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JUDUL: THE EFFECT OF THIDIAZURON ON THE PROLIFERATION OF Phalaenopsis gigantea PROTOCORM

Ijazah: PLANT TECHNOLOGY

SESI PENGAJIAN: 2004/2006

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THE EFFECT OF THIDIAZURON ON THE PROLIFERATION OF *Phalaenopsis gigantea* PROTOCORM

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DISSERTATION SUBMITTED AS PARTIAL FULFILMENT FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

PLANT TECHNOLOGY PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

2007

DECLARATION

I hereby declare that this thesis is result of my own research except for quotations as cited in the references.

April 20, 2007

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ACKNOWLEDGEMENTS

First of all, I would like to thank to God for His mercy, guidance and patience towards my spiritual growth and walk with Him in these three years. I would like to express my sincere appreciation to my honorable project supervisor Prof. Madya Datin Dr. Mariam Abdul Latip for her assistance, advice and guidance throughout the duration on this project.

My special appreciation to Ms. Rosmah Murdad who had contributed to the success of this project. I also express my gratitude to all the lab assistants of Tissue Culture, Genetic, Plant technology and Phisiology Laboratory, especially Ms.Roseline, without them my project might not be completed successfully.

I also take this opportunity to thank my lab mates especially Lai Hui Ting and those who had contributed to the success of this project. My special thanks to my loving friends Keetanjaly, Sri Swastika, Thirushala Devi, Manikodi, Puvaneswari, Prssana and not forgetting my course mates on their support.

Lastly, I would also like to express my deepest and warmest sense of thanks and appreciation to my family, for which their love, encouragement and support made it possible for me to complete my project.

LILLY MALAR GOVINDASAMY

2007

ABSTRACT

This study was conducted to determine the effects of coconut water (CW) and Thidiazuron (TDZ) on proliferation of *Phalaenopsis gigantea* protocorms. The protocorms used for this study were obtained from previously initiated seed germination. Protocorms was segmented vertically and cultured on NDM basal medium containing different concentrations of TDZ (0, 0.1, 0.2, 0.3 and 1.0 mg/L) and coconut water (0% and 15% v/v). The One-way ANOVA showed a significant difference among all the treatments media studied on day 120. Protocorm segments exhibited the highest percentage of proliferation on a medium containing 15% (v/v) CW and 0.1 mg/L TDZ (68.00 ± 16.43) with an average range of 3-30 new protocorms formed per protocorm segment. Protocorm cultured on a medium containing 15% (v/v) CW and 1.0mg/L TDZ produced the highest number of new protocorms in the range of 0-40 per segment with an average percentage of proliferation 20.00 ± 21.21. Among the concentrations, the best protocorm proliferation was obtained in the combination of 15% (v/v) CW and low concentration (0.1mg/L) of hormone TDZ.

ABSTRAK

Kajian penentuan kesan air kelapa dan Thidiazuron (TDZ) terhadap proliferasi protokom orkid *Phalaenopsis gigantea* telah dijalankan. Protokom yang digunakan dalam kajian ini adalah protokom yang dikultur sebelumnya. Protokom dipotong kepada 2 segmen secara menegak dan dikultur dalam medium NDM sebagai medium basal. Kepekatan air kelapa 0%, 15% (v/v) dan 0.1, 0.2, 0.3, dan 1.0 mg/L TDZ telah digunakan sebagai jenis rawatan yang berlainan. Medium NDM tanpa sebarang komponen tambahan digunakan sebagai kawalan. Ujian ANOVA 1-hala telah menunjukkan perbezaan yang bererti di antara kesemua media rawatan yang dikaji. Media rawatan yang menunjukkan purata peratus proliferasi yang tertinggi adalah media NDM + 15% air kelapa + 0.1 mg/L TDZ iaitu (68.00 ± 16.43) dan julat pertambahan protokom baru per segmen adalah di antara 0-30. Segmen protokom yang dikultur dalam media rawatan 15% (v/v) air kelapa + 1.0mg/L TDZ menghasilkan jumlah protokom baru yang tertinggi dimana julatnya adalah 0-40 per segmen tetapi dengan purata peratus proliferasi yang rendah 20.00 ± 21.21. Secara keseluruhannya, hasil proliferasi yang terbaik didapati daripada rawatan yang mengandungi kombinasi air kelapa 15% (v/v) dan kepekatan Thidiazuron sebanyak 0.1mg/L.

