PROLIFERATION OF *Phaleonopsis gigantea* PROTOCORM SEGMENTS ON NDM MEDIA SUPPLEMENTED WITH 6-BENZYLAMINOPURINE (BA)

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THIS DISSERTATION IS SUBMITTED AS A PARTIAL REQUIREMENT T0 OBTAIN THE BACHELOR OF SCIENCE DEGREE WITH HONOUR

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DECLARATION

I hereby declare that the result of this final year project is fully my own work except the quotations that I had clearly stated the sources

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1. SUPERVISOR

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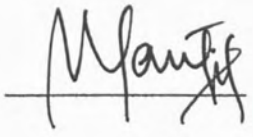
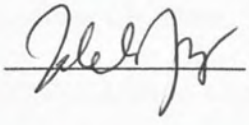


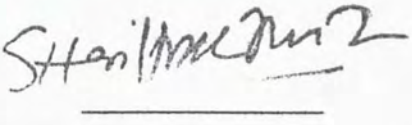
2. EXAMINER 1

(Dr. Zaleha Abdul Aziz)



3. DEAN

(SUPT/ KS Prof. Madya Dr. Shariff A. Kadir S. Omang)



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ABSTRACT

This research was conducted to determine the optimum level of the hormone cytokinin 6-benzylaminopurine (BA) for the maximum proliferation of *P. gigantea* protocorm segments. NDM as the basal medium was supplemented with 11 different concentrations of hormone BA (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/l BA). Each medium was replicated 4 times with 10 protocorm segments per replicate. The experiment was carried out for 84 days in the Tissue Culture Laboratory of UMS by using complete random experimental design (CRD). From the result obtained, the average percentage protocorm segments proliferated were approach to maximum after 70 days of culture initiated. However, the statistical analysis did not show significant different between the treatments. After 70 days of culture, high percentage of proliferation (40%) occurred in T5 (NDM+2.0mg/l BA) while only (10%) proliferated in T9 (NDM+4.0mg/l BA). PLBs formed per segment in T5 (NDM+2.0mg/l BA) ranged 17-36 after 70 days of culture. In the experiment, T2 (NDM+0.5mg/l BA) also showed high percentage of protocorm segments proliferated (37.50%) after 70 days of culture, however high percentage of dead segments (20.00%) also occurred in this medium.

ABSTRAK

Eksperimen ini dijalankan bagi menentukan tahap optimal hormon sitokinin 6-benzylaminopurine (BA) yang sesuai untuk proliferasi yang maksimum bagi segmen protokom *P. gigantea*. NDM merupakan medium asas yang ditambah dengan 11 jenis kepekatan hormon yang berlainan (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/l BA). Setiap medium direplikasi sebanyak 4 kali dan setiap replikasi mengandungi 10 segmen protokom. Eksperimen ini telah dijalankan selama 84 hari di dalam Makmal kultur tisu,UMS dengan menggunakan rekabentuk eksperimen rawak lengkap. Daripada keputusan yang diperolehi, didapati purata peratus segmen protokom berproliferasi adalah mendekati maksimum selepas 70 hari pengkulturan dilakukan. Walaubagaimanapun statistik analisis tidak menunjukkan perbezaan yang significant antara rawatan pada hari tersebut. Selepas 70 hari pengkulturan dilakukan, didapati purata peratus proliferasi yang tinggi (40%) berlaku dalam T5 (NDM+2.0mg/l BA) manakala hanya (10%) proliferasi berlaku dalam T9 (NDM+4.0mg/l BA). Julat bilangan JSP yang terbentuk pada setiap segmen dalam T5 (NDM+2.0mg/l BA) adalah 17-36 selepas 70 hari pengkulturan dilakukan. Dalam eksperimen ini, walaupun T2 (NDM+0.5mg/l BA) menunjukkan purata peratus proliferasi yang tinggi (37.50%) selepas 70 hari pengkulturan dijalankan, namun peratusan kematian yang tinggi (20.00%) juga berlaku dalam medium ini.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Varians
BA	N^6- benzyl adenine
BAP	6- benzylaminopurine
CRD	Completely Randomized Design
CITES	Convention on International Trade of Endangered Species
DMAP	Dimethylaminopurine
Fe-EDTA	Ferric ethylene diamine tetraacetic acid
L	Liter
mg	milligram
mg/L	milligram per liter
ml	milliliter
MS	Formula medium Murashige and Skoog
NaOH	Natrium Hydroxide
NDM	New Dogashima Medium
PLB	Protocorm-like body
VW	Formula medium Vacin and Went
XER	Formula medium Experimental Ernst Robert
%	Percentage
°C	Degree Celcium

