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UNIVERSITI PUTRA MALAYSIA

ESTABLISHMENT OF A REGENERATION SYSTEM THROUGH CALLUS FORMATION AND GENETIC ANALYSIS BASED ON RAPD FOR DETECTION OF SOMACLONAL VARIATION IN *DENDROBIUM* SERDANG BEAUTY

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MASTER OF SCIENCE
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By

ALIREZA KHOSRAVI

This thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

OCTOBER 2008

DEDICATION

I would like to dedicate my thesis to my parents, who are very encouraging and contributing much in all my life. Meanwhile, I would also like to dedicate to my brother, for his continuous support throughout the duration of my study.

Thanks God

Alireza Khosravi

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

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OCTOBER 2008

Chairman: Associate Professor Mihdzar Abdul Kadir, PhD

Faculty : Agriculture

Flowers of the *Orchidaceae* family are highly variable in shape, color and smell, which make orchids the most important cut flowers in the market. Among the orchid varieties, *Dendrobium* hybrids have high regards in the cut flower industry. This study was aimed at establishing a plant regeneration system of *Dendrobium* Serdang Beauty and the characterization of colchicine-induced mutation by RAPD.

The first part of the study was to develop a plantlet regeneration system for *D.* Serdang Beauty. Callus was induced from protocorm- like bodies (plbs), and cultured on media supplemented with different auxins of various concentrations for plantlet regeneration.

Highest fresh weight in callus induction and proliferation was obtained on MS medium containing 1.5 mg/L IBA.

Calli proliferated on medium supplemented with 1.5 mg/L IBA were used as explants for plantlet regeneration. The highest percentage of plantlet regeneration was obtained in treatments with 1 mg/L KIN and 1.5 mg/L NAA (90%).

In the acclimatization study, plants were cultured *in vivo* in different media under the same environment. All media gave high percentage of plant survival. Medium M1 (charcoal), M2 (charcoal mixed with broken rock) and M5 (sawdust mixed with charcoal) produced the highest percentage of plant survival (100%).

Following the callus induction study, calli obtained were cultured on media containing different concentrations of colchicine (0, 5, 10, 20 and 25 mg/L). Colchicine treatment at 5 mg/L significantly gave the highest fresh weight of regenerated plantlets (3.43 g).

Subsequently, the resulting calli from colchicine treatments were analyzed for somaclonal variation and characterized using RAPD. The study indicated that somaclonal variation existed and was polymorphic in nature. Based on the analysis, *D.* Serdang Beauty V showed 25% differentiation with the mother plant. In a further analysis, *D.* Serdang Beauty V was also characterized with other *Dendrobium* species. *D.* Serdang Beauty V showed high dissimilarity with other *Dendrobium* varieties.

In conclusion, the result showed that IBA in low concentration was effective to induce and proliferate more callus. In the regeneration study, KIN alone or in combination with other auxin was useful to regenerate more plantlets. Meanwhile in the acclimatization study, charcoal was useful to growth of *Dendrobium.* Also colchicine induced mutation method was useful for the production of mutated *Dendrobium* and the RAPD technique appeared to be useful for the detection of variation between species and varieties.

Abstrak tesis yang dikemukan kepada Senat University Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

PENGASASAN SISTEM REGENERASI MELALUI PEMBENTUKAN KALUS DAN ANALISIS GENETIK MENGIKUT KAEDAH RAPD INTUK PENGECAMAN KEPELBAGAIAN KLON-SOMA DALAM *DENDROBIUM* SERDANG BEAUTY

Oleh

ALIREZA KHOSRAVI

OCTOBER 2008

Pengerusi: Associate Professor Mihdzar Abdul Kadir, PhD

Fakulti: Pertanian

Bunga dari keluarga *Orchidaceae* mempunyai kepelbagaian bentuk, warna dan wangiannya yang terserlah, dan ini menjadikan keratan bunga orkid sebagai keratan bunga terpenting di pasaran. Diantara varieti-varieti orkid yang dipasarkan, orkid *Dendrobium* merupakan varieti yang diutamakan dalam industri tersebut. Kajian ini bertujuan membentuk satu sistem regenerasi pokok bagi orkid *Dendrobium* Serdang Beauty dan mengenalpasti mutasi yang dihasilkan oleh kesan rawatan kolcisina, melalui teknik RAPD.

Bahagian pertama kajian ini adalah untuk pembentukan sistem regenerasi *D.* Serdang Beauty. Kalus yang dihasilkan dari plbs (protocom-like bodies), dikultur dalam media

yang mengandungi pelbagai kepekatan auksin untuk menggalakkan regenerasi. Pembentukan kalus dan berat segar yang tertinggi didapati pada MS media yang mengandungi 1.5mg/L IBA. Kalus ini telah digunakan sebagai eksplan dalam kajian regenerasi. Peratus regenerasi pokok dari kalus yang tertinggi didapati dari media MS a yang mengandungi 1 mg/L KIN dan 1.5 mg/L NAA (90%).

