UNIVERSITI PUTRA MALAYSIA

GENETIC VARIATION AND CLONAL PROPAGATION OF SUPERIOR GENOTYPES OF SELECTED Acacia SPECIES

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By

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Chairman : Professor Nor Aini Ab Shukor, PhD
Faculty : Forestry

The *Acacia* species was introduced into Malaysia in the late 1960's for timber production. Plantations of *Acacia* species gain interest as a major timber species in the 1960's especially in Peninsular Malaysia along with *Hevea brasiliensis*. The product in forestry ventures is often sawn timber, which requires trees to be in good conditions such as straight, single-stemmed and defect-free trunks for maximum utilization. Production of such quality wood is another big problem in forestry plantation since trees grown in plantation of some high-value temperate and tropical hardwood species tend to produce low value, short butt logs and bolts due to crooked stems, low fork heights and delayed shedding of lower branches.

In this context, a research study was initiated to select and recommend the best performing tree species or provenances suited for timber production in Malaysia with respect to growth and other characteristics. Species/provenance/progeny test was conducted on four species of *Acacia* namely, *A.mangium*, *A.auriculiformis*, *A.crassicarpa* and *A. aulococarpa* with four provenances for each species from two main regions of Papua New Guinea and Queensland. The growth of the provenances was monitored in terms of some quantitative and qualitative characteristics to evaluate the genetic variation and growth performance of a base breeding population. The study showed that there were significant differences ($p< 0.05$) between species, provenance and progenies for their growth performance. There were also significant differences between provenances within regions and progenies within provenances in all quantitative and qualitative traits tested in this study.

Generally, with regard to the growth, *A.mangium* performs better compared to other *Acacia* species in all of the aspects tested and it was followed by *A.crassicarpa*, *A.aulococarpa* and *A.auriculiformis*. Generally provenances and progenies selected from Papua New Guinea excelled those from Queensland both in quantitative and

qualitative characteristics. Among the top performing progenies of *Acacia* species are CG 1854 of (Bensbach WP) and KN000107 (SW of Boset WP) of *A.mangium*, BVG2609 (Bensbach WP) of *A.crassicarpa*, BVG 00835 (WP Morehead) and MM1016 (Arufi E Morehead WP) of *A.aulococarpa* and JSL363 (Wenlock River) and BVG 2657 (Bansbach) of *A.auriculiformis.*

Three best performing clone (genotypes) were then chosen based on their phenotypic characteristic for clonal propagation of superior tree species through traditional and modern techniques. Vegetative propagation was attempted as initial pretreatment stage of rejuvenation of mature sources through forced flushing, stem cuttings and trunk decapitation. *A. mangium* and *A. auriculiformis* respond well to force flushing by having highest survival percentage (87.7% and 90%, respectively) together with bud breaking and sprout growth. Whereas, *A.aulococarpa* and *A.crassicarpa* only recorded 52.2% and 31.1% of survival percentage. Rooting ability of stem cuttings, feasible and mean root number and root length increase at juvenile stage for all species studied. Rooting ability of mature cuttings decreased and bud breaks occurred only for few days eventually died, and did not respond to the treatments of growth regulators. Rooting ability of young stem cuttings of *A. mangium* (83.3%) and *A.auriculiformis* (76.6%) was better compared to that of *A.crassicarpa* and *A aulococarpa* with only 48% and 68.8%, respectively. Investigation was also done for the use of coppice materials as an alternative source for *in vitro* propagation of mature sources. 12 year-old trees of selected *Acacia* species were felled to the height of 1.0m and 1.5m. Vigorous production of sprout or coppice was noted on the stumps of trees of all species except of *A.crassicarpa*. The greatest copping ability in terms of survival rate of stumps was observed on *A.auriculiformis* with 83.8% followed by, *A.mangium, A.aulococarpa and A.crassicarpa* with 75.0%, 40.0% and 1.67 %, respectively. *A.crassicarpa* produces a very low number of sprouts with mean of 0.03 and mean of 0.09 for sprout length.

