**UNIVERSITI PUTRA MALAYSIA**

# *SYSTEMATICS AND CUTTINGS ROOTING PERFORMANCE OF CEMPAKA (Magnolia SPP. PLUM. EX L., MAGNOLIACEAE) IN PENINSULAR MALAYSIA*

**TAN SIN HOONG**

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# SYSTEMATICS AND CUTTINGS ROOTING PERFORMANCE OF CEMPAKA (*Magnolia* SPP. PLUM. EX L., MAGNOLIACEAE) IN PENINSULAR MALAYSIA

**By**

**TAN SIN HOONG**

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

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**SYSTEMATICS AND CUTTINGS ROOTING PERFORMANCE OF CEMPAKA (*Magnolia* SPP. PLUM. EX L., MAGNOLIACEAE) IN PENINSULAR MALAYSIA**

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**TAN SIN HOONG**

**July 2020**

**Chair: Associate Professor Thohirah Lee Abdullah, PhD**
**Faculty: Agriculture**

*Magnolia* Plum. ex L. (Magnoliaceae) is a genus of medicinal ornamental trees and shrubs with around 300 species. In Peninsular Malaysia, four species are cultivated while 15 species are wild. Propagation of *Magnolia* is generally difficult due to seed dormancy and slow rooting.

In study of rooting performance of *Magnolia champaca*, the effects of types of cutting (softwood, semihardwood) and indole-3-butyric acid (IBA) concentrations (0, 6000, 12000, 18000 mg/L) were investigated. Significant differences were found among the types of cutting and IBA concentrations. Almost all semi-hardwood cuttings failed to root. For the softwood cuttings, significant differences in rooting percentage and length of root were found, but not for number of roots. Cuttings treated with 12000 mg/L IBA produced the highest rooting percentage of 66.7% and longest mean length of roots at 7.5 cm, but the treatment is not significantly different with other IBA concentrations used. In the experiment involving *M. alba*, the effect of ethephon treatments (0, 1 day) and IBA concentrations (0, 3000, 6000, 9000 mg/L) were tested on softwood cuttings. The treatments were not significantly different. Rooting percentage of 40% was obtained in cuttings treated with 9000 mg/L IBA regardless of the ethephon treatment, however, its effect is not significantly different from the others. Histological study on softwood cuttings of *M. alba* found that root primordia originated from between secondary phloem and callus.

Twenty-nine individual trees representative of 5 wild *Magnolia* species from the forests of Peninsular Malaysia were documented morphologically, mapped and accessioned. The species found are identified as *M. elegans*, *M. liliifera*, *M. macklottii* var. *beccariana*, *M. praecalva* and *M. villosa*. Propagation trials successfully collected 3 accessions of 2 wild species and 2 additional taxa cultivated in Thailand as rooted

cuttings. Accordingly, *ex situ* collection of *M. liliifera* and *M. villosa* were successfully made.

Forty-two ISSR markers were screened and consequently 25 markers were selected to study 26 *Magnolia* accessions, which produced 223 bands with 100% polymorphism. Dendrogram generated showed similar relationships with previous studies based on chloroplast DNA sequences. The number of ISSR markers informative for discrimination of infraspecific variation in *M. champaca*, *M. alba*, *M. macklottii* var. *beccariana*, *M. liliifera* and *M. villosa* studied are 12, 13, 21, 25 and 23 respectively. Species-specific bands were found in *M. alba* (primer UBC807), *M. champaca* (UBC817), *M. macklottii* var. *beccariana,* (primers UBC815, UBC827, i2 and ISSR-B) and *M. villosa* (primer UBC855)

In conclusion, rooting performance of cuttings was investigated using *M. champaca* and *M. alba* and the cutting method was applied to collect new germplasm of wild native *Magnolia.* The documentation process of magnolias discovered new morphological variation and showed that there could be new findings based on observation of living plant in the forest. Protocols for elucidation of *Magnolia* genetic diversity, molecular identification and genetic relationship developed will be useful for future study and management of these valuable plants.

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

**SISTEMATIK DAN PRESTASI PENGAKARAN KERATAN BATANG CEMPAKA (*Magnolia* SPP. PLUM. EX L., MAGNOLIACEAE) DI SEMENANJUNG MALAYSIA**

Oleh

**TAN SIN HOONG**

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Cempaka (*Magnolia* spp.) merupakan satu genus pokok dan pokok renek hiasan dan ubatan yang mempunyai lebih kurang 300 spesies. Empat spesies ialah tanaman hiasan dan lima belas spesies boleh dijumpai liar di Semenanjung Malaysia. Pembiakan cempaka secara amnya mencabar disebabkan kedormanan biji benih dan pengakaran yang mengambil masa yang lama.

