UNIVERSITI PUTRA MALAYSIA

MASS CLONAL PROPAGATION OF SELECTED BAMBOO GENOTYPE FOR CONSTRUCTION PURPOSES IN MALAYSIA

LEE PAY CHIANN

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By

LEE PAY CHIANN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2020

DEDICATION

To my late grandmother,
Choi Poh Lin

To my parents,
Lee Yoke Ying & Lee Kuan Sui

To my sisters,
Ann & Herng

For your continuous support and endless love.

[To ***Hippo***, ***Fafa*** & ***Batman***, for your purest souls]

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

MASS CLONAL PROPAGATION OF SELECTED BAMBOO GENOTYPE FOR CONSTRUCTION PURPOSES IN MALAYSIA

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LEE PAY CHIANN

July 2020

Chair : Nor Aini Ab Shukor, PhD
Institute: Tropical Forestry and Forest Product

Malaysia is one of the leading exporters of tropical timber products in the world, to fulfil the market demand for construction materials as world population is expected to reach 9.7 billion in year 2050, natural forest stand will not be sufficient. Forest plantation development programme established in 2005, and bamboo has been recommended as one of the species to produce timber quality material. In 2018, the global export of bamboo-based products amounted at RM 287.2 billion and Malaysia was able to export RM 9.97 million worth of bamboo and bamboo products. The timber species and sufficient stock of planting material has always been the biggest concern to produce quality timber for forest plantation. Thus, to mitigate these issues, identifying potential species such as bamboo and technology to mass produce these identified genotypes must be assessed. The objectives of the current research were to evaluate the physical and mechanical properties as well as to develop a suitable *in vitro* protocol for mass clonal propagation of *Bambusa vulgaris* 'Striata', *Bambusa vulgaris* and *Dendrocalamus asper*.

Three matured culms of bamboo were sampled randomly from two selected clumps each, further divided into three equal lengths of 3 m each marked as bottom, middle and top. Samples were taken from nodal and internodal parts from each levels of height for properties evaluation. Physical properties included moisture content, density and shrinkages while three-point bending test was used to evaluate flexural modulus and flexural strength. The properties were all significantly different between species at $p \leq 0.05$, except the modulus of elasticity (MOE) were non-significant. The highest moisture content was observed in *B. vulgaris* 'Striata' (55.28 %) and the lowest in *D. asper* (33.13 %). For both *B. vulgaris* 'Striata' and *B. vulgaris*, moisture content was the highest at the top level (56.32-58.08 %), and also at internodal section (54.60-57.66 %). *D. asper* has highest moisture content at the bottom level (38.71 %) and nodal section (34.53 %). Density was the highest in *D. asper* (0.93 g/cm^3), the lowest

in *B. vulgaris* 'Striata' (0.70 g/cm^3). Higher density was observed at bottom and middle level for *B. vulgaris* 'Striata' (0.69-0.73 g/cm^3) and *B. vulgaris* (0.77-0.83 g/cm^3), while highest density was observed at top level in *D. asper* (0.94-0.97 g/cm^3). Node had higher density in *B. vulgaris* 'Striata' (0.71-0.74 g/cm^3) and *D. asper* (0.92-0.94 g/cm^3), while *B. vulgaris* had the same density for node and internode (0.74-0.82 g/cm^3). Tangential shrinkage of bamboo strips was recorded the highest in *B. vulgaris* 'Striata' (23.52 %) and the lowest in *D. asper* (1.45 %). Highest shrinkage was observed at middle and top levels for *B. vulgaris* 'Striata' (25.33-26.29 %) and *B. vulgaris* (16.45-19.44 %), at bottom level for *D. asper* (1.40-2.66 %). Node shrunk greater in *B. vulgaris* 'Striata' (32.01-32.48 %) and internode shrunk greater in *D. asper* (1.52-2.30 %). The highest MOE was observed in *D. asper* (12,262 MPa), and the lowest in *B. vulgaris* 'Striata' (11,890 MPa). Highest MOE was observed at top level in *B. vulgaris* (12,968-13,841 MPa) and *D. asper* (14,276-14,519 MPa). Internode had higher MOE in *D. asper* (13,785-14,595 MPa) and *B. vulgaris* (97-106 MPa), while node had higher MOE in *B. vulgaris* 'Striata' (106-124 MPa). The highest modulus of rupture (MOR) was observed in *D. asper* (119 MPa) and the lowest in *B. vulgaris* (97 MPa). Highest MOR obtained from bottom level of *B. vulgaris* (99-113 MPa), bottom and middle in *B. vulgaris* 'Striata' (115-116 MPa), top level for *D. asper* (134-140 MPa). Internode sections had higher MOR than node for both *B. vulgaris* (97-106 MPa) and *D. asper* (147-161 MPa), while *B. vulgaris* 'Striata' was on the contrary (106-124 MPa). Both *B. vulgaris* and *B. vulgaris* 'Striata' have showed as reliable resources to be considered as potential plantation species like *D. asper*.

