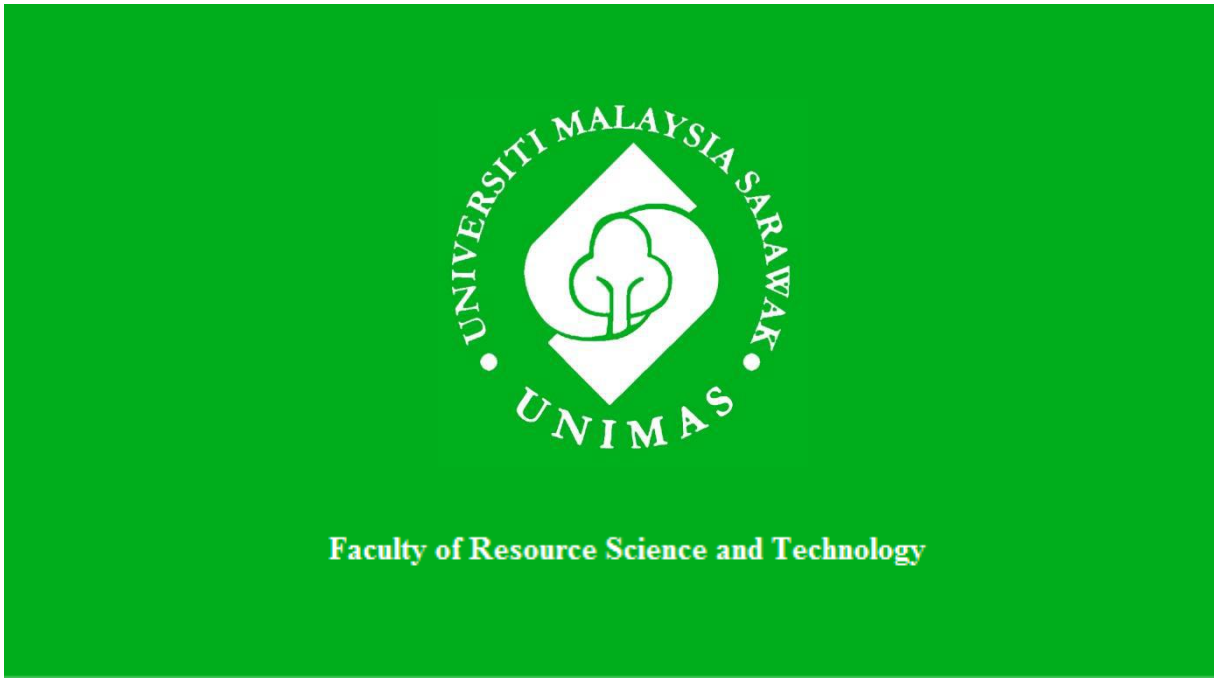

ESTABLISHMENT OF AXENIC EXPLANTS AND CALLUS CULTURE OF *SWIETENIA MACROPHYLLA* KING (TUNJUK LANGIT)

NOR ASHIKIN BINTI ISMAIL

Bachelor of Science with Honours
(Plant Resource Science and Management)
2015

UNIVERSITI MALAYSIA SARAWAK

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Final Year Project Report ☑

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Establishment of Axenic Explants and Callus Culture of *Swietenia macrophylla* King (Tunjuk Langit)

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This report is submitted in partial fulfilment of the requirement for the degree of Bachelor of Science with Honours in Plant Resource Science and Management

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LIST OF ABBREVIATIONS

B5	Gamborg B5 medium
BAP	N6-benzylaminopurine
Benomyl	Methyl [1-[(butylamino) carbonyl]-1H-benzimidazol-2-yl] carbamate
cm	centimeter
CRD	Completely randomized design
HCl	Hydrochloric acid
IBA	Indole-3-butyric acid
KOH	Potassium hydroxide
m	meter
mg/l	milligram per litre
mm	millimeter
MS	Murashige and Skoog medium
NaOCl	Sodium hypochlorite
PGR	Plant growth regulator
WPM	Wood Plant Medium
($Ca[OCl]_2$)	Calcium hypochlorite
2,4-D	2,4- dichlorophenoxy acetic acids

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Establishment of Axenic explants and Callus Culture of *Swietenia macrophylla* King (Tunjuk Langit)

