UNIVERSITI PUTRA MALAYSIA

PRELIMINARY GENETIC MANIPULATION STUDIES ON MUSKMELON (Cucumis melo L.) USING PROTOPLAST TECHNOLOGY

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By

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Thesis Submitted in Fulfilment of the Requirements for
the Degree of Master of Science in the Faculty of
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TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	percentage
bp	base pairs
M	molar
ml	millilitre
mM	millimolar
2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
ABA	Abscissic Acid
BAP	6-benzylaminopurine
CAT	chloramphenicol acetyltransferase
CPW	CPW Salts Solution (cell and protoplasts washing solution)
CV	cultivar(s)
g	gram (s)
fr.	fresh weight
GA3	Gibberellic acid
GA4	Gibberellin A4
GUS	β-glucuronidase
h	hour
IAA	Indole 3-acetic acid
IBA	Indole-3-butyric acid
kb	Kilobase
Kinetin	6-furfurylaminopurine
kV	kilo Volts
min	minutes
MU	4-methylumbelliferone

MUG	4-methylumbelliferone-β-D-glucuronidase
NAA	1-napthaleneacetic acid
NADP	nicotinamide adenine dinucleotide phosphate
NFT	Nutrient Film Technique
rpm	revolution per minute
v/v	volume/volume
w/v	weight/volume
wt	weight
Zeatin	6-(4-hydroxy-3methylbut-2-enylamino) -purine
1 µL	10^{-6}L or 10^{-3}ml
1 nanogram	10^{-9}g or 10^{-6}mg
1 picomole	10^{-9}mole or 10^{-6}mmole
µF	micro Farad
UV	ultraviolet
µs	microsecond
dH_2O	double distilled water
ms	millisecond
Amp	Ampicillin
Ori	Origin of replication
Nos ter	Nopaline synthase gene termination
EtOH	ethanol
var	variety
Temp	temperature
nm	nanometer
µm	micrometer
EtBr	Ethidium bromide

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

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TAN SIANG HEE

June 1994

Chairman : Norihan Mohd. Saleh, Ph.D.

Faculty : Food Science and Biotechnology

In this study, parallel attempts have been made to initiate somatic embryos from two different explants on various hormone combinations and the results obtained were then applied to establish plant regeneration system for protoplast-derived macrocalli. Simultaneously, optimisation of plant transformation system using electroporation techniques (direct uptake) were carried out to facilitate future research on the genetic improvement of muskmelon.

Somatic embryos were successfully initiated from Birdie radicle region and Hami melon cotyledon using MS medium containing 1.0 mg/L 2,4-D, 2.0 mg/L NAA and 0.1 mg/L BAP and medium gave 2.0 mg/L NAA and 0.1 mg/L BAP, respectively. The somatic embryos developed into normal plantlets after subculturing onto hormone-free MS media solidified with 0.4% (w/v) Gelrite®.

Both Birdie and Hami melon protoplasts were successfully isolated using cotyledons of seven day *in vitro* seedlings incubated in different enzyme concentrations. Birdie melon explants were incubated in 0.05% (w/v) pectolyase Y23 and 1.0% (w/v) cellulase RS while Hami melon explants were incubated in 0.025% (w/v) Pectolyase Y23 and 1.0% (w/v) Cellulase RS for 1.5 hours.. Subsequently, Birdie protoplast which were cultured onto semi-solid (0.09% Gelrite®) medium containing $^1/_2$ strength MS supplemented with 0.1 mg/L NAA and 0.5 mg/L BAP gave the highest number of macrocolonies formation (60.96 number of colonies/field) after 30 days in culture, while Hami melon protoplast gave highest number of macrocolonies formation (30.48 number of colonies/field) in liquid culture containing $^1/_2$ strength MS supplemented with 0.5 mg/L NAA and 1.0 mg/L zeatin. Further experiments were conducted to establish the regeneration system for the macrocalli with addition of auxin and cytokinin, sucrose, growth regulators and amino acids. The results obtained showed that the macrocalli had potential to regenerate into plantlets on two types of medium (0.5 mg/L and 2.0 mg/L BAP). However, further studies must be performed to obtain a more suitable regeneration media from protoplast-derived macrocalli.

In this study, genetic transformation of muskmelon protoplasts were carried out using electroporation system. Using pBI221 as a standard, various parameters for electroporation were optimised and the GUS activities were assayed. Pulse strength of 3.0 kV/cm, single pulse, 10.0 µs pulse length, 25.0 µg/ml plasmid DNA and 15 hours incubation time were optimum for efficient DNA uptake and transient assay. Treatment of the protoplast with external stimulus such as UV irradiation in the presence of different plasmid constructs showed that the plasmid pYCN01 and

pYCN02 containing different length of PSPAL promoter fragment gave higher GUS activity compared to the CaMV 35S promoter. The results indicate that ultra violet irradiation of the protoplasts was at least partly responsible for the activation of PAL promoter.

Abstrak tesis yang telah dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat untuk ijazah Master Sains

KAJIAN PRELIMINARI MANIPULASI GENETIK TEMBIKAI WANGI MENGGUNAKAN TEKNOLOGI PROTOPLAS

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Kajian ini telah dilakukan dengan tujuan mengaruh embrio somatik menggunakan dua jenis eksplan yang berlainan yang dikulturkan di atas media MS yang mengandungi kombinasi hormon yang berbeza dan keputusan yang diperolehi telah digunakan untuk menjana-semula kalus makro yang diperolehi daripada sistem kultura protoplas. Sistem transformasi tumbuhan melalui elektroporasi juga telah dioptimumkan bagi memudahkan kajian manipulasi genetik seterusnya ke atas pokok tembikai wangi.

Embrio somatik telah berjaya diaruh daripada radikel `Birdie' yang dikulturkan di atas medium MS yang mengandungi 1.0 mg/L 2,4-D, 2.0 mg/L NAA, 0.1 mg/L BAP dan kotilidon `Hami melon' dikulturkan di atas medium yang mengandungi 2.0 mg/L NAA dan 0.1 mg/L BAP. Embrio somatik yang

dipindahkan ke atas medium MS tanpa hormone yang ditambah dengan 0.4% (w/v) Gelrite® telah menghasilkan anak pokok yang normal.

Kedua-dua protoplas 'Birdie' dan 'Hami melon' telah berjaya dipencilkan menggunakan kotilidon daripada pokok *in vitro* berumur tujuh hari yang dieramkan di dalam enzim yang berbeza iaitu 0.05% (w/v) pectolyase Y23 dan 1.0% (w/v) cellulase RS untuk 'Birdie') selama 1.5 jam dan 0.025% (w/v) Pectolyase Y23 dan 1.0% (w/v) Cellulase RS untuk 'Hami melon'. Selepas itu protoplas Birdie telah dikulturkan ke atas medium separa pejal (0.09% w/v) Gelrite®, yang mengandungi $^1/_2$ kepekatan nutrien MS, 0.1 mg/L NAA dan 0.5 mg/L BAP dan telah berjaya membentuk koloni macro yang terbanyak (60.96 bilangan koloni/bidang) selepas 30 hari dikulturkan. Manakala `Hami melon' membentuk koloni makro terbanyak (30.48 bilangan koloni/bidang) dalam cecair yang mengandungi $^1/_2$ kepekatam nutrein MS, NAA (0.5 mg/L) and zeatin (1.0 mg/L). Kajian selanjutnya telah ditumpukan ke arah menjana pertumbuhan anak pokok daripada makro kalus dengan menggunakan berbagai kombinasi auksin dan sitokinin, sukrosa, pengatur tumbesaran dan asid amino. Keputusan diperolehi menunjukkan bahawa dua jenis medium yang mengandungi 0.5 mg/L BAP dan 2.0 mg/L BAP mempunyai potensi untuk mengaruhkan pembentukan anak pokok. Walaubagaimanpun kajian seterusnya perlu dijalankan untuk memperolehi media yang lebih sesuai untuk penjanaan-semula anak pokok daripada protoplas.

Dalam kajian ini, manipulasi genetik juga dilakukan ke atas protoplas yang dipencilkan daripada kotilidon `Birdie' dengan menggunakan kaedah electroporasi. Berbagai parameter telah dikaji untuk mengoptimakan parameter untuk