Dalam kajian terhadap cempaka kuning (*Magnolia champaca)*, kesan jenis batang keratan (batang lembut dan separa-keras) dan kepekatan IBA (0, 6000, 12000, 18000 mg/L) telah diujikaji. Perbezaan yang ketara telah dikesan antara rawatan-rawatan jenis batang keratan and kepekatan IBA. Hampir semua keratan separa-keras tidak dapat berakar. Bagi keratan lembut, perbezaan ketara dapat dikesan dalam peratusan pengakaran dan purata kepanjangan akar tapi tidak dapat dikesan dalam bilangan akar. Keratan lembut yang dirawat dengan 12000 mg/L IBA telah menghasilkan peratusan pengakaran dan kepanjangan akar yang paling tinggi, iaitu 66.7% dan 7.5 cm, tetapi ianya tidak berbeza secara signifikan dengan kepekatan IBA yang lain. Dalam eksperimen ke atas cempaka putih (*Magnolia alba*), keratan batang lembut telah dirawat dengan ethephon (0 atau 1 hari) dan IBA (0, 3000, 6000, 9000 mg/L). Rawatan tidak mendatangkan kesan yang berbeza dengan ketara. Peratusan pengakaran sebanyak 40% telah dicapai dalam keratan yang dirawat dengan 9000 mg/L IBA tidak kira jenis rawatan ethephon, tetapi ia tidak berbeza dengan signifikan berbanding dengan rawatan yang lain. Kajian histologi ke atas keratan batang lembut cempaka putih memdapati bahawa tunas akar berasal dari bahagian antara floem dan kalus.

29 individu pokok yang mewakili 5 spesies cempaka telah dijumpai dalam hutan-hutan Semenanjung Malaysia dan dicatat ciri-ciri morfologi dan nombor koleksi. Spesies tersebut ialah *M. elegans*, *M. liliifera*, *M. macklottii* var. *beccariana*, *M. praecalva* dan

*M. villosa.* Percubaan pembiakan telah berjaya mengumpul 3 individu yang mewakili 2 spesies liar Semenanjung Malaysia dan 2 taxa yang lain dari Thailand sebagai keratan batang yang telah berakar. Dengan ini, koleksi *ex situ M. liliifera* dan *M. villosa* berjaya dibangunkan.

Empat puluh dua penanda ISSR telah diuji dan 25 penanda telah dipilih untuk mengkaji kepelbagaian genetik 26 koleksi cempaka. 223 jalur telah dihasilkan dengan 100% polimorfisme. Dendrogram yang dihasilkan menunjukkan hubungan genetik yang mirip dengan hasil kajian jujukan DNA kloroplas. Bilangan penanda yang berguna untuk pencerapan variasi intraspesifik dalam *M. champaca*, *M. alba*, *M. macklottii*, *M. liliifera* dan *M. villosa* yang dikaji adalah 12, 13, 21, 25 dan 23 masing-masing. Jalur yang khusus kepada spesies telah dikenalpasti untuk *M. alba* (primer UBC807), *M. macklottii* var. *beccariana* (primer UBC815, UBC827, i2 dan ISSR-B) dan *M*. *villosa* (primer UBC855).

Kesimpulannya, kajian tentang prestasi pengakaran keratan batang telah dijalankan untuk cempaka kuning dan cempaka putih dan diaplikasikan untuk pengumpulan germplasm baru untuk spesies cempaka dalam hutan. Dokumentasi spesies cempaka telah menjumpa variasi morfologi yang baru dan menunjukkan bahawa hasil kajian yang baru dapat dijumpa berdasarkan permerhatian pokok hidup dalam hutan. Protokol untuk penghuraian kepelbagaian genetik, pengecaman molekular dan perhubungan genetik yang dibangunkan boleh dimanfaatkan dalam kajian lanjutan dan pengurusan pokok-pokok yang berharga ini.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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I hereby confirm that:

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| | |
|---|---|
| AFLP | Amplified Fragment Length Polymorphism |
| ANOVA | Analysis of variance |
| BAP | 6-Benzylaminopurine |
| *Blu* | *Blumiana* |
| Ca | Callus |
| cf. | confer |
| Co | Cortex |
| CTAB | Cetyltrimethylammonium bromide |
| DNA | Deoxyribonucleic acid |
| EMR | Effective multiplex ratio |
| F | Fiber |
| FH | Fraser's Hill |
| *Gwi* | *Gwillimia* |
| *Gy* | *Gynopodium* |
| IBA | Indole-3-butyric acid |
| ISSR | Inter-simple sequence repeat |
| IUCN | International Union for Conservation of Nature |
| *Ma* | *Manglietiastrum* |
| MA | *Magnolia alba* |
| MC | *Magnolia champaca* |
| ME | *Magnolia elegans* |
| MF | *Magnolia figo* |
| MI | Marker index |
| ML | *Magnolia liliifera* |

| | |
|---|---|
| MM | *Magnolia macklottii* |
| MO | *Magnolia coco* |
| MP | *Magnolia praecalva* |
| MS | Murashige and Skoog |
| MT | *Magnolia* sp. Thai |
| MV | *Magnolia villosa* |
| MX | *Magnolia* cf. *liliiflora* |
| NAA | Naphthalene-1-acetic acid |
| NPB | Number of polymorphic band |
| NSB | Number of scored band |
| PCR | Polymerase chain reaction |
| PH | Penang Hills |
| Pi | Pith |
| PIC | Polymorphic information content |
| PP | Primary phloem |
| PPB | Percentage of polymorphic band |
| PRF | Permanent Reserved Forest |
| PVP | Polyvinylpyrrolidone |
| RAPD | Random Amplification of Polymorphic DNA |
| RCBD | Randomized Complete Block Design |
| rcf | Relative centrifugal force |
| RP | Resolving power of primer |
| SP | Secondary phloem |
| SX | Secondary xylem |
| *Taq* | *Thermus aquaticus* |

| | |
|---|---|
| TPA | Totally Protected Area |
| WNJ | Weighted Neighbour Joining |
| *Yul* | *Yulania* |

# CHAPTER 1

# INTRODUCTION

Malaysia is a mega-biodiverse developing country committed in sustainable development. With an estimate of 15000 plant species native to Malaysia, Malaysia possesses an immense wealth of plant resources that can be developed for sustainable utilization. In line with the ratification of Convention on Biological Diversity in 1994, Malaysia has since then formulated and implemented many policies related to the conservation and sustainable utilization of plant resources. Among them, the National Landscape Policy has provided the framework and action plans to conserve green environments and to improve green infrastructures and landscape industry towards the goal of a "beautiful garden nation" (National Landscape Department, 2011). National Strategy for Plant Conservation and National Policy on Biological Diversity were formulated for the documentation, public education, sustainable utilization and conservation of the rich plant resources of Malaysia.

The origin of floristic diversity of Malaysia is complex and includes local and mainland Asian elements. An entity of perhumid rainforest have long existed in Malaysia since middle Eocene (around 48-38 million years ago) and persisted in forest patches during drier, colder epochs (Morley, 2018). A portion of these plants persisted and diversified *in situ* in the heterogeneous physical environments of Malaysia and formed local floristic elements called Sundaic elements. Among these plants are multiple lineages of *Magnolia* belonging to ancient flowering plant family Magnoliaceae (Nie *et al.*, 2008).

*Magnolia* Plum. Ex L. is a genus with 300 species of timber- and essential oil-producing highly ornamental and medicinal shrubs and trees. They are native to tropical and subtropical forests of Asia and America and a few species are widely cultivated. 25 *Magnolia* species are native to Malaysia, ranking 7$^{th}$ among countries with the most magnolia species (Rivers, Beech, Murphy & Oldfield, 2016). Notable species includes the yellow and white *cempaka*, which are straight-bole trees with cylindrical crown, producing large, strongly fragrant flowers used in perfumery, while also possessing various medicinal properties (Jaishree & Shabna, 2011). Elsewhere, magnolias are intensely bred as ornamental, such as various species as small trees that flower without leaves in the spring, and southern magnolia *M. grandiflora* with flowers reaching 30cm across (Gilman & Watson, 1994). Magnolia bark extract from *M. officinalis* is widely used in traditional chinese and japanese medicine, and have found modern applications as antibacterial (Ho *et al.*, 2001) and anticancer agents (Lee *et al.*, 2011). Many species share or produce similar bioactive phytochemicals (Song & Fischer, 1999). *M. montana*, also present in Malaysia, is used as the preferred timber for the construction of Acehnese traditional house in Indonesia (Fauna & Flora International, 2019).