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ABSTRACT

Swietenia macrophylla King or big-leaf Mahogany was mainly distributed from Mexico to southern Amazon region of Brazil and it is belongs to Meliaceae family. It is known as large deciduous tree and has been used for many purposes which mainly for medicinal. *S. macrophylla* seeds were used to relieve pain, diabetes and also hypertension in Malaysia. *S. macrophylla* is mostly propagated by seeds but this technique is not very successful. The objectives of this study are to establish an effective procedure for *in vitro* propagation of the *S. macrophylla* by using plant growth regulators. Leaves from grown seedling of *S. macrophylla* were used as the explants. Results showed most of the explants were found damaged and contaminated by treatment of 15% Clorox® concentration. 100% axenic culture were found by establish an effective surface sterilization protocol which involved dipping explants first in 70% ethanol for 30 seconds followed by agitating the leaf explants in 10% Clorox® concentration for 15 minutes, rinsed at least three times with sterilized distilled water before culture in full strength MS medium. The experiment on callus induction was carried out relatively successful. Result showed that 2,4-dichlorophenoxyacetic acid (2,4-D) at 1.5 mg/l is effective for the induction of callus formation. It was found that the 2,4-D alone has potential effect as plant growth regulator for callus induction of this plant species. The study of BAP and IBA in combination for shoot and root proliferation was conducted but there is no any sign of shoot and root produced after a month of sub-culturing. Such protocol needs to be further study in order to improve the potential and modified by using other types of plant growth regulator.

Key words: *Swietenia macrophylla* King, *in vitro*, surface sterilization, callus induction, shoot and root proliferation.

ABSTRAK

Swietenia macrophylla King atau “big-leaf Mahogany” (Tunjuk Langit) kebanyakannya ditemui dari Mexico ke rantau selatan Amazon di Brazil dan diklasifikasikan dalam keluarga Meliaceae. Ia dikenali sebagai pokok daun luruh yang besar dan telah digunakan untuk pelbagai tujuan terutamanya untuk perubatan. Biji benih *S. macrophylla* telah digunakan untuk melegakan kesakitan, kencing manis dan juga tekanan darah tinggi di Malaysia. *S. macrophylla* kebanyakannya disebarkan oleh biji benih tetapi teknik ini tidak begitu berjaya. Objektif kajian ini adalah untuk mewujudkan prosedur yang berkesan untuk pembiakkan *in vitro S. macrophylla* dengan menggunakan hormon pertumbuhan. Daun dari anak benih *S. macrophylla* digunakan sebagai eksplan. Keputusan eksperimen menunjukkan kebanyakan eksplan didapati rosak dan tidak axenic oleh rawatan 15% Clorox®. 100% eksplan axenic dihasilkan dengan mewujudkan protokol permukaan pensterilan yang melibatkan eksplan direndam dalam 70% etanol selama 30 saat diikuti oleh mengagitasi eksplan dalam 10% Clorox® selama 15 minit, dicuci sekurang-kurangnya tiga kali dengan air suling yang steril sebelum di kultur dalam MS media. Eksperimen induksi kalus telah dijalankan dengan jayanya. Keputusan menunjukkan bahawa 2,4-D pada 1.5 mg/l berkesan dalam induksi pembentukan kalus. Keputusan mendapati bahawa asid ‘2,4-dichlorophenoxyacetic’ (2,4-D) sahaja mempunyai kesan yang berpotensi sebagai hormon pertumbuhan untuk induksi kalus bagi spesies tumbuhan ini. Kajian gabungan antara BAP dan IBA dalam percambahan pucuk dan akar juga dijalankan tetapi tiada tanda pucuk dan akar yang dihasilkan dalam masa sebulan selepas pengkulturan. Protokol itu memerlukan kajian lanjut untuk meningkatkan potensi dan diperbaiki dengan menggunakan hormon pertumbuhan yang lain.

Kata kunci: *Swietenia macrophylla* King, *in vitro*, permukaan pensterilan, induksi kalus, percambahan pucuk dan akar.

CHAPTER 1

INTRODUCTION

Swietenia macrophylla King or big leaf mahogany belongs to Meliaceae family. It is mainly grows in Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru and Venezuela. The species has been widely planted mainly in Southern Asia and the Pacific such as India, Indonesia, Philippines and Sri Lanka. It has also been introduced into West Africa (Soerianegara and Lemmens, 1993).

S. macrophylla has commercial value for industrial plantations as well as for reforestation and afforestation in Indonesia. Martawijaya et al. (2005) reported that the species is a relatively soft and has intermediate weight. The structure of the wood showed an attractive looks and can be easy to work in making furniture. The seed also has medicinal importance such as anti-oxidant, anti-microbial, anti-inflammatory, anti-HIV, anti-ulcer, anti-fungal, anti-malarial and also anti-diarrhoeal effects (Eid et al., 2013). The seeds of the *S. macrophylla* also have an ability to treat dysentery, cancer, coughs, intestinal parasitism and also fever. According to Suzuki et al. (2008), the leaves can be used as dye the extract from the bark can be acts as an astringent for wounds and also apply for tanning.