Rejuvenated mature explants were further subjected to *in vitro* conditions for mass production of improved materials for establishment of efficient *in vitro* protocol for *Acacia* sp. Decontamination of field collected materials was conducted as an initial stage in shoot initiation stage using some methods optimized in preliminary study. Most effective sterilization in term of average clean culture percentage (>70%) was recorded in 0.1% $HgCl_2$ for 5 minutes for *A.mangium, A.auriculiformis, A.mangium* 'Superbulk' and *A*. hybrid and 0.1% $HgCl_2$ for 10 minutes for both *A.crassicarpa* and *A.aulococarpa.* Incorporation of 0.1g/l of fungicide Benomyl with 50mg/ml of antibiotic streptomycin further enhanced the survival rate and percentage of clean culture up to 80%-100%. Multiple shoot production was obtained from all species of *Acacia* on Murashige and Skoog (MS) medium supplemented with 2.0 mg/l benzyladenine (BA) plus 0.5 mg/l of NAA. It was also noted that greater shoot production occurred with combination of plant growth regulators with additives. The maximum shoot number and shoot length was produced in medium supplemented with 2.0 mgl^{-1} benzyladenine (BA) + 0.5 mgl^{-1} of NAA + activated charcoal (0.1% w/v) combined with 100 mgl^{-1} $AdSO_4$. It produced maximum number of 9.0 shoots per explant with 3.51 cm in length. Shoots were then elongated and rooted in an optimized condition and further acclimatized to nursery condition.

Another study was initiated to evaluate and identify sequence markers which gave phylogenetic information to be used to infer relationship within *Acacias* at a fine level. Primer designed based on second intron of *LEAFY* gene of *A.mangium* amplified the specific region with single band except for *A*. hybrid. The amplified regions were sequenced to reveal the species relationship within selected *Acacias*. Result revealed that non coding region of the second intron of LEAFY gene is more variable and can be used as marker for phylogenetic studies at lower taxanomic levels.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

VARIASI GENETIK DAN PEMBIAKAN KLON GENOTIP TERBAIK SPESIS AKASIA TERPILIH

Oleh

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Spesies *Akasia* telah diperkenalkan di Malaysia pada lewat tahun 1960-an untuk pengeluaran kayu. *Akasia* dipilih sebagai spesis kayu utama terutama di Semenanjung Malaysia bersama dengan *Hevea brasiliensis*. Produk dalam penghasilan kayu gergaji sering memerlukan pokok dalam keadaan yang baik seperti batang induk yang lurus, serta bebas kecacatan batang untuk penggunaan yang maksima. Pengeluaran kayu berkualiti adalah satu masalah besar di dalam perladangan hutan yang ditanam dengan spesies kayu keras sederhana dan tropika cenderung untuk menghasilkan batang yang bernilai rendah, ruas pendek dan bolt kerana batang yang bengkok, ketinggian cabang rendah dan kelewatan dalam penanggalan cabang rendah.

Kajian ini telah dimulakan untuk memilih dan mencadangkan spesies pokok yang terbaik dengan asal usul yang berkaitan dengan pertumbuhan dan ciri-ciri lain yang sesuai untuk pengeluaran kayu di Malaysia. Ujian spesis / provenan / progeni telah dijalankan ke atas empat spesies Akasia iaitu, *A.mangium*, *A.auriculiformis*, *A.crassicarpa dan A. aulococarpa* dengan empat provenan bagi setiap spesies daripada dua kawasan utama Papua New Guinea dan Queensland. Pertumbuhan provenan telah dipantau dari segi beberapa ciri-ciri kuantitatif dan kualitatif untuk menilai perubahan dan pertumbuhan prestasi populasi genetic. Kajian ini menunjukkan bahawa terdapat perbezaan yang ketara ($p <0.05$) antara spesis, provenan dan progeni prestasi pertumbuhan mereka. Terdapat juga perbezaan yang ketara antara provenan di dalam kawasan dan progeni dalam provenan untuk semua sifat-sifat kuantitatif dan kualitatif yang diuji

Secara umumnya, dengan mengambil kira pertumbuhan, *A.mangium* membesar lebih baik berbanding dengan spesies Akasia yang lain dalam semua aspek yang diuji dan ia diikuti oleh *A.crassicarpa*, *A.aulococarpa* dan *A.auriculiformis*. Provenan dan progeni yang dipilih dari kawasan Papua New Guinea adalah lebih baik dari

Queensland dari segi pertumbuhan dalam semua ciri-ciri kuantitatif dan kualitatif. Di antara progeni terbaik spesies Akasia adalah CG 1854 daripada (Bensbach WP) dan KN000107 (SW dari Boset WP) daripada *A.mangium*, BVG2609 (Bensbach WP) daripada *A.crassicarpa*, BVG 00835 (WP Morehead) dan MM1016 (Arufi E Morehead WP) daripada *A.aulococarpa* dan JSL363 (Wenlock River) dan BVG 2657 (Bansbach) daripada *A.auriculiformis*.