Nodal segments (2.5-3.0 cm) from lateral branches were collected as explant for *in vitro* propagation. The explants pre-sterilized in Benomyl (0.1 %), Streptomycin (0.2 %), Boric acid (1.0 %) for 60 minutes had significantly reduced the percentage of microbial contamination, 15-20 % of aseptic culture was able to produced. In surface sterilization, 0.1 % of mercuric chloride ($HgCl_2$) was observed to be the best compound to produce clean and viable cultures. The exposure time of 10, 8, and 5 minutes were obtained as the best to produce highest percentage of aseptic culture for *B. vulgaris* 'Striata' (73 %), *B. vulgaris* (67 %) and *D. asper* (85 %) respectively. The explants were then inoculated into MS media supplemented with 6-Benzylaminopurine (BAP). BAP (0, 1, 2, 3, 4, 5, 10 mg/L) were tested for *B. vulgaris* 'Striata' and *B. vulgaris*, and BAP (0, 1, 3, 5, 10, 15, 20 mg/L) were tested for *D. asper*. MS media supplemented with 5 mg/L of BAP was optimum for *B. vulgaris* 'Striata' and *D. asper* while 3 mg/L was optimum for *B. vulgaris* in bud breaking stage. 50 % of bud breaking was achieved in *B. vulgaris* 'Striata' with mean number of shoots of 1.8 and mean length of 3.01 cm. For *B. vulgaris*, 65 % of bud break with 1.23 shoots of 4.08 cm were produced. 70 % of bud break with 1.74 shoots of 1.37 cm length of shoots were recorded for *D. asper*. The protocol for *in vitro* propagation achieved for bud breaking is effective and further development are needed for future tests.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PEMBIAKAN KLON SECARA BESAR-BESARAN BAGI GENOTIP BULUH TERPILIH UNTUK TUJUAN PEMBINAAN DI MALAYSIA

Oleh

LEE PAY CHIANN

Julai 2020

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Malaysia merupakan salah satu pengeksport utama produk kayu tropikal di dunia, hutan semula jadi tidak mencukupi bagi memenuhi keperluan permintaan pasaran bahan pembinaan di mana populasi sedunia dianggarkan akan mencapai 9.7 bilion pada tahun 2050. Program ladang hutan telah ditubuhkan pada tahun 2005, dan buluh juga telah disyorkan sebagai salah satu spesis untuk menghasilkan sumber kayu berkualiti. Pada tahun 2018, produk berasaskan buluh telah mencapai eksport global sebanyak RM 287.2 bilion, dan Malaysia berupaya mengeksport buluh dan produk buluh yang bernilai RM 9.97 juta. Pilihan spesis dan stok bahan penanaman yang mencukupi merupakan isu yang sentiasa dipertimbangkan bagi ladang hutan. Justeru, bagi menangani isu-isu tersebut, spesis yang berpotensi seperti buluh dan teknologi menghasilkan genotip ini dalam skala yang besar perlu dinilai. Objektif penyelidikan ini adalah untuk menilai ciri-ciri fizikal dan mekanikal serta menubuhkan protokol *in vitro* yang sesuai bagi pembiakan klon *Bambusa vulgaris* 'Striata', *Bambusa vulgaris* dan *Dendrocalamus asper*.

