UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF AGROBACTERIUM-MEDIATED FOR CHI2;1 PROMOTER ANALYSIS OFLLYCOPERSICON ESCULENTUM ON MT11

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FBSB 2005 27

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By

SITI SUHAILA BT. A. RAHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

May 2005

DEDICATED TO

My Husband,
Donald Lee Bernovich II @ Muhd. Uthman b. Abdullah

And

My Parents,
Tuan Hj. A. Rahman b. Abu
&
Puan Hjh. Siti Zainun bt. Hj. Mohd. Yasin

Living a life in the world today takes everything you got,
Taking a break from all your worries sure will help a lot,
Wouldn't you like to get away?
Sometimes you want to go where everybody knows your name,
And they always glad you came,
You want to go where people know troubles are all the same,
You want to go where everybody knows your name,
You want to go where you can see people are all the same,
You want to go where everybody knows your name.

Cheers. I learn a lot more about life in these last few years than the rest of my living…

Life is a journey,
Make the best with what you have,
Enjoy the moments,
Keep good health,
Be useful.

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

DEVELOPMENT OF *AGROBACTERIUM*-MEDIATED FOR Chi2;1 PROMOTER ANALYSIS OF *LYCOPERSICON ESCULENTUM* ON MT11

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SITI SUHAILA BT. A. RAHMAN

May 2005

Chairman: Associate Professor Norihan bt. Mohd. Saleh, PhD

Faculty: Biotechnology and Biomolecular Sciences

Improvement of *Agrobacterium*-mediated transformation system (AMT) in MT11 cultivar is required to achieve higher efficiency of gene transfer to produce transgenic plants of MT11. Various concentrations of kinetin and zeatin were tested for cultivar MT11 and VF36 explants, up to 100% of the explants are capable of producing calli from cotyledon and hypocotyls. Kinetin at 5 mg/L was highly significant ($p<0.01$) in terms of the largest number of shoot regeneration from cotyledons explants of MT11 cultivar. The use of 2 mg/L zeatin was highly significant ($p<0.01$) for shoot elongation of American variety, VF36.

The addition of ascorbic acid (pre- and post- inoculation with *Agrobacterium*) and acetosyringone were significant ($p<0.01$) for cell recovery and in enhancing the development of putatively transformed plantlets of MT11 and VF36 tomato cultivar. The combined interactions between pBY4.1 and acetosyringone was found to be highly significant ($p<0.01$) in terms of number of putatively transformed plants obtained. Kanamycin at 100 mg/L is the most suitable minimal inhibition concentration to be use in the transformation. *Agrobacterium tumefaciens* strain LBA4404 is more suitable to be use in the transformation system compared to GV2260 strain for MT11 cultivar.

The PCR amplification technique determined that the full length of Chi2;1 promoter (1336 bp) and the deletions: pBY2.3 (226 bp), pBY4.1 (435 bp), pBY6.1 (616 bp) and pBY8.1 (865 bp) showed the 1.8 kb GUS genes after gel electrophoresis. The pistils from the transgenic plants showed the expression of GUS genes blue stained in the transmitting tract, except for pBY0.5 construct (58 bp) and the control. Southern blots analysis showed pBY4.1 deletion had the strongest signal. Seedlings from pBY4.1 flower were tested and confirmed that the foreign integrated genes is inherited.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBANGUNAN PERANTARA *AGROBACTERIUM* BAGI ANALISA PROMOTER Chi2;1 *LYCOPERSICON ESCULENTUM* TERHADAP MT11

Oleh

SITI SUHAILA BT. A. RAHMAN

Mei 2005

Pengerusi: Profesor Madya Norihan bt. Mohd. Saleh, PhD

Fakulti: Bioteknologi dan Sains Biomolekular

Pembangunan sistem perantara transformasi *Agrobacterium* disarankan untuk pokok tomato kultivar MT11 bagi mencapai tahap pemindahan gen yang tinggi dan menghasikan pokok MT11 transgenik. Eksplan kotiledon daripada tomato kultivar MT11 dan VF36 mempamerkan pertumbuhan kalus yang tinggi dengan penggunaan pelbagai kepekatan hormon kinetin dan zeatin sehingga 100%. Kultivar MT11 memberikan nilai perbezaan yang beerti ($p<0.01$) bagi regenerasi pucuk dengan kinetin pada kepekatan 5 mg/L. Bagi kultivar VF36 pula, kepekatan 2 mg/L zeatin memberikan nilai yang beerti tinggi ($p<0.01$) bagi regenerasi pucuk. Penggunaan 'acetosyringone' dan asid askorbik (sebelum dan selepas inokulasi dengan *Agrobacterium*) adalah bahan penggalak terbaik

yang dengan nilai perbezaan nyata beerti ($p<0.01$) untuk kultivar MT11 dan VF36 dalam meningkatkan kadar transformasi dan masa pemulihan yang singkat. Kajian mendapati interaksi di antara plasmid mengandungi serpihan promoter pBY4.1 dan 'acetosyringone' adalah kombinasi terbaik dengan nilai perbezaan nyata tinggi ($p<0.01$) dalam menghasilkan pokok transformasi. Kanamycin pada tahap 100 mg/L adalah kepekatan yang paling sesuai digunakan dalam system transformati. Kombinasi penggunaan *Agrobacterium* LBA4404 dan antibiotik karbenisillin adalah lebih baik berbanding *Agrobacterium* GV2260 bagi kultivar MT11.

Teknik amplifikasi PCR mendapati kesemua pokok transgenik mengandungi promoter daripada PY2 (jujukan lengkap promoter Chi2;1 (1336 bp), pBY2.3 (226 bp), pBY4.1 (435 bp), pBY6.1 (616 bp) dan pBY8.1 (865 bp) mengandungi gen 1.8 kb GUS. Penggunaan larutan pengikat GUS menampilkan ekspresi gen GUS berwarna biru pada pistil pokok MT11 transgenik kecuali pBY0.5 (58 bp) dan pengawal. Teknik 'Southern blot' menunjukkan pBY4.1 mempamerkan isyarat gen 1.8 kb GUS yang paling terang. Biji benih dari pokok pBY4.1 juga menunjukkan gen GUS diwarisi oleh anak pokok tersebut.

ACKNOWLEDGEMENTS

Praise to Allah, The All Merciful, and The All Compassionate for He has guided me and provided strength and comfort throughout the undertaking the completion of my research.

The success achieved in this study is attributed to the cooperation of the following persons:
1. Associate Professor Dr. Norihan bt. Mohd. Saleh, as Supervisor for the project, I truly appreciate the guidance for the completion of the research and the thesis. You do care about students and thank you for always helping me. Associate Professor Dr. Suhaimi b. Napis and Dr. Faridah bt. Qamaruz Zaman as Committee Member, thank you for the support and being my friends.

2. My husband, Donald Lee Bernovich II @ Muhd. Uthman Bernovich bin Abdullah. Thank you for your love and support and for believing that I can do this! May Allah bless our marriage.

3. My wonderful parents: Tuan Hj. A. Rahman b. Abu and Puan Hjh. Siti Zainun bt. Hj. Mohd. Yasin. My sister and my best friend, Shukriah Aini; and her family. Thank you for your help and support, and all the fun we had together! I am truly the luckiest little sister ever. My bros: Md. Kharir and Md. Zamri and their families – I love you guys!

4. My colleagues at Universiti Putra Malaysia, Tropbio Research Sdn. Bhd. and Forest Research Institute Malaysia. I could not thank you enough everyone that has been helping me in this study: Assoc. Prof. Dr. Harikrishna and Dr. Ho Chai Ling: thank you for giving me the chance to be in your research team, it meant so much to me when you put your trust; Choong Chieh Wean, Lee Pick Kuen and Siti Habsah: just to name a few but truly significant people in my lab life back then! How can I ever thank you guys? My seniors : Meta Sritua Arif, Law Sen You, lab technicians and at administration level (at faculty and Graduate School Office) that help in giving me a better understanding about working smart. Special thank you to Ummi Kalthum and Haslinda who are always there for me whenever I need a friend and lending me a helping hand. I really appreciate the time that you took from yourself to help another person, that's noble. May Allah bless you throughout your life, Ummi and Linda. Seha, I cannot thank you enough! I am truly blessed to have friends like all of you. I would also like to thank you Dr. Kodiswaran in FRIM, I always see you as my mentor. I learn a lot and see the world (and myself) in a better way. Thank you.

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AMT	*Agrobacterium*-mediated transformation
A	ampere
bp	base pair
BAP	6-benzylaminopurine
ChiP	Chi2;1 promoter
chv	chromosomal genes
CM	callus induction medium
cv.	cultivars(s)
C.V.	coefficient of variation
/D	dark treatment
DNA	deoxyribonucleic acid
2,4-D	2,4-dichlorophenoxyacetic acid
DMSO	dimethyl sulfoxide
EtBr	ethidium bromide
GUS	Beta-glucuronidase gene
g	gram(s)
mg	milligram(s)
hpt	hygromycin phosphotransferase gene
IAA	indole-3-acetic acid
INT	intron
kb	kilobase
LB	Luria Bertani
L	liter

L/	light treatment
M	molar
MCS	multiple cloning site
MIC	minimal inhibition concentration
mg	milligram
ml	milli liter
mM	milli molar
uM	micro molar
uL	micro liter
ug	microgram
MS	Murashige and Skoog medium/media
NAA	naphtaleneacetic acid
nm	nanometer
NOS ter	nopaline synthase gene terminator
nptII	neomycin phosphotransferase genes
OD	optical density
onc	oncogenes
ORI	origin of replication
PCR	polymerase chain reaction
rol	auxin-synthesizing genes
rpm	revolution per minute
35SCAMV	35S ribosomal subunit of the cauliflower mosaic virus
SD	standard deviation
SE	shoots elongation
SP	shoot primordial

TFL	tobacco feeder layer
UV	ultraviolet
vir	virulence genes
v/v	volume per volume
X-gluc	5-bromo-4-chloro-3-indoly-glucuronide powder or solution

CHAPTER 1

INTRODUCTION

1.1 Plant Genetic Manipulation

Plants play important roles in satisfying the requirements of other life forms; either directly or indirectly, from low to higher organisms including human beings. It provides food, oxygen, fiber, flowers, aromas and therapeutic medicines. Over the past decade, plant cultivation has made use of various technologies, including plant breeding, which involves the crossing of plant species to protect and select for desirable traits. Breeders have developed sophisticated crossing schemes, which increased the yield of crops such as rice, wheat and maize. However, plant breeding is slow, time-consuming and laborious (Watson *et al.*, 1992). It also requires the utilization of large land areas, a present rarity in many developing countries where land area is shrinking due to over population (Beversdorf, 1993).

The introduction of Genetic Engineering (recombinant DNA and gene transfer) technique as well as plant cell and tissue culture techniques in 1970's, have provided an attractive alternative to conventional plant breeding and accelerate the production of new plant varieties with desirable traits (Cocking and Davey, 1987). From 1982 onwards, when the first single gene was successfully transferred, progress has been rapid. At present, important crops such as alfalfa, corn, cotton, flax, potato, soybean, sugar beet,