

**TRANSIENT TRANSFORMATION OF *ONCIDIUM* TAKA BY
PARTICLE BOMBARDMENT**

By

WEE CHIEN YEONG

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

May 2006

DEDICATED TO:

Dad (deceased), mum, brother, sister in law and Charles who always have faith in me,
my dear who is always there for me.

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the Degree of Doctor of Philosophy

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Chairman: Professor Maziah Mahmood, PhD

Faculty: Biotechnology and Biomolecular Sciences

Oncidium Taka was used in this study for its importance as cut flower in Malaysia floriculture industry. Protocorm like bodies (PLBs) that proved to have vigorous propagation and regeneration capacity were induced from shoot tips and flower buds in MS hormone free medium. Subsequently, callus induction by using different concentrations of auxins (2,4-D, dicamba, picloram, IAA and NAA) was investigated in dark condition. Picloram in a concentration of 40 μM was found to be the most effective for inducing callus from PLBs. However, the initiated callus was maintained and proliferated in medium consisted of combination hormones of 50 μM picloram and 20 μM kinetin. Both PLBs and callus were used as target tissues in the genetic transformation study.

Regeneration study of these calli was carried out in half strength MS medium under light condition. The calli regenerated by intervening PLB stage and the shoot primordial was developed as early as 44 days after culture. Besides that, media composition and different concentrations of BAP were examined to enhance plant regeneration and plant growth from PLBs. Half strength MS medium supplemented with full strength B5 vitamin, 3 % sucrose and 20 μ M BAP was found to be efficient for plant regeneration from PLBs. Apparently, better plant growth can be promoted in full strength MS medium supplemented with full strength B5 vitamin, 2 % sucrose and 20 μ M BAP.

The effectiveness of hygromycin as selective agents to inhibit growth of PLBs and callus was evaluated. Toxicity effect of hygromycin was found rapid and effective to kill the untransformed PLBs and callus in low concentration at 25 mg/L and 10 mg/L, respectively.

GFP as a non-destructive, early and rapid detection reporter system was used in this transformation studies. The p35S-*sgfp*-TYG-nos GFP plasmid was determined to give the highest transient expression in

both target tissues among five different plasmids evaluated. The fluorescent signals expressed with elevated level of green fluorescent on nuclei were observed using Leica Stereo-fluorescence System MZ FLIII with GFP 2 filter set that masked out chlorophyll. The highest numbers of transient expression achieved on day two post-bombardment for both target tissues.

The physical and biological parameters affecting DNA delivery, based on the highest scorable transient GFP expression and minimal tissue dislocation upon impact, were optimised. The physical parameters optimised in helium pressure, distance from stopping plate to target tissue, vacuum pressure, size of microcarrier, quantity of DNA and effect of CaCl₂ and Spermidine were found no different between PLBs and callus. As for biological parameters tested, PLBs in sizes of 5 – 6 mm had significantly higher transient expression compared to calli. Seven days old calli with one day pre-culture period and fourteen days old PLBs that pre-culture on the same day gave the highest transient expression. The quantity of DNA per bombardment used was 1.5 µg for both target tissues to achieve the highest transient expression.

The bombarded tissues were subsequently transferred to hygromycin containing medium for a minimum of six months selection. The survived and proliferated putative transformed tissues were subjected to molecular analysis. Insertion of the transgenes (*gfp*, *gus A*, *hpt II* and *chs*) into host genome was confirmed using PCR and Southern

Blot analysis. Transformation efficiency was determined at 1.5 % to 1.67 % and 1.67 % to 2.5 % when using PLBs and callus, respectively as target tissue. In addition, PCR analysis showed co-transformation frequency of the un-linked genes from different plasmids was 40 % to 67 %.

**Abstrak tesis yang dikemukakan kepada Senat Universiti Putra
Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah**

**TRANSFORMASI TRANSIEN *ONCIDIUM* TAKA SECARA
LEDAKAN PARTIKEL**

Oleh

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Oncidium Taka digunakan sebagai bahan penyelidikan dalam kajian ini kerana kepentingnya sebagai bunga keratan dalam industri floral kultur di Malaysia. Protocorm like bodies (PLBs) yang dibukti mempunyai kapasiti propagasi dan regenerasi dinamik diinduksi daripada pucuk dan kudup bunga dalam media MS tanpa hormon. Induksi kalus menggunakan lima jenis hormon (2,4-D, dicamba, picloram, IAA dan NAA)

dalam pelbagai kepekatan diuji dalam keadaan gelap. Hormon picloram dalam kepekatan 40 μM didapati paling efektif untuk induksi kalus daripada PLBs. Kalus dikekalkan dan dilipat-gandakan dalam media mengandungi kombinasi hormon 50 μM picloram dan 20 μM kinetin. Kedua-dua PLBs dan kalus digunakan sebagai tisu sasaran untuk kajian genetik transformasi.