Tiga klon berprestasi terbaik (genotip) kemudian dipilih berdasarkan ciri fenotip mereka untuk tujuan pembiakan klon spesies pokok yang unggul melalui teknik pembiakan tradisional dan moden. Pembiakan tampang telah dilakukan sebagai peringkat rawatan awal untuk menjuvinasikan sumber matang melalui 'force flushing', keratan batang dan penebangan batang. *A. mangium* dan *A. auriculiformis* bertindak balas dengan baik untuk 'force flushing' dengan mempunyai peratusan kemandirian yang tertinggi (87.7% dan 90%) dengan pertumbuhan putik dan pucuk. Manakala, *A.aulococarpa* dan *A.crassicarpa* hanya merekodkan 52.2% dan 31.1% peratus kemandirian. Keupayaan pengakaran keratan batang boleh dilaksanakan dan purata bilangan akar dan panjang akar dapat ditingkatkan dengan penggunaan sumber yang dijuvenasikan untuk semua spesies Akasia. Keupayaan pengakaran menurun bagi sumber matang dan pertumbuhan putik berlaku hanya untuk beberapa hari dan mati. Ia juga tidak bertindak balas kepada semua rawatan pengalak pertumbuhan. Keupayaan pengakaran keratan batang muda *A. mangium* (83.3%) dan *A.auriculiformis* (76.6%) adalah lebih baik berbanding dengan *A.crassicarpa* dan *A aulococarpa* dengan hanya 48% dan 68.8% masing-masing. Kajian juga telah dijalankan untuk penggunaan sumber dari pucuk muda sebagai sumber alternatif untuk pembiakan *in vitro* sumber matang. Spesis pokok Akasia yang berumur 12 tahun telah dipilih dan ditebang dengan ketinggian 1.0 m dan 1.5 m. Pengeluaran pucuk yang baik diperhatikan pada dari segi kadar kemandirian pada tunggul pokok daripada semua spesies kecuali *A.crassicarpa*. Peratus keupayaan percambahan tunggul dari segi kadar kemandirian yang tinggi diperhatikan pada *A.auriculiformis* dengan 83.8% diikuti oleh, *A.mangium*, *A.aulococarpa* dan *A.crassicarpa* dengan masing-masing mempunyai 75.0%, 40.0% dan 1.67%. *A.crassicarpa* menghasilkan jumlah pucuk yang sangat rendah dengan purata 0.03 dan purata 0.09 untuk kepanjangan pucuk.

Sumber yang dijuvenasikan seterusnya didedahkan kepada keadaan *in vitro* untuk pengeluaran besar-besaran bahan yang terpilih bagi pembentukan protokol *in vitro* yang berkesan untuk spesis Akasia. Pembersihan sumber yang dikumpulkan dari lapangan telah dijalankan sebagai peringkat awal permulaan percambahan pucuk dengan menggunakan beberapa kaedah yang dioptimumkan dalam kajian awal. Pensterilan yang paling berkesan dari segi peratus purata kultur bersih (> 70%) dicatatkan pada 0.1% $HgCl_2$ selama 5 minit untuk *A.mangium*, *A.auriculiformis*, *A.mangium* 'Superbulk' dan *A.* hybrid dan 0.1% $HgCl_2$ selama 10 minit untuk *A.crassicarpa* dan *A.aulococarpa*. Penambahan 0.1g / l racun kulat Benomyl dengan 50mg / ml antibiotik streptomycin mempertingkatkan kadar survival dan peratusan purata kultur bersih sehingga 80% -100%. Pengeluaran pucuk yang banyak telah diperolehi daripada semua spesis Akasia pada media Murashige dan Skoog (MS) yang diperkaya dengan 2.0 mg / l benzyladenine (BA) serta 0.5 mg / l. NAA. Didapati pengeluaran pucuk yang terbaik berlaku dengan gabungan pengawal pertumbuhan tumbuhan dengan bahan tambahan. Bilangan pucuk dan panjang pucuk yang maksimum dihasilkan dalam media ditambah dengan 2.0 mg/l benzyladenine (BA) + 0.5 mgl-1 NAA + arang aktif (0.1% w / v) digabungkan dengan 100 mgl/l $AdSO_4$. Ia menghasilkan 9.0 bilangan pucuk maksimum per eksplan dengan kepanjangan 3.51 cm. Pucuk telah dipanjangkan dan diakar dalam keadaan optimum dan selanjutnya didedahkan di luar untuk menyesuaikan diri kepada keadaan nurseri.