CHAPTER 1

INTRODUCTION

1.1 Preface

Orchids can be found all over the world between latitude 68° north and 56° south, except in the polar regions and in the driest regions (Fadelah Abdul Aziz *et al.*, 2001). The orchid flower can be so popular is properly because of the unique flower of the species comparing to other plants. The orchid flower is belongs to the Orchidaceae family which is probably the largest family among the higher flowering plants with 22,000 to 35,000 species in about 700 to 800 genera (Fadelah Abdul Aziz *et al.*, 2001). According to Arditti, 1967, orchid can be found the most in the tropical regions. Malaysia with a geological history of millions of year is a natural home to a vast variety of orchids. There are up to 3,000 species orchid of the most attractive, beautiful and mysterious orchids being found (Fadelah Abdul Aziz *et al.*, 2001). Unfortunately, the number of orchid species is reducing due to over-logging activity for agricultural development purpose (Abdul Karim & Hairani Haris, 1989).

Phaleonopsis, 'moth orchids' is the most beautiful flowers among the long arching sprays plants in the world (Chen & Chang, 2004). Since the flowers they produced were resemble to a flight of moth at twilight, therefore from there it get its name as *phaluna* in Greek meaning 'moth', and *opsis* meaning 'resembling'. In Philippines, they are called *mariposa* or butterflies while in Indonesia they are known as the Moon Orchid, *Anggerek boelan* (Teoh, 1980). This genus is widely distributed throughout Southeast Asia with a few species extending northwards to Taiwan and Sikhim and southwards to Australia and the Pacific. On the other hand, this genus also has its economic value for pot plant and cut flower production (Chen & Chang, 2004).

Phaleonopsis gigantea is an orchid that known locally as "elephant ear" due to its large and enormous leaves. It is an endemic orchid that can only being found in Borneo. It is also one of the wild orchid species that have been category as endangered species in the list of Appendix II of the Convention on International Trade in Endangered Species (CITES), that restricted trading of this species. It is named for its gigantic leaves that easily exceed 60 centimeters in cultivation and *P. gigantea* is the largest *Phalaenopsis* species (Peter, 2003). Due to the high demand of this plant in the horticulture trade, therefore it is easily brought to extinction purely from collection to meet the demand. Moreover, it is a slow growing plant. *P. gigantea* arguably is the best *Phalaenopsis* species use to produce award-winning hybrids therefore it is shine as parent in today modern *Phaleonopsis* species hybridizing (Peter, 2003).

Phaleonopsis is an important ornamental orchid that producing almost perfect cut flower, but it is difficult to propagate vegetatively (Griesbach, 1983). Propagation is generally by *in vitro* seed germination but this method can not produce seedlings that are uniform (Ishii *et al.*, 1998). As a result, propagation through tissue culture has been desired. In 1960, Professor Georges Morel discovered a method of obtaining several thousands of virtually identical specimens from a single mother plant, even of a rare or unusual hybrid species, without recourse to seed (Arditti & Ernst, 1993).

Proliferation of protocorm and Protocorm-Like Bodies (PLBs) is increasing the number of orchid plantlets that produce few seeds which may also not germinate well or clone that may be difficult to propagate through tissue culture methods (Rosmah *et al*, 2005). The word protocorm was being used by Melchior Treub in Kebun Bunga Bogor (Bogor Botanical Gardens), Indonesia to describe a stage in moss development. At present this term is used to describe the small spherical tuber-like bodies formed by germinating orchid seeds (Arditti & Ernst, 1993). In order for the protocorm to proliferate, the basal medium usually will be supplemented with hormone cytokinin, benzyl adenine (BA). Moreover, cytokinin being found to be able to induce proliferation and increase the proliferation effect when using synthetic cytokinin, kinetin (Ernst, 1975). Therefore, this experiment was conducted to determine hormone concentration that needed for protocorm segments of *P .gigantea* to proliferate optimally.

