

PLANT REGENERATION AND OPTIMIZATION  
OF PARTICLE BOMBARDMENT PARAMETERS  
FOR GENETIC TRANSFORMATION OF  
BORNEO SWORD (*Aglaonema simplex*)

MA NYUK LING

MASTER OF SCIENCE  
UNIVERSITY MALAYSIA TERENGGANU

2008

9/6916

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Perpustakaan Sultanah Nur Zahirah  
Universiti Malaysia Terengganu (UMT)



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SF 457.7 .L5 2008



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UNIVERSITI MALAYSIA TERENGGANU (UMT)  
21030 KUALA TERENGGANU

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BORNEO SWORD (*Aglaonema simplex*).**

**MA NYUK LING**

**Thesis Submitted in Fulfillment of the Requirement for the  
Degree of Master of Science in the Faculty of Science and Technology  
University Malaysia Terengganu.**

Abstract of thesis presented to the senate of University Malaysia Terengganu in fulfilment of the requirement for the degree of Master of Science.

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BOMBARDMENT PARAMETERS FOR GENETIC TRANSFORMATION OF  
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**MA NYUK LING**

**DECEMBER 2007**

**Chairperson : Associate Professor Aziz Ahmad, Ph.D**

**Member : Professor Maziah Mahmood, Ph.D  
Cha Thye San, Ph.D**

**Faculty : Faculty of Science and Technology**

Aquatic plants become popular and important as ornamental in water garden as well as in aquarium. *Aglaonema simplex*, an aquatic plant native to Malaysia, is commonly known as Malayan Sword or Borneo Sword which has reddish leaves when young, and mid to dark green when mature. Plant regeneration from rhizome and callus culture, and genetic transformation using particle bombardment were developed for this plant. The purpose of this study is to add value on this plant for enhancing the market and aesthetic value.

Plant regeneration from rhizome and rhizome-derived-calli were investigated using several types of plant growth regulators (PGRs). The plant regeneration from rhizome were successfully obtained in MS media added with BAP, 2ip, and zeatin after two

months of culture. All rhizome cultured on  $3 \text{ mgL}^{-1}$  of BAP was formed plant with the highest number of plantlets. Calli obtained from treatment with  $2 \text{ mgL}^{-1}$  of dicamba were yellow compact, green compact and whitish frangible dicamba. Plants were successfully regenerated from yellow-compact and green-compact calli cultured on half strength of MS medium added with  $3 \text{ mgL}^{-1}$  and  $2 \text{ mgL}^{-1}$  of TDZ, respectively. No shoot formation was formed on whitish-frangible calli. Four months were required to obtain complete plantlets from the calli. On the whole, 48% of the total calli were successfully forming shoots.

The bombardment transformation parameters for both rhizome and calli were successfully optimised using green fluorescent protein (GFP, 35s-sgfp-TYG-nos) as the reporter gene. The optimum condition for calli transformation including: 12 cm distance between the target tissue and stopping screen, 1100 psi of acceleration pressure, 1.0  $\mu\text{m}$  of gold particle size, 27 Hg of vacuum pressure, two times of bombardment, spermidine as precipitation agent, 8  $\mu\text{g}$  of plasmid DNA, and 4 days of pre-culture period. Meanwhile the optimum condition for transformation of rhizome were 6 cm distance between the target tissue and stopping screen, 1000 psi of acceleration pressure, 1.6  $\mu\text{m}$  of gold particle size, 24 Hg of vaccum pressure, one times of bombardment, spermidine as precipitation agent, 8  $\mu\text{g}$  of plasmid DNA, and 4 days of pre-culture period. The insertion and integration of the transgene into the plant genome was confirmed through molecular analysis such as PCR and Southern Blot. A specific fragment of GFP band of PCR product with approximately 750 bp

was obtained similar size with GFP gene. Southern blot analysis showed that one to two copies of gene were integrated into genome of putative transformed calli, and one to four copies into putative transformed rhizome tissue. Genomic transformation system for rhizome and calli tissue of *A.simples* using particle bombardment method were successfully achieved.

DISCLAIMER 2007

Pengaruh

Professor Muzahid Ahmad, Ph.D

Abdi

Professor Marjan Hashimuddin, Ph.D

Che Chey Sam, Ph.D

Teknologi

Sains dan Teknologi

Pembelahan akar yang semakin popular dan penting sebagai tambahan bahan baku untuk produksi sepatu, alat-alat rumah tangga, kerupuk, roti dan kripik. Malah ada yang dicampurkan dalam minuman. Pada bagian akar ini terdapat sel-sel yang berpotensi untuk berubah menjadi sel-sel yang aktif dan berfungsi. Sel-sel ini dapat dikenali dengan adanya perubahan morfologis dan transformasi genetik yang merupakan hasil dari pengaruh faktor-faktor eksternal dan internal. Dapat juga diambil untuk dimanfaatkan sebagai sumber genetik dalam penelitian dan pengembangan teknologi mutagen. Pengaruh teknologi ini pada akhirnya akan memberikan kontribusi bagi peningkatan kualitas dan kuantitas produksi akar dan sel-sel akar.

Pengaruh teknologi ini akan memberikan kontribusi bagi peningkatan kualitas dan kuantitas produksi akar dan sel-sel akar. Pengaruh teknologi ini pada akhirnya akan memberikan kontribusi bagi peningkatan kualitas dan kuantitas produksi akar dan sel-sel akar.