Nowadays, it was been recorded that the species was almost extinct in some countries like Ecuador, Colombia, Panama, Costa Rica, Bolivia, Mexico, Belize and Brazil. Lugo (2002) reported that the species was declined in Guatemala, Peru, Nicaragua and Honduras. The problem is exacerbated by the short viability of Mahogany seed and hard to store. Tissue culture has been used in the production of quality seedling of most medicinal plants for example Victório et al. (2012) studied the *in vitro* propagation of *Calendula officinalis* which

has high medicinal value and cosmetics purposes. Next, development of adventitious shoots of *Betula platyphylla* var. *japonica* was also successful as stated by Vaario et al. (1995). Tissue culture method is a potential method to produce quality planting stock for large scale planting. This study is proposed to examine the possibility of *in vitro* propagation of this species for production of axenic culture. Hence, the specific objectives of the present study are:

1. To establish a working surface sterilization protocol for *S. macrophylla* leaf explants.
2. To determine the effect of 2,4-D on callus induction.

CHAPTER 2

LITERATURE REVIEW

2.1 *Swietenia macrophylla* King

2.1.1 The species origin

Swietenia macrophylla King was distributed from Mexico to Bolivia and central Brazil (refer **Figure 2.1**) (Lamb, 1966). It is known as big leaf mahogany and a tropical tree species that native to Central and South America. *S. macrophylla* be the largest plantations in South and Southeast Asia and the Pacific region. This species is also widely used for avenue planting in some Asian countries such as Indonesia, India and Sri Lanka. The first introduction of *S. macrophylla* into any country is to Indonesia in 1870 with seeds from India (Mayhew and Newton, 1998). Between 1897 and 1902, it was planted as an ornamental and cultivated in plantations in Java. The species distribution usually corresponds to tropical dry forests with about 1000-2000 mm annual precipitation. Lamb (1966) also reported that *S. macrophylla* also grows in humid and subtropical zones in a many types and conditions of soil.

The taxonomic classification and nomenclature of *S. macrophylla* is as follow:

Kingdom: Plantae

Phylum: Magnoliophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Sapindales

Family: Meliaceae

Subfamily: Swietenoideae

Genus: Swietenia Jacq.

