

Benjawan Suwannate 2009: Transformation of Ethylene-Related Genes into *Dendrobium* 'Pompadour' Using pMAT21 Vector. Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Ms. Orawan Chatchawankanphanich, Ph.D. 140 pages.

Parameters affecting gene transformation into *Dendrobium* 'Pompadour' by pMAT21 vector containing *gus* gene as reporter gene and *ipt* gene as selectable gene were studied. The optimum conditions were used of *Agrobacterium* strain EHA105 at concentration of  $\sim 4.1 \times 10^7$  cfu/ml, co-cultivation between *Agrobacterium* and PLBs added with celite 4.5 g/l by vortex mixer for 30 min and use of cefotaxime 200 mg/l for growth suppression of *Agrobacterium* after transformation. In this study, ethylene-related genes which are *ACS* and *ACO* genes were transformed into *Dendrobium* 'Pompadour' by pMAT21 vector to generate marker free transgenic orchid. Both *ACS* and *ACO* genes were divided into 3 regions including 5' region, central region and 3' region that was named as *ACSA*, *ACSB*, *ACSC* for *ACS* gene and *ACOA*, *ACOB* and *ACOC* for *ACO* gene, respectively. Each region was cloned into pMAT21 vector in antisense orientation for gene transformation into orchid by *Agrobacterium*. The transformed PLBs developed extreme shooties within 3-4 months after transformation. Then, normal shoot developed from shooties within 6-8 months after transformation. From Southern blot analysis of normal shoots, it showed that five and two putative transgenic orchids transformed with AS-*ACSB* and AS-*ACOB* contained only one copy of transgene, respectively. While, orchids transformed with AS-*ACOA* contained 2-4 copies of transgene. All of these putative transgenic orchids were free of marker gene. From analysis of gene expression at RNA level, two transgenic lines of orchid transformed with AS-*ACSB* which are line AS-*ACSB*-203A and line AS-*ACSB*-208A showed high level of expression of antisense *ACSB*, but expression of endogenous sense *ACS* was low. The results were the same in two transgenic lines of orchid transformed with AS-*ACOB* which are line AS-*ACOB*-10B and line AS-*ACOB*-11B. Therefore, transformation with AS-*ACS* or AS-*ACO* cloned in pMAT21 vector could produce marker-free transgenic orchids containing AS-*ACS* or AS-*ACO* transgenes.

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Thesis Advisor's signature

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