Faculty of Resource Science and Technology

EFFECT OF SHADING ON GROWTH OF STOCK PLANTS AND SUBSEQUENT ROOTING OF CUTTINGS OF *AQUILARIA MICROCARPA* BAILL.

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Effect of Shading on Growth of Stock Plants and Subsequent Rooting Of Cuttings of *Aquilaria microcarpa* Baill.

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No portion of the work referred to this report has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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LIST OF ABBREVIATION

UNIMAS	University Malaysia Sarawak
NPK	Nitrogen Phosphorus Potassium
S0	0% shading
S1	50% shading
S2	75% shading
RWR	root weight ratio
SWR	shoot weight ratio
RSR	root shoot ratio
K-IBA	Potassium-indole-3-butyric acid
H0	treatment without hormone
H1	treatment with K-IBA
H2	treatment with Seradix 1
mg/ L	milligram/ Liter
ANOVA	Analysis of Variance
SPSS	Statistical Package for Social Sciences

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Effect of Shading on Growth of Stock Plants and Subsequent Rooting of Cuttings of *Aquilaria macrocarpa* Baill.

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ABSTRACT

The study on the growth performance of *Aquilaria microcarpa* Baill. under different light intensity and subsequently used as rooting of cuttings was conducted in University Malaysia Sarawak (UNIMAS) External Laboratory. Growth of stock plants was observed for duration of 16 weeks and then for cuttings in ten-week duration. Under the shading experiment, stock plants were kept under 0%, 50%, and 75% of shades. Stock plants under 50% shading showed the greatest mean height increment recorded (12.87 cm). This was followed by 75% shading (12.66 cm) and 0% shading (7.11 cm). Stock plants that showed the highest mean diameter increment was grown under 50% shading too (2.17 mm), followed by 75% shading (1.75 mm) and 0% shading (1.11 mm). Stock plants from 75% shading obtained the highest mean root weight ratio (0.38 ± 0.04 g/g) while stock plants from 50% shading had the highest mean shoot weight ratio (0.53 ± 0.04 g/g). Stock plants from 75% shading also recorded the highest root shoot ratio (0.78 ± 0.01 g/g). Cuttings taken from stock plants under 75% shade showed the highest rooting percentage (6.67%). The rooting percentage of cuttings taken from 0% and 50% shades were recorded the same which was 5% when the experiment terminated. Cuttings from 75% shading had 15% rooted in treatment without hormone, 5% rooted in treatment with K-IBA 200 mg/L.

Keywords: *Aquilaria microcarpa* Baill., growth performance, shading, cuttings, hormones

ABSTRAK

Kajian ini adalah untuk melihat prestasi pertumbuhan <u>Aquilaria microcarpa</u> Baill. di bawah keamatan cahaya yang berbeza dan seterusnya digunakan untuk pengakaran keratan batang daripada stok pokok. Kajian ini dijalankan di Makmal Luar Universiti Malaysia Sarawak (UNIMAS). Pertumbuhan stok pokok diperhatikan untuk 16 minggu dan seterusnya pengakaran keratan batang diperhatikan selama sepuluh minggu. Untuk experimen teduhan, stok pokok diletak di bawah teduhan yang berbeza iaitu 0%, 50% dan 75%. Stok pokok di bawah 50% teduhan menunjukkan penambahan ketinggian yang paling banyak di mana penambahan min direkodkan ialah 12.87cm, diikuti 75% teduhan (12.66 cm) dan 0% teduhan (7.11 cm). Stok pokok yang menunjukkan penambahan min diameter yang paling tinggi ialah stok pokok yang diletakkan di bawah teduhan 50% (2.17 mm), diikuti dengan 75% teduhan (1.75 mm) dan 0% teduhan (1.11 mm). Stok pokok di bawah teduhan 75% mendapat min nisbah berat akar yang paling tinggi (0.38 ± 0.04 g/g) manakala stok pokok daripada teduhan 50% mendapat min nisbah berat pucuk yang paling banyak (0.53 ± 0.04 g/g). Stok pokok yang diambil daripada teduhan 75% juga merekodkan nisbah akar dengan pucuk yang paling tinggi (0.78 ± 0.01 g/g). Keratan batang yang diambil daripada stok pokok di bawah 75% teduhan menunjukkan peratus pengakaran yang paling tinggi (6.67%). Peratus pengakaran keratan batang yang diambil daripada stok pokok di bawah 0% dan 50% teduhan adalah sama iaitu 5% semasa experimen ini ditamatkan. Keratan batang yang menghasilkan peratus akar yang paling banyak ialah keratan yang tidak dirawat dengan hormone (15%) dan diikuit dengan keratan yang dirawat dengan K-IBA 200mg/L.

