

Rakchanok Koto 2006: Transformation of *CPACO* Antisense into *Dendrobium* Orchid.  
Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology,  
Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Sermsiri Chanprame,  
Ph.D. 152 pages.  
ISBN 974-16-2739-4

*Dendrobium* 'Bom17' and *Dendrobium* 'Earsakul' were used for the transformation of *ACO* antisense via *Agrobacterium tumefaciens*. The recombinant plasmids, pCAMBIA 1301a*ACO1* and pCAMBIA 13121a*ACO2* were constructed to contain reverse orientation of *CPACO1* or *CPACO2* cDNA from papaya and *hygromycin phosphotransferase (hpt)* as the plant selectable marker gene under the control of 35SCaMV promoter. Each plasmid was transferred to *A. tumefaciens* strain AGL-1 by electroporation technique.

For the transformation experiment, 2 mm thick of PLBs derived transversely thin cell layers (tTCLs) were sonicated 6 seconds for wounding and were pre-cultured in VW liquid medium supplemented with 15 % coconut water and 1 % sucrose for three days. The tTCLs were then immersed in the suspension of *A. tumefaciens*. ( $5 \times 10^8$  cell/ml,  $OD_{600} \approx 1$ ) in VW liquid medium, for 60 min. For co-cultivation, the tTCLs were transferred onto fresh VW solid medium supplemented with 200  $\mu$ M acetosyringone for 2 days. The elimination of *Agrobacterium* and the selection of transformants were performed in two steps, solid and liquid medium. Firstly, the infected tTCLs were selected on VW solid medium supplemented with 30 mg/l hygromycin and 250 mg/l cefotaxime for 1 month and then transferred into VW liquid medium supplemented with the same concentration of both antibiotics for 2 months. For plantlets regeneration, the PLBs derived from served tTCLs were cultured on VW solid medium supplemented with 15 % homogenated potato 1 % sucrose and 0.2 % activated charcoal. The above procedures yielded 7 putative transformed lines of *Den.* 'Bom17' and 3 putative lines of *Den.* 'Earsakul' when transformed via antisense *CPACO1* construct while in the antisense *CPACO2* construct 4 and 3 putative lines were obtained from these 2 orchid cultivars, respectively.

All of putative transformants were subjected to PCR analysis of *hpt* gene and the result demonstrated the present of 800 bp of *hpt* gene in all transformants. The Southern blot analysis of *hpt* gene revealed the 2-4 copies of *hpt* gene incorporated into the *Dendrobium* genome. ACC oxidase activities and ethylene production measurements were studies on young leave of the transgenic plants and the results indicated that five transgenic lines had lower level of both enzyme activity and ethylene production than that of untransformed line.

Rakchanok Koto

Student's signature

Sermsiri Chanprame 30 / 10 / 06  
Thesis Advisor's signature