### **FRIM PROCEEDINGS NO. 21**

## PROCEEDINGS OF REGIONAL WEBINAR ON *EX-SITU* CONSERVATION AND CARBON SEQUESTRATION POTENTIAL OF RED LIST TREE SPECIES

## 20 - 21 October 2021 Kuala Lumpur

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### **Editors**:

Jeyanny Vijayanathan Ho Wai Mun Rozita Ahmad Nor Hasnida Hassan Farah Fazwa Md Ariff Tang Lai Kuen Noor Shahirah Mohd Ibrahim Mohd Zaki Abdullah









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#### PREFACE

The declining biodiversity of flora and fauna worldwide has prompted the commencement of the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species, or the IUCN Red List in 1964, to further assess the status of these plants and animals in terms of size and stability of a species' populations and habitat. The body provides us with global information that are used by policy-makers, academicians, NGOs and the public to understand the risks of extinction through various categories from 'Least Concern' to 'Critically Endangered' species. This year, FRIM with the support from ASEAN-ROK Forest Cooperation Project (AFoCo) through the project on "Domestication of endangered, endemic and threatened plant species in disturbed terrestrial ecosystems in Malaysia and Thailand" is organising a Regional Webinar themed "*Ex-situ* Conservation and Carbon Sequestration Potential of Red List Tree Species" from 20 - 21 October 2021.

Overwhelming oral and poster presentations have been received deliberating on current issues on *ex-situ* conservation, production of quality planting materials, plantation development and carbon sequestration potential of Red List species. This proceeding includes 3 keynote papers from distinguished international and local speakers, 27 oral and 8 poster papers to be presented in this webinar. I would like to express my sincere gratitude to all the reviewers for their time and efforts. The quality of these papers is a tribute to the authors and also to the reviewers who have guided the necessary improvements. It is envisaged that this publication will serve as useful references for all the stakeholders, especially for young and aspiring scientists, including graduate and post graduate students.

On behalf of the Scientific and Technical Committee, I wish to thank all presenters for sharing their findings. I hope this conference will succeed in creating an appropriate forum to bring together scientists, academicians, agronomists, managers and also policy-makers to share knowledge and information, to discuss and exchange their views and experiences in the quest to educate and inspire awareness, formulate policies and facilitate on-going *exsitu* conservation, quantification of carbon sequestration potential and plantation development strategies of IUCN Red List Threatened Species.

DR. JEYANNY VIJAYANATHAN Chief Editor Scientific Committee Regional Webinar on "*Ex-situ* Conservation and Carbon Sequestration Potential of Red List Tree Species

19 October 2021

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## AFoCO restoration and reforestation initiatives and their contributions to *ex-situ* conservation and climate change adaptation

Calderon RL

Secretariat for Asian Forest Cooperation Organization (AFoCO), Seoul, Republic of Korea Corresponding author's email: contact@afocosec.org / soozin.ryang@afocosec.org

The Asian Forest Cooperation Organization (AFoCO) is a treaty-based intergovernmental organization aiming to strengthen forest cooperation by transforming proven technologies and policies into concrete actions in the context of sustainable forest management (SFM) to address the impacts of climate change. Since the initial phase, AFoCO has been committed to facilitating the transfer and translation of best policies and experiences into site-specific actions where these are most needed. AFoCO promotes and undertakes action-oriented forest cooperation programmes on SFM, biodiversity conservation, maintenance and enhancement of ecosystem services, as well as reforestation and forest rehabilitation; climate change mitigation and adaptation activities and supporting REDD+ initiatives; and the reduction of deforestation, forest degradation, desertification, land degradation, and mitigation of the impacts of forest-related disasters.

Through its member-driven approaches, 26 various contextualized projects have been developed and implemented to achieve its mission. In particular, three projects showed AFoCO's ex-situ interventions and the perceived impacts in climate change adaptation. Firstly, a project entitled "Establishment of Forest Genetics Research Center for Restoration of Major Species in Cambodia" which demonstrates that *ex-situ* conservation is closely related to climate change adaptation by contributing to SFM and biodiversity. An 18 ha demonstration forest site of the project presents its potential to be utilised as a reforestation living lab for addressing the negative impacts of climate change in forests and the forestry sector. There is a strong demand from AFoCO Member Countries to improve on the operation of clonal/seedling seed orchards with long-term tree breeding programmes. For sustainability, it is critical to establish a systematic seed and seedling supply system for the roadmap to forest genetic resource management. Likewise, more comprehensive professional human resources is needed not only for tree genetic resource management but also for forest disease and pest control, soil analysis, laboratory works, etc. Secondly, a project entitled "Domestication of Endangered, Endemic, and Threatened Plan Species (EETS) in Disturbed Terrestrial Ecosystems in Malaysia and Thailand" is remarkable with the restoration of abandoned areas using selected EETS that indicating the possibilities of replicating these interventions under similar conditions. It is further expected to become a creative and scientific living lab for germplasm contributing to climate change adaptation (CCA). For upscaling these works at the regional level, the EETS planted needs viewed as a deliberation in biodiversity conservation rather than being regarded as a plantation project. In this regard, cost-benefit analysis on biodiversity conservation may be a recommendable action to provide a more proper valuation assessment of the project. The third project entitled "Research on Forest Enrichment Using High-Value Native Species in Hoa Binh Province, Viet Nam" has recently been launched. Its 2 ha demonstration site in a natural forest is expected to be utilised as a living labon community resilience through SFM by using high-value native species. Assessment on community resilience through SFM may include psychological wellbeing indicators, a practical alternative to quantify the value of forests.

## *Ex-situ* conservation of endemic, endangered and threatened tree species in an ex-tin mine - An experience of planting and tending practices

#### Ang LH

Head, Forest Plantation Programme, Forest Research Institute Malaysia (FRIM), 52109 Kepong Selangor

 $Corresponding\ author's\ email:\ anglh@frim.gov.my,\ tree friend 2023@gmail.com$ 

The mixed forest stands grown on a 121.4 ha ex-tin mine in Tin Tailings Afforestation Center (TTAC) is a model plot for rehabilitation of ex-tin mines. The TTAC has three distinct soil properties and classified as sand, slime and sandy slime tailings. Greening technologies developed for each type of tailings were recorded. The greened ex-tin mine is further enriched with endemic, endangered and threatened tree species (EETS) after the site qualities are improved. The success of the *ex-situ* conservation of the EETS in TTAC outlines three important principles in *ex-situ* conservation of EETS for similar site properties in other terrestrial degraded sites. Currently degraded lands that are available for *ex-situ* conservation of EETS could be classified as poor, medium and good site according to their site properties. The three principles could be applied through adoption and adaptation for *ex-situ* conservation of EETS on the three sites.

**1.0 Introduction.** The successful establishment of man-made mixed forest stands in Tin Tailings Afforestation Center (TTAC) is a model for rehabilitation of ex-tin mines in the tropics (Ang et al. 2014). Ex-tin mine is an impoverished site for tree growing. The poor site properties of the denudedexmine are limiting factors for growing trees. The limiting factors for growing rainforest tree species especially the endemic, endangered and threatened tree species (EETS) for reclamation and rehabilitation efforts on ex-tin mine include the poor soil properties and adverse microclimate (Ang, 1994). The planting technologies developed for successfully greened TTAC can be adopted, and/or, adapted accordingly to create a suitable environment for tree growing at different sites (Ang et al. 1999). This paper aims to highlight three main inter-connecting factors of growing EETS for ex-situ conservation on different site properties include [I] Know the site properties and growing needs of EETS [II] Develop suitable site preparation methods, and lastly [III] Engage good planting and tending practices.

2.0 Know the site properties and growing needs of EETS. Acquiring baseline information is necessary prior to create a suitable microclimate for the planting. The background information includes characterization of site properties such as soil, water, microclimate and existing vegetation (Ang et al. 1994). After having the reliable baseline information of the site properties and the knowledge of the physiological traits of EETS that are to be introduced, careful approach of site preparation aims to ameliorate the site properties for growing EETS are to be developed (Maruyama et al. 1997). The scientifically sound site preparation (Ang et al. 2014; Ang et al. 2018) will ensure sustained growth of EETS (Maruyama et al. 1997). This involves also the timing to introduce EETS after modification of the microclimate (Ang et al. 1999). Suitable EETS are to be introduced to the improved site. Generally, three types of site properties for growing EETS can be classified. These are poor, medium and good sites. 2.1 The poor site includes terrestrial ecosystems that are severely degraded and the original soil composition was destroyed. The degraded sites are often barren and have adverse site properties for the natural regeneration to occur. These poor sites are lack of native species and have low tree diversity index. 2.2. The medium site has natural succession but the soil properties were not altered in the process of degradation. 2.3 Lastly, the good site includes less than 50% disturbance in natural vegetation. The opening created in the site is not more than 0.5 ha and the soil properties are not disturbed.

**3.0 Develop suitable site preparation.** The site preparation is to be tailored made for the three classes of the site properties namely poor, medium and good sites. Suitable site preparation methods are to be developed in a cost-effective manner. Hence, unnecessary inputs are to be avoided for site improvement to have suitable site preparation and its effectiveness in creating a suitable growing environment for EETS. Site improvement on each class of site properties is to be developed accordingly. 3.1 Site preparation for poor sites such as ex-mines, BRIS (Beaches Ridges interspersed with Swales) and decking site will normally involving high inputs such as loosening of soils, inputs of good mineral soils, and construction of irrigation or drainage for optimum water management. Introducing the EETS may need to wait for a decade or two until the improved site has achieved the suitable site properties for their establishment and growth. 3.2 Site preparation for planting EETS on the medium site is less intensive and often the EETS can be directly planted without much improvement of the site properties. These are the sites like secondary forest, abandoned farmland, and slope (>35%) in agricultural plantations. The site preparation can be line planting or gap planting for secondary forest and abandoned farmland. The slope planting will be more costly as it involves machinery to create suitable terrace. The design of the terrace will promote conservation of water and reduction of surface run-off. The loosening of soils in the medium site would need to be carried out but only to the depth of 0.5 m after the planting lines or gap were prepared. 3.3 Site preparation for planting EETS on the good sites involves no mechanization except for making the access roads to the plots. These sites are logged-over forests or fragmented forests in the alienated land excluding decking sites and forest roads or forest plantations that are to be converted as ex-situ conservation sites. Line planting should be prepared following the existing logging trails. Small backhoe is to be employed to clear the natural regeneration so that a clear planting line can be established on the logging trails. If the logged-over forest was left for more than three years, then it is good that vegetation survey is to be carried out. This will help to introduce EETS that are not found in the natural regeneration process.

4.0 Good planting and tending practices. Best planting practice for all the sites involves establishment of suitable planting lines according to the slope and also the direction of the sun. Planting line is preferably prepared across the gradient of the slope and facing the east-west direction. This is to reduce surface run-off and also tapping maximum sunlight in the morning. The planting points should be marked at 4 m apart within the row and 5 m apart between rows for 4 x 5 m spacing. The size of the planting hole is proportion to the size of the potting bag. The size of planting hole should be dug at the ratio of 3:1 to the size of the potting bag. The planting site must not be mechanically impeded more than 1.5 MPa. The planting hole preparation must also take into considerations of the standing water table level and the soil composition. The planting hole is to be dug two days before planting. Soil conditioner will be applied to ameliorate the extreme acidity of the planting site. The amount of soil conditioner used would depend on the soil acidity. The planting stock would need to be site conditioned for a month to three months before planting. However, the planting stock can be directly planted if the planting is carried out under the nurse stand, and/or, watering can be carried out. After the planting, staking is to be done to ensure apical growth especially for the young seedlings and sapling that have top height less than 2 m. The success of growing suitable EETS at improved planting sites will be materialized only if suitable tending has been practiced in the first five years after planting. Tending practice aims to provide a conducive growing environments for the EETS to achieve sustainable growth. Sustainable growth of the newlyplanted EETS at a site could only be achieved when there is sufficient available soil water during thefirst week after planting and also in the subsequent dry periods. In addition, suitable size of opening for receiving optimum sun light for photosynthesis must be provided. Other tending activities include to provide sufficient available soil space for root development, provide balance nutrients for healthy growth and administer a good pests and diseases control. 4.1 Good tending practice for poor site. After site improvement, the newly planted EETS would require 7 days of watering if there is no rain, especially for the open planting. Watering would need to be carried out after two dry days and the amount of water needed would be about 20-30 L per planting point. The water source for watering of the EETS is preferably within 1 km. In a 3.6 ha plot, using a 7-Horsepower water pump will have enough power to deliver water through synthetic rubber hoses of 2" diameter to each planting point.

Soil loosening to a depth of 5 to 10 cm at 50 to 75 cm radius from the collar diameter needs to be carried out monthly in the first three years after planting. A good loosening of soils will create a circular ring with a depth of 10-15 cm at the edge of radius. This is to improve the infiltration capacity of the soil and prevent water loss from surface runoff during heavy rains. In addition it also removes the suppression of weeds and climbers. However, when the surface roots of the tree has emerged in the loosened soils. Loosening of soils should not be practised anymore as it will destroy the surface roots. Blanket weeding would need to be carried out at one to three-month intervals depending on the openness of the planting site and the regeneration ability of the weeds. Normally, grass cutting is carried out and followed by a round of weedicide application for Imperata cylindrica and Dicranopteris linearis infestation sites. The weedicide application is best to be carried out 15 days after mechanical weeding. Environmental friendly systemic weedicide should be used. Fertilizer application is also needed for all sites. However, fertilizer regime is different for poor, medium and good sites (Ang et al. 2006). A formula of a mixed organic and inorganic fertilizers at a ratio (2.7:1) by weight is used for growing EETS on ex-tin mines. The organic fertilizer is chicken manure at 80% maturity and fortified with trace elements. Whereas, the inorganic NPK compound fertilizer (16:16:16) with trace elements are to be mixed with the organic fertilizer. The well-mixed fertilizers are to be applied at the rates of 120 g and 240 g per point to seedlings and saplings, respectively. The frequency of a mixed fertilizer application is at 3 month-interval. Watering should be carried after the fertilizer application if it is without rain. The fertilizer application is best to be carried out a day after heavy rain (precipitation >daily evapotranspiration). The fertilizer must be applied at 5 cm away from the stem collar and evenly distributed within the 50-70 cm radius of the loosened soils. The fertilizer normally will be trapped by the loosened soils but some granules of the fertilizer shall be washed and collected in the circular ring-like depression during heavy rain. This will ensure zero loss of granular fertilizer from surface runoff especially for the planting points on the slope. For the EETS grown on medium and good sites, same fertilizer regime should be used but only until three years after planting. Pests and diseases control. Pests that are common to forest plantation are wildlife such as wild boar and monkey. The destruction caused by wild boar to the newly planted EETS can be as high as 30% mortality within the first year after planting. Subsequently, the wild boar continues to damage saplings by debarking them within the first 10 years after planting. Trees grown to a diameter at breast height > 10 cm will suffer rarely from the attack of the wild boars. Monkey is another pest other than wild boar but they normally only damage the young shoots of the saplings and some seedlings. This has caused retarded growth. Stem borers are also one of the pests but they prefer only certain EETS especially, Aquilaria malaccensis. The stem borers can be controlled by insecticide such as Dominion 2L Imidacloprid which is a systemic insecticide. It kills the borers in all parts of the infested tree by sending the active-compound namely 1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-2 imidazolidinimine through root uptake that eventually distributes to the entire tree. Other diseases are rare for the EETS grown on the improved sites. Normally, insect pests and diseases attack the stressed trees, and seldom on trees grow on medium and good sites.

**5.0 Conclusive Remarks.** A sustainable growth of EETS on all three sites would depend on three important related factors namely good planning, sound technical know-how, and vigilant monitoring of each process involves in the establishment phase. Technical know-how of site preparation or modifying of site properties to create a suitable environment for tree growth is crucial to ensure the success of ex-situ conservation of EETS.

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# Current development in biotechnology for *ex-situ* conservation of Red List tree species

Mohd Zaki A

Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia Corresponding author's email: zaky@frim.gov.my

Biotechnology is simply defined as a technology based on biology, capitalising on cellular and biomolecular processes to help improve the welfare of human beings and their environment. It has been applied in various fields including in agriculture, food processing, medicine, bioremediation and more recently in forestry. Biotechnology, thus, is an indispensable tool that provides solutions to problems by the development of breakthrough technologies or products. Advances in agriculture and forestry through biotechnology are primarily aimed at producing novel capabilities for instance increased yields yet with reduced costs or losses besides improved resilience against pests and diseases as well as climate change. In this context, Forest Research Institute Malaysia (FRIM) has recognised the importance of biotechnology with the inception of the Forestry Biotechnology Division to carry out research and development, commercialisation and innovation in biotechnology for the advancement of the forestry industry across the value chain from seed to plantation. The purpose of this paper is to present development in biotechnology that are currently undertaken by FRIM for conservation, protection and management of germplasm particularly for Red List tree species. Beginning from seed, cryopreservation and artificial seeds are some of the techniques in seed technology being used to preserve tropical species with recalcitrant seeds, sporadic lowering and fruiting, and seeds with no dormancy. These techniques are unfortunately not possible for all timber species, therefore requiring alternatives like tissue culture for mass propagation. The temporary immersion system technology has further accelerated the ability of research facilities in FRIM towards large scale micropropagation of important Red List species like agarwood and commercial species like Eucalyptus sp. which are closely monitored for their performances in trial plots. Since the 10<sup>th</sup> Malaysia Plan in 2010, the Division has been actively collecting Red List forest species throughout the Peninsular Malaysia with particular interest in dipterocarps for ex-situ conservation as gene banks for future exploration. Besides timber species, valuable medicinal herbs have also been collected, selected for desirable phenotypes and chemical constituents, and mass propagated. Partnership with local communities have succeeded in developing the selected cultivars for commercial production generating additional income for the farmers. Another remarkable achievement of the Division is the recent findings in population and ecological genetics in plants that has revealed new perspectives underlying evolution and diversification especially for dipterocarps that are constantly under threat due to over-exploitation, and loss or fragmentation of habitats. Deciphering of dipterocarp genome for the first time has also taken the dipterocarp research to another milestone. In addition, plant DNA profiling and barcoding have elevated R&D in genetics to a new era in Malaysia with the development of capacity in forestry forensics. In summary, biotechnology is a dynamic and emerging tool that is transforming industries and impacting lives. With the support of the Malaysian government, current and future developments in biotechnology are set to catalyse the forestry sector by providing solutions and playing invaluable roles in conservation and sustainable management of forest resources.

#### Reforestation of degraded Kerapah forest - A case study from Brunei Darussalam

Wardah HT<sup>1</sup>, Faizah HM<sup>2</sup>, Hazimah HMD<sup>1</sup>, Salwana MJ<sup>1</sup> & Rahayu SS $^{*1}$ 

<sup>1</sup>Institute for Biodiversity and Environmental Research, Universiti Brunei Darussalam, Jalan Tungku Link, BE 1410, Brunei Darussalam <sup>2</sup>Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, BE 1410, Brunei Darussalam

\*Corresponding author's email: rahayu.sukri@ubd.edu.bn

Bornean heath forests are high conservation value ecosystems that are increasingly facing threats from man-made activities. Kerapah forests are a sub-type of heath forests that are waterlogged and are often regarded as a transition between heath and peat swamp forests. Within Brunei Darussalam, Kerapah forests often exist as isolated patches and degraded Kerapah forests are in need of rehabilitation. This study focused on reforestation of a degraded *Kerapah* forest under the Brunei ASEAN-Korea Environmental Project. Three 15 m x 50 m plots were established in 2015 on a degraded Kerapah forest in the Lumut area, Belait District that was logged over in 2012. Saplings of five native timber tree species (Agathis borneensis, Anisoptera reticulata, Dryobalabonops rappa, Shorea albida and Shorea rubra) were planted. Planted saplings were monitored for their growth and survival over a period of 20 months. Our results showed that D. rappa saplings recorded significantly highest survival and growth rates (Table 1), followed by A. borneensis. Saplings of Anisoptera reticulata recorded good survival rate but slow growth which was contrary to S. albida saplings. We found that S. rubra saplings were not suitable for use as a reforestation species for degraded *Kerapah* forests due to their complete mortality (Figure 1). These findings can be applied for suitable forest management and rehabilitation practices, especially in terms of species selection for planting and maintenance methods, for other degraded Kerapah forests in Brunei Darussalam and within Borneo.