Satu lagi kajian telah dilakukan untuk menilai dan mengenalpasti penanda urutan maklumat filogenetik yang akan digunakan untuk membuat kesimpulan mengenai hubungan antara Akasia pada tahap yang baik. Primer yang telah direka berdasarkan intron kedua gen *LEAFY A.mangium* telah mengamlifikasi kawasan yang dikehendaki dengan band tunggal kecuali *A.* hybrid. Rantau yang diamlifikasi telah disusun untuk mendedahkan hubungan dalam spesies Akasia yang terpilih. Keputusan ini mendedahkan kawasan bukan pengekod intron kedua gen *LEAFY* ini adalah lebih bervariasi dan boleh digunakan sebagai penanda untuk kajian filogenetik taxanomi pada tahap yang lebih rendah.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

µg/ml	microgram per millilitre
µM	microMolar
℃	degree centigrade
2,4-D	2,4-dichlorophenoxyacetic acid
ABA	abscisic acid
AdS	adenine sulphate (anhydrous)
ANOVA	analysis of variance
BAP	6-benzylamina-purine
cm	centimetre
dbh	diameter at breast height
df	degree of freedom
g	gram
g/l	gram per litre
GA_3	gibberellic acid
HCI	hydrochloric acid
$HgCl_2$	mercuric chloride
IAA	indole-acetic-acid
IBA	indole-butyric-acid
Kin or K	kinetin (6-furfurylaminopurine)
kPa	kilopascal (unit for pressure)
L	litre
LB Agar	Luria Bertani agar
LSD	least significant different
m	metre
M	molar
mg	milligram
mg/l	milligram per litre
min	minute
ml	millilitre
mm	millimetre
Ms	mean of square
MS	Murashige and Skoog medium (1962)

MW	molecular weight
NAA	1-naphthaleneacetic acid
NaOCl	*sodium hypochlorite*
NaOH	natrium hydroxide
PDA	potato dextrose agar
pH	negative logarithm of the hydrogen concentration
ppm	part per million
SDW	sterile distilled water
UV	ultraviolet
v/v	volume over volume
w/v	weight over volume

CHAPTER 1

GENERAL INTRODUCTION

The tropical rainforest has long been recognized as a repository of genetic resources and is important to world's economy and for preserving the ecosystem. Malaysia, as in any developing countries has converted some of its natural forest areas for agricultural, industrial, recreational and urban development uses, but still retains 44.04 % (5,803,213 ha) of the total land area (13,184,629 ha) under forested land area in Peninsular Malaysia. Out of this, 83.52% of total forested land (4,933,787 hectares) is designated as Permanent Forest Estate (PFE) by legislation which is under sustainable management with 387,828 hectares falls under plantation land area. Approximately 10.84 million hectares of the PFE is production forest with the remaining 3.49 million hectares being protection forest (Forestry Department of Malaysia, 2014).

Due to the rapid shift in the economy of this country and increasing population growth, greater expectation are being placed on the natural forest to function as production area to fulfill the country's economic benefit (generally associated with timber harvesting) and in the meantime as a protection area for conservation of flora and fauna. Concern for the sustainability of natural forest has become a major issue now since Malaysia has traditionally relied on its timber from natural forest. In order to maintain its biodiversity and genetic resources from our natural forest, more timber has to be produced from other resources. Due to its declining trend in the domestic tropical timber production along with conversion of rubber plantation to oil palm, there is a pressing need for Malaysia to ensure wood security for its important and significant wood-based sectors (**Figure 1.1**). Several options such as reliance on the Sustainable Forest Management (SFM) only will not be enough or sufficient to readily provide raw material for drastically developing the industries of the country based on primary and secondary wood. To ensure a long term supply of raw materials for downstream industries, viability of establishing forest plantation is the only practical strategy to overcome this supply and demand paradox.

In addition, the reduction of available harvestable forests from natural distribution of virgin forest due to the above mentioned purposes of land utilization and food security has led to the introduction of several fast-growing tree species for establishment of forest plantation programme. The needs for the establishment of plantations in Malaysia have been emphasised in the National Forestry Policy 1978 (revised 1002) and encouraged the establishment of quality timber forest plantation as a startegy to overcome insufficient raw material for downstream industries.