Tiga batang buluh yang matang disampel secara rawak dari dua rumpun masing-masing, seterusnya dibahagikan kepada tiga bahagian yang sama (3 m) dan ditandakan bawah, tengah dan atas. Sampel diambil daripada bahagian node dan internode bagi setiap ketinggian untuk penilaian ciri-ciri. Ciri-ciri fizikal termasuk kandungan lembapan, ketumpatan dan pengecutan sementara 'three-point bending test' dijalankan untuk menilai modulus lenturan dan kekuatan lenturan buluh. Semua ciri-ciri kecuali 'modulus of elasticity' (MOE) menunjukkan perbezaan ketara di antara species pada $p \leq 0.05$. Kandungan lembapan tertinggi terdapat dalam *B. vulgaris* 'Striata' (55.28 %) dan terendah dalam *D. Asper* (33.13 %). Bagi *B. vulgaris* 'Striata' dan *B. vulgaris*, kandungan lempaban tertinggi di paras atas (56.32-58.08 %), dan juga pada internode (54.60-57.66 %). *D. asper* mempunyai kelembapan tertinggi pada paras bawah

(38.71 %) dan node (34.53 %). *D. asper* mempunyai ketumpatan yang paling tinggi (0.93 g/cm^3), dan *B. vulgaris* 'Striata' paling rendah (0.70 g/cm^3). Ketumpatan tertinggi terdapat pada paras bawah dan tengah bagi *B. vulgaris* 'Striata' (0.69-0.73 g/cm^3) dan *B. vulgaris* (0.77-0.83 g/cm^3), manakala pada paras atas dalam *D. asper* (0.94-0.97 g/cm^3). Node lebih tumpat bagi *B. vulgaris* 'Striata' (0.71-0.74 g/cm^3) dan *D. asper* (0.92-0.94 g/cm^3), manakala sama bagi node dan internode dalam *B. vulgaris* (0.74-0.82 g/cm^3). Pengecutan tangen dicatatkan tertinggi dalam *B. vulgaris* 'Striata' (23.52 %) dan terendah dalam *D. asper* (1.45 %). Kecutan tertinggi berlaku pada paras tengah dan atas bagi *B. vulgaris* 'Striata' (25.33-26.29 %) dan *B. vulgaris* (16.45-19.44 %), pada paras bawah bagi *D. asper* (1.40-2.66 %). Node mengecut lebih dalam *B. vulgaris* 'Striata' (32.01-32.48 %) dan internode mengecut lebih dalam *D. asper* (1.52-2.30 %). 'Modulus of elasticity' (MOE) tertinggi tercatat dalam *D. asper* (12,262 MPa), dan terendah dalam *B. vulgaris* 'Striata' (11,890 MPa). MOE tertinggi terdapat pada paras atas dalam *B. vulgaris* (12,968-13,841 MPa) dan *D. asper* (14,276-14,519 MPa). Internode mempunyai MOE lebih tinggi dalam *D. asper* (13,785-14,595 MPa) dan *B. vulgaris* (97-106 MPa), manakala lebih tinggi dalam node bagi *B. vulgaris* 'Striata' (106-124 MPa). 'Modulus of rupture' (MOR) tertinggi tercatat dalam *D. asper* (119 MPa) dan terendah dalam *B. vulgaris* (97 MPa). Paras bawah *B. vulgaris* mempunyai MOR tertinggi (99-113 MPa), *B. vulgaris* 'Striata' (115-116 MPa) pada paras bawah and tengah, *D. asper* (134-140 MPa) pada paras atas. Internode mempunyai MOR lebih tinggi bagi *B. vulgaris* (97-106 MPa) dan *D. asper* (147-161 MPa), manakala *B. vulgaris* 'Striata' mempunyai MOR lebih tinggi pada node (106-124 MPa). Kedua-dua *B. vulgaris* dan *B. vulgaris* 'Striata' telah menunjukkan potensi untuk dipertimbangkan sebagai spesis ladang hutan