Species: *Swietenia macrophylla* King

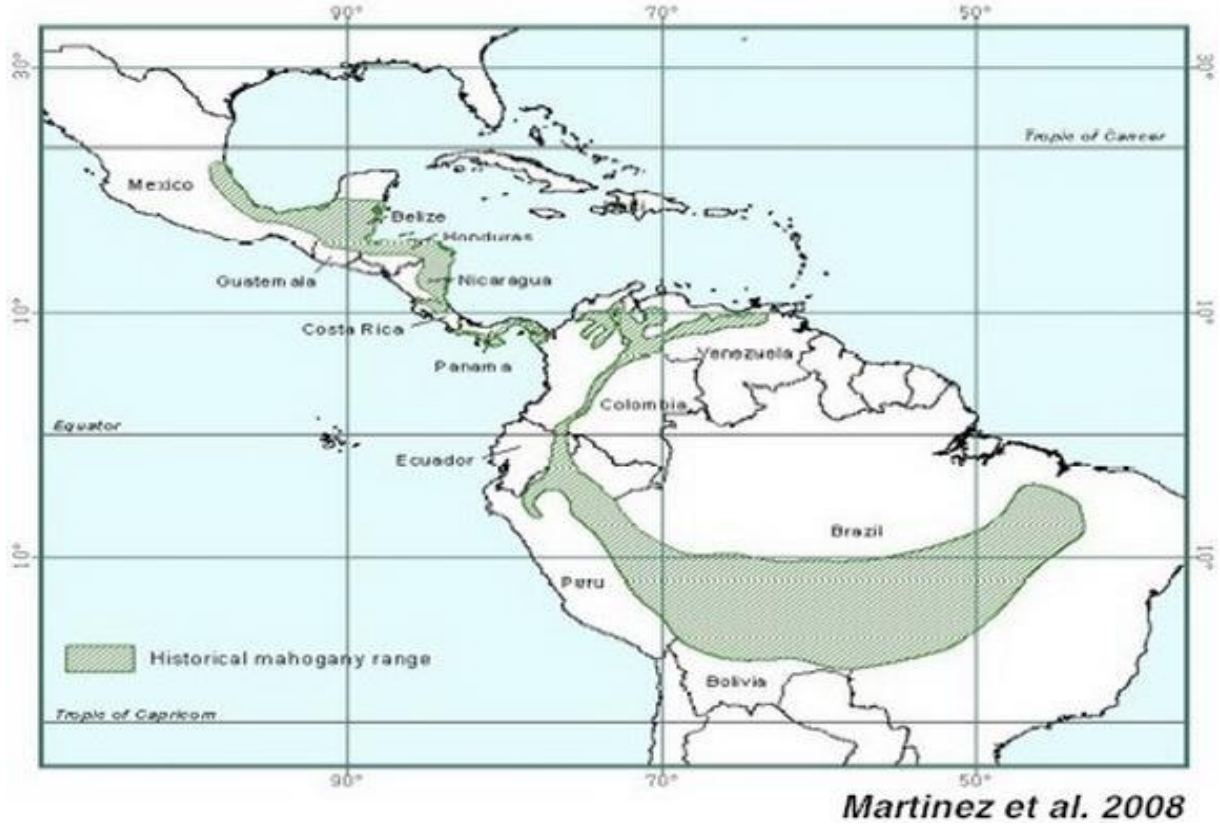

Figure 2.1 Map distribution of *Swietenia macrophylla* from Mexico to south eastern Amazonian (Martinez et al., 2008)

2.1.2 Description of *Swietenia macrophylla* King

Swietenia macrophylla is a large deciduous tree with an umbrella-shaped crown. It belongs to Meliaceae family, subfamilies of Swietenoideae. The common name in Indonesia is mahoni while in other countries are bara mahauni, bara-mahagoni, mahagni (Bangladesh), Brazilian mahogany tree, Colombian mahogany tree, Dominican mahogany, Honduras mahogany, Mexican mahogany tree, Peruvian mahogany tree, Spanish mahogany, West Indian mahogany (England), mahok, mahonie (Netherland), mogno (Portugal), mahokkani-bailek (Thailand) (Agroforestry Tree Database (1993); Soerianegara and Lemmens, 1993).

Soerianegara and Lemmens (1993) reported that the species can reach heights up to 30 m and diameter at breast height (DBH) of more than 1.5 m. The trunk is straight and cylindrical. The crown of young tree is narrow, but old tree have a broad, dense and highly branched crown. The outer bark of older trees is scaly and brownish-grey to reddish-brown, and the inner bark is red-brown or pinkish-red. The leaves are usually paripinnate or imparipinnate, leaves shape lanceolate or ovate. The leaflets are asymmetrical, 5–12 cm long and 2–5 cm wide, with a whole margin and an acute or acuminate apex (Soerianegara and Lemmens 1993; Schmidt and Joker, 2000). The flowers are unisexual, 0.5–1.0 cm in length, and are borne in large, branched inflorescences including both male and female. The fruits are capsular, oblong or ovoid, 11.6–38.7 cm in length, 6.7–12.0 cm in diameter and light grey to brown with 4–5 valves (**Figure 2.2**). Each fruit contains 22–71 developed seeds. The seeds are samaroid, bulky at their base, 7–12 cm long and 2–2.5 cm wide including the wing (**Figure 2.3**) (Soerianegara and Lemmens, 1993).

Figure 2.2 The internal arrangement of seeds of *S. macrophylla* (Source: Krisnawati et al., 2011)

Figure 2.3 The seeds of *S. macrophylla* (Source: Krisnawati et al., 2011)

2.2 Economic importance

According to Lamb (1966), *Swietenia macrophylla* King are suitable for timber production especially in making furniture or veneers because it is strong in weight and easily worked. The wood of this species also in good quality and can also be used for construction materials for example plywood, paneling, cabinet, framing, flooring and so on.

In addition, because of the limonoids and its derivatives content in the seeds of *S. macrophylla*, it has been discovered to have anti-inflammatory, anti-mutagenecity and anti-tumor activity (Guevera et al., 1996), anti-malaria (Soediro et al., 1990), anti-microbial (Maiti et al., 2007), and anti-nociceptive activity (Das et al., 2009). It is also useful in health commercially products to improve blood circulation and skin condition. In Malaysia, the seeds are used traditionally to treat hypertension, diabetes, and relieve pain (Goh & Kadir, 2011). In Indonesia, *S. macrophylla* seeds have been used as treatment of diabetes, hypertension, and malaria (Kadota et al., 1990).