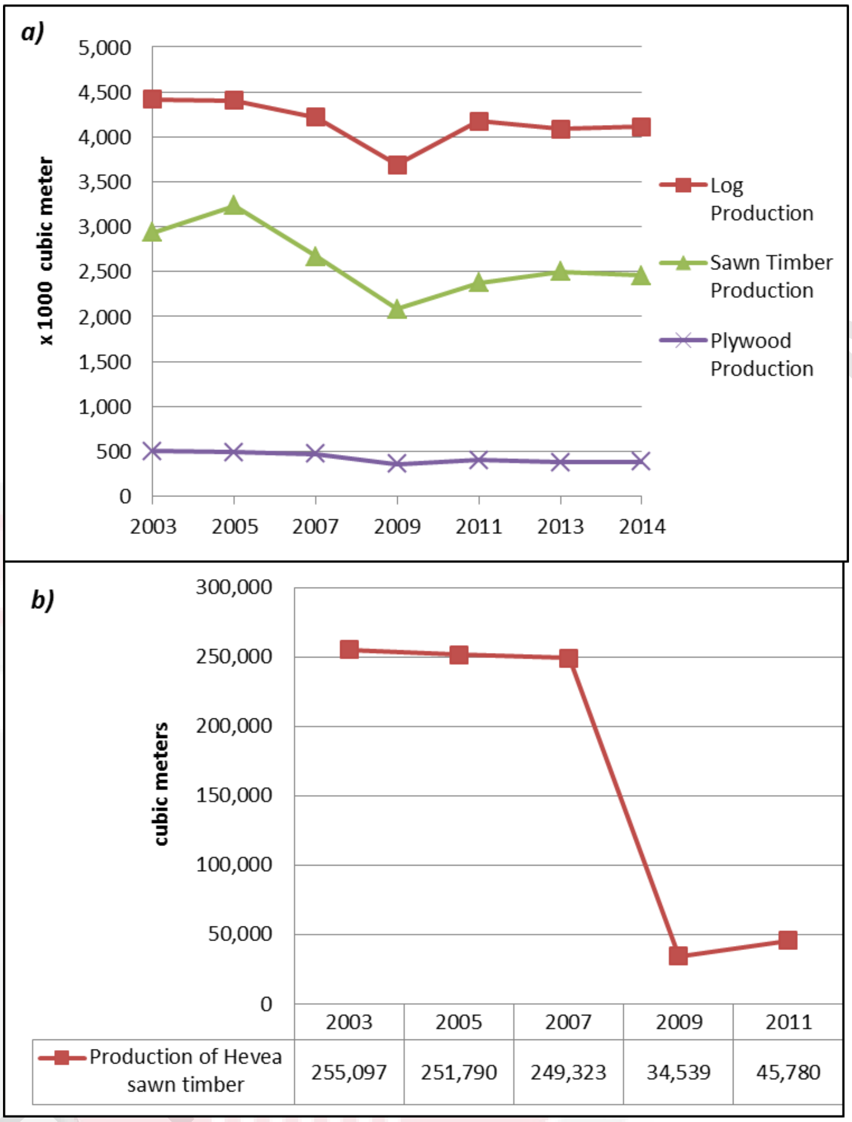

Figure 1.1: a) Production of plywood, sawn timber and logs in Malaysia between 2003-2014; and b) Production of sawn timber from *Hevea* logs in Malaysia between 2003-2011 (Forestry Department of Malaysia, 2014).

The long period of harvestable rotation age hinders private investment for establishing forest plantation. Estimates of present and future merchantable sawntimber volume have been attempted but still the assumption of a particular minimum volume of merchantable sawntimber greatly depends on the supply which genetic and environment interaction and none can predict the phenotype of a particular tree after some prolong period. Given such shortfall, return on the investment will be less than predicted by financial analysis. Furthermore, the genetic diversity of insects herbivory and the low stand density which contribute to the formation of poor quality of harvestable wood need further research to quantify the importance of the interactions between various factors (Scowcroft *et al.* 2010).

Introduction of some species for development of large scale forest plantation area in Malaysia depends totally on the objectives of the plantation and the knowledge as well as the management of the species in forest tree breeding perspective. Forest trees, especially indigenous forest species, pose a long generation period which has been the major obstacle in traditional tree breeding in Malaysia. In addition, the high heterozygosity, especially trees being propagated by seeds, hampers tree improvement through conventional breeding techniques. Thus, mass clonal propagation of superior trees/clones for forest plantation along with accelerated tree improvement programme for sustainable production of wood supplies is necessary for effective reforestation and management of forest resources.