**Table 1:** Mean relative growth rates  $(\pm SE)$  of planted saplings of *Agathis borneensis, Anisoptera reticulata, Dryobalanops rappa* and *Shorea albida* in terms of diameter (RGR<sub>diameter</sub>), height (RGR<sub>height</sub>) and number of leaves (RGR<sub>leaves number</sub>) for a 20-month study period (from mid-June 2015 until mid-January 2017). Different letters represent significant differences in RGR values between species as analysed using one-way ANOVA and Tukey's test. *Shorea rubra* data were excluded because none of the saplings survived after December 2015.

Tree species	RGR <sub>diameter</sub>	RGR <sub>height</sub>	RGR <sub>leaves number</sub>
	$(mm mm^{-1} month^{-1})$	(cm cm <sup>-1</sup> month <sup>-1</sup> )	(unit month <sup>-1</sup> )
Agathis borneensis	$0.108 \pm 0.087$ <sup>a</sup>	$0.108 \pm 0.078$ <sup>a</sup>	$0.158 \pm 0.116^{a}$
Anisoptera reticulata	$0.055 \pm 0.031^{a}$	$0.079 \pm 0.052$ <sup>a</sup>	$0.133 \pm 0.074$ <sup>a</sup>
Dryobalanops rappa	$0.148 \pm 0.026$ <sup>b</sup>	$0.211 \pm 0.048$ <sup>b</sup>	$0.366 \pm 0.060^{b}$
Shorea albida	$0.076 \ \pm 0.039 \ ^a$	$0.101 \ \pm 0.069 \ ^a$	$0.190 \ \pm 0.088 \ ^a$



**Figure 1:** Survival percentage of *Agathis borneensis, Anisoptera reticulata, Dryobalanops rappa* and *Shorea albida* from mid-June 2015 until mid-January 2017 and *Shorea rubra* from mid-June 2015 until mid-December 2016. Values are means  $\pm$  SE, and different letters represent significant differences in mean survival percentage values obtained at the final census in mid-January 2017 as analysed using one-way ANOVA and Tukey's test.

#### **Conserving Indonesian threatened plants: A role of botanic gardens**

Widyatmoko D

Bogor Botanic Garden/Research Center for Plant Conservation, National Agency for Research and Innovation (BRIN) Jl. Ir. H. Juanda No. 13 Bogor 16122 Indonesia. Corresponding author's email: didik\_widyatmoko@yahoo.com

Despite occupying only 1.3% of the world's land surface, Indonesia represents c.10% of the world's flowering plants. The Indonesian archipelago supports 238 species of Dipterocarps (comprising 34% of the world's total), 2,197 species of ferns (21%), c.5,500 species of orchids (20.5%), 477 species of palms (20%) and 159 species of bamboos (13%). Indonesia's palm diversity is the highest in the world of which 53% are endemic. However, at the same time, many Indonesia's plant species face high threats and pressure, and are on the verge of extinction. There is a total of 856 threatened species in this country by 2021 of which members from Dipterocarpaceae, Lauraceae, and Zingiberaceae families become the three largest contributors of the endangerment. Contribution of Indonesia to the world's plant species rarity and extinction reaches nearly 4%. Almost 20% (comprising 166 species) of the Indonesia's threatened plants have been planted and accessible in the Indonesian Botanic Gardens (IBGs). However, this figure is still far from achieving the Target 8 of the GSPC-CBD. Habitat loss, over exploitation, and environmental pollution have become the most serious threats to plant diversity. In Indonesia, botanic garden is one of the best practices in ex-situ conservation in linking conservation to sustainable usage of plant diversity. This paper elaborates the roles of IBGs in conserving and managing Indonesia's threatened, endemic plant species. As the success of any biodiversity conservation and utilization efforts depends on people's concern and awareness, IBGs become very strategic destinations for promoting such efforts as the gardens (Bogor, Cibodas, Purwodadi and Bali) are visited by approximately three million people annually (before the onset of Covid-19). IBGs also have significant roles in plant conservation due to the accommodation of more than 100,880 living plant specimens (mostly native) belonging to nearly 7,000 flowering plant and fern species. Rafflesia horsfieldii, the very rare-endemic parasitic giant flower, has been successfully grown at Bogor Botanic Garden after a long time of trials and treatments making it a prominent ex-situ conservation achievement. Such achievement would also make ex-situ conservation effort meaningful to everyone. In addition, during the last two decades the IBGs have been collecting threatened-endemic species throughout the Indonesian archipelago as well as bringing some of them back to the wild through re-introduction programme.

#### Malaysia Red List: Conservation status of trees in Peninsular Malaysia

Yong WSY\* & Chua LSL

Forest Research Institute Malaysia, 52109 Kepong, Selangor. \*Corresponding author's email: wendy@frim.gov.my

Malaysia is as one of the megadiverse countries in the world with an estimation of 15,000 taxa of vascular plants comprising angiosperms, gymnosperms and pteridophytes. Peninsular Malaysia with an area of 132,000 km<sup>2</sup> has recorded 9,030 vascular plant taxa comprising 248 families and 1,651 genera. Thus, managing such rich natural resources is a challenging task for Malaysia considering the need to balance between resource conservation and socio-economic development. Red List assessment is a very useful tool in priority setting for conservation planning. It measures extinction risk of each taxa to provide baseline information for national priorities setting and conservation planning for both species-based and ecosystem-based conservation approaches. The first national Red List for plants, Peninsular Malaysian Dipterocarpaceae, was published in 2010. Then, the second national Red List, Malaysia Red List: Plants of Peninsular Malaysia, Vol. 1, was published in 2021. This latest publication listed the conservation status for 1,292 indigenous plant taxa including 706 tree taxa from 44 families and 123 genera. Out of these 706 tree taxa assessed, 207 taxa are categorised as threatened of which 39 taxa are listed as Critically Endangered (CR), 68 taxa listed as Endangered (EN) and 100 taxa listed as Vulnerable (VU). To date, at least 12 taxa of the 207 threatened tree taxa are conserved in High Conservation Value Forest (HCVF) established by state forestry department and 72 taxa are represented in *ex-situ* collection such as botanical gardens and arboreta. In-situ conservation is recognised as the most effective conservation measure for threatened tree conservation. Ex-situ conservation measure, such as establishment of ex-situ germplasm collection, complements in-situ conservation measures to ensure long-term success of conservationefforts for threatened species.



Figure 1: Distribution of IUCN categories in ten largest tree families assessed (Yong et al. 2021).

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# *Ex-situ* conservation of teak and carbon sequestration of different forest types in Myanmar

Thaung NO<sup>\*1</sup>, Aung ZM<sup>2</sup>, Aung MM<sup>2</sup> & Aye YY<sup>2</sup> <sup>1</sup> Forest Department of Myanmar, <sup>2</sup>Forest Research Institute, Myanmar \*Corresponding author's email: tnoo71@gmail.com

The Republic of the Union of Myanmar is geographically located in Southeast Asia between latitudes 9°32'N and 28°31'N and longitudes 92°10'E and 101°11'E. The total area of Myanmar is 676,577 km<sup>2</sup>. Myanmar still has forest cover of about 42.19% of the country's total land area (FRA, 2020). Total population of Myanmar has reached 53.9 million in 2015 and its growth rate is 1.52%. As a matter of fact, over 70% of the country's total populations are rural and dependent on forest resources for basic needs such as food, fodder, fuel, and shelter. Forest areas under the management of Forest Department can be classified into two categories, Permanent Forest Estate- PFE (reserved forests and protected public forests) and protected areas system (PAS). There are 42 globally threatened tree species in Myanmar and in-situ conservation of rare and threatened tree species are conducted especially in PAS. Among the 42 globally threatened tree species, 16 tree species on the IUCN Red List are assessed and listed as Critically Endangered (CR), 16 tree species as Endangered (EN), 10 tree species as Vulnerable (VU) (NBSAP-2015). The commercially important tree species are mainly selected for *ex-situ* conservation which may conserve for long term repository of genetic material and further tree improvement programme. Forest Department has been conducting the genetic conservation of some valuable species such as Tectona grandis (Teak), Pterocarpus macrocarpus (Padauk), Xylia xylocarpa (Pyinkado), some Dipterocarpus species, Pinus spp. (Htinyu), Gmelina arborea (Yemane) and some mangrove species. Total teak forest estateacross the world is about 34.79 million ha including 27.9 million ha of natural teak forest and the rest are planted forests. Myanmar has the largest area of natural teak forest of about 16.5 million ha (Gvi & Tint 1998). Management of teak forest in Myanmar uses Myanmar Selection System (MSS), which has been the principal management system applied for managing sustainable harvest and use of plant resources from natural forests with 30 annual coupes and a 30-year felling cycle since 1856. Due to the felling of desirable phenotype, genetic diversity of teak has degraded. In this perspective, the Modified Myanmar Selection System (MMSS) integrates modifications not only on yield regulation but also conservation of their genetics. Based on geographic genetic structure of teak for conservation of the teak genetic materials, some tree improvement programmes are being conducted such as mapping tree breeding zone, plus tree selection, establishment of Clonal Seed Orchards, Provenance Trials and Teak Hedge Gardens in different geographic genetic zones. Nowadays, climate change is a global issue and forests play multiple roles in climate change mitigation because of their ability to absorb and store large quantities of atmospheric carbon through the process of photosynthesis. On the contrary, carbon emissions occur during deforestation or forest degradation. In order to contribute to the REDD+ discussion by constructing baseline carbon emission estimates from deforestation, carbon stocks in different forest types are estimated. Forest Department has been conducting *in-situ* and *ex-situ* conservation for sustainable forest management and climate change mitigation. 42 globally threatened tree species are assessed in Myanmar, *ex-situ* conservation of these species are limited. Currently, Forest Department has been conducting *ex-situ* conservation of some commercial tree species and *in-situ* conservation of threatened tree species in natural forests.

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#### **Corporate-led conservation program for Philippine Red List tree species**

Malabrigo Jr. PL\*<sup>1,2,3,4,5</sup>, Umali AGA<sup>1,4</sup>, Tobias AB<sup>1,4,6</sup>, Eduarte GT<sup>4,6</sup>, Gibe RC<sup>5</sup>, De Guzman Jr. EG<sup>5</sup>, Solatre JS<sup>5</sup>, Barcena AV<sup>5</sup> & Barstow M<sup>7</sup>

<sup>1</sup>Department of Forest Biological Sciences, College of Forestry and Natural Resources, University of the Philippines Los Baños, Laguna, 4031, Philippines

<sup>2</sup>Museum of Natural History, University of the Philippines Los Baños, Laguna, 4031, Philippines

<sup>3</sup>Land Grant Management Office, University of the Philippines Los Baños, Laguna, 4031,

Philippines

<sup>4</sup>Pro-Seeds Development Association Inc., Los Baños, Laguna, 4031, Philippines <sup>5</sup>Energy Development Corporation, Pasig City 1605, Philippines

<sup>6</sup>Graduate School, University of the Philippines Los Baños, Laguna, 4031, Philippines

<sup>7</sup>Botanic Gardens Conservation International, Richmond TW9 3BW, United Kingdom

\*Corresponding author's email: plmalabrigo@up.edu.ph

The Philippines is one of the 17 mega-diverse countries in the world and is considered as one of the most important countries for conserving diversity of life on earth. Its exceptional floristic richness makes it a land of innumerable tree species of economic and ecological significance. However, with only 7% of its original forest cover remaining, Philippine trees as well as the associated plants in the forests, are also the most threatened in the planet. The most updated Philippine Red List for Plants (2017) included 984 threatened taxa including 153 premium tree species, more than half of these are endemic to the country. Recognizing the urgency to save the remaining populations of these threatened trees, the Energy Development Corporation (EDC), the leading renewable energy company in the country, initiated a noble tree conservation programme called BINHI (Filipino term for seedling). It is the country's first comprehensive private sector-led conservation programme that focuses on threatened trees. Using science-based criteria primarily considering the ecological and economic importance, EDC came up with 96 priority threatened trees for conservation. BINHI followed five key steps in trying to prevent the extinction of Philippine threatened trees namely: 1) tree inventory and documentation; 2) propagation; 3) establishment of future mother trees; 4) protection of natural habitat; and 5) advocacy and awareness campaign. Since the program's implementation in 2008, EDC managed to discover and protect several new populations of these threatened trees (Malabrigo et al. 2017; Malabrigo et al. 2016). The company's state of the art vegetative material recovery facility has already developed protocols for the mass propagation of species through cuttings with survival rate ranging from 85-100%. More importantly, with the help of different organisations (i.e. academic institutions, government agencies, civil society organisations, private sectors), BINHI was able to mainstream the planting of the previously unpopular threatened trees to urban landscaping. To date, with the help of 195 partner organisations (about 80% are academic institutions), EDC has planted about 10,000 threatened trees all over the 16 regions in the Philippines. Many of the planted seedlings among EDC's partner organisations are now source of seeds and other planting materials for further mass propagation. All botanical information gathered in the course of programme implementation, including new distributions, phenology, and propagation technology were published in a book "BINHI Tree for the Future" to disseminate the project learning and experiences to a larger public. Apart from ex-situ conservation, BINHI also focuses on the survey and protection of the remaining natural populations of the threatened species. BINHI was able to confirm the existence of all the 96 priority species through its gargantuan exploration efforts. Some of the priority threatened trees were known only from the type locality stated in the original publication of the species and were never documented for more than a century. Through its awareness and information campaign, as well as close collaboration with the local stakeholders with jurisdiction over the threatened tree populations, a number of local policies and provincial ordinances were already in place to strictly protect the fragile populations of the red list trees.

With its long record of comprehensive population survey of the priority threatened species, many of which are endemic trees, EDC also became the first and only organisation in the Philippines accredited to conduct the IUCN red list assessment. Recognising the relentless efforts of EDC in the population inventory and conservation of Philippine red list trees, Botanic Gardens Conservation International (BGCI), the secretariat and coordinator of IUCN Global Tree Assessment (GTA), initiated a partnership with EDC for the red listing of all the Philippine endemic trees. The partnership officially started through the signing of the Memorandum of Agreement between EDC and BGCI on 23 May 2019. To date, the GTA Philippines team, guided by IUCN Red List criteria, have already completed the red list assessment of 800 Philippine endemic trees. Remarkably, our red list assessment for the 800 endemic trees showed significant differences with the Philippine red list. For instance, we assessed 229 trees as critically endangered while only 49 were assessed the same in the Philippine Red List. Even more surprising, 190 of the critically endangered species were categorized as other wildlife species (equivalent to least concern) in the Philippine red list. It is important to note that the Philippine Red List is not just a list of species' conservation status, but it is also a national policy issued as an Administrative Order by the Department of Environment and Natural Resources (DAO 2017-11), wherein all species categorised as threatened are ban for collection and/or trade. Our assessment which is based on the current data and population surveys of Philippine endemic trees suggests the necessity for the scrupulous revision of the Philippine Red List to protect the true threatened species in the country.

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#### The Species Recovery Programme under Singapore's City in Nature Vision

#### Hassan I

#### National Parks Board, Singapore Corresponding author's email: hassan\_ibrahim@nparks.gov.sg

Despite being highly urbanized, Singapore is home to a wide range of biodiversity. A Species Recovery Programme was introduced in 2015 by the National Parks Board (NParks) which outlines plans to coordinate, strengthen and intensify the biodiversity conservation efforts in Singapore. It aims to conserve native flora and fauna by targeting endemic, rare or threatened native species in Singapore through reintroduction, habitat enhancement and protection efforts. The main aim of the recovery programme is to increase populations of these targeted species amidst the present environmental and developmental changes occurring in Singapore. It also garners support and help of volunteers including academia and the nature community. The Species Recovery Programme is part of the Nature Conservation Masterplan, which charts the biodiversity conservation strategies in the medium to long term. This masterplan is an integral part of Singapore's City in Nature vision, building on what Singapore has achieved as a biophilic City in a Garden. This vision is one of the pillars under the Singapore Green Plan 2030, to create a sustainable and liveable city for the residents. Enhancing biodiversity and infusing greenery into the urban planning and development can lead to ecological, climate and social resilience for Singapore.

#### Ex-situ conservation in disturbed terrestrial ecosystems in Thailand

Boonsermsuk S

Royal Forest Department, Bangkok Thailand 10900 Corresponding author's email:sapolboonsermsuk@gmail.com

The project "Domestication of Endangered, Endemic and Threatened Plant Species in Disturbed Terrestrial Ecosystems in Malaysia and Thailand" has been conducted since 2016. Thailand, a total of 13 endangered, endemic and threatened plant species (EETS) were planted, namely Cotylelobium lanceolatum, Dalbergia cochinchinensis, Dalbergia oliveri, Aquilaria crassna, Neobalanocarpus heimii, Vatica diospyroides, Dalbergia cultrata, Magnolia rajaniana, Parkia sumatrana, Dillenia ovata, Magnolia sirindhorniae, Millettia leucantha and Aquilaria malaccensis. There are 2 sites for the project in Thailand. One of the sites is located in Mae Moh Coal Mine, at Lampang province in the northern part of Thailand which is an arid area. The second site is located at Takuapa, Phang Nga province in the southern part of Thailand which is a humid area where tin mining was carried out and has been restored for a while. In each site, there are two planting plots. In the first planting plot of Mae Moh, the height growth of six species planted is shown in Figure 1 while the height growth and survival of trees in second plot with 12 species is shown in Figure 2. In Takuapa, the height growth of four species planted in each of the first and second planting plots are shown in Figures 3 and 4. In conclusion, it was found that M. rajaniana and P. sumatrana adapted better to the high humidity areas in the south compared to arid areas in the north with low survival rates. Cotylelobium lanceolatum and N. heimii cannot adapt to the conditions of arid areas while V. diospyroides is a plant that can adapt to both conditions. Dalbergia cochinchinensis is a species that was found to grow well in drought. It was found that the selection of suitable EETS to the climate of the planting site is very important. Native species or species that grows in similar climatic conditions should be screened and selected first. Nurse tree species such as banana that provides shade and humidity to the EETS should be planted in advance. Based on unexpected changes in climate conditions such as extreme droughts, it is recommended that there should be enough water supply, covering of ground area above roots with rice straw to maintain moisture, and using shading nets to reduce the intensity of the light. While in the areas at risk of flooding, the area should be adjusted higher and installation of drainage channels to divert flow of rainwater.



Figure 1: Height growth of six species in first plot at Mae Moh



Figure 2: Height growth and survival of trees in second plot at Mae Moh (until June 2021)



Figure 4: Height growth of second plot in Takuapa

#### The conservation journey of Sabah's endemic Dipterocarps: Dipterocarpus ochraceus

Richard M<sup>1</sup>, Khoo E<sup>\*1</sup>, John S<sup>1</sup>, Reuben N<sup>1</sup>, Joan P<sup>1</sup> & Colin M<sup>2</sup>

<sup>1</sup>Forest Research Centre, Sabah Forestry Department, P.O. Box 1407, 90715 Sandakan, Sabah,

Malaysia

<sup>2</sup>Faculty of Tropical Forestry, Universiti Malaysia Sabah, Kota Kinabalu 88400, Sabah, Malaysia \*Corresponding author's email: eyen.khoo@sabah.gov.my

Currently, there are a total of 181 species of Dipterocarps in Sabah that spans across nine genera, of which six species are Sabah endemic. Many of these species had their conservation status assessed in 1998 based on data collected from the 1930s to late 1990s. In 2009, an effort to reassess the IUCN Red List status and distribution pattern of these Dipterocarps was initiated. Prime on the list were six Sabah endemics and one of them was *Dipterocarpus ochraceus* (Keruing Ranau). Due to the rarity of collections, certain morphological features remained to be unknown, such as the inflorescence and flowers of this species. Based on herbarium records, the last collection was in the 1960s from two sites in the Ranau District. Since then, the species was predicted to have lost 68% of its original habitat due to land use changes and natural disasters. The reassessment initiative subsequently snowballed into a journey of niche models generation, extensive surveys, recording of new populations, genetic and seedling recruitment studies, establishment of *ex-situ* collections and most important of all fostering long term interdepartmental and institutional collaborative efforts.