Penyelidikan regenerasi kalus telah dijalankan dalam setengah kekuatan media MS tanpa hormon bawah keadaan bercahaya. Kalus digenerasi melalui peringkat PLB dan membentuk pucuk primordia seawal 44 hari lepas kultur. Selain daripada itu, komposisi media dan kepekatan BAP diuji untuk mempercepatkan regenerasi dan tumbesaran pokok daripada PLBs. Setengah kekuatan media MS dilengkapi dengan vitamin B5, 3 % sukrosa dan 20 μM BAP ditentukan efisien untuk regenerasi pokok. Tumbesaran pokok dapat dipertingkatkan dalam penuh kekuatan media MS yang dilengkapi dengan vitamin B5, 2 % sukrosa dan 20 μM BAP.

Kecekapan hygromycin sebagai agen pemilihan untuk merencat pertumbuhan PLBs dan kalus telah dinilai. Kesan toksik hygromycin terhadap PLBs dan kalus adalah cekap dan efektif berdasarkan keupayaannya untuk membunuh tisu-tisu sasaran dengan kepekatan yang rendah, 25 mg/L (PLBs) dan 10 mg/L (kalus).

GFP sebagai sistem pelapor yang membolehkan pengesanan awal dan cepat serta tidak memudaratkan digunakan dalam kajian transformasi ini. Plasmid GFP p35S-*sgfp*-TYG-nos ditentukan untuk menunjukkan paling tinggi pengekspresan sementara GFP pada

hari kedua selepas tembakan atas kedua-dua tisu sasaran. Pemerhatian isyarat floresen yang ekspresi dengan kelebihan floresen hijau pada nukleus dijalankan dengan menggunakan Leica Stereo-fluorescence System MZ FLIII yang dilengkapi dengan set penapis GFP 2.

Pengaruh faktor-faktor biologi dan fizikal dalam pemindahan DNA juga telah dioptimalkan berdasarkan kepada skor tertinggi ekspresi floresen dan minima kerosakan impak. Parameter fizikal yang dioptimalkan termasuk tekanan gas helium, jarak antara piring penghenti ke tisu sasaran, tekanan hampagas, saiz pembawa mikro emas dan kesan kalsium klorida dan spermidin terhadap “pemendapan” DNA dan pembawa mikro didapati tidak berbeza antara PLBs dan kalus. Sebagai parameter biologi yang diuji, 5 – 6 mm PLBs, umur tujuh hari kalus dengan satu hari pre-kultur dan umur 14 hari PLBs yang pre-kultur pada hari yang sama dan penggunaan 1.5 µg DNA setiap kali tembakan menunjukkan ekspresi sementara GFP yang paling tinggi.

Tisu-tisu sasaran didedahkan kepada pemilihan selepas tembakan sekurang-kurangnya enam bulan atas media pemilihan yang mengandungi hygromycin. PLBs yang rintang diasingkan dan ditumbuhkan menjadi pokok. Kehadiran transgen (*gfp*, *gus A*, *hpt II* and *chs*) dalam pokok-pokok demikian seterusnya dibuktikan melalui analisa PCR dan Southern Blot. Efisiensi transformasi telah ditentukan pada 1.5 % to 1.67 % dan 1.67 % to 2.5 % dengan menggunakan PLBs dan kalus sebagai tisu sasaran masing-masing. Tambahan lagi, analisis PCR menunjukkan frekuensi co-transformasi untuk gen-gen dari plasmid berlainan adalah antara 40 % hingga 67 %.

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I certify that an Examination Committee has met on 10 May 2006 to conduct the final examination of Wee Chien Yeong on her Doctor of Philosophy thesis entitled “Transient Transformation of *Oncidium* Taka by Particle Bombardment” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

WEE CHIEN YEONG

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