1.2 Research Objective

To determine the optimum level of hormone BA for proliferation of *P. gigantea* protocorm segments.

1.3 Justification

The study was conducted to develop a protocol to proliferate protocorm segments effectively before heading for clonal propagation of *P.gigantea*. This species was a popular ornamental plant and having high demand in horticulture trade. Moreover, it had already been listed as one of the endangered species in Sabah. Therefore, conservation must be done in order to manipulate them. So far, propagation by shoot-tip culture was unsuitable as this method will kill the mother plant which already limited in number. Naturally, *P.gigantea* produced fewer offshoots due to limited seed produced and also limited vegetative part to be used as explant therefore proliferation of protocorm segments were a method to induce more PLBs. Previous study had been done with intact protocorm proliferation in the same treatments. However, there were no promising numbers of PLBs formed. Hence, more study was needed to find a reliable and more efficient method.

CHAPTER 2

LITERATURE REVIEW

2.1 Orchid Distribution

Malaysia appears to be an ideal place for the growth of many different orchid species, ranging from the tiny *Bulbophyllum* species to the gigantic *Grammatophyllum* species which is the largest of all orchid although there are still many wild orchid that are still unidentified (Fadelah Abdul Aziz *et al*., 2001). There are about 12 genera of orchid that are commonly being grown in Malaysia such as *Aerides*, *Arachnis*, *Asocentrum*, *Cattleya*, *Dendrobium*, *Doritis*, *Oncidium*, *Phaleonopsis*, *Rhynchostylis*, *Renanthera*, *Vanda* and *Vandopsis*. In the same time, there are more than ten species of orchid hybrids been produced such as *Ascocenda, Aranda, Bandacinis* and lot more by the orchid breeders (Kamal & Shariff, 2002). All of these orchid hybrids have been used by many orchid growers from many places and that gives Malaysia an international reputation as the centre of producing orchid hybrid.

In the Borneo Island, there are about 3000 species that can be found and of these 34.4% were found to be endemic (Chan *et al.*, 1994). Moreover, there are 290 species being found under threat and endangered (Alphonso, 1987). This is mainly due to human activities such as over-logging, illegal collection of wild orchids and the clearing of lands for agricultural purposes. Therefore, there is an urgent need to conserve the Borneon wild orchid species to prevent them from totally disappears. Many efforts have been done such as preserved forest or establishment of orchid collection centre such as Tenom Orchid Center in Sabah.

2.2 Orchid General Characteristic

All kinds of orchids have a few features in common such as they have immense quantities of microscopic seeds which mostly lacking in nutritive reserves; their germination and development only make possible by the presence of a fungus that is parasitic; the flower structure consisting of 3 sepals and 3 petals while one of petal modified into a lip (labellum) and a single central stamen (except for the Cypripedioideae); the protocorm which subsequently becomes a modified plantule; the pollen that usually formed into waxy masses which known as pollinia and lastly is that the pollination of orchids is generally carried out by a specific insect for each species but there is also some rare cases (e.g *Dendrobium sophronites*) the pollinator is a bird or a bat (Leroy-Terquem & Parisot, 1993).

Generally, there are two types of orchid which are the terrestrial and epiphytie orchids. The terrestrial orchids are the orchids that grows on the ground and they can be found usually in the temperate country. The epiphytic orchids are the orchids that grows on trees or rock but not on the ground which usually marked as parasitic plant and can be found mostly in the tropic country.

Basically, there are two types of growing habit which are monopodial and sympodial orchids. Monopodial is the orchid that has one main stem and grow taller every year. As the stem lengthens, the new leaves usually added on the top while the aerial roots formed occasionally along the stem. The flower stalk emerge from the aerial side of the leaves while the flower borne laterally and successively from the older nodes toward the younger nodes. Examples for this growing habit are *Phalaenopsis*, *Arachnis*, *Vanilla*, *Aerides*, *Vandopsis*, *Vanda*, *Phyncostylis*, *Ascocentrum* and *Trichoglottis*. Sympodial is the orchid that has creeping ground stem or rhizome that send out shoot which eventually develop into stem and leaves. Every year one or two shoot from the lower part of the frontal pseudobulb producing a new portion of rhizome and a new pseudobulb after flowering. Flowers are formed at the terminal or at the sides of the stem. *Cattleya*, *Dendrobium*, *Oncidium*, *Coelogyne*, *Bulbophyllum*, are the examples of orchid with sympodial growth habit.

