# THE EFFECTS OF NAPHTHALENEACETIC ACID AND BENZYLAMINOPURINE ON THE PROLIFERATION OF *PHALAENOPSIS GIGANTEA* PROTOCORMS

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A DISSERTATION SUBMITTED AS PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN PLANT TECHNOLOGY

PLANT TECHNOLOGY PROGRAMME
SCHOOL OF SCIENCE & TECHNOLOGY
UNIVERSITY MALAYSIA SABAH



MARCH 2005

PUMS 99:1

**UNIVERSITI MALAYSIA SABAH**

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JUDUL: THE EFFECTS OF NAPHTHALENEACETIC ACID AND BENZYLAMINOPURINE ON THE PROLIFERATION OF PHALAENOPSIS GIGANTEA PROTOCORMS.

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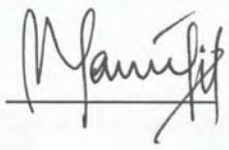
   **(Associate Prof. Dr. Mariam Abdul Latip)** 

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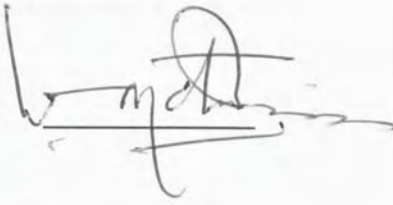
   **(Associate Prof. Dr. Wan Mohamad bin Wan Othman )** 

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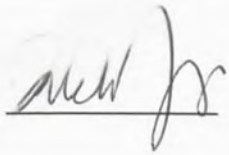
   **(Dr. Zaleha Abdul Aziz)** 

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   **(Associate Prof. Dr. Amran Ahmed)** _____

## ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation and deepest gratitude to his supervisor Associate Professor Dr. Mariam Abd. Latip for her guidance, advice and assistance during the course of this thesis as well as the preparation of this manuscript.

Gratitude is also expressed to Ms Rosmah Murtad for her guidance and advice during the course of conducting my laboratory experiment. I would also like to take this opportunity to thank our laboratory assistant Mrs. Rokiah, Ms Christina and the other laboratory assistant that were involved in this experiment.

Finally, I would like to thank my family and friends for their help and support. These are vital to the success of this final year project.

## ABSTRACT

Protocorms of *Phalaenopsis gigantea* were cultured on Experimental Robert Ernst (XER) media supplemented with naphthaleneacetic acid (NAA) in concentrations of 0.1 mg $l^{-1}$ and 1 mg $l^{-1}$ and benzylaminopurine (BAP) in concentrations of 1 – 20 mg $l^{-1}$. These were added to the basal media either singly or in combinations. A total of 13 treatments were produced. The media containing 0.1 mg $l^{-1}$ NAA generally gave better result in terms of proliferation rate (16.07%) and the number of protocorm-like bodies (PLB) produced (0-6). Proliferation rate were reduced while shoot and leaf formations increased with increasing concentrations of BAP. Survival rate of protocorms were unusually low on all treatments with hormone free media (control) having the better survival rate among the treatments.

## ABSTRAK

Protokorm daripada *Phalaenopsis gigantea* telah dikultur menggunakan media Experimental Roberts Ernst (XER) yang ditambah dengan asid naftalena asetik (NAA) dalam kepekatan 0.1 mg $l^{-1}$ atau 1 mg $l^{-1}$ dan benzilaminopurin (BAP) pula pada kepekatan 1 – 20 mg $l^{-1}$. Rawatan kepekatan hormon ini diberi secara berasingan ataun secara kombinasi. Sejumlah 13 rawatan telah dihasilkan dengan menggunakan kombinasi berlainan antara hormon NAA dan BAP. Secara am, media yang mengandungi 0.1 mg $l^{-1}$ NAA menunjukkan keputusan yang baik dari segi proliferasi protokorm (16.07%) dan bilangan jasad seperti protokorm (JSP)) yang terhasil (0-6). Kadar proliferasi didapati menurun sementara pembentukan pucuk dan daun meningkat dengan setiap peningkatan kepekatan BAP. Kadar kemandirian protokorm adalah rendah dan hanya media kawalan tanpa hormon menunjukkan kadar kemandirian yang lebih memuaskan jika dibandingkan dengan rawatan-rawatan lain yang terlibat.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Varians |
| BA | $N^6$-benzyl adenine |
| BAP | 6-benzylaminopurine |
| CRD | Complely Randomized Design |
| CITES | Convention on International Trade of Endangered Species |
| DMAP | dimethylaminopurine |
| EDTA | ethylene diamine tetraacetic acid |
| GI | growth index |
| IAA | indoleacetic acid |
| IAA-ala | indoleacetyl alanine |
| IAA-gly | indoleacetyl glycine |
| IBA | indolebutyric acid |
| KNA | potassium naphthaleneacetate |
| MS | Murashige-Skoog |
| NAA | naphthaleneacetic acid |
| NAA-ala | naphthaleneacetic alanine |
| NAA-gly | naphthaleneacetic glycine |
| NDM | New Dogashima Medium |
| PLB | protocorm like bodies |
| ppm | part per million |
| SPSS | Statistical Package of Social Science |
| TDZ | thidiazuron |
| XER | Experimental Robert Ernst |
| VW | Vacin-Went |