***Kata kunci**: <u>Aquilaria microcarpa</u> Baill., prestasi pertumbuhan, teduhan, keratan batang, hormon*

1.0 INTRODUCTION

Aquilaria microcarpa Baill. is a species of agarwood that belongs to the family of Thymelaeaceae. This species is native to South and Southeast Asia, which can be found from the foothills of the Himalayas to the rainforests of Papua New Guinea. Mohd Farid, Mohd Rusli, Lim, and Alias (2010) suggested that the agarwood species are plants that producing a resinous wood that widely used as incense for most of the religious ceremonies, medicinal purposes and aromatic preparation. This non-forest type timber is among the most valuable tree species found in Sarawak and is having high prices and demands worldly. Due to the high demand and shortage of supply for agarwood with specific to *Aquilaria* species, the prices increase drastically. According to Forestry Department (cited in Norman Botes, 2008), prices of gaharu for super grade, grade A, grade B, grade C, grade D and grade E in 2007 were RM 25000 per kg, RM 20000 per kg, RM 18000 per kg, RM15000 per kg, RM 8000 per kg and RM 3000 per kg respectively. Other grades ranged between RM 500 to RM 1000 per kg while gaharu chips were RM 5 to RM 100 per kg.

The high demand for agarwood has lead to uncontrolled and illegal felling of the species in the natural forests. Hence to protect the species, World Conservation Monitoring Center (1998) has considered agarwood as vulnerable species (IUCN Red List of Threatened Species, 2011). In Malaysia, the indiscriminate cutting of the species has resulted to the species being listed under Appendix II of CITES (Convention on International Trade in Endangered Species) in 1995 and trading of *Aquilaria* spp is controlled under CITES protection (Mostafa, 2006). In Malaysia, efforts are intensified to plant the species in large scale. Large scale planting needs a large quantity of planting stocks. However, poor germination, insufficient supply of seedlings and unpredictable flowering season often hampered the progress of planting. Recalcitrant seeds (short viability) and problem of collecting seeds on the forest floor add to the shortage of planting stock for *Aquilaria* plantation establishment.

Agarwood or gaharu is naturally propagated by seed. However seed requires optimum condition to germinate. A gaharu tree will only flower at the age of 4 and flowering is unpredicatable. The other alternative method is vegetative propagation by rooting of cuttings. Although various factors are known to affect the ability of cuttings to root, the technique has been used in many fast growing species. Micropropagation can be used as propagate method but contamination remain the main obstacle. Stock plant manipulation such as shading is known to affect plant growth. According to Holley (2009), the application of light intensity based on different shading to the plant will have different growth response. Process of transpiration become less when the stock plant of applied with moderate shading. Heavier shading however, will reduce photosynthesis to a greater degree and result in weak stock plant.

As different shading influence the ability of stock plant to grow, it indicates that the effect will be on subsequent rooting of cutting too. Rooting of cuttings will increase in some difficult to root woody species if stock plants are pretreated under shades (Hartmann, Kester, Davies & Geneve, 2002). Hence, the objectives of this study are:

i) To examine the effect of shading on growth of stock plants of *Aquilaria microcarpa* Baill.

ii) Subsequently to determine the effect of stock plant shading on rooting of cuttings

iii) To determine the effect of rooting hormone on cuttings

In spite of this, there is no published report on the effect of shade on stock plant and subsequently rooting of cuttings of *Aquilaria microcarpa*. Light intensity is an environmental factor that can be controlled and can be study in order to manipulate the growth of stock plant.

2.0 LITERATURE REVIEW

2.1 Taxonomy and Distribution

Thymelaeaceae family is subdivided into four subfamilies, which are Aquilarioideae, Gilgiodaphnoideae, Gonystyloideae and Thymelaeoideae. Apart from Gilgiodaphnoideae, the other three subfamilies have their representatives in Sabah and Sarawak. Aquilariodeae and Thymelaeoideae have leaves without translucent glandular dots while Gonystyloideae has leaves with translucent glandular dots. Flowers of Aquilariodeae members have short cylindrical calyx tube or free of sepals. Gonystyloideae has flowers with short or inconspicuous calyx tube while Thymelaeoideae's flowers are with funnel-shaped or cylindrical calyx tube. These characteristics are used to distinguish among the different subfamilies (Tawan, 2004).

