significantly hindered root growth, resulting in poor nutrient and water acquisition.

Table 1. Effects of ripping on plant height increment during 10 – 13 months growing period

Plant species	Treatments	Plant height (cm) in the month of												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Mangium	Rip	37.11	42.01	47.73	65.18	85.28	104.86	120.41	146.43	166.62	197.92	-	-	-
	No-Rip	28.49	33.44	43.04	53.04	67.37	75.80	81.67	93.90	104.81	117.83	-	-	-
Sengon	Rip	23.07	39.52	77.58	110.65	173.13	208.17	228.19	245.92	318.62	282.62	431.93	453.88	527.63
	No-Rip	18.95	28.36	46.09	62.72	92.96	112.12	118.48	151.20	174.92	208.48	233.31	254.15	288.86
Turi	Rip	44.78	67.79	121.51	166.47	227.67 NS	275.59	290.39	321.16	353.54	410.60	444.68	488.63	532.69
	No-Rip	41.18	60.44	97.69	132.85	173.90	209.68	219.78	244.39	278.49	313.30	342.38	372.65	402.91
Sungkai	Rip	8.41 NS	10.45 NS	17.34	25.71	30.88 NS	32.15 NS	32.97 NS	36.37 NS	40.04 NS	42.71 NS	44.62	46.65	48.56 NS
	No-Rip	6.79	7.96	12.02	16.03	119.41	21.06	21.66	24.25	29.64	31.63	33.90	36.44	38.57

Notes:
Rip = Ripped-soil; No-Rip = Non-ripped soil
Values between rows in the same column of each Plant, except those followed by NS, are significantly different at 5% level of confidence.

Table 2. Effects of ripping on stem diameter increment during 10 – 13 months growing Period

Plant species	Treatments	Stem diameter (cm) in the month of												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Mangium	Rip	0.35NS	0.54	0.82	1.11NS	1.52NS	1.76	2.02	2.33	2.54	2.90	-	-	-
	No-Rip	0.33	0.44	0.69	1.00	1.21	1.37	1.49	1.67	1.85	2.09	-	-	-
Sengon	Rip	0.47	0.76	1.47	2.02NS	2.94	3.39	3.83	4.16NS	5.13	5.80	6.59	7.22	7.36
	No-Rip	0.43	0.60	0.99	1.15	1.72	2.02	2.06	2.63	2.97	3.48	3.92	4.32	4.67
Sungkai	Rip	0.49NS	0.64	0.96 NS	1.29 NS	1.41 NS	1.51 NS	1.57 NS	1.62 NS	1.69 NS	1.72 NS	1.77 NS	1.81 NS	1.86 NS
	No-Rip	0.66	0.59	0.78	0.95	1.04	1.18	1.23	1.30	1.40	1.44	1.48	1.57	1.64
Turi	Rip	0.66NS	1.67NS	2.98	3.55	4.66	5.75	5.85	6.95	7.67	8.14	8.61	9.02	9.01
	No-Rip	0.66	1.46	2.28	2.76	3.71	4.68	4.70	5.71	6.44	6.91	7.52	7.21	7.48

Notes :
Rip = Ripped-soil ; No-Rip = Non-ripped soil
Values between rows in the same column of each Plant, except those followed by NS, are significantly different at 5% level of confidence.

Table 3. Effects of endomycorrhizal inoculation on plant height increment during 10 – 13 months growing Period

Plant species	**Treatments**	**Plant height (cm) in the month of**												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Mangium	Mi	37.11	42.26	49.91	65.18	83.37 NS	98.88 NS	111.16 NS	134.33 NS	154.27 NS	179.88 NS	-	-	-
	No-Mi	28.49	33.44	49.87	53.04	69.29	81.76	90.93	106.00	117.16	135.58	-	-	-
Sengon	Mi	20.49 NS	35.75 NS	66.48 NS	93.26 NS	146.55 NS	176.83 NS	193.31 NS	212.78 NS	274.84 NS	328.32 NS	373.18 NS	388.93 NS	452.51 NS
	No-Mi	21.53	33.14	57.19	80.10	119.54	143.46	153.36	184.34	218.70	262.79	292.06	319.10	363.98
Sungkai	Mi	6.36 NS	8.83 NS	14.14 NS	20.57 NS	26.03 NS	28.51 NS	29.63 NS	32.68 NS	38.09 NS	41.19 NS	43.47 NS	45.99 NS	48.20 NS
	No-Mi	8.83	10.03	15.22	21.16	24.26	24.70	25.00	27.95	31.59	33.14	35.05	37.11	38.94
Turi	Mi	43.50 NS	61.92 NS	106.11 NS	145.35 NS	194.32 NS	237.19 NS	249.99 NS	274.10 NS	306.08 NS	351.05 NS	387.82 NS	426.31 NS	465.50 NS
	No-Mi	43.45	66.31	113.09	153.96	207.25	248.26	260.19	291.45	325.95	372.85	399.22	434.97	470.10

Notes :
Mi = Endomycorrhizal-inoculated plants ; No-Mi = Non- Endomycorrhizal-inoculated plants
Values between rows in the same column of each Plant, except those followed by NS, are significantly different at 5% level of confidence.

Table 4. Effects of endomycorrhizal inoculation on stem diameter increment during 10 – 13 months growing Period

Plant species	**Treatments**	**Stem diameter (cm) in the month of**												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Mangium	Mi	0.37	0.54	0.82	1.15	1.51	1.76	1.98	2.30	2.56	2.91	-	-	-
	No-Mi	0.31	0.44	0.69	0.96	1.23	1.40	1.53	1.70	1.83	2.07	-	-	-
Sengon	Mi	0.45NS	0.70NS	1.27NS	1.66NS	2.47NS	3.01NS	3.05NS	3.55NS	4.42NS	5.10NS	5.77NS	6.29NS	6.58NS
	No-Mi	0.45	0.66	1.18	1.51	2.18	2.41	2.83	3.24	3.67	4.18	4.74	5.25	5.59
Sungkai	Mi	0.49NS	0.59NS	0.84NS	1.10NS	1.23NS	1.39NS	1.45NS	1.49NS	1.57NS	1.62NS	1.65NS	1.74 NS	1.80
	No-Mi	0.50	0.63	0.90	1.14	1.23	1.31	1.35	1.42	1.51	1.55	1.60	1.64	1.71
Turi	Mi	0.65NS	1.49NS	2.44NS	3.07NS	4.11NS	5.13NS	5.21NS	6.24NS	7.00NS	7.44NS	7.98NS	8.19NS	8.26NS
	No-Mi	0.67	1.64	2.72	3.24	4.26	5.29	5.33	6.41	7.11	7.60	8.15	8.04	8.23

Notes :
Mi = Endomycorrhizal-inoculated plants ; No-Mi = Non- Endomycorrhizal-inoculated plants
Values between rows in the same column of each Plant, except those followed by NS, are significantly different at 5% level of confidence.

Field observations found that roots of plants on non-ripped soils were concentrated in the top layer of the soil, and partly exposed to the soil surface. Consequently, these plants were susceptible to water stress and wind force.

At 13 months from planting, effects of endomycorrhizal inoculation on plant growth were not significant, except for mangium. The inoculated mangium plants grew better, especially in the first four months. Although statistically not significant, there were also interactions among treatments. Both mangium and sengon grew better on ripped soil; however, effects of endomycorrhizal inoculation on these plants were more pronounced on the non-ripped soil. On the other hand, these effects were not found for turi growth.

Table 5 shows that, in general, soil properties of mine spoils were slightly improved after 10 months of legume planting, especially available P and K concentration and bulk density. Bulk densities of ripped soils were consistently lower than those of non-ripped soils, resulting in consistently low total porosities of the non-ripped soils. Numerically, the bulk densities for both ripped and non-ripped soils were not so high compared to those found prior to planting. This means, regardless the ripping treatment, the presence of growing plants was a factor in reducing soil compaction. Part of the reason was probably that growing roots of both tree legumes and understory plants (weeds) had loosened the soil structure of mine soils.

Table 5. Some soil properties under 10-month old growing stands

Plant Species	Treatments	Total N (%)	Avail-P (ppm)	Exch.-K (me/`100g)	Org-C (%)	pH (H_2O)	CEC (e/100g)	C/N	BV (g cm^{-3})	BJ (g cm^{-3})	Total Porosity (%)
Mangium	Rip	0.13	18.60	1.28	4.08	5.33	22.70	31.38	1.39	2.09	33.51
	No-Rip	0.13	19.25	0.98	3.38	5.73	20.69	26.00	1.40	1.89	25.86
Sengon	Rip	0.14	28.62	1.10	5.32	5.55	21.44	38.00	1.37	1.97	30.53
	No-Rip	0.16	20.58	2.33	4.25	5.36	22.31	26.56	1.47	2.01	26.56
Sungkai	Rip	0.14	17.77	1.02	4.11	5.40	20.17	29.36	1.45	2.07	30.04
	No-Rip	0.16	18.77	1.13	4.74	5.45	19.47	29.63	1.50	1.98	24.17
Turi	Rip	0.12	20.14	1.10	5.85	5.32	22.18	48.75	1.28	2.15	40.37
	No-Rip	0.17	17.07	1.23	4.97	5.50	21.76	29.24	1.42	2.01	22.77

Notes :

Rip = Ripped-soil ; No-Rip = Non-ripped soil

Tot-N = Total N, Avail-P = Available P, Exch.-K = Exchangeable K, Org-C = Organic C, CEC = Cation Exchange Capacity ; BV = Bulk Density, BJ = Particle Density.

Biological properties of the soils indicated that growing plants had significantly improved biological activities in the soil. Populations of bacteria, fungi, and mycorrhizal fungi in the planted soils were, respectively, at least 40, 100 and 1000 times higher than those in soil prior to planting (Table 7). Accumulation of litter might also have enhanced biological activity in the soil. Field observation showed that leaf litter of turi took less than two weeks to completely decompose, suggesting that biological processes in the soil had been very active. Litter production for two plant species - sengon and turi - is shown in Table 6. Within 8 weeks, sengon produced more litter than turi. However, soil analysis showed that this difference had not significantly contributed to the differences in soil properties under these two plants.

Table 6. Litter Weight of Sengon and Turi Stands

Plant Species	**Treatments**	**Plant Parts**	**Litter weight (gm^{-2}) at sampling time**					*Total Litter*
			Nov.6,1998	**Nov.21st, 1998**	**Dec.5th, 1998**	**Dec. 18,1998**	**Jan.3.,1999**	**($g\ m^{-2}$)**
	Rip	Leaves	34.81	21.41	23.92	15.36	17.03	112.53
		Twigs	27.49	29.91	17.09	8.16	12.81	95.16
Sengon	No-Rip	Leaves	14.90	3.92	6.16	8.25	4.60	37.83
		Twigs	6.24	2.86	3.59	1.39	4.90	19.16
	Total							**264.16**
	Rip	Leaves	4.43	6.57	8.58	9.26	7.64	36.48
		Twigs	17.80	7.57	5.85	4.76	7.09	43.07
Turi	No-Rip	***Leaves***	4.05	6.30	9.48	10.44	5.58	35.85
		Twigs	10.29	6.94	5.37	3.49	6.66	32.75
	Total							**148.15**

Notes:
Rip. = Ripped-soil
No-Rip = Non-ripped soil

Table 7. Type and population soil microbes in mine soil and forest soil

Type of Microbes	**Soil depths**	**Population**	
		Mine soil *)	**Forest soil **)**
Bacteria	0 – 30 cm	71.75×10^4	162.00×10^4
(cel/g dry soil)	30 – 60 cm	90.97×10^4	139.60×10^4
Fungi	0 – 30 cm	7.11×10^4	5.80×10^4
(cfu/g dry soil)	30 – 60 cm	5.33×10^4	2.00×10^4
Mycorrhizal fungi	0 – 30 cm	77.15	91.10
(spore/g dry soil)	30 – 60 cm	49.65	35.64

Notes : *) Average values of 18 samples
**) Average values of 5 samples

Possibility of Timber Estate Development

From the findings above, it is apparent that mined soils in Bengkulu could support forest growth. However, the following must be considered. First, due to compaction, soil ripping would be necessary to support root growth. Second, to accelerate rebuilding soil fertility, revegetation with legume plants for a year or more would be beneficial. Third, like other soils, additions of organic matter, inorganic fertilizers and water would be necessary.

The performace of sengon indicated that this species would be the best choice for a forestry plantation under the test conditions. Turi could also be considered.

Since the infrastructure of roads and inspection trails is already developed, the planning of mined-site rehabilitation could be designed also as a timber estate scheme. Therefore, revegetation of mined land may also provide this option for future land management. One problem that may be encountered relates to the size of the mined areas. Some areas have enough space to support a minimum timber estate unit, but some may be too small for this usage. Thus, future landscape management has to be defined prior to development – e.g., whether to strive for revegetation only or to also aim at developing a productive timber estate.

Conclusion

1. Revegetation using legume tree crops showed better growth on ripped soils; however, inoculation with endomycorrhiza only affected early growth of *Acacia mangium*.
2. Tree planting both improved certain soil properties (e.g., the contents of organic Carbon and total Nitrogen were increased) and accelerated the growth of other understory species.
3. With proper land management and rehabilitation using timber species, some leftover coal mining areas can be reclaimed and developed for forestry production.

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Strengthening Tree Farming Activities to Reduce Pressure on Natural Forests and Support Sustainable Timber Production

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Abstract. Forestry plays an important role in Indonesian economic development. Hundreds of forestry companies are involved in this sector, supported by the Indonesian government. There are millions of smallholder farm families that depend on forest resources to meet their needs. Though they have a great potential to develop community- based industrial timber plantations and reduce pressures on natural forests, there has been only minor support from the government for their tree farming activities.

Increased involvement of communities is very important for sustainable forest development. Community tree planting activities will supply raw materials for forest industry, reduce pressures on natural forests and support genetic conservation.

Farmers may have great interest in tree planting activities but they often lack experiences in that area. They are usually unaware of the importance of matching species with site, germplasm quality, and proper seed and tree management. There are several strategies for strengthening their tree farming activities - increasing farmers' awareness in using high quality germplasm and strengthening their access to supply; strengthening the capacities of communities for procurement, distribution, and dissemination of germplasm; strengthening capacities of communities for tree planting activities; and providing infrastructure to support tree planting activities.

Introduction

Forestry sectors play important roles in the economic development of Indonesia. Until very recently, commercial timber production was primarily based on harvesting natural forests. The industry demands for timber have increased to the point that pressure on natural forests is very great to the point that although the efficiency of extraction can be increased, the demands simply cannot be totally supplied by harvesting natural forests. The Ministry of Forestry issued hundreds of forest concession licences to state forestry or private forestry companies for managing natural forests but there were few successes, if any, under this system. Natural forests were not well managed and deforestation rates and loss of forest genetic resources were very high. The Ministry of Forestry reassessed this policy and revoked many forest concession licenses.

Because of these and many other environmental issues, there is a general awareness within the broad forestry sector that timber production can no longer be based solely on harvesting natural forests. To address this issue, the government promoted reforestation and afforestation activities for industrial timber production in Government Regulation No. 7 issued in 1990. Under this program, all forestry companies – state and private, domestic and international - are involved in the development of industrial timber plantations facilitated by the Government of Indonesia with soft loans. Although support from the government was relatively high, the resultant conditions of the industrial timber plantations in Indonesia were not so good. In fact, the development of industrial timber plantations was not at all comparable to the projected demand for industrial timber.

There are million of smallholder farm families that depend on forest resources to make their livelihood. They have a great potential to support industrial timber plantation demands and reduce pressures on natural forests through on-farm tree cultivation and domestication. Tree domestication is simply defined as the process of bringing trees into human use. It is human who initiate and direct evolutionary processes, i.e., selection and breeding, which change the gene frequencies in certain tree populations. Tree domestication may occur within or outside of forests. Tree domestication must therefore be seen as a continuum – from the natural or “pristine” state, to management of trees in forests, to cultivation and semi-domestication on farms, to monoculture plantations of advanced generation breeding lines (Simmons 1996). On farms, tree cultivation and domestication must be promoted to complement and support other forms of sustainable timber tree production. Initially, there must be a new conceptual framework and subsequent policy development to forward that goal. In the past, large timber companies received support from the government and the forests were not managed properly. The farmers should receive more support to make their tree-farming systems more competitive.

As tropical forests disappear, there is a tendency to increase tree-planting activity on farmlands, especially in the area where land ownerships are small. In some areas the number of trees grown on farms equals those planted through industrial or government reforestation programs. This represents an existing complementary timber production system to meet demands of the forest company. And, by optimising that system, pressures on natural forests can be reduced and forest genetic resources can be protected in natural ecosystems (*in situ* conservation) while still other tree species can be conserved through on farm cultivation and domestication (*ex situ* conservation).

On Farm Tree Cultivation - Genetic Conservation and Timber Production

Many efforts have been made to conserve plant genetic resources. Two general approaches can be distinguished – *in situ* and *ex situ* conservation. These two approaches are complementary efforts to reduce genetic erosion. To date, most forest genetic conservation efforts have focused on *in situ* conservation – conserving genetic resources through protecting natural habitats. This approach is not always as successful as we intend it to be. Deforestation and loss of genetic resources still occurs in many of these protected places – especially in southern hemisphere countries where forests are the main resource for economic development. Here, the *in situ* approach typically follows the western model of national parks, but most likely fails to achieve objectives. Forests are continually changing as they grow and regenerate, and the people who use them are also changing as they grow and reproduce. More flexible models for conservation are now becoming popular and the distinction between protected areas, forest areas, and even agricultural areas are becoming blurred (Freeman 1999).

Ex situ conservation of forest genetic resources is thus of vital importance, and this is an approach that can be implemented as part of tree cultivation and domestication programs on farms. From experience in agriculture, farmers have great influence on genetic conservation. Although intensive utilization may lead to a relative reduction of genetic variation, in many cases farmers have made great contributions to maintaining biodiversity and conserving genetic resources through their cultivation of indigenous species. They have brought wild species into cultivation and bred them by an iterative process of selection and improvement. Plant genetic resources have a complex and highly important function for the farming community - to produce food, medicines, fuel, building materials, fodder, and to restore soil fertility, among others. In the long term, however, the most important factor in reversing genetic erosion will be in strengthening the community-based system of conserving local genetic resources and also the local knowledge about them.

Under this system, foresters would not be the sole authorities on forest genetic conservation. Other stakeholders - NGOs and farmers – should also be involved. Valuable genetic resources have been conserved and improved by indigenous peoples throughout the world and although modern science has given attention to only a small fraction of these, they have contributed greatly to our abilities to sustain human life and society. Many plant species that are cultivated or collected by farmers are still not known to modern science or have even been identified as to their potential. In this context, linking the scientist and the farmer is of vital importance and NGOs may play an important part in this.

Numerous NGOs are currently working with farmers and while they have a strong sociological link, most are much weaker in technical capacity. The NGOs capacities lie mainly in the concept of empowering the local community, providing self-reliance, and alleviating poverty and they try to build the capacity of the local communities so that they can conceptualise and articulate their needs. NGOs are also often concerned with identifying, testing, adapting, and disseminating locally- appropriate technologies. If both scientists and NGOs could collaborate with farmers in the conservation and distribution of genetic resources, valuable indigenous knowledge about genetic resources management can be used and sustained. While researchers contribute science-based knowledge to develop genetic resource management, farmers can contribute the indigenous knowledge they have and NGOs can develop opportunities to exchange these experiences and systematise the process of validating and adopting the knowledge. The challenge is how to create conditions that are conducive for mutually exchanging and synthesizing experience, promoting and facilitating implementation, and encouraging the participation of all stakeholders.

In the past, their governments ignored millions of smallholder farm families, who possessed a potential to assist in sustainable forest development. Farmers cultivated numerous kinds of trees – timber, fruit and multi purpose tree species. Lists of species cultivated by farmer in Nusa Tenggara, Lampung, and Yogyakarta are shown in Table 1 as reported by ICRAF partners from those areas. Timber tree species – *Tectona grandis*, *Swietenia macrophylla*, and *Paraserianthes falcataria* – are among the farmers' priority species (Mulawarman & Roshetko 2000). If surveys were conducted in other areas, the list would become longer. Only few of this species – the commercial species – are cultivated and managed by commercial companies. The rest - valuable timber species that are becoming scarce in natural forests - are cultivated, managed and conserved by farmers. On-farm tree cultivation can thus serve timber production needs as well as reforestation and conservation goals. In some areas there is much interest in planting trees - the number of trees grown on farms equals those planted through industrial or government reforestation programs. Therefore, the "farmer contribution" to genetic conservation, reforestation, and timber production is definitely worth considering.

Table 1. Species identified by ICRAF partners

	Local name	Species	Remarks
•	Asam jawa	*Tamarindus indica*	Fruit
•	Alpokat	*Persea americana*	Fruit
•	Aren	*Arenga pinnata*	Fruit
•	Bambang lanang	*Michelia* sp	Wood
•	Coklat	*Theobroma cacao*	Fruit
•	Eucalyptus	*Eucalyptus* spp	Wood
•	Gaharu	*Aquilaria*	Wood
•	Gamal	*Gliricidia sepium*	MPTS
•	Gmelina	*Gmelina arborea*	Wood
•	Jambu mete	*Anacardium occidentale*	Fruit
•	Jati	*Tectona grandis*	Wood
•	Jelutung	*Dyera costulata*	Wood
•	Jengkol	*Archidendron pauciflorum*	Fruit/vegetable
•	Jeruk	*Citrus* sp	Fruit
•	Johar	*Cassia siamea*	Wood
•	Kaliandra	*Calliandra calothrysus*	MPTS
•	Karet	*Hevea brasiliensis*	Resin/Wood
•	Kayu afrika	*Maesopsis eminii*	Wood
•	Kayu manis	*Cinnamamum* sop	Spice
•	Kedawung	*Parkia javanica*	Fruit/MPTS
•	Kemuning	*Murraya paniculata*	Fruit
•	Kelapa	*Cocos nucifera*	Fruit
•	Kemari	*Aleurites molucana*	Fruit
•	Kopi	*Coffea* sp	Fruit
•	Mangga	*Mangifera indica*	Fruit
•	Mangium	*Acacia mangium*	Wood
•	Mahoni	*Swietenia mahogany*	Wood
•	Melinjo	*Gnetum gnemon*	Fruit
•	Mimba	*Azadirachta indica*	Medicinal
•	Nangka	*Artocarpus heterophyllus*	Fruit/Wood
•	Papaya	*Carica papaya*	Fruit
•	Petai	*Parkia speciosa*	Fruit/vegetable
•	Pulai	*Alstonia scholaris*	Wood
•	Rambutan	*Nephelium lapaceum*	Fruit
•	Rotan manau	*Calanus manan*	Rattan
•	Sawo	*Chrysophyllum caimito*	Fruit
•	Sengon	*Paraserianthes falcataria*	Woods/MPTS
•	Sirsak	*Annona muricata*	Fruit
•	Sonokeling	*Dalbergia latifolia*	Wood
•	Sungkai	*Peronema canescens*	Wood
•	Tembesu	*Fagraea fragrans*	Wood
•	Trembesi	*Albizia saman*	Wood/MPTS
•	Turi	*Sesbania grandiflora*	MPTS
•	Ulin	*Eusideroxylon* spp	Wood

Strengthening On-Farm Tree Cultivation

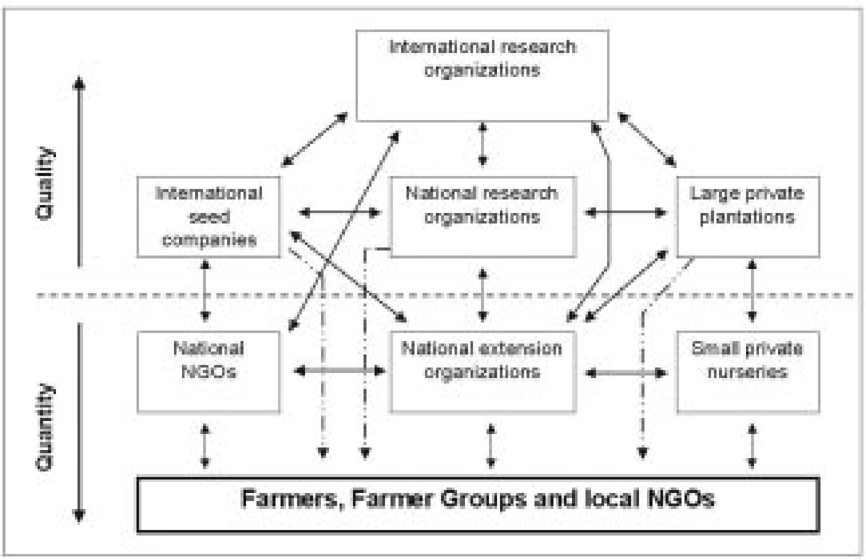
Although farmers may have a high interest in tree farming activities, they often lack experience as tree planters. They are commonly unaware of the importance of matching species with site, germplasm quality, and proper seed and tree management. This can result in an over-reliance on species less appropriate for a site or the use of inferior germplasm (Roshetko 2000). Among these shortcomings, lack of proper germplasm and access to market are most often the greatest constraints. The success of tree cultivation depends on the availability of adapted, high quality germplasm in the form of seeds, seedlings, or cuttings. If sources of germplasm and markets for products are unavailable, interest in tree cultivation will quickly fade for obvious reasons.

Farmers are strongly interested in income generation and farmers' preferences for different species reflect and depend on the market for their products. Farmers always value indigenous species highly and would prefer to grow them, but because of scarcity of seed and inability to access markets they are often discouraged from doing so. Consequently, they usually plant species for which both market and planting material are available (Lawrence 1999). As reported by Mulawarman and Roshetko (2000) timber trees such as *Tectona grandis*, *Swietenia macrophylla*, and *Paraserianthes falcataria* – are among farmers' priority species in Indonesia because they can be sold easily and the planting material can be readily procured. There should be collaborative efforts from all stakeholders to provide good markets and planting materials. And another issue is the lack of clear policies that would attract the interests and energies of farmers to plant and market trees. At present there is a void here.