## Conservation of *Abies koreana*, an endangered tree species declining due to climate change in Korea

Lim JH\*1 & Lim HY2

<sup>1</sup> Forest Ecology Division, National Institute of Forest Science (NiFoS), Republic of Korea
 <sup>2</sup> Forest Bioinformation Division, National Institute of Forest Science (NiFoS), Republic of Korea
 \*Corresponding author's email: limjh@korea.kr

Korean fir (Abies koreana) is endemic in several high mountains in southern part of Korea and valuable because of good tree shape, cone color and so on. In Korea, mass mortality of evergreen conifers including pines in lowlands, and firs and spruces in high altitude have occurred since the late 1990s. Mass mortality and dieback occurred due to combined factors of seasonal climate, trend of climate change, phenology and mycorrhizal fungi. Winter is cold and dry until spring, but the temperature is increasing faster than other seasons with no increase of precipitation. The events of overlapping of warm temperature and severe drought in winter and early spring season have been more frequent and severe particularly in 1998, 2007, 2009 and 2014. As evergreen conifers have leaves in winter, they consume carbohydrates during respiration and the amount increases with increasing temperature. However, photosynthesis decreases due to drought and mycorrhizal fungi are not active until early spring. Our temperature test on 10 ectomycorrhizal fungi revealed their mycelial growth is initiated at 8°C and above. Korea Forest Service surveyed the status of seven endangered subalpine evergreen needle-leaved tree species including Korean fir in 2017-2018 and conducted monitoring every two years. It is distributed mainly in Mount Jiri, Mount Halla and Mount Dukyu, and small populations in six other mountains. The area is about 6,939 ha and estimated number of trees is about 2.65 million. Tree decline index (opposite to tree health) of Korean fir was 29% and increased to 33% after two years. Regeneration of Korean firs is very poor and the tree size structure is a bell-shaped skewed to the left, not a reverse J-shaped. In order to conserve the Korean fir populations, we are trying to do both *ex-situ* and *in-situ* conservation activities. For *in-situ* conservation, we are looking for 1) how to help natural regeneration, and 2) how to restock the populations to enhance genetic diversity and maintain genetic identity. To increase natural regeneration, we are conducting tests on sites to determine suitable time and canopy size to provide light considering reducing shade tolerance by tree age or size. Enhancement of genetic diversity is important to maintaining populations and resistance against uncertain future stresses on trees including insects, pests and diseases. When we plant such endangered species to increase populations, we have to collect seeds considering genetic diversity and identity. For Korean fir, we collect seeds separately between Mt. Halla in Jeju Island and inland populations because the genetic distance is high between the two. In Mt. Halla, we have to collect seeds from trees at least 10 m apart, and 30 m apart in Mt. Jiri to increase genetic diversity. We successfully restocked 1,350 trees in Mt. Geamwon as a pioneer project.



Figure 1: Distribution of Korean fir populations.



Figure 2: Successfully restocked Korean fir trees in Mt. Geumwon considering genetic diversity and identity.

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#### Conservation management of an agarwood producing and CITES listed species Aquilaria malaccensis (Thymelaeaceae)

Lee SL\*, Nurul Farhanah Z, Lee CT, Tnah LH, Ng CH, Ng KKS, Lau KH & Chua LSL Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia \*Corresponding author's email: leesl@frim.gov.my

*Aquilaria malaccensis* (Thymelaeaceae) is the main source of high-grade agarwood in Southeast Asia. Aggressive collections and trade activities over the past decades have put great pressure on the natural stands and raised concerns over the long-term survival potential of *A. malaccensis*. This study aimed to evaluate the genetic diversity and phylogeography of *A. malaccensis* based on 942 individuals sampled from 35 forest reserves throughout Peninsular Malaysia. By utilizing 12 microsatellite loci, the present study revealed high levels of genetic diversity in *A. malaccensis*. The mean number of alleles ( $A_a$ ) was 5.414, ranging from 3.333 (Batu Papan) to 8.000 (Sungai Udang) whereas the gene diversity ( $H_e$ ) was 0.537, ranging from 0.447 (Batu Papan) to 0.642 (Lenggor). The estimated  $F_{ST}$  was 0.081, implying that 91.9% of the total genetic diversity was partitioned within populations. The cluster analysis divided the populations into two major clusters: Cluster Kedah-Perak and Cluster Kelantan-Johor (Figure 1). Similarly, the haplotype distribution map based on seven chloroplast regions also divided the populations into two main clusters. Implications of the study to establish conservation and management guidelines of *A. malaccensis* are discussed.



**Figure 1**: (a) Relationship among populations determined using Bayesian analysis via STRUCTURE. (b) Neighbor-joining tree based on genetic distance. (c) Based on STR markers, *A. malaccensis* in Peninsular Malaysia is partitioned into two genetic clusters, i.e. Kedah-Perak and Kelantan-Johor.

#### The limestone orchid flora of the northern Peninsular Malaysia

Shahrul Nizam AB\*, Farah Alia N & Mohd Farhan R

School of Biological Sciences, Universiti Sains Malaysia (USM), 11800 USM, Penang, Malaysia \*Corresponding author's email: shahrulbakar96@gmail.com

The state of Kedah covers a total area of about 9,425 km<sup>2</sup>, bordered up north by Perlis and Thailand, Penang in the southwestern and Perak in the south part of the state. Kedah has a forested area of 330,585 ha (Wan Mohd Jaafar et al. 2020). Since 1939, botanical studies on the flora of limestone hills have been carried out due to their remarkable characters in appearance and uniqueness of their vegetation types. However, these botanical excursions focused mainly on the more accessible limestone hills of the north and west Peninsular Malaysia (Henderson, 1939). Limestone hills also have proven to be a hotspot for high level of endemicity (Clements et al. 2006). The inaccessibility from the unique yet rough topography with highly variable climatic and edaphic conditions have evince the development of high diversity and endemism on limestone formations (Kruckeberg & Rabinowitz, 1985; Clements et al. 2009). As reported by Chin (1977), about 21% of the plant species occurring on the karst habitat in Peninsular Malaysia are endemic species. Limestone hills too hosts many interesting plant species that are found to be as new to science, such as Vatica najibiana Ummul-Nazrah (Dipterocarpaceae), Gymnostachyum kanthanense Kiew (Acanthaceae), Meiogyne kanthanensis Ummul-Nazrah & J.P.C. Tan (Annonaceae) and Vatica kanthanensis Saw (Dipterocarpaceae) (Ummul-Nazrah et al. 2018; Tan et al. 2014). In recent study done by Rafidah et al. (2020), they discovered Sohmaea teres, a herb belonging to the family Fabaceae during a field study in Gunung Pulai, Kedah which later was described as a new record for Peninsular Malaysia. Although botanical surveys have been started since decades ago, these new findings show that limestone hills in Peninsular Malaysia, particularly in the state of Kedah is still understudied in a great extent. Documentation on the orchid flora from limestone hills in the northern part of Peninsular Malaysia is still lacking, with limited amount of body literature available. Thus, there is an urgent need to fill in the knowledge gap, so that proper documentation on the diversity of orchids from this particular limestone habitat can be prepared. This documentation will later serve as a fundamental reference for future conservation efforts in protecting the limestone orchid species and their unique habitat. Granted that being environmentally rich in biodiversity, however, only few studies on the flora of limestones hills in Kedah have been conducted. The lack of attention and report over time would only result in more habitat loss. The obscure species that could be new to science will be facing threats that are common to see in limestone hills such as quarrying and forest clearing for agriculture by locals. The diversity of limestone orchids in Kedah is poorly studied, with records on endemic and rare species are limited due to the harsh topography that caused inaccessibility to the areas. Reports on the forest cover change in Kedah has shown a decrement in total forested area by 31,583 ha of terrestrial forest and 1,807 ha of mangrove forest due to land use practices such as land clearing, development of oil palm plantation, access road, housings and water bodies. In the period of 29 years, deforestation of different purposes has taken about 9% of Kedah's forest cover between the year of 1988 and 2017 (Wan Mohd Jaafar et al. 2020). The loss of wild orchid species in limestone forest is also assignable to the destructive human activities including the recalcitrant orchid collectors that harvest them from the wild. The surge in illegal collecting is very concerning towards preserving these rare and endangered orchids as they are being exported for sale to orchid amateurs (Yee et al. 2005). Included in the main initiatives of this study is to identify the conservation priorities of any species in the family Orchidaceae that are possibly threatened for extinction. To prevent any rare and endangered species from being drifted away together with any events of disfigurement to limestone hills, a substantial study should take place promptly. Thus, this research aims to evaluate the species richness of orchids, by focusing on limestone hills located in the northern region of Kedah. Protecting the orchids *in situ* in their natural habitat is the best approach of conservation. However, for species that is recognized to be continuously threatened by habitat lost, ex-situ conservation is the best immediate measure to prevent the species from extinct locally. The initiatives on orchid germplasm conservation is another best approach in conserving genetic

resources of the limestone orchids. In Malaysia, the Malaysian Agriculture Research and Development Institute (MARDI), is the only known reference body that has embarked on the Orchid Breeding Programme which include germplasm collection as one of the ways to preserve and protect wild orchids from being endangered or extinct. Thus, the need to document the diversity of orchids in the less-explored limestone hills in Kedah is critical, as more and more forests are facing threats from logging. Immediate conservation acts are needed to be implied more efficiently to secure the well-being of our bio-diverse forests. Several *ex-situ* conservation approaches such as germplasm collections and cryopreservation have been determined to protect these rare and threaten species from the relentless form of disturbances such as whether legal or illegal logging, over-collection, conversion of land use and many others.

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#### Low-temperature seed storage of forest tree species

Nashatul Zaimah NA\*, Nadiah Salmi N, Nor Asmah H, Noraliza A & Nor Rashidah M Seed Technology Laboratory, Biotechnology Programme, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia \*Corresponding author's email: nashatul@frim.gov.my

Malaysian forests are dominated by tall dipterocarp trees which make up most of the canopy. The family Dipterocarpaceae, consists of almost entirely of recalcitrant seeds which means they are short-lived, sensitive to desiccation and termed as a so called 'difficult to store' seeds. Recognising the importance of *ex-situ* maintenance of plant genetic resources, the FRIM Seed Technology Laboratory has carried out various research activities to support the *ex-situ* conservation of Malaysian forest tree species using low-temperature storage methods. In this method, several applications are available; for short-term, seeds can be stored at  $+5^{\circ}$ C and storing seeds at freezing temperatures (-20°C or -80°C) allows medium-term storage but special laboratory freezers are required. On the other hand, cryopreservation in liquid nitrogen (-196°C) is also effective for longterm storage. Preserving seeds at low-temperatures allow reduction in metabolic activity and this assists in medium and long-term preservation of seed plant genetic resources. In all studies performed, only ripe seeds were used. The seeds were desiccated under the laminar flow or by using silica gel to a range of moisture content (mc) between 5 and 25% and subjected to either storage in a deep freezer (-20°C), direct plunge into liquid nitrogen at -196°C. The seeds were stored in liquid nitrogen for one month to a year and thawed under the laminar flow or rapidly immersion in a 40°C water bath. Meanwhile, storage of dipterocarp seeds was conducted in a 20°C cold room. Viability of the seeds in these studies was tested by germinating the seeds on paper in petri dishes at 28-30°C. Table 1 and 2 present the results of low-temperature storage studies on tropical forest tree seed conducted by the FRIM Seed Technology Laboratory from 1992 to present.

Species	Storage	Method	Optimum mc	Germination
	temperature		(%)	(%)
Acacia mangium	-196°C	Direct plunge	7.6	76
Bambusa arundinacea	-196°C	Direct plunge	10.7	73
Cassia spectabilis	-196°C	Direct plunge	16.9	83
Dendrocalamus membranaceus	-196°C	Direct plunge	8.5	53
Dendrocalamus brandisii	-196°C	Direct plunge	7.3	48
Dyera costulata	-196°C	Direct plunge	6.2	90
Lagerstroemia floribunda	-196°C	Direct plunge	9.8	87
Leucaena leucocephalla	-196°C	Direct plunge	9.6	79
Pterocarpus indicus	-196°C	Direct plunge	4-6	90
Swietenia macrophylla	-196°C	Direct plunge	5-8	63
Tectona grandis	-196°C	Direct plunge	7-9	90
Adenanthera pavonina	-20°C	Deep freeze	9.7	96
Albizia falcataria	-20°C	Deep freeze	6.6	100
Alstonia angustiloba	-20°C	Deep freeze	5.0	81
Dialium platysepalum	-20°C	Deep freeze	10.6	37
Lagerstoremia speciosa	-20°C	Deep freeze	12.8	24
Melia axederach	-20°C	Deep freeze	12.4	43
Tectona grandis	-20°C	Deep freeze	6-9	97

 Table 1: Summary of seed storage experiments in liquid nitrogen (-196°C) and freezer (-20°C) of non-dipterocarp species

At low moisture content (<17%) those seed species survive cryopreservation (-196°C) exhibit high germination percentage of >60% (Table 1). These seeds are categorised as orthodox in which they can be dehydrated to low moisture content of <5% and tolerate freezing thus allow *ex-situ* conservation. Small size orthodox seeds mostly benefit cryopreservation as less space is needed and the seeds can be stored for unlimited time. This is especially important for threatened species, species with very little seed and species of economically value. At FRIM, a cryopreservation plot has been established with the purpose to support further future research related to genetic stability of seedlings and trees raised from cryopreserved seeds. Currently, two species have been planted i.e. *Dyera costulata* and *Khaya ivorensis*.

Species	Duration (weeks)	Germination (%)
Hopea helferi	7	100
Hopea odorata	7	100
Hopea nervosa	3	50
Hopea subalata	4	73
Shorea argentifolia	2	30
Shorea curtisii	3	10
Shorea dasyphylla	3	40
Shorea glauca	2	65
Shorea leprosula	6	85
Shorea materialis	2	38
Shorea ovalis	4	98
Shorea parvifolia	2	62
Shorea roxburghii	4	85 - 100

 Table 2: Summary of low-temperature seed storage experiments (cold storage 20°C) of dipterocarp species

Table 2 indicates the survival of dipterocarp seeds at cold storage (20°C). High germination (> 40%) was observed for most species. Storage of *Hopea helferi*, *H. odorata* and *Shorea leprosula* seeds can be extended to more than 6 weeks with high germination retained. It is expected that storage duration could be extended for these species as the germination percentage is still high after 6 weeks in storage. According to Harrington (1972), aging occur during storage initiating a reduction in quality and eventually seed lost viability if storage conditions are not suitable. It is suggested to store recalcitrant seeds in moist condition at  $\geq 10^{\circ}$ C for less than 1 year (De Vitis et al. 2020). Cryopreservation of truly recalcitrant seeds are challenging and success is very limited (Elliot et al. 2013). Recalcitrant seeds are sensitive to desiccation and reduction of moisture content to below 20-30% and exposure to freezing temperature cause cells injury (Walter et al. 2013). It is recommended that this study is to be continued in the future when more seeds are available.

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## Tissue culture as an alternative solution to production of planting materials for Red List Threatened species

Nor Hasnida H\*, Mohd Saifuldullah AW, Muhd Fuad Y, Nazirah A, Rozidah K, Rohani A, Sabariah R, Naemah H, Rukiah M, Ris Amirah AM & Tengku Nurul Munirah TA Tissue Culture Laboratory, Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia. \*Corresponding author's email: hasnida@frim.gov.my

Plant tissue culture technique commonly used to propagate seedlings and involves the cultivation of small pieces of tissue and aseptic organs in culture media and under controlled environmental conditions, and is becoming an increasingly important tool for both scientific and commercial applications in recent years. This method has many advantages over conventional vegetative propagation as it produces a large number of pathogen-free plants in a relatively short time with high uniformity. The success of micropropagation depends on several factors, such as the composition of the nutrient medium, the culture environment and the genotype of the plant species (Kumar & Reddy 2011). The easiest way to propagate plants in vitro is the axillary shoot propagation method. The axillary buds are treated with hormones to ward off dormancy and sprout. Then the shoots are separated and rooted to get complete plantlets. Alternatively, shoots are used for further propagation (Gregory & Hubstenberger, 1995). This technique was used for the establishment of tissue culture protocol for Red list species in our laboratory such as Aquilaria malaccensis (Figure 1) and A. hirta (Figure 2) for planting material production. Both species are common species of Aquilaria found in Peninsular Malaysia. In Malaysia, A. malaccensis is locally known as Karas meanwhile A. hirta is known as Chandan or Chandan Bulu. Both species can produce agarwood, the heavy fragrant wood that can be used as a stimulant, tonic and carminative medicine. Collection of these species from Malaysian forests is becoming increasingly difficult as it is threatened with extinction due to indiscriminate collection and over-exploitation for commercial purposes. Therefore, there is an urgent need for assistance in the preservation and existence of this particular species, as well as in the conservation of its germplasm. The use of biotechnology, such as tissue culture technique, is a better alternative for the above purposes, especially for these species with a very short dormant period (Chang et al. 2002).



**Figure 1**: Production of *Aquilaria malaccensis* planting material (A) Shoot multiplication in MS basal medium containing 1.0 mg/L BAP (B) *In vitro* rooting in MS basal medium supplemented with 1.0 mg/L IBA (C) Plantlets growth in the nursery.



**Figure 2**: Production of *Aquilaria hirta* planting material (A) Shoot multiplication in MS basal medium containing 0.1 mg/L BAP (B) *In vitro* rooting in ½ MS basal medium containing 1.0 mg/L IBA (C) Plantlets growth in the nursery

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# Good nursery practices: A simple guide for production of high-quality planting materials

Siti Salwana H\*, Syajariah S, Abdul Rrazak S & Ahmad Fuad Z

Main Nursery Branch, Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor. \*Corresponding author's email: salwana@frim.gov.my

There is a great demand for high-quality planting materials for Malaysia Red List plant species, which are mostly used for reforestation programmes in degraded forest lands. Hopea helferi (Giam lintah bukit) is currently classified as a vulnerable species on the Malaysia Red List due to a dramatic population decline. Therefore, the selection of this species in reforestation initiatives has the potential to increase the species' population in Malaysia. Besides, the conservation strategies have been implemented to maximise survival during outplanting and to reduce mortality of this species by using high quality planting materials, mainly seedlings raised in nurseries, as nursery management and practices have a direct impact on their success. Due to poor nursery practices, most nurseries have been experiencing a shortage of high-quality planting materials which causes seedlings to have retarded growth with small root systems, resulting in a high mortality rate. The conservation and reforestation activities attempted have had numerous failures because of this issue. Therefore, nursery producers should prioritize producing high-quality seedlings over quantity. Numerous factors contribute to seedling quality at nursery including environmental, nursery facilities and cultural practices. The term "best practices" refers to the proper selection of growing and potting media as well as container types. These practices are important in manipulating growth, development and flowering of seedlings (Deogade et al. 2020). Therefore, all these aspects should be supervised to ensure that all operations are performed on time and implemented correctly to promote optimum seedling growth. Hence, in this paper, we aim to propose a simple guide for producing high-quality seedlings of forest species through good nursery practices by selecting suitable container types and ratios for potting medias. Hopea helferi seedlings were grown in two container types (polybags and root trainers) with varying potting media ratios (M1, M2, M3 and M4). The results showed that H. helferi seedlings grown in root trainers with potting media at M2 ratio significantly improved their growth performance compared to other treatments (Figure 1). This was because the potting media (M2 ratio) provided the highest nutrient availability for root uptake, hence promoting seedling growth (Deogade et al. 2020). Besides, root trainer raised seedlings are better alternatives to polybags in many aspects including root patterns as they alter the rooting fibrosity and volume of the seedling, which in turn allow them to support better root growth and greater survival during field planting rather than in polybags. However, the seedlings grown in polybags that consisted of the same ratio of potting media (M2) showed slow growth performance and a weak root system (Figure 2). This was because of an inappropriate container type that led to root balling and circling affected by rounded polybag's bottom shape. Furthermore, the stunted seedling growth in this treatment might also be due to shallow taproot that reduced the ability of the roots to absorb water and nutrients from the soil caused by a deformed root growth pattern. Consequently, it inhibited plant survival and growth due to time spent in polybags (Doran, 1997). Thus, the implementation of good nursery practices such as selection of suitable potting media such as at M2 ratio filled in root trainers in nursery are able to produce good quality seedlings by improving the growth performance of H. *helferi* seedlings at nursery stages. Hence, high-quality planting materials can be produced and made available for large-scale commercial production and successful reforestation projects in degraded forests.