Most of the plantations were established during the first phase of the Compensatory Forest Plantation Programme between 1982 and 1988. Since 1989, annual planting rates have not exceeded 7 000 ha. In order to achieve the target, government provided allocation for the first phase of implementation (2006-2010) and about 40,000 hectares of forest plantation was identified throughout the country in Sabah, Sarawak, Johor and Pahang (Ministry of Palntation Industries and Commodities, 2005). Since then, more forest plantations in Malaysia have been established annually in terms of total area planted with fast growing tree species. A total of 387,828 hectares of forest plantation was successfully established by the year 2014 compared to only 73,957 hectares in 2002 (**Figure 1.2**)

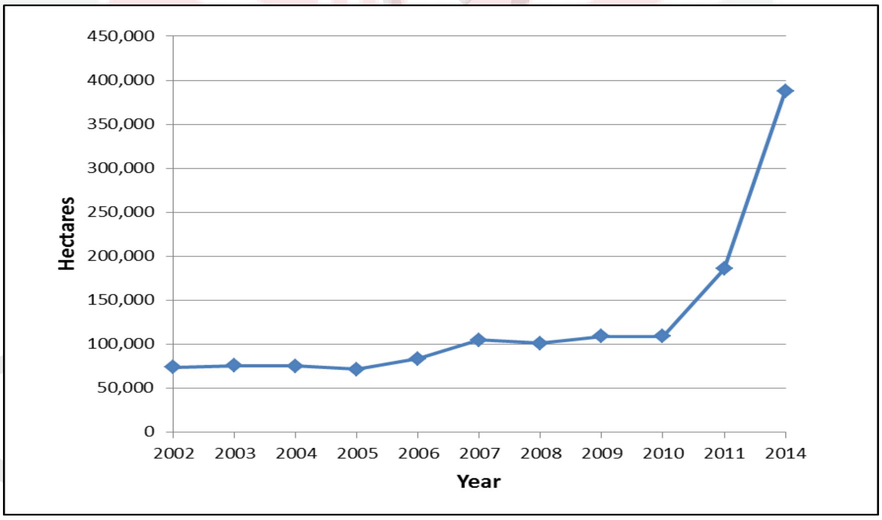

Figure 1.2: Forest plantation established in Permanent Reserved Forest in Malaysia from 2002-2014.

Another important variable for assessing the wood supply and market potential of plantations is species distribution. By thorough perambulation of both public plantation and private field throughout Malaysia, eight plantation tree species have been identified and selected as potential tree species for commercial forest plantation programme by Malaysian Timber Industry Council (MTIB, 2007). One of the main species highlighted in the selection of fast growing hardwood species is *Acacia* sp. (*Acacia* hybrid) among other timber species such as Teak (*Tectona grandis*), Rubber (*Hevea brasiliensis*), Sentang (*Azadiractha excels*a), African Mahogany (*Khaya* *ivorensis*), Kelempayan (*Neolamarckia cadamba*), Batai (*Paraserianthes falcataria*) and Binuang (*Octomeles sumatrana*). For example, of the 6 600 ha established in Johore, 6 470 ha were planted with *Acacia mangium*, whereas another 38 ha, 54 ha and 2 ha were planted with teak (*Tectona grandis*), Sentang (*Azadirachta excelsa*) and rubber respectively.

Malaysian government is striving to establish plantations comprised of fast-growing trees and one of their main goals to achieve this purpose is to establish species, provenance and progeny trial all over the country to choose for appropriate species that fits well with our environmental conditions. There are more than 600 species of tropical timbers in the world where most of them are commercially valuable in the international trade of plywood, roundwood, sawnwood and veneer (Chudnoff 1984; GTWPTN 2011, ITTO 2011; USDA FPL, 2011). Among all the fast growing tropical tree species, *Acacia* species seems to be most viable for plantation forestry and agroforestry needs of tropical Asia and Africa. It has been recommended as one of the most priority species for extensive development in tropical lowlands (Woo *et al*.1997; Phi, 2009; Pijut *et al.* 2012). The *Acacia* species was introduced into Malaysia in the late 1960’s for timber production.

Plantation of *Acacia* species gain interest as a major timber species in the 1960’s especially in Peninsular Malaysia after failures in introduction of tropical pines. Once the plans for constructing the paper mill were scuttled in Malaysia, pine plantations were left unmanaged and some of the plantation area shifted to meet for more appropriate needs because pines were initially planted for the purpose of pulp production (FAO,2002). *Acacia mangium* became one of the most extensively planted species in Malaysia along with *Hevea brasiliensis* and some trial plots have been initiated to study their performance under tropical environment. Almost 68.5% of the total plantation areas in Peninsular Malaysia are planted with *Acacia* species followed by *Hevea* species with 20.6% and others (**Figure 1.3**)