If farmers do not have access to improved germplasm, they will plant whatever they can get regardless of its quality, as reported by Mulawarman and Roshetko (2000). Farmers mainly fulfill their planting material needs by collecting natural seedlings. They also procure seed from local seed trees or buy from local seed collectors. Unfortunately, both farmers and local commercial seed collectors are generally unfamiliar with proper seed collection seed storage methods. Seeds are collected from limited numbers of mother trees and there are no quality criteria for selecting these. Poor quality trees may be targeted for seed collection because of close proximity to the collector or the farm and, once collected, seed may not be properly cleaned or stored. This chain of sub-optimal conditions results in low quality seed lots. Even when farmers have sufficient technical skills and follow collection and storage protocols, locally available seed may be of low genetic or physical quality due to the degraded condition of local forests. In these cases local seeds may be far inferior to the seed available from national or international agencies. Unfortunately, farmers have limited knowledge of and access to these central seed resources (Roshetko 2000) as shown in Figure 1, which depicts a generalized model of germplasm pathways in Indonesia and elsewhere in Southeast Asia.

One low cost approach to seed procurement is on-farm seed sources, a system gaining in popularity as a farmer-oriented approach to improving seed quality and supply. Small-scale seed sources can be established to produce sufficient improved seed to satisfy local need. It needs smallholder support and involvement in establishing seed banks, seed exchanges and a selection and breeding programme. This farmer-oriented system could be managed privately, by NGOs, or under community control. The decentralized seed farmer could concentrate on producing seed and selling it locally. The village-based seed farm would need extension-like advice from a decentralized seed inspection unit. When they have become familiar with the techniques, the farmers could assume the maintenance of an improvement program.

Figure 1. Model of germplasm pathways in Southeast Asia (Harwood *et al.* 1999)



To address some of the seed-related problems mentioned above, ICRAF-Winrock International-IFSP have been conducting a project "*Strengthening germplasm security for the farmers and NGOs in Indonesia*" to provide technical assistance to NGOs and communities (Roshetko 2000). The objectives are to increase the availability and use of high quality seed and the adoption of appropriate seed collection, handling, and storage methods by the NGOs and smallholder communities. Activities and outputs could include the following:

1. Surveys, meetings and participatory appraisals to identify NGO/smallholder current pathways, as well as their seed technology awareness, capacity and constraints.
2. Participatory training activities, field visits to relevant germplasm-related research or production sites, and workshops. These activities will focus on the constraints and possible solutions identified above.
3. Fact sheets, manuals, training curricula, workshop proceedings, seed orchard guidelines, and seed source directories in English and Indonesian as well as translations of appropriate documents into Indonesian.
4. Testing the suitability of guiding the formation of an NGO- or smallholder-based seed procurement/diffusion entity with appropriate links to Forest Seed Centre (*Balai Perbenihan Tanaman Hutan*, BPTH).
5. Participatory smallholder and NGO research activities, including: a) appropriate smallholder tree farms demonstrating the advantages of superior quality seed and the importance of correct seed collection, storage and handling practices; and b) the design, composition, management, and establishment of seed orchards and production areas.

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Miscellaneous
(Posters and Voluntary Papers)

Conservation of Soil Microbial Diversity from the Tropical Rain Forests: Its Importance to Plantation Forestry Development and for Future Biotechnology

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Abstract. Among terrestrial ecosystems, tropical rain forests provide the most complex ecosystem supported by a massive belowground microbial diversity. However, deforestation activities and ecosystem disturbance endanger this biodiversity. Compared to that in macro-organisms (fauna or vegetation), research on diversity of soil microbes in the tropical rain forest is still in its infancy. There is obvious lack of knowledge about their structure and their role in supporting forest productivity. Moreover, despite their vast applications in commercial biotechnological processes, knowledge on their potential use is still limited. Most groups of this soil microbial diversity remain unknown and unexplored and, therefore, still untapped.

An understanding of the mechanisms of (i) plant-soil microbe interactions and (ii) the role of soil microbes in nutrient or soil organic matter transformation and cycling may provide insight on how soil microbes contribute to sustained forest productivity. Conventional approaches using taxonomical or phylogenetical methods are limited due to our inability to link their diversity with their ecological functions. Evidence of frequent horizontal gene-transfers among microbes in soils also another barrier. In short, microbial diversity is a complex issue. Recently, however, trends in using functional diversity to study this group of organisms are starting to produce results.

Extravagant metabolic diversity and inability to cultivate (unculturability properties) most soil microbes present major constraints to applying culture-based collection methods during a conservation program. In this regard, molecular-based techniques seem to be promising tools for diversity assessment, conservation programs (soil microbes as a soil meta-genome) and future uses in biotechnology. It is suggested that the land area covered by *in situ* conservation of above ground macro-organism biodiversity also serves as the most important source of soil microbial diversity. Therefore, a linkage between *in situ* conservation of faunal and floral biodiversity and that of the associated soil microbes is proposed.

A global collaboration is urged to ensure sustained utilisation of this mutual diversity and to avoid any extinction of potentially valuable gene resources, especially since tropical rain forest ecosystems are located in the less-developed regions of the world.

Introduction and Scope of the Discussion

There are significant roles for microbial diversity due to the capacity of these organisms to: (i) produce novel and potentially important biotechnological products, (ii) recycle nutrients and energy, (iii) detoxify hazardous wastes, (iv) provide new insights into how life is possible in some extreme biological niches, and (v) sustain agricultural and forestry practices. It is also widely accepted that the first steps in biotechnological activities start from exploring biodiversity and genetic variability (Rondon *et al.* 1999b, Cowan 2000).

Most of these beneficial microorganisms are those that inhabit soils; however, despite their essential roles, we currently know about very few soil microorganism taxa, while the rest of them are still relatively obscure because of significant problems with culturing them *in vitro.* Meanwhile, extensive disturbances caused by human activities in nearly all terrestrial ecosystems have threatened their biological components, including microbial diversity. Therefore, adequate study of microbial diversity becomes increasingly important if we are to avoid extinctions at all levels of biological diversity. Furthermore, adequate information on specific microbial communities at a given location may be useful for further considerations of whether or not such habitats should be preserved or conserved areas (Kennedy & Gewin 1997).

This review aims at describing soil microbial diversity values. An appreciation of the importance of conserving microbial diversity and why this should be implemented are emphasized. Possible or potential values both to plantation forestry and to future biotechnology programs are also explained. Methodological barriers to attaining microbial diversity are also briefly discussed including proposals for using methods mainly based on functional approaches as well as on more recent nucleic acid-based techniques. Aspects relating microbial conservation to current activities to conserve macro-organisms (flora or fauna) are considered. Due to limitations in financial support and human resources, research addressing soil microbial diversity in the less-developed countries should be finely focused and not merely follow trends in the developed countries. A proposed strategy will complement this last suggestion.

Importance of Conservation Activities on Microbial Diversity in the Tropical Rain Forests

Among all other terrestrial ecosystems, tropical rain forests provide the most complex ecosystem, consisting of diverse niches formed by their cascade of vegetation canopies. These ecosystems are supported by massive levels of belowground microbial diversity. A good example is obtained from studies in the Amazon region, which is the most intensively studied of all the tropical rain forest ecosystems. There, research indicates that within one observation plot, 15,000 animal species, 40% of the world's fresh-water fish species, and over 5,000 tree species were found. Using culture independent methods, in a preliminary study using an universal small-subunit rRNA primer, Borneman & Triplett (1997) found 100 sequences of bacteria that were classified as novel organisms, previously unrecorded.

Because these diverse ecosystems are located in countries mostly of less-developed status, appreciation of this diversity has been insufficient due primarily to major conflicts of interest that merely relate to the immediate utilization of currently valuable forest resources, especially timber. Moreover, human resources and infrastructures to access these values of diversity have been unavailable, due to limited scientific understanding and absence of direct incentives that clearly link diversity and conservation with economical return.

Nowadays, we see the utmost devastation of such ecosystems by activities that mainly relate to timber extraction, land conversion, and forest fire. Damage to such ecosystems is increasing. The ITTO (International Tropical Timber Organization) reported that approximately two million hectares of tropical forest ecosystems have been destroyed, annually. A crash programme, should therefore be proposed to mitigate all of the biodiversity values of these valuable systems to avoid their irreversible extinction.

Importance of Soil Microbial Diversity for Plantation Forestry in the Humid Tropics

The poor nutrient status of soils in the humid tropics that is usually associated with extended weathering conditions validates nutrient uptake through internal nutrient cycling mechanisms. These inherently infertile soils have nearly no nutrient reserves and almost all elements required for above ground vegetation are derived from current standing organic matter input or from previous vegetation. These nutrients are concentrated on the thin surface of organic-influenced soils. The turnover rates of organic input and soil organic matter are very fast due to warm temperatures and water availability year-round, even in logged-over areas where erosion is evident. In addition, the nutrient holding capacity of these soils is very weak; hence, most of available nutrients are at risk to be leached out or fixed. This implies a requirement for an efficient nutrient absorption mechanism to support ecosystem productivity and sustainability, that is only available through some biological means.

There is general acceptance that soil microbes play significant roles in nutrient acquisition in such nutrient-poor ecosystems. In fact, enormous types and numbers of soil micro-organisms play important roles during nutrient acquisition in such ecosystems; however, in this paper only two major groups will be discussed.

Ectomycorrhizal associations in dipterocarp forests

An important feature of the dipterocarp family is its close association with ectomycorrhizal fungi during nutrient uptake. In addition to expanding the root system through mycelial nets, these symbiotic fungi allow nutrients to be absorbed in organic forms. Nutrient acquisition without inorganic transformation controls nutrient loss through leaching and fixation.

Similarly, indigenous vegetation should be adapted to these rainforest sites. As indigenous species, *Dipterocarpaceae* seem best suited to these conditions. Their growth rate is relatively low as it often occurs under nutrient-depleted conditions. The foliage contains relatively high contents of polyphenols and lignin, which are less degradable, thereby avoiding nutrient loss through rapid decomposition when returned to soils as litter-fall. This mechanism may also allow utilisation of nitrogen via protein-polyphenolic complexes mediated by ectomycorrhizae, similar to infertile agro ecosystems that mimic polyphenol-rich forests, as reported by Northup *et al*. (1998) and by Hattenschwiler and Vitousek (2000) where, in particular ecosystems, ectomycorrhizal fungi play an essential role during nutrient acquisition.

Ectomycorrhizal diversity in dipterocarp forests has attracted several studies. In general, initial abnormal tree growth is usually associated with an absence of ectomycorrhizal association due to forest burning, abandoned logged-over areas leading to grassland encroachment indicating mycorrhizal dependence. This also occurs during introduction to exotic regions. This is true for *Pinus merkusii*, a pine species indigenous to humid tropical regions. Usually this group of fungi has a narrow-host range, and therefore specificity or compatibility is one aspect to be considered.

Despite their ability to be vegetatively propagated in laboratory media, forced association between vegetative inoculum of ectomycorrhizal fungi and dipterocarp hosts have not been successful. Unlike ectomycorrhizal associations in sub-tropical or temperate regions, clonal association between host and symbiont is still absent in the ectomycorrhizal fungi found on dipterocarps. This technical barrier limits the advancement of studies on dipterocarp – ectomycorrhizae associations. So far, those studies have been restricted to: inoculation effects (using non-vegetative inoculants), morphological diversity / taxonomy, ecology, and nutritional aspects. These contrast with studies in the sub-tropical or temperate regions where techniques to synthesize mycorrhizal formation using vegetatively defined inoculant are routine protocols, allowing the physiological and molecular aspects of the associations to be addressed. In the latter situation, an appreciation of the fungal diversity is attained.

Nevertheless, inability to form an aseptic ectomycorrhizal association in dipterocarp species does not restrict its practical applications, since soil or spore inoculation have proven effective at the nursery or field scale. However, it is speculated that the requirement for vegetatively defined inoculant of ectomycorrhizal fungi will be evident in the future, where commercial dipterocarp plantations are to be established on various sites using clonal propagation. Efforts to successfully employ ectomycorrhizal association using vegetative inoculant are therefore crucially needed to assess the functional diversity of this group of fungi. Opportunities to study this diversity are now possible by 'culture-independent approaches' using molecular techniques. These procedures allow associations between particular species or isolates of the fungi and dipterocarp species or clones to be investigated. Thus, information on dominant fungal species that associate with particular host species/genotypes on particular sites and also their phylogenetic relatedness may be obtained without an aseptic mycorrhizal synthesis with a defined vegetative inoculant. A sequence database for the identification of ectomycorrhizal fungi in the temperate regions is already accessible for public use (Bruns *et al*. 1998), even though databases for fungi collected in tropical regions are still unavailable.

Today, conserving ectomycorrhizal diversity is a crucially important activity due to the vast destruction of tropical rain forests through improper logging. Forest-fire also presents a threat to the survival of these fungi in forest soils. There is evidence indicating that the failure of dipterocarp plantation establishment in disturbed areas may be due to absence of ectomycorrhizal populations. It is suggested that due to their narrower host range, the diversity of these ectomycorrhizal fungi is naturally unique and is related to their site heterogeneity and/or their geographical distributions, paralleling the diversity of their host dipterocarp species. This diversity may also be similar to that occurring in rhizobia (Lie *et al*. 1987, Karyanto *et al.* in this proceeding).

Arbuscular mycorrhizal fungi

Unlike those in ectomycorrhizal associations, fungi belonging to the arbuscular mycorrhizal fungi (*amf*) have a broader host range and thus an ability to associate with diverse groups of host plant. The ubiquity of *amf* is reflected by their associations with more than 80% of all terrestrial vascular plants species. It was estimated that these associations have been established 353-462 million years ago during land colonization by plant species. This parallel development between *amf* and vascular plants suggests a vital role for *amf* in vegetation development.

Despite its obligatory growth habit (grows only in the presence of a host), an ability to aseptically synthesize isolated *amf* spores to host plants provides an advantage over the dipterocarp ectomycorrhizae. Interaction between *amf* isolates and host plant genotypes have been well documented, leading to an appreciation of the importance of their diversity. The benefit of this association may not be limited to nutrient capture, but also in wider mechanisms of protection from root pathogens (Newsham *et al*. 1995).

Box 1 explains that productivity and vegetation diversity can be determined by *amf* diversity and that may indicate that the current rate of decline in biodiversity threatens ecosystem stability if components other than vascular plants, such as *amf*, are not protected and conserved, also.

In addition to beneficial aspects, it is recognised that microbes, especially those that are pathogenic, also represent a major controlling factor in ecosystem stability. Dramatically altered forest ecosystems by pathogens may be found in various regions, such as in US, Western Europe, Australia and East Africa (fungal blights caused by *Endothia parasitica* on chestnut trees in the US, elm trees in western Europe by *Ceratocystis ulmi,* and *Eucalypts* attacked by *Phytophthora* fungi in Australia). Again, despite their ability to dramatically alter the diversity and species composition of ecological communities, soil microbes, in this case as pathogens, have only occasionally received attention as agents of ecosystem function and change.

Importance of Soil Microbial Diversity for Biotechnology

For millennia, microorganisms have produced useful biological materials in medicine, industry, and agriculture. Over the last five decades, we have seen the commercial production of secondary metabolites, tapped from soil microorganisms, known today as 'mining-out' of microbial resources. Products such as antibiotics, anticancer drugs, antifungal compounds, immunosuppressive agents, enzymes, enzyme inhibitors, antiparasitic agents, herbicides, insecticides, and growth promoters present significant commercial products in worldwide industry. For example, the bulk enzyme market worldwide was valued at US$ 720 million in 1990 and is still being expanded. Strong R&D funding support for this development; for example, during its first 25years, total expenses for this R & D amounted to US$10 billion.

Box 1

Structure of vegetation and ecosystem stability are determined by diversity of soil microbial communities:
An example from mycorrhizal association

Since its first report in the previous century (1800s), research focused on plant/tree – mycorrhizae associations has increased dramatically. Today these symbiotic associations are among the most intensively studied of biological phenomena, especially in the areas of physiology, ecology, and molecular biology. Reports appear regularly in many scientific journals. However, little knowledge has been available on functional impacts at an ecosystem level until van der Heijden *et al.* (1998) clearly demonstrated that below-ground diversity of arbuscular mycorrhizal fungi (amf) was a major factor contributing to the maintenance of plant diversity and ecosystem function By using several amf species inoculated on different host plants, they found that diversity of amf species determined several vegetation parameters such as: *Simpson's* diversity index and plant performance when grown on conditions simulating two ecosystems - *European calcareous grassland* and *North American old-field* ecosystem. Similar phenomena were also found in an arid ecosystem in Australia (Patrick O'Connor-*personal communication*). An opposing explanation, i.e., where aboveground vegetation determines amf diversity, was reported by Johnson *et al.* (1992). They insisted that even closely related host plants might cause divergence in amf composition. Whichever of these two explanations may viable, both sets of evidence indicate strong interactions of plant communities leading to ecosystem stability. It was suggested that successful conservation management requires an understanding of these mechanisms (van der Heijden *et al.* 1998).

While research on diversity of amf has been increasing, even its molecular aspects, the basis for its variability and functional aspects in the ecosystem are lesser known (Sanders *et al.* 1999, Koide 2000). As hypothesized by van der Heijden *et al* (1998), an increase of amf diversity may extend spatial limits of fungal hyphae where P in soils could be exploited, Smith *et al* (2000) proved a functional complementary mechanisms that linking between amf diversity as revealed by hyphal distribution and its P-acquiring capacity in differing host plant species. Amf species *Scuttelospora calospora* was best suited for plants whose roots are extensively distributed. On the other hand, *Glomus caledonium* could acquire P both far from and in close proximity to the roots (Smith *et al.* 2000). They suggested that redundant fungal hyphae that are spatially non-complementary to the host rooting system might explain why growth response is not always obtained during mycorrhizal associations. These findings significantly contribute to understanding the linkage between diversity and ecological consequences in mycorrhizal symbioses.

Most useful microbial secondary metabolites come from microorganisms that inhabit soils, especially actinomycetes taxa, *Bacillus* spp., mycobacteria, and pseudomonads (Kobinata & Osada *et al*. 1998, Rondon *et al*. 1999a). However, most microbes in nature have not yet been discovered, either by using culture-based approaches or by culture-independent techniques. The diversity of soil microbes is still largely unexplored, and it is suggested to be much greater than was thought before (Rondon *et al*. 2000).

Approaches for assessing soil microbial diversity

There has been a long history of trying to measure the richness and diversity of the soil microbial community in a given environment. The main obstacle is the fact that soil microbial communities are characterised by complex components that cannot be cultivated using conventional plate-culture. A gram of soil may contain more than 5,000 different taxa of microorganisms, and most of them (more than 95%) are viable but not culturable (Torsvik *et al*. 1990 & 1994). Improved laboratory media that are specific to a particular microorganism have been available, but again, the target organism is limited. Several methods based on physiological characteristics, especially by detecting catabolizing ability of several known compounds, have been used to overcome inherent constraints during cultivation of unknown groups of organisms, such as *Biology system* (Zak *et al*. 1994) and *Catabolic Response Profile* (CRP) (Degens & Harris 1997, Degens 1997, 1998, 1999). Even though both approaches were useful for 'fingerprinting' soil microbial diversity among different land-uses (Winding 1994, Degens and Vojuodic-Vukovic 1999), little information was available to interpret these 'values of diversity'. Moreover, the outcome of these methods was limited to the compounds used during the test. Nonetheless, these are the only simple methods that are currently available to examine functional diversity, where genetic diversity is directly linked to physiological traits.

Fatty acid pattern of microbial phospholipids and lipopolysaccharides (PFLAs) is yet another method used to fingerprint soil microbial diversity. The method is based on the fact that the composition of total microbial fatty acids is taxonomically specific. Classification of soil microorganisms therefore could be conducted by subdividing the fatty acids according to their chain structure, degree of saturation, and substitution by specific 'signaturePFLAs' (Zelles *et al*. 1992, Zelles & Bai 1993). Despite high resolution, it is difficult to interpret diversity as revealed by this approach.

Nucleic Acid Based-methods

The three most commonly used methods above produce non-repeatable results, since the outcome data is qualitative and only enables comparative analysis. As a result, no precise taxonomic descriptions would be available. Moreover, the system only measures the metabolically active components. In the case of PFLA measurements, the microbial components that exist in the minute population may not be detected. Likewise, it is not possible to create a universal standard of data to be compared, since the measurements are inter-laboratory dependent.

With the advent of molecular biology, various nucleic acid based-methods have been available to ecologists. For their appropriate application, the choice of methods should be based on the nature and capabilities of each method. While starting from the same materials (DNA extract from environmental samples), the target and the resolution of the analysis would be different. Box 2 summarizes the various nucleic acid-based techniques that are commonly used to study molecular microbial ecology.

Box 2

Table 1. Molecular methods used to examine soil microbial diversity

methods	**Comments**
16S rRNA gene sequence analysis	Cloning required; provides identification of members of community
in-situ hybridisation	Require certain probes; labour intensive
DNA-RNA re-association kinetics	Provides a global view of genetic complexity of sample
ARDRA	More useful for simple communities; useful for comparative analysis
PCR amplification or expression cloning	Functional diversity target
BAC libraries	Permanent archive of genetic information from sampled environment; phylogenetic and functional diversity
Flow cytometry	Enumeration micro-organisms
RNA dot of slot blot	Representation of metabolically active members of a community
SSCP	Comparative analysis
%GC content	Global view of community diversity
T-RFLP	Comparative analysis
DGGE or TGGE	Used to monitor enrichment, comparative analysis

Abbreviation: **ARDRA**, amplified ribosomal DNA restriction analysis**, BAC**, bacterial artificial chromosome; **DGGE**, denaturing-gradient gel electrophoresis; **SSCP**, single-strand conformational polymorphisms; **TGGE,** temperature-gradient gel electrophoresis; **T-RFLP**, terminal-restriction-fragment-length polymorphisms.

(Source: Rondon *et al*. 1999)

Nucleic acid isolation and extraction from environmental samples

Without culturing, diversity of un-culturable soil microbes could be assessed by nucleic acid information. Some methods have been developed to provide quick and easy steps for nucleic acid isolation and extraction from environmental samples (Torsvik et al. 1990, 1994, 1996, Zhou et al. 1996). The extracted DNA, which is a complex mixture of microbial community origin, may be used for direct amplification or for nucleic acid hybridisation. Purification from humic acids or clay interactions were the most difficult tasks in the past; however, in some cases extraction (e.g., using full CTAB or phenol-chloroform) is not always necessary, especially for PCR template (Thies et al. 1999). Simple methods of soil DNA extraction were developed by Zhou et al. (1996) thanks to commercial development of soil DNA extraction kits by which extraction could be completed within half an hour, even if sample with high content of humic acids (e.g., MoBio™ products).

Polymerase Chain Reactions (PCR)

Molecular methods have revolutionised the study of microorganisms in situ, none more so than PCR, since its specificity and its ability to detect the presence of very low target organisms within a large background of other microbial populations (Ward et al. 1995). The development of this method as a powerful tool is supported by the development of target-specific primers. However, random primers (such as arbitrary – primer with G: C> 50%) could be used for generating random PCR (RAPDs).

PCR produces a fingerprint or profile for any particular target genome. Nevertheless, there are limitations to this technique and the fingerprint pattern of the PCR product sequence may vary due to subtle variation during the amplification conditions.

Further analysis of PCR products

Further electrophoresis of PCR products in polyacrylamide gels also provides a finer resolution of discrimination on DNA sample diversity. Unlike agarose gel, polyacrylamide gel can distinguish the electrophoretic mobility of macromolecules on the basis of their shape, which is beyond the capacity of routine hybridisation methods.

Several methods including single-strand conformational polymorphism (SSCP) (Dewit & Klatser, 1994), denaturing gradient gel electrophoresis (DGGE) (Sheffield et al. 1989), temperature gradient gel electrophoresis (TGGE) (Wartell et al. 1998), and hetero-duplex mobility assays (HMA) (Espejo & Romero 1998) can be used for single-base discrimination of amplified DNA sequences. DGGE and TGGE detect DNA sequence diversity based on their stability toward denaturing conditions. Sequences differing in G+C content or neighbouring nucleotide interaction may produce different banding-patterns. For denaturing conditions, DGGE uses chemical agents (eg. urea) while TGGE utilises temperature. Given the ease of high-resolution outcomes, both methods have been widely used for comparison of DNA structure (especially based on 16S rDNA – PCR products) within complex microbial communities (Heuer et al. 1999).

Diversity of thermal denaturation and reassociation of soil microbial DNA

Soil DNA may provide genetic information about the non-culturable bacteria in soil. DNA heterogeneity can be determined by thermal denaturation and reassociation. Denaturation of homologous, single-stranded DNA follows second-order reaction kinetics. The fraction of denatured DNA is usually expressed as a function of the product (Co_t) of DNA nucleotide concentration (Co) in moles per liter and the reaction time ($_t$) in seconds. Under defined conditions Co_t for a half-completed reaction ($Co_{t1/2}$) is proportional to the genome size or the complexity of DNA. The complexity is defined as the number of nucleotides in the DNA of a haloid cell, without repetitive DNA. The diversity can be expressed by using $Co_{t1/2}$ as a diversity index (Torsvik et al. 1990).

Molecular microbial diversity using small-subunit rRNA genes (SSU rRNA)

The limitations for culture- or physiological- based measurements of soil microbial diversity are results much less than real or estimated population. Developments in molecular ecology provide nucleic acid-based methods that do not require cell cultivation and/or physiological measurement. The methods then revolutionise the present concept of diversity that also covers non-culturable organisms (Amann *et* al. 1991, Amann *et al.* 1995, Ward *et al.* 1995).

