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The Thesis Submitted for the Degree of Ph.D. of Science

Plant Breeding and Biotechnology

Change in petal color of African violet (*Saintpaulia ionantha*) via genetic manipulation of genes involved in pigment biosynthetic pathway

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Abstract

Generating novel ornamental cultivars with different flower colors is one of the major goals in the industry of flowers and ornamental plants. African violet (*Saintpaulia ionantha)* is commercially well-known and available in different colors except for the yellow flower. The present research aimed to change in petal color of A. violet via genetic manipulation of genes involved in pigment biosynthetic pathway aurone. First, in order to obtain the best combination for the growth and propagation of African violet *in vitro*, the effect of different explants of four African violet cultivars was evaluated on MS medium containing different concentrations of NAA+BAP and IBA+BAP hormones for direct regeneration. The results of this section showed that the leaf explant was the best sample and MS medium containing 1 mg/l^{-1} IBA+1 mg/l^{-1} BAP was the best combination for in vitro regeneration of all genotypes. Then, the gene encoding *