UNIVERSITI PUTRA MALAYSIA

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By

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To Almighty God for His blessings.

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LIST OF ABBREVIATIONS

AP	:	2-amino-purine
bp	:	base pair
BU	:	5-bromo-uracil
BUdR	:	5-bromo-deoxynridine
°C	:	degree(s) Celsius
cm	:	centimetre(s)
^{60}Co	:	cobalt-60
CTAB	:	cetyltrimethylammonium bromide
CV	:	coefficient of variation
DES	:	diethyl sulphate
DF	:	degree of freedom
DNA	:	deoxyribonucleic acid
EDTA	:	ethylenediaminetetraacetic acid
EMS	:	ethyl methanesulphonate
g	:	gram(s)
Gy	:	Gray(s)
Gy/s	:	Gray(s) per second
HCl	:	hydrochloric acid
HNO_2	:	nitrous acid
hr(s)	:	hour(s)

IPMS	:	isopropyl methanesulphonate
kb	:	kilobase
LB	:	Luria-Bertani
LD	:	lethal dose
M	:	molar(s)
MeV	:	megaelectron volt(s)
min	:	minute(s)
MINT	:	Malaysia Institute for Nuclear Technology Research
ml	:	mililitre(s)
mM	:	milimolar(s)
MNH	:	N-nitroso-N-methyl urea
MNU	:	N-nitroso urea
ng	:	nanogram(s)
NaCl	:	sodium chloride
NaOH	:	sodium hyroxide
NH_2OH	:	hydroxylamine
nm	:	nanometre(s)
nM	:	nanomolar(s)
NPK	:	sodium:phosphorous:potassium (ratio)
PCR	:	polymerase chain reaction
pH	:	hydrogen ion concentration

PLBs	:	protocorm-like bodies
psi	:	pound(s) per square inch
PVP	:	polyvinylpyrrolidone
RAPD	:	random amplified polymorphic DNA
RCBD	:	Random Complete Block Design
RNA	:	ribonucleic acid
rpm	:	revolution(s) per minute
SCARs	:	sequenced characterised amplified regions
SDS	:	sodium dodecyl sulphate
sec	:	second(s)
SSC	:	saline-sodium citrate
TAE	:	Tris-acetate-EDTA
TE	:	Tris-EDTA
Tris-HCl	:	Tris(hydroxymethyl) aminomethane hydrochloride
UV	:	ultraviolet
V	:	volt(s)
VW	:	Vacin and Went's formulation medium (modified)
μg	:	microgram(s)
μl	:	microlitre(s)
γ	:	gamma

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Agricultural Science.

EFFECTS OF GAMMA IRRADIATION ON *IN VITRO* CULTURES OF SELECTED ORCHID HYBRIDS

By

ADRAIN LING CHIENG KUANG

February 1999

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

Faculty : Agriculture

Mutation breeding is an alternative strategy to conventional breeding. The physical mutagen, gamma (γ) radiation, has long been used in various mutation breeding programmes. In this connection it is necessary to determine the optimum dose before starting any mutation breeding programme.

In vitro cultured protocorm-like bodies (PLBs) of 2 orchid hybrids, *Mokara* Chark Kuan and *Dendrobium* Jacky were used to study the effects of γ-irradiation and to determine the optimum dose. The PLBs were irradiated with γ-rays at different doses of 0, 25, 50, 75, 100, 125 and 150 Grays (Gy) in Set I

and 0, 20, 40, 60 and 80 Gy in Set II of the experiment. A series of observations on the effects of γ-irradiation were recorded. Effects on genomic DNA were also observed.

The present study showed that irradiation resulted in various degrees of lethality to *in vitro* cultures of both *Mokara* Chark Kuan and *Dendrobium* Jacky. Decrease in survival with increasing doses of γ-irradiation was significantly observed. Optimum dose of γ-irradiation for *in vitro* cultures of *Mokara* Chark Kuan and *Dendrobium* Jacky were estimated to be in the range of 20-40 Gy and 60-70 Gy respectively.

Regeneration of plantlets was found to decline when higher irradiation doses were applied. Irradiation also has inhibited regeneration of *in vitro* cultures of both hybrids.

In general, fresh weight, dry weight and dry weight gain of *in vitro* cultures of *Mokara* Chark Kuan and *Dendrobium* Jacky decreased with increasing of γ-irradiation doses. *In vitro* cultures of *Mokara* Chark Kuan irradiated at 40 Gy were found to have higher fresh weight than those irradiated at 20 Gy but still lower than the control. Higher fresh weight, dry weight and

dry weight gain were observed in cultures of *Dendrobium* Jacky irradiated at 80 Gy.