Aquilaria spp. is classified under the subfamily of Aquilarioideae. There are approximately 15 species of *Aquilaria* all over the world. Having 15 species in this genus, according to Tawan (2004), there are five species found occurring in Borneo. In Sabah and Sarawak, nine genera with 45 species are recorded. Members in Thymelaeaceae in Sabah and Sarawak found to be occurred in mixed dipterocarp, fresh water, peatswamp and kerangas forest. They are normally found at low to medium altitudes. Some can be found in lowland to lower montane forests. They can grow on a wide range of soils, including poor sandy soil (Fairul, 2008).

There are only four species of *Aquilaria* found in Malaysia, which are *A. beccariana*, *A. malaccensis*, *A. microcarpa* and *A. hirta*. According to Anderson (1980), *A. beccariana, A. malaccensis* and *A. microcarpa* are found in Sarawak. *Aquilaria* has several vernacular names. It is known as gaharu in Malaysia and Indonesia, jin-koh in Japan, Sasi in India, Agor in Bangladesh and Ch'en Xiang in China. In Sarawak, as mentioned by

Dinggai (1998), it is often known as Gaharu engkaras (Malay), Tengkaras (Iban), Lako (Punan) and Tengala (Berawan). *Aquilaria microcarpa* is locally known as engkaras or gaharu and is one of valuable timber species.

Aquilaria can be found in India, Indo-China, Myamnar, Sumatra, Singapore and throughout Borneo in lowland mixed dipterocarp forest at altitudes to 200 m. It is commonly found in primary forest, secondary forest, mixed dipterocarp forest and kerangas forest. It is distributed in lowland to lower montane forests at altitudes to 1700 m (Tawan, 2004). Alfian's study (2005) recorded that *Aquilaria* commonly has smooth marginal, shiny surface of adaxial and delicate hair on abaxial surface. Even though all species have similar characteristics, they can be differentiated by the characteristics of fruits. For instance, the fruit of *A. beccariana* has a pear shape whereas *A. microcarpa* is heart shaped.

2.2 Uses of Agarwood

Aquilaria is an important source of agarwood or gaharu. Gaharu is the resin-containing heartwood of infected trees. It appears in varies colors ranging from blackish to light brownish (Mostafa, 2006). The trees are wounded by harvesters to induce secretion of resins. The wounds will heal and the wood around the old wounds are cut off for use. This in turn causes the formation of new wounds and more resin secretion. Old and dying trees are cut down and heartwoods are harvested for medicinal use. In Asia, fragrance produced by burning the wood is widely used as incense for religious and ceremonial purposes, especially the Chinese and Indians. Some believe that smoke can chase away evil spirits. In western, Chinese and Indians medicines, the incense is used against cancer, especially thyroid gland (Tawan, 2006). In China, it is used as sedative against asthma, colics and diarrhea and also as aphrodisiac and carminative.

Besides that, the grated wood is included in preparations used to cure smallpox, rheumatism and also illness during and after childbirth. *Aquilaria* herbs relieve spasms in digestive and respiratory systems and can be used to lower fevers. The well known agarwood oil is an essential oil extracted from agarwood. The oil is used in luxury oriental perfumery (Chua, 2008). In pathological condition, the extraction powder from *A. agallocha* can produce fragrant perfume (Mostafa, 2006). The whitish and light wood of several species of *Aquilaria* is the main source of karas timber. This type of timber is highly prized and prevalent for making decorative cabinets and various types of interior decorative items. It is also suitable for veneer, plywood, block-board and particle-board manufacture. Although less common, agarwood can be carved into sculptures and beads (Barden, Noorainie, Mulliken, & Song, 2002). The timber of undiseased trees is very light and is suitable for making boxes, light indoor construction and veneer. The silvery inner bark is highly valued for its strength and durability and is used for making ropes and cloth (Tawan, 2004). Malaysia and Indonesia are major exporters of Gaharu which trades in form of wood, chunks, chips and dust. Products of Gaharu such as essential oil, perfume, incense and medicinal sources are exported (Mostafa, 2006).

2.3 Stock Plants as Source of Cuttings

Stock plants are parent plants where cuttings are taken from. They must be of a defined and well-characterized genotype where they are free of disease and pest. Stock plants that suitable to be used are at juvenile or young phase which being characterized by three criteria: absence of potential to shift from vegetative growth to reproductive maturity and formation of flowers, specific morphological and physiological traits where leaf shape, thorniness, vigor and disease resistance are included, and having greater ability of the juvenile phase to regenerate adventitious roots and shoots.