In eubacteria, rRNA genetic loci include 16S, 23S, and 5S genes, which are separated by two internal transcribed spacer (ITS) regions. DNA sequences in the 16S-23S spacer are known to exhibit a great deal of sequence and length variation (Jensen *et al.* 1993). Previous researchers have shown that the presence of uncultured microorganisms can be recognised using analyses of the small-subunit rRNA genes. SSU rDNA clonal libraries are constructed by using DNA extracted from soil. SSU rRNA genes are amplified using the universal SSU rDNA primers. The PCR products are then sequenced. The sequences are compared to sequence databases and statistically analysed. The microbial diversity is illustrated by a phylogenetic tree analysis of 100 SSU rDNA sequences (Borneman & Tripplet 1997, Borneman *et al.* 1996)

Box 3

Accessing soil microbial diversity and function as a soil meta-genome: A novel approach

A method has been developed to capture genetic information of unassessed-unculturable organisms based on direct isolation of their DNA from soils or environmental samples followed by cloning into a vector to construct an environmental DNA library. This method encompasses limitations owing to the difficulties in culturing, measuring metabolic or physiological nature of un-culturable soil microbes. BACs (bacterial artificial chromosomes) vector was developed from *E. coli* especially its single-copy plasmid F factor that enables cloning large (>300 kb) segments of DNA. Additionally, the recombinant vectors then can be maintained stably within *E. coli* or in other host systems (Shizuya *et al.* 1992). Since the whole DNA fragment can be cloned, the entire pathway of biosynthesis can be detected. Thus, this method may provide the means of detecting natural products produced from a BAC library made from soil DNA. Furthermore, by labelling the BAC libraries, this method also provides specific probes for examining soil microbial diversity in a given environment (Rondon *et al.* 1999). This technique also emerges as a new approach to phylogenetic studies of microbial communities without culturing (Hugenholtz *et al.* 1996, 1998).

Practical aspects of this system have addressed expressing the characters that are conferred by large DNA fragments or multi-gene control. For example, using DNA extracted from soils, Rondon *et al.* (2000) found clones that expressed DNase, antibacterial, lipase, and amylase; of them the DNase and the antibacterial were synthesized by genes that were previously unknown (novel). By using a similar approach, Cottrell *et al.* (1999) isolated chitinase from uncultured marine microorganisms, while Henne *et al.* (1999) found genes conferring utilization of 4-hydroxybutyrate from an environmental DNA library.

Combination between an advent of soil DNA isolation and amplification technology, expression of unknown DNA sequences within the soil especially derived from un-cultured organism becomes more realistic. Since genes required for secondary metabolite production along with the accessory resistance and regulatory genes are often clustered in one contiguous segment on the chromosome of the producer organisms, BAC libraries offer a new source for natural-product discovery, that may commercially viable (Cottrell *et al.* 1999, Rondon *et al.* 2000). Application of this approach to exploit currently un-retrievable resources of soil microbial mega-diversity in tropical rain forest ecosystems may offer hopes for biotechnology that produces economically important materials.

The DNA sequence itself would never be meaningful and might be continued by observing which sequences are expressed. Alternatively, by *in vitro* expression on the artificial chromosome and then tracing their metabolic products or activity, it would be feasible to reveal functional measurement of un-culturable biodiversity. However, there are still lots of challenges to determine the specific function of gene products, since there are complex gene-gene or gene-environment interactions occurring. Ultimately, completion of this task may lead to identification of all of the genes and their protein products.

The current development of gene expression studies as well as the functionality of the gene products (especially proteins) offer the further opportunity of exploiting functional benefits of biodiversity. Fortunately, current methods have been allowing determination of the linear sequences of chromosomes and identification of short portions of cDNA clones of mRNAs [expressed sequence tags (ESTs)]. Microarray technology provides the complete system allowing the simultaneous measurement of gene expression (especially its transcription) in different conditions combined by the appropriate documentation in databases (Brazma *et al*. 2000). By similar methods, the possible function of the un-culturable soil organisms could be easily studied - which conditions may stimulate actions and which genes would be involved. Knowing specific conditions for each gene's expression, the potential role of un-culturable organisms may be explored including potential use such as function in soil or what bioactive agents they produce. However, measuring total genome expression across diverse environmental conditions is one of the most difficult tasks that molecular biologists have. Box 4 gives an example of an effort where exploration of soil microbial diversity was incorporated into an integrated activity of diversity characterization in a particular region.

Box 4
ATBI (All Taxa Biodiversity Inventory) as a model for ecosystem-based biodiversity inventory.

The previous studies in microbial biodiversity have been limited to the clinical area (pathogens) or other groups proven to be of benefit either in agro-forestry, industry or medicine. Therefore, world culture collections and information are restricted to the microorganisms devoted to those purposes. As the result, appropriate attention to the microorganism groups that are currently unknown or of potential benefit is negligible.

The model for comprehensive biological aspect inventory in a particular ecosystem was initiated at Pennsylvania University, giving the first habitat that covered 50,000 ha of undisturbed ecosystem in the tropics, named as ATBI (All Taxa Biodiversity Inventory) (Janzen & Hallwachs *cit.* Tiedje 1995). Since the infancy of such activities, there were several significant considerations to make this challenging project feasible. First, a comprehensive approach should be practiced to examine the biodiversity groups that are currently ignored, not only opportunities to recognise novel species, but even novel genera or family levels. Second, as there are many opportunities for novel symbiotic organisms invention, a proper sampling strategy that involves diverse groups of hosts should be proposed. Third, it should be borne in mind that a definition of a niche or habitat that might be 'rich' in diversity in order to define sampling strategy efficiently. Moreover, the consequences of massive data produced should be also anticipated by providing database systems that are well structured, easily accessed, and global network-compatible.

Table 3. General scheme for total diversity characterisation at ATBI project

Habitat	**Approach**	**Remove redundancy**	**Coarse characterisation**	**Fine characterisation**
Soil, plant, insect, animal, sediment, water	Culture (specialist)	REP-PCR	FAME	Group specific methods
	Total DNA extract	Hybrid substraction	rRNA family	16/18s rRNA sequence
	microscopy	Image comparison	Morphological group	Specific characters of taxa

Sharing the Outcomes

Data collected from microbial diversity explorations would be unique. It would be anticipated that reference data would not be available if novel species or taxa are to be described. On the other hand, without corresponding to an available database, it would be possible to obtain overlapped outcomes, which are inefficient. The advent of molecular biology has been also backed-up by established databases which are globally accessible (Fields 1992, Blake & Bult 1996). By using the available databases, people who study this issue could always share, refer to, or communicate their data.

Molecular techniques produce data in the form of banding patterns or nucleic acid sequences. Unlike the sequence, a banding pattern is not ordinary, therefore to get communicable outcomes, procedure and data analysis should be standardised. Several software systems are available such as GelCompar (Applied Maths, Kartrijk, Belgium), Diversity Database (BioRad, Hercules, CA, USA), Molecular Analyst (BioRad, Hercules, CA, USA), RFLP Scan (Scananalytic, Billerica, MA, USA) and Dendron (Soltech, Inc. Oakdale, USA).

Moreover, current computer technology offers abilities both in database and research tools (Blake & Bult 1996). Likewise, most databases can be accessed through the Internet, providing a quick and easy global data inventory. Box 5 lists the current available global databases:

Box 5

Table 2. Molecular biology database

Types of Database	**URLs (WWW addresses)**	**Resource**
Molecular sequence databases	http://www.ncbi.nlm.nih.gov/	National Center for Biotechnology Information GenBank molecular sequence database
	http://.gdb.org/Dan/protein/owl.html	OWL : a non-redundant protein sequence database
Specialised molecular information databases	http://www.gdb.org http://www.tigr.org	GDB Genome Data Base EGAD Expressed Gene Anatomy Databse
Taxonomic databases and resources	http://muse.bio.cornell.edu/ http://phylogeny.arizona.edu/tree/phylogeny.html	Biodiversity and Biological Collections WWW Server
Other biological resources	http://www.wdcm.riken.go.jp:80/	World data Center on Microorganisms

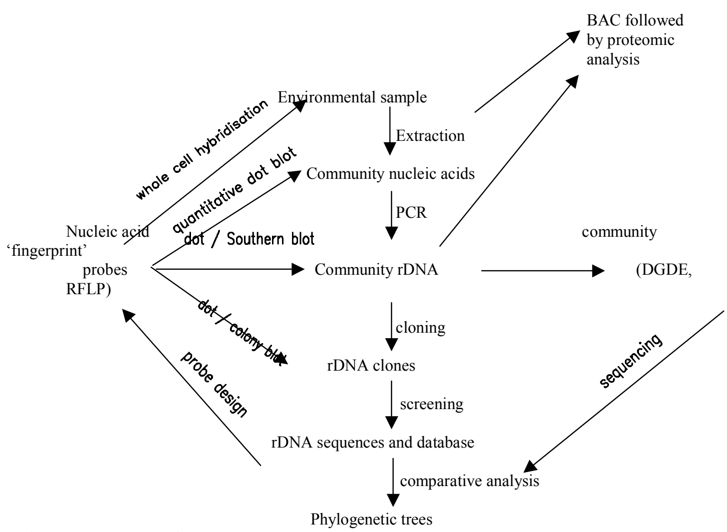
Future Considerations on a Global Biodiversity Strategy

Considering its future potential, the conservation of soil microbial diversity in ecosystems is a major task not only for its individual nations, but also for international societies that might benefit from use of the biodiversity. For such activities, scientific understanding of diversity on the molecular level, how to maintain diversity (primarily for *ex-situ* conservation), as well as how to assess its potential use becomes an obligatory task.

There should be economic reasons for exploring soil microbial diversity, since these activities would need huge investments. Consequently, the surveys of biodiversity should consider the applied aspects for human welfare. Detailed information on diversity *per se* would not be meaningful unless it was supplemented by potential use. For example, collection of antibiotic- producing actinomycetes would be much more useful than just a collection of actinomycetes without any information on their economic characters. Likewise, the scope of biodiversity is beyond the present coverage or the current knowledge. For that reason, as much as possible, functional biodiversity is much preferable. However, too much emphasis on functional aspects would ignore its potential value for additional explorations. Accordingly, the conservation strategy would combine both of these visions.

Box 6

Schematic representation of the components of multiplex cloning techniques, exploring DNA diversity into possible functional bioactive gene products which are of potential commercially valued.



(source: Cowan 2000)

Evidence of interest in international collaborations on the sustainable use of biodiversity was provided at the Convention on Biological Diversity (Earth Summit in Rio de Janeiro 1992) bringing global attention to this matter. Omitting the financial issues, a fair system of collaboration could be established between "host" or "source" tropical countries and commercial manufacturers that might economically exploit the advantages of microbial biodiversity. A situation such as that between Merck Co. and a Costa Rican non-governmental organization where Merck Co. is involved in funding biodiversity research exploration and the potential future properties and benefits are of mutual advantage and benefit could be adopted. There are, however, several non-profit international initiatives that address biodiversity research, as shown in Box 7.

Box 7

Table 4. International initiatives responsible for global biodiversity activities

United Nations agencies UNEP (United Nations Environment Programme) UNESCO (United Nations Educational, Scientific and Cultural Organization) FAO (Food and Agriculture Organization of the United Nations) UNDP (United Nations Development Programme)UNIDO (United Nations Development Organisation)
Non-governmental international organisation IUCN (International Union for Conservation of Nature and National Resources or IUCN The world Conservation Union) WRI (World Resource Institute) ICSU (International Council of Scientific Unions) SCOPE (cientific Committee on Problems of the Environment) IUMS (International Union of Microbiological Sciences) IUBS (International Union of Biological Sciences) WCMC (World Conservation Monitoring Center)
Intergovernmental bodies The World Bank CGIAR (Consultative Group on International Agriculture Research) ICRAF (International Center for Research in Agroforestry) CIFOR (Center for International Forestry Research) CAB International
International programme and activities GEF (Global Environment Facility –World Bank, UNDP, UNEP) UNCED (United Nations Conference on the Environment and Development) Agenda 21 Global Biodiversity Strategy (WRI-IUCN-UNEP) GBA (UNEP Global Biodiversity Assessment) Diversitas (IUBS-SCOPE-ENESCO)

(Source : Heywood 1995)

However, to make the activities run effectively and efficiently, appropriate strategies should be proposed. The infrastructure and human resources would become major issues. Basically, the establishment of site-based molecular biology laboratories devoted to these activities is far too expensive, but perhaps a nationally based centre or institute in each of the countries bearing with tropical rain forest ecosystems would be more realistic. Each institute would be responsible for the collection and exploration of soil microbial diversity in their own regions, unlike the current situation where all of the microbial collections are based in the developed countries' sites. Once the methods have been established and become routine protocol, the obstacles for human resource availability are eliminated.

Current technology provides powerful means for assessing soil microbial diversity in the molecular level, not only allowing assessment of un-culturable organisms, but also producing a universal database (of nucleic acid sequences) for international communication in both scientific and commercial perspectives. Most of the databases may be freely accessed, providing the chance for developing countries to get some of the advantages. Moreover, efforts to link the genetic sequences with their functions would become reality by application of microarray (DNA chips), expression within bacterial artificial chromosomes (*BACs*), and activities in the protein engineering areas. The use of these methods would promote the utilisation of mega-diversity in the soils, which would also cover their conservation as well as their sustainable exploitation.

Last but not least, tropical humid regions housing this mega-soil diversity should focus on global initiatives in biodiversity supported by the current advances in the molecular technologies. Furthermore, the technologies-holder might share the products and profits. A combination of a fair global policy supported by the advances in molecular technology would ensure sustainable interest in and appreciation of tropical soil biodiversity and protect it from extinction by human expansion. Figure 1 simplifies some of the biological possibilities of utilising DNA diversity for possible commercially valuable products.

Conclusion

Microorganisms represent a major part of the genetic diversity that is crucial to maintaining the sustainability of ecosystems. Mega-diversity of tropical rain forests, including that of their soil microbial communities is widely acknowledged. The role of microbes in maintaining sustained forest productivity is also appreciated; however, this role may be declining due to the current levels of forest devastation that are frequently irreversible. Efforts on *ex situ* or *in situ* conservation of macro-organisms (flora or fauna) have been a routine protocol to mitigate the process and/or to rescue the diversity from extinction as well as to sustain their use. Unfortunately, the role of soil microbial diversity in those conservation activities has received less attention. It is suggested that present threats to ecosystem stability and sustainability can only be stopped if the entire ecosystem, including soil microbial diversity, are protected and conserved.

In addition to forestry uses, the role and potential of soil microbial diversity in future biotechnological processes have attracted much interest. Even so, there is an obvious obstacle to their accurate assessment, since most organisms cannot be studied properly using current methods due to their un-culturability. To overcome this barrier, *ex situ* conservation methods that consist of collections of permanently preserved living cultures of microorganisms should be complemented by *in situ* approaches to ensure the conservation of currently un-culturable taxa. It is speculated that in the future, these sources of diversity may contribute as important non-timber products of tropical rainforests. However, the skills required to exploit these values are beyond those of foresters, so integrated research activities should be conducted. Protected sites demarcated for *in situ* conservation activities for tropical tree species would also offer ideal sites for *in situ* conservation of soil microbial diversity.

Novel approaches using molecular methods offer many new opportunities to access these un-culturable sources of microbial diversity. Moreover, they also promise functional outcomes on how the diversity could be utilised. Limitations of sophisticated equipment and highly-skilled scientists and technicians required for investigating, identifying, and utilising these microbial resources in the less-developed tropical countries could be augmented through mutual international collaborations.

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Additional Activities to *Ex situ* Conservation of *Paraserianthes falcataria*: Development of its Rhizobial Symbiont

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Abstract. Due to its potential source as a more sustainable raw material for future plywood industries, *Paraserianthes falcataria*, a nitrogen-fixing fast-growing tree species indigenous to the eastern part of Indonesian archipelago has been attracted to many studies. This activity on isolation and selection of its microsymbiont partner, rhizobium, was aimed to support the previous *ex situ* conservation and on going activities on tree improvement program of *P. falcataria*, especially that addressed to ultisol soils.

One hundred and thirty seven isolates of rhizobia associated with *P. falcataria* were isolated from the collected soil samples from the various geographical regions of Indonesia. Most of them are classified as fast-growing rhizobia. Based on the soil's type, they were identified as ultisols, histosols, vertisols and entisols. Selection of the isolates performance was conducted on an ultisol using seed-source collected from a family of *P. falcataria* as host plant, in order to control error caused by the heterogeneity of the seed source. Growth parameters of the seedling were assessed for analysing the rhizobial performance. Furthermore, aluminium tolerant of the isolates which was suggested to limiting the early stage of the symbiotic association was also studied. In addition, cluster analysis was used to propose the diversity among the isolates. Association with different families/provenances of host tree was used to clarify the possible genetic interaction between provenances/families and isolates of rhizobia.

The host biomass increment due to association with the isolates ranged –54% to 983%. The isolates collected from ultisols tended to produce better response. It is suggested that unlike nitrogenase activity, total biomass and biomass of root nodules could be used as the appropriate parameters during the rhizobial screening/selection. A modified synthetic aluminium stress medium was proposed as the rapid methods and early screening procedure for Al tolerance of these isolates. *Ward* method dendrogram indicated that the isolates collected from the similar regions tended to be grouped. The genotype interaction during this symbiotic associations were evident, therefore it is strongly suggested to consider host tree genotype during selection of rhizobial isolates.

Introduction

Due to its potential source as a more sustainable raw material for future plywood industries, *Paraserianthes falcataria*, a nitrogen-fixing fast-growing tree species indigenous to the eastern part of Indonesian archipelago has been attracted to many studies. This activity on isolation and selection of its microsymbiont partner, rhizobium, was aimed to support the previous *ex situ* conservation and on going activities on tree improvement program of *P. falcataria*, especially that addressed to ultisol soils. Ultisols were chosen for their abundance in Indonesia and for their multi-constraints properties to plant productivity.

Objectives

1. To establish a culture collection of rhizobial isolates associated with *P. falcataria* from various regions in Indonesia
2. To select those isolates using a representative ultisol
3. To confirm whether genetic interaction between the host trees and rhizobial isolates were evident
4. To confirm whether physiological trait on Aluminium tolerance contributed to the rhizobial isolates performance on ultisols
5. To explore diversity of the collected rhizobial isolates

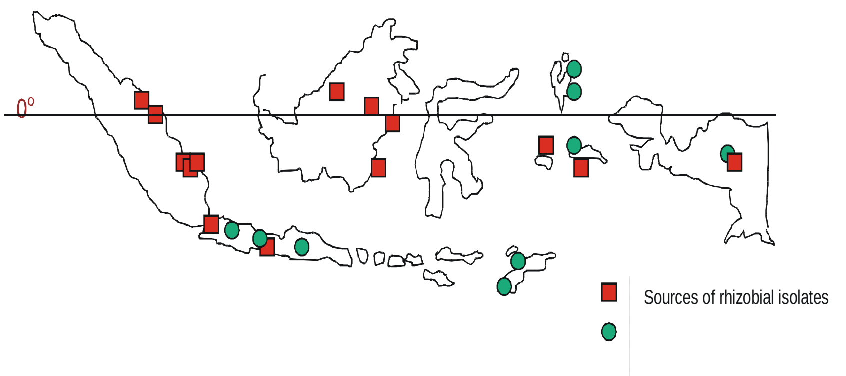
Materials and Methods

Soils were sampled from the top 20 cm surface of each site, then aseptically transferred into *P. falcataria* seedlings grown on a Leonard-jar, for nodulation. Two (randomly selected) nodules in each soil sample were cultured. Selection of the isolates were conducted by inoculating *P.f.* aseptic seedling grown on an ultisol, and the following parameters were considered :(i) total biomass, (ii) height, (iii) diameter, (iv) percentage of increment from the un-inoculated control, (v) nodule number, (vi) nodule dry weight, and (vii) specific nitrogenase activity. *In vivo* screening of the aluminium tolerance were conducted on different synthetic or non-synthetic media. Intrinsic antibiotic resistance (IAR) pattern assays using five antibiotics (erythromycine, ofloxacine, rifampicine, ciprofloxacine, chloramphenicol and tetracycline differing in concentration) combined with a cluster analysis were used to explore the isolates diversity. Inoculation of the three selected rhizobial isolated from the diverse regions (*PfLn*U 9.2; *PfLnU*16.2 and *PfUJ*) into nine families of *P.f.* from 3 provenances/ land-races (Morotai provenance, Cigudeg West Java landrace and Wamena provenance) were used to confirm the present of genotype interactions between rhizobial isolates and *P.f.* Two kg of sn ultisol collected from Kentrong, West Java was used for growing media.

Results and Discussion

A hundred and seventy isolates of rhizobial associated with *P. f.* were collected from 18 sites distributed from diverse regions of Indonesia. Most of the isolates are classified as fast-growing rhizobia, but several slow-growing isolates were also noted. The source of the isolates were not only limited from the natural distribution of *P.f.* in Indonesia but also covering the regions where the commercial plantation of this species may be developed. The soils samples were collected from plantation or natural distribution of *P.f.*, as suggested by Woomer *et al*. (1988) that soils with high population of rhizobial symbionts were found in the sites where their associated hosts occurred. It was expected that the isolates with higher genetic diversity were collected from the natural distribution of this legume species. However, we also successfully collected the rhizobial isolates from exotic regions to this species where no *P.f.* was planted, as in the western geographical areas of Indonesia (Figure 1). This suggests that cross-inoculation indigenous groups of rhizobia able to associate with *P.f.* were ubiquitous in Indonesian soils.

Figure 1. Sources of rhizobial isolates collected and *P. falcataria* seed sources used during this study



Several types of soils were included as sample sources during the exploration of the rhizobial isolates to anticipate future *P.f.* plantation on soils other than ultisols. It was shown that generally, the isolates collected from these acid mineral soils produced better host growth response grown on an ultisol as shown in Figure 2. Natural adaptation to a multiple limiting factors (high Al, Fe, Mn; low P, Ca, Mg, Mo, B, Co) as typical characteristics of these ultisols was suggested to the main reason for this better performance. This agreed to Fliss *et al*. (1993), Sprent (1994) and Wood (1995) who suggested that a survival and adaptability was much more important traits of rhizobia grown on acid soils. This led to the development of an *in vivo* Al tolerance screening protocol prior plant-inoculation tests, as this would minimize time during selection of large number isolates. Several media were used, however a modified of aluminium rich semi-synthetic agar media (Gemell *et al*. 1993) was proven to be useful to recognize aluminium tolerance. Based on the tolerance to aluminium, they were divided into 3 classes: tolerant, moderate and sensitive. It was found that this *in vivo* tolerance to aluminium could be used to predict the performance of the isolates when associated with *P.f.* on an ultisol, as the tolerance category corresponded to their growth response on an ultisol (Figure 3 and 4). On the average, the growth response from inoculation the Al tolerant isolates was at 400%, on the other hand, an average at 200% were obtained from the inoculation of the Al sensitive isolates. A value at 300% of biomass increment from the non-inoculated control was proposed to be a baseline that distinguishing an aluminium tolerant from those sensitive isolates. Based on this criteria, percentage of the aluminium tolerance isolates that collected from histosols, entisols and ultisols accounted for 11%, 5.3% and 69%, respectively. However, among the most Al tolerant isolates, 2 of them were isolated from histosols, suggesting that the tolerant isolates were unnecessarily collected from ultisols. Therefore, we suggested that source of soils were only an indicative consideration during strategy of this isolation.

Figure 2. Growth response of the collected rhizobial isolates to *P. falcataria* grown on an ultisol. Each dot or square or triangle represents one isolate of rhizobia

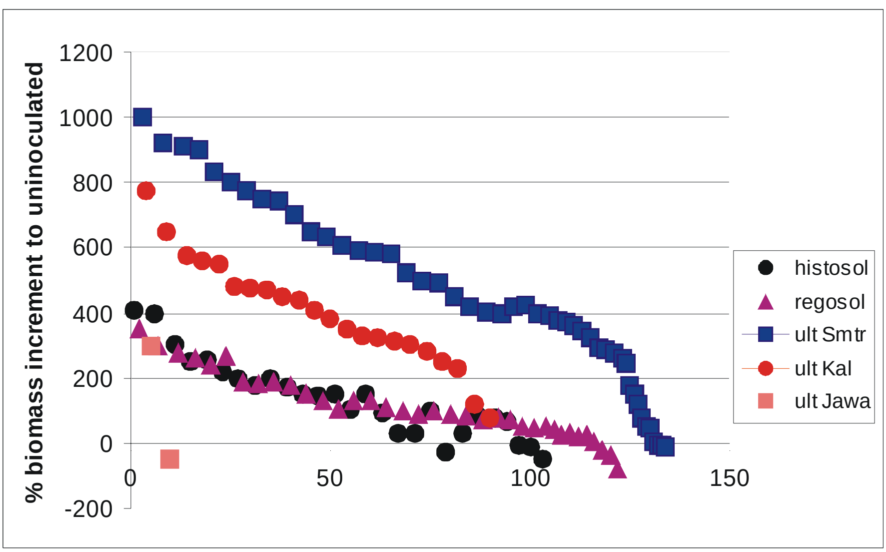


Figure 3. Root nodules formed by various isolates or rhizobia differing in Al tolerance

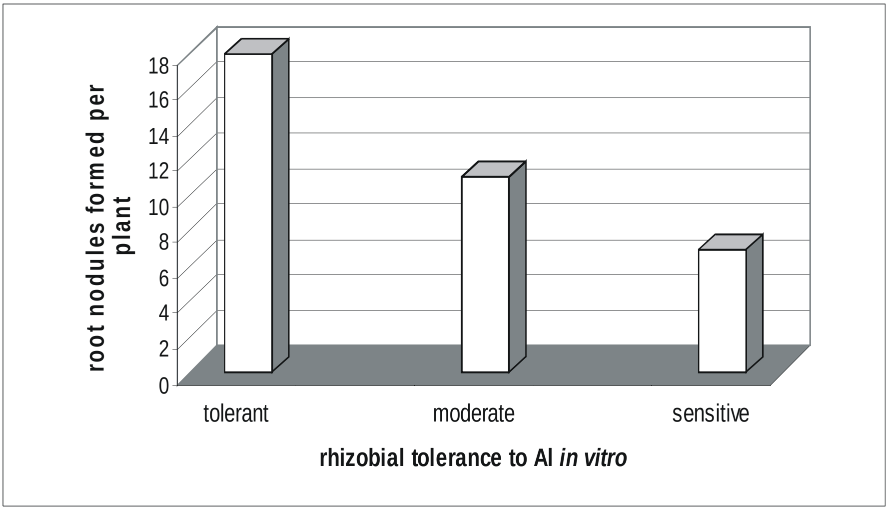
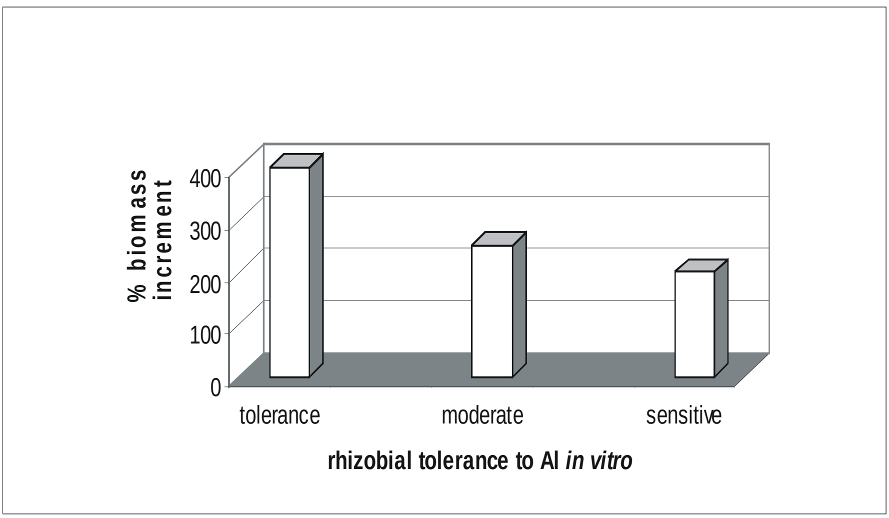


Figure 4. Biomass increment by various rhizobial isolates differing in Al tolerance



A simple observable parameter is important consideration during selection of rhizobial isolates in association with their host plant. Among the various growth parameter, we noted that root nodule number, root-nodule dry weight and biomass increment of the inoculated plant were the most meaningful parameters used during the selection of the isolates. It was cautious that the host plant height, despite of its simplicity for undisturbed measurement, was not appropriate parameter for this selection. As also reported by Sprent (1994), the activity of nitrogen fixation (as measured as acetylene reduction activity) did not indicate strong correlation to their biomass, possibly this physiological trait was only measured its potential rather than its actual value. It also suggested that heterogeneity of the host genotypes may contribute to an inconsistent measurement of this trait. The importance of biomass and nodule during selection were also similar to those noted in other tropical tree species, such as *Acacia mangium* and with *Leucaena leucocephala* (Karyanto-unpublished). We recommended a direct inoculation into an ultisol as a straight-forward procedure after the *in vivo* screening, rather than by using a sand culture supplied with controlled nutrient solution. It was noted that the growth response obtained from an artificial media (sand culture) did not represent the isolates performance on ultisols (Karyanto-unpublished). It was suggested that a multi-constraint nature of ultisol could not be simply imitated by artificially stress conditions.