# CHAPTER 1

# INTRODUCTION

## 1.1 Preface

Almost 10% of the world's orchids can be found in the island of Borneo alone with the number of species recorded so far at around 2500-3000 (Lamb, 1990; Rao, 1995). In Sabah alone there are 1500-2000 species of orchids. However, out of the total, 290 species have been found to be in danger of extinction (Rao, 1992).

*Phalaenopsis gigantea* is an epiphytic orchid known locally as "elephant ear" due to its large and enormous leaves. *P. gigantea* is listed on appendix I of the Convention on International Trade in Endangered Species (CITES), which restrict trading of this species (Lamb, 1991). They are currently under threat from the demand of mature plants in horticulture trade and massive deforestation. This may have been due to human activities such as over-logging, illegal collection of wild orchids and the clearing of lands for agricultural purposes. For instance, the clearing of Sabah's tropical forest has been recorded to be as high as 300 000-400 000 ha/year and there is an estimate of 30% of Sabah's forest which is still in pristine condition (Soepadmo, 1992)

Due to the difficulty for seedlings to reach maturity, wild plants tend to be collected to meet the demand, and *P. gigantea* could easily be brought to extinction purely from collection. *Phalaenopsis*, being a monopodial orchid, has difficulty to propagate vegetatively. Therefore, suitable micropropagation methods had been desired for rapid mass production to overcome the shortage of this orchid species in the wild. This study is undertaken as proliferation of protocorms is often the only means of increasing the number of plantlets.

The application of tissue culture technique is a valuable and viable method in preserving and conserving the depleting sources of orchids especially orchids that are endemic to the island of Borneo. With proper research, the culturing of orchids has the potential of improving our knowledge on certain species of orchids especially those that are under threat from extinction.

## 1.2 Research Objective

To determine the effects of Naphthaleneacetic acid (NAA) and Benzylaminopurine (BAP) on the proliferation of *Phalaenopsis gigantea* protocorms.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Orchid Distribution

Orchid is a member of the Orchidaceae family which is the largest in terms of numbers out of 350 angiosperm family that exist on earth. Orchidaceae consist of 750 genus and around 25 000 species spreading widely across the world from the sub-Artic, equatorial and all the way through to the sub-Antarctic regions. It is estimated that around 148-450 orchid genus can be found in Borneo alone, with the number of species recorded at around 2500-3000 (Lamb, 1990; Rao, 1995). In Sabah, there are 138 genus of orchid with the number of species around 1500-2000. Out of the total, 107 genus or 1200 species of orchids can be found around the area covering Mount Kinabalu alone (Lamb, 1991).

## 2.2 General Characteristics of Orchids

Orchids can be classified as simpodial and monopodial based on its vegetative growth. The growth of simpodial plants occurs with the seasonal lengthening of its rhizome to form multiple offshoots. Monopodial plants however, have the ability to grow all year round.

Most orchids are hermaphrodite whereby both its anther and stigma are found on the same flower. There are also certain species of orchids that are dioecious with both male and female flower existing on the same plant. Monoecious orchids are plants where male and female flower are found separately on different individual plant. In general, orchids have three sepals and three petals. The sepals are either connected or otherwise to form a chin. The petals are larger and more vibrant in colour; which normally form a structure known as labellum. Different species have distinctive size, colour and shape of labellum.

Orchid seeds are normally small and powdery-like with the number of seeds in a pod ranging from a few hundreds to a few thousands depending on the species. However, most seeds are unable to germinate or reach maturity in its natural habitat; only less than 5% are able to germinate in the wild (Rao, 1995).

## 2.3 *Phalaenopsis*

The genus *Phalaenopsis* comprises about 70 species. It comes from the subfamily *Vandoidea*. Species in this genus have also been known as *Polychilus*, *Polystylus*, *Stauritis*, *Stauroglottis*, and *Synadena* (Baker, 1991).