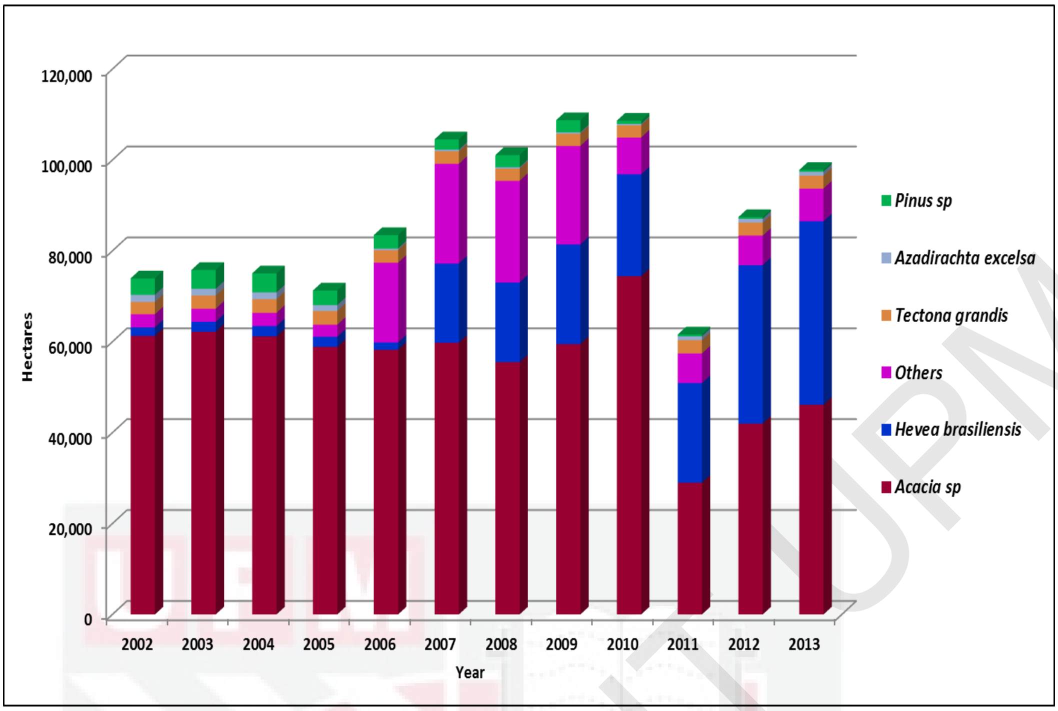

Figure 1.3: Forest plantation establishment according to species distribution in Malaysia from 2002-2013
(Source: Forestry Department of Peninsular Malaysia, 2013)

The results from international trials of *Acacia* species and provenances (Nor Aini *et al.* 1994; Zhang and Yang, 1996; Luangviriyasaeng and Pinyopusarerk, 2002; Kha, 2003; Phi, 2009) have shown that *Acacia* species is a multipurpose tree species, being fast growing and suitable for various purpose such as timber production, pulp and paper production and other end products such as furniture (Turnbull *et al,* 1998). Products and sawntimber often require trees to acquire appropriate characteristics such as straight, single-stemmed and defect-free trunks for maximum utilization (Nor Aini, 2004; Eldoma *et al.* 2015). Production of such quality wood is another big problem in plantation forestry since trees grown in plantation of some high value tropical hardwood species tend to produce low value, short butt logs and bolts due to crooked stems, low fork heights and delayed shedding of lower branches (Phi, 2009). Results from existing *Acacia* plantation in Malaysia indicated that most trees especially of *Acacia auriculiformis* fork very heavily which lead to the formation of multiple leaders and some forks are so close to the ground that they will produce little to no merchantable wood (Woo *et al.* 1997; Nor Aini *et al.* 1994; Mahat, 2007; Phi, 2009; Scowcroft *et al.* 2010).

On the contrary one of the most established *Acacia,* i.e. *A.mangiu, is* commonly assumed as the miracle tree, which suffers badly due to the incidence of root rot, heartrot and phyllode rust. Until now there are still no solutions for these diseases eventhough many researches have been done on this species. Unlike agricultural products, which can be produced for sale within a short period of time (from a few months to one to two years), most forest trees can only be harvested after 15 years. Rotations depend on the management objective and future markets are inherently uncertain. Despite the high economic, ecological and cultural value of this multipurpose tree species, there are still no documented examples of planted stands of *Acacia* species in Malaysia that have been through a full silvicultural rotation or tree breeding, i.e. establishment, stand improvement, harvest and re-establishment of superior trees, etc.