A sub-optimal dosage of the basal fertilizer was required during these *in vitro* selection. Without these applied nutrient, nodulation were very poor. In contrast, an excessive nutrient application suppressed the potency of the tolerant isolates, therefore could not distinguish the Al tolerant from the sensitive ones. Phosphorous was shown to be major component in this basal fertiliser, in addition, nitrogen should be completely omitted since it may inhibit early step of the nodulation.

Genotypic interaction between host plant and rhizobial isolates were examined by inoculating the nine families derived from three different provenances/landraces of *P.f.* with the three selected isolates of rhizobia as shown in Figure 5 and 6. An interaction was shown by statistical analysis of the following parameters: (i) host plant biomass, (ii) number of root nodule, and (iii) biomass of root nodule. Host plant diversity at both family and provenance/land-race levels as well as the isolate of rhizobia contributed to this interaction. The best response was achieved by a compatible interaction, on the other hand, reduction on plant growth caused by incompatibility during *P.f.* - rhizobia association was apparent. It was interesting to note that the growth response of a *P.f.* provenance collected from Morotai island was negative. The associations was characterized by small and under-developed nodules formation accompanying with negligible or even undetected of nitrogenase activity. Genotypic interaction between host and rhizobia are frequently reported (Pvovorov *et al*. 1994, Josephson *et al*. 1991), but limited informations have been available in tree species. Galiana *et al.* (1996) reported this evidence in rhizobia – *Acacia mangium* symbiosis. We, therefore, strongly recommended that genotype of host plant is considered during the selection of rhizobial isolates.

Figure 5. Interaction between provenances of *P. falcataria* and rhizobial isolates on an ultisol

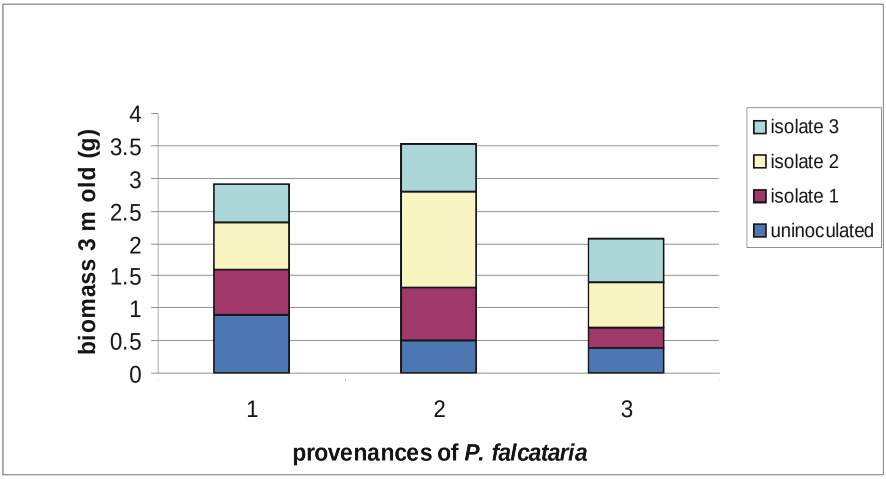
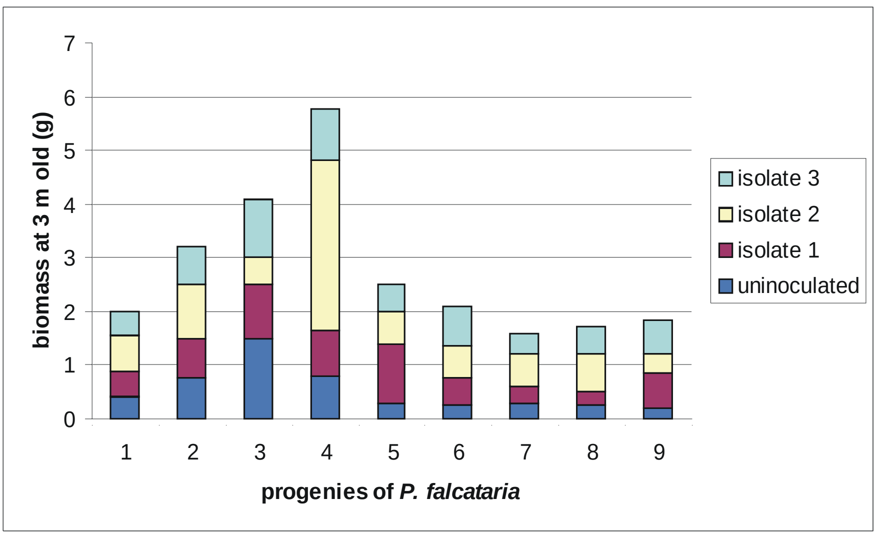
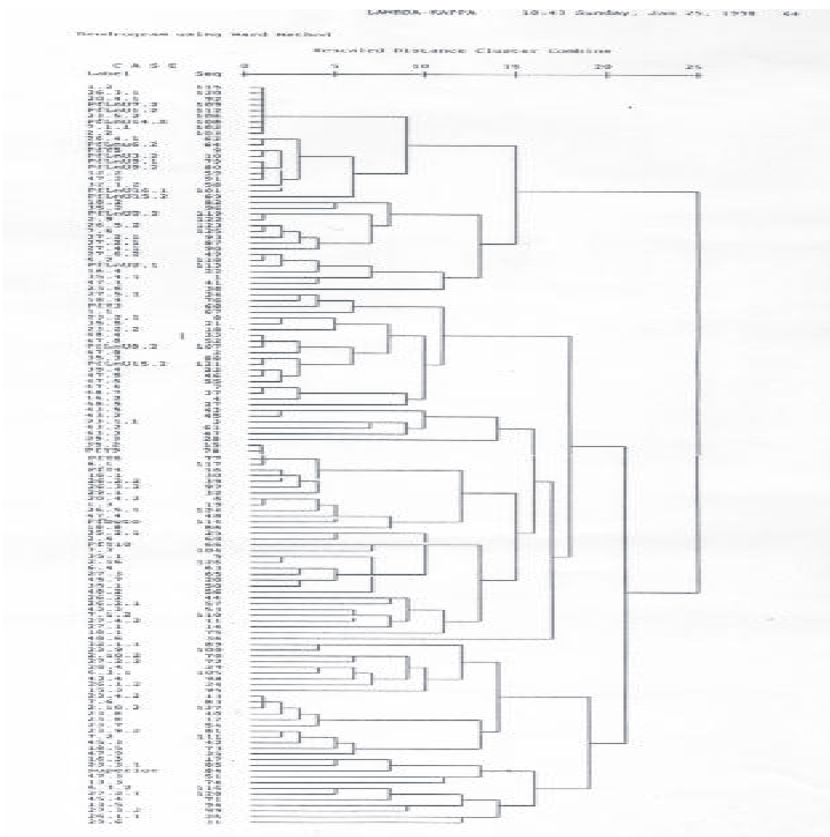


Figure 6. Interaction between rhizobial isolates and progenies of *P. falcataria* on an ultisol



Intrinsic Antibiotic Resistance (IAR) were useful during characterization of the isolates. Cluster analysis using the *Ward's minimum* method was used for classifying the isolates. This multivariate analysis indicated that the isolates collected from the similar region tended to be clustered, even though in some cases the patterns were not obvious (Figure 7). We noted that the isolates collected from Molucca and East Kalimantan were separately clustered. Geographical distribution in rhizobial isolates could be distinguished using phylogenetic grouping as reported by Haukka *et al*. (1998). In addition, the aluminium tolerant isolates were also tended to be grouped. For example a cluster (2.2) consists of 64.5% of the Al tolerant isolates. Despite its simplicity, it is suggested that antibiotic resistance profile were similar to those obtained by plasmid profiling, since this trait is plasmid-borne (Chan *et al*. 1988). However, a molecular methods (especially biodiversity based on rDNA sequence) is still to be done in order to reveal possible relatedness/phylogenetic among these isolates collected from the various geographical regions.

Figure 7. Diversity of the rhizobial isolates associated with *P. falcataria* collected from various regions in Indonesia as revealed by Intrinsic Antibiotic Resistance (IAR) and presented as dendrogram analysed with Ward's minimum method.



Suggestion and Future Plan

1. Due to its multi-nutrients deficiency, further application of the rhizobia symbiotic of this tree species should be integrated into other nutrient managements, for example by combining with arbuscular mycorrhizal fungi. This symbiotic association should be treated as one component, among other component, that contributing to an integrated nutrient management during development of *P.f.* on ultisols.
2. Strong evidences on genotypic interaction between rhizobial isolates and the provenances/progenies of the *P. falcataria* cautions the practical application of this associations. The host trees proven their site adaptability (using genetic trials) offer a suitable material for developing an improved rhizobial component.
3. More analytical studies on the field competitiveness traits of the promising rhizobial isolates should be undertaken by using a reporter gene.
4. Under-developed and early senescent root nodules in the several host-isolate interactions suggesting an evidence of genotypic interaction that may attracting further studies on molecular mechanisms of these symbiotic associations.

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Mycorrhizal Fungal Population in an Over-Burned Tropical Rain Forest in East Kalimantan

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Abstract. Dipterocarp species are the most important tree species of tropical rain forests in Southeast Asia. Mycorrhizal fungi affect growth and establishment of these species. When the tropical rain forests are destroyed by fire, the population of mycorrhizal fungi might change drastically.

Populations of mycorrhizal fungi in an over-burned tropical rain forest in East Kalimantan, in which the latest fire occurred in mid-1998, were assessed in February 2001. Observations were conducted on three permanent plots that represented unburnt (Control), lightly burnt (LB) and severely burnt (SB) sites. At the time of this study, the SB site was occupied mainly by ferns and macarangas. Ferns and macarangas also occupied part of LB site, while the unburnt Control remained occupied by dipterocarp trees.

Observations of fruiting bodies and ectomycorrhizal roots revealed that the ectomycorrhizal fungi were present only on the unburnt site and on that part of the LB site where the host trees remained. None were found on the SB site. Two genera, namely *Russula* and *Hydnum,* were found on the unburnt site, while *Boletus* and *Hydnum* were on the LB site. In contrast to ectomycorrhizal fungi, endomycorrhizal fungi were found on all sites. The spore numbers of endomycorrhizal fungi were similar on all sites, while levels of endomycorrhizal infection varied. Greatest infection level was on the control site, with a lesser level on LB and the lowest on the SB site.

The absence of ectomycorrhizal fungi on the SB site might inhibit efforts to revegetate this area with dipterocarps. However, this needs to be investigated further as the soil might contain wind-blown spores originating from the neighbouring dipterocarp trees. The inoculum potential of this soil is being assessed in the Faculty of Forestry, Gadjah Mada University, using a baiting experiment.

Background

Dipterocarps are the most important tree species of tropical rain forests in Southeast Asia. Growth of dipterocarp trees was reported to be associated with ectomycorrhizal fungi (eg. Ogawa 1992, Kikuchi 1996, Lee *et al*. 1997). Growth and survival of dipterocarp seedlings were also enhanced by the presence of ectomycorrhizal fungi (eg. Lee & Alexander 1994, Kikuchi *et al* 1996, Yasman 1996).

Populations of mycorrhizal fungi can be drastically affected by forest fire (eg. Amaranthus & Trappe 1993, Parke *et al.* 1984, Harvey *et al*. 1980, 1981). Since tropical rain forests often suffer from fire, there is a need to assess mycorrhizal fungal populations in an over-burned tropical rain forest.

This paper reports a preliminary study to assess the effect of forest fire on the population of mycorrhizal fungi in a tropical rain forest in East Kalimantan, and discusses the importance of inoculating dipterocarp seedlings before planting them on over-burned forests.

Literature Review

Mycorrhizae

Mycorrhizae are mutualistic-symbiotic associations between fungi and roots of vascular plants. The fungi benefit by the supply of carbon - particularly monosaccharide sugars such as glucose and fructose – and other essential substances such as vitamin B and certain amino acids from the hosts. The plant, in return, gains fungal-absorbed nutrient elements, secondary metabolites, and soil moisture (Harley & Smith 1983, Kropp & Langlois 1990, Mukerji *et al.* 1991).

Smith and Read (1997) classified mycorrhiza into seven groups based on structural characteristics and taxonomic classification of both fungal and plant symbionts. However, only ectomycorrhizae and endomycorrhizae - or arbuscular mycorrhizae (AM) - are the most prevalent types of mycorrhizae in nature (Maronek *et al*. 1981).

The ectomycorrhizal association is characterised by the presence of a fungal sheath or mantle which develops to surround the root; a Hartig net, which is a network of hyphae growing intercellularly in the epidermal-cortical region of the host root (Harley & Smith 1983, Wilcox 1982); and the presence of hyphal systems connecting the ECM to both the soil and fruiting bodies of the fungi, forming the ECM (Harley & Smith 1983).

The structure of the mantle, which covers the root, is highly variable (Warcup 1990). It may vary from loose wefts of hyphae partially covering the root to a dense pseudoparenchymatous sheath completely enclosing the root tip (Harley 1969). The sheath thickness also varies, from 6 mm to 65 mm (Kope & Warcup 1986). In the well-developed ECM, the mantle can constitute 20 - 40 percent of the total ectomycorrhizal root weight (Smith & Read 1997).

The mantle sheath plays important roles in the enhancement of the absorption surface of the host to allow increased water and nutrients, provision of a physical barrier to soil-borne pathogens, and storage of beneficial substances including polyphosphate, glycogen, lipids and perhaps proteins. The morphology of the mantle sheath also sometimes allows identification of the symbiont species of field-collected ectomycorrhizae (Smith & Read 1997) as shown by Chilvers (1968), Ingleby *et al*. (1990), and Agerer (1987-1993, 1995).

The structure of the Hartig net also varies. Generally it consists of a complicated fan-like or labyrinthine branching hyphae, which results from repeatedly and prolifically branching hyphae (Smith & Read 1997). The net may only enmesh the epidermal layer, as found in eucalypt-ectomycorrhiza (Chilvers & Pryor 1965, Malajczuk & Hingston 1981, Malajczuk *et al*. 1984), or it may fill the intercellular space of the cortex as found in the ectomycorrhiza of most conifers. The Hartig net rarely penetrates the cortical cells.

The Hartig net is believed to have a role in the exchange of nutrients between the fungus and host as the two symbionts come into close contact (Marks & Foster 1973). The repeatedly and prolifically branched Hartig net provides a very large surface area for that contact (Harley & Smith 1983).

Ectomycorrhizal roots have different gross morphology from uninfected roots of similar age (Bowen 1980). Infected roots are usually thicker, branched in a number of forms, lack root hairs, and are different in colour (Kendrick & Berch 1985, O'Dell *et al*. 1993), although ectomycorrhizal roots which are not short and without thickened morphology may also occur.

Endomycorrhizae, in contrast to ectomycorrhizal associations, do not alter the morphology of plant roots. Endomycorrhizal fungi grow intra- and intercellularly in the cortical cells but do not usually form a fungal network outside the roots as extensive as in ectomycorrhiza. Endomycorrhizae are often called vesicular-arbuscular mycorrhiza (VAM) because the fungi form characteristic, large, thick-walled vesicles in the roots and invade the cortical cells to form arbuscules which are tree-like growths of the fungi that mediate interchange of metabolites between fungi and host plant. Vesicles are apical or intercalary swelling of hyphae that contain lipids and function as reserve organs. Since vesicles are not always formed in endomycorrhizal associations, the term arbuscular mycorrhiza (AM) is often used instead of vesicular-arbuscular mycorrhiza (VAM) (Smith & Read 1997).

Forest fire

Fire in forests can be a good servant or a bad master. As a good servant, fire can be used as a means of eliminating unwanted fuel, providing easier access to forest products, and clearing land for other uses. A bad master because fire can destroy valuable resources, add carbon dioxide to the atmosphere, and kill or injures people (Landsberg-no date).

Burning following clear cutting is widely practised to clear land before trees are re-planted or forests are converted to plantations such as rubber and oil palm. This method of land clearing is economical, requiring only one third of the cost of land clearing by mechanical methods (Saharjo & Watanabe 1999). A good burning can also provide easy access during planting, kill seeds and coppice shoots of weeds, and make available nutrients such as P, K, Ca and Mg in the "A" horizon.

When the burning become uncontrolled, however, fire can be a big disaster. In 1997, for example, forest fire destroyed at least 2 million ha of forest in Indonesia. Haze spread into the atmosphere causing air pollution in Indonesia and the neighbouring countries such as Malaysia, Singapore, Thailand, Philippines, Brunei, and Papua New Guinea. The highest air pollution index (API) of 859 was recorded in Malaysia. It has been estimated that this forest fire contributed approximately 20% of the recent increase in the greenhouse gasses, primarily by releasing CO_2 and CH_4 (Saharjo & Watanabe 1999).

Effect of forest fire on mycorrhizal fungal population

Forest fire has been reported to reduce endomycorrhizal fungal populations (Dhillion *et al*. 1988, Klopatek *et al*. 1988, Vilarino & Arines 1990, Wicklow-Howard 1989). Mycorrhizal inoculum potential observed following burning was extremely low (Anderson & Menges 1997). Severe forest fire can affect endomycorrhizal fungi by changing soil conditions and by directly altering endomycorrhizal proliferation. Rashid *et al.* (1997) found a similar number of total spores, but a lower number of viable endomycorrhizal fungal propagules in soil from a burnt forest than from the nearby unburnt control forest at Margalla Hills near Islamabad. Their results also indicated that after fire, mycorrhizal activity was initiated from endomycorrhizal hyphae in the roots of dominant shrubs. This indication was supported by Bellgard *et al.* (1994) who suggested that endomycorrhizal fungi maintained their viability in the underground organs of plants in New South Wales, Australia. After the fire, these re-established the arbuscular mycorrhizal associations.

Similarly, populations of ectomycorrhizal fungi can be affected by forest fire. Severe forest fires in the Siskiyou Mountains of southern Oregon USA in 1987 reduced inoculum potential of ectomycorrhizal fungi resulting in a slight formation of ectomycorrhiza in *Pseudotsuga* seedlings grown in the following season (Amaranthus & Trappe 1993). Similar low levels of ectomycorrhizal fungal propagules after a forest fire were previously observed by Parke *et al.* (1984) and Harvey *et al*. (1980, 1981).

Fire also changes mycorrhizal fungal diversity, as shown by Torres and

Honrubia (1997). By following fruiting body formation of fungi within two years after fire, they found that fruiting bodies in the burned sites were typically ascomycetes, while basidiomycetes were strongly reduced. The sclerotia extracted from soils were mainly *Cenococcum*. The number of sclerotia of this fungus was also greater in burnt stands than in unburnt stands (Torres & Honrubia 1997). Similarly, Warcup (1990) and Miller *et al.* (1998) found that ascomycete fungi forming E-strain ectendomycorrhizae were the dominant post-fire fungi.

Procedures

The experiment was conducted in a conservation forest, Bukit Bangkirai, in East Kalimantan. The last fire in this forest occurred in mid-1998. Three permanent plots representing unburnt control (C), lightly burnt (LB), and severely burnt (SB) sites, were established. At the time of this study (February 2001), the SB site was occupied mainly by ferns and macarangas. Part of the LB site was also occupied by ferns and macarangas while some other parts remained occupied by dipterocarp trees. Each plot was divided into (10 x 10) m^2 subplots.

Fruiting bodies were collected from all subplots. Fresh fruiting bodies were photographed and the characters were recorded. A "fungal herbarium" was made using a coal-fueled drier.

To assess the presence of endomycorrhizal fungi, soil samples (approx. 5x5x10 cm^3) were collected from the center of 5 randomly selected subplots using a shovel. At the Laboratory of Forest Biotechnology, Faculty of Forestry-GMU, spores of endomycorrhizal fungi were extracted from the soil samples using wet sieving and decanting methods.

To assess mycorrhizal infection, another set of soil samples were collected. At the camp, the roots were extracted from soil samples and stored in 50% ethanol. At the Laboratory of Forest Biotechnology, Faculty of Forestry-GMU, the roots were cut, cleared, and stained with 0.05% (w/v) Tryphanblue lactoglycerol (1:1:1 distilled water, lactic acid, glycerol). After de-staining with 50% glycerol, the intensity of endomycorrhizal infection was determined following the method of Giovannetti and Mosse (1980). The intensity of ectomycorrhizal formation was determined by counting the number of ectomycorrhizal root tips and non-mycorrhizal root tips.

Results and Discussion

Fruiting bodies of ectomycorrhizal fungi were present only on the unburnt (control) site and on those parts of the LB site where the host trees remained.

None were found on the SB site, confirming the detrimental effect of fire on an ectomycorrhizal fungal population. Fruiting bodies of *Russula* and *Hydnum* were found on the unburnt site while on the LB site B*oletus* and *Hydnum* were found (Figure 1). Fruiting body production was generally low. On the unburnt control site, fruiting bodies of *Russula* were found only on 2 subplots where the fruiting bodies emerged in cluster. In one subplot only 2 fruiting bodies were found, and in another subplot there were 4 fruiting bodies. Similarly, fruiting bodies of *Hydnum* were found in 2 different subplots, where 4 fruiting bodies were collected from each subplot. On the LB site, 2 fruiting bodies of *Boletus* and 4 fruiting bodies of *Hydnum* were collected from 1 subplot. On this subplot several dipterocarp (Meranti Merah, Meranti Putih, and Keruing) poles (height > 3m, diameter <20 cm) were present.

*Figure 1.*Fruiting bodies of ectomycorrhizal fungi found in unburnt control and lightly burnt site in Bukit Bangkirai, East Kalimantan. A: *Hydnum*, B: *Boletus*, C: *Russula*

A.

B.

C.

Several ectomycorrhizal fungal genera have been found to be associated with dipterocarps, including *Amanita, Russula, Boletus, Scleroderma, Pisolithus, Tricholoma,* and *Lepiota* (Lee 1998). However, in this study, only 2 genera of common dipterocarp associates (*Russula* and *Boletus*) and 1 additional genera (*Hydnum*) were found. A fruiting body of *Amanita* was found in the unburnt forest some distance away from the observation plot.

Similar to the pattern found for presence of fruiting bodies, ectomycorrhizal infection occurred on the unburnt control and LB sites. The intensity of infection, however, was low (Table 1). In this study, root samples collected using a soil corer were used. Roots of dipterocarps were not separated from roots of other plants. The roots from several subplots were not dipterocarp roots and these roots did not bear ectomycorrhiza. Since all root samples were considered, the average intensity of infection appears low. When roots from the soil core containing ectomycorrhiza only were considered, intensities of infection as great as 76% could be obtained.

Table 1. Mycorrhizal associations in an over-burned tropical rainforest in East Kalimantan

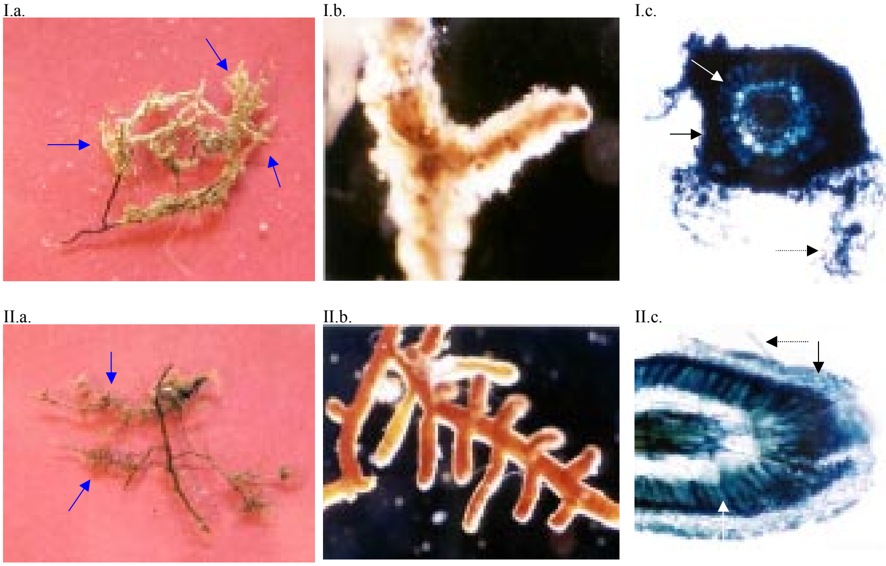
Plot	Ectomycorrhizal infection (%)	Spore Number of Endomycorrhizal Fungi				Endomycorrhizal Infection (%)
		53 μm	75 μm	125 μm	Total	
Control	15.5	4	12	3	19	34.6
LB	17.8	3.8	11.3	7.1	22.2	16.7
SB	0	4.5	13.8	11	29.3	13.3

Table 2. Distribution of ectomycorrhiza within each subplot in unburnt control and lightly burnt control site

Plot	Sub Plot	No of soil core with ectomycorrhiza (from 3 soil cores assessed)	Range of Ectomycorrhizal Infection (%)
Control	B2	1	13
	B9	3	17 – 41
	F5	0	0
	H2	0	0
	H9	2	54 – 76
LB	B2.	0	0
	B5	3	36 – 62
	D3	1	37
	F2	0	0
	F5	3	24 – 64

Two types of ectomycorrhizal roots: (a) irregularly pinnate with abundant formation of extramatrical hyphae, and (b) monopodial pinnate without significant amount of extramatrical hyphae were obtained (Figure 2).

