



UNIVERSITI PUTRA MALAYSIA

**INDUCTION AND PLANT REGENERATION OF CALLUS IN
DENDROBIUM**

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FP 2000 15

**INDUCTION AND PLANT REGENERATION OF CALLUS IN
*DENDROBIUM***

By

CYNTHIA PSYQUAY COSSALL

**Thesis Submitted in Fulfilment of the Requirements for the Degree
of Master of Agriculture Science in the Faculty of Agriculture
Universiti Putra Malaysia**

March 2000



DEDICATION

ESPECIALLY FOR....

**MUMMY AND DADDY
TIL DEATH DO US PART**

I LOVE YOU

**RAYMOND
SHELLA
ANTHONY
RICHARD**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Agriculture Science.

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Chairman : Associate Professor Saleh bin Kadzimin, Ph.D.

Faculty : Agriculture

The present study examines the effects of some selected chemicals and physical treatments on protocorm-like body (plbs) of orchid hybrid, *Dendrobium* Kasem White. This is an attempt at initiating embryogenic callus, and setting conditions optimal for its maintenance and regeneration into plbs and hence plantlets. This protocol was initiated to establish a system for the transfer of genetic material through genetic engineering technology.

The study was conducted in four main parts, a set of preliminary studies, callus induction, maintenance and regeneration. The critical concentration of picloram and kinetin for the survival of wounded and unwounded plbs were obtained from the preliminary studies. Optimal concentration of picloram and kinetin for unwounded plbs ranged from 0 to 1.0 mg/l. For wounded plbs the



concentration ranged from 0 to 0.5 mg/l and 0 to 1 mg/l for picloram and kinetin respectively.

Callus-like tissue formation was observed from unwounded plbs cultured on $\frac{1}{2}$ MS supplemented with 0.6 to 0.9 mg/l picloram and 0.3 to 0.6 mg/l kinetin treatments. The best concentrations were 0.75 mg/l picloram and 0.60 mg/l kinetin. Induction was achieved in the 40-day dark treatment. Wounded plbs produced callus-like tissue on 0.1 mg/l picloram + 0.8 mg/l kinetin and 0 mg/l picloram + 1.0 mg/l kinetin treatments after 30 days of culture in the dark.

Callus-like tissue remained viable in both solid and liquid medium of $\frac{1}{2}$ MS supplemented with 0.75 mg/l picloram and 0.6 mg/l kinetin, cultured in the dark.

Through several modifications of medium, the callus-like tissue regenerated into plbs when the medium was devoid of hormones and cultured in the light. Regenerated plbs formed shoots and roots upon transfer to medium with IBA and BA.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains Pertanian.

**PENGGALAKAN DAN PENJANAAN SEMULA PERTUMBUHAN
KALUS *DENDROBIUM***

Oleh

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Kajian telah dijalankan untuk mengkaji kesan beberapa rawatan kimia dan fizikal terhadap protokom hibrid orkid *Dendrobium* Kasem White. Kajian ini berusaha untuk menghasilkan sel-sel kalus yang embrionik dan mengujudkan keadaan persekitaran yang optima bagi pemeliharaan dan penjanaan semula kalus kepada protokom dan seterusnya kepada anak pokok. Protokol ini dibentuk untuk mencipta satu sistem pemindahan gen melalui teknologi kejuruteraan genetik.

Kajian dibahagikan kepada empat bahagian iaitu kajian-kajian permulaan, penggalakan kalus, pemeliharaan kalus dan penjanaan semula kalus. Kepekatan kritikal pikloram dan kinetin untuk pertumbuhan protokom terluka dan tak luka didapati dari kajian-kajian permulaan.

Kepekatan optima pikloram dan kinetin bagi pertumbuhan protokom tak luka telah diperolehi dari 0 hingga 1.0 mg/l pikloram dan 0 hingga 1.0 mg/l. Kepekatan optima pikloram dan kinetin bagi protokom terluka adalah masing-masing 0 hingga 0.5 mg/l dan 0 hingga 1.0 mg/l.

Pembentukan tisu yang menyerupai kalus berlaku pada protokom tak luka yang dikultur pada $\frac{1}{2}$ MS yang mengandungi 0.6 hingga 0.9 mg/l pikloram dan 0.3 mg/l hingga 0.6 mg/l kinetin. Kepekatan 0.75 mg/l pikloram dan 0.6 mg/l kinetin merupakan kepekatan optima yang untuk penggalakan kalus dari protokom tak luka yang dikulturkan selama 40 hari di rawatan gelap. Protokom terluka mengeluarkan tisu yang menyerupai kalus selepas 30 hari di rawatan gelap pada rawatan 0.1 mg/l pikloram + 0.8 mg/l kinetin dan 0 mg/l pikloram + 0.1 mg/l kinetin.

Tisu yang menyerupai kalus kekal hidup pada medium cair dan beku dalam rawatan $\frac{1}{2}$ MS dengan 0.75 mg/l pikloram dan 0.6 mg/l kinetin dan dikultur di dalam gelap.

Melalui pengubahsuaian medium, penjanaan tisu yang menyerupai kalus berlaku pada medium tanpa hormon dan pendedahan kepada cahaya. Protokom yang disubkultur ke medium dengan rawatan

IBA dan BA menghasilkan pembentukan anak pokok yang berdaun dan berakar.