In the present study, polymorphic DNA was detected by 2 random primers via polymerase chain reaction (PCR) between 7 DNA samples of *Mokara* Chark Kuan. Variations caused by repeated subculture and irradiation were detected by random amplified polymorphic DNA (RAPD) analysis. Homology search using 2 sequences (pAL108 and pAL202) from polymorphic bands as query sequences revealed close similarity to retrotransposons. No polymorphism could be detected by the primers used in RAPD analysis of *Dendrobium* Jacky DNA samples.

Abstrak tesis ini diserahkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk penganugerahan ijazah Master Sains Pertanian.

KESAN RADIASI GAMMA KE ATAS KULTUR *IN VITRO* HIBRID ORKID TERPILIH

Oleh

ADRAIN LING CHIENG KUANG

Februari 1999

Pengerusi: Profesor Madya Saleh bin Kadzimin, Ph.D.

Fakulti : Pertanian

Pembiakan mutasi ialah satu strategi alternatif kepada pembiakan konvensional. Mutagen fizikal, sinaran gamma (γ), telah lama digunakan dalam pelbagai program pembiakan mutasi. Dalam hubungan ini, penentuan dos optimum adalah penting sebelum memulakan sesuatu program pembiakan mutasi.

Struktur sel berbentuk protokom (PLBs) daripada 2 hibrid orkid, iaitu *Mokara* Chark Kuan dan *Dendrobium* Jacky yang dikultur secara *in vitro* telah digunakan untuk mengkaji dos optimum dan kesan sinaran γ ke atas kultur *in*

vitro. PLBs telah didedahkan kepada sinaran γ pada dos yang berlainan, iaitu 0, 25, 50, 75, 100, 125 dan 150 Gray (Gy) dalam Set I dan 0, 20, 40, 60 dan 80 Gy dalam Set II. Pemerhatian ke atas kesan-kesan radiasi telah direkodkan. Pemerhatian kesan radiasi ke atas DNA juga dilakukan.

Kajian ini menunjukkan bahawa radiasi γ menyebabkan beberapa tahap kematian kepada kultur *in vitro Mokara* Chark Kuan dan *Dendrobium* Jacky. Bilangan PLBs yang hidup terus menurun dengan peningkatan dos radiasi. Dos optimum untuk kultur *in vitro Mokara* Chark Kuan dan *Dendrobium* Jacky adalah masing-masing dianggarkan di antara 20-40 Gy dan 60-70 Gy.

Pertumbuhan semula anak pokok didapati merosot apabila dos radiasi yang lebih tinggi digunakan. Radiasi juga didapati merencatkan pertumbuhan semula anak pokok kedua-dua hibrid.

Secara amnya, berat basah, berat kering dan peningkatan berat kering kultur *in vitro Mokara* Chark Kuan dan *Dendrobium* Jacky didapati menurun dengan peningkatan dos radiasi γ. Walau bagaimana pun, rawatan 40 Gy bagi *Mokara* Chark Kuan didapati memberi berat basah yang lebih tinggi daripada rawatan 20 Gy tetapi masih kurang daripada kawalan. Berat basah, berat kering

dan peningkatan berat kering yang lebih tinggi telah diperhatikan pada rawatan 80 Gy bagi *Dendrobium* Jacky.

Dalam kajian ini, DNA polimorfik telah dikesan oleh 2 primer rambang menerusi 'polymerase chain reaction' (PCR) di antara 7 sampel DNA *Mokara* Chark Kuan. Variasi yang disebabkan oleh subkultur yang berulangkali dan radiasi telah dikesan oleh 'random amplified polymorphic DNA' (RAPD). Pencarian homologi ke atas 2 turutan (pAL108 and pAL202) daripada jalur-jalur polimorfik telah menunjukkan darjah persamaan yang tinggi kepada retrotransposon. Tiada polimorfisma dapat dikesan pada sampel DNA *Dendrobium* Jacky dalam RAPD oleh primer yang digunakan.

CHAPTER I

INTRODUCTION

Orchid is one of the major cut flower types grown in numerous tropical countries. It has become an important export commodity in Malaysia, Singapore and Thailand contributing major shares in the floriculture industries of these countries. In Malaysia, orchids have contributed about 40% of the total value of cut flower production in 1995. With the increasing affluence of the local population and that of the importing countries, demand for orchids is expected to increase.

Orchid gains its popularity for its wide range of varieties producing an array of shapes, colours and sizes. However, many of the existing hybrids have been in the market for more than 15 years, with some more than 20. With ever changing tastes and preferences of consumers, there is an urgent need for new and better varieties of orchids. Nevertheless, creation of such varieties through conventional breeding and selection has always been difficult, costly and time-consuming, making it a venture less attractive to many. In addition, improving already established varieties through conventional means is often hampered by genetic sterility.