UNIVERSITI PUTRA MALAYSIA

INDUCTION AND PLANT REGENERATION OF CALLUS IN DENDROBIUM

CYNTHIA PSYQUAY COSSALL

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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

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DEDICATION

ESPECIALLY FOR....

MUMMY AND DADDY
TIL DEATH DO US PART

I LOVE YOU .....

RAYMOND
SHEILLA
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.

INDUCTION AND PLANT REGENERATION OF CALLUS IN *DENDROBIUM*

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March 2000

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 ½ 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 ½ 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

CYNTHIA PSYQUAY COSSALL

Mac 2000

Pengerusi : Profesor Madya Saleh Kadzimin, Ph.D.

Fakulti : Pertanian

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 pemelihraan 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 ½ 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 ½ 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

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

I certify that an Examination Committee met on 30 March, 2000 to conduct the final examination of Cynthia Psyquay Cossall on her Master of Agriculture Science thesis entitled "Induction and Plant Regeneration of Callus in *Dendrobium*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows :

WONG KAI CHOO, PhD.
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

SALEH BIN KADZIMIN, PhD.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

MARZIAH BT. MAHMOOD, PhD.
Professor
Faculty of Scince and Environmental Science
Universiti Putra Malaysia
(Member)

K. HARIKRISHNA, PhD.
Associate Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

MOHD. GHAZALI MOHAYIDIN, PhD,
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia

Date : 24 MAY 2000

This Thesis submitted to the Senate of Universiti Putra Malaysia and was accepted as fufilment of the requirements for the degree of Master of Agriculture Science.

KAMIS AWANG, PhD,
Associate Professor
Dean of Graduate School,
Universiti Putra Malaysia

Date : 13 JUL 2000

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that if it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

CYNTHIA PSYQUAY COSSALL

Date : 29 MAY 2000

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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 achived 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 altrernative 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.