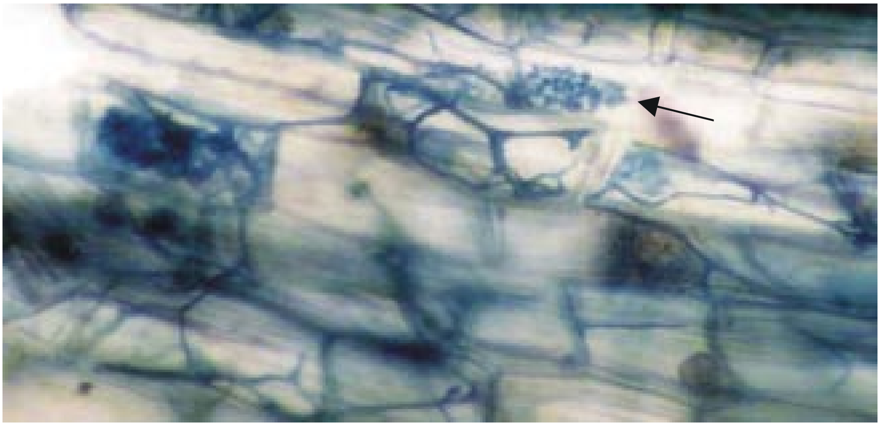
Figure 2. Two typical ectomycorrhiza commonly found in Bukit Bangkirai, East Kalimantan. I: Irregularly pinnate with abundant formation of extramatrical hyphae II: Monopodial pinnate without significant amount of extramatrical hyphae. a,b: Gross morphology of the ectomycorrhiza (blue arrow: ectomycorrhizal root tips), c: Cross & longitudinal section of ectomycorrhizal root (black arrow: mantle; white arrow: Hartig net; dashed arrow: extramatrical hyphae)



The presence of ectomycorrhizae on the unburnt control and the LB sites probably supports the statement that ectomycorrhizal associations are obligatory for dipterocarps (Yasman 1996). The HB site that has been regenerated to many shrub and tree species but not to dipterocarps did not contain ectomycorrhizae.

In contrast to ectomycorrhizal fungi, endomycorrhizal fungi were found on all sites. The spore numbers of endomycorrhizal fungi were similar on all sites, although on the SB site numbers were slightly greater (Table 1). The genera commonly found were *Glomus* and *Acaulospora*. Endomycorrhizal infection varied, being greatest on the control site, less on the LB site, and least on the SB site (Table 1). In these associations only arbuscules were found (Figure 3). No vesicle was observed in the roots.

Figure 3. Arbuscules (arrow) formed in the root cells of an infected root



The presence of endomycorrhizal fungi on severely burned sites probably reflected their capability to form associations with a broader host range than ectomycorrhizal fungi. It was reported that endomycorrhizal associations occurred in species of most plant families, either herbaceous or woody species. Other scientist after compiling publications on mycorrhizal research, estimated 67% of Angiospermae species and 71% of tropical plant species are capable of forming endomycorrhizal associations. Along with the establishment of vegetation on the SB site, endomycorrhizal fungi also were re-established. Bellgard *et al.* (1994) and Rashid *et al.* (1997) suggested that in a forest fire endomycorrhizal fungi might maintain their viability in the underground organs of plants and re-grow as these plants re-sprouted.

The absence of ectomycorrhizal fungi on the SB site might inhibit efforts to revegetate this area with dipterocarps. However, this possibility needs further investigation, as the soil might contain wind-blown spores originating from the dipterocarp trees remaining on neighbouring plots. Pasture paddocks that were long without eucalypt trees contained spores blown in from mature eucalypt stands in the surrounding hills (Nurjanto 2000). The inoculum potential of these soils is currently being assessed in the Faculty of Forestry, Gadjah Mada University using a baiting experiment. Endomycorrhizal associations in dipterocarps were not usually observed, except in two studies in Thailand and Malaysia (Lee 1998). Further investigation is required to assess the role of endomycorrhizal fungi in assisting dipterocarp seedling establishment in an over burned forest.

Conclusions

1. Populations of ectomycorrhizal fungi were significantly affected by fire.
2. Ectomycorrhizal fungi were present only on the unburnt and the LB sites where the host dipterocarp trees remained.
3. Endomycorrhizal fungi that had broad host range were found on all sites. These fungi might re-establish with the establishment of vegetation.

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Population Genetic Study of *Shorea leprosula* Using RAPDs (Random Amplified Polymorphic DNAs)

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Abstract. DNA analysis of *Shorea leprosula* was conducted to evaluate genetic diversity within and between populations, and genetic relationships among populations in Indonesia. Samples were collected from 44 trees from 3 populations; i.e., Jambi (Sumatera), Tanah Grogot (Southern East Kalimantan), and Mahakam River (Northern East Kalimantan). Samples from Jambi were collected from 2 sub-populations, namely PT Dalek Hutani Esa and PT Sadarnila. H_e value within population was high (0.310) in the Jambi population (Sumatra) but low in both of the Kalimantan populations (0.257 and 0.247 in Sungai Mahakam and Tanah Grogot populations, respectively). The values within subpopulations were lower than that within the Jambi population but higher than those within the Kalimantan populations. The G_{ST} value was 0.152 among 3 populations. The values between two regions (Sumatra and Kalimantan), between populations in the same region (Sungai Mahakam and Tanah Grogot in the Kalimantan region), and between subpopulations (Dalek and Sadarnila in the Jambi population, Sumatra) were 0.088, 0.080, and 0.065 respectively. The mean value of genetic distances between individuals (*d*) within populations was 0.341, lower than the mean between populations (0.393), and between regions it was the highest (0.406), as expected. However, the mean value within subpopulations (0.355) was higher than that between subpopulations (0.353) or within populations (0.341). The UPGMA dendrogram of each individual formed two major clusters. Cluster 1 consisted of only the individuals in the Kalimantan region, but cluster 2 consisted of both the Kalimantan and Sumatra regions.

Introduction

Shorea leprosula Miq. is distributed naturally in Peninsular Thailand, Peninsular Malaysia, Sumatra, Kalimantan, Bangka, and Belitung. *Shorea leprosula* Miq., which is synonymous with *Hopea maranti* Miq. (1861) and *Shorea maranti* (Miq.) Burck (1887) is one on the commonest light meranti timbers.

This species is common on well-drained or swampy sites on clay soils below 700-m altitude and develops large to very large trees up 60-70 m tall, with clear boles to 35-42 m. Diameters may go to 175-255 cm, with buttresses up to 2-5 m high. In Indonesia, *Shorea leprosula* Miq. is considered as a light red meranti with lightweight, hard wood and it is a commercially important species.

Information on genetic diversity within and between populations is important for an *ex situ* conservation program. Such information for *S. leprosula* in Indonesia, however, is almost non-existent. The objectives of this study were to evaluate the genetic diversity within and between populations and evaluate the genetic relationships of *Shorea leprosula* populations to support *ex situ* conservation and tree improvement of this species in Indonesia.

Materials and Methods

Plant material

Forty-four wildlings were randomly selected from the 3 different populations of *Shorea leprosula* in Sumatra (Jambi) and Kalimantan (Northern and Southern areas of East Kalimantan), Indonesia, for this study. Leaf samples were taken from the wildlings and used for DNA analysis. The structure of the population was arranged by region (Sumatera and Kalimantan), population (Jambi, Tanah Grogot, and Sungai Mahakam) and sub-population (PT Dalek Hutani Esa and PT Sadarnila) (Table 1) and this same organization was used in the data analysis.

Table 1. Population origin of trees used in this study

No.	Population Origin	No of trees
1.	Kalimantan Region	
	Population Tanah Grogot	12
	Population Sungai Mahakam	12
2.	Sumatera Region	
	Population JambiSub-population PT Dalek Hutani EsaSub-population PT Inhutani V	1.010

DNA extraction

Total genomic DNA was extracted using a modified protocol (Shiraishi and Watanabe 1995) of the CTAB method (Murray & Thompson 1980). Template DNA was purified by the Wizard Clean Up System (Promega).

RAPD Analysis

A total of 120 arbitrary decamer primers (Operon Technologies) were used for first primer screening. Of these primers, 40 were selected for second primer screening. Finally, 16 primers with high reproducibility and clear bands were chosen for the population study.

Data analysis

Population analysis

RAPD bands were scored as present (1) or absent (0), and a (1/0) data matrix was constructed. Frequency of alleles was calculated by the square root method. Proportion of genetic diversity residing among populations (*Gst*) was calculated according to Hartl and Clark (1989), and genetic diversity (*He*) according to Nei and Li (1979).

Individual analysis

The UPGMA cluster analysis was carried out using PHYLIP ver. 3.57c and genetic distance (*D*) was calculated according to Nei and Li (1979).

Results

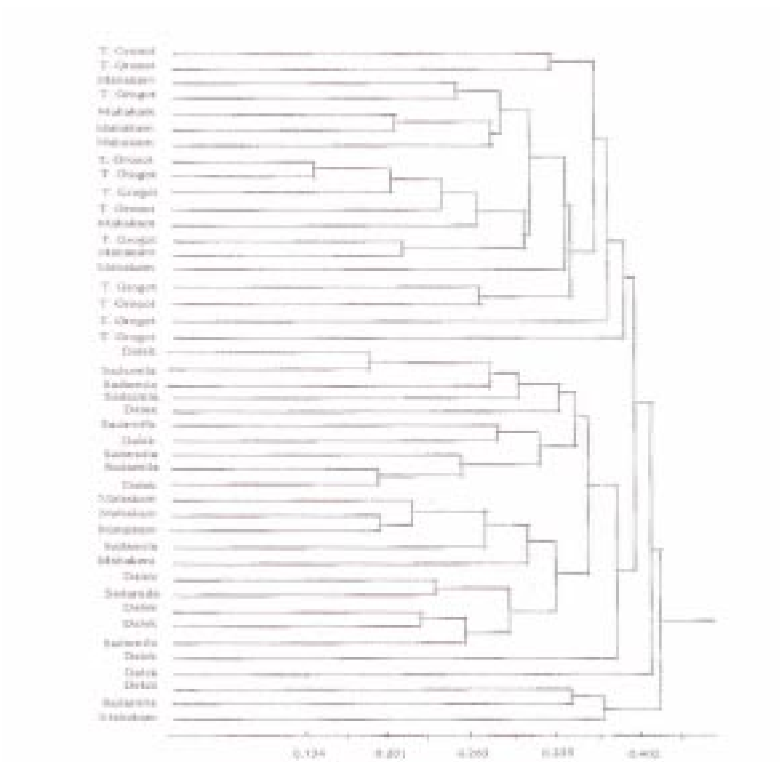
Seventy polymorphic RAPD bands were obtained from 13 primers. The genetic parameters calculated from the RAPD data are summarized in Table 2. Heterozygosity (H_e) within population was high (0.310) in Jambi population (Sumatra) but low in both of the Kalimantan populations (0.257 and 0.247 in Sungai Mahakam and Tanah Grogot populations, respectively). The values within subpopulations were lower than that within the Jambi population but higher than those within the Kalimantan populations. The G_{ST} value was 0.152 among 3 populations. The values between the two regions (Sumatra and Kalimantan), between populations in the same region (Sungai Mahakam and Tanah Grogot populations, Kalimantan region), and between subpopulations (Dalek and Sadarnila subpopulation in Jambi population, Sumatra) were 0.088, 0.080, and 0.065 respectively.

Genetic distances between individuals (*d*) were also calculated. The mean value within populations was 0.341; lower than the mean of between populations (0.393), and between regions the mean value was the highest (0.406), as expected. However, the mean value within subpopulations (0.355) was higher than that of between subpopulations (0.353) or within population (0.341). The UPGMA dendrogram of each individual is shown in Fig. 1. Although the divergence was not clear, two major clusters were observed. Cluster 1 consisted of only the individuals in Kalimantan region, but cluster 2 consisted of both the Kalimantan and Sumatra regions.

Table 2. Summary of genetic parameters of *S. leprosula*

	H_e	H_t	H_s	D_{ST}	G_{ST}
Species (3 population)	-	0.320	0.271	0.049	0.152
Between Region (Sumatera vs. Kalimantan)	-	0.320	0.292	0.028	0.087
Within Sumatera Region	-	-	-	-	-
Jambi Population	0.310	-	-	-	-
Between Sub-population	-	0.310	0.290	0.021	0.066
Sub-pop PT Dalek Hutani Esa	0.291	-	-	-	-
Sub-pop PT Sadarnila	0.289	-	-	-	-
Within Kalimantan Region	-	0.274	0.252	0.022	0.082
Sungai Mahakam Population	0.257	-	-	-	-
Tanah Grogot Population	0.247	-	-	-	-

Figure 1. UPGMA dendrogram of each individual of *S. leprosula*



Discussion

The genetic diversity of *Shorea leprosula* was examined at 4 different levels of hierarchy, i.e., species level, regional level, population level, and subpopulation level. The heterozygosity (H_e) tends to be higher at the higher hierarchy levels (species = 0.320 > region = 0.310 or 0.274, region = 0.274 > population = 0.257 or 0.247, population = 0.310 > subpopulation = 0.291 or 0.289).

The genetic diversity of both populations in the Kalimantan region was much lower than that in the Jambi population in Sumatra, or even lower than that of the subpopulations in the Jambi population (Table 2). The dendrogram (Fig. 1), however, showed a countertrend; i.e., all individuals in the Jambi population were in the same cluster, but individuals in the Kalimantan populations were not in the same cluster. The detailed observation of genetic distances suggested that there are some groups consisting of genetically similar individuals but there are also genetically distant individuals, especially in the Mahakam population. Therefore, the lower heterozygosity values in the Kalimantan populations may be a consequence of several related individuals.

The genetic differentiation (G_{ST}) was slightly larger between regions (Sumatra vs. Kalimantan) than between populations in the region (Tanah Grogot vs. Mahakam populations, both in Kalimantan), and was slightly larger between populations than between subpopulations (Dalek vs. Sadarnila in the Jambi population). This was supported by the individual analyses. Individuals in the same population or subpopulation did not compose a single cluster, but the individuals in the same region composed an almost single cluster. Therefore, it could be concluded that the genetic differentiation tends to reflect the geographic distance in this species.

The genetic differentiation (G_{ST}) among the three populations was higher than in other outcrossing tropical trees. On the contrary, Lee *et al.* (1996) reported low genetic differentiation of *S. leprosula* in Peninsular Malaysia. The high genetic differentiation in this study was, however, caused by the low genetic diversity level in the two Kalimantan populations. Therefore, larger numbers of populations should be investigated to obtain the general trend of genetic differentiation of *S. leprosula* in Indonesia.

Genetic conservation for breeding purposes aims at obtaining variable genetic materials rather that simply preserving the genetic composition of a species. Our results indicated that the geographically distant populations could possess different genetic compositions, and that the genetic diversity within populations is lower than the species' total diversity. It is therefore advisable to sample trees from several populations in the *ex-situ* conservation plots.

Acknowledgements

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Study on Reproductive Phenology of *Eucalyptus pellita:* Flowering Pattern, Breeding System and Pollination Mechanism of *Eucalyptus pellita* F. Muell on Wanagama Education Forest, Yogyakarta

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Abstract. Observation on the reproductive phenology of *Eucalyptus pellita* was done in the three-years and five-months old *E. pellita* plantation at Wanagama Educational Forest, Gunungkidul, Yogyakarta. The study site located in the upland of Gunungkidul District about 40 km south of Yogyakarta. Rising 150-200 m above sea level, 7° North Latitude – 8° South Latitude, it classified as C Schmidth and Fergusson climatic type (Anonymous, 1996). Base rocks are mostly limestone and napal, and soils are generally shallow, formed from latosol with clay textured.

The study began on December 1995 and finished on June 1997. Observation was done on the flowering, pollination and fruiting of *E. pellita*, that involved several activities: (I) observation on flowering pattern and the stages of floral development, and (ii) observation on reproductive biology i.e. breeding system and pollination mechanism.

The *flowering pattern* and the stages of floral development were detected by observation on (i) physical and morphological changes of reproductive organs, (ii) the developmental stages of reproductive organs, and (iii) the time and duration of each developmental stage. Study on *reproductive biology* was concerned with breeding system and pollination mechanisms of plant. Observation on *breeding system* were achieved by controlled pollination using artificial self and out-cross pollination followed up by monitoring of seed set and seedling growth. Study on *pollination mechanism* was focused on receptive stages of flower and plant-pollinator interaction.

E. pellita spent 302 days to develop the reproductive organs, from the floral initiation until the ripening of fruit and seeds dispersal. There were 145 days needed to reach *anthesis*. Depending on the time of maturity of sexual organs, it is categorized as *protandry dichogamy* type. Anther dehiscence starts just before anthesis, and stigma reach maximal receptivity 3 days later. Floral initiation began in January, peak flowering reached in March-April where transition from rainy season to dry season took place, and ripening of fruit and seed dispersal occurred in September. Naturally, *E.pellita* seemed to have outcrossing mechanism because of the *protandry dichogamy* flowering type. The seed came from self-pollination still found because of the *self-fertile* mechanism, but the inbreeding depression suppressed the growth at the seedling level. There was the broad range on the kind of flower visitors including the three families from order Hymenopthera: Apidae, Vespidae, and Formiciidae. From those flower visitors, it seemed that the bee of Apidae was the most effective pollinator. The primary attractant of the flowers were foods: nectar and pollen. On *E. pellita* flowers that had *dish-and-brush shaped* structure, it seemed that secondary attractant dominating visual appearance was the color of filament as *perianthium*. The optimal activity was detected on duration April-May, from 06.00 am to 02.00 pm, with the peak of activity on 08.00 to 11.30 am.

Introduction

As well as being of research interest in helping to unravel the complex functioning of tropical forest ecosystem, studies on forest phenology could play an important role in developing silvicultural practices to restore natural forest ecosystem and to conserve biodiversity (Elliott *et al.* 1994). Information about gene flow and reproductive biology is of critical importance in designing suitable breeding strategies for tree improvement programs (Bawa & Hadley 1990).

Central to any breeding program is knowledge of the pattern and degree of genetic variability within and between species populations. Data on population genetic structure also provide the basis of adequate sampling for *ex situ* conservation, and for suitable design of reserves for *in situ* conservation of forest genetic resources (Bawa & Hadley 1990, Ghazoul 1997). Here, patterns of genetic variation in populations are largely determined by pollination mechanisms of plants (Bawa & Hadley 1990, Griffin & Sedgley 1989).

Review on phenology also needed as the first step of long term breeding program. Information on the flowering pattern and reproductive biology, including breeding system and pollination mechanism, must be well understood in the beginning of the breeding activity for tree improvement purpose (Owens *et al.* 1991, Bawa & Hadley 1990). Phenological data could be used to plan seed production program. This program requires such information to stimulate flowering, enhance flower, fruit and seed productivity, managing pollination, plan seed harvesting and determine several problem such as breaking the seed dormancy (Bawa & Hadley 1990).

Eucalyptus pellita is a commercial timber species naturally found in Australia and Papua. Its natural distribution extends from Cape York Peninsula, Queensland and Bateman's Bay – New South Wales. It also found naturally in north west of Papua New Guinea and south east of Papua (FAO 1979), and in several places in South America (Harwood 1992). In its native place, *E. pellita* commonly used for building construction, and it has been used since long time ago as materials in charcoal industry in Brazilia. Recent research shows that *E. pellita* also good for pulp (Harwood 1992). Tree improvement programs in several places of Australia and South America involve *E. pellita* as one of the target for hybridization activity. This species has good capability to breed with other species to form the combination of selected traits (Eldridge 1993).

Considering the difficulty of *E. pellita* to be reproduced vegetatively, sexual reproduction is the way to regenerate this species. So it is very important to pay attention to its phenology with the following aspects: flowering pattern and stages of floral development, and the reproductive biology that include breeding system and pollination mechanism.

Study Site

The study was conducted at Wanagama Education Forest, Yogyakarta, from early December 1995 to late June 1997.
Wanagama Forest – established in 1966 – is a research station covering about 600 ha of land. This area belongs to Faculty of Forestry, Gadjah Mada University; located in the upland of Gunungkidul District about 40 km south of Yogyakarta, 150-200 m above sea level, 7^o North Latitude – 8^o South Latitude, it is classified as C Schmidth and Fergusson climatic type. Base rocks are mostly limestone and napal, and soils are generally shallow, formed from latosol with clay textured (Anonymous 1988).

Materials and Methods

A series of studies were done using three-years and five-months old *E. pellita* plantation at Compartment 14 of Wanagama Education Forest.

The flowering pattern and stages of floral development

Three trees were selected for this observation. Ten (10) flower buds distributed on upper, middle and lower part of the crown were randomly chosen and identify within each selected tree.
The stages of floral development were then determined by (i) physical and morphological changes of reproductive organs, (ii) the developmental stages of reproductive organs, and (iii) the time and duration of each developmental stage. Each physical and morphological change, that will be used to define and classify the developmental stages of reproductive organs, were recorded and illustrated by photograph. From these observations, the flowering pattern was defined.

Reproductive biology

Study on reproductive biology was concerned with *breeding system* and *pollination mechanisms*.

Observation on breeding system was achieved by controlled pollination on two selected trees as male and female parent. Within each selected tree, controlled pollination was done using artificial selfing, out-crossing, and open pollination, followed up by monitoring of several parameters: ratio of fruit/flower, ratio of seed/ovule, seed efficiency, reproductive success, seed viability, and the growth of seedlings.

Study on pollination mechanism was focused on the receptive stages of flower and plant-pollinator interaction. Receptive stages of flower were observed from morphological sign of receptive flower, period of receptive stage, sign and duration of anthesis, and the peak of flowering season. Study on plant-pollinator interaction was done by observing the pollination pattern and behavior, the kind and quantity of pollinators, and the optimal period of pollination.

Result and Discussion

The flowering pattern and stages of floral development

The inflorescence of *E. pellita* has 7 single flowers: 1 flower grows in the central axis of inflorescence, and the others grow laterally surround the central axis. The morphology of *E. pellita* flowers formed from *hypanthium/inner operculum* as the modification of *sepals* or *petals* or both of them, and the bounded *filament* covered by *outer operculum* that will be fell as the *anthesis* take place. Before anthesis, the *hypanthium* and *outer operculum* connected by the thick line called *line of dehiscence*. The structure of *E. pellita* flower is a *dish-and-brush shaped* with many *filaments* as the *perianthium*.

Classified by physical and morphological changes, it was observed that there were 4 developmental phases of the reproductive organ: (1) floral initiation and development of floral bud, (2) development of individual flower prior to *anthesis*, (3) pollination and fertilization, and (4) development of fruit to ripening and dispersal of seed. Within each phase there were several developmental stages.

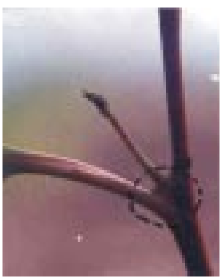
E. pellita totally spent 302 days to develop its reproductive organ, from floral initiation until the ripening of fruit and seeds dispersal. Table 1 shows the developmental phase of reproductive organs of *E. pellita*, and each phase explained more in Figure 1 to 4.

