EFFECTS OF THE COCONUT WATER AND NAA ON THE GROWTH AND DEVELOPMENT OF *Dendrobium hamaticalcar* protocorm

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

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DECLARATION

I hereby declare that this thesis is the result of my own research, except for certain quotations and references that have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any degree.

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ABSTRACT

This experiment was carried out to determine the effects of coconut water and NAA hormone on the growth and development of *Dendrobium hamalticalcar* protocorm. Nine treatments were used and for each treatment, three replicates were used. *D. hamalticalcar* protocorms after 180 days in germination medium were used as explants. Medium XER was used as basal medium. The cultures were placed in bright room which has 24 hours light (2.5mW/cm² light intensity) at 22±3 °C. Coconut water with 5%, 10%, 15% and 20% (v/v) concentrations and 0.5 mgL⁻¹, 1.0 mgL⁻¹, 1.5 mgL⁻¹ and 2.0 mgL⁻¹ of NAA concentrations were used as additives in this study. Based on the result, medium with 0.5 mgL⁻¹ of NAA was the most suitable medium on the growth and development since the medium producing a significant highest (p<0.05) growth index of 523.33. The treatment with 0.5 mgL⁻¹ of NAA showed promising result where the percentage of root formation was the highest in this treatment which is 48.33 %. XER medium supplemented with 5% (v/v) CW and with 20% (v/v) CW were found to be the most suitable media for leaf formation of *D. hamalticalcar* protocorm with percentage of 56.67% and 55.00%, respectively. Medium with 2.0 mgL⁻¹ of NAA doesn't give any positive result because this medium has the lowest growth index (390.00). The less died protocorm was found in medium supplemented with 1.5 mgL⁻¹ of NAA with the percentage of 1.67%. Media added with 0.5 mgL⁻¹ of NAA in XER media was found able to stimulate the growth and development of *D. hamalticalcar* protocorm which form plantlet in short period of time in 100 DAC. To improve the growth and development, it was recommended to use different types of organic additive with different concentration and also combination of different organic additives and hormone such as media with NAA and CW combination at different concentration.
Kajian Tentang Kesan Air Kelapa dan NAA ke atas Pertumbuhan dan Perkembangan protokom *Dendrobium Hamalticalcar*