**Figure 1**: The growth performance of *Hopea helferi* seedlings planted in different potting media at M1, M2, M3 and M4 ratios (from left to right) by using polybags (front) and root trainers (back)



Figure 2: Root structure of *Hopea helferi* seedlings planted in potting media at ratio of M2 in root trainer (left) and polybag (right)

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### Improvement programme and *ex-situ* conservation of selected *Shorea* spp.

Nor Fadilah W\*, Farah Fazwa MA & Mohd Zaki A

Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia \*Corresponding author's email: norfadilah@frim.gov.my

The timber industry and wood-related products have been a crucial pillar of the Malaysian economy. In 2019 the exports of timber increased from MYR 22.3 billion to MYR 23 billion from the previous year. This trend is expected to continue, in line with increasing global demand. However, current harvesting practices focus on obtaining timber products from natural forests. Depending solely on natural forests to satisfy this demand will lead to rapid deforestation and loss of biodiversity. Therefore, the Plant Improvement Programme under the Forestry Biotechnology Division of Forest Research Institute Malaysia (FRIM) has taken initiatives to conduct tree improvement studies to develop high-quality planting materials of desirable forest tree species in an effort to reduce dependency on natural forests. Tree improvement programme is an essential part of forest management operations. Utilising genetically best trees with a combination of good silvicultural practices would ensure optimum yield. This paper summarizes the improvement programmes conducted on three selected Shorea species which are Shorea leprosula (Meranti tembaga), Shorea roxburghii (Meranti temak nipis) and Shorea parvifolia (Meranti sarang punai). According to Malaysia Plant Red List, both S. leprosula and S. parvifolia are categorised under Least Concern (LC), whereas S. roxburghii is categorised under Near Threatened (NT). These three species were chosen based on their high value timber and accessibility. The improvement programmes were approached via the selection of plus trees. Plus tree is defined as a selected tree that has been graded for the sources on production for further breeding study (Hettasch et al. 2002). However, the genetic superiority of the selected plus trees needed to be tested. But, the probabilities of the progenies from selected plus trees to have good genotype is high due to reasonable heritability. Seeds collected from the selected plus trees were grown and planted in the progeny trial. Conceptually, in the progeny trial, the seedlings are planted in the replicated field trial. Growth performances are evaluated regularly. Moreover, the established trial plots can be converted into a Seedling Seed Orchard (SSO) in the future. SSO is an orchard whereby only the selected trees will remain and the inferior trees will be removed. The trial plots establishment have also increased the number of field gene banks and seeds collection, consequently empowering the nation's germplasms bank. The improvement programme for S. leprosula has started in 1998 with the establishment of a progeny trial plot at Ulu Sedili Forest Reserve, Johor. The trial plot was established with 40 half-sib families originated from five populations and laid out in a randomised complete block design (RCBD) with a total planted progenies are 1,280 making the total area of 2.05 ha (Figures 1-3). Current findings showed the growth variations among the 40 half-sib families were significantly high for important economic traits such as diameter at breast height (DBH), total height (HT) and clear bole height (CBH). The highest recorded Mean Annual Increment (MAI) at the age of 20 years old for DBH was 1.68 cm yr-<sup>1</sup>, while the highest MAI for HT was 1.3 m yr-<sup>1</sup>. In addition, genetic diversity assessment revealed a high ranged of Heterozygosity (He), 0.67 to 0.72 (based on 5 populations), whereas the overall He was 0.71. The He values indicated that the established plot consists of a very diverse gene pool. The way forward for this research is to convert the trial plot into an SSO. Quantitative and qualitative data were already evaluated, genetic diversity and relatedness of the 40 half-sib families have also been assessed. The selected trees (superior) will remain in the plot as seed trees, whereas the inferior trees will be cut down to allow pollination only to occur among the selected trees. Second, the improvement programme for S. roxburghii has started in 2017. Seeds were collected from 27 halfsib families. The progeny trial plots were established in 2018 at three FRIM's research stations (SPF), SPF Mata Aver (Perlis), SPF Setiu (Terengganu) and SPF Jeli (Kelantan) (Figures 4-6). The progeny trial plots were also laid out in RCBD, with a total of 3,240 seedlings; SPF Jeli (864 seedlings), SPF Mata Ayer (1,296 seedlings) and SPF Setiu (1,080 seedlings). Currently, the growth data were actively being collected. The early assessment indicated that there were high variations among the

trial Site, Family and Site by Family interactions. Survival rates of the three trial plots were also very promising (81.9% - 96.4%). Since this study is still at an early phase, it would be biased to assess the MAI but the study conducted by Ho et al. (2018) recorded the MAI of 3.9 cm. yr<sup>-1</sup> for DBH and 2.8 m. yr<sup>-1</sup> for HT at the age of 6 years old. This is significantly faster than *S. leprosula* and considerably fast growth for indigenous species. Based on the MAI of DBH, it is expected that the rotation period for *S. roxburghii* could be decreased to 20 to 25 years with an expected minimum DBH of 30 cm. Whereas the improvement programme for *S. parvifolia* is relatively new, having only started in 2019. Currently, planting materials for the establishment of the progeny trial plot are actively being prepared.



Figures 1-3: Progeny trial plot of *Shorea leprosula* at the age of 20 years old located at Ulu Sedili Forest Reserve



Figures 4-6: Progeny trial plots of *Shorea roxburghii* at the age of two years old located at SPF Jeli, SPF Mata Ayer and SPF Setiu (respectively)

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## Vegetative propagation of some selected threatened tree species indigenous to Malaysia for conservation purposes: A review

Kumar SM\*, Farah Fazwa MA & Mohd Zaki A

Herb and Tree Improvement Branch, Plant Improvement Programme, Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia \*Corresponding author's email: sures@frim.gov.my

Threatened tree species are restricted to a small, endemic range with few remaining reproductive individuals. These species suffer from many threats such as illegal logging, habitat destruction, invasive species, pollution, and climate change. Many of the remnant populations have been reduced drastically and natural regenerating capabilities have been impaired. Conservation of these species often requires the rebuilding of the depleting population within the known endemic range of these threatened species or by the development of commercial plantation forests. The ability to propagate and successfully reintroduce the threatened species into their natural habitats is one of the important components of conservation efforts. The majority of the threatened species are in the dipterocarp family where standard seed storage techniques for long-term storage conservation and maintenance of genetic diversity are not possible due to the recalcitrant nature of the seeds. The new knowledge on the development of successful propagation techniques for each species allows sustainable conservation through the production of an unrestricted number of new propagules. In this context, the adoption of alternative methods for vegetative propagation from stem cuttings might be a viable solution. However, the propagation using hardwood stem cuttings is hampered by factors intrinsic to the planting material such as the age of the mother tree, collection time of the cuttings, phytohormone concentration, or exogenous factors such as other growing conditions of the cutting. Manipulation and modification of these influencing factors through propagation trials can assist in the production of planting materials with enhanced rooting and development from the cuttings. To answer the growing demand for saplings by the nursery industry for application in reforestation projects some technically simple and inexpensive vegetative propagation methods through stem cuttings were developed by Forest Research Institute Malaysia (FRIM). Propagation research conducted at FRIM has opened up some protocols that may expand the planting stock production in support of these goals. The primary aim of the study is to gather data on various propagation work done by FRIM researchers to identify appropriate techniques and modifications on controlling factors for recommendations on how the propagation techniques can be further improved. A thorough review has been done on research work documented by Aminah Hamzah (1990-2013), one of the leading researchers in the field of vegetative propagation in FRIM. Her works were predominantly focused on some of the important factors influencing the rooting ability of woody stem cutting, such as rooting facilities, rooting media, source of cutting material, type, and treatment of cuttings. The percentage of rooting and number of roots produced per cutting indicated that they are highly variable among species (Figure 1). For comparison, five threatened tree species, Shorea bracteolata, Shorea pauciflora, Shorea parvifolia, Hopea odorata, and Neobalanocarpus heimii have been selected and reviews were made on the propagation techniques attempted for planting stock production. Materials for cuttings were mainly collected from juvenile stems of seedlings, rooted cuttings, orthotropic shoots of pruned plants. Stem cuttings were ranged from 1.2 cm to 5.0 cm with a diameter of 1.2 mm to 4.5 mm. Leafy stem cuttings with at least two active nodes were treated with different concentrations and a combination of rooting hormones. Indole-butyric acid (IBA) or Naphthalene acetic acid at different levels and without any hormone application. All plants were planted on cleaned river sand in an enclosed mist propagation system. All species responded differently to hormone treatments. Application of rooting hormone aided the rooting ability of stem cuttings however, moderate to difficult-to root species such as H. odorata and S. bracteolata were reported to be able to produce vigorous healthy roots without any application of rooting hormone under controlled nursery conditions. The number of roots produced from each cutting was positively correlated to the cutting size, the longer and bigger cuttings produced a greater number of roots. Cuttings from juvenile materials gave better rooting and survival percentages in all the tested species.

Survival and rooting percentages of 65% to 95% were recorded for all species. Generally, coppice materials collected from the 7-year-old stock plant of *Shorea pauciflora* responded better in terms of survival rate, growth, and rooting percentage (95%) compared to the other species. Based on the review, it can also be concluded that longer and bigger cuttings produced a greater number of roots. All species tested are amendable to vegetative propagation through stem cuttings. Results indicated that through vegetative propagation of stem cuttings, a continuous supply of planting stock of threatened species can be made available for the planting program. The implication of these findings for conservation and plantation development are further discussed and more advanced propagation trials are attempted to further propagate other threatened tree species.



Figure 1: Rooting ability of stem cuttings of some selected threatened tree species under nursery condition in FRIM (Source: FRIM nursery record) Note: CR= Critically Endangered, EN= Endangered, VU= Vulnerable

## Growth and yield of Chengal (Neobalanocarpus heimii) planted in Bukit Lagong, FRIM campus, Selangor, Malaysia

Amir Saaiffudin K\*, Dasrul I, Rosdi K & Zawiah N

Plantation Management Branch, Forest Plantation Programme, Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM) Kepong, Selangor, Malaysia \*Corresponding author's email: saaiff@frim.gov.my

Neobalanocarpus heimii or locally known as Chengal is listed as an 'Endangered' in the IUCN Red List (2021). Neobalanocarpus hemii was found to be naturally growing in Peninsular Malaysia and southwards to Singapore (Desch, 1941) and the northern part of Thailand, however the populations in Singapore are believed to be extinct (IUCN, 2021). Records of planting heavy hardwood species in West Malaysia dated back to 1880s, when concerns for the potential loss of desired species was expressed in Hill (1900). Continuation from the previous efforts, the first N. hemii plot in FRIM was planted in 1932 at Field 11. Unfortunately, records of the performance of survival and growth were not well documented. A planting trial plot was established in December 2011 at Field 48, Bukit Lagong, FRIM (3° 14' N and 101° 38' E). Mean daily temperatures of the area ranged from 27° to 32°C with annual rainfall of 2,000 - 2,900 mm. The average slopes were 10 - 15° and the area was formally the trial planting site of *Pinus* and *Araucaria* species. Soil at the site was identified to be Rengam series which was derived from granite parent material. The soil pH ranged from 3.7 to 4.1 while the clay content ranged between 31-55%. The soil is well-drained with good permeability (Jeyanny et al. 2019). The planting stocks seeds were collected from Field 11, FRIM and sown in a nursery bed before potted in 8" x 12" polybags filled with potting media of topsoil and peat with 2:1 ratio. The seedlings were tended in the nursery for eight months under 50% netting shade and consequently thereafter two months with 0% shading before field out planting. Planting site was clear felled using tractors and the slashed piles were removed. An area of 0.5 ha (50 x 100 metres) was cleared by removal of all trees and shrubs. The surrounding plants at site were < 2 m tall. A total of 300 seedlings with the average mean height of 0.45 m were planted with the initial density of 833 trees ha<sup>-1</sup>. Measurements were done at 6 months intervals in the first year and 12 months afterwards. The variables measured were total height and diameter at breast height (DBH) at 1.3 m above ground when the heights of tree reached above 2 m. Competing vegetation in the plots were cleared mechanically using brush cutter. The frequency of plot maintenance works varied with an average of four intervals annually. The species showed consistent increase in DBH and height annually, at 3.46 cm and 3.73 m at 6-years after planting to an average of 6.4 cm and 5.11 m at 10-years after planting. The survival rate for the stand was 69% at age 10 years. The inventory of the stand and stock from 2017-2021 is shown in Table 1. The growth data from this study showed that the initial early growth of diameter and height of this species is slow but Foxworthy (1927) stated that rapid growth will take place upon DBH reaching 25 cm, and maintained until the trees reach 80 cm DBH. Ng (1974) reported the mean annual diameter increment (MAId) of 0.96 cm/year whereby equivalent to 38.4 cm DBH at 40 years after planting in trial plots of Kepong, Selangor. His work reports the growth potential of N. heimii under planted forest conditions. A study carried out in Chikus Forest Reserve, Perak showed that *N. heimii* can be planted in large open planting lines with widths ranging from 18.5 to 33.3 m (Hiroshi et al. 2003). Kenzo (2011) stated that this species preferred low light conditions of less than 20% canopy openness in Ayer Hitam F.R. Selangor. Neobalanocarpus heimii is known for its premium wood quality and high demand in wood industries. Establishing a permanent plot of the species outside or away from its natural habitat is important to provide a protective enclosure for cultivation and conservation to ensure continuity.

Age (Years)	N (stems ha <sup>-1</sup> )	Max/Min Dbh (cm)	Dbh (cm)	MAId (cm)	Max/Min Ht (cm)	Ht (m)	MAIh (m)	<b>S</b> (%)
6	580	0.5/8.3	3.46	0.35	0.64/6.69	3.73	0.37	70%
7	575	1.0/10.2	4.87	0.49	2.00/8.90	4.3	0.43	69%
8	574	1.8/11.9	5.73	0.57	2.16/10.10	4.91	0.491	69%
10	574	2.4/13.5	6.35	0.64	2.4/10.2	5.11	0.511	69%

Table 1: Stand and stock table of Neobalanocarpus heimii stand in Field 48 FRIM

Note: Dbh: diameter at breast height; MAId: mean annual increment of diameter; MAIh: mean annual increment of height; Ht: height; S: Survival



Figure 1: Tree heights and DBH of Neobalanocarpus hemii as observed from 2017-2021

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## Growths and carbon stock of Dipterocarp trees planted on abandoned mining area in Phangnga Forestry Research Station

Wasan C\*, Jetsada W, Ladawan P & Punyanuch A

Faculty of Forestry, Kasetsart University 50 Ngamwongwan Road Ladyao Chatuchak, Bangkok, 10900 Thailand \*Corresponding author's email: fforwsj@ku.ac.th

Dipterocarp trees play important roles in ecological service of tropical forests. In this study, we aimed to investigate the growth and estimate the carbon stocks of six dipterocarp trees, Dipterocarpus alatus Roxb., Dipterocarpus gracilis Blume, Hopea odorata Roxb., Shorea gratissima (Wall. ex Kurz) Dyer, Shorea roxburghii G. Don, and Parashorea stellata Kurz, planted on abandoned mining area in the Phangnga Forestry Research Station, southern Thailand. The dipterocarp seedlings were planted under a 6-year-old Acacia mangium plantation. The survival rate, diameter at root collar  $(D_0)$ , diameter at breast height (DBH), total height (H) and carbon stock of 1-, 5- and 9-year-old dipterocarp trees were investigated. The results revealed that H,  $D_0$  and DBH of 9-year-old H. odorata and S. roxburghii were the highest, having the values of 7.71 and 7.60 m, 10.14 and 10.45 cm, and 7.38 and 6.59 cm, respectively. Maximum survival percentage was recorded for S. gratissima and H. odorata (95%), followed by S. roxburghii (93.33%). Carbon stocks of S. roxburghii was the highest (24.63 ton/ha). Dipterocarp trees are best planted alongside nurse trees as the nurse trees create suitable microclimate for the survival and growth of the trees (Norisada et al. 2005; Sakai et al. 2014). The findings suggested that S. roxburghii should be considered for restoring abandoned mining land, since it recorded preferable growth with high carbon stocks. In addition, mixed plantation of fast-growing trees and Dipterocarp trees should be recommended for rehabilitating degraded areas, for improving forest structure and diversity, and for providing ecological services.



Figure 1: Survival rates of six dipterocarp trees



Figure 2: Carbon stocks of six dipterocarp species planted on abandoned mining area



Figure 3: Diameter at root collar (D<sub>0</sub>), DBH and total height of six dipterocarp trees

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## Conservation of threatened tree species through establishment of germplasm bank

Ahmad Fauzi MS\*, Aini Izzati AS, Zainol A & Zainol A

FRIM Research Station Perlis, Tree Improvement Programme, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia \*Corresponding author's email: afauzi@frim.gov.my

Population of tree species that are classified as threatened needs to be effectively managed and maintain in *in-situ* and *ex-situ* conservation as planned in the Malaysia National Strategy for Plant Conservation published in 2009. Threatened species are defined as any species which are vulnerable to being at risk of endangerment in the near future. The establishment of germplasm banks is one of the method for *ex-situ* conservation. This conservation method is widely applied by government and non-government entities towards achieving target 9 in which 60% of threatened plant species in ex situ collection within the country, and 10% of them included in recovery and restoration programmes. The International Union for Conservation of Nature's IUCN Red List of Threatened Species which was established in 1964 and Malaysia Red List version 2021 defines all species that are facing risks of extinction as Threatened. Threatened species are categorized as Vulnerable (VU), Endangered (EN) and Critically Endangered (CR). A project title 'Enhancement of germplasm bank or Pemantapan Bank Germplasma under RMK 11 (Eleventh Malaysia Plan), was carried from 2017 - 2020 to address this issue. The main objective was to collect 100 species of potential forest genetic resources for conservation, production of new cultivars and authenticated raw materials. Plants Checklist is based on list of taxa categorized as threatened in Malaysia (Chua et al. 2010) which has identified 92 species throughout Peninsular Malaysia. Collections are based on previous assessment or primary existing phenology reports from in-house monitoring, Forest Health and Conservation, Forest Biodiversity Department, Forest Research Institute Malaysia (FRIM) and Forestry Department of Peninsular Malaysia. Species collected are in the form of seeds, wildings or seedlings in polybags from reliable sources such as from the State Forestry Department Nurseries and FRIM, Kepong which includes compulsory information such as plant identification, geolocation or area collected, dates and sources e.g. seed, wilding or established planting materials (polybags). These information will be used for live asset management and tree certification. Planting materials are germinated, potted and raised in nursery at FRIM Mata Ayer Research Station accordingly with their requirements to an appropriate size before planting to selected plots in compartment 17 & 23. Mata Aver Forest Reserved, Perlis. A total of 78 species were collected in 4 years' duration. Priorities were given to Dipterocarpaceae family to archive the 100 species. A total of 72 dipterocarps were collected and the balance of 6 belongs to other families. Planting materials are now categorized using Malaysia Plant Red List 2021 listed in the latest version and are relevant to this country. The collection consists of 31 threatened species including CR, EN and VU and 47 species are in others categories. As for now, a total of 475 numbers of dipterocarp and non-dipterocarp seedlings which included 27 threatened and 35 in other categories have been planted on ground in Compartment 17 & 23, Mata Ayer Forest Reserved, Perlis with a planting distance of 4 x 4 meter. Planting activities were done in raining season (August - December) to ensure good survivability. Maintenance of plots were done periodically following silvicultural practices. The success of the establishment of germplasm bank for threatened species depends on proper planning, execution and collaboration with relevant governmental and non-governmental agencies. Identifying tree location, flowering seasons and availability of wildings is based on monitoring, phenology report from relevant authorities and from other reliable sources. Decision to use resources, tools and methods are based on types of planting materials to be collected and site accessibility. Proper handling of planting materials from site to nursery will increase the survivability of planting materials. Raising the planting materials with good nursery practices will ensure they will have a better chance to survive in field planting plots. Last but not least, proper planting technique and viable planting materials will achieve the desired goal. Mata Aver Forest Reserve will benefit from this project to become monsoon tropical forest rich with dipterocarp threatened species.