Table 1. Developmental phase of reproductive organ of *E. pellita*

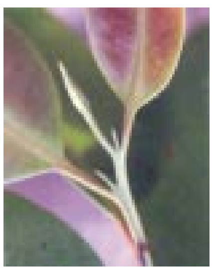
Developmental phase		Time required
Phase 1: Floral initiation and development of floral bud		
Stage 1	Differentiation of reproductive apex to form peduncle and floral bud	29 days
Stage 2	Swell and burst of bud to maximum size	17 days
Stage 3	Abscission of the enclosed bud, shows the inflorescence with 7 individual flowers	12 days
Phase 2: Development of individual flower prior to anthesis		
Stage 1	Abscission of the enclosed outer operculum	39 days
Stage 2	Swell and burst of flower to maximum size	25 days
Stage 3	The change of color from green to light yellow	23 days
Stage 4	Anthesis occur by the opening of outer operculum	5 hours
Phase 3: Pollination and Fertilization		
Stage 1	Developmental process from anthesis to pollinated flowers	5 days
Stage 2	Morphological ccchange from flower structure to fruit structure	19 days
Phase 4: Development of young fruit to ripening and dispersal of seed		
Stage 1	Enlargement of young fruit to maximum size	65 days
Stage 2	Development of fruit to ripening and dispersal of seed	63 days
T O T A L		**302 days**

Figure 1. Floral initiation and development of floral bud

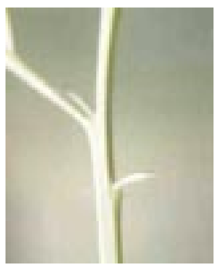
1a



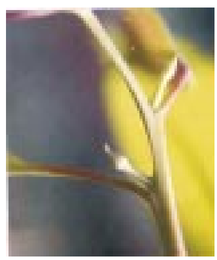
1b



1c

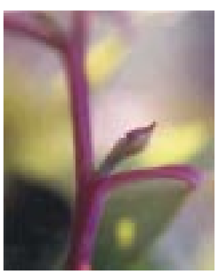


1d

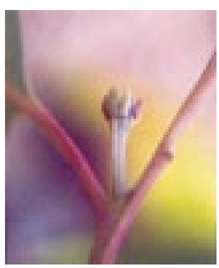


1e

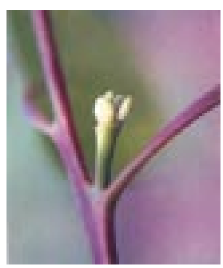
1f



1g

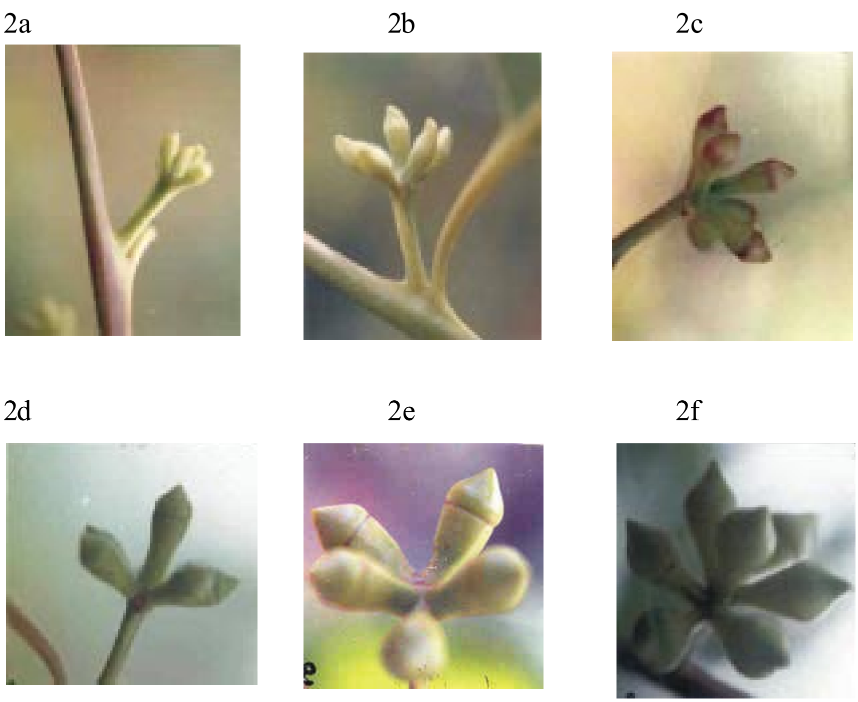


1h



The first stage in the flowering process is floral induction (evocation), when the vegetative meristem was switched into reproductive meristem. While floral initiation is the first morphological change detected by the appearance of reproductive bud (Owens *et al.* 1991, Griffin & Sedgley 1989). Compared with vegetative apex, the visible reproductive bud of *E. pellita* shows the difference on the shape and position of growth, where the reproductive bud has the dome shaped and always growth axillary (1a), while the terminal apex remains vegetative. The reproductive bud elongated (1b) and during 29 days it differentiated into peduncle and bud (1c and 1d), but the enlargement of floral bud became complete 17 days later (1e). Griffin and Sedgley (1989) stated that in the plants that bear the flowers in inflorescence, the individual flower parts do not differentiate until the inflorescence branches have formed. The early floral meristem produces secondary meristem which then initiate the individual flowers along the inflorescence branches. During 12 days later, the thick layer covering floral bud were abscissing (1f and 1g), finally fall down and shows the inflorescence of E. pellita with 7 single flowers (1h).

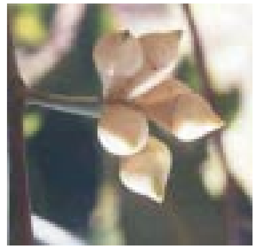
Figure 2. Development of individual flowers prior to *anthesis*



2g

2h

2i



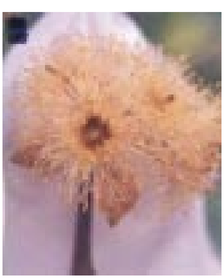
2j

2k

Each single flower covered by the thick layer (2a) that dried slowly (2b) and finally fell down within 39 days (2c). The green single flower clearly shows the part of inner operculum, outer operculum (hypanthium) and pedicle (2d). Flower continued swollen (2e) to maximum size (2f) within 25 days, followed by the change of color from soft green to mature green. Within 23 days later the green color change to light yellow (2g) and mature yellow (2h). The stage of mature yellow is the morphological sign to start the emasculation treatment on the controlled pollination activity; marked by the appearance of calycine ring as the dark-bold line connected the hypanthium and outer operculum. Slowly, the outer operculum become dry and shrink, and its color change from yellow to brown (2i).

Figure 3. Pollination and fertilization

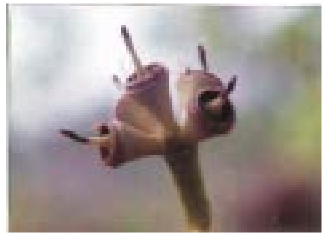
3a



3b

3c

3d

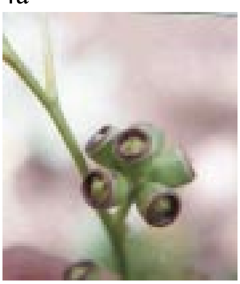


By the dried of calycine ring, outer operculum released slowly and finally fell down within 5 hours, spread out the bounded filament to be the blooming flowers (3a). Within 12-24 hours later, the filament fully erected became the fully blooming flower (3b), and the mature anthers split to disperse the mature pollen. Three days after anthesis, stigma become fully receptive, that morphologically indicated by: I) appearance of maximal secretion that covered the surface of stigma, ii) erection of the bending stylus, iii) swell and burst of stigma, and iv) the change of stigmatic color from light green to bright yellow.

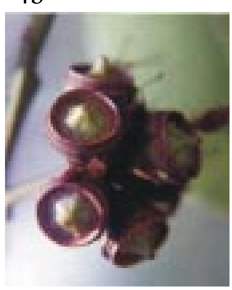
Within 19 days the pollinated flower change its structure to be the structure of young fruit: hypanthium, four triangle-shaped valves, disc, calycine ring, and staminal ring (3c and 3d).

Figure 4. Development of fruit to ripening and dispersal of seed

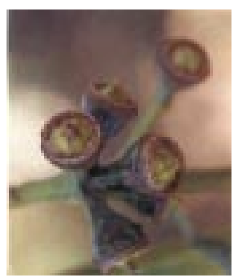
4a



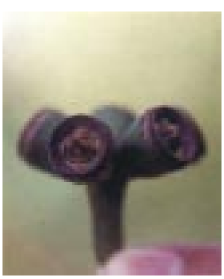
4b



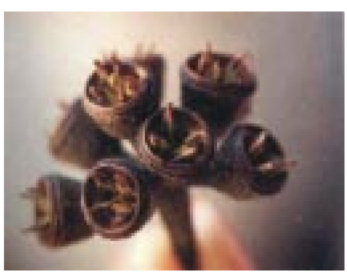
4c



4d



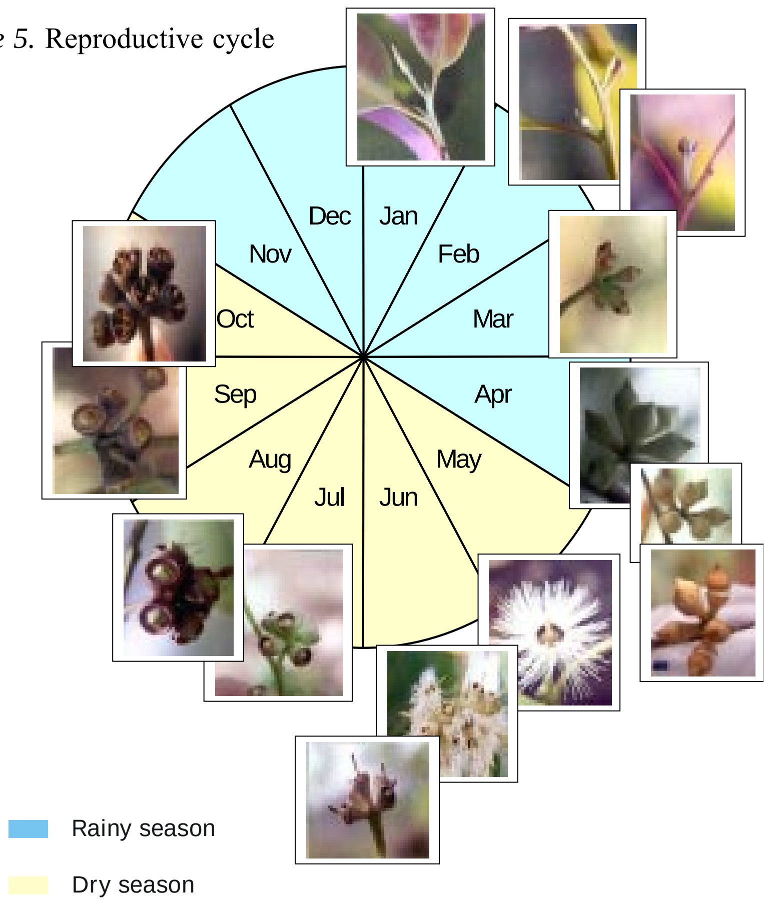
4e



During 65 days of enlargement process of fruit to reach the maximum size, the color of hypanthium changes from light green (4a) to mature green (4b), and finally become dark green (4c). Within 63 days later, the decrease of moisture content and the shrinkage of tissues caused the hardening of hypanthium as the color change to be woody brown (4d). The line of dehiscence appeared clearly between the dry and woody valve, indicated the maturity of seeds. For the seed collectors, this is the morphological sign to begin the seed picking and collection. Within a day, the dry and woody valves will be splitting and spread out the mature pollen.

From these observations, the flowering pattern was determined. Floral initiation began in January, peak of flowering was reached in March-April where there was a transition from rainy season to dry season, and ripening of fruit and seed dispersal occurred in September. Figure 5 shows the reproductive cycle of *E. pellita* in its population on Wanagama Education Forest, Yogyakarta.

Figure 5. Reproductive cycle



Reproductive biology

Breeding system

Controlled pollination was done using artificial selfing, out-crossing and open pollination. The parameters of fruit/flower, seed/ovule, seed efficiency, reproductive success, seed viability, and the growth of seedlings were then measured and compared as shown on Table 2 and Figure 6.

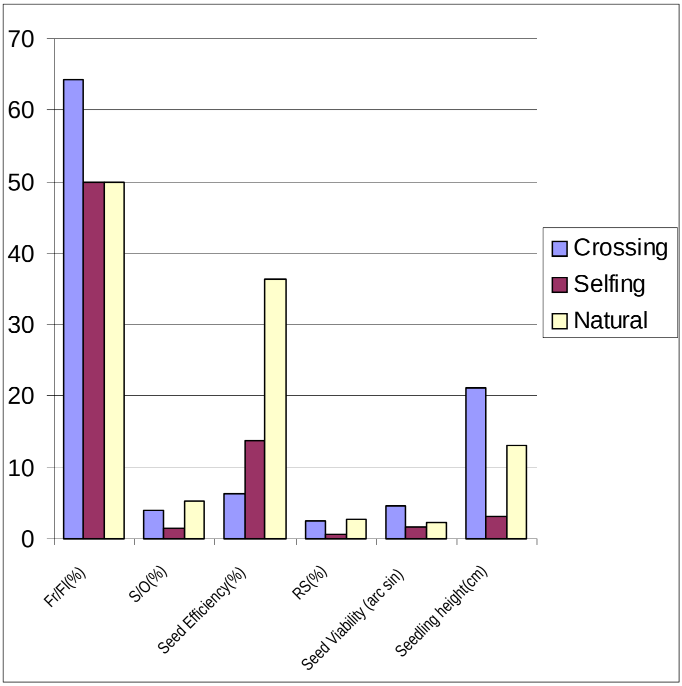
There was no significance on the value of fruit/flower, but artificial selfing showed the lowest value on ratio of seed/ovule. Natural pollination showed the highest value of seed efficiency while artificial selfing was the lowest. The same phenomenon found in the value of reproductive success, where artificial selfing had the lowest value. Artificial crossing had the highest value both in the value of seed viability and height of seedling, while artificial selfing was the lowest. In nature, *E.pellita* seemed to have outcrossing mechanism because of the *protandry dichogamy* flowering type. However, seeds from self-pollination were found due to its *self-fertile* mechanism, but it was indicated that the inbreeding depression suppressed the growth at the seedling level.

Table 2. The value of Fruit/Flower, Seed/Ovule, Seed Efficiency, Reproductive Success, Seed Viability, and the growth of seedling of three pollination treatments

	Fr/Fl(%)	S/O(%)	Seed Efficiency(%)	RS(%)	Seed Viability (arc sin√percent)	Seedling height(cm)
Crossing	64.25^a	3.95^a	6.25^a	2.57005^a	4.559^a	21.07556^a
Natural	50^b	5.29^a	36.415^c	2.71316^a	2.305857^b	13.05571^b
Selfing	50^b	1.525^b	13.79^b	0.707475^b	1.639714^b	3.157143^c

Note: The value with the same letter shows no statistical significance

Figure 6. The value of Fruit/Flower, Seed/Ovule, Seed Efficiency, Reproductive Success, Seed Viability, and the growth of seedling of three pollination treatments



Pollination mechanisms

Among tropical forest trees, gene flow and genetic diversity is largely mediated by pollinators and and animal seed dispersers (Ghazoul 1997). Here, patterns of genetic variation in populations are largely determined by pollination mechanisms of plants (Bawa & Hadley 1990, Griffin & Sedgley 1989).

In this research, study on pollination mechanism was focused on the receptive stages of its flower and plant-pollinator interaction.

Receptive stages of flower

Aspects of receptive stages of flower observed morphological sign of receptive flower, period of receptive stage, sign and duration of anthesis, and the peak of flowering season.

Depending on the time of maturity of sexual organs, *E. pellita* is categorized as *protandry dichogamy* type. Dichogamy is a natural outcrossing mechanism where the female and male organs mature at the different time. It is protandrous when male organs mature before female organs (Griffin & Sedgley 1989).

Anther dehiscence starts just before anthesis, and stigma reach maximal receptivity 3 days later. Prior to anthesis, it was detected that some anthers have been splitting, and the mature pollen grain – bright yellow, copious, sticky, and present on a big aggregate – have spread out. As the outer operculum fell down, the bounded filament slowly erected, and the mature anthers dehisce and split to shed the mature pollen. Within 12 to 24 hours the bounded filament were fully opened, and the flowers were fully bloomed. When this male organ reach maturity, the female organ has not fully receptive yet. It can be indicated by several morphological sign: stigma has not swell and burst to maximal size yet, there was still a little secretion found on the surface of stigmatic area, and the bending stylus still continue the elongation process.

During 3 days, the surface of stigmatic area become more sticky and copious because of the maximal volume of secretion – a medium for pollen germination and tube growth. Color of stigma became brighter as the result of the thickening of pistillate tissues, that shows the readability of stigma to secrete the secretion (Eldridge *et al.* 1993).

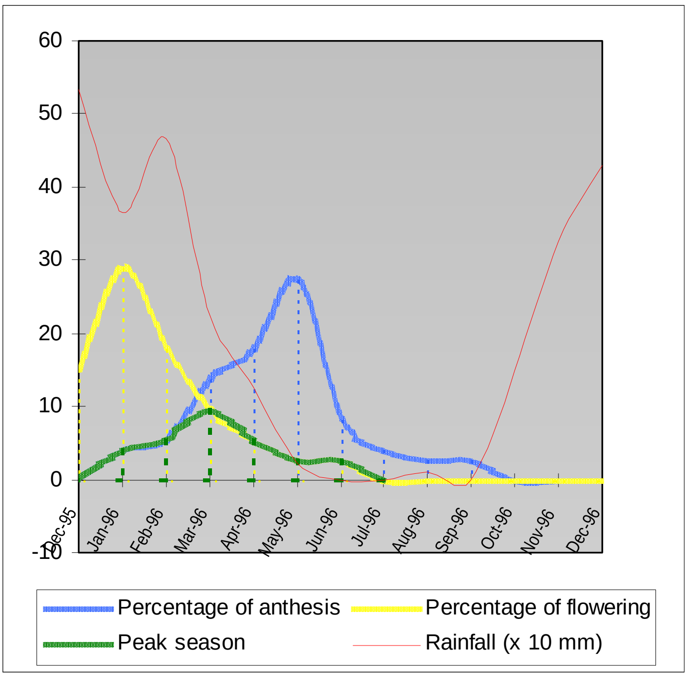
The swollen stigma and the erection of bending stylus were the result of the developing transmitted tissues inside, that formed to prepare the formation of pollen tube (Griffin & Sedgley 1989). Stigma remains receptive for 3 to 7 days after anthesis, and the end of this stage can be indicated by the dried of female organ.

In temperate region, the flowering time of *E.pellita* is strongly dependent on photoperiod and day-length. It seemed that flowering in tropical region is dependent more on water availability, that is strongly related with the two main seasons: dry and rainy season. Larcher (1995) stated that in tropical regions where rainy and dry season occur, the phenophases are linked to the hydro-periodic alternation. The rainy season is the main growth period where the vegetative activities took place. The transition from rainy season to dry season always related with the change on climatic condition such as air temperature, soil temperature, relative humidity, and water status.

Floral initiation of *E. pellita* began in January. Peak flowering reached in March-April where transition from rainy season to dry season took place. Anthesis occurred in May where there was almost no rain, and ripening of fruit and seed dispersal occurred in September-October, the end of dry season.

Explaining this phenomenon, it was stated that in many cases, the low temperature stimulated floral induction, where apical meristem programmed to change into lateral meristem. After this, the higher temperature needed when lateral meristem began the activity to form the floral primordia and started the floral initiation processes (Larcher 1995, Griffin & Sedgley 1989). In March-April when there was transition from rainy to dry season, peak flowering season occurred as a result of the change of climatic conditions.

Figure 7. Peak flowering season of *E. pellita* in its population on Wanagama Education Forest



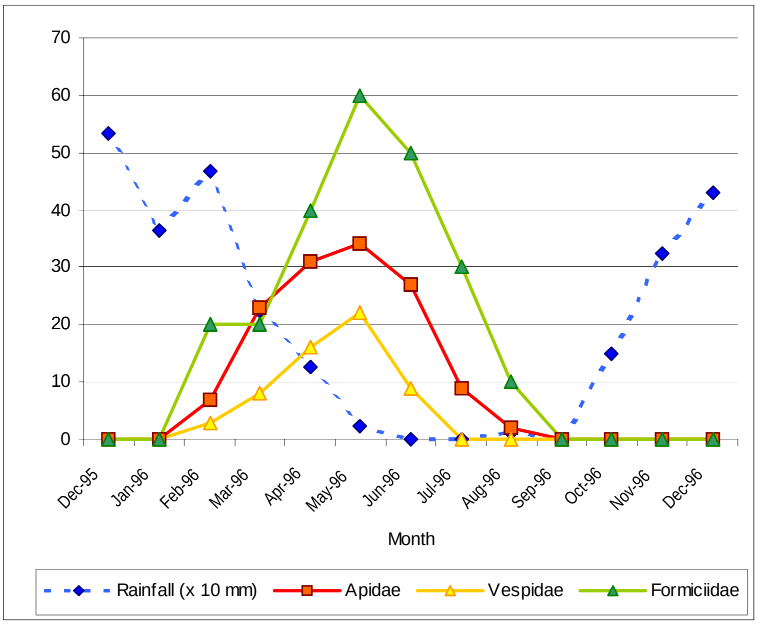
Plant-pollinator interaction

For effective management of pollination, it is necessary to understand the functioning of the crop as a population, or indeed in the case of biotically pollinated species, as an even more complex ecological system (Griffin & Sedgley 1989, Owens *et al.* 1991).

During one year (December 1995-December 1996) the 10 x 10 m plot was made to conduct a series of observation on the pollination pattern and behavior, kind and quantity of pollinators, and the optimal period of pollination.

Most of eucalypt are biotically pollinated (Eldridge *et al.* 1993). There was the broad range on the kind of flower visitors including the three families from order Hymenopthera: Apidae, Vespidae, and Formiciidae. Figure 8 shows the number of flower visitors and the optimal period of plant-pollinators interaction

Figure 8. The number of flower visitors and the optimal period of plant-pollinators interaction



According to Smith (1970) in Griffin and Sedgley (1989), the effective pollinator has to hold several characteristics: make regular visits to flowers at the time of pollen shed and/or receptivity, visit many flowers on many trees but remain constant to the crop species, carry significant loads of viable pollen, make frequent contact with stigmas, and be present in sufficient numbers to effect all necessary pollen transfer.

From flower visitors found in *E. pellita* plantation at Wanagama, it seemed that the bee of Apidae was the most effective pollinator. It made regular visits to flowers at the same time with flowering season, with the peak activity April-May – coincides with the peak of anthesis. The optimal activity was detected from 06.00 am to 02.00 pm, with the peak of activity at 08.00 to 11.30 am.

Foraging behavior of Apidae were kept to one species during a visit, and prefer to forage near to the hive (Bawa & Hadley 1990, Griffin & Sedgley

1989). Such foraging behavior also enhancing possibility to make frequent contact with stigmas. The hairy body of Apidae, and the structure of feet with special basket, enhancing its capability to carry significant loads of viable pollen. Most of this family conducted their activity in a colony, so they always in sufficient numbers to effect all necessary pollen transfer.

The primary attractant of *E. pellita* flowers was food: nectar and pollen. Formiciidae and Vespidae made visit for nectar, but Apidae took both nectar and pollen.

All of visitors on *E. pellita* were diurnal pollinators that conduct the activity in the daytime, so the visual attraction is the important means whereby attracted them to the plant from a distance. On *E. pellita* flowers that had *dish-and-brush shaped* structure, it seemed that secondary attractant dominating visual appearance was the color of filament as *perianthium*.

Conclusion

E. pellita spent 302 days to develop the reproductive organs, from the floral initiation until the ripening of fruit and seeds dispersal. There were 145 days needed to reach *anthesis*. Depend on the time of maturity of sexual organs, it is categorized as *protandry dichogamy* type. Anther dehiscence starts just before anthesis, and stigma reach maximal receptivity 3 days later. Floral initiation began in January, peak flowering was reached in March-April where there was transition from rainy season to dry season, and ripening of fruit and seed dispersal occurred in September.

In nature, *E. pellita* seemed to have outcrossing mechanism because of the *protandry dichogamy* flowering type. The seeds from self-pollination were found due to its *self-fertile* mechanism, but it was indicated that the inbreeding depression suppressed the growth at the seedling level.

There was a broad range of the kind of flower visitors including the three families from order Hymenopthera: Apidae, Vespidae, and Formiciidae. From those flower visitors, it seemed that the bee of Apidae was the most effective pollinator. The primary attractant of the flowers were foods: nectar and pollen. On *E. pellita* flowers that had *dish-and-brush shaped* structure, it seemed that secondary attractant dominating visual appearance was the color of filament as *perianthium*. The optimal activity was detected on duration April-May, from 06.00 am to 02.00 pm, with the peak of activity on 08.00 to 11.30 am.

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Revolving Cutting Technique (RCT) for Producing Cutting Material of Meranti Without Establishing a Hedge Orchard

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Abstract. The use of hedge orchards to produce cutting material for meranti species has many problems such as low sprouting ability and slow growth of the shoots. The revolving cutting technique (RCT) was developed to produce cutting material without establishing a hedge orchard. Instead of using material from hedge orchards, cutting material is taken from nursery-raised seedlings. The multiplication process is repeated to their offspring again and again before planting. Normally, meranti planting stock is ready to be planted at 8 months. This new technique postponed planting time by 4 months to allow new shoot growth from seedlings. Rooting percentages for *Shorea leprosula* and *S. selanica* cuttings derived from RCT were 89 % and 83 %, respectively. Mortality of the seedlings after their shoots were taken was less than 1 %. Height of new shoots 16 weeks after cutting for *S. leprosula* and *S. selanica* were 26.8 cm and 15.8 cm, respectively, which was a suitable size for out planting. These results indicate that RCT is an attractive alternative to hedge orchards for producing cutting materials.

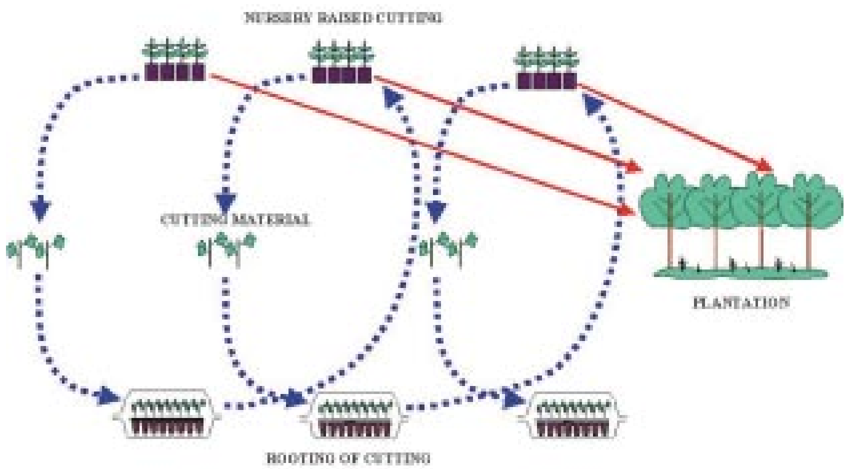
Introduction

Vegetative propagules have been increasingly used for establishment of commercial forest plantations, especially for some fast growing species such as *Acacia mangium*, *Eucalyptus* spp and *Gmelina arborea*. Hedge orchards are commonly established to produce cutting materials (scions) of fast growing species. Unlike fast growing trees, the use of hedge orchards for meranti poses some problems due to low sprouting ability and slow growth of shoots.

Harvesting of shoots from hedge orchards of fast growing trees such as *A. mangium* can be done once per month, and from a single tree in a hedge orchard dozens of shoots can be obtained in each harvest. Unlike *A. mangium* however, meranti can only produce 2 – 4 shoots from a single tree in a hedge orchard, and the harvesting interval is at least 4 months. In addition, establishing a 100 ha plantation of a fast growing tree like eucalyptus requires only 1 ha area of hedge orchard (Zobel & Ikemori 1983), but for the establishment of a meranti plantation a much larger area of hedge orchard is needed and the management of meranti hedge orchards becomes costly and inefficient.