Toogood (1999) suggested that a stock plant is grown purely to provide cutting materials. The stock plant should be healthy, mature and vigorous. Diseased plants should be avoided especially those infected by virus as diseases can be passed on to cuttings. According to Bachman (2008), stock plant should be of true-to-type and free of pests which ensure production of high quality cuttings. However, the healthy stock plant must be sufficient to ensure quantities of cutting materials meet the production goals. There are lots of studies on stock plants. In order to maximize cutting propagation, selection and maintenance of stock plant is necessary.

Besides that, environmental condition and physiological status of stock plant are often manipulated in relation to light, shading, nutrient level, temperature, and water status (Hartmann et al., 2002). Research by Costa Jr, Scarpare Filho and Bastos (2003) on stock plant of *Psidium guajava* L. found out those cuttings from 30% shading provided the best result of rooting as the plants growing with plenty of sun. Stock plants are maintained in good condition by manipulating their growth management practices and environmental conditions within acceptable parameters to produce high quality cuttings (Bachman, 2008). Light, shading, nutrients and potting media are some factors that will affect growth of stock plant which will influence results of rooting of cuttings of stock plants.

2.3.1 Light

Light is the key factor for plant growth. Photosynthesis enables plant metabolism processes to take place and provides energy. Light intensity levels can have a significant effect on photosynthesis rate which directly related to plant's ability to grow. Plants grow in response to light. Phototropism will be either positive or negative. Positive phototropism is a movement towards light while negative phototropism is movement away from light. According to research by Ipor, Tawan and Tagor (cited in Mostafa, 2006), the higher the light intensity, the higher number of leaves produced. Lower light intensity caused less number of leaves but increase in the size of leaves. Lower light intensity however can induce the plant growth. Bjorkman (1968) recorded that reducing light intensity to certain level caused the increasing of stem extension in *Solidago virgsuwea* L..

In spite of this, further reduction of light will suppressed the plant growth. In natural condition, differences in height and direction of growth usually being associated with light intensity. Hence, growth of seedlings depends on the morphological response of plant to shade. Shade can be created with the growth of leaves. Charles-Edwards, Doley, and Rimmington (1986) stated that capacity of every leaf to grow will decrease because the new leaves shade it. According to Hale and Orcutt (1987), plant adapt to shade comes in two ways; (1) Increase of leaf area to minimizes the use of metabolites, (2) Decrease in amount of transmitted and reflected light. Undeniably, light is vital for plant to survive as plant utilizes light directly in biomass production through photosynthesis. Shade will affect the growth of stem and leaf (Hart, 1988).

The amount of light reaches the plant can be manipulated by the creation of shading. Hence, shading is the process of light exclusion throughout plant growth. Biran and Halevy (1973) suggested that shading of whole plant decreased rate of photosynthesis and growth therefore decreased the number of available cuttings. Based on their research, shading did not improve rooting in ‘Lavender Perfection’ but partial shading on ‘Choot Hashani’ showed improvement on rooting. Kawase (1965) reported that shading may decrease rooting (cited in Biran & Halevy, 1973). Pacholczak and Szydlo (2010) proved that shading positively affected rooting of *Cornus Alba* L. cultivars. Cuttings from stock plants shaded under 50% light showed the best rottings. An experiment by Maynard and Bassuk (1990) showed that etiolation or shading increased initial and maximum rooting capacities.

Herman & Hess in 1963 found out that shading promoted meristematic character of tissue (cited in Biran & Halevy, 1973).

2.3.2 Nutrients

Nutrients are essential elements in plant growth. Soil nutrients can be divided into two major groups, macro elements and micro elements. Macro elements are nutrient elements that needed by plant in large concentration, such as nitrogen, potassium, magnesium, phosphorus and sulphur. On the other hand, micro elements such as chlorine, copper, iron and manganese are needed by plant in small concentration. Requirement for nutrients vary with species, plants' age and parts, and soil type used (Tisdale & Nelson, 1983). Essential elements must be necessary for a complete, normal plant development and growth through a full life cycle. The elements must have no substitute that can effectively replace and functioning within the plants for a complete, normal plant development and growth through a full life cycle.

In early stage of planting, plant needs much nutrient for growing and resistance from disease. Seedlings need fertilizer to ensure growth consistent as most of the soils having deficiency in nutrients though soil itself has different nutrients. Poole and Chase (1991) found out that stock plants and cuttings of *Epipremnum aureum* were affected by nitrogen rate. The highest nitrogen rate which was 112.5mg N/6 inch pot/ week produced relatively good growth. Experiment by Qazi, Nadeem, Nadia, Ishfaq, and Asia (2005) showed that fertilizer treatment with 30:20:20g NPK gm/m^2 performed well on *Zinnia elegans* compared to treatments with lower nitrogen application.