**ABSTRAK**

Kajian ini dilakukan untuk menguji pengaruh air kelapa dan hormon NAA terhadap pertumbuhan dan perkembangan protokorm *Dendrobium hamalticalcar*. Sembilan rawatan digunakan dengan masing-masing tiga replikasi. Protokorm *D. hamalticalcar* yang berumur 180 hari selepas percambahan digunakan sebagai eksplan. Kajian dijalankan menggunakan media asas XER dengan percambahan air kelapa pada kepekatan 5%, 10%, 15% dan 20% (v/v) atau 0.5, 1.0, 1.5 dan 2.0 mgL⁻¹ NAA. Melalui hasil kajian didapati bahawa media dengan 0.5 mgL⁻¹ NAA adalah paling sesuai untuk pertumbuhan dan perkembangan protokorm *D. hamalticalcar* dengan indeks pertumbuhan tertinggi iaitu 523.33. Rawatan 0.5 mgL⁻¹ NAA juga menunjukkan peratus pembentukan akar yang tinggi dengan purata 48.33%. Rawatan yang mengandungi 5% (v/v) dan 20% (v/v) air kelapa mengalakkan pembentukan daun pada protokorm *D. hamalticalcar* dengan peratus masing-masing sebanyak 56.67% dan 55.00%. Manakala media yang mengandungi 2.0 mgL⁻¹ NAA kurang sesuai untuk pertumbuhan dan perkembangan protokorm *D. hamalticalcar* kerana nilai indeks pertumbuhan yang sangat rendah. (390.00) walaupun selepas 100 hari pengkulturan. Secara keseluruhan, didapati media dengan penambahan 0.5 mgL⁻¹ NAA menggalakkan pertumbuhan dan perkembangan di samping mengekalkan kemandiran protokorm *D. hamalticalcar*. Walau bagaimanapun, masih terdapat aspek kajian berkaitan pertumbuhan dan perkembangan *D. hamalticalcar* perlu dijalankan untuk menghasilkan media yang lebih efektif. Antaranya ialah menggunakan pelbagai jenis kompleks tabii dengan kepekatan yang berbeza dan juga kombinasi kompleks tabii dan hormone seperti media kombinasi NAA dan CW pada kepekaan yang berbeza.
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<tr>
<td>ABA</td>
<td>Abscisic acid</td>
<td>Abscisic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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</tr>
<tr>
<td>B</td>
<td>Boron</td>
<td>Boron</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
<td>Calcium</td>
</tr>
<tr>
<td>CRD</td>
<td>Complete Randomised Design</td>
<td>Complete Randomised Design</td>
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<tr>
<td>CW</td>
<td>Coconut Water</td>
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<td>DAC</td>
<td>Days After Culture</td>
<td>Days After Culture</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>NAA</td>
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<td>PGR</td>
<td>Plant growth regulator</td>
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<td>SPSS</td>
<td>Statistical Package for Social Science</td>
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<td>Tricarboxylic acid</td>
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<tr>
<td>v/v</td>
<td>Volume over volume</td>
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<tr>
<td>XER</td>
<td>Experimental Ernst Robert formula</td>
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Orchid plant family is the largest family which contains about 650 to 800 genus (Dillon, 1963) in Borneo Island. There is about 2500 to 3000 species (sp.) of orchid that can be found there. Out of that species 34.4% of the sp are endemic (Chan et al., 1994) and 290 species are now under treat because of the deforestation and development that are going on in Borneo Island. Borneo’s orchids are also endangered, result of the loss of natural habitat from fire, forest damage, and illegal logging. Increased exploitation of the forests of West Borneo, including gold mining and illegal burning, has led to the certain extinction of hundreds of orchid species. According to a Global Forest Watch (2002) report’s, Indonesia is experiencing one of the most dramatic losses of forestland in the world. Reports showed that the current rate of loss, Borneo's forests could vanish completely by 2010. Economic factors, including illegal collecting and selling of wild orchids by domestic or foreign ‘orchid hunters’, along with increasing consumer demand for orchids, also contribute to the endangerment of Borneo’s native orchids (Alphonso, 1987).

The endangerment and loss of orchid affects the cut flower industry where orchid plays an important role in cut flower industry. Long known for their beauty and fragrance, orchids produce spectacular blossoms that often last for days, even weeks. Depending on the species, these blooms can be removed from their plants and used as cut flowers in many different ways, including stemmed flowers in a vase, a cluster of blossoms in a corsage, or as a single-flower boutonniere.
Examples of the wild species that can be found in Sabah are *Cymbidium atropureum*, *C. pubescens*, *C. finlaysoniana*, *C. rectum*, *C. dayanum*, *Denrobium linguella*, *D. Sanguinolentum*, *Paphiopedilum lowii* and more of wild orchids. Most of the species that found in Sabah and Sarawak are threatened and including *Dendrobium hamaltcalcar*.

*Dendrobium* orchids are highly valued for their fantastic range of variation and long lasting flowers. *Dendrobium* is one of the most important orchids known for its beautiful colored flowers. *Dendrobium hamalticaclar* is among the famous wild orchids of *Dendrobium* sp. in Borneo because the sp. is considered as a symbol for conservation of endangered plants. It is often found as an epiphytic on trees with thick robust roots. Conservation of orchids is now a matter of universal concern.

Generally, orchids are propagated both asexually and sexually. But the traditional asexual propagation is extremely slow which can give rise to 2-4 plants per year (Nasiruddin *et al.*, 2003). Therefore, tissue culture techniques have been proven to be an important tool in the micropropagation of plants, especially in the multiplication of quality clones and virus free plants. The application of plant growth regulators in tissue culture also offers opportunities to conserve and multiply such species for the mass scale production for commercial purposes (Decruse *et al.*, 2003).