In order to have a good productive plantation management programme for a long term, there is an urgent need to have sufficient supply of genetically improved trees for timber production. The possibilities of successful application of traditional plant breeding methods to woody trees species have their limitations due to long regeneration or reproductive cycles (Fillatti *et al.*1987). There are few plantation trees with desirable stem form, especially if they originate from bulk seed lots collected with or without regard to stem form or timber quality. Even though there is a genetic component to good stem form and volume yield, other factors which are commonly associated with environmental factors often hinder their expression. Progeny via seed sources as a product of open pollination failed to develop better height growth of clear bole, then their parents due to wide variation of many characters from one sexual generation to another. For example, Brewbaker (1997) found that almost 90% of *Acacia Koa* trees established from 500 seed accessions produced only multi-stem progeny which is non-profitable if the objective of the plantation is to produce sawntimber. In addition, despite having maternal parents with good stem form, 55-71% trees of the same species grown in plantation developed major forking problems in the trunk within 0.3 m of the ground or at diameter of breast height (Daehler *et al.* 1999).

Management interventions in terms of traditional tree breeding alone do not ensure the establishment of quality sawntimber plantation trees without introduction of modern plant breeding. Plantation established through seeds shows a wide variation among and within species in the field due to heterozygous nature of the parents. In addition, traditional vegetative propagations such as cuttings, air layering, grafting, etc. of superior selected genotypes are very poor or in many cases not possible in any advanced tree improvement programmes as age of these elite trees become a limiting factor (Gupta, 1988; Haliza *et al.* 2012).

Taking these factors into consideration, vegetative propagation in the form of micropropagation offers great potential for mass propagation of superior clone. Modern plant breeding through tissue culture has been introduced as early as 1920 for mass production of commercially important timber trees (Krikorian, 1982; Dixon, 1985; Torrey, 1985). Optimized micropropagation protocol along with controlled culture growth is believed to produce the next generation of propagules containing high frequency of genes of favouring characters (provided the gene effects are additive) compared to the one propagated by seeds (Rao and Riley, 1994). The importance of having clonal material to study the heritability of tree form as well as other traits of interest has been stressed by many authors for some economically important tree species including some *Acacia* species (Karnosky, 1981; Glover *et al.* 1991; Vengadesan *et al.* 2003; Giri *et al.* 2003; Merkle and Nairn, 2005; Ishii, 2006; Phi, 2009; Girijashankar, 2011; Haliza *et al.* 2012; Pijut *et al.* 2012). The role and importance of having tissue culture as a solution for production of quality propagules from superior tree species has also been emphasized by several researches (Yanchuk, 2001; Jain and Ishii, 2003; Varshney and Anis, 2014).

Modern vegetative propagation offers a great impact on advanced tree improvement programmes which includes propagation through existing meristem such as shoot and nodal explants, direct and indirect organogenesis and somatic embryogenesis. In all of these cases, adequate protocol for mass propagation of *Acacia* species has been established and optimized, and in some cases propagated tissue cultured plantlets have even been introduced into the field. (Mittal *et al*, 1989; Yashoda *et al*, 2004; Girijashankar, 2011; Haliza *et al.* 2012; Banerjee, 2013).Most micropropagation techniques of tropical hardwood species including of *Acacia* species have been mainly limited to short term studies using juvenile plant sources which is not favourable in advance tree improvement programme and they are limited on seeds as explant sources (Haliza *et al.* 2012; Pijut *et al.* 2012). However production and multiplication of material from matured trees with superior characteristic seems to be problematic (Girijashankar, 2011).

Therefore, taking these limiting factors into consideration, this work has been developed to evaluate the potential of mature explants to be used as initial plant source for optimization of regeneration protocol for some selected *Acacia* species. With the foregoing considerations in mind, the present study was conducted to fulfill the following objectives;

1. To evaluate the growth performance of four *Acacia* species and the selection of plus trees as sources for propagation work.
2. To develop a standard rejuvenation method for mature *Acacia* species as source for micropropagation.
3. To develop standard workable protocols for micropropagation of selected *Acacia* species, viz. *A.aulococarpa, A.auriculiformis, A.crassicarpa, A.* hybrid *(A. auriculiformis* x *A. mangium), A. mangium and A. mangium* 'Superbulk'.
4. To determine the optimum conditions for the *in vitro* development and the multiplication from the various propagules (development of buds, shoots and callus).
5. To determine the optimum conditions for rooting of shoots and acclimatization.
6. To assess the genetic diversity of selected plus trees using second intron of *Acacia mangium* LEAFY gene.

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