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LIST OF SYMBOLS / ABBREVIATIONS

%	-	Percentage
=	-	Equal
<	-	Less than
X	-	Multiple
M	-	Molar
Mm	-	Milimetre
Cm	-	Centimetre
m	-	metre
ml	-	Milimetre
mg	-	Miligram
mg/ml	-	Miligram per milimetre
µm	-	Macrometre
µl	-	Microliter
mg/L	-	Miligram per liter
g/L	-	gram per liter
g	-	Gram
Kpa	-	Kilopascal
Ca	-	Calcium
H	-	Hydrogen
HCl	-	Hydrochloric acid
K	-	Kalium
Mg	-	Magnesium
Mn	-	Manganase

NH_4	-	Ammonium
NO_3	-	Nitrate
OH	-	Hydroxide
Na	-	Natrium
SO_4	-	Sulfate
H_2O	-	Water
PO_4	-	Phosphate
Fe	-	Ferum
°C	-	Degree of Celsius
sp.	-	Species
P.gigantea	-	*Phalaenopsis gigantea*
NDM	-	New Dogashima Medium
TDZ	-	Thiadizuron
CW	-	Coconut Water
CRD	-	Completely Randomized Design
SPPS	-	Statistical Package of Social Science
ANOVA	-	Analysis of Variance
CITES	-	Convention in Trade on Endangered Species of Flora and Fauna

CHAPTER 1

INTRODUCTION

The Orchidaceae is one of the largest and most diverse flowering plant (Angiospermae) of Orchids family (Talukder *et al.*, 2003). Taxonomically, they represent the most highly evolved family among monocotyledons with 600-800 genera and 25,000-35,000 species thousands of hybrids all around the world (Arditti, 1984). Orchids exhibit an incredible range of diversity in size, shape and colour of their flowers. They are most pampered of the plants and occupy top position among all the flowering plants valued for cut flower production and as potted plants. They are known for their longer lasting and bewitchingly beautiful flowers which fetch a very high price in the international market.

Orchid species, the backbone of some of our potential hybrids are to be found throughout Malaysia, but with our rapid urbanization this is not true these days. There are orchids which resemble moths (*Phalaenopsis*), butterflies (*Oncidium papillo*), the slippers of Aphrodite or moccasins (*Paphiopedilum* and *Cypripedium*), dancing ladies (*Oncidium*), spiders (*Brassia*), scorpions (*Arachnis*) and bees (*Ophrys*) (Soon, 1980).

P. gigantea is native to Borneo and named for its gigantic leaves that easily exceed 60 centimeters in cultivation (Arditti, 1977). *P. gigantea* is the largest *Phalaenopsis* species. The massive leaves are pendent, leathery, broadly rounded, pale silver green and shiny on both surfaces. *P. gigantea* 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 (Arditti, 1982). It is found on trees along comparable streams but high up in the horizontal out stretched limbs, a hundred feet above the ground, where there is breeziness.

Plant tissue culture is a broad term which means the growing or cultivation of plantlets or plant parts in an artificial culture medium under aseptic conditions (Hodgson *et al*, 1991). The orchid industry was one of the most benefited with this technique because it is the only means of mass propagating orchids.

Nutritional requirements for optimal growth of a tissue *in vitro* may vary with the species. There are many nutrient formulae used in orchid culture such as Knudson C medium, Vacin & Went medium (VW) and Murashige & Skoog medium (MS) (Abdul Karim & Hairis, 1989). The addition of organic supplements such as banana, coconut water and potato extract in the culture medium is one of the modifications made in the culture of orchid to accelerate the growth of orchid. (Arditti, 1982). Therefore in this project, a cytokinin hormone called TDZ 1-Phenyl-3-(1,2,3-thiadiazol-5-yl)-urea or commonly known as Thidiazuron and coconut water are used to observe their effects on protocorm proliferation in both individually and in combination.

P. gigantea is listed 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). A range of symbiotic seed germination techniques and tissue culture procedures under artificial conditions is carried out with an aim to protect these endangered species from complete extinction which are suffering from over collecting and continuous loss of their natural habitats.

Conventionally, *Phalaenopsis* is a monopodial orchid which is difficult to propagate vegetatively. Even propagation through tissue culture has been desired, using shoot-tip may lead to the loss of the mother plant. Callus initiation and culture from flower stalk buds, root tips and leaves is still poorly defined in their subculturing and subsequent morphogenesis (Tokuhara & Mii, 1993). Alternative methods such as seed derived protocorms developed in sterile culture have provided useful and easily handled research material and it is expected that more details of seedling physiology will become available from their continued use.

The research objective is to examine the effect of TDZ hormone and coconut water on proliferation of *P. gigantea* protocorms in both individually and combination. This study is a part of our trial research to establish an appropriate protocol for *in vitro* culture to obtain the best treatments which induced high proliferation of protocorms. The findings of this study would be beneficial to researchers, commercial propagators, orchid growers, etc in mass propagation of orchids.

CHAPTER 2

LITERATURE REVIEW

2.1 Orchid

History tells us that the first appearance of the word "orchis" was in the Greek manuscript, Enquiry into Plants, written by the renowned philosopher Theophrastus, pupil of Plato and Aristotle, somewhere between 370 and 285 B.C. (Withner, 1959). The word orchid comes from the term orchis, meaning testis referring to the paired underground bulbs of the Mediterranean orchid plants. Orchids, the most beautiful flowers in god's creation, comprise a unique group of plants.