Micropropagation of orchids is the most frequently used convenient technique for their exploitation as a major trade in developed countries (Sagawa and Kunisaki, 1982). It is the only method currently available (Goh *et al.*, 1992) which initially starts in early 1960 (Bose *et al.*, 1986). Orchids can be rapidly propagated through tissue culture techniques by using shoot tips (Saiprasad *et al.*, 2002), leaf (Chen *et al.*, 2001), and stem nodes (Pathania *et al.*, 1998). Rotor (1949) for the first time tried to propagate *Phalaenopsis* clonally using flower stalk buds. Other research reports on the micropropagation of orchids through tissue culture of leaf (Tanaka, 1987), root tips (Tanaka *et al.* 1976) internodal section of flower stalk and lateral buds from young flower stalks (Ichihashi, 1992) are available but none of these methods proved to be effective commercially in producing lots of plantlets in a short period.

One of the techniques often used in the conservation of orchids is by using tissue culture or in vitro culture. This technique can produce new orchid progeny
quickly, clean and under controlled environment. This technique can also produce new orchid progeny in the desired amount. Tissue culture technique is a technique in which the explants such as leaf stem or plant cell placed on the media for the growth and development in aseptic and sterile conditions (Musatafa Kamal, 2002). Through this technique, high-quality orchids can be produced. This study is important to produce larger amounts of *D. hamalticaclar* for economic purposes and also for cut flower industry. Therefore, the present study was undertaken to investigate the effect of complex additive and growth regulators concentration on the growth and development of *D. hamalticaclar* tissue culture and plantlet regeneration of *D. hamalticaclar*.

1.2 Justification

This study is about the effect of the coconut water and naphthaleneacetic acid (NAA) on growth and development of *D. hamalticaclar*. This experiment is conducted to see the effect of different concentration of coconut water and NAA on growth and development of *D. hamalticaclar* protocorm. The growth and development of orchid plant by asexual and sexual propagation technique are slower compare to the tissue culture technique. The amount of produce plant that produced from asexual and sexual propagation is lower than the tissue culture. Other than that, producing orchid plant by sexual and sexual propagation easily infected by pest and disease problems. Tissue culture technique reduces the pest and disease problem and also helps us to produce the plants in larger amount in one time. *D. hamalticaclar* orchid is one of the important plant in cut flower industry because of the uniqueness of the flower. Increasing the number of plants will increases the production rate and also increases the economic sector in cut flower industry.
1.3 Objective

The objective of this study is to determine the effect of coconut water and NAA on growth and development of *D. hamaticalcar* protocorm.
2.1 Orchid distribution In World

The Orchidaceae is one of the largest families in the plant kingdom, consisting of over 25,000 documented species, some 800 subspecies and at recent count, around 110,000 registered hybrids. Most species occur in subtropical and tropical region of Asia, South and Central America, but this diverse and adaptable family of flowering plants is found all around the globe, except for the polar region and most arid deserts. Certain orchids live high in the rainforest canopy clinging to the branches of a host tree, some grow on the forest floor, while others have adapted to living in rock crevices or decaying organic matter.

While climate change and human interference in natural habitats are contributing to the decline and in some cases, extinction of certain orchid species, exploration in the depths of the rain forest and previously unexplored parts of Asia and South America are revealing exciting new discoveries. Furthermore, new hybrids are being produced all the time.

2.2 Orchid distribution In Malaysia

In Malaysia, there are 700 genus’s, 20000 – 25000 species and at least 100,000 hybrid orchids can be found here (Hew et al., 2002). Approximately 1000 new hybrids produced
annually. Malaysia started to produce own crossbreed orchids since the beginning of the 20th century, but the industry developed during 1960.

There are about 12 genera of orchids commonly grown in Malaysia which is Aerides, Arachnis, Ascocentrum, Cattleya, Dendrobium, Doritis, Oncidium, Phalaenopsis, Renanthera, Rhynchostylis, Vanda and Vandopsis. The results from the crossbreeding give Malaysia an international reputation as a center of crossbreeding orchids (Kamal & Shariff, 2002).

