Faculty of Resource Science and Technology

VEGETATIVE PROPAGATION OF *AQUILARIA MICROCARPA* BAILL. (GAHARU) BY STEM AND BRANCH CUTTINGS

Choo Yung Yung

Bachelor of Science with Honours
(Plant Resource Science and Management)
2013

VEGETATIVE PROPAGATION OF *AQUILARIA MICROCARPA* BAILL. (GAHARU) BY STEM AND BRANCH CUTTINGS

CHOO YUNG YUNG

This report submitted in partial fulfillment of the requirement for the Degree of Bachelor of Science with Honours in Plant Resource Science and Management

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK

2013

APPROVAL SHEET

Name of candidate: Choo Yung Yung

Matric no.: 26116

Title of dissertation: Vegetative Propagation of *Aquilaria microcarpa* Baill. (Gaharu) by Stem and Branch Cuttings

……………………………………….

(Prof. Dr. Hamsawi bin Sani)

Supervisor

……………………………………….

(Dr. Rebecca Edward)

Coordinator of Plant Resource Science and Management

Faculty of Resource Science and Technology

DECLARATION

I am Choo Yung Yung, final year student of Plant Resource Science and Management hereby declare that this thesis is my own work and effort with the guidance of my supervisor, Professor Dr. Hamsawi bin Sani. No portion of the work referred to this report has been submitted in support of an application for any other degree, university or institution of higher learning.

…………………….................

Choo Yung Yung (26116)

Plant Resource Science and Management

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

ACKNOWLEDGEMENT

First of all, I would like to give thanks to GOD for the continuous blessing and guidance to me to complete this thesis.

I also would like to express my sincerest gratitude to my supervisor, Professor Dr. Hamsawi bin Sani for his excellent advices, guidance, caring, and supports from the very beginning until the completion of this project.

Million of thanks to all supportive friends especially Lau Mee Cheng, Chong Seow Chen, Cheah Bin Pin, Lo Nyuk Thin, Anis Khalida binti Abd Halim, Fong Yin Mei, and Cherrie Lim Han Rou for their helping hand, provide transports during experiments, companionship, and give their best suggestions.

Last but not least, I would like to devote the utmost thankfulness to my lovely and caring family especially my parents: Choo Kuong Hock, Koh Ing Chien, brother and sisters for their loves, encouragement, supports and prayers during the whole process of learning in UNIMAS.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PGR	Plant Growth Regulators
mg/L	Milligram per liter
IBA	Indole-3-butyric acid
IAA	Indole-3-acetic acid
NAA	1-Naphthalene acetic acid
ANOVA	Analysis of Variance
GLM	General Linear Model
cm	Centimeter
P	P-value
X^2	Chi square
SD	Significant different
NSD	No Significant different

Vegetative Propagation of *Aquilaria microcarpa* Baill. (Gaharu) by Stem and Branch Cuttings

Choo Yung Yung

Plant Resource Science and Management
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

A study on vegetative propagation of *Aquilaria microcarpa* Baill. (Gaharu) by using stem and branch cuttings method under different stock ages plant of *Aquilaria microcarpa* and Plant Growth Regulators (PGR) was carried out at the Green house, East Campus of University Malaysia Sarawak (UNIMAS). The objectives of the study were (i) to examine the effect of stock plant age on rooting of cuttings, and (ii) to examine the effect of PGR on rooting. *Aquilaria* sp. has high commercial value in the production of incense, perfume and medicines. Due to the high demand of *Aquilaria* sp., it has been over exploited in the natural forests. The populations of *Aquilaria* sp. are decreasing rapidly and become endangered through uncontrolled harvesting. Vegetative propagation through stem cuttings is an effective way to control the number of *Aquilaria* sp. populations. In the study, four different ages of stock plant were used. They are i) 6 months old coppice from two years old seedling, ii) two years old potted seedlings, iii) three years old coppice from 8 years old stock plant, and iv) 4 years old of seedling. 0 mg/L, 50 mg/L, 100 mg/L and 200 mg/L of Indole-3-butyric acid (IBA) were used to examine which concentration is more effective to enhance rooting in stems and branch cuttings. 2 years old potted seedlings of cuttings was the best age of plant stock to produce highest rooting success percentage, number of root formed, and rooting length. 6 months old coppice from 2 years old seedlings of cuttings was the best ages of plant stock to produce the highest shoot percentage, number of shoots produced, and shoot length. Cutting treated with 0 mg/L of IBA was the best PGR to produce highest rooting success, number of root formed, root length, shoot percentage and number of shoot produced.

