

Bodin Phadungsawat 2015: Transformation of *crtW* Gene for Astaxanthin Production in Tobacco, Petunia and Calibrachoa Using *Agrobacterium tumefaciens*. Master of Science (Horticulture), Major Field: Horticulture, Department of Horticulture. Thesis Advisor: Associate Professor Thunya Taychasinpitak, M.S. 63 pages.

Agrobacterium-mediated gene transfer method for transferring *crtW* gene was investigated in tobacco (*Nicotiana tabacum*), *Petunia hybrida* and *Calibrachoa × hybrida* by using *Agrobacterium tumefaciens* strain EHA105, harboring a plasmid containing the *crtW* gene from *Brevundimonas* sp. strain SD212 under 35S promoter with kanamycin resistant gene, used as a selectable marker. Co-cultivation was performed by soaking leaf disks of each plant species in *A. tumefaciens* solution with 20 ppm acetosyringone and cultured on MS medium containing 20 ppm acetosyringone for three days in the dark, and selection was carried out on selective MS medium containing 200 ppm kanamycin. Inoculated leaf explants of tobacco were regenerated to whole plants, whereas inoculated leaf explants of Petunia and Calibrachoa were produced putative transformed callus tissue that demonstrated orange color. Transformed tobacco plants showed astaxanthin accumulation in leaf, stem, sepal, corolla tube, glandular hair, and nectary. TLC analysis revealed astaxanthin accumulation in the transformed tobacco plants (leaf, sepal, corolla tube, glandular hair, and nectary) and the transformed callus tissues of Petunia and Calibrachoa. HPLC analysis indicated that the transformed tobacco leaves contained astaxanthin 0.496 and 0.383 mg/g FW. The mean of chlorophyll *a*, chlorophyll *b* and total carotenoids content determined by spectrophotometry showed significantly difference between transformed and non-transformed tobacco leaves that chlorophyll *a*, chlorophyll *b* and total carotenoids content in transformed tobacco leaves was higher than non-transformed tobacco leaves. Presences of *crtW* gene in the transformed tobacco leaves, Petunia and Calibrachoa calluses were validated by PCR, and results from RT-PCR confirmed *crtW* expression in the plant tissues.

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Thesis Advisor's signature

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