This paper introduces a technique called the *revolving cutting technique* (RCT), for producing meranti scions without establishing hedge orchards. In this technique, nursery-raised cuttings are used as a source of cutting materials. Multiplication is started by cutting juvenile shoots of nursery-raised cuttings, and using these as cutting materials. Shoot harvesting was done once on each cycle to prevent quality degradation of planting materials. Four months after harvesting, the nursery-raised cuttings are ready for out planting. Normally, meranti cuttings are ready to be planted at 8 months old. This technique postponed the planting time by 4 months to allow new shoots to grow from cuttings. Sustainable cutting production can be achieved by repeating the process shown in Figure 1 as required.

Figure 1. Scheme of revolving cutting technique (RCT)



Material and Methods

Two sources of cutting materials of *Shorea leprosula* and *S. selanica* originated from wildlings and nursery-raised cuttings were used in this study. Only autotropic shoots were used. The age of wildlings was unknown, and the age of the nursery-raised cuttings was 8 months old with a height average of 40 cm. About 20 cm of upper (autotropic) shoot was taken from the donor trees (ortets), and shoots were cut into pieces of scion about 8 cm long with two leaves. The scions were planted in pot-trays of 5 x 9 compartments containing a mix of coconut fiber and paddy husk, and placed in a greenhouse with a fog cooling KOFFCO system (Subiakto *et al.* 1999).

Parameters measured in this study were rooting percentage of the tested scions and shoot growth. Rooting percentage of the scions was assessed in the green house, and shoot growth was measured in the nursery.

Results and Discussion

Rooting percentage of cuttings derived from RCT of both *S. leprosula* and *S. selanica* was better than those from wildlings (Table 1). This phenomenon may be attributed to scion juvenility (Bonga 1982). Scions from wildlings were collected from different-aged ortets, whereas scions from RCT were collected from nursery-raised cuttings averaging 8-months-old.

Table 1. Rooting percentage of cuttings from RCT and wildlings

Species	Source of scions	No. of tested scions	Rooting %
S. leprosula	RCT	5940	89
	Wildlings	10935	77
S. selanica	RCT	5175	83
	Wildlings	13230	73

Two weeks after shoots were harvested, new shoots started to emerge. Shoots of *S. leprosula* grew faster than those of *S. selanica*. Figures 1 and 2 show shoot growth of harvested seedlings. Sixteen weeks (about 4 months) after shoot harvesting, heights of new shoots of *S. leprosula* and *S. selanica* were 27 cm and 16 cm, respectively. With initial height (after cutting) of both species averaging 15 cm, the total height of *S. leprosula* and *S. selanica* then becomes 42 cm and 31 cm, respectively, a suitable size of meranti planting stock for out planting (Kimura & Nishiyama 1999).

Figure 2. Growth of new shoots of S. leprosula and S.selanica after shoots cut

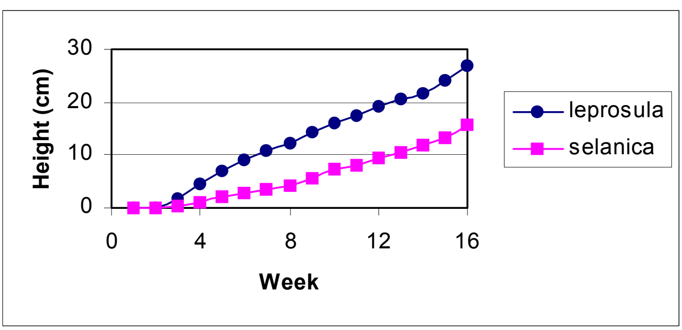


Figure 3. Shoot development of *S. leprosula* (upper), shoot development of *S. selanica*, 4 weeks after cut (bottom left), and shoot development of *S. selanica*, 16 weeks after cut (bottom right)

Conclusion

The revolving cutting technique (RCT) can minimize the cost of producing cutting materials of meranti. For large-scale operational cuttings production, this technique is commercially competitive with the establishment of hedge orchards.

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Evaluation of a Progeny Test of *Eucalyptus Urophylla* S.T. Blake Against the Leaf Blight Disease

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Abstract. A study to evaluate the resistance of the progeny test of Eucalyptus urophylla S.T. Blake to leaf blight disease has these objectives: (1) measuring the genetic variation between families, (2) calculating the genetic correlation of the disease with height and diameter growth, and (3) determining the best family based on disease intensity tests.

The study was conducted on the progeny test in Sebulu (East Kalimantan). The progenies tested were 183 half-sib families derived from 16 provenances. The test designed was Randomized Complete Block with six blocks as replicates, 4-tree plots of progeny in each block. Trees were planted at 3.5 x 3.5 m spacing.

Results showed that variability in crown destruction by leaf blight disease was significant between families within provenances, for interaction of family blocks in the provenances, and between individual trees. Narrow sense heritability estimates for crown destruction were moderate (h^2= 0.53) for family, and low (h^2=0.012) for individual tree. Genetic correlations between crown destruction by leaf blight disease and height or diameter growth of the tree were moderate and low, i.e., –0.53 and –0.20, respectively. Based on disease intensity, families 601 and 740, derived from Tebalvalli and Wasbilla provenances, were the best families in Sebulu at 16.70%.

Introduction

Ampupu trees (*Eucalyptus urophylla*) have been planted over large areas of Indonesia for pulpwood production including Sumatra (Aceh, North Sumatra, and Jambi) and Kalimantan (West, East, and South Kalimantan). This species also has a large natural distribution in Indonesia, i.e., on Flores, Lembata, Adonara, Wetar, Alor, Pantar, and Timor Islands, and also on Timor-Timur, occurring at elevations between 150 and 1700 m above sea level (Doran & Kleining 1979). Numerous provenance and progeny trials of the species have been established in small-scale experimental plantations at many locations.

In Sebulu, Samarinda, and East-Kalimantan, three- year-old Ampupu trees in provenance and progeny trials have been largely unsuccessful because of the leaf blight disease, which is characterized by yellowish to brownish necrotic lesions of variable sizes on the leaf surface. It was proven that one of the causal agents of the blight is *Macrophoma* sp.

The disease tends to start on the lower, older foliage and as it progresses, moves upward on the diseased plant. This disease frequently results in premature and often severe defoliation and severe repeated attacks, with or without defoliation, can result in stunted growth of the host (Brown 1993).

In a preliminary study, we noticed that severity of leaf blight disease within the progeny trials was variable. Some trees and progenies were disease-free, while others were severely infected. It was thought necessary to more carefully evaluate resistance of progeny of *E. urophylla* against leaf blight disease in that trial. The results of this evaluation would then provide data on family/progeny genetic resistance to leaf blight disease in that area.

Material and Methods

The study was conducted at the progeny test in Sebulu. Progenies tested were 183 half-sib families derived from 16 provenances. Test design was Randomized Complete Block with six blocks as replicates, 4-tree plots of each progeny in each block. Trees were planted at 3.5 x 3.5 m spacing. Traits observed were Disease Intensity (DI) based on the index score of crown destruction as calculated by the Chester formula (1959). Considering disease intensity, levels of resistance were categorized as highly resistant (DI=0-10%), resistant (DI=11-20%), somewhat resistant (DI=21-30%), moderately resistant (DI=31–60%), somewhat susceptible (DI=61-70%), susceptible (DI=71-80%), and highly susceptible (DI=81-100%).

Evaluation of resistance in the three-year-old *E. urophylla* progeny trial in Sebulu was conducted from May to September 1995.

The Statistical Analysis System (SAS) program GLM was employed to analyze (ANOVA) Disease Intensity data. Variance components were also estimated by the SAS program package.

Results and Discussion

Heritability

Narrow sense heritabilities (h^2I) were estimated by the formula (Namkoong 1981):

$$h^2I = \frac{4\sigma^2 f}{\sigma^2 e + \sigma^2 fb + \sigma^2 f}$$

Where: $\sigma^2 e$ = Variance component of error

$\sigma^2 fb$ = Variance component of family interaction with block

$\sigma^2 f$ = Variance component of family

These h^2 estimates ranged from 0.52 to 0.53 for family, and 0.012 for individual tree. These results again illustrate the moderate degree of additive genetic control of the expression of partial (quantitative) resistance to leaf blight caused by *Macrophoma* sp. in *E. urophylla* families.

Correlations between leaf blight incidence and height and diameter growth

Expected genetic gain was strongly influenced by genetic correlation. Covariance analyses of genetic correlations between leaf blight incidence and height and diameter growth were computed by the formula (Zobel & Talbert 1984)

$$r_G(x,y) = \frac{\sigma^2 f(xy)}{\sigma^2 f(x) * \sigma^2 f(y)}$$

where:

$\sigma^2 f(xy)$ = Variance component of height or diameter and crown destruction by leaf blight disease

$\sigma^2 f(x)$ = Variance component of family for character of crown destruction, and

$\sigma^2 f(y)$ = Variance component of family for character of height or diameter

Table 1. Genetic correlation between crown destruction by leaf blight disease and height and diameter growth in progeny trial of *E. urophylla*

No	Character	$\sigma^2 f(x)$ and $\sigma^2 f(y)$	$\sigma^2 f(xy)$	r_G
1	Crown destruction	0.0053		
	and		0.1337	-0.53
	Height	0.1609		
2	Crown destruction	0.0053		
	And Diameter		0.0338	-0.20
		0.1187		

The negative genetic correlation between crown destruction and height was greater than that for diameter, indicating that crown destruction by leaf blight disease could cause reduction in tree height more than in diameter. This phenomenon is highly important, especially if we are to simultaneously select parent trees for more than one character.

Analysis of variance for leaf spot disease intensity on the three-year-old *E. urophylla* progeny trial again yielded highly significant F-values for blocks and families. A Duncan's Multiple Range Test of means of disease Intensity is presented in Table 2.

In general, the level of resistance of all families originating from 16 provenances ranged from highly susceptible to resistant. No one family showed high resistance to leaf blight disease. Families from one provenance also showed variation in levels of resistance. More than 50% of those families were moderately resistant to leaf blight. Families number 740 and 601 originated from Wasbilla provenance (Pantar island) and Month of Tebalvalli (Alor island) were resistant to leaf blight (DI=16.70%). There was no geographic or altitudinal pattern of leaf blight resistance in the provenance. The level of resistance was related more closely to family. Thus, one needs to select the best families in a tree improvement program.

Table 2. Disease intensity and level of resistance some families of *E. urophylla* to leaf blight disease

No	Provenance/island	Number of family	Disease intensity (%) *)	Level of Redistance **)	No	Provenance/island	Number of Family	Disease indensity(%) *)	Level of Redistance **)
1.	M.Egon (Flores)	101	46.07 c	M	6	M.Boleng (Adonara)	501	83.30e	H.S
	400-890 m	102	45.84c	M		915-1025 m	503	29.63b	R.R
	Above sea level	103	45.83c	M		Above sea level	504	29.72b	R.R
		104	40.82b	M			505	44.41b	M
		107	57.42c	M			506	42.24b	M
		108	39.87b	M			507	41.62b	M
		109	43.73b	M			508	50.00c	M
		110	52.75c	M			509	47.22c	M
		112	24.97b	R.R			511	56.10c	M
		119	44.10c	M			515	46.64c	M
		122	70.83d	R.S			516	91.70e	H.S
		124	83.35e	H.S	7	M. Tebalvali (Alor)	601	16.70a	R
		127	67.37c	R.S		800-1200 m	612	38.90b	M
2	Sokaria (Flores)	292	53.47b	M		Above sea level	628	46.66c	M
	1140-1240 m	294	38.67b	M			629	56.36c	M
	Above sea level	290	46.52c	M	8	M. Kalabahi (Alor)	632	58.32c	M
		297	53.22c	M		450-850 m	633	56.32c	M
		147	27.80b	R.R		Above sea level	636	42.50b	M
		148	33.30b	M			637	40.00b	M
3	M.Mandiri (Flores)	236	62.50b	R.S			643	45.83c	M
	500-600 m	237	27.77b	R.R			646	38.34b	M
	Above sea level	238	39.82b	M			647	44.23b	M
		243	52.78c	M			648	34.44b	M
		244	34.02b	M			649	39.57b	M
		245	46.53c	M			651	41.87b	M
		246	36.33b	M	9	M. Delaki (Pantar)	730	45.80c	M
		249	38.90b	M		680-780 m	731	55.36c	M
		250	34.88b	M		Above sea level	732	83.30e	H.S
4	M. Wukoh (Flores)	218	57.62c	M			733	44.18b	M
	580-900 m	263	36.10b	M			734	57.22c	M
	Above sea level	265	52.08c	M			736	41.66b	M
		267	57.87c	M			737	40.46b	M
		268	48.62c	M			738	46.21c	M
		269	59.72c	M	10	Wasbilla (Pantar)	740	16.70a	R
		272	52.10c	M		750 – 800 m	741	39.58b	M
		279	57.63c	M		Above sea level	742	41.67b	M
		280	62.85c	R.S			743	58.78c	M
		281	56.40c	M			747	47.68c	M
		282	62.02c	R.S			749	49.98c	M
		284	70.35d	R.S			750	54.38c	M
		285	70.37d	R.S			751	52.07c	M
		286	66.90d	R.S			752	66.67c	R.S
		287	35.18b	M			753	53.56c	M
		288	67.80d	R.S			754	33.30b	M
		291	52.08c	M			755	75.00d	S
5	M.Lewotobi (Flores)	304	50.00c	M			756	60.75c	R.S
	450-550 m	305	45.13b	M			757	63.34c	R.S
	Above sea level	309	51.95c	M			758	67.86d	R.S

Notes:
*) Means with some letter are not significantly different (P>0.05) as determined by Duncan's
Multiple Range Test
**) HR = Highly resistant, R = Resistant, R.R = Somewhat resistant, M = Moderately resistant,
R.S = Somewhat susceptible, S = Susceptible, H.S = Highly susceptible

Conclusions

- Variability of crown destruction by leaf blight disease was significant between families within provenances, for interaction of family blocks within provenances (family block x provenance), and between individual trees.
- Narrow sense heritability estimates for crown destruction were moderate (0.53) for family and low (0.012) for individual tree. Genetic correlations between crown destruction by leaf blight disease and height or diameter of the tree were moderate and low; i.e., – 0.53 and - .20, respectively.
- Based on the disease intensity, families 601 and 740 (16.70%) derived from Tebalvalli and Wasbilla provenances, were the best families in the Sebulu test.

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Plantations in Experimental Forests for *Ex Situ* Conservation

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Abstract. As a part of Borneo, Kalimantan is floristically rich, especially in dipterocarp species of which there are 267, a much higher number than is found on other islands. However, due to continuous deforestation caused by human activities and natural disasters such as forest fires, natural habitats and the biodiversity of Kalimantan's tropical forests are being degraded. Without any positive conservation effort, some valuable species will disappear in the near future. Ex situ conservation is one of the effective conservation alternatives that may be applied. Though actually designed for research purposes, some of the experimental plantations developed by the Forestry Research Institute Samarinda (FORIS) in its experimental forests at Samboja and Sebulu in East Kalimantan, can be considered as ex situ conservation areas.

Unfortunately, development of experimental plantations has encountered some serious problems due to land use conflicts, forest fire, lack of awareness of importance of these plantations, budget limitations, and inadequate planning and coordination.

Introduction

As a part of Borneo, Kalimantan has a high diversity of plants. Whitmore et al. (1990) recorded 84 families on the island with at least one "big tree species" (defined as either 35 cm dbh or over, or at least 20 m tall). These Borneo trees represent 382 genera and 1902 species, and this number does not include many trees of the Annonaceae, Lauraceae, Myrtaceae, and Rutaceae families. Many of the commercially important Dipterocarp species grow on Borneo. According to Ashton (1982), there are 267 dipterocarp species on Borneo and 155 of these are endemic to Kalimantan. Unfortunately, this unusually diverse flora may change in the near future due to degradation of natural forest ecosystems caused by improper management, forest fires, and other natural disasters.

Currently, natural forests in the country are generally characterized by these disturbances, which affect the ecosystems by creating soil erosion, flooding, drought, climatic modifications, and other negative influences. And additional pressures and disturbance by people living adjacent to forest areas and by other forest users who are seeking short-term benefits cannot be easily resolved. All of these disturbances will influence the endangered status of some species as well as general plant diversity, because in tropical forests the habitat of a certain species may depend absolutely on the existence of other species. As an extreme example, *Rafflesia* would disappear following the extinction of *Tetrastigma*. It is believed that without any serious efforts to improve forest management and conservation, some tree species that are currently over exploited may disappear in the near future.

Realizing that rapid depletion of forest areas and the destruction of habitats are happening not only in the production forests of concession areas, but in protected forests and conserved areas as well, significant conservation programs should be implemented to avoid further degradation and extinction of genetic resources. *Ex situ* conservation is one among several approaches that should be considered as an alternative solution.

Ex situ Plant Conservation as an Alternative

The government of Indonesia has established several types of conservation areas, such as national parks, game and nature reserves, or sanctuaries. The government has also established protected forest areas to maintain water resources and soil fertility. Unfortunately, many of those areas are under the process of degradation in terms of both their size and quality. Human activities and natural disasters, such as timber extraction, shifting cultivation, community settlement, and forest fires, are threatening the existence of those conserved and protected areas. Rapid destruction of those areas will indeed cause economic, environmental, and social losses to the country in many forms, such as soil erosion, local climate change, and permanent loss of biodiversity. Since conservation areas were mostly established based on their unique biodiversities, the destruction of those areas may cause losses of valuable and unique genetic material.

To avoid the loss of valuable and unique genetic material from conserved areas that are being degraded, the *ex situ* conservation method may become an effective tool. In contrast to *in situ* conservation, which employs on site conservation methods (FAO 1989 cited by ITTO and RCFM 2000), *ex situ* conservation involves the removal of individual plants or propagating materials such as seed, pollen, and tissues, from their original habitats. These plants and/ or materials are then grown or conserved in gene banks, plantations, or other live collections, such as arboreta, botanical gardens, seed stands, or seed orchards.

Ex situ Conservation in Experimental Forests

The current *ex situ* conservation activities in Kalimantan have not been appropriately recorded and documented. In many cases, institutions or organizations do not even realize that their activities actually have tremendous impacts in relation to plant conservation methods and programs.

In the past, most of the *ex situ* conservation activities in Kalimantan were created to provide stock plants of target species that are economically important for production of fruits, nuts, and/or timber. During that period, there was already a general awareness that many of these target species were becoming rare and difficult to find in adequate numbers in their natural habitats. A good example is a quite large area of tengkawang (*Shorea stenoptera* and *S. macrophylla*) plantations in Sanggau, West Kalimantan, planted by the local Forestry Service in 1971/1972, 1983/1984 and 1992/1993. These plantations were actually developed to produce seeds in adequate numbers for subsequent planting activities. At present, these plantations have formed dense forests that provide genetic materials for further development of tengkawang plantations. Moreover, these plantations have also become sources of additional income for local communities through the collection of tengkawang's nuts.

There are also many arboreta and plant collections in Kalimantan that were initially developed by universities, research, and training institutions for educational and research uses. However, the stands currently play a role as *ex situ* conservation areas for many tree species originating not only from Kalimantan but also from other parts of Indonesia.

The Forestry Research Institute Samarinda (FORIS) has two experimental forests located in Samboja and Sebulu in the Province of East Kalimantan. The area of experimental forest in Samboja is about 3000 hectares, while in Sebulu it is about 2900 hectares. Most of FORIS's experimental plantations have been developed at those two sites. Although the plantations were developed using various scientific techniques and with different research objectives, some of them may be considered as *ex situ* conservation areas because the planting material was brought from many sites around Indonesia, although mostly from Kalimantan.

Species Priority

Depending on the objectives of the biodiversity conservation project, selection of species or plant diversity is one of the most important tasks in order to achieve the program goals. Whitmore (1990) indicated that *ex situ* conservation will only be able to conserve a tiny fraction of the species in the forests. However, by applying appropriate techniques and selection processes, *ex situ* conservation will be very helpful and valuable to protect some target species from extinction.

Since FORIS must prioritize its activities so as to promote appropriate research on tropical forests in Kalimantan, thus the institute has concentrated its conservation research on developing species collection and seed source plantations. The species selected for these plantations have generally been those producing commercial timber, such as dipterocarp species, sungkai (*Peronema canescens*), ulin (*Eusideroxylon zwageri*), and other valuable species found in Kalimantan, such as fruit trees and medicinal plants.

Since 1992, FORIS in cooperation with PT Kutai Timber Indonesia (an Indonesian private forestry company) and Sumitomo Forestry (a Japanese forestry company) has established about 600 hectares of tree plantations in Sebulu. Various species have been planted for the rehabilitation of logged-over forests as well as for species collection. With financial support from ITTO, the institute has developed 64 hectares of dipterocarp plantations and planted 51 hectares of fast growing species to demonstrate silvicultural options for the restoration of burnt forest areas in Samboja. Unfortunately, almost all of the experimental plantations in Samboja dan Sebulu were destroyed by fire in 1997 and 1998.

As mentioned earlier, plantations of dipterocarp species in Sebulu were initiated in 1992 with the main objective to rehabilitate logged over forests. Planting material was brought from Lampung (Sumatra) and from Samboja and Berau in East Kalimantan, and about 200-250 seedlings of each species were planted. Data on growth of those dipterocarp plantations in the Sebulu Experimental Forest still surviving after 1998's forest fire are presented in Table1.

Table 1. Growth of six dipterocarp species in the experimental forest at Sebulu, East Kalimantan, eight years after planting.

Shorea johorensis					***Shorea lamellata***			
	Planted	1	2			Planted	1	2
Measurement Date	92/01/28	99/12/27	00/12/16		Measurement Date	92/01/28	99/12/27	00/12/16
Days after planting		2890	3245		Days after planting		2890	3245
Average Height (cm)		672	788		Average Height (cm)		409	387
Max Height (cm)		1097	1310		Maximum Height (cm)		987	1235
Min Height (cm)		150	66		Minimum Height (cm)		45	10
Average Diameter (cm)		7.1	8.3		Average Diameter (cm)		4.3	4.7
Maximum Diameter(cm)		12	13		Maximum Diameter(cm)		14	16
Minimum Diameter (cm)		1	2		Minimum Diameter (cm)		1	0.8
Shorea leprosula					***Shorea parvifolia***			
	Planted	1	2			Planted	1	2
Measurement Date	92/01/28	99/12/27	00/12/16		Measurement Date	92/01/28	99/12/27	00/12/16
Days after planting		2890	3245		Days after planting		2890	3245
Average Height (cm)		801	898		Average Height (cm)		801	898
Maximum Height (cm)		1259	1410		Maximum Height (cm)		990	1198
Minimum Height (cm)		234	55		Minimum Height (cm)		497	645
Average Diameter (cm)		11.3	13.4		Average Diameter (cm)		11.3	13.4
Maximum Diameter		22	26		Maximum Diameter(cm)		15	16
Minimum Diameter (cm)		3	3		Minimum Diameter (cm)		1	8
Shorea pauciflora					***Shorea seminis***			
	Planted	1	2			Planted	1	2
Measurement Date	92/01/28	99/12/27	00/12/16		Measurement Date	92/01/28	99/12/27	00/12/16
Days after planting		2890	3245		Days after planting		2890	3245
Average Height (cm)		462	535		Average Height (cm)		460	551
Maximum Height (cm)		758	932		Maximum Height (cm)		839	943
Minimum Height (cm)		202	209		Minimum Height (cm)		20	64
Average Diameter (cm)		5.0	6.2		Average Diameter (cm)		4.7	6.4
Maximum Diameter(cm)		11	14		Maximum		11	14

Source: PT Kutai Timber Indonesia and Sumitomo Forestry (2001) unpublished.

To provide material to rehabilitate the burnt area of the Samboja Experimental Forest, in 1999 FORIS started developing a 4.5 hectares plantation of 10 dipterocarp species; these are *Shorea leprosula, S. stenoptera, S. lamellata, S. parvifolia, S. johorensis, S. balangeran, S. ovalis, S. seminis, S. pauciflora* and *S. selanica*. About 180 seedlings of each species were planted with seedlings brought from Bogor (West Java) and several sites in East Kalimantan, such as Sangkulirang, Berau, Pasir, and Gunung Meratus. Based on evaluations12 months after planting, the plantation is in good condition and has grown well. On average, more than 90 % of seedlings have survived, with average heights presented in Table 2.

Table 2. Height of 10 dipterocarp species in the experimental forest at Samboja, East Kalimantan, 12 months after planting.

	Average height (cm) at various widths of planting strips		
	1 m strip	1.5 m strip	2 m strip
Shorea leprosula	35	43	13
Shorea stenoptera	56	71	7
Shorea lamellata	22	21	13
Shorea parvifolia	25	28	10
Shorea johorensis	34	33	12
Shorea balangeran	25	38	13
Shorea ovalis	28	35	11
Shorea seminis	27	35	10
Shorea pauciflora	25	22	9
Shorea selanica	24	37	13

Source: Omon (2000)

Problems with *Ex situ* Plant Conservation

After the economic crisis and "reformation" era, land use conflicts in Kalimantan have occurred almost daily, even in areas assigned and demarcated as experimental and educational forests. This situation makes security assurance on the areas of experimental plantations one of the most significant problems faced by FORIS. Illegal tree cutting and land encroachment for agricultural activities are threatening the existence and integrity of experimental forests both in Samboja and Sebulu.

Other major problems faced by the institute in relation to the development of plantations for experimental as well as for conservation purposes can be described as follows:

- Forest fire. Experimental forests were established in or surrounded by secondary forests, which are very sensitive to fire, especially during the dry season. Therefore, the plantations developed in those areas face a serious fire threat, annually.
- People's unawareness of the importance of these plantations. The rapid destruction of conservation areas in Indonesia indicates that people's level of attention to the protection and development of conservation areas is very low. It seems that most people, including some government officials and some in the private forestry community, have not been really aware of the purposes and long-term benefits of plant conservation.
- Limited funds. Similar to other state research institutes in Indonesia, FORIS operates with a very limited budget to develop and manage its facilities, including experimental plantations. This condition will indeed affect the physical quality of the plantations being developed, as well as quality of information gathered.
- Inadequate planning and coordination. Although an experimental forest represents an excellent potential site for establishing *ex situ* conservation areas, so far there have been no collaborative programs in plant conservation between FORIS and local universities or other conservation units under the Ministry of Forestry, such as Nature Conservation Offices (*Balai Konservasi Sumberdaya Alam*) and National Park Stations (*Balai Taman Nasional*).