Keywords: *Aquilaria microcarpa* Baill., stem and branch cuttings, IBA, PGR

ABSTRAK

Kajian tentang pembiakan vegetatif pokok gaharu (Aquilaria microcarpa Baill.) dengan menggunakan kaedah keratan batang dan cabangan di bawah umur dan hormon yang berbeza telah dijalankan di Rumah Hijau, Kampus timur UNIMAS. Objektif kajian ini dijalankan untuk mengenalpasti kesan umur dan hormon terhadap pengakaran keratan batang. Aquilaria sp. mempunyai nilai komersial yang tinggi dalam pengeluaran minyak wangi dan ubat-ubatan. Oleh sebab permintaan tinggi terhadap Aquilaria sp. menyebabkan berlebihan eksploitasi dalam hutan semula jadi. Penuaian yang tidak terkawal ini mengakibatkan populasi Aquilaria sp. menjadi semakin berkurangan dan terancam. Pembiakan vegetatif melalui keratan batang adalah satu cara yang amat berkesan untuk mengawal populasi Aquilaria sp. Dalam kajian ini, empat umur yang berbeza telah digunakan. Mereka adalah i) Belukar berusia 6 bulan dari 2 tahun anak benih lama, ii) Anak benih berumur 2 tahun, iii) Belukar berusia 3 tahun dari pokok lama berusia 8 tahun, dan iv) Anak benih berumur 4 tahun. Indole-3-butyric asid (IBA) seperti 0 mg / L, 50 mg / L, 100 mg / L dan 200 mg / L telah digunakan untuk mengkaji kepekatan yang lebih berkesan untuk meningkatkan perakaran pada keratan batang dan cabangan. Keratan dari anak benih berumur 2 tahun mempunyai kejayaan yang tertinggi dalam peratusan perakaran, bilangan akar terbentuk, dan kepanjangan akar. Keratan dari belukar berusia 6 bulan dari 2 tahun benih lama adalah umur yang terbaik untuk menghasilkan peratusan pucuk, bilangan pucuk, dan ketinggian pucuk. Keratan yang dirawat dengan 0 mg / L IBA merupakan PGR yang terbaik untuk menghasilkan peratusan perakaran, bilangan akar terbentuk, kepanjangan akar, peratusan pucuk dan bilangan pucuk dihasilkan.

Kata kunci: *Aquilaria microcarpa Baill., keratan batang dan cabangan, IBA, PGR*

1.0 INTRODUCTION

Aquilaria sp. is a non-timber species belongs to Thymeleaceae family. It also can be known as gaharu or karas (Malay), aloewood, agarwood, or eagle wood (English), chen-xiang (Chinese), and Engkaras (Iban). For the purpose of this study, *Aquilaria microcarpa* Baill. was used due to its availability. In Malaysia, *A. microcarpa* is mostly distributed in Sabah and Sarawak (Chua, 2008). It can grow on various habitats which from the lowland to upland tropical forest (Eghenter, 2005). The genus itself is very popular in resin production. Resin produced from *Aquilaria* sp. gives huge contribution to the economy of a country since they are really valuable. Higher demand in market has caused excessive felling of the trees by the locals, thus significantly reduced the tree population in natural forest. Hence, all *Aquilaria* species have been listed under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II (Lata, 2007, Lim & Noraine, 2010, STIDC, 2008). In Sarawak, all *Aquilaria* species are placed under the protected species in Sarawak's Wildlife Protection Ordinance (1998).