Segmen nod (2.5-3.0 cm) dari dahan sisi digunakan sebagai eksplan untuk pembiakan *in vitro*. Eksplan dipra-sterilkan dalam Benomyl (0.1 %), Streptomycin (0.2 %) , Asid boric (1.0 %) selama 60 minit telah mengurangkan pencemaran mikrob scara ketara, 15-20 % kultur bersih dapat dihasilkan. Untuk pensterilan permukaan, 0.1 % mercuric chloride ($HgCl_2$) didapati paling berkesan bagi menghasilkan kultur aseptik. 10, 8 dan 5 minit masing-masing didapati terbaik bagi mensterilkan dan menghasilkan kultur bersih bagi *B. vulgaris* 'Striata' (73 %), *B. vulgaris* (67 %) dan *D. Asper* (85 %). Eksplan seterusnya dikulturkan dalam media MS yang ditambah dengan 6-Benzylaminopurine (BAP). BAP (0, 1, 2, 3, 4, 5, 10 mg/L) diuji untuk *B. vulgaris* 'Striata' dan *B. vulgaris*, manakala BAP (0, 1, 3, 5, 10, 15, 20 mg/L) diuji untuk *D. Asper*. Media MS ditambah dengan 5 mg/L BAP adalah optimum bagi *B. vulgaris* 'Striata' dan *D. asper*, manakala 3 mg/L adalah optimum bagi *B. vulgaris* pada tahap pertunasan. 50 % eksplan *B. vulgaris* 'Striata' bertunas dan 1.80 pucuk dengan 3.01 cm telah dihasilkan. Untuk *B. vulgaris*, 65 % eksplan telah bertunas dan menghasilkan 1.23 pucuk dengan kepanjangan 4.08 cm. Sebanyak 70 % eksplan dan jumlah 1.74 pucuk dengan 1.37 cm kepanjangan telah direkodkan untuk *D. asper*. Protokol pembiakan *in vitro* yang tercapai untuk pertunasan adalah berkesan dan perkembangan protokol diperlukan bagi pengajian yang seterusnya.

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Thank God.

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

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
$HgCl_2$	Mercuric chloride
HCl	Hydrochloric acid
IBA	Idole-3-butyric acid
M	Molar
MC	Moisture content
Mg/L	Milligram per litre
MS	Murashige & Skoog's medium
MOE	Modulus of elasticity
MOR	Modulus of rupture
NAA	Naphthalene acetic acid
NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
SDW	Sterile distilled water
VB	Vascular bundle

CHAPTER 1

INTRODUCTION

1.1 Background

Tropical rainforest is one of the most productive types of forests in the world, especially those in Southeast Asia, which believed to be the oldest and among the most biologically diverse forest in the world. Malaysia, a tropical country, was once almost entirely covered with forests, now with 67.6 % remained is facing deforestation issue every year (FAO, 2019). Clearance of natural forests in Malaysia is not only for development of housing area to accommodate the expanding population, it is also for timber extraction in order to generate national income. Besides timber extraction, land cleared for industrial palm oil and rubber plantation was also contributing to deduction in forested area (Miyamoto, 2020).