It is obvious that the development and establishment of conservation areas are not easy tasks. However, considering the rapid rate of degradation of tropical forests in Indonesia, the establishment of *ex situ* conservation areas should be seriously considered. If such programs of genetic conservation are not planned and implemented, the country's future generations will not have a chance to benefit from – or perhaps even to see - some plant species that may actually have even higher values to those generations than they have at present.

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Plantation Forests in East Kalimantan

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Abstract. The degradation of the natural forest has indeed threatened the existence of wood-based industries in East Kalimantan. After the forest fire disaster, the natural forest in the region is only able to produce about 2.5 million m3 of logs per year. In the meantime, East Kalimantan already has 200 units of wood based industries (lumber, plywood and other wood panels) with annual capacity of 6.4 million m3, and one pulp mill with a capacity of 550,000 tons per annum (Robian 2001). This significant gap between supply and demand of logs being produced from the region's natural forests has contributed greatly to the massive degradation of the natural forests at both the provincial and national levels. One effort to reduce increasing pressures on natural forests in East Kalimantan has been the establishment of plantations. By 1999/2000 about 1.4 million hectares of state forestland in the region have been allocated to 32 companies to develop forest plantations. However, to date, less than 500 thousand hectares have been planted. To establish the plantations, most of the companies have depended upon the reforestation fund (dana reboisasi) and used fast growing tree species, mainly as raw material for pulp and wood panel industries. The establishment of planted forests in East Kalimantan has not been attractive as a long-term business because of many problems, mainly land use conflicts, lack of seed availability, and frequent forest fires.

Introduction

Natural tropical forests in Indonesia have experienced a great deal of pressure in the last three decades. Since the 1960's when intensive, large-scale logging activities were introduced in Sumatra and Kalimantan, the tropical forest has played very important roles to support the development of the country's economy and the improvement of the people's welfare. In the earlier stages logging activities were concentrated on timber extraction for export. However, in an effort to increase the value of forest products and to create more job opportunities, in the early 1980's the government of Indonesia enacted and enforced various policy packages to reduce export of raw products from the forest. These policies successfully stimulated the growth of wood-based industries in the country, such as lumber, plywood, molding, and furniture.

Unfortunately, the rapid growth of these wood-based industries has greatly contributed to the massive degradation of the country's natural forests. This occurs due to an imbalance between the actual demands for raw material by the industries, and the ability of the forest resources to provide timber on a sustainable basis. According to the European Union (2000), wood-based industries in Indonesia require about 63 million m3 of roundwood annually. But data reported by the Indonesian Ministry of Forestry (MOF), as cited by Kompas (2001), have shown that such industries need about 80 million m3 of logs per year. Meanwhile, the sustainable timber production of the country's natural forests is less than 30 million m3 per year.

The gap between supply and demand of timber has caused widespread illegal logging practices, not only in the area of production forests, but also in the forests designated as major protection and conservation areas. Indeed, those illegal practices have become the principal cause of rapid deforestation in the country. The Minister of Forestry mentioned that of 114 million hectares of state forest areas in Indonesia, about 37 million hectares are in the "heavily degraded" condition, and about eight million hectares of that are in the area of national parks and protected forests (Media Indonesia 2000).

To reduce pressures on natural forests and to meet high demand for timber, in 1984 the MOF initiated the development of industrial plantation forests, and during the period of 1989-1994 the program established 100,000 hectares of plantations per year (Fatawi & Mori 2000). For developing the plantations, the Ministry recommended 21 groups of 43 trees as priority species, such as groups of dipterocarps, pines, teak, mahogany, eucalyptus, akasia (*Acacia mangium*), sungkai (*Peronema canescens*), agatis (*Agathis borneensis* and *A. hamii*), sengon (*Paraserianthes falcataria*) and gamelina (*Gmelina arborea*).

Besides meeting needs for wood demand, the establishment of planted forests will provide other environmental benefits. The plantations will serve not only as carbon absorbers but also as *ex-situ* conservation areas, at least before being harvested. Plantations of fast growing species, such as akasia, eucalyptus, sengon, and gamelina, might play such roles only in 8 to 15 years, but plantations of slower growing species, such as teak, mahogany, and dipterocarps could have a longer period (more than 30 years).

This paper aims at summarizing the development of plantation forests in the province of East Kalimantan. It also describes some related problems in establishment of plantation forests in this region.

The Forest and Forest Industries in East Kalimantan

The total land area of East Kalimantan Province is about 21.1 million hectares, and state forestland occupies about 70 % of the total area. The latest data shows that the province has about 14.8 million hectares of state forest lands, consisting of 9.7 million hectares of production forests and 5.1 million hectares of protected forestlands, including national parks, nature reserves, and protected forests (Robian 2001).

Most state forestlands in East Kalimantan are natural lowland dipterocarp forests with a high proportion of valuable tree species producing high quality timber. Consequently, when intensive forest exploitation was introduced in the late 1960s, the natural production forests in the region became the main source of logs for both exports and domestic wood-based industries. According to the MOF, cited by Fatawi and Mori (2000), during the past 20 years annual log production in East Kalimantan has been about three to five million m3, which accounts for about 20 % of the national log production.

Unfortunately, the rapid degradation of natural forests in East Kalimantan has diminished the sustainable timber production of the region. Robian (2001) showed that during the period 1974-1979, log production from East Kalimantan was 43.6 million m3, or about 8.7 million m3 per year. But production dropped to only about 5.5 million m3 per year during the period of 1994-1999, and to only 2.2 million m3 in the year 2000. Gemmingen (2001) estimated that during the period 1983-1996, the annual rate of deforestation in East Kalimantan was about 90 thousand hectares, mainly caused by over exploitations and forestland encroachments.

The deforestation rate has tended to increase, particularly after local authorities were allowed to issue permits for small-scale concessions to local communities - up to 100 hectares for each permit. Ironically, the deforestation and stand degradation occurred not only in the production forests but also in the areas supposed to be conserved and protected, such as national parks, nature reserves, and protected forests. The condition of natural forests in the region has become worse after the forest fires of 1997 and 1998. A study by the Institute of Forest Fire Management (IFFM) indicated that the total land in East Kalimantan affected by these forest fires was approximately 5.2 million hectares, and that about 2.3 million hectares of this are in the areas of forest concessions (Gemmingen 2001).

The degradation of the natural forest has threatened the existence of wood-based industries in East Kalimantan because this forest is the main source of raw material for the industries. After the forest fire disaster, it was estimated that the natural forest would only be able to produce about 2.5 million m3 logs per year (Gemmingen 2001). In the meantime, East Kalimantan has 200 units of wood based industries (lumber, plywood and other wood panels) with annual capacity of 6.4 million m3, and one pulp mill with a capacity of 550,000 tons per annum (Robian 2001). These data indicate that the region has been experiencing a significantly wider gap between supply and demand of logs produced from natural forests. In order to maintain the sustainability of raw material for timber industries, the establishment of good quality plantation forests is considered to be the most appropriate option.

Plantation Forests In East Kalimantan

Data presented by the Regional Forestry Office (2001) have shown that by 1999/2000, about 1.4 million hectares of state forestland in East Kalimantan were allocated to 32 companies to develop plantation forests. However, to date less than 500 thousand hectares have been planted, consisting of about 291 thousand hectares for pulpwood production, 77 thousand hectares for timber, and 120 thousand hectares for both pulpwood and timber. And, the latest plantation was developed in relation to transmigration programs (see Table 1 in the Appendix). Most of the companies have depended upon the reforestation fund (dana reboisasi) to establish the plantations in the area of 10 forestry districts, using 15 recommended tree species (see Table 2 in the Appendix).

Plantation forests in East Kalimantan have been developed mostly using fast growing species to supply raw material for the pulp and wood panel industries, especially particle-board and medium density fiberboard (MDF). The following brief summary describes the recent condition of most planted forests in East Kalimantan.

ITCI Hutani Manunggal, a private forest company in partnership with Inhutani I (a state owned company), has planted *Acacia mangium, Eucalyptus deglupta, E. urophylla,* and *Paraserianthes falcataria* for pulpwood. By 1997 the planted area reached about 87,000 hectares, but about 54,000 hectares were burned in 1997 and 1998. Recent information indicated that about 50,000 hectares of the burnt area have been replanted, and the average diameter of the plantation has now reached 13 cm with an average height of 14 meters.

Surya Hutani Jaya, another private company also in partnership with Inhutani I, started establishing plantations for pulpwood in 1989. The total area allocated to the company is 183,300 hectares located in the District of Kutai (at the central part of East Kalimantan). By April 2001 about 115,000 hectares were planted, mainly with *Eucalyptus urophylla, E.deglupta, Acacia mangium,* and *Paraserienthes falcataria.* The plantation established in 1994 has had an average diameter of 9 cm with an average height of 10 meters.

Tanjung Redeb Hutani, Adindo Hutani Lestari and Kiani Hutani Lestari are private companies in partnership with PT Inhutani I, which have been granted areas in Berau (northern East Kalimantan) for developing plantation forests. Those companies are using *Acacia mangium* as a primary species to produce pulpwood. Other fast growing species, such as eucalyptus, gamelina, and sengon have also been planted on small portions. The seeds used have been brought mostly from Palembang (South Sumatra) and Bogor (West Java). Data collected from Tanjung Redeb Hutani shows that the 8 years old plantation of *akasia* had an average diameter of 22 cm and an average height of 20 meters.

Inhutani I is a state owned forest company which in 1984 started the development of planted forests in Long Nah (located in the District of Bulungan in northern East Kalimantan), and in Batu Ampar (about 30 km north of Balikpapan city) in 1989. Unlike the other companies mentioned earlier, Inhutani I has established plantations for timber production. The species used for the plantation are dipterocarps, namely *Shorea leprosula, S. johorensis, S. parvifolia, S. pauciflora, S. selanica* and *S. smithiana.*

Problems and Suggestions

The establishment of plantation forests in Indonesia seems to be far from succesful at this point. Although started in 1984, the forests planted for industrial purposes in the country have been slowly developed. According to the Directorate of Plantation Forests of the MOF, as cited by Manurung and Sukaria (2001), up to December 2000 total areas allocated by the MOF to 175 companies for plantation establishment have reached 7.8 million hectares. However, only about 1.85 million hectares of plantations have been established, of which about 1.17 million hectares are for pulpwood production.

Unfortunately, a greater part of the planted forests have not been available for harvesting. Manurung and Sukaria (2001), citing data of the MOF in March 2001, mentioned that in 2000 log production from planted forests was only 3.8 million m3. In the meantime, pulp mills and wood-based industries in the country require raw material equivalent to roundwood of about 25 million m3 and 63 million m3, respectively (Manurung & Sukaria 2001, and European Union 2000) and the country’s natural forests are only able to produce less than 30 million m3 of roundwood per annum. These conditions explain why the natural forests in Indonesia are being rapidly degraded.

Despite the high demand for roundwood by pulp and wood-based industries in the country, business sectors have not been interested in making “real” investments to establish plantations for log production. According to the opinion of many analysts, the large part of those companies developing plantation forests are only interested in two things: (1) to utilize the reforestation fund as a loan from the government with a very low, even zero, interest rate; (2) to get timber by utilizing harvesting permits to convert natural forest areas allocated for plantation development. It seems that those opinions are in close agreement with the facts that the establishment of plantations has diminished significantly after the reforestation loans were terminated temporarily, and many natural forest areas have been abandoned after clearing.

The establishment of man-made forests has not been attractive for business sectors mainly because there are no long-term assurances of security and certainty for such businesses. Many conflicts and claims have been made by local communities on the forest areas allocated for plantations, particularly during the last three years.

Another major problem is in relation to the productivity of the established plantations. Most of the plantations have not shown impressive results in producing roundwood. For instance, the 8 years old plantations of *Acacia mangium* established by Surya Hutani Jaya and Tanjung Redeb Hutani have produced only 100-150 m3 of logs per hectare, where such plantations are expected to produce more than 250 m3 of logs per hectare. That low productivity has occurred because most of the planted forests have been developed using low quality and uncertified seeds, and poorly selected sites for plantations. According to plantation developers, the low quality, unrecognized seeds have been used because sources of good quality certified seeds in the country are limited and not adequate to meet their demand. Up to present the plantation developers have mostly relied on imported seeds.

Considering that the secondary forests and grasslands of ilalang (*Imperata cylindrica*) in Kalimantan are very sensitive to fire during the dry season, forest fire has also become a major threat for planted forests. The report made by IFFM in 1999 indicated 880,000 hectares of planted forests in East Kalimantan were burned during fire disaster in 1997 and 1998 (Gemmingen 2001).

In order to make the establishment of plantation forests more attractive for business and to improve the productivity and quality of the future forest areas, in both natural and planted forests, the following actions are suggested:

- The government, both central and local authorities, have to provide policies and rules which give long-term security and certainty to the forestry business, especially to avoid land-use conflicts on the areas of state forestland designated for plantation;
- The government has to allocate, protect, and manage an adequate area of natural state forests to be conserved as sources of seeds and seedlings;
- Good quality seed sources, such as seed stands and seed orchards, should be established by both the forest companies and forestry authorities, and these should also receive appropriate support from well designed research programs on tree improvement and silviculture;
- Converting natural forests to plantations should be terminated, and man-made forests should be established only in grasslands and degraded production forests. The less degraded secondary or logged over production forests should be rehabilitated by applying enrichment planting techniques using merchantable tree species, preferably local species;

- The establishment of planted forests in Kalimantan should be aimed at tree plantations of mixed merchantable species, especially mixed dipterocarp species.

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In Situ Conservation of Ebony (*Diospyros celebica* Bakh.)

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Abstract. Geographically, ebony lies between 1^0 - 4^0 S and 119^0 E – 120^0 W. It is an endemic species of Sulawesi, Indonesia. Due to its beautiful and unique texture, it is heavily utilized and its presence in natural forests is threatened.

There are many silvicultural systems for ebony; however, there are many obstacles to its conservation and culture. For example, seed should be stored less than 14 days. Naturally adapted seedlings are the best choice for planting in the field, especially for maximum growth rate. To improve the initial rate of ebony's growth, seedlings with sprout length of no more than 2 cm are the preferred planting material.

Species conservation and restoration efforts in ebony aim at managing the species in natural forests and also efforts to take advantage of its special growth characteristics.

Introduction

Ebony is one of the endemic tree species of Sulawesi. Nowadays, its presence in natural forests is endangered. Ebony wood is beautiful, strong, and resilient making it valuable and highly sought after.

Many attempts have been made to regenerate ebony. However, these have been generally unsuccessful due to lack of knowledge about the ecology and adaptations of ebony.

Environmental factors vital for plant ecosystems include proper elevation, soil texture, water supply, rainfall, and slope condition. Soerianegara (1967) observed that the soil texture in ebony forests is permeable and that it will tolerate dry conditions, clay texture, and limestone soils. *In situ* conservation of ebony is essential and should be based on adapted habitats.

Range and Importance

Geographically, ebony is distributed between 1^0 – 4^0 S and 119^0 - 120^0 E in mountain ranges running north to south on Sulawesi. Sagala (1994) determined that on Sulawesi there were approximately one million hectares of ebony forest spread mainly across Bolaang, Mongondow, Donggala, Poso, Parigi (Central Sulawesi), Maros, Gowa, Barru, Sidrap, Mamuju, Luwu (South Sulawesi), and

Gorontalo (North Sulawesi) (Santoso, 1997). Ebony is also found in Halmahera and Morotai, North Maluku (Soerianegara 1967).

In the Kalaena Nature Preserve, one of the natural habitats, it was found that ebony stocking had these Importance Values; seedling 35.80 %, sapling 24,94 %, pole 16.62 %, and was tree 5.77 % (Seran *et al.* 1988). Kiding Allo and Sallata (1991) surveyed 127 different sites where the Importance Value of ebony seedlings was highest of all classes (66.57 %).
Considering the above, there is optimism for the future with plenty of ebony regeneration. Without any major disturbances, ebony could naturally regenerate its habitat.

In Situ Conservation

Habitat

Ebony grows and spreads in the Wallacea zone of Sulawesi's forests. Generally, there are three main requirements influencing ebony sites; i.e., water supply (rainfall), temperature (elevation), and soil texture.

One ebony habitat in the Kalaena Nature Preserve demonstrates natural topographic conditions for the species; sloping and hilly, slope=3–60 %; rainfall A type; average temperature=20^0–25^0C; and humidity=85–90%. Soils are brownish yellow podzolic with clay texture. Ground water low on surface and medium in depth, correlated with low organic material. Commonly, ebony is also found in the C or D type rainfall (1500 mm per year); and on soils that are limey, sandy, clay, or rocky. Elevation 400 m asl (Gintings 1990). Data obtained from several ebony habitats in Sulawesi showed that range of elevation=60 m asl (Maros) to 450 m asl (Poso); range of rainfall=1230–2750 mm per year; range of temperature=25.5–27.5^0 C; range of humidity=83–93%; range of light intensity=197–452 luxmeters; range of slope=10- 30^0; soil texture=red yellow to brownish yellow podzolic. Layers of soil were shallow and rocky, effective rooting was 45–60 cm (see Table I).

Table 1. Condition of Ebony Habitat at Several Sites in Sulawesi

Condition	Mangkutana	Mamuju	Maros	Poso
Elevation (m asl)	180	240	60	450
Temperature (°C)	25.5	27.5	26.5	27
Humidity (%	83	95	98	97.5
Light Intensity (lux)	197	452	298	449
Slope (°)	30	25	25	10
Effective Root (cm)	45	55	55	60

Ebony Characteristics

Ebony is a straight, clear-stemmed tree with heights up to 40 meters and diameters of up to 100 cm. It is not unusual for trees to produce clear boles of up to 20 meters. Buttresses about 4 meters high can be found on older ebony trees.

In the forest, ebony trees can easily be recognized by their unique appearance; its bark is black or charcoal and scaly. Its tree form or architecture is triangular. Young trees have very leafy canopies.

Flowering and fruiting season at several habitats demonstrated that flowers will be produced starting in June and will continue until September. Ripe fruits can be taken in November. The color of ebony flowers is white and its sepal color is hairy brown. The color of unripe fruit is green and ripe fruit is yellowish green. Flesh of the fruit is soft, sweet, and aromatic. There are usually 3–5 brown seeds in each fruit. There are 5–10 fruits on one stem. It is important to realize that not all ebony trees will produce fruit; only wide-canopied trees will produce fruits.

***In Situ* Conservation**

Natural Seedlings

Ripe fruits will fall onto the forest floor where microclimatic conditions under ebony trees are conducive to germination. In addition, these conditions also favor the development of the fungus *Peniulliopsis clavariaeformis.* It is pathogenic on ebony seedlings. A month after seeds germinate, a great number of infected seedlings will die.

Seran *et al.* (1990) found that canopy cover and slope did not influence one year-old ebony seedlings. Under these conditions of reduced light, growth rate was 4.79 cm in height and 0.16 cm in diameter. Larger canopy gaps and greater light intensity would not increase seedling growth (Al Rasyid 2001). However, Sidiyasa (1988) and Sallata *et al.* (1988) observed that under more open canopies there were more seedlings.

Association with Other Species

Environmental factors influence species colonization, survival, and growth. Based on floral analyses of several ebony habitats, the species has a number of associates on different habitats. Data obtained from five ebony habitats in South and Central Sulawesi showed that of 42 species associates representing 23 families, the Rubiaceae family is the most frequently seen on ebony habitats. In Luwu and Maros Regency, ebony habitats were dominated by ground flora, i.e., palm trees and forest areca nut. In this case, these other species will have an effect on microclimatic conditions on the ebony sites.

Development Habitat

One of the conservation techniques often attempted for endangered species is to establish and manage conservation area(s). Nowadays, ebony develops in remote forests and highly protected conservation areas such the Kalaena Nature Reserve and the Faruhumpenai Nature Reserve in the Luwu Regency. In this area, there are three species of Diospyros: *Diospyros borneensis, D. buxifolia,* and *D. celebica.* Seran *et al.* (1988) determined *D. celebica* was the most dominant *Diospyros.* It showed that without any disturbance, this ebony might be well developed in its natural habitat.

Ebony stands in forest will give an impression that natural seedlings are plentiful in the fruiting season. Nevertheless, this reproduction will decline as the stand matures to sapling and pole stages. Silvicultural techniques are needed to maintain healthy young stands.

Kiding Allo and Sallata (1991) reported that to conserve natural seedlings, when they are two centimeters high they should be removed to the nursery. Larger seedlings are not worth saving. In the nursery, seedlings should be covered by plastic.

Seran *et al.* (1992) suggested that the canopy should be opened to 80% to favor diameter and height growth of seedlings. Horizontal canopy opening could increase height of seedlings growth to 9.6 cm per ten months, and vertically opening canopies yielded seedlings of 8.1 cm in ten months.

Conclusion and Suggestion

1. Ebony (*Diospyros celebica* Bakh.) natural seedlings are generally adequate on most sites. For example, at the Luwu habitat, the Importance Value of seedlings was 35.8%, for saplings it was 24.9%, and for poles it was 16.6%. The lowest IV was that of trees (5.8%).
2. Silvicultural actions such as rotation in nursery improved diameter and height growth. The growth rate of year-old ebony seedlings was 4.8 cm and diameter 0.16 cm.
3. Generally, common habitats at four location showed that average elevation is 230 m asl, temperature 26.6^{0}C, humidity 93.4%, light 349 luxmeter, slope 22^0, and soil texture yellow-red podzolic.

4. The Rubiaceae family was the most frequent associate of ebony in several habitats.
5. It is suggested that to obtain quality seed, proper methods of collection should be known. It is important to protect seed and seedlings from the fungus *Peniulliopsis clavariaeeformis.*

Figure 1. Nutrient Content in Ebony Habitat in Several Locations

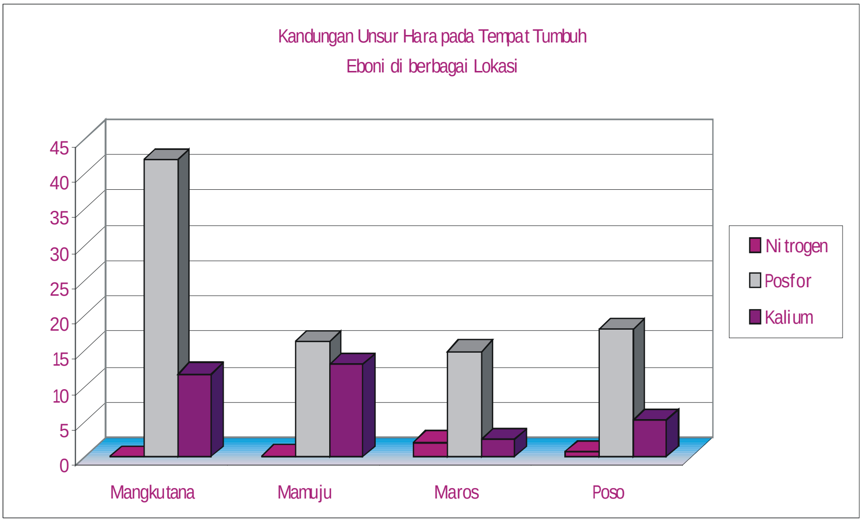

Figure 2. Nutrient Content in Ebony Habitat in Several Locations

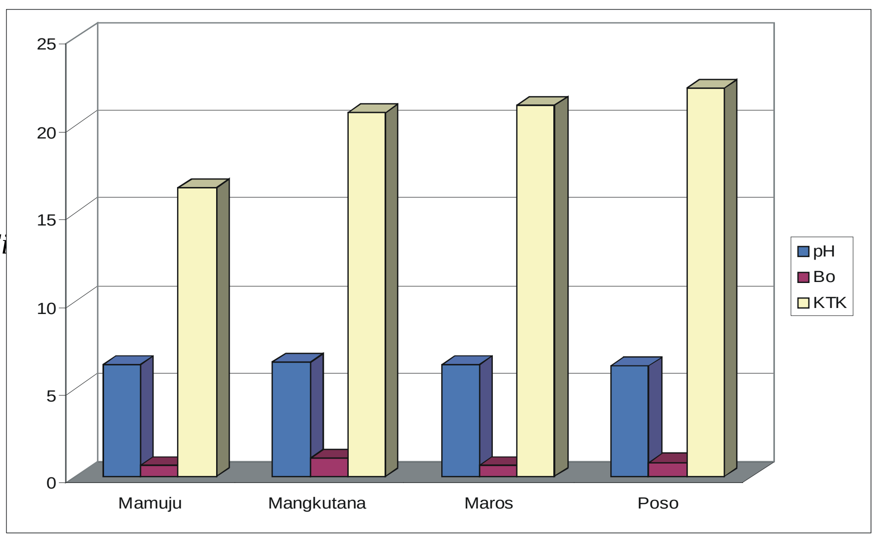

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Figure 4. Abundance Ebony Nature Seedlings Under Mother Tree

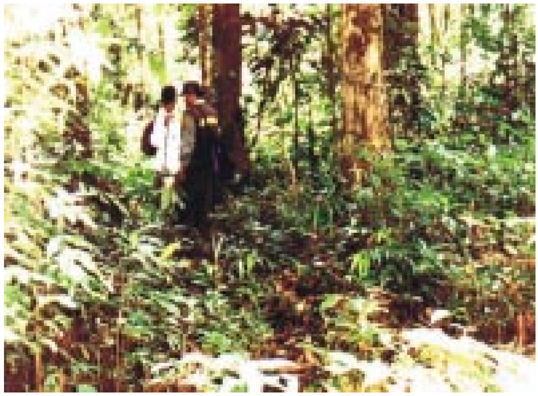

Figure 5. Straight Ebony Tree (Height is able to obtain 40 m high)

Figure 6. Shape of Ebony Flower

Figure 7. Shallow Soil Layer of Ebony

Figure 8.

Ebony Root Shape (less than 30 cm long)

Shallow and Rocky Soil Layer of Ebony Habitat at Camba District, Maros Regency

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International Conference on *ex situ* and *in situ* Conservation of Commercial Tropical Trees Yogyakarta - Indonesia, 11-13 June 2001

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