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

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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 mgL^{-1} of BAP was formed plant with the highest number of plantlets. Calli obtained from treatment with 2 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 mgL^{-1} and 2 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 tranformation including: 12 cm distance between the target tissue and stopping screen, 1100 psi of acceleration pressure, 1.0 μm of gold particle size, 27 Hg of vacuum pressure, two times of bombardment, spermidine as precipitation agent, 8 μg 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 μm of gold particle size, 24 Hg of vaccum pressure, one times of bombardment, spermidine as precipitation agent, 8 μg 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.

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)*

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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

mgL^{-1} BAP telah menghasilkan pokok dengan bilangan anak pokok tertinggi. Kalus yang dihasilkan daripada 2 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 mgL^{-1} atau 2 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. Keseluruhannya, 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, 1.0μm saiz zarah emas, 27 Hg tekanan vakum, dua kali pembedilan, spermidine sebagai agen pemendakan, 8 μg 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 μm saiz zarah emas, 24 Hg tekanan vacum, satu kali pembedilan, spermidine sebagai agen pemendakan, 8μg 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.