In 2019, Malaysia had achieved the highest GDP (Gross Domestic Product) for the last five years, with a total of RM 1,420.5 billion (DOSM, 2020). Out of it, with a contribution of 7.2 %, at a total value of RM 101.3 billion was from agriculture sector. Forestry and logging had been an essential sub-sector for agriculture sector that contributed at an average of 6 billion MYR yearly, from 2015 to 2019. In 2015, the gross output value of forestry and logging sub-sector was RM 7,552.2 million, 76 % was contributed solely by logging industry, marked a gross output of RM 5,774.6 million (DOSM, 2017). It shows that there has been a constant demand of Malaysian timber. It was not surprised when Malaysia as one of the leading exporters of tropical timber products in the world, supplying majority to Asian countries as well as the US and EU. Value generated from exporting major timber products attained RM 22.5 billion in year 2019, the major products were wooden furniture (40.6 %), plywood (15.1 %) and sawn timber (15.0 %) (MTIB, 2019). Based on the biennial report by ITTO (2019), 20 million m^3 of tropical logs produced in 2018, 12 % were exported, and remaining was for domestic consumption. In order to accommodate such a large supply of timber, excessive logging from natural forest stands has led to serious scenario of deforestation.

Recent Country Report from Malaysia in Global Forest Resources Assessment (FRA) 2015, total forested area in Malaysia was 21,097,000 ha, including 14,503,000 ha of Permanent Reserved Forest, 4,735,000 ha of State Land Forest and 1,859,000 ha of National Parks and Wildlife and Bird Sanctuary. Report showed that, there is an average loss of 7,200 ha of forested area yearly from 1990-2015 (FAO, 2015). Malaysia had the world's highest rate of forest loss between 2000 and 2012, according to a new global forest map developed in partnership of Mongabay.com with Google. Malaysia's total forest loss during the period amounted to 47,278 km^2 (14.4 %) of its year 2000 forest cover (Rhett, 2013). Malaysian government's major concern is the remaining forested lands are facing the threats of unsustainable logging plus illegal exploitation of forest product, The loss of natural forest land in large scale has caused negative effects

like loss of biodiversity richness, causing endangered species to extinct, soil erosion further leading to landslides and lightning flood, causing warmer climate, decreased cloud formation thus water supply reduced, and eco-tourism decreased and causing losses of national revenue (WWF, n.d.).

In an effort to reduce pressure on native forest as a source for raw materials and to ensure its continuous availability for the domestic timber industry, the Malaysian Government is encouraging the development of large-scale commercial forest plantations. In line with this policy, in March 2005, Ministry of Plantation Industries and Commodities (MPIC) was entasked to pursue an aggressive programme for the development of forest plantations in Malaysia. Under this programme, the Ministry has targeted to develop 375,000 ha of forest plantation at an annual planting rate of 25,000 ha per year for the next 15 years. Upon success, every 25,000 ha of plantation is expected to produce 5 million m^3 of timber. In line with the plantation programme, some fast-growing timber species were recommended. Among nine selected, Rubberwood (Timber Latex Clone) and Acacia spp. (mangium /hybrid) are the major species. Other fast-growing timber species recommended are *Tectona grandis* (Teak), *Azadirachta excelsa* (Sentang), *Khaya ivorensis*/ *Khaya senegalensis* (Khaya spp.), *Neolamarckia cadamba* (Kelempayan/ Laran), *Paraserianthes falcataria* (Batai), *Octomeles sumatrana* (Binuang) and five selected commercial bamboo species, they are *Gigantochloa scortechinii* (Semantan), *Gigantochloa Levis* (Beting), *Gigantochloa wrayi* (Beti), *Dendrocalamus asper* (Betong) and *Phyllostachys nigra* (Hitam) (MPIC, 2005).