Malaysia produces its own orchid hybrid Vandaceous in 1960. Apart from Vandaceous species Malaysia also produce Aranda species such as Aranda Wendy Scott and Aranda Christine. This species was produced and propagated by Mr. L. E. Wong from Pulau Pinang and sold out throughout Malaysia. In the 1970s, commercial cultivation of orchids such as the production of orchid hybrids, planting seeds and tissue culture services performed in Thailand. In the 1980s, the growth of interest in commercial cultivation of orchids in Malaysia had culminated in which several areas in Selangor and Johor has been developed for orchids planting (Nuraini, 1987)

2.3 Orchid Status in Borneo

In Borneo Island, there is about 2500 to 3000 species of orchid that can be found here. From that species 34.4% of them are endemic. According to the International Union for Conservation of Nature and Natural Resources (ICUN), about 200 species of orchid that found in Sabah and Sarawak are categorized as endangered species and they are rarely found in Borneo Island (Abang and Gombak, 1990). This was the result of the unplanned development and the climate change. Global warming is one of the factors that cause the extinction which is one of the concerns. Climate changes cause the orchid hard to adapt to its natural adaptation and habitat. This cause most of them to die and extinct. They are also endangered as result of the loss of natural habitat from fire, deforestation, and illegal logging (Lamb, 1991). Increased exploitation of the forests of West Borneo, including gold mining and illegal burning, has led to the certain extinction of hundreds of orchid species. According to a Global Forest Watch 2002 report, Indonesia is experiencing one of the most dramatic losses of forestland in the world (Matthews, 2002)
2.4 *Dendrobium* Sp.

*Dendrobium* is a large genus of orchids. It contains about 1,200 species and can be found in south, east and Southeast Asia such as Philippines, Borneo, Australia, New Guinea, Solomon Island and also in New Zealand. They can be grown in high altitude area to lowland area and even desert area (Wellsy, 2008). *Dendrobium* can grow fast throughout summer and stop during winter time for rest. Reproduction is usually through seed, but a few species reproduce asexually through keikis produced along the stem, usually after flowering and sometimes as a result of injury to the growing tip (Talukder *et al.*, 2003).

*Dendrobium* species are either epiphytic, or occasionally lithophytic. This species adapted to a wide variety of habitats, which is from the high altitudes in the Himalayan Mountains to lowland tropical forests and even to the dry climate of the Australian desert. *Dendrobium* is sympodial orchids develop pseudobulbs, which vary in length from a few centimeters to two metres long. Most grow into long reedlike stems. Some appear densely covered with short white or black hairs such as *D. infundibulum*. The axillary inflorescence varies in length from insignificant to 1m long, and can carry from a few to as many as 100. Deciduous species carry their leaves for one to two years then typically flower on leafless canes, while canes of evergreen species usually flower in the second year and can continue to flower for a number of years.

2.4.1 *Dendrobium hamaticalcar*

*Dendrobium hamaticalcar* is originated from Kalimantan which includes Section Calcarifera. The common name or meaning of *Dendrobium hamaticalcar* is knowns as The Hook-Shaped Spur Dendrobium The color of the flower is brown color on inside the flower and bright yellow outside of the labellumare branched with serrated edge. The size of the flower from horizontal is around 1 cm, but can reach 3 cm in length. It’s grows in the lowland area that has medium intensity of the sun.

It is also found in Sabah, Borneo in lowlands and foothills at elevations of 400 to 900 meters as a large sized, hot growing epiphyte with arching stems that are slightly thicker towards the apex and are enveloped by violet flushed leaf-bearing sheaths and carrying thin, broad, ovate, acute, basally clasping and articulate to the
leaf sheaths that blooms in the fall on a pendulous, 4 to 9 flowered, .8 to 1.8" (2 to 4 cm) long inflorescence arising from the nodes towards the apex of the cane-like stem.