ACKNOWLEDGEMENTS

The author would like to express her gratitude to Associate Professor Dr. Saleh bin Kadzimin, of the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM) for his advice and supervision throughout the course of her studies. She also would like to express her appreciation and thanks to her co-supervisors, Professor Dr. Marziah bt. Mahmood of the Department of Biochemistry and Microbiology, Faculty of Science and Environmental Studies, UPM and Dr. K. Harikrishna of the Department of Biotechnology, Faculty of Food Science and Biotechnology , UPM.

Her thanks are also due to Associate Professor Dr. Ghizan Saleh, Dr. Sayed Mohd. Zain Sayed Hassan, Dr. Maheran Abdul Aziz of the Department of Plant Science and Dr. Anuar Rahim of the Department of Land Management, UPM for their advice and other contributions to her studies.

Special thanks also to due to Encik Abdul Rahman bin Sidam, Cik Haryati bt. Jamsari, Puan Norshila bt. Abdul Jalil, Cik Aniza bt.



Che Ngah and Cik Salimah bt. Ali of the Plant Tissue Culture Laboratory of the Department of Crop Science for their invaluable help in the running of the experiments.

Her thanks are also due to Encik Anib Ali and Puan Maiminah bt. Tahir of the Plant Breeding Laboratory, to Encik Aminnudin Hashim and Mr. Wong Chee Ching of the Floriculture Laboratory, to Puan Salmi Yaacob of Biometry Laboratory and Encik Baharin of the Photography Unit of the Faculty of Agriculture.

The author also would like to thank Pn. Azilah bt. Abdul Jalil, Puan Sulika Madhavan and Mr. Ho Oi Kuan of the Microscopic Unit, Institute of Bioscience, UPM for their assistance in electron microscopic work.

Last but not least, her thanks to Dr. Ahmad Tarmizi bin Hashim, Mr. Khairul Naim Adham, Ms. Florencen C. Ginibun, Mdm. Kathreen Sherit, Mr. Philip Sipen, Mr. Adrain Ling, Mdm. Janna Ong, Mdm. Sarong Muring, Ms. Wong Lay Yieng, Mdm. Sam Yen Yen, Mr. Hendry Joseph, Ms. Rebecca Ranty, Ms. Nancy Ngaran, Mdm. Jenes Anyi, Ms. Mary Ahin, Ms. Ida Suryani bt. Md. Sidik, Cik Norazlina Noordin and Ms. N. Sivajothi for their kind support.



The author would like to take this opportunity to dedicates this thesis to her friends, Ms. Valerie Ngau, Mdm. Regina Leong, Mdm. Judy Henry, Ms. Helena Banun Valentine, Ms. Trinkka Slan, Ms. Diana Lucas Sadin and Ms. Lucyana Dominic Ritay.

The author dedicates her thesis to her second family, Mr. and Mrs. Md. Noh Manap of Sri Serdang, Selangor.



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LIST OF ABBREVIATIONS

- % - Percentage
- RM - Ringgit Malaysia
- mg/l - milligramme per litre
- ° C - degree Celcius
- p.s.i. - pound per square inch
- rpm - rate per minute
- plbs - protocorm-like bodies
- MS - Murashige and Skoog's
- VM - Vacin and Went

CHAPTER I

INTRODUCTION

Orchids belong to the largest family of flowering plants. There are about 100 genera consisting of about 800 species of orchids in Malaysia (Holttum & Enoch, 1991; MARDI, 1991; Teo, 1985). The flowers have long captured the interest of orchid growers because of their great diversity in shapes and colour. The passion which started as a hobby has now become a million-dollar industry, in countries like Malaysia, Thailand, Singapore, Indonesia and Philippines.

The orchid industry, which started as a small-scale activity, has become an important export earner contributing about 40% of the total value of cut flower production in 1995. The general economic outlook for the industry appears to be positive. The high demand for orchids seems to continue with the growing affluence of the population.

Dendrobium is by far the largest genus in the orchid family and mostly grown in commercial farm's. It is one of the leading cut and potted orchid hybrids grown in the tropics.



Breeding of *Dendrobium* using sexual hybridization is restricted due to the long generation time and lack of useable genetic variability. Hybridization could only occur within intra-generic boundaries, that is *Dendrobium* could only be crossed with another *Dendrobium*. It has not always been possible to obtain full hybrids between desired individuals because of sexual incompatibility. In recent years, genetic engineering has played a vital role in producing new hybrids where foreign genetic material could be introduced via the use of several systems of genetic transformation such as particle-bombardment. This may provide an alternative to improve *Dendrobium* hybrids genetically.

Transformation of *Dendrobium* protocorm-like bodies (plbs) using particle bombardment was achieved by Kuehle and Sugii (1992). However, chimerism may be a problem using plbs as target tissue. In order to recover non-chimeric plants, callus as an alternative could be used as target cells.

Callus is a mass of proliferating unorganized cells. Naturally, wounded plant cells produce callus for wound healing. Growing a mass of callus on a semisolid or liquid suspension is widespread in *in vitro* growth studies. The culture of callus provides a system for the study of differentiation, morphogenesis and plant regeneration. It may also provide an alternative to improve plants genetically.