### 2.3.1 Distribution

*Phalaenopsis* climate ranges from tropical to subtropical. They are distributed from the regions of the Himalayas through to Thailand, Indo-China, Malaysia, Indonesia, New Guinea and Australia. Northwards, its distribution extends upwards to Taiwan and Southern

China. The Philippines has the highest numbers of species from the genus *Phalaenopsis*, having recorded 42 species and some 36 varieties (Baker, 1991).

### 2.3.2 Plant and Flower Information

All *Phalaenopsis* are monopodial and most are epiphytic. The main shoot of a monopodial plant will grow continuously for the rest its life. Most *Phalaenopsis* leaves are rich green, firm and succulent. However some species have limp, notched, wavy, recurved, grey-green, or purplish leaves. Normally, the leaves are elliptic and are 13 cm long with cupped near the based. Inflorescence of *Phalaenopsis* plants is about 15 cm long. The spikes are somewhat zigzag (Baker, 1991).

*Phalaenopsis* flowers can often last for 2-5 months. The number of flowers produced is around 8 per season. The small, star-shaped flowers are 1.2 cm across and its colour ranges from cream to white. The sepals are larger than the petals, and both are somewhat pointed. The lip is triangular in shape (Baker, 1991).

*Phalaenopsis* seeds normally mature sufficiently to be viable for use in pod culture about 85 days after pollination. In 110-120 days, the seeds are fully mature and the fruits open. Changes in the capsule appearance may indicate that seeds are ready for sowing immediately; otherwise they will become contaminated when the fruits open and will require sterilization before being sown (Baker, 1991).

## 2.4 *Phalaenopsis gigantea*

*P. gigantea* is listed on Appendix I by the Convention on International Trade of Endangered Species (CITES) as a highly endangered species due to illegal collection and its slow rate of growth (Lamb, 1991). Trading of this species are restricted by CITES.

### 2.4.1 Distribution

*P. gigantea* habitat can be found in Sabah around the Merutai and Tiger Mountain areas, where it is rare or nearly extinct. It is also found on the west side of the Crocker Mountain range in Sarawak and West Kalimantan. *P. gigantea* can be found in primeval forest areas of Java and is reportedly easy to cultivate at an elevation of 152 m (Baker, 1991). Besides, *P. gigantea* had also been discovered in the Tawau district of Sabah (Lamb, 1978).

### 2.4.2 Plant and Flower Information

*P. gigantea* is a very large monopodial epiphyte. In nature, the leaves are 56-91 cm long and are leathery and shiny with 5-6 leaves per plant. The plant has one or more inflorescence while the pendent spikes are 15-38 cm long and usually branch once or twice (Baker, 1991).

They have in between 20-30 flowers. However, a single inflorescence may have nearly 100 flowers. They are 3.8-7.0 cm across, star-shaped and fleshy. The colours of the flowers may range from greenish white to yellow, with closely spaced brown or maroon spots. The white lip is marked with bright reddish purple. However this plant is difficult to breed and takes 8-12 years to bloom (Baker, 1991).

## 2.5 Propagation of *Phalaenopsis*

*Phalaenopsis* can be propagated by seed germination through *in vitro* culture. Although germination rate of *Phalaenopsis* are lower than other species. Fertilization takes place within two and a half to three months after pollination and its capsule can be harvested subsequently and culture of their content one to two months prior to ripening (Ernst, 1984). This method of culturing can be termed as "embryo culture". Embryo culture has the following advantage such as being able to reduce the time between pollination and flasking of seeds; the reduction of adverse effect on plants due to fruit setting; the salvation of fruits from complex hybrids before they abort and the external portion of the fruit can be sterilized without harming the embryo. Many of the published seed germination media are sufficient for *Phalaenopsis* seeds, such as Knudson C medium (Ernst, 1984).

*Phalaenopsis* can also be propagated vegetatively. *Phalaenopsis* are monopodial and usually does not form side shoots. In cases where the monopodial axis has been severed or the crown of the plant injured, one or more shoots may arise from basal leaf axils. Certain *Phalaenopsis* species such as *P. leuddemanniana* tend to produce plantlets from buds of its flower stalk (Arditti & Ernst, 1993).

Asexual propagation *in vitro* or in other words, the micropropagation of *Phalaenopsis* had been used throughout the years for the mass rapid clonal propagation of commercial cut-flowers cultivars of orchids. For example, the clonal propagation of *Phalaenopsis* by flower stalk with axillary buds by Intuwong *et al.* (1972), meristems culture by Intuwong and Sagawa (1974), shoot tip culture of flower stalk buds by Zimmer

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