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# GC-MS analysis of agarwood using different type of inoculums in Malaysia plantation

Mustapa MZ<sup>\*1</sup>, Alias MA<sup>1</sup>, Saripah Salbiah SAA<sup>2</sup>, Mastura I<sup>2</sup>, Yuhanis MB<sup>2</sup>, Chee FW<sup>3</sup> & Rozita  $Y^2$ 

<sup>1</sup>Faculty of Forestry and Environment, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

<sup>2</sup>Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia <sup>3</sup>Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris,

35900 Tanjong Malim, Perak, Malaysia

\*Corresponding author's email: zamakhsyary@mtib.gov.my

This study aims to determine the chemical composition of all essential oils and conclude the best inoculum based on the average of accumulated yield of agarwood resin. The essential oils were produced from agarwood of A. malaccensis species by using different commercial inoculums labelled with A, B, C, D and E. The agarwood resins were obtained from dark colour trunk (part) of this species after inoculated with different inoculums and then separated from non-resinous wood or trunk (white colour). Agarwood resin that produced in the trunk was processed into an essential oil via hydrodistillation process. Inoculum C produced the highest yield of agarwood resin, 6.0 kg per tree, meanwhile, inoculum B and E produced the least amount of agarwood resin, about 200 g per tree. The quality of essential oils was measured by the existence of the aromatic compounds using GC-MS analysis. The results indicated a similar presence of sesquiterpene group in all essential oils. Two compounds were identified as 10-epi- $\gamma$ -eudesmol (11.21%) and  $\alpha$ -cadinol (20.59%) which exhibited the highest abundance compound in essential oil after injected with Inoculum D. In addition, 4-phenyl-2-butanone compound was recorded the least composition in all essential oils. These compounds were expected to contribute a pleasant odour and aroma of essential oils. As a conclusion, the current finding indicates the promising potential inoculums in producing agarwood resin.

# Biomass accumulation and carbon sequestration potential of *Hopea odorata* on problematic sites in Malaysia

Ho WM\*, Sik HS & Noor Shahirah MI

Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia \*Corresponding author's email: howaimun@frim.gov.my

The high demand for new planting sites for timber species has led to the establishment of plantations on poor soils. *Hopea odorata* has been identified as one of the most promising plantation species in Malaysia for its excellent growth, ease of management, potential uses and promising economic benefits. It has also been popularly used in reforestation programmes, as an ornamental tree and also as a shade tree. At this moment, *H. odorata* is still listed as a vulnerable species under the 2021 Malaysia Plant Red List and also the IUCN Red List (2017). This study aimed to determine the growth and quantify the capability of seven-year-old *H. odorata* to sequester carbon at problematic sites, an ex-tin mine in Bidor, Perak and BRIS soil in Setiu, Terengganu. The height and diameter of all trees in the stands were measured at breast height (DBH). A destructive sampling method was used in which nine trees were felled from each site. Tree components (root, trunk, branch and leaf) were separated. The fresh weight of the tree components was determined on site. Samples of each component were dried and the total dry weight of each tree was estimated. The wood specific gravity (WSG - oven-dried wood over green volume) was determined from discs cut from the stems. Figures 1 and 2 show the linear regression of the natural logtransformation of DBH and the total above-ground biomass for Bidor and Setiu, respectively, with a 95% confidence interval (CI).



Figure 1: Fitted line with confidence interval (95%) bands for Bidor

Figure 2: Fitted line with confidence interval (95%) bands for Setiu

Trees in Setiu and Bidor recorded high survival rates of 95% and 98%, respectively despite the harsh environmental conditions and unfavorable microclimate. Nonetheless, the growth performance in Bidor was significantly better than in Setiu (Table 1). We tested several allometric relationships between independent and dependent variables. The independent variables were DBH and WSG whereas the dependent variable was the dry weight of the total aboveground biomass (TAGB). These models were also compared with published models used for estimation of TAGB in tropical forests (Chave et al. 2005; Basuki et al. 2009). All computation and analyses were carried out with the R statistical software.

Table 1: Relative growth rates of the	he /-year-old	Hopea odorata planted	in Bidor and Setiu	
	n	Bidor	Setiu	
Relative height growth (m year <sup>-1</sup> )	100	$0.96\pm0.31^{\rm a}$	$0.81\pm0.13^{\text{b}}$	
Relative dbh growth (cm year <sup>-1</sup> )	100	$1.80\pm0.53^{\rm a}$	$1.33\pm0.26^{b}$	

Note: n – number of trees, dbh – diameter at breast height, different letters in same row denote significant differences at P<0.01 according to Student's T-test.

Table 2: Model description for the estimation of the total above-ground biomass of 7-year-old Hopea odorata in Bidor and Setiu

Model	Allometric equation	Adjusted r <sup>2</sup>	RSE	AIC
B1	$ln(TAGB) \sim a + b*ln(DBH)$	0.9395	0.1774	-1.8498
B2	$ln(TAGB) \sim a + b*ln(DBH) + c*ln(WSG)$	0.9294	0.1916	0.1500
<b>S</b> 1	$ln(TAGB) \sim a + b*ln(DBH)$	0.9242	0.1336	-6.9571
S2	$ln(TAGB) \sim a + b*ln(DBH) + c*ln(WSG)$	0.9116	0.1443	-4.9573

Note: The statistical analyses are significant at 95% confidence interval. \*\*\*p < 0.001; \*\*p < 0.01; \*p < 0.05; and non-significant, ns > 0.05. B: Bidor, S: Setiu, TAGB: total aboveground biomass based on dry weight (kg/tree), DBH: diameter at breast height (cm), WSG: wood density (g/cm<sup>3</sup>), RSE=Standard error of residual

The inclusion of WSG values in the equations of B2 and S2 were found to reduce the adjusted  $r^2$  and increased the RSE for both Bidor and Setiu (Table 2). However, Akaike Information Criterion (AIC) was used for model selection where by the optimal model will minimize the AIC value. The lowest AIC value was found in models B2 and S2 for H. odorata in Bidor and Setiu, respectively. In addition, the upper and lower limits of the CI for all models fit within the CI range of the observed values. Contrary to the findings of Rutishauser et al. (2013), tree height inclusion in the Bidor model actually increased the AIC of our study (data not shown). Models that only use DBH are reliable because additional explanatory variables such as WSG merely increase the indicators' goodness of fit for the equations. For reasons of cost and time constraints, the inclusion of WSG or other variables such as height and commercial bole height must also be taken into consideration. Therefore, the models B2 and S2 are used in this work to estimate the biomass and carbon sequestration potential of *H. odorata* at these two sites. Assuming 625 trees ha<sup>-1</sup>, the total biomass of *H. odorata* in Bidor was almost three times larger than Setiu (Table 3). In Setiu, the trees have used more than 35% of the total biomass for the development of a more extensive root system, probably due to poor water retention and supply. The carbon stock was estimated assuming that the biomass contains 50% carbon. Due to the large differences in the total biomass, the carbon stocks of the trees in Bidor and Setiu were also significantly different. Based on our estimate, each *H. odorata* tree can contribute to the absorption of approximately 19 and 7 kg CO<sub>2</sub>e per year in Bidor and Setiu, respectively. With a global average carbon price of US \$2 /t CO<sub>2</sub>e, Bidor would generate an additional income of US \$24 ha  $^{-1}$  yr  $^{-1}$ .

in Bidor and Setiu								
Site	TAGB (t ha <sup>-1</sup> )	Root (t ha <sup>-1</sup> )	TB (t $ha^{-1}$ )	$C (t ha^{-1})$	$CO_2e(t ha^{-1}yr^{-1})$			
Bidor	33.90	12.59	46.49	23.25	12.18			
Setiu	10.72	6.00	16.72	8.36	4.38			

Table 3: Biomass allocation and carbon sequestration potential of 7-year-old. Hopea odorata

TB: Total biomass, CO<sub>2</sub>e: Carbon dioxide equivalent

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## Mean annual carbon storage capacity of selected IUCN Red List threatened species established on an ex-tin mine in Peninsular Malaysia

Jeyanny V\*, Ang LH, Rozita A, Ho WM, Tang LK & Muhammad Asri L Forest Plantation Programme, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia \*Corresponding author's email: jeyanny@frim.gov.my

Tropical timber species are important for carbon sequestration as they are able to sequester carbon in their living biomass, deadwood, roots, litterfall and soil. Despite measures related to sustainable forest management, the supply of tropical timber species are dwindling due to illegal logging and other factors. This has forced most of the commercially important species into the IUCN Red List, mainly Shorea sp., Neobalanocarpus hemii, Dryobalanops aromatica and Aquilaria malaccensis. In the recent years, FRIM aware of this scenario has taken strategic steps in rehabilitation of degraded areas, such as tin tailings with these species to provide a germplasm pool as well as for conservation purposes. The study site is located at Bidor, Perak N 4° 05' 22.9"; E 101° 14' 07.3", with an average monthly rainfall of 283-290 mm, average daily maximum temperature of 34 to 35°C and average mean daily minimum temperature of 22 to 24°C. In early May 2012, selected species from the lists, namely Shorea roxburghii, S. platycados, S. ovalis, Neobalanocarpus hemii, Dryobalanops aromatica and Aquilaria malaccensis which were 30 to 50 cm in height were planted at 2 m x 2 m planting. A total of 36 trees were planted for each species but a portion of the plots were used for this study. Planting holes (30 cm radius x 50 cm depth) were filled with 60 g 80% chicken manure and 10 g NPK (15:15:15) and 1 kg of burnt rice husk ash. Fertilization was carried out every three months at the rate of 60 g chicken manure and 120 g of NPK (15:15:15) for the first two years. Watering and weeding were carried out for the first year and second year, respectively. Wildings of Macaranga gigantea were removed to eliminate nutrient competition periodically. The tree height and diameter at breast height were recorded annually from 2012 to 2020. The latest data was collected in December 2020. In early September 2021, soil core rings and litter fragments were collected at 0 - 30 cm depth and on surface to estimate C stocks. The carbon stocks in above and belowground biomass were calculated using allometric equations for aboveground biomass (Chave et al. 2005) and belowground biomass (Pearson et al. 2005) depending on the wood density of each species. A stoichiometric conversion factor of 3.67 (44/12) was used to convert C to carbon dioxide equivalents (CO2e). Our results displayed that the listed species were either vulnerable (VU) or critical (CR) according to the IUCN Red List for a total of 77 trees measured from the subplot. Based on our results, the highest carbon stocks were recorded by S. platycados with a total aboveground biomass of 30.98 tC ha<sup>-1</sup> and belowground biomass of 6.70 tC ha<sup>-1</sup>. Values for A. malaccensis and D. aromatica recorded similar biomass trends (Figure 1). Shorea platycados was reported to have high mean DBH at 16.6 cm for a 6.5 year old tree in a natural forest condition and at 6.5 cm (5 years old) in a rehabilitated ex-tin mine (Widiyatno et al. 2014; Ang et al. 2017) displaying its adaptability in harsh environmental conditions to serve as a C stock. Our results for A. malaccensis is in consensus with Majid et al. (2021), which showed C stocks were close to 30 tC ha<sup>-1</sup> for 14 cm DBH in natural conditions similar to our median value (13.5 cm). The lowest biomass was recorded by N. hemii, 6.6 tC ha<sup>-1</sup> in aboveground biomass and 1.71 tC ha<sup>-1</sup> in belowground biomass due to its slow annual growth increments (Ang et al. 2017). The soil C stocks at given depth and litter C stocks were 67.26 and 0.496 tC ha<sup>-1</sup>, respectively. Values for soil C stocks were two folds higher compared to a lowland secondary forest in Pahang (Jeyanny et al. 2015) at given depth. The total aboveground biomass stands at 112.60 tC ha<sup>-1</sup> and 25.53 tC ha<sup>-1</sup>, respectively. The surveyed area records an estimation of 138.13 tC ha<sup>-1</sup>, or an annual allocation of 17.26 tCO<sub>2</sub>e yr<sup>-1</sup>. Based on the average price for Forestry & and Land Use Category (USD 4.3 t CO<sub>2</sub>e), the area has the potential for carbon financing at USD 74 ha<sup>-1</sup> yr<sup>-1</sup>. This study indicates that degraded areas like ex-tin mines despite harsh conditions, have great potentials serving as an *ex-situ* germplasm conservatory of red list species, restore ecological values by enriching back the C reservoir as a nature based solution for sustainable forestry.



Figure 1: Carbon stocks in aboveground and belowground biomass of selected IUCN Red List species

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# *Gonystylus bancanus* (ramin melawis) and *Shorea platycarpa* (meranti paya): A review of threatened tree species of peat swamp forest in Peninsular Malaysia

Mohd Afzanizam M<sup>\*1</sup>, Azian M<sup>1</sup>, E Philip<sup>1</sup> & Jeyanny V<sup>2</sup>

<sup>1</sup>Climate Change and Forestry Programme, Forestry and Environment Division, <sup>2</sup>Forest Plantation Programme, Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia \*Corresponding author's email: mohafzanizam@frim.gov.my

Gonystylus bancanus (Miq.) Kurz from the Thymelaeaceae family and Shorea platycarpa Heim from the Dipterocarpaceae family are among important indicator species in peat swamp forests. Both Gonvstylus bancanus (ramin melawis) and Shorea platycarpa (meranti paya) are listed as critically endangered (CR) on the IUCN Red List of Threatened Species ver 2.3, 1998. G.bancanus has also been re-evaluated and assessed for the latest ver 3.1 (2018). The ecological importance of both native trees is that they are restricted to wetland environments with harsh conditions where inland trees cannot thrive due to seasonality with prolonged dry spells and / or water inundation during the tropical monsoon season. Hence, these two species are only endemic to peat swamp ecosystems. Given that they are listed as 'critically endangered' on the IUCN Red List of Threatened Species, it is vital to conserve both species to ensure their survival and significant growing populations, particularly in peat swamp forests. The aims of the study are (1) to conduct biomass and carbon stock assessment studies of G. bancanus and S. platycarpa under natural/pristine conditions and (2) to quantify changes in biomass and carbon stocks in tropical peat swamp forests in 2016 and 2018. The study area is located at Compartment 75, Pekan Forest Reserve, in the southeast of Pahang, Malaysia. Compartment 75 is 200 hectares in size, currently classified as "production forest" by the Forest Department of Pahang. For the analysis of the population profile, twenty-five (25) plots of 20 m x 20 m were established. All trees  $\geq$ 10 cm DBH were measured for stand structure and density. Three basic forest stand metrics are the tree basal area, the volume of the trees and the stand density. The allometric equation for calculating biomass uses three variables: diameter-at-breast height (DBH), height (H) and wood density (WD) (Manuri et al. 2014). The carbon stock was calculated by multiplying the sum of the biomass by the C fraction. The C fraction of the dry matter in the biomass used for this study is 0.47 (IPCC, 2006). Based on the species composition (Table 1), G. bancanus and S. platycarpa have a higher stocking density and a higher total volume compared to other trees given in Table 2. Stocking densities refer to the frequency of occurrence of a particular species at a given time. Field observation on S. platycarpa for example, is considered big and mature tree with aDBH of 15.8 to 102 cm and a height of 10.9 to 28.7 m. DBH for G. bancanus ranged from 21.1 to 73cm and the height from 18 to 36.4 m. The floristic richness and rarity of the species on the Red List will further enhance knowledge on prioritizing flora conservation in peat swamp forest. In the Peninsular Malaysia, 260 plant species have been identified from the Pekan peat swamp forest alone (Latif, 2005). The accumulation of biomass during stand development was accompanied by C accretion stored at the study site under pristine conditions as given in Table 2. With time, increments and decrements in biomass C stocks are influenced by many factors. A series of diameter increment function should be developed to model the growth of Ramin melawis and Meranti paya in the near future. In addition to the tree diameter, the mortality rate and the stand recruitment (new growth) should be assessed simultaneously to determine the long-term forest productivity and stand dynamics to contribute to carbon sequestration potential.

No.	Trees Species	Stocking density (stems ha <sup>-1</sup> )	Total basal area $(m^2 ha^{-1})$	Total volume (m <sup>3</sup> ha <sup>-1</sup> )
1.	Gonystylus bancanus (ramin melawis)	17	4.68	83.15
2.	<i>Shorea platycarpa</i> (meranti paya)	14	3.54	55.41

 Table 1: Red listed flora composition at Pekan Forest Reserve (Compartment 75) during assessment year 2019

**Table 2**: Stand inventory (SI) of Gonystylus bancanus and Shorea platycarpa at Pekan ForestReserve (Compartment 75) during assessment year 2019

Trees Species	Year	Total biomass (t ha <sup>-1</sup> )	Total carbon stock (tC ha <sup>-1</sup> )	Increment/Decrement
Gonystylus bancanus	2016	35.45	13.87	11.22 t ha <sup>-1</sup>
(Raminmelawis)				(for biomass)
	2018	48.93	19.15	5.28 tC ha <sup>-1</sup>
				(carbon stock)
Shorea platycarpa	2016	34.94	16.42	7.83 t ha <sup>-1</sup>
(Meranti paya)				(for biomass)
	2018	32.11	15.09	1.33 tC ha <sup>-1</sup>
				(carbon stock)

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## Aboveground and belowground biomass and carbon stock from dry dipterocarp forest in primary dry dipterocarp forest, Nakhon Ratchasima province, Thailand

 Phongthep H<sup>1\*</sup>, Wittanan T<sup>1</sup>, Phuvasa C<sup>2</sup>, Jeyanny V<sup>3</sup> & Mohd Afzanizam M<sup>4</sup>
 <sup>1</sup>Department of Environment, Faculty of Environmental Culture and Ecotourism, Srinakharinwirot University, Bangkok, Thailand
 <sup>2</sup>Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand
 <sup>3</sup>Forest Plantation Programme, Forest Research Institute of Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia
 <sup>4</sup>Climate Change and forestry Program, Forest Research Institute Malaysia (FRIM), 52109 Kepong Selangor Malaysia
 \*Corresponding author's email: phongthep@g.swu.ac.th

Forest is an important carbon sink and source that contains most of the terrestrial aboveground organic carbon as large amounts of carbon are stored in the biomass and soil. After plant dies, carbon is released from the decomposition of the biomass. Thus, rare or endangered tree species have imported components of forest ecosystems and play a crucial role in management and conservation. Accurate forest carbon estimates are therefore urgently needed to support efforts to conserve forests, particularly Red List tree species. In dry dipterocarp forest in Thailand has more Red List tree species, for example, Pterocarpus macrocarpus Kurz, Dalbergia cana Graham ex Kurz, and Dalbergia cochinchinensis Pierre, etc. Understanding the carbon stock in the Red List species in the dry dipterocarp forest are crucial for evaluating their carbon sink capacity, climate feedback and the global carbon cycle. However, although the examples underscore the importance of site conditions, they also show that there are different patterns which need to be studied first regionally and then individually in order to adequately manage and protect different tree species in different regions, especially in view the presence of the Red List tree species. The objectives of this study were to quantify the carbon stock and CO<sub>2</sub> mitigation in aboveground biomass at dry dipterocarp forest (DDF) and its responses to environmental factors. The study site was located within Sakaerat Environmental Research Station, Nakhon Ratchasima province, North Eastern Thailand (latitude: 14° 30' 29.80" N, longitude: 101° 56' 58.50" E). This area is situated at 390 m elevation above sea level and is part of the Sakaerat Biosphere Reserves with a total area of 79.61 km<sup>2</sup> and a DDF area of 11.80 km<sup>2</sup> (1,180 ha), accounting for 14.8% of the total area. The average annual precipitation and air temperature were 1260 mm and 20.44°C, respectively. The average maximum temperature was 30.39°C and the minimum temperature was 21.18°C. The methodology for the carbon stock and CO<sub>2</sub> reduction were estimated by allometric equations. The results found that the average plant height and diameter-at-breast height (DBH) at 130 cm above the ground were  $11.77 \pm 2.82$  m and  $12.25 \pm 1.77$  cm, respectively, in January 2019, and at the end of January 2020 they were  $11.72 \pm$ 2.28 m and  $12.99 \pm 2.25$  cm, respectively. While the average total carbon stock in the stem, branch and leaf for a year were  $59.06 \pm 2.99$ ,  $13.13 \pm 0.75$ , and  $1.84 \pm 0.06$  tC ha<sup>-1</sup>, respectively. The carbon stock rate at the DDF for one year was 6.63 tC ha<sup>-1</sup>. The CO<sub>2</sub> elude from the biomass stock through forest conservation was about 24.33 tCO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>. Thus, tree planting and conservation are good components for CO<sub>2</sub> mitigation through carbon capture in plant biomass.