Orchids are among the biggest and most diverse of the flowering plant families, with over 800 described genera and 25,000 species (Singh & Voleti, 1995). Some sources give 30,000 species, but the exact number is unknown since classification differs greatly in the academic world. The class Angiospermae, or the flowering plants, is customarily divided into two subclasses, the Monocotyledonae (monocots) and the Dicotyledonae (dicots).

Although the specialized flower structure conforms to a standard plan, the vegetative parts are shown great variation, a large number of them being epiphytes, or

terrestrial and a few saprophytes and leafless in nature. Those orchids which are most popular today are the epiphytic tropical species (Lamb, 1990).

2.2 Orchid distribution

Orchid, popular name for members of the Orchidaceae is a family of perennial herbs widely distributed in both hemispheres (Tom & Marion, 1994). Orchids are a cosmopolitan family found above the Arctic Circle, in southern Patagonia and even on Macquarie Island, close to Antarctica. They are absent only from open water and from true deserts. Majority of the cultivated orchids are native of tropical countries and occur in their greatest diversity in humid tropical forest of South and Central America, Mexico, India, Ceylon, Burma, South China, Thailand, Malaysia, Philippines, New Guinea and Australia (Chan *et al.*, 1994).

A rough distribution of orchids in Eurasia is about 40-60 genera, North America: 20-30 genera, Tropical America: 300-350 genera, tropical Africa: 125-150 genera, tropical Asia: 250-300 genera and in Ocenia: 50-70 genera. Malaysia has about 220 genera, consist of 1750 wild species (Abdul Karim & Hairis, 1989). Borneo is also known as the Orchid Island (Chan *et al.*, 1994) where it has 2500 to 3000 species of orchid. In Sabah, 138 genus of orchid is found with around 1500 to 2000 species. Out of the total, 1200 species of orchids can be found around the area covering Mount Kinabalu (Lamb, 1991).

2.3 Characteristics of Orchid

Naturally, the Orchids share many features with related groups of monocots, scattered vascular bundles, parallel leaf venation, flower parts in threes, an inferior ovary and so on (Kamal, 1989). Their size and shape can be an aid in identifying the orchid, since it reflects the taxonomic position. Species that typically bask in sunlight, or grow on sites which can be occasionally very dry and have thick leathery leaves. Orchids are truly flowers of superlatives. The basic orchid flower is composed of three sepals in the outer whorl, and three petals in the inner whorl. The medial petal is usually modified and enlarged then called the labellum or lip, forming a platform for pollinators near the center of the corolla. Together except the lip, they are called tepals (Withner, 1959).

The stem of an orchid determines the habit of the species. Each type of stem can grow in two ways, monopodial ("one-footed") growth and sympodial ("many-footed") growth (Soon, 1980). In monopodial, the new shoots grow upwards from a single stem, originating in the end bud of the old shoots. It then produces leaves and flowers along this stem. The stem of these orchids can reach a length of several meters. Examples of Orchids in this category are *Vanda*, *Vanilla*, *Arachnis*, *Phalaenopsis* and many more.

In sympodial, the plant produces a series of adjacent shoots which grow to a certain size, bloom, and then stop growing, to be replaced by the next growth. Plants of this type grow laterally rather than vertically, following the surface of their support. The growth continues by development of new leads with their own leaves and roots

sprouting from or next to those of the previous. *Cattleya* and *Dendrobium* falls in this category of orchids (Arditti, 1977).

2.4 Conservation of Orchid

In Southeast Asia today orchid hybrids predominate in private collections and commercial orchid nurseries and species that were grown extensively before the war are now hard to find (Fighetti, 1993). The three well known orchids of Sarawak, *Dimorphorchis lowii*, *Phalaenopsis violacea* and *Vanda dearei* are all threatened species now, as is *Phalaenopsis gigantea* of Sabah (Chan *et al.*, 1994). The conservation of orchid species in Southeast Asia is a complex and difficult problem.

Scientists, conservationists and many members of the general public are now well informed about the escalating losses of biological diversity throughout the world. Relative to other plant families, there is evidence that orchids are subject to high levels of threat, through both natural and anthropogenic causes (Hutchings, 1989).

In 1990, a series of workshops was initiated to bring together scientific information on aspects of orchid biology and conservation of orchids to discuss future research, management and conservation. There are many ideas for the protection of orchid species but as far as Malaysia is concerned, the measures to be adopted for the protection of orchid species are the allocation of more areas as nature reserves, proper legislation for the control of export of orchid species, educating the citizen on the importance of conservation and lastly the role of the Botanic Gardens in the pursuit of conservation (Kamal & Shariff, 2002).

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