Dalam kajian aklimatisasi anak pokok yang terhasil telah dibiasakan pertumbuhanmya di nurseri dalam pelbagai media tanaman yang dikaji. Semua media menghasilkan peratus penyuaian pokok yang tinggi. Medium M1 (kayu arang), M2 (kayu arang dengan pecahan batu) dan M5 (habuk kayu dengan kayu arang) menghasilkan peratus penyuaian pokok yang tertinggi (100%).

Menuruti ujian induksi kalus, kalus yang dihasilkan telah dikultur dalam medium mengandungi berbagai paras kepekatan kolcisina 0 (kawalan), 5, 10 dan 15 mg/L. Rawatan 5 mg/L kolcisina menghasilkan purata berat basah pokok yang tertinggi (3.43 g).

Seterusnya, teknik analisis RAPD telah digunakan untuk mengenalpasti variasi somatik dalam kalus hasil dari rawatan kolcisina. Analisis tersebut menunjukkan bahawa perbezaan *D.* Serdang Beauty V (dari kesan rawatan kolcisina) dari pokok induknya adalah 25%.

Seterusnya, dalam analisis terakhir, pelbagai genera orkid telah dibandingkan dengan *D.* Serdang Beauty V. Di sini, *D.* Serdang Beauty V menunjukkan perbezaan yang tinggi dari varieti-varieti tersebut tetapi masih mengekalkan tahap persamaan yang tinggi dengan pokok induknya.

Kesimpulannya, aplikasi rawatan IBA pada kepekatan rendah berkesan dalam pembentukan dan pertumbuhan kalus. Kajian regenerasi pokok dari kalus pula berkesan dibawah rawatan KIN sahaja atau bersama auxin. Seterusnya, kajian penyuaian pokok-pokok tersebut paling berkesan dalam medium arang kayu. Rawatan kolcisina untuk menjanakan mutasi merupakan teknik yang berkesan dalam menghasilkan variasi dalam *Dendrobium* dan teknik RAPD merupakan teknik yang sesuai untuk mengenalpastikan perbezaan antara spesis dan varieti.

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I certify that an Examination Committee has met on ---------- to conduct the final examination of Alireza Khosravi on his Master Science thesis entitled "Establishment of a Regeneration System through Callus Formation and Genetic Analysis Based on RAPD for Detection of Somaclonal Variation in *Dendrobium* Serdang Beauty" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1981 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Member of Examination Committee are as follows:

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Date: 12 February 2009

DECLARATION

I declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted to any other degree at Universiti Putra Malaysia or at any other institutions.

ALIREZA KHOSRAVI

Date: 27 February 2009

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LLIST OF ABBREVIATIONS

A260:	Absorbance at 260nm in spectrophotometer
A280:	Absorbance at 280nm in spectrophotometer
AFLP:	Amplified Fragment Length Polymorphism
ANOVA:	Analysis of variance
Asc:	*Asco*
BA:	N^6 –benzyladenine
BAP:	N^6 –benzylaminopurine
bp:	Base pair
CTAB:	Cethyltriaminobromide
DAF:	DNA amplification fingerprinting
D.:	*Dendrobium*
DGGE:	Denaturing gradient gel-electrophoresis
dH_2O:	Distilled deionized water
DNA:	Deoxyribonucleic acid
dNTP:	Deoxynicotinamide triphosphate
DOA:	Department of Agriculture
EDTA:	Ethylenediamine tetra-acetic acid
Ethanol:	Ethyl alcohol
FAO:	Food and Agriculture Organization of the United Nation

GMO:	Genetically Modified Organisms
HCl:	Hydrochloric acid
hrs:	Hours
IAA:	Indole-3-Acetic Acid
IBA:	Indole-3-Butyric Acid
IPUC:	International Union of Pure and Applied Chemistry nomenclature
ISSR:	Inter Simple Sequence Repeats
Kb:	Kilo base
KIN:	6-Fururylaminopurine
M:	Molar
$MgCl_2$:	Magnesium chloride
Min:	Minute
mM:	Milimolar
MS:	Murashige and Skoog
NAA:	1-Naphthaleneacetic Acid
NaCl:	Sodium chloride
ng:	Nanogram
OD:	Optical Density
Onc:	*Oncidium*
PCR :	Polymerase Chain Reaction
pH:	Negative logarithm of hydrogen ion concentration

Phil:	*Philonopsis*
Plbs:	Protocorm-like bodies
RAPD:	Random Amplified Polymorphic DNA
RFLP:	Restriction Fragment Length Polymorphisms
RNA:	Ribonucleic acid
RNAase:	Ribonuclease
rpm:	Revolution per minute
SCARs:	Sequence characterized amplified region
SSR:	Simple Sequence Repeats
STSs:	Sequence-Tagged site
Taq:	Thermus aquaticus
TDZ:	*N*-phenyl-*N*_-1,2,3-thiadiazol-5-yl urea
TE:	Tris-EDTA
Tris:	Tris aminoethane
USDA:	United State Department of Agriculture
UV:	Ultraviolet
v/v:	Volume for volume
Van:	*Vanda*
w/v:	Weight for volume
µg:	Microgram
µl:	Microliter