### Wood density variations and carbon sequestration rate of Red List trees

Hamdan O

Geoinformation Program, Forestry and Environment Division Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor Corresponding author's email: hamdanomar@frim.gov.my

Tropical forests, especially dipterocarps trees species contain more biomass than any other biome on earth. The mass of carbon in trees is governed by the volume and density of their wood. Since wood density is largely determined by taxonomic identity this challenge is greatest in tropical forests with tens of thousands of tree species. However, the abundance of tree from Dipterocarpaceae family is depleting. Dipterocarps have supplied most of the tropical hardwoods traded in domestic and international markets since the early 1970s. The Dipterocarpaceae family comprises 17 genera and around 500 species. The family is pantropical, spreading from Colombia and Venezuela to Guyana in South America to Africa and the Indo-Malayan region of Asia. In Malaysia, the dipterocarps are the most abundant constituents among the upper stories of the evergreen lowland rainforest. The family is most dense in the highest size classes in places where it is floristically the richest. At their edaphic optimum, dipterocarps can comprise more than 80% of the emerging individuals and up to 40% of below canopy trees (Chua et al. 2010). 92 dipterocarp taxa (56.1%) found on the peninsula are of a threatened category nationwide; 22 of them are endemic to the peninsula. Of the 92 taxa, eleven are not threatened on the peninsula, but are threatened in Sabah and Sarawak. Thirteen taxa are threatened on the peninsula but not in Sabah and Sarawak. Of the fifteen critically endangered (CR) taxa, six are endemic to the Peninsular Malaysia. Of the 35 endangered (EN) and 42 vulnerable (VU) taxa, 10 and six, respectively, are endemic to Peninsular Malaysia. Trees from Dipterocarpaceae family are known to have the best quality wood in the world. Most of the trees from this family have high values, both in terms of wood density and price. The density of wood varies depending on type and growth environment of the trees. The parts of the tree also have different densities: branches usually have a lower wood density than the trunk. The density of the wood is also one of the important parameters for determining the aboveground biomass (AGB) and the carbon stock of the trees. Although Chave et al. (2004) found that the most important source of error in estimating the AGB currently lies in the choice of the allometric model, but the stand-level specific wood gravity still explained 45.4% and 29.7% of the total variation in the AGB (Slik, 2006). Wood specific gravity also known as wood density, measures the amount of actual wood material in a unit volume of wood. It is the ratio of a mass of oven-dry wood divided by the green volume of the wood. The common unit of wood density is g cm<sup>-3</sup> or kg m<sup>-3</sup>. Wood density varies depending on the species of trees, and normally wood quality is classified based on the wood density, i.e., heavy hardwood, medium hardwood, light hardwood, and softwood. There is a growing need for accurate estimates of tree biomass on a large spatial scale. Carbon stocks are also recognized as one of the most important bioclimatic variables in balancing the global climate and are always associated in climate change impact assessment and mitigation measures. Wood density is an important part of these biomass estimates. There is a growing need for accurate estimates of tree biomass on a large spatial scale. Carbon stocks are also recognized as one of the most important bioclimatic variables for global climate compensation and are always included in the impact assessment and mitigation measures of climate change. Wood density is an important part of these biomass estimates. The average wood density of trees in Southeast Asia is 0.574 (±0.151) g cm<sup>-3</sup>. This average value was calculated from 3,648 species, which produced measures of wood density in the range from 0.080 to 1.095 g cm<sup>-3</sup> (Chave et al. 2009). This was further elaborated by Rosaizan and Gang (2013), who found that the average value for dipterocarp trees was at 0.451 for trees with a diameter at breast height (DBH) of 10-30 cm and 0.564 for trees > 30 cm in DBH. Although the carbon stock in dipterocarp trees is believed to have a higher composition than others, the growth rate of these trees is slow. Consequently, the rate of growth will somehow affect the rate of carbon sequestration by these trees. King et al. (2007) and Ludwig et al. (2002) came to the conclusion that the growth increment of dipterocarps species in Malaysia was between 2.0 to 5.0 mm yr<sup>-1</sup>. This is far slower

than the other non-dipterocarp and fast-growing species, which has faster growth of more than 1 cm yr<sup>-1</sup>. However, under controlled environment and treated planting, growth can be faster even for dipterocarp species, such as described by Ahmad Zuhaidi et al. (2004) that the growth increment for Dryobalanops aromatica was around 0.96-1.09 cm yr<sup>-1</sup> for trees  $\geq$  30 cm in DBH. Osunkoya et al. (2007) and King et al. (2005) stated that the wood density has a negative proportion to the annual growth rate of trees (Figure 1). This means that the higher the wood density, the slower the growth rate. The photosynthetic process is slow, which makes carbon sequestration very slow, but ultimately these trees stored much more carbon than trees with low wood density and fast-growing species. The species that has low wood density also tend to have a high mortality rate and die quickly at early age (i.e., 10-20 years) compared to species with high wood density which can live longer (i.e., up to hundreds of years) [Table 1]. These relationships were further elaborated by Phillips et al. (2019), who found that wood density, trunk volume and biomass are all inter-related to each other, as described in Figure 2. In summary, a list of carbon sequestration rate is produced based on the Red List dipterocarp species from Chua et al. (2010) by using allometric equation from Chave et al. (2004) and the wood density values from Zanne et al. (2009) [Table 2]. It is obvious that dipterocarp species store more carbon than that of non-dipterocarp species. In contrast, the rate of carbon sequestration in fast-growing species is much faster than in dipterocarp species.



Figure 1: Patterns of covariation among plant traits using species as independent data points: (a) stem mortality versus wood density; (b) stem growth versus wood density; (c) stem mortality versus stem growth

Table 1: Relationship b	between wo	od density,	growth rate,	carbon	sequestration,	mortality
		and lifespa	in of trees			

Wood Density	Carbon Stock	Growth	Carbon	Mortality	Lifespan
$(g \text{ cm}^{-3})$	$(Mg C ha^{-1})$	Rate	Sequestration	(%)	(Years)
	-	$(mm yr^{-1})$	$(Mg C ha^{-1} yr^{-1})$		
High	High	Slow	Slow	Low	Long
Low	Low	Fast	Fast	High	Short



Figure 2: Direct measurement of tropical trees shows that wood density and size each independently control biomass

IUCN Status	Family/Species	Wood Density (g cm <sup>-3</sup> )	Growth Rate* (mm yr <sup>-1</sup> )	Carbon Stock** (kg C tree <sup>-1</sup> )	Carbon Sequestration (kg C tree <sup>-1</sup> yr <sup>-1</sup> )
	Dipterocarpaceae				
CR	Dipterocarpus coriaceus	0.59	3	332	8.6
-	Hopea auriculata	0.74	3	420	10.9
	Parashorea globosa	0.58	3	329	8.6
	Shorea hemsleyana	0.65	3	369	9.6
	Shorea lumutensis	0.92	3	522	13.6
EN	Anisoptera marginata	0.53	3	299	7.8
	Dipterocarpus dyeri	0.64	3	363	9.4
	Dipterocarpus sublamellatus	0.65	3	369	9.6
	Dryobalanops beccarii	0.50	3	286	7.4
	Hopea apiculata	0.86	3	488	12.7
	Hopea bracteata	0.56	3	315	8.2
	Shorea blumutensis	0.72	3	408	10.6
	Shorea curtisii	0.53	3	299	7.8
VU	Anisoptera costata	0.55	3	314	8.2
	Dipterocarpus costatus	0.76	3	428	11.1
	Dipterocarpus kerrii	0.61	3	346	9.0
	Hopea glaucescens	0.71	3	403	10.5
	Hopea johorensis	0.55	3	312	8.1
	Shorea foxworthyi	0.82	3	463	12.0
	Shorea johorensis	0.39	3	218	5.7
	Shorea johorensis	0.41	3	233	6.0
	Shorea materialis	0.75	3	423	11.0
	Vatica maingayi	0.78	3	440	11.4
NT	Shorea roxburghii	0.70	5	397	17.3
	Non-Dipterocarpaceae				
VU	Swietenia macrophylla	0.52	5	295	12.8
NT	Intsia palembanica	0.66	5	374	16.3
LC	Hevea brasiliensis	0.47	10	265	23.3
NT	Agathis borneensis	0.38	5	217	9.5
LC	Acacia mangium	0.51	10	287	25.4
LC	Macaranga gigantea	0.29	10	164	14.5
LC	Macaranga beccariana	0.29	10	164	14.5
n.a.	Koompassia malaccensis	0.76	5	431	18.8

**Table 2**: Rate of carbon sequestration by Red List dipterocarp and the selected non-dipterocarp trees listed under International Union for Conservation of Nature (IUCN)

\*Assuming growth rate at 3 mm yr<sup>-1</sup> for slow-growing, 5 mm yr<sup>-1</sup> for intermediate, 10 mm yr<sup>-1</sup> for fast-growing species.

\*\*Calculation based on tree at DBH of 30 cm

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## Assessment of carbon sequestration potential of Red List tree species in Tekam free air CO<sub>2</sub> enrichment plot at Tekam Forest Reserve

Azian M<sup>\*1</sup>, Nik Norafida NA<sup>1</sup>, Nizam MS<sup>2</sup>, Wan Mohd Shukri WA<sup>1</sup>, Mohd Puat D<sup>3</sup>, Samsu Anuar N<sup>4</sup> & Mohd Zarin R<sup>5</sup>

<sup>1</sup>Forestry and Environment Division, Forest Research Institute Malaysia, 52109, Kepong, Selangor

<sup>2</sup>Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor

<sup>3</sup>Forest Management Division, Forestry Department of Peninsular Malaysia, Jalan Sultan Salahuddin, 50660 Kuala Lumpur

<sup>4</sup>Pahang Forestry Department, 5<sup>th</sup> floor, Kompleks Tun Razak, Bandar Indera Mahkota, 25990 Kuantan, Pahang.

<sup>5</sup>District Forest Office, Jerantut Batu 1, Jalan Benta, 27000 Jerantut, Pahang. \*Corresponding author's email: azyan@frim.gov.my

The global average atmospheric carbon dioxide is higher today than it has ever been in the last 800,000 years. The CO<sub>2</sub> content will continue to rise in the coming years. Therefore, 2021 is expected to be the first year on record that sees  $CO_2$  levels of more than 50% above pre-industrial levels. A free-air CO<sub>2</sub> enrichment (TekamFACE) experiments was established at Compartment 84, Tekam Forest Reserve, Jerantut, Pahang to study the effects of elevated CO<sub>2</sub> on plants and ecosystems grown under natural conditions. Recognizing Malaysia as a megadiverse tropical country that is rich in resources and its conservation of which is frequently and substantially challenged by socioeconomic priorities (Yong et al. 2021). Therefore, our aim was to look at the conservation status of the tree species in TekamFACE and their ability to store carbon in the higher  $CO_2$  concentration environment. The TekamFACE system was built on a 25 x 25 m plot in the shape of a hexagon, 6 m long on each side and 12 m high. All tree species 15 cm or more in height were measured, identified and compared to the IUCN list to evaluate the conservation status. Species classified as critically endangered (CR), endangered (EN) and vulnerable (VU) are collectively referred to as threatened. A total of 1,108 individuals represented by 84 species from 25 families were listed on the Red List of Threatened Species. Only one species, Aquilaria malaccensis (Thymelaeaceae) has been classified as critically endangered on the Red List while nine species of Aglaia leptantha (Meliaceae), Cynometra malaccensis (Fabaceae), Enicosanthum fuscum (Annonaceae), Horsfieldia sucosa (Myristicaceae), Horsfieldia fulva (Myristicaceae), Palaquium hexandrum (Sapotaceae), Payena lucida (Sapotaceae), Pouteria malaccensis (Sapotaceae) and Shorea longisperma (Dipterocarpaceae) were classified as near threatened. Five species of Beilschmiedia dictyoneura (Lauraceae), Gonystylus maingayi (Thymelaeaceae), Hopea glaucescens (Dipterocarpaceae), Kokoona littoralis (Celastraceae) and Palaquium maingavi (Sapotaceae) were classified as vulnerable and the remaining 69 species were categorized as least concern on the Red List (Table 1). Yong et al. (2021) listed 1,353 taxa in the threatened category of the IUCN Red List. Thus, 6.21% of the threatened species of Peninsular Malaysian occurred in the study area.

Table 1: The number of species in TekamFACE plot categorized in IUCN conservation status

IUCN Red List Status	Number of Species
Critically endangered (CR)	1
Near threatened (NT)	9
Vulnerable (VU)	5
Least concern (LC)	69

Among the species on the TekamFACE IUCN Red List, a greater percentage of the total biomass was accounted by the above ground biomass, corresponding to 83% (232.12 t ha<sup>-1</sup>), while the remaining 17% (47.39 t ha<sup>-1</sup>) was credited to the root biomass. The total carbon that was sequestered and stored in the biomass of the TekamFACE IUCN Red List species was 131.46 tC ha<sup>-1</sup>, equivalent to  $482.03 \text{ t } \text{CO}_2 \text{ ha}^{-1}$ . Based on 25 families encountered, Dipterocarpaceae achieved the highest total biomass and carbon storage with 218.17 t ha<sup>-1</sup> and 102.54 tC ha<sup>-1</sup>, respectively, with an equivalent of 375.98 t CO<sub>2</sub> ha<sup>-1</sup>. This was followed by Fabaceae with a biomass of 39.4 t ha<sup>-1</sup> and a carbon stock of 18.52 tC ha<sup>-1</sup> (Table 2). Dipterocarpaceae contributed the most in the carbon stock because their trees are larger in diameter and heights compared to other families (Nor Farika et al. 2018). The estimates of biomass and carbon density obtained in this study suggest that the species on the IUCN Red List in TekamFACE not only require more attention and additional consideration for the purpose of biodiversity conservation but also represent a large part of carbon stocks in terrestrial ecosystems and they play an important role in regulating global carbon and nutrient cycles. The assessment of these species will continue as this study (TekamFACE) will continue to monitor whether they can maintain their presence and the value of carbon stocks as CO<sub>2</sub> levels in the environment continue to rise.

Family	Above-ground	Root	Total biomass	C stock	CO <sub>2</sub> equivalent
		(t ha <sup>-1</sup> )		$(tC ha^{-1})$	$(t CO_2 ha^{-1})$
Dipterocarpaceae	181.05	37.12	218.17	102.54	375.98
Fabaceae	32.70	6.70	39.40	18.52	67.90
Sterculiaceae	5.00	1.03	6.03	2.83	10.39
Myristicaceae	4.05	0.83	4.88	2.30	8.42
Icacinaceae	1.91	0.39	2.30	1.08	3.96
Chrysobalanaceae	1.62	0.33	1.95	0.92	3.36
Burseraceae	1.32	0.27	1.59	0.75	2.74
Lauraceae	1.28	0.26	1.54	0.72	2.65
Olacaceae	0.70	0.14	0.85	0.40	1.46
Phyllanthaceae	0.51	0.11	0.62	0.29	1.07

 Table 2: Total biomass, carbon stock and equivalent CO2 of ten leading families with IUCN conservation status of tree species at TekamFACE

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### Enhancing carbon sequestration potential by *ex-situ* plantations

Chauhan A<sup>\*1</sup> & Clarke A<sup>2</sup>

<sup>1</sup> Sage Himalaya Consultancy, India <sup>2</sup> University of Central Lancashire, United Kingdom \*Corresponding author's email: achauhan189@gmail.com

Carbon dioxide, a by-product of wood and fossil fuel burning, is the main greenhouse gas contributing to global warming. The impact of climate change mainly caused by greenhouse gases in Peninsular Malaysia, among others, could pose a serious threat to the ecosystem and coastal communities (flora and fauna) due to the rise of sea level and tidal inundation. Based on the IUCN's 2008 Red List, Malaysia is home to 1,141 threatened species, including plants and animals. A total of 567 plant species of the 1,600 Peninsular Malaysia plant species assessed on the Malaysia Red List have been classified as threatened (CBD). Threats to biodiversity in Malaysia include threats to ecosystems and species, such as land development, pollution, poaching, encroachment, climate change and invasive alien species. The main drivers of these threats are: economic growth; increase demand for food and agricultural land. Developed seedlings from critically endangered and vulnerable species need to be established and maintained in the natural habitats in which the species were originally found. To target biological sequestration in Figure 1, seed gene banks for forest species were set up which are not appropriate as most of the plants produce recalcitrant seeds that cannot be stored for long. Research is carried out at various institutions around the world to explore the possibilities of using cryogenic and in vitro techniques for long-term gene conservation of tree species. This can also be achieved by using native microalgae species instead of common species to expedite the acclimatization period and ease-out the ex-situ biological carbon dioxide fixation process due to the robust and conducive environment for optimal growth of the species (Yahva et al. 2020). Moreover, ex-situ conservation practices can be accelerated by introducing regular monitoring of habitats with high conservation and socio-economic values, public participation, planting campaigns and restoration of degraded habitats. The degraded land and habitats need to be restored first through planting of grasses and later with seedlings of the species to natural succession and promotion of medicinal plants (MPs) in cultivation: prioritization of the high value medicinal plants for the conservation and socio-economic development (agroforestry, plantation of fruits, fodder of economic species) of the inhabitants of the region would contribute to ecosystem services. The initial task should include surveying degraded land available for plantation. The quick and fast method is to take aerial photograph with GIS techniques using satellite or drone. Then look for ground truthing and identify those areas (Ramli et al. 2021). The next step is to determine the species and their quantum requires to be reintroduced. The requisite perfect technique for the regeneration of such species that are on the Red List needs to be implemented. The planting material in the form of seeds and seedlings needs to be made available. Sowing of seeds and planting of seedlings should be done during suitable rainy season. The protection of regeneration and plantations can be achieved through public campaigns and participation of primary stakeholders and local institutions. It is necessary to protect the regeneration and seedlings, and to perform causality replacement until the seedlings able are to grow independently. The methods can be further developed according to a detailed impact assessment carried out on land available for planting.



Figure 1: Carbon sequestration methods (Yadav & Mehra, 2021)

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## Survival and growth of Aquilaria crassna, Cotylelobium lanceolatum, Neobalanocarpus heimii and Vatica diospyroides grown on ex-mines in two climatic zones at 42 months after planting

 Whuangplong P\*<sup>1</sup>, Nongnuang S<sup>1</sup>, Chadthasing B<sup>1</sup>, Nuchit S<sup>1</sup>, Intasen M<sup>1</sup>, Ratcharoen W<sup>1</sup>, Chandang W<sup>2</sup>, Tang LK<sup>3</sup>, Ho WM<sup>3</sup> & Ang LH<sup>3</sup>
 <sup>1</sup> Royal Forest Department, Bangkok Thailand 10900
 <sup>2</sup> Kasetsart university, Bangkok Thailand 10900
 <sup>3</sup> Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia
 \*Corresponding author's email:pyf49@hotmail.com

Four endangered, endemic and threatened species (EETS) were planted at an ex-mine located in dry climatic zone of Lampang Province, Northern Thailand and humid climatic zone of Phang Nga Province, Southern Thailand. The soil of northern plot is a well-drain loam but the southern plot is an impoverished sand, hence, northern plot has better soil properties for growing plants than the southern plot. The survival count and growth parameters were measured for the four EETS including *Aquilaria crassna, Cotylelobium lanceolatum, Neobalanocarpus heimii* and *Vatica diospyroides*. Both plots were tended with similar tending activities including loosening of soils, weeding and fertilizer applications. The survival and growth performances of the four EETS were compared at 42 months after planting. The findings show that all four EETS had better survival and vegetative growths in humid southern plot than the cold and dry northern plot despites growing on the poorer soils of sand tailings (Table 1 and 2). Both top height and relative height growths of the four EETS were due mainly to severe die-back of apical shoot during its dry period and in cold climate, especially for *C. lanceolatum* and *N. heimii*. The best adapt EETS to dry climatic zone is *V. diospyroides* but *A. crassna* grown well only in the humid southern plot.