Around half of *Magnolia* species around the world are threatened with extinction due to habitat loss, and many are included in conservation programs due to their ethnobotanical significance and pressure from overexploitation (Rivers *et al.*, 2016). However, most species in Malaysia are little-known, rare and understudied trees

restricted to primary forests (Nooteboom, 2012). Due to the sparse distribution pattern of wild magnolia species in Malaysia (Manokaran *et al.*, 1992), widespread difficulty in the genus to propagate vegetatively (Ranney & Gilooly, 2014) and dormancy and low productivity of seeds (Kameneva & Koksheeva, 2013), conservation and horticultural use of native Malaysian *Magnolia* species is very limited. Despite their ornamental and medicinal potentials, and global proposition and efforts in conserving native *Magnolia* species in *ex situ* collections worldwide (Botanic Gardens Conservation International, 2008), interest in collecting and conserving wild *Magnolia* species in Malaysia remains lacking.

In regular practice, woody plants are vegetatively propagated by cuttings, layering and grafting. The cuttings method for instance, relies on the formation of new roots from segments of the plant, induced by endogenous or exogenous plant hormones responsible for the formation of new root meristemoids and then primordia (Pop *et al.*, 2011). Auxins such as indole-3-butyric acid (IBA) are the main phytohormones controlling rhizogenesis, however, many chemicals such as ethylene, cytokinins, jasmonate and nitric oxide are involved in this complex process (Pacurar *et al.*, 2014). Feasibility to vegetatively propagate woody plants, especially for selected cultivars or varieties, enables their use in horticulture (Davies *et al.*, 1994). Among the cultivated species in Malaysia, arborescent *M. champaca* and *M. alba* are difficult to propagate by cuttings and layering. In contrast, the shrubby *M. coco* and *M. figo* are readily propagated by layering and cuttings respectively. Preliminary observations suggested that rooting of evergreen *Magnolia* might require young softwood and extremely high auxin concentrations (Ranney & Gillooly, 2014). Recent discovery of complex interactions involving many chemicals during the process of adventitious rooting may also guide the improvement in vegetative propagation of difficult-to-root *Magnolia* species and varieties. All in all, improvement is needed in vegetative propagation of tropical *Magnolia* species for their utilization, collection and conservation.

Tremendous advances in the molecular biology achieved in recent decades, including the discovery of the polymerase chain reaction (PCR), made genetic materials in organisms widely accessible for study. Many techniques were developed and pioneered new fronts of genetic research. It is now possible to study the characteristics of genes and genomes, genetic diversity and genetic relationships of organisms, genotype-phenotype correlation, and molecular process involving genes. Applications were found in agriculture, diagnosis, and the science and conservation of natural resources.

DNA sequences dominated in the systematic studies of *Magnolia* to clarify their genetic relationships. Molecular markers based on amplifying DNA fragments through polymerase chain reaction (PCR) are widely used to study the genetic diversity of *Magnolia* populations and species (Cires *et al.*, 2013), and to a lesser extent in other plants, interspecific relationships (Iruela *et al.*, 2002). One of these molecular markers is inter-simple sequence repeats (ISSR) marker, which relies on the ubiquitous genomic simple sequence repeats (e.g. ACACACAC…, GTTGTTGTTGTT…) as targets to amplify DNA segments. The ISSR markers are hypervariable with additional advantages such as low-cost, high reproducibility, and not requiring sequences of target genome to use. The data produced by ISSR markers can be used to study genetic

diversity, molecular profiling and genetic relationships (Ng & Tan, 2015). Understanding the genetic aspects of *Magnolia* in Malaysia using molecular marker techniques will guide their *ex situ* collection, *in situ* conservation, utilization and improvement.

To summarize, the main problems in *Magnolia* research in Malaysia are difficulty in propagation especially conventional propagation by cuttings, lack of work and inventory database on native *Magnolia*, and the need for and lack of genetic tools for various purpose including taxonomy, systematics and conservation management.

To overcome the mentioned constraints in *Magnolia* research in Malaysia, a few objectives are proposed in this study:

i. To assess the rooting performance of *M. champaca* and *M. alba* cuttings treated with ethephon, different IBA concentrations and types of cuttings.
ii. To locate, identify, map for basic floristic database and conduct cutting propagation trials on the native *Magnolia* species from the forests of Peninsular Malaysia
iii. To study the genetic diversity and genetic profiling of *Magnolia* species in Peninsular Malaysia using ISSR markers.

The justifications of these research objectives are for better horticultural knowledge in propagating *Magnolia* trees vegetatively, establishing basic floristic database on these precious species, introduction of new native species into cultivation, landscaping and *ex situ* conservation, and developing the genetic apparatus for studying *Magnolia* populations and species. Therefore, this study is relevant not only to researchers in horticulture, plant conservation and molecular biology, but also to ornamental plant producers, landscapers, *in situ* and *ex situ* conservation site managers and plant breeders.

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