This orchid mainly distributed in Sabah, Borneo. The plant size is about 30 to 45 cm. The size of the flower is around 2.5 to 3.5 cm per plant. These species will flowers during the summer season and also during fall. The flowers can stand for 15 to 20 days and has no fragrance. It requires moderate sunlight and frequent watering. The cultivation is hard where its need to be handle carefully.

Figure 2.1  *Dendrobium hamaticalcar* J.J. Wood & Dauncey 1993
Source  Petrus Kurniawan, 2009
2.5 Plant Propagation

2.5.1 Vegetative Propagation

There are two ways to propagate the plants which can be in sexual or asexual. Sexual reproduction involves the fertilization of egg (ovule) with the pollen that produces seeds. Sexual propagation involves two parent plants to produce young plant. It is just use a part of plant tissue from the parent plants. It is known as multiplication of parent plant. The new plant that grown from the parent plant also known as clone. This process called cloning.

2.5.2 In vitro Propagation

In vitro propagation or tissue culture is one widely used in agricultural system. Micropropagation is a process of multiplication of plant using plants parts such as leaf, roots and shoot. It’s been widely used in scientific study in plant growth and development. This technique used to culture plant organs such as tip of shoot and root, leaf or stem tissue which will culture in media that contain nutrient in controlled facilities (Hussey, 1980).

Orchid is one of the plants that widely used in tissue culture technique. Nowadays, commercial companies have their own culture lab for the purpose of culturing. Some foliage plants have been produced through tissue culture techniques. The production result of some plants using this technique showed better results than using conventional methods of propagation (Hussey, 1980).

2.6 Orchid Tissue Culture

Plant propagation can be done in two ways, which are asexual and sexual ways. Sexual propagation requires the use of plant seed where it requires pollination and gamete attachment. The asexual way is different than the sexual way because it involves the production of progeny which has same characteristic has the mother plant. Asexual is the best method to produce progeny that has same characteristic as the mother plant. Micro propagation is the one of the asexual that used to propagate orchid since 50 years ago and become popular during this time (Prasad and Kumar, 2003).
Micropropagation is true to type propagation to selected genotype. In vitro is cell culture, pieces of tissue or organ in aseptic in either glass vials or Petri dishes. A piece of plant tissue fragments capable of growing new plants naturally because every cell in the plant tissue contains the same genetic material that contains in mother cell. They are 'totipotent' which means every cell in the plant has the potential to grow into complete plants (Prasad and Kumar, 2003). This method is applied in the production plant that is almost extinct.

*Dendrobium draconis* Rchb. f., a dragon orchid, is one of the most popular wild orchids in Thailand. It has a high economical value in flower markets throughout the world because of its attractive flower. This species is propagated by separation of shoots in which the rate of proliferation is low. Tissue culture has played an important role in multiplication of several commercially important orchids for many years using various plant parts such as shoot tip, leaf, stem, flower stalk, or root segment. Recently, thin cross-sections (TCSs) of actively growing tissues such as shoots, stem nodes or protocorm-like bodies (PLBs) have been successfully used by different workers for plantlet regeneration in orchids (Nayak et al., 2002). TCS explants containing only few layers of cells are capable of regeneration either roots, flower bud or shoot (Cantrill et al., 2001). This culture system was proved to be more efficient than other conventional in vitro culture methods with regard to the total output of plantlets of orchids as well as in other plants such as *Aranda Deborah*, *Dendrobium candidum*, *Rhynchostylis gigantea*, *Lilium spp.*, *Sorghum bicolor* and *Phalaenopsis gigantea* (Zhao et al., 2007).

2.7 Orchid Growth Requirements

Orchid can grow in natural environment in the shade of big plants or trees. But some orchid lovers, they build shade house or orchid house to protect the orchid plants from direct sun and rains. The shade house will be built with complete requirement that required by the orchid plants.

In orchid culture, it is different than growing orchid in natural environment. In tissue culture, they grow well if they get the proper requirements that orchid need. Each orchid species has their own requirement which depends on their species. To achieve sustainable growth as the original habitat, we must adjust the environment as
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