Table 1: Average growth rate and mean relative growth rate of 4 EETS in Takua Pa and Mae Moh

Tree species	Survival (%)		He	ight (m)	RGR	
	Takua Pa	Mae Moh	Takua Pa	Mae Moh	Takua Pa	Mae Moh
Aquilaria crassna	$44.00 \pm 20.40$	19.33±16.43	2.49±0.39	$0.99 \pm 0.57$	0.12	0.02
Cotylelobium lanceolatum	$69.00 \pm 08.25$	$18.00 \pm 03.58$	$1.24\pm0.23$	$0.34 \pm 0.07$	0.10	-0.03
Vatica diospyroides	76.00±07.30	62.33±16.27	$2.59\pm0.51$	$1.41\pm0.13$	0.09	0.04
Neobalanocarpus heimii	$56.00{\pm}11.78$	$02.33 \pm 02.34$	$1.63\pm0.34$	$0.34\pm0.34$	0.07	-0.02



Figure 1: Annual rainfall comparing between sites at Mae Moh site and Takua Pa

	Plot site			
Properties	Mae Moh	Takua Pa		
Soil level	0-50 cm	0-15 cm	15-30 cm	> 30 cm
рН	7.59 (slightly alkaline)	5.21(strongly acid)	5.92(moderately acid)	5.90(moderately acid)
OM (%)	$6.20 \pmod{\text{moderately low}}$	3.65(low)	0.37(very low)	0.19(very low)
Avai.P(mg/kg)	2.15 (low)	$211.90 (very \ high)$	5.51(low)	1.83(very low)
Exch.K(mg/kg)	126.28 (high)	18.15 (very low)	3.74 (very low)	2.36(very low)
Exch.Ca(mg/kg)	13,559 (high)	974.63(very low)	127.05(very low)	99.76(very low)
Exch.Mg (mg/kg)	804 (low)	114.76(very low)	65.24(very low)	2.51(very low)
Exch.Na (mg/kg)	63.43 (very low)	31.24(medium)	21.70(low)	18.94(very low)
CEC (cmol/kg)	18.87 (moderately high)	7.01(moderately low)	1.88(very low)	1.44(very low)
BS (%)	405 (medium)	70.69 (medium)	64.08(medium)	47.96(medium)

Table 2: Average values of chemical soil properties of sites at Mae Moh and Takua Pa

Humid climate zone at Takua Pa plot site and dry climate zone at Mae Moh plot site displayed high difference of average annual rainfall of five years. Takua Pa plot site displayed 3,170.4 mm, while Mae Moh plot site 1,063.4 mm (Figure 1). Both plots also had soil texture differences, whereby Takua Pa plot site was sandy, acidic, low organic matter, very low potassium but very high phosphorus while Mae Moh plot site was clayey, weakly alkaline, very low organic matter, very high potassium and low phosphorus. The results of two plot sites show that *C. lanceolatum* and *N. heimii* are not suitable to be domesticated in dry climate zone but it can be domesticated in humid climate zone even though in disturbed areas. *Aquilaria crassna* Pieere ex. Lecomte recorded the highest relative growth rates compared to others.

#### Seed quality testing for several threatened dipterocarps

Noraliza A\*, Nashatul Zaimah NA, Nor Asmah H & Fadzlinah Z

Seed Technology Laboratory, Forestry Biotechnology Division, Forest Research Institute Malaysia \*Corresponding author's email: noraliza@frim.gov.my

The Dipterocarpaceae family dominates tropical rainforests including Peninsular Malaysia. Tropical rainforests play a vital role in timber production, biological conservation, carbon sequestration and global climate regulation (Beer et al., 2010). The reduction in forest cover is leading directly to the endangerment and local extinction, including dipterocarp trees. One of the factors that contribute to the extinction is high demand for dipterocarps in the international tropical timber market which play an important role in the economy of many Southeast Asian countries. In addition, dipterocarp trees also produce other non-timber products like resins and oleoresins. Forest degradation in dipterocarp forest is affecting conservation status of dipterocarps. Dipterocarps that are involved in supra-annual mast flowering events will reproduce in gregarious mast-fruiting events once every 2-11 year (Ashton et al. 1988). The seeds are recalcitrant and if not handled properly, their viability will be reduced after collection. Mast flowering and fruiting are visually stunning and can be measured in harvest records for dipterocarp species, as we saw this year at Forest Research Institute Malaysia (FRIM), Kepong, Selangor, Malaysia. Most dipterocarp species are categorised as endangered or critical threatened by the loss of significant tracts of forest, according to the IUCN Red List of Threatened Species (IUCN, 2017). Clearly, given these values and dangers, the group needs improved management in terms of population maintenance and conservation. Some of the collected dipterocarp species in FRIM are protected species with status as in Table 1.

Species	Conservation status	Last assessed
Dipterocarpus elongatus	Critically endangered (CR)	1998
Dipterocarpus semivestitus	Critically endangered (CR)	1998
Hopea sangal	Vulnerable (VU)	2017
Shorea argentifolia	Least concern (LC) (population trend decreasing)	2019
Shorea macroptera	Least concern (LC) (population trend decreasing)	2017
Shorea multiflora	Least concern (LC) (population trend decreasing)	2018

#### Table 1: Conservation status of dipterocarps collected in FRIM

The origin of seed lots were collected from FRIM. Seed testing is required to comprehend the various components of seed's physical and biological features. This technique will either determine the quality of planting-ready seeds or discover a seed quality issue. Seed germination (GT) and moisture content (MC) are factors that can determine the overall seed viability and moisture of seed lot.

This study focuses on seed germination and moisture content in six dipterocarps. Results are as shown in Figure 1.



Figure 1: Germination and moisture content test of dipterocarps seeds

Seed procurement efforts are frequently hampered by a lack of knowledge about suitable seed handling techniques, particularly for seeds that quickly lose viability (recalcitrant). Species with recalcitrant seeds that dominate the climatic tropical forest are the most essential in restoration programmes, but significant amount of high-quality seeds are difficult to get by. Despite the large number of seeds accessible, the majority of them have died, demonstrating that stubborn (recalcitrant) seed characteristics remain an issue in seed procurement.

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## Preliminary findings on soil properties in the natural *Rafflesia* habitat and experimental plot in Gerik, Perak

 Nur Hafiza AH\*<sup>1</sup>, Rozita A<sup>1</sup>, Mohamad Fakhri I<sup>1</sup>, Muhammad Asri L<sup>1</sup> & Noorsiha A<sup>2</sup>
 <sup>1</sup>Soil Management Branch, Forest Plantation Programme, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia
 <sup>2</sup>World Heritage Site, Forestry and Environment Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia
 <sup>\*</sup>Corresponding email: nurhafiza@frim.gov.my

In 1997, Rafflesia was listed on the IUCN Red List of threatened plants being classified as endangered (R. manillana), vulnerable (R. keithii and R. pricei), rare (R. cantleyi, R. kerrii and R. zoolingeriana) and intermediate (R. hasseltii). However in 2016, the status of R. manillana was changed to critically endangered. *Rafflesia* spp are holoparasitic plants, they have no roots or leaves and are dependent on a certain species of the *Tetrastigma* vine as a host for their growth. *Rafflesia* spp. are found in the rainforests of Southeastern Asia including Malaysia (Toda & Masuda, 2014). Preserving their natural habitat will help endangered species continue their existence in the world. Understanding the state of their environment, such as the soil, will conserve these species by replanting them in a different area. However, little information is available about the soil properties of the Rafflesia habitat. This paper provides information about the selected soil properties of its natural habitat and is compared with those of the established plot in Gerik, Perak. The description of the study area is shown in Table 1. Composite soil samples were taken at two different depths; 0-25 cm and 25-50 cm. Soil samples were collected in the experimental plots in the *Tetrastigma* planting areas and also in the natural habitat where *Rafflesia cantleyi* was actively bloomed. The experimental site with *Tetrastigma*, EXP1 and EXP2, were established in 2018. The collected soil was sent to the laboratory for various soil analysis. Soil pH was measured in a pH meter using a 1 : 2.5 ratio of soil to water. The total nitrogen (N) in the soils was determined by Kjedahl digestion followed by distillation and titration process. Organic carbon (C) was analyzed using the Wakley and Black titration method. Available phosphorus (P) was based on Bray and Kurtz No. 2 procedure. The exchangeable potassium (K) from the soil was extracted by the 1N ammonium acetate leaching method and its concentration was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES).

Plot	Description
EXP1	Experimental plot of <i>Tetrastigma</i> planting
EXP2	Experimental plot of <i>Tetrastigma</i> planting
NAT1	Natural habitat of <i>Rafflesia</i>
NAT2	Natural habitat of <i>Rafflesia</i>

 Table 1: Description of the study plot

The results of the soil properties of the study plots, NAT and EXP are shown in Table 2. The soil in the natural habitat and the experimental plots showed pH values in the range of 3.97 - 4.35 and 3.98 - 4.28, respectively, indicating acidic soils. It has been observed that the pH value increases with depth in all the study plots. The addition of organic material from plants could lead to the formation of organic acids and thus lower the pH of the soil. Sánchez (1976) stated that increases in soil pH with depth may be related to the decrease in organic matter and this is agreeable with the organic carbon result here. Carbon and nitrogen levels in Nat and EXP plots were generally low and their levels decreased with depth. The first 25 cm have almost 50% more C compared to the underneath soil layer in all the study area. The same pattern was observed for nitrogen and this was consistent with other findings that N is related to C content (Jupri, 2011). However, it was found that the soil in the NAT1 and NAT2 plots had the same N concentration (0.10%) over the entire depth of the soil.

This could be related to the fact that the N content in the natural habitat has stabilized over time and the optimal growth of *Tetrastigma* was achieved. The available P in the soil at all the study locations was found to be generally very low thus the soil can be considered as low fertility soil. This is expected because the soil condition is acidic, where the available P is closely related to the soil pH. However, it was observed that the P levels in the NAT plots were higher compared to the EXP plots. This tallies with low C in NAT plots which could be due the decomposition of organic material that releases nutrients to the soil. However, P, total N and C in the soil of the study plots in NAT and EXP were below sufficient levels required for plant growth. An almost similar concentration of K was found along the soil depth in the NAT and EXP plots. The levels of exchangeable K in the soil were also very low in all study areas, which was below the adequate range. The findings of the study show that the host plant *Tetrastigma* can grow in acidic soil with low presence of N, P, C and K content. The organic C and nitrogen in the soil from the experimental area were slightly higher, but less P compared to their natural habitat. This study will continue to investigate the microbial population in the soil that can influence the growth of *Tetrastigma* as an important host for the growth of *Rafflesia*.

Depth	pН	Ν	Organic C	Avail. P	Exch. K			
	-	(%)	(%)	(ppm)	(cmol/kg)			
	EXP1							
0 - 25 cm	4.21	0.15	1.13	0.73	0.02			
25 - 50 cm	4.35	0.08	0.46	0.35	0.03			
		EXI	P2					
0 - 25 cm	3.97	0.11	1.10	1.15	0.02			
25 - 50 cm	4.03	0.08	0.69	0.68	0.02			
NAT1								
0 - 25 cm	4.14	0.10	1.09	1.25	0.02			
25 - 50 cm	4.28	0.10	0.55	1.23	0.02			
NAT2								
0 - 25 cm	3.98	0.10	0.86	1.75	0.02			
25 - 50 cm	4.03	0.10	0.53	1.55	0.02			

**Table 2**: Soil properties at nature and experimental plot in Gerik, Perak

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## Increasing tree diversity index through *ex-situ* conservation of endangered, endemic and threatened tree species on an ex-tin mine in Peninsular Malaysia

Tang LK\*, Ho WM & Ang LH

Ecophysiology Branch, Forest Plantation Programme, Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia. \*Corresponding author's email: tlkuen@frim.gov.my

Restoration of extremely degraded sites has a common goal like other ecosystem restoration projects which is the recovery of lost biodiversity (Bakker et al. 2020). Ex-tin mine limits the colonisation processes and also colonizer species (Mitchell, 1959; Kochumen, 1966; Kochumen & Ng, 1977). Due to lack of seed source and dispersal agents of climax forest tree species that are classified under IUCN Red List of Threatened Species. A planting project was carried out under a regional project sponsored by ASEAN-ROK Forest Cooperation Project (AFoCo). The study was carried out in Tin Tailing Afforestation Center, Forest Research Institute Malaysia Research Station at Bidor, Perak. The plantation plot was established in 2001. The ex-tin mine was improved through providing a proper drainage with 2 m width and 2 m depth. After watered-logged slime tailing was dried, the site was loosened to a depth of 50 cm depth from the surface and the planting bed raised to a height of 30 cm above the ground level. Later, the improved site was planted with three timber tree species. All the tree species in the plot higher than 2 m top height and diameter at breast height > 1 cm were identified and tagged. The distribution of each tree was positioned using a Global Positioning System (GPMAP60CSx). The species were further divided into three groups namely natural regeneration (NRSP), plantation species planted in 2001 (PLSP) and endemic, endangered and threatened tree species introduced in 2019 (EETS). The diversity index of the three groups of tree species in the greened ex-tin mine was determined using Shannon Index (Shannon, 1948). The H and EH values of NRSP, NRSP+PLSP, and NRSP+PLSP+EETS were computed for comparison of their diversity index and its evenness.



**Figure 1**: The blue dot represents the existing nurse trees and natural regeneration, the green dot represents the introduced endemic, endangered and threatened tree species (EETS) and the red dot represents the dead EETS

The distribution map of the nurse stands and EETS are shown in Figure 1. Mortality of EETS is indicated in the GPS distribution map.

An assumption is made that the naturally regenerated species group (NRSP) after site improvement could be established with and without the planted three timber species. The nurse stand was further enriched with 18 EETS at 2019. The assessment of Shannon diversity index (H) was made and the changes of H value of NRSP with PLSP and the addition of EETS are as shown in Table 1. The increase of H and EH taking into the consideration of the planted forest tree species (PLSP) with the natural regeneration (NRSP) was 33.1 and 23.2% respectively. The addition of EETS had increased H and EH of the forest plantation and the natural regeneration by 36.9 and 24.6% respectively. The planting of PLSP and EETS had increased the H and EH by 82.2 and 53.6%, respectively.

**Table 1**: The changes of Shannon diversity index (H) and Shannon evenness index (EH) incomparing between the tree groups NRSP (Natural regeneration), PLSP (planted plantation species<br/>at 2001) and EETS (planted endemic, endangered and threatened tree species in 2019)

Types	Species	Quantity	Н	EH	Change H (%)	Change EH (%)
Type 1 NRSP	20	772	1.69	0.56	0	0
Type 2 NRSP + PLSP (Type 2 vs Type 1)	26	1315	2.25	0.69	33.1	23.2
Type 3 NRSP+PLSP+EETS (Type 3 vs Type 2)	44	2932	3.08	0.86	36.9	24.6
Type 3 vs Type 1					82.2	53.6

The enrichment planting had increased 40.9% on the species richness of the nurse stand. The forest plantation or nurse stand without the enrichment planting with EETS had Shannon Index (H) slightly higher than the range of secondary forests 1.29 to 1.50 in the tropics (Khairil et al. 2011). The natural regeneration has increased the species diversity of the forest plantation of only six species into a mature secondary forest. The natural regeneration accounted for the 24 tree species found in the nurses stand (Table 1). Introducing 1,650 trees comprising 18 EETS to the 3.6 ha plot in 2019 had increased the H value of the nurse stand to 3.08. The EETS had mortality of 2% leaving only 1617 stems. There was an increase of 36.9% in H value of the nurse stand after EETS were introduced. However, the Shannon evenness index (EH) increased only by 24.6% (Table 1). The lowland dipterocarp natural forests had H value range of 3.38 to 5.40 depending on forest types and their site properties (Suratman et al. 2010). The planting of EETS through the project does not increase the H value to the level of the natural forests. The 3.6 ha greened ex-tin mine plot shall be the seed production area of the EETS. The H and also EH of the enriched stand may increase with time. However, the species richness of the stand could only be increased through seeds brought by avian seed dispersers or further planting of more EETS.

The *ex-situ* conservation of the 18 EETS had increased about 36.9% of the Shannon Index of the greened ex-tin mine. The Shannon Evenness Index (EH) was increased to from 0.69 to 0.89 after including the 18 EETS. The Shannon index (H value) of conservation plot is now greater than the secondary forest but lower than the natural forest. The species richness is now at 44 species. More importantly, the ex-tin mine can also now be a seedproduction area of the EETS.

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# Preliminary study on tissue culture protocol development for *Neobalanocarpus heimii* (Chengal)

Ris Amirah AM\*, Nor Hasnida H, Muhammad Fuad Y, Saifuldullah AW, Nazirah A, Rozidah K, Rohani A, Sabariah R, Tengku Nurul Munirah TAR, Naemah H & Rukiah, M *Tissue Culture Laboratory, Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia* \*Corresponding author's email: risamirah@frim.gov.my

Neobalanocarpus heimii, locally known as Chengal is a well-known heavy hardwood timber species, it is also categorized as vulnerable (VU) in Peninsular Malaysia due to the extreme demand for its timber and poor regeneration of the species. Chengal is considered the number one wood (classified as heavy hardwood) of Malaysia and export of logs is prohibited due to its scarcity. It is classified by IUCN as 'vulnerable' but not critically endangered (Chua, 2010). Therefore, with the increasing demand for wood in Malaysia's furniture industry, it is timely to consider and explore the possibilities of conservation programs for the species. FRIM has made proactive initiative to produce tissue culture plants of N. heimii for the production of planting material purposes. Establishment of clean culture is a very important stage for tissue culture protocol development. This study aims to find the best technique of surface sterilisation protocol for the establishment of N. heimii clean culture. In this study, seeds were used as explants and two different methods of surface sterilisation were used. N. heimii seeds (Figure 1) were collected at FRIM, Kepong. Seeds were thoroughly washed under running tap water with liquid detergent and immersed in 70% Ethanol for 3 minutes, and rinsed with sterile distilled water for three times. For method A, seeds were then immersed in 60% Clorox® for 25 minutes, rinsed with sterile distilled water then dried and cultured into MS basal medium containing 0.1 mg/L Benzyl amino purine (BAP). In method B, after rinsing with sterile distilled water, seeds were immersed in 60% Clorox® for 25 minutes, then rinsed with sterile distilled water. After that, seeds were cleaned further with 10% Clorox® for 10 minutes and then the seeds were flamed about 5 seconds before being cultured into MS basal medium supplemented with 0.1 mg/L BAP. All cultures were incubated in the growth room with conditions of  $22 \pm 2^{\circ}C$ , 2000 Lux and 16-hour light. Observations on culture contamination and germination were recorded every week. After three weeks in culture, results showed that Method A produced 71.2 % clean culture whereas Method B produced 66.7% clean culture. However, germination rate for both treatments were 100% (Table 1). The clean culture and germinated seeds of N. heimii were shown in Figures 2 and 3. Seeds of N. heimii started to germinate as early as three days after cultured. Method A produced higher percentage of clean culture compared with Method B. All types of explants including seeds, spadices or spathes and leaves have been frequently surface sterilised with ethanol(70%) (Teixeira da Silva et al. 2015). The result of this study showed that, three minutes' exposure time to 70% of ethanol is considered long enough, however was not effective in removing contaminants from the seeds of N. heimii. This may be due to the decontaminant toxicity as the concentration increased (Rodrigues et al. 2013). Double surface sterilization with Clorox® may also be toxic to the explants. Therefore, in the next study, the exposure time to 70% ethanol might be reduced from three minutes with using only single surface sterilisation. N. heimii germinated seeds obtained will be used as explants for further shoot multiplication study.