2.3.3 Potting Media

Potting media is important in growing plants. A good potting media must meet all the basic of plant requirements. According to Pettinelli (n.d.), potting media is supplying plants with support, good drainage, adequate air circulation, and storage of water and nutrients. Potting media that commonly used are peat, coconut coir dust, clay soils and sphagnum moss. Most of the potting media come in mixtures where perlite or bark is mixed together (Kala, Rosenani, Fauziah, & Thohirah, 2009). They (Kala et al., 2009) carried out an experiment on composting oil palm wastes and sewage sludge for use in potting media of ornamental plants. Shredded oil palm wastes were mixed with sewage sludge in three different ratios (1:0, 3:1 and 4:1) and adjusted to 60% moisture content. The result showed that oil palm wastes with sewage sludge at 4:1 ratio to be the most optimum compost as potting media for ornamental plants.

2.4 Stem Cuttings

Vegetative propagation is a common propagation method used to produce a plant identical in genotype with mother plant. New roots or shoots can be regenerated on stems, leaves or roots. Cuttings, grafting, layering, air-layering and micropropagation are methods of vegetative propagation. Cuttings propagation is the most important means for clonal regeneration of many crops. Adventitious root formation is the requirement to successful cuttings propagation (Hartmann et al., 2002). Propagation by cuttings produces a true-to-type progeny and avoids bud or graft incompatibilities. Besides that, it is economically least expensive and less complicated to implement. There are three types of cuttings which are leaf cuttings, stem cuttings and root cuttings. Leaf cuttings are commonly used for house plants while root cuttings are for plants that are suckers in nature. Hence, for *Aquilaria microcarpa* Baill., the most suitable cuttings is stem cuttings.

Stem cuttings can be divided into four groups according to nature of stem tissue: hardwood, semi-hardwood, softwood and herbaceous (Hartmann et al., 2002). The segments of shoots containing lateral or terminal buds are obtained. Under proper conditions adventitious roots will develop and produce independent plants. A softwood cutting is taken from the soft, succulent or evergreen species before tissues become matured and lignified. Softwood cuttings generally root easier and quicker, about two to five weeks, but required more attention (Hartmann et al., 2002). Softwood cuttings stress easily, hence, it is important to collect the cutting material early in the day and keep in moist and cool so that it turgid all the time. A cutting was considered rooted if it possessed one or more roots >1mm long (Maynard & Bassuk, 1992).

2.4.1 Factor Affecting Rooting of Cuttings

2.4.1.1 Cutting Position

Research by Soundy, Mpati, du Toit, Mudau & Araya (2008) indicated that apical cuttings rooted earlier than basal cuttings but after 15 to 20 days of establishment, both rooted similarly. The result showed basal cuttings of Fever Tea had thicker stem circumferences and more number of leaves compared to apical cuttings. Al-Salem & Karam (2001) dipped the bases of basal and terminal cuttings of *Arbutus andrachne* in solutions of IBA acid form (5-48mM) as he suggested that position of cutting on the branch did not affect rooting.

2.4.1.2 Age of Stock Plant

In the experiments with rooting of cuttings from various plant species, it was found that the younger the stock plant, the better rooting capacity of its cuttings. Kadman (1975) in his preliminary experiments with rooting of avocado cuttings found out that cutting taken from young seedlings of 3 to 6 months old rooted easily within a relatively short period of time in a suitable rooting medium under artificial mist spray. In the experiment by Henry, Blazich and Hinesley (1992), it showed rooting of cutting reduced with increased tree age of *Juniperus virginiana* L. although cuttings from 40-year-old trees retained substantial rooting capacity. Maynard and Bassuk (1992) suggested that percent rooting and root counts declined with increasing cutting age.

2.4.1.3 Stock Plant Manipulation

Stock plants are manipulated to maximize the rooting potential prior to taking cuttings. Examples of manipulation include shading and nutrients. High irradiation of stock plants of *Chrysanthemum* ‘Horim’ resulted in high number of roots per cutting (Borowski, Hagen & Moe, 1981). Howard found out that percent rooting and root count were proportional to shade level (cited in Maynard & Bassuk, 1992). Different fertilizer level will affect the growth of stock plant and subsequently affect the rooting of cuttings. Qiu, Zhang, Wang and Liu (2008) in their experiment suggested that nitrogen plays an active and important role in root-microorganism communication. Interaction intensity of plant-microorganism appears positive with a high nitrogen nutrient medium. Light intensity has been shown to influence rooting potential of stock plant shoots used for cutting (Knox & Hamilton, 1982). In their experiment, optimum rooting of cuttings of *Berberis* resulted when stock plants grown at 70% light. However, the number of roots per cutting of