Realizing the depletion of agarwood trees population, many interested parties tried to plant the species trees at commercial scale. However, poor supplies of seedlings usually slow down the effort of increasing tree population. *Aquilaria* sp. seeds have poor germination as they loss moisture content rapidly (He, Qi, & Hu, 2005). While according to Chua (2008), species of *A. malaccensis* are found to produce seeds after 7 to 9 years while other *Aquilaria* species can only produce seeds once in their life cycle. Besides, the hard coat seed itself causing slow and prevent germination to take place. Nevertheless, the available seed is not assured to be viable until develop into growing seedlings. As it is not reliable to plant seed that having sort of difficulties in germination, vegetative propagation become the potential alternative technique to produce the quality planting stocks.

Hence, the objectives of this study were to i) examine the effect of stock plant age on rooting of cuttings and, ii) examine the effect of Plant Growth Regulators on rooting.

2.0 LITERATURE REVIEW

2.1 Genus Aquilaria

According to the statistics of The Plant list (2010), Thymelaeaceae family consists of 52 plant genera. There are 2574 species ranking for the Thymelaeaceae family, but only 776 species are accepted (The Plant list, 2010). *Aquilaria* genus is one of the most important gaharu-producing trees in the Thymelaeaceae which have 15 species of temperate and tropical region trees. Among the 15 species, only 8 species are known to produce gaharu (Ahmad Junaidy, 2008). They are *A .microcarpa* Baill., *A. beccariana* van Tiegh*., A. hirta* Ridl., *A. malaccensis* Lamk., *A. agallocha* Roxb., *A. khasiana* Hallier, *A. crassna* Pierre ex Lecomte, and *A. grandiflora* Benth. Throughout Malaysia, agarwood-producing tree species were found such as *A. beccariana, A. hirta*, *A. malaccensis* and *A. microcarpa* (Chua, 2008). Tawan (2004) mentioned in Pusaka (Sept-Oct, 2008) that at least three tree species have been identified to be most valuable in Sarawak which are *A. microcarpa, A. beccariana*, and *A. malaccensis.*

2.1.1 Growth habit

Aquilaria sp. is a medium-sized tree. Normally, the height range is about 15-25m tall, but some species might reach up to 40m tall. While the diameter of *Aquilaria* sp. is about 50-60 cm, and some species might achieve up to 250cm (Donovan & Puri, 2004). In Malaysia, the average diameter growth rate of *Aquilaria* sp. in the native forests is quiet low, eg. a mean of 0.33 cm per year, but the fastest growing larger specimens are reported might grow to 0.8-1 cm per year (La Frankie, 1994). *Aquilaria* sp. is found growing in the plain hill slopes and ridges of up to 750m in both primary and secondary lowland dipterocarp forests (Jantan, 1990). In Sarawak, *Aquilaria* sp. is growing in the lowland mixed dipterocarp forest at altitude up to 270 m. Besides that, *Aquilaria* sp. can grow on wide range of soils type which including poor sandy soil. According to CITES (2004) and Barden, Noorainie, Mulliken and Song (2000), the *Aquilaria* sp. able to live at rocky, sandy or calcareous, well-drained slopes and ridges and land near swamps. *Aquilaria* sp. is suitable growth in hot, rainy, humid tropical and southern subtropical monsoon climate. Typically they grow between altitudes of 0-850 m, and up to 1000 m in locations with average daily temperatures of 20-22^{o}C (Wiriadinata, 1995). *Aquilaria* sp. has good shade-tolerant ability when young and can regenerate in almost pure patches underneath their mother tree (Beniwal, 1989).

2.1.2 Flowering habit

During the age of 5-6 years, *A. malaccensis* tree will start to produce flowers and fruits especially in the dry season (Adelina, 2004). Due to dry season at Sumatera all the time, *A. malaccensis* tree is flowering and fruiting twice in a year. The color of *Aquilaria* sp. flower is white or yellowish green with fragrant. It contains both androecium and gynoecium. Fruits are green color in the egg-shaped capsule. There are two seeds in a fruit. Mature fruits are blackish brown in color. Besides, seeds are ovoid shape, blackish brown color and densely covered with red-brown hair (Adelina, 2004). Seeds are recalcitrant. When seeds are dried until the moisture content is between 35% and 20%, their viability start drops. However, moisture content below 20% may rapidly accelerate the viability of seeds. Thus, seeds of *Aquilaria* sp. cannot be stored for long period and it is recommended to sow shortly after harvest. In a good seed year, *Aquilaria* sp. tree can produce about 1.5 kg of seeds (Adelina, 2004).

