



UNIVERSITI PUTRA MALAYSIA

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

SITI SUHAILA BT. A. RAHMAN.

FBSB 2005 27



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PROMOTER ANALYSIS OF *LYCOPERSICON ESCULENTUM* ON MT11**

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*
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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 transformasi. 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.

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LIST OF ABBREVIATIONS

| | |
|------------|---|
| AMT | <i>Agrobacterium</i> -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) |
| <i>hpt</i> | 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 |
| <i>NOS ter</i> | nopaline synthase gene terminator |
| <i>nptII</i> | neomycin phosphotransferase genes |
| OD | optical density |
| <i>onc</i> | oncogenes |
| ORI | origin of replication |
| PCR | polymerase chain reaction |
| <i>rol</i> | auxin-synthesizing genes |
| rpm | revolution per minute |
| 35SCAMV | 35S ribosomal subunit of the cauliflower mosaic virus |
| <i>SD</i> | standard deviation |
| SE | shoots elongation |
| SP | shoot primordial |

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



| | | |
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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 (Beverdors, 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,