Surface sterilisation method	Clean culture (%)	Germination (%)
А	71.15	100
В	66.67	100

Table 1: Percentage of clean culture and germination of Neobalanocarpus heimii seeds explants



Figure 1: Neobalanocarpus heimii seeds



Figure 2. Clean culture of Neobalanocarpus heimii



Figure 3: Germinated seed of Neobalanocarpus heimii

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## Biomass quantification of Chengal seedlings planted using innovative planting technique in logged-over forest

Farah Shahanim MM\*, Nurcahaya Khairany MA & Zahirah MT Forest Research Institute of Malaysia (FRIM), 52109, Kepong, Selangor, Malaysia \*Corresponding author: farahshahanim@frim.gov.my

Enrichment planting is a technique in which seedlings of preferred timber trees are planted in the under-storey of existing logged-over forests and then given preferential treatment to encourage their growth (Paquette et al. 2009). However, previous studies have shown that the survival and growth rate of seedlings using enrichment planting has been very poor and the costs of maintenance was high, due to the need for repeated post-planting treatments. As a result, an improved planting technique was applied to sustain excellent plant growth with minimum post-planting tending. Other than that, forest biomass accounts for over 45% of terrestrial carbon stocks, with approximately 70% and 30% contained within the above and belowground biomasses, respectively (Ngo et al. 2013). As far as Malaysia is concerned, the majority of the carbon in the forest is stored in aboveground biomass (AGB) and soil. Meanwhile, understorey trees namely trees with diameter of breast height (DBH) more than 1.0 cm and lower than 10 cm, contributes to (1.0 - 3.0%) of the total forest biomass (Ngo et al. 2013) which also includes saplings from Dipteorcarpaceae family. Little knowledge is known on understorey trees contribution towards forest biomass of seedlings especially those of planted Dipterocarp species namely Chengal. Therefore, the study in this chapter has the following objectives; 1) to determine the growth parameters of Chengal seedlings and 2) to quantify biomass of those seedlings; both planted using innovative techniques. The study site chosen was a 1.73hectare plot in Compartment 89B, Tekai Forest Reserve, Jerantut, Pahang. A total of 540 Chengal seedlings were planted using improved planting technique developed by Raja Barizan & Shamsudin (2008). Planting spacing was 3 m x 3 m. A semi-mechanized planting method which uses the vehicle of track tires skid steer loader model 753 or 773 (Bob-Cat) attached with a hydraulic auger size 36 inches (90 cm) diameter were applied. Prior to planting, Chengal seedlings were hardened through slow hardening process. Big and fit saplings were planted and fertilised during planting with slow release fertilizer (SRF) and goat dung. For biomass quantification measurements, the allometric equation model developed by Kirby & Potvin (2007) was used. This model was developed for forest type for saplings above 1 cm and below 5 cm of basal diameter (BD).

> Above ground biomass = exp  $[3.965 + 2.383 \ln (BD)]$ BD = basal diameter (cm)

The total root biomass was indirectly estimated as 24% of the above-ground biomass of trees above 1 cm BD (Niiyama et al., 2010). Growth parameter measurements were done at 12th, 22nd and 44th month after planting in the field using a height pole and a caliper. The data was analysed using Statistical Package for the Social Sciences (SPSS). The survival of Chengal seedlings planted was high with 88% of survival percentage. The increments for growth parameters were significant for all the months at p<0.05 (Figure 1a and 1b). Improved planting techniques which put a high emphasis on selecting only quality and fit saplings with application of SRF have contributed to the significant increment of growth performance of Chengal stands. These mean values are considered moderate and acceptable for a heavy hardwood and slow growing species, since other dipterocarp species which are mainly fast growing species, such as Hopea sp and Shorea sp, record values which are comparable to the values observed in this study (Okuda et al. 2013). Above ground and root biomass, of Chengal seedlings showed a significant difference at p<0.05 (Table 1). The addition of combination fertilizer exhibited higher biomass production for AGB and root biomass. A number of studies have shown an increase in the biomass of Dryobalanops species, by at least 30%, with additions of N, P and K to more than 200% on degraded soils (Dong et al. 2014). The addition of organic fertilizer also increased the water holding capacity and reduced the incidence of leaching thereby making more nutrients available to the soil (Eifediyi et al. 2010).



Figure 1a & b: Mean height and diameter of Chengal stands planted in Tekai Forest Reserve

Month	AGB	Root biomass
12 month	$17.61 \pm 2.46$	$4.23\pm0.59$
22 month	$40.81\pm6.13$	$9.79 \pm 1.47$
44 month	$100.05\pm22.34$	$24.01\pm5.36$
r 1 .		1 20 11.

 Table 1: Mean for growth and biomass quantification Chengal seedlings

*Mean values*  $\pm$  *standard deviation with* n = 30 *seedlings* 

This study has shown that the survival, growth performance and biomass quantifications of the shade tolerant Chengal species, which has a very slow growth rate, can be improved and increased using innovative planting techniques compare to conventional methods.

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## Assessment of zinc layered hydroxide hybrid with nitrate and phosphate anion on the growth performance of selected forest tree seedlings under nursery trial

Rozita A\*<sup>1</sup>, Nor Farhana K<sup>1</sup>, Mohd Zobir H<sup>2</sup>, Siti Salwana H<sup>1</sup>, Tumirah K<sup>1</sup>, Zulkharnain Z<sup>2</sup>,

Wan Rasidah K<sup>1</sup> & Norhayati H<sup>3</sup>

<sup>1</sup>Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia
 <sup>2</sup>Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
 <sup>3</sup>Universiti Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak, Selangor, Malaysia
 \*Corresponding email: rozita@frim.gov.my

Fertilizers are essential at the beginning of planting to promote the growth and root development of forest wood species. However, they are not fully utilized by plants because they are lost through leaching and evaporation causing environmental pollution. The incorporation of nitrogen and phosphorus sources into layered metal hydroxide host matrices can minimize the environmental problem. Layered metal hydroxides, such as zinc layered hydroxide (ZLH), as a controlled release material, can store the active ingredient in its interlayer area, creating a nanohybrid material with high stability that reduces solubility and degradation. The active ingredient can be released from its nanohybrid at its new target through the anion exchange process and the partial dissolution of the ZLH layer (Hussein et al. 2012; Cursino et al. 2010). In addition, ZLH can be a source of zinc to the soil, thereby improving the soil's micronutrients, which is beneficial for plant growth. In this study, zinc layered hydroxide (ZLH) nanohybrids containing plant nutrient sources were evaluated as controlled release fertilizers. This is carried out through a plant growth study in the FRIM nursery to investigate the effect of the ZLH with nitrate and phosphate anion on the growth of selected forest tree seedlings. In this experiment, Neolamarckia cadamba, known locally as Kelempayan, was used. This fast-growing tree species is a lightweight hardwood and is widely used in lightweight construction, plywood, and paper manufacturing. In the nursery trial, Kelempayan seedlings were tested with four different types of treatment. Treatment T1 was a control with no chemical application. Treatment T2 consisted of commercial fertilizer and T3 was applied with raw chemicals or sources of nitrate and phosphate. While the T4 treatment uses nanohybrids of zinc layered hydroxide nitrate (ZLH-N) and zinc layered hydroxide phosphate (ZLH-P). Destructive sampling was carried out four months after the treatment and the biomass yield was measured. The levels of N and P in samples of kelempayán leaves were analyzed by microwave digestion and the inductive couple plasma - optical emission spectrometer (ICP-OES) method to determine the uptake of nutrients by the plants. The results of the plant growth test in the nursery showed that treatment 4 with ZLH-N and ZLH-P had the highest biomass yield of Kelempayan (33.6 g) compared to other treatments (Figure 1). The control recorded the lowest value of 16.2 g, while T2 and T3 achieved biomass weights of 26.2 and 19.4 g, respectively. The nutrient concentration in the leaf samples from T4 showed the highest nutrient content with 2.2% N and 0.24% P (Figure 2). Control plant samples received the lowest levels of N (1.5%) and P (0.16%). This pattern is consistent with the highest biomass reading observed with a similar treatment, T4. This could be contributed to the zinc content of the nanohybrid and the controlled release properties of ZLH-N and ZLH-P, which improve nutrient uptake and biomass yield. Due to the ZLH anion exchange capacity, nitrate and phosphate anions could be released from their respective ZLH-N and ZLH-P to the soil environment. Nitrate and phosphate were released from their nanohybrid and exchanged with anions in the soil medium and then taken up via the roots of the plant. The results show that ZLH could be used as a host for controlled release formulation of plant nutrient source.



Biomass weight, g

Figure 1: Biomass yield of Kelempayan seedlings after four months application with different treatments





T1= Control; T2= Commercial Fertilizer; T3= Raw chemical; T4= ZLH-nitrate and ZLHphosphatenanohybrid

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### Carbon removal and emission from forestry sector: Adding value to protect Red List tree species

Norsheilla MJC

Climate Change Program, Forestry and Environment Division Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor Corresponding author's email: norsheilla@frim.gov.my

Forest land is important as a huge potential carbon removal or sequestration along with balanced climatic conditions (IPCC, 2006). Large-scale carbon removal from the forest area has recently been highlighted as a large part of climate change mitigation measures, which limit global warming to below 2°C and an additional 1.5°C (UNFCCC, 2021). The world's forests are estimated to have a net carbon source of 1,100 Gt C (Gigatonnes carbon) (IPCC, 2000). In 2010, the Food and Agriculture Organisation of the United Nations (FAO) estimated total carbon stocks less than 652 Gt C. In another study by Pan et al. (2011), bottom-up carbon stocks and fluxes from data and inventory rated using long-time field observations are estimated to be  $861 \pm 66$  Gt C with a similar breakdown: 42% in living biomass, 8% in dead wood, 5% in litter and 44 percent in soil. Moreover, 55% is found in tropical forests, 32% in boreal forests and 14% in temperate forests. Tropical and boreal forests are similar in terms of carbon density [242 and 239 tonnes of carbon per hectare (tC ha<sup>-1</sup>), respectively] while temperate forests are about 40% lower (155 tC ha<sup>-1</sup>). Although tropical and boreal forests store more carbon, the trade between biomass and soil is very different: In tropical forests, 56% of carbon is stored in biomass and 32% is stored in the soil, while in boreal forests, only 20% is stored in biomass and 60% are stored in the soil. Terakunpisut et al. (2006) also reported that tropical rainforests have the greatest potential of carbon removal and have consequences for evergreen mixed forests or deciduous forests. However, due to population growth and the need for social development, there may be disturbances in forest areas. These human disturbances such as forest fires, harvested trees, drained organic peatlands and the conversion of forest land to other land can lead to a reduction in land area, have increased carbon emissions and adversely affect the presence of IUCN Red List tree species. Rare or endangered species are important components of forest ecosystems and play an important role in management and conservation (Saw et al., 2010). In order to protect the tree species on the Red List, extensive literature is required to examine the potentials, opportunities, risks and trade-offs of dependencies in relation to carbon removal and to understand the value of forests. According to the third biennial update report (BUR3, 2020), Malaysia covers an area of 330,345 km<sup>2</sup> (million ha) and is one of the few forest-rich-tropical countries left with around 55.2% or 18.24 million ha of the total area remained forested. Malaysia has defined forest land as a minimum mapping unit (MMU) which is 0.5 ha, the minimum crown cover is 30% and the minimum height at maturity is 5 m (KeTSA, 2020). To date, Malaysia has consistently retained more than 50% of its land mass as a forest. Forest land in Malaysia is owned by individual states and can be divided into three administrative groups: (i) forest reserves, (ii) state forests, (iii) national parks and wildlife reserves. In 2016, inland forest made up the majority of forest areas in Malaysia (88.29%), followed by planted forests (5.51%), mangrove forests (3.35%) and peat swamp forests (2.84%). Of the total forest area, there are 5.773 million ha on the Peninsular Malaysia, 4.558 million in Sabah, and 7.910 million in Sarawak (KeTSA, 2020). The country's forest land can absorb 259,146.03 Gg CO<sub>2</sub>eq per year and reduce the net amount of national greenhouse gas emissions from 334,634.51 Gg CO<sub>2</sub>eq to only 75,488.48 Gg CO<sub>2</sub>eq. (BUR3, 2020). Are we aware of this, and in some cases, we may find it difficult to identify the best reason to protect forests? By estimating the carbon removal and emissions on site, we can create added value for the protection of species on the Red List. The simple question most people ask is why do we need to protect the forest? What is the absolute amount of carbon left in a place at a specific time? Which trees store the most carbon? What are the fastest growing trees and longest living trees? To address these uncertainties, we are using data collection to measure carbon and understand carbon storage in specific forests. This paper aims to provide some of these scientific insights on carbon removal and Red List debate. We suggest that greater involvement in critical research on carbon removal can help

bridge the knowledge gap. The review of previous carbon removal experiences speaks directly to the current possibilities, limitations, barriers and conditions for negative emissions and should incorporate current research and policy promises if we want to avoid past mistakes. A historical perspective also helps us understand the continuities and discontinuities in the development of discourses and practices on emissions and is an important tool in defining future research and the policy agenda on the subject. The methodology used in this study is described in Table 1. Hamdan et al. (2017) reported a carbon estimate of 264.32 Mg C ha<sup>-1</sup> in inland forests, including 79% living biomass, 18% soil, 2% deadwood and 1% litter. In addition, an estimated carbon of 416.27 Mg C ha <sup>1</sup> in peat swamp forests with 49 percent living biomass, 45 percent soil, 5 percent deadwood and 1 percent litter. On the other hand, the estimated carbon of mangrove forests is 264.89 Mg C ha<sup>-1</sup>, with 69% living biomass, 21% soil, 8% deadwood and 2% litter (Figure 1). Our study estimated the average carbon removal of 970 tCO<sub>2</sub>eq ha<sup>-1</sup> in inland forests, 1,527 tCO<sub>2</sub>eq ha<sup>-1</sup> in peatswamp forest and 972 tCO<sub>2</sub>eq ha<sup>-1</sup> in mangrove forest. The result demonstrates the role of forests in carbon removal or sequestration. However, there is a gap in the study, as the carbon emissions at this location are not calculated minus the carbon removals. In fact, estimates of carbon removal and emission depend on the type of forest and the age of the forest, depending on the size class of the tree and the disturbances of the activity. Strict protection and conservation of these forests, including Red List tree species, toremove atmospheric CO<sub>2</sub>, can increase carbon uptake in natural forests. The Forest Research InstituteMalaysia (FRIM) is the implementing agency that leads the carbon stocks and greenhouse Gas Inventory for land use, land use change and forestry (LULUCF) sector. The aim is to measure and understand trends in carbon removal and greenhouse gas emissions in order to develop strategies for reducing emissions and climate change. If enough carbon is sequestered, and emissions reduced, thegreenhouse effect will be reduced in the future and incidence of climate change-related extreme weather events will be reduced. The goal of reducing global greenhouse gases would not have been possible without significant contributions from forest areas. Strengthening forest management for all types of forest is important to increase carbon removal or sequestration.



Figure 1a: Proportion of carbon pools in the inland forest

Figure 1b: Proportion of carbon pools in the peat swamp forest



Figure 1c: Proportion of carbon pools in the mangrove forest

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#### **Appendix 1: Methodology**

### Carbon Stock estimation for five (5) carbon pools

#### AGB for inland forest

1102 Ior mana Iorest	
The allometric function of trees applied in the calculation of standing biomass can be	
expressed as in Equation 1 (Kato et al. 1978). The key parameter is dbh.	
1/H = 1/(2.0  'D) + 1/61	(1)
Where $H = total$ tree height and $D = dbh$ . From the values of D and H, the dry mass	
values of stem (Ms,), branches (Mb) and leaves (Ml) of the tree are estimated.	
Ms = 0.0313 (D2H) 0.9733	(2)
Mb = 0.136 $Ms 1.070$	(3)
1/Ml = 1/(0.124Ms0.794) + 1/125	(4)
Therefore, the total aboveground biomass for a tree is a summation of Ms, Mb and Ml.	
According to Chave et al. (2014), the allometric function of a tree can be expressed as	
$AGB = [exp (-1.803 - 0.976E + 0.976 \ln (\rho) + 2.673 \ln(D) - 0.0299 [\ln(D)]2]$	(5)
Where,	
AGB = Aboveground biomass (kg/tree)	
E = bioclimatic variable (average value of -0.1 for Peninsular Malaysia)	
$\rho$ = wood specific gravity/ wood density, 0.57 g/cm3 (Reyes et al. 1992)	
D = dbh	

#### AGB for peatswamp forest

AGB of trees in peat swamp forests was adopted from Chave et al. (2005) and can be	
expressed as	
AGB = 0.65 * exp(-1.239 + 1.98 * ln(D) + 0.207 * ln(D)2 - 0.0281 * ln(D)3	(6)

#### AGB for mangrove forest

Tree biomass in mangroves was calculated by using allometric equation that was developed by Komiyama et al. (2007) for common species in the mangroves of Peninsular Malaysia. The allometry can be expressed as  $Wt = 0.251\rhoD2.46$  (7) Where Wt is the dry weight of the aboveground component,  $\rho$  is wood density (CIFOR) and D is diameter at breast height (dbh). The calculated biomass was converted into C

by using a biomass expansion factor of 0.47.

**Belowground biomass (BGB)**: Comprises of living coarse and fine roots of trees. The BGB is an important part of the total forest biomass after AGB, representing up to 25% of the total biomass. Equation adopted from Niiyama et al. (2010) was used to estimate the BGB in this study.

#### BGB for inland and peatswamp forest

BGB = 0.023\* D2.59Where WB is belowground biomass and D is dbh.

#### **BGB** for mangrove forest

For mangrove forests, the BGB is calculated based on allometry that was developed by Komiyama et al. (2007), which can be written as  $Wr = 0.199\rho \ 0.899 \ D \ 2.22$ 

Wr is the root biomass, which can be referred to as belowground biomass,  $\rho$  and D is wood density and diameter at breast height (dbh), respectively. The calculated biomass was converted into C by using a constant factor of 0.47.

(7)

(8)

(9)

c) Deadwood: Coarse Woody Debris (CDW) involves large pieces of standing and lying dead wood. Depending on the forest type, stage of succession, land use history and management practices, CDW can be a significant contributor to the total AGB. Calculation of carbon for this category was based on the following equation: Carbon stock = (Volume  $\times$  wood density) \* 0.47 (10)Where, wood density is either solid = 0.57, intermediate = 0.39, and rotten = 0.21

d) **NTV and litter**: the oven-dry mass per area of this category was obtained from the samples that have been collected in the field. The carbon stock calculation was based on the following equation:

Carbon = Dry mass  $\times 0.4$  (IPCC default factor)

Soils: Samples were oven-dry at a constant temperature of 115°C for about 24 hours and the dry mass was used to obtain bulk density. Samples were then analysed at the soil laboratory for OC by using the combustion method, after pre-treatment to remove carbonate. Soil OC (%) was then multiplied by soil bulk density and soil depth to obtain total soil carbon stock per unit area.

Soil Carbon = OC (%) x Bulk density x depth (30 cm)

(12)

(11)

#### Carbon removal and emission estimation

Carbon removal (tonne  $CO_2eq$ ) = Biomass (kg) x 0.001 x 3.67

### SUMMARY

The Regional Webinar themed "Ex-situ **Conservation and Carbon Sequestration** Potential of Red List Tree Species" held from 20-21 October 2021 was a success that showcased key players and relevant stakeholders whom shared their relevant knowledge and presentations on the subject of declining population of Red List Threatened Species. This proceeding summarizes the various case studies from the local and international stakeholders on concerted efforts on addressing planting materials, plantation technology, ex-situ conservation and carbon sequestration potential of these threated species.