2.2 Distribution of *Aquilaria sp.*

Blanchette, Shoreview, Beek and Amsterdam (2009) mentioned that, *Aquilaria* trees are native to Asia ranging from Northern India to Vietnam and Indonesia. Barden *et al.* (2000), Donovan and Puri (2004) point out that, *Aquilaria* trees are widely distribute in the south and south-east Asia to the Pacific. Besides, they are diverse in Malaysia, India, Myanmar, Sumatra, Singapore, and Philippines (Chua, 2008). According to Rahimi (2007), Agarwood tree species are widely distributed in the natural forest of Peninsular Malaysia, Sabah and Sarawak. The Third National Forest Inventory (NFI–3) conducted between 1991 and 1993 for Peninsular Malaysia showed that *Aquilaria* sp. occurred in both logged and primary forests (Chin, Nor Akhiruddin, Samsuanuar, Yong, Hasnuddin, & Mohd Nashir, 1997).

2.3 Resin of *Aquilaria* sp.

Resin or agar oil is obtained from the infected agarwood tree. This infection will be occurred after natural or mechanical injured of stem (Ahmad Junaidy, 2008, Donovan and Puri, 2004) or present of larvae or fungal species that called as *Zeuzera confera* or *Cytosphaera mangiferae* inside the stem (Lata, 2007, NEDFi, n.d.). The larvae will make tunnels inside the tree trunks to allow fungus enters inside the plant through the vertical hollow or zigzag tunnel which caused initial sites of infections (Ahmad Junaidy, 2008, NEDFi, n.d.). This infection will gradually and slowly spread to all sides of trees. Under natural conditions, only 10% of the trees may be affected by fungus (Ahmad Junaidy, 2008). Then, formation of agarwood is the resinification of accumulated oleoresin due to the action of microorganisms. Due to the accumulation of infection oleoresins in the infected wood and later become odoriferous (Ahmad Junaidy, 2008, NEDFi, n.d.). During the initial stage of infections, brown streaks will appear in the plant tissue. Accumulation of oleoresins increased the infection rate as well as aging of infection. As more of infections of oleoresins are deposited, the color intensity of infected wood increases and finally it become dark or black due to increase in concentration (Lata, 2007, NEDFi, n.d.). Resin can be formed in 7-8 years time if there is more insect infestation in the infected area (Ahmad Junaidy, 2008). If the infection starts at the age of 5-6 years, then total of 10 years age may get sufficient gaharu in a plant for commercial (NEDFi, n.d.). *Aquilaria* sp. also might be taken 20 years or older to produce resin under natural condition and more than 50 years to have the highest concentration of resin. The degree of resin saturation was determined the market value of *Aquilaria* sp. (Donovan & Puri, 2004). True gaharu is heavier than water.

2.4 Economic importance of gaharu

Gaharu is one of the most valuable non-timber forest products in Sarawak (STIDC, 2008). Mucharromah (2011) mentioned that, "Gaharu has a high economical value compared to other forest products". It has high commercial value production in the incense, perfume and medicines products (Barden *et al*., 2000, Eghenter, 2005, Lim & Noorainie, 2010, Nasima & Ananta, 2008). The best grade of gaharu can release fragrant scent without being heated or burnt. However, distillation process is needed on the low grade of gaharu to get the resin (Mucharromah, 2011). Buddhists, Hindus and Muslim widely used the gaharu in the form of essential oil or incense sticks for religious purposes (Ahmad Junaidy, 2008, Eghenter, 2005) such as beliefs rituals and religious ceremonies. This essential oil usually extracts or made up from low quality of gaharu.

On the other hand, gaharu is useful in medicine sector. Gaharu contain more than 12 chemical components that can be extracted. Gaharu is widely used in general pain reducer, dental pain, kidney, and rheumatism medicine (Adelina, 2004). In traditional East Asian medicine, gaharu provides the flow of *qi*, relieve pain, arrest vomiting by warming the stomach, and to relieve asthma (Anonymous, 1995). Wood without or low content of resin can be used as boxes, interior or veneer. The inner fibrous bark can be used as raw material for clothing and ropes (Adelina, 2004). Furthermore, gaharu trees play important role as environment protector. It increases ground water absorption, strengthens soil, prevents landslide occur, and produce photosynthesis to support animals and plants life (Mucharromah, 2011).

According to Chua (2008), gaharu can be traded in the various forms such as whole plants or seedlings, logs to chips, flakes, oil and spent powder waste. Only 10 cm length of raw gaharu in trade can be considered as chips, fragments, shavings and splinters. Today, the international trade of gaharu involves at least 18 countries (Lim & Noraine, 2010). Middle East, Taiwan, Japan and Korea are the major importing countries while United State (US) and Europe are the minor importing countries (Chua, 2008). Lata (2007) mentioned that, Indonesia and Malaysia are the main exporter of gaharu from 1995 to 1997. At the same time, Singapore acts as major entrepot or transshipment center for both import and export (Chua, 2008). Due to the high economic value of gaharu, Sarawak government is encouraging the local people to cultivate gaharu even know there is less guideline given from agriculture sector (Lepun, Khairulmazmi & Nor Azizah, 2011).

2.5 Current status of *Aquilaria sp.*

In the past of 1970s, only a small quantity of gaharu was traded in the Apo Kayan area of Borneo (Eghenter, 2005). In 1980, the best quality of gaharu was trade for USD $20 per kilogram (Jessup & Peluso, 1986). Lately, prices for the best grade of gaharu have been increased about twenty times. Therefore, gaharu collecting activity was started popular among the population of outside collectors and traders. The number of outside collector and the levels of exploitation have been increased. This exploitation might not be exhausted the resources in the natural forest, but it might effect on the income and life of local collectors. Most of them are forced to move to others areas or islands before the depleted of resources (Eghenter, 2005). Some of the local people were competed with each others to get the same resource. Due to the occurrence of economic and social problems, local government had started to opposite and against the outsiders depletes the resource. They were organized a customary law which is to exact user fee to the outsiders before enter on a forest expedition. Today, all gaharu species has been have been listed under CITES Appendix II (Lata, 2007, Lim & Noraine, 2010, STIDC, 2008) to control and sustain the quantity of gaharu and its trade. A license and removal pass is required before commercial harvesting of gaharu (Chua, 2008). The objective of removal pass is to verify the entire fee payments have been settled and they are harvested log from a licensed area.

Even though gaharu species have been listed under CITES, but the quantity is still not enough to fulfill the demand of market. Low germination rate of *Aquilaria* sp. seeds is the major problem because seeds are rapidly loss their moisture content (Tasen, Tangmitcharoen, Thakeaw, & Ogata, 2009). Besides, the hard coat seed itself causing slow and prevent germination to take place. This may affect the availability of the products and the local gaharu economy.

2.6 Vegetative Propagation

Plant propagation is an artificial or natural process which increases the number of plants (McMahon, Kofranek & Rubatzky, 2007). Plants can be propagated through two methods that are sexual and asexual method. Sexual method can be propagated using seed while asexual method can be propagated using vegetative. According to Haapala (2004), vegetative propagation is a technique which able to multiple the best possible stock plants into new identical plant. Vegetative propagation is the production of clone plants from somatic part of a plant without using seeds (Jonip, 2008). Vegetative propagation has offers several advantages. The first advantage of vegetative propagation is able to maintain the superiority genetics and selected characteristics of the individual plant (Haapala, 2004, Messen, Leakey & Newton, 2001). Second, vegetative propagation is more stable to maintain similar genetic characteristics with their parent plants (Firmansyah, 2007) compared with sexual propagation. This is due to new plants produced from sexual propagation may not necessary to have similar characteristics with their parent plant. Open fertilization may change their original genetic characteristics and produce better or worst plant genetic than their parent plants. Next, vegetative propagation is able to combine more than one genotype into a single quality plant. Two or more different genotype plants under the same genus or species can be propagated or combined to form a better quality plant. Besides that, vegetative propagation is easier and more rapid method in plants compared to sexual propagation. Vegetative propagation does not need to undergo pollination and fertilization process which may consume time. Other than that, vegetative propagation produces disease free plant and reduces the variability of seeds. However, vegetative propagation is a method widely used in agriculture and horticulture sector but rarely in forestry (Haapala, 2004). Vegetative propagation can be classified into several types that are cutting, grafting, budding, layering and micropropagation. Hartmann, Kester, Davies

Jr., and Geneve (1997) mentioned that vegetative propagation can be used in different ways such as cuttings, tubers, bulbs, corms, pseudopulps, rhizomes, tuberous roots and stems, grafting or budding.

There are four types of cutting can be classified that are stem cutting, leaf cutting, root cutting, and leaf-bud cutting. Stem cutting is a cutting of shoot segments that contain lateral or terminal buds. According to Barden, Halfcare and Parrish (1987), stem cutting can be divided into four types which are softwood cutting, herbaceous cutting, hardwood cutting, and semi-hardwood cutting. Types of stem cuttings used are depending on the species and stage of their branches or twigs (Brennan & Mudge, 1998). Softwood cutting is taken from soft, succulent and new spring growth of deciduous plants. This cutting requires leaves for rooting and to prevent it from wilt and die. However, herbaceous cutting is used from succulent herbaceous plants such as chrysanthemum, coleus, geranium, carnation, and many tropical house plants. This cutting is better placed in a rooting medium in a warm (75^oF to 80^oF), draft free environment and misted (McMahon, Kofranek & Rubatzky, 2007). Haapala (2004) mentioned that hardwood cutting is taken from dormant woody stems with lateral buds. According to Barden *et al.* (1987), hardwood cutting is taken from deciduous and narrow-leaved plants. This cutting does not require leaves for rooting. Deciduous hardwood is better made in late winter or early spring while narrow-leaved hardwood is best made in early winter. The last cutting is semi-hardwood. This cutting is usually for woody, broad-leaved, and evergreen species. Semi-hardwood cutting is suggested placed in a well lighted, humid location to minimize water loss from the leaves (McMahon *et al.*, 2007).

However, stem cutting propagation is often the preferred method for plant propagation in forestry and agroforestry (Leakey *et al.*, 1990) because it is the simple, inexpensive, rapid and effective way to produce a clone of a particular parent plants (Khan & Daud, 1994).

2.7 Factor affecting rooting of cuttings

The ability of cuttings to produce adventitious roots and shoots is depending on the internal and external factors. The internal factors that can be affecting the cuttings are age of stock plant, cuttings position, presence of leaves and PGR levels while the external factors are temperature, water, nutritional status, carbon dioxide and light (Hartmann, Kester, Davies & Geneve, 2002).

2.7.1 Age of stock plant

According to Darus Ahmad, Thompson and Pirrie (1990), Ky-Dembele, Tigabu, Bayala, Savadogo, Boussim and Oden (2011), age of the stock plants is one of the most important factors affecting rooting of cuttings. There is a lot of evidence to support that the ability of cuttings to produce adventitious roots decrease with the increase of age of plants. According to Haapala (2004), old plant is usually more difficult to multiple compare to juvenile characteristics. Awang, Sandrang, Mohamad and Selamat (2011), Darus Ahmad *et al.* (1990), Raviv, Reuveni and Goldschmidt (1987) mentioned that, the rooting percentage declined with increasing age of stock plants. Older or mature plants have low rooting ability while juvenile stock plants have higher rooting ability. Thus, increasing age of plants may decrease the root length, survival rate and rooting speed of cutting. Darus Ahmad *et al.* (1990) reported that, 6 and 12 month old stock plant cuttings of *Acacia mangium* rooted faster and produced higher rooting percentages than 18 and 24 month old stock plants cuttings. While, cuttings from 1 year old of *Acacia auriculiformis* seedlings