2.3 The Genus *Phaleonopsis*

Phaleonopsis is a monopodial genus that does not usually form offshoots (Arditti & Ernst, 1993). The popularity of *Phaleonopsis* orchids has increased greatly in the last ten years which having immense commercial value in the horticultural world (Jose, 2005). *Phaleonopsis* come from the lowlands and are epiphytes growing on trees close to streams with only a few exceptions. They are shade-loving plants that requiring a light intensity of only 1000 foot-candles and responded well to fluorescent light culture and cultivation by the window sill (Teoh, 1980). As they are simple to grow therefore they are excellent orchid for beginner. The attractive flowers can last for 2 to 3 months, and continuous blooming can be achieved by good culture and judicious pruning of the flower spike (Teoh, 1980).

Phaleonopsis is monopodial plants with large succulent leaves on an extremely short stem (Teoh, 1980). There are two major subdivisions in *Phaleonopsis* which are the *Euphaleonopsis* and *Stauroglottis*. *Euphaleonopsis* is the orchid which have large and round flower with the petals larger than the sepals. The lip have well appendages at the tip that resembling antennae. Members of this group have branching sprays of flowers that well displayed as a cascade. For example *P.amabillis* var. *grandiflora*, *P. schillerana* and *P. sanderana*. *Stauroglottis* is the orchid that have small colourful which usually spotted or barred. The petals are in the same size or smaller than the sepals and the lip is

devoid of appendages which is shaped like a spade or anchor and some species the lip is decorated with hair or calli. The special characteristic of the lip is the important criteria in identify the individual species in the group. Examples of the orchid categoried in this group are *P. amboinensis*, *P. cochlearis*, *P. cornu-cervi*, *P. gigantea*, *P. fuscata* and *P. gersenii*.

2.4 *Phaleonopsis gigantea*

In Sabah the natural habitats of *P. gigantea* are in Merutai and Tiger Mountain areas of Sabah but it is also found on the west side of the Crocker Mountain range in Sarawak and West Kalimantan. It is a slow growing plant but is reportedly easy to cultivate at an elevation of 500 feet (152m) from the sea level (Baker, 1990). The plants usually have five to six leaves that are pendent, leathery, broadly rounded, pale silver green and shiny on both surfaces of leaves that hanging from the stem of the plant. The long arching flower of this species is the most fascinated path. The flower stem usually is pale yellow green in color and emerge from the leaf axils. The inflorescence usually branch once or twice. The flower has a natural spread of about 5 centimeters with the record of 97 flowers on a single spike that open simultaneously. The blossoms often can last for 2 to 5 months if not spoiled by the water on the flower. The sepals and petals of the flower are equal in size with brightly color of yellow or white as background that usually overlapping and heavily marked with raised red-brown spots or brotches that can be feel with our fingers (Peter, 2003).

P. gigantea is the species that being used to produce award-winning hybrids. So far, it is used in 140 first-generation hybrids and is in the background of 1,187 hybrids going back seven generations, with more than 500 plants awarded (Peter, 2003). This plant has a rest period during the winter season.

2.5 Propagation of Orchid

Orchids are inherently slow-growing plants (Arditti, 1977). It may be propagated either sexually or asexually. Orchid propagated sexually is the process where pollination happens either by using hand pollination or natural pollination and fertilization occur subsequently. There is a fact that orchid pollination usually seldom or not happen at all.

Propagation asexually is the propagation that consists of traditional asexual propagation such as division of rhizome, top cutting and a more advance method such as tissue culture. Consequently, the traditional method can hardly fulfill the needs of market, therefore tissue culture is more preferred (Montakan, 2005). Moreover, orchids that propagated both vegetatively and sexually produced distinct variation in the offspring (Talukder *et al.*, 2003). As a matter of fact, in order to get true-to type plants, clonal propagation *in vitro* is the only mean (Talukder *et al.*, 2003).

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