In recent years, the use of bamboo as a whole or value-added product has risen globally. According to INBAR, total global export of bamboo-based products was valued at US$ 68.8 billion (RM 287.2 billion) in 2018, with an increment of 12.8 % compared to 2017. Malaysia was able to export RM 9.97 million worth of bamboo and bamboo products in 2018. (“Govt to introduce”, 2019). Looking at the promising industry, Primary Industries Ministry is scouting at possibilities of establishing more commercial nurseries to ensure an adequate supply of planting materials for bamboo plantation. Statistics for bamboo stands in Malaysia was not up to date since the last report in year 2005 for global FRA, country report on bamboo resources. The reported area of bamboo stand was 677,000 ha, accounted for 11 million tonnes of bamboo resources. In the recent press conference, the then Primary Industry Minister Teresa Kok revealed that bamboo cultivation in Peninsular Malaysia has amounted to 329,000 ha, 31 % of total acreage in Malaysia, whereas Sarawak has 45 % and Sabah 24 %. (“Bamboo industry must transform”, 2018). 58 mill bamboo clumps were covering the 31 % of cultivation in Peninsular Malaysia, 15 million clumps are in Perak. At the two-day event of Malaysia World Bamboo Day, Teresa Kok also mentioned that, at the end of year 2019, a nursery, TC lab and advancement in R&D were expected to produce 100,000 quality bamboo seedlings monthly of supply for bamboo plantation in Lahat (“Move to boost bamboo industry”, 2019). The expanding demand of this fast growing, energy saving material will not risk clearing of the natural forest, instead, it creates job opportunities via the developing of bamboo plantation.

1.2 Problem Statement

With the increasing global population, which is expected to reach 9.7 billion by the year 2050 (United Nation, 2020), the use of non-conventional building materials is crucial to provide a sustainable development to protect the environment. By cutting down the vast usage of cement and steel in construction industry will substantially reduce carbon emission that cause global warming. The bamboo has been introduced in regions such as the Central America for replacing conventional building material. Even though the characteristics of fast-growing, high-yielding and easily renewable of bamboo is attractive, the strength and durability for it to be commonly adapt in construction usage need more investigation. Lack of study concerning the mechanical properties of bamboo is one of the factors that is affecting low utilization of bamboo in construction.

Conventionally, bamboos are propagated through seeds, offsets and culm cuttings. However, it has been known for long that propagation through seeds is not feasible for bamboo due to the fact that most of the bamboo species have extremely long and unpredictable flowering cycle. Following with their monocarpic nature, poor seed setting, low seed viability, plus consumption of seeds by wild animals, it is impractical to produce through seeds. Alternatively, vegetative propagation through clump divisions, rhizomes, offsets and culm cuttings are techniques being practiced but these methods have a downside when the objective of mass scale propagation is the be meet. According to Gielis & Oprins (2002), most of the conventional techniques are useful for the production on smaller scale (up to 10,000 plants per year), so as to obtain large scale propagation by achieving 100,000 to 500,000 plants per year, classical techniques are inadequate, thus tissue culture is the only viable method.

1.3 Objectives

This experiment is carried out with the aim to clonally propagate selected bamboo genotypes in Malaysia for construction purposes in large scale basis by i*n vitro* methods. The specific objectives of this research are:

i) to evaluate the physical and mechanical characteristics of *Bambusa vulgaris*, *Bambusa vulgaris* 'Striata' and *Dendrocalamus asper* for their potential as plantation species;

ii) to develop a workable protocol of micropropagation of selected genotype of bamboo species.

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Lee Pay Chiann, born on December 25th, 1986, is the second daughter of three in the family from Ampang, Selangor. She was Chinese Educated in primary school and continued her secondary education in an all-girls school. After obtaining her SPM (Malaysian Certificate of Education) in 2003, she chose to take up STPM (Malaysian Higher School Certificate) as her pre-university programme.

She discovered her great interest in environmental issues and biology, especially into the studies of plants. She then decided to pursue her Bachelor of Forestry Science in Universiti Putra Malaysia, with the minor study of Urban Forestry. Graduated in 2009 with thesis entitled “Assessment of Leaf Miners Associated with Landscape Trees in Putrajaya, Selangor”.

LIST OF PUBLICATIONS

Lee, P. C., Kumar, S., & Nor Aini, A. S. 2018. In Vitro Regeneration of Bamboo Species. *Pertanika Journal of Scholarly Research Reviews*, 4(3): 80-88.

Lee, P. C., Kumar, S., & Nor Aini, A. S. 2020. Rooting Ability of *Gigantochloa scortechinii* Through Branch Cuttings. *The Malaysian Forester*, 83(1): 64-72.

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