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

**REGENERASI POKOK DAN PENGOPTIMUMAN PARAMETER BEDILAN  
MIKROPROJEKTIL UNTUK TRANSFORMASI GENETIK PEDANG  
BORNEO (*Aglaonema simplex*)**

**MA NYUK LING**

**DISEMBER 2007**

**Pengerusi : Profesor Madya Aziz Ahmad, Ph.D**

**Ahli : Profesor Maziah Mahmood, Ph.D  
Cha Thye San, Ph.D**

**Fakulti : Sains dan Teknologi**

Tumbuhan aquatik menjadi semakin popular dan penting sebagai tumbuhan hiasan di taman air dan aquarium. *Aglaonema simplex*, tumbuhan aquatik asli kepada Malaysia yang dikenali umum sebagai Pedang Malayan atau Pedang Borneo, mempunyai daun muda kemerahan dan menjadi hijau gelap apabila matang. Regenerasi pokok daripada rizom dan kalus kultur, dan transformasi genetic menggunakan bedilan mikroprojektil telah dibina untuk tumbuhan ini. Tujuan kajian ini adalah untuk menambah nilai tumbuhan bagi meningkatkan pasaran dan nilai kecantikan.

Regenerasi pokok daripada rizom dan kalus terbitan daripada rizom telah dikaji dengan menggunakan beberapa jenis pengawalatur tumbesaran tumbuh. Regenerasi pokok daripada rizom telah berjaya dihasilkan dalam medium MS yang ditambah dengan BAP, 2ip, and zeatin selepas dua bulan dikulturkan. Semua rizom dikultur atas

$\text{mgL}^{-1}$  BAP telah menghasilkan pokok dengan bilangan anak pokok tertinggi. Kalus yang dihasilkan daripada  $2 \text{ mgL}^{-1}$  dicamba adalah kuning padat, hijau padat dan putih gembur. Pokok telah dapat diregenerasi dengan jayanya daripada kalus kuning padat dan hijau padat yang dikultur atas medium MS separuh kekuatan yang ditambah dengan  $3 \text{ mgL}^{-1}$  atau  $2 \text{ mgL}^{-1}$  TDZ masing-masing. Tiada pembentukan pucuk telah terbentuk pada kultur kalus putih gembur. Empat bulan diperlukan untuk regenerasi pucuk daripada callus kepada tumbuhan lengkap. Keseluruhananya, 48% daripada jumlah kalus telah berjaya membentuk pucuk.

Parameter transformasi bedilan untuk rizom dan kalus telah berjaya dioptimumkan dengan menggunakan green fluorescent protein (GFP) 35s-sgfp-TYG-nos sebagai sistem pelapor. Parameter optimum bagi transformasi kultur termasuk: jarak 12cm antara tisu sasaran dengan skrin penghenti, 1100 psi tekanan pecutan,  $1.0\mu\text{m}$  saiz zarah emas, 27 Hg tekanan vakum, dua kali pembedilan, spermidine sebagai agen pemendakan,  $8 \mu\text{g}$  plasmid DNA dan empat hari subkultur sebelum pembedilan. Manakala parameter optimum untuk rizom transformasi ialah jarak 6 cm antara tisu sasaran dengan skrin penghenti, 1000 psi tekanan pecutan,  $1.6 \mu\text{m}$  saiz zarah emas, 24 Hg tekanan vacum, satu kali pembedilan, spermidine sebagai agen pemendakan,  $8\mu\text{g}$  plasmid DNA dan empat hari subkultur sebelum pembedilan. Kemasukan dan integrasi gen asing ke dalam genom tumbuhan boleh disahkan dengan menjalankan analisis molekul seperti PCR dan Southern Blot. Serpihan khusus jalur GFP daripada produk PCR dengan lebih kurang 750 bp diperolehi dimana adalah sama saiznya

dengan gene GFP. Analisis Southern Blotting menunjukkan satu hingga dua salinan gene telah berintegrasi ke dalam genomik calon transformasi kalus dan satu ke empat salinan ke dalam calon transformasi rizom yang dibedil. Sistem transformasi genetik untuk kalus dan rizom *A.simplex* yang menggunakan kaedah mikroprojektil bedilan telah berjaya dihasilkan.

I would like to thank my dear advisor for his support and guidance during the research process. I would like to thank him for all his effort in the review of this thesis and his suggestions on how I could improve it.

I would like to thank my dear supervisor for my postgraduate studies, Prof. Dr. Masih Mahmood, which gave me a lot of supports and help me pass the hard exam of UPM. Besides, for advice, assistance, guidance and motivation, he always helps students in their life and thesis but also guides them to be a better and successful person. Again, special thanks to Prof. Dr. Masih who provided me the OFF opportunity to this study. To Dr. Che Tahir bin Idris, I would like to say that will never underestimate the huge efforts made by him for this project and, my acknowledgement to my supervisor gave me a lot of support, assistance and continual encouragement.

Here I would like to acknowledge an amazing group of individuals whose technical suggestion, comments, ideas and feedbacks they bestowed upon my GenBank submission, Huda, Andeen, Odilia, Shu, Firdaus, Dely, Kali Kurni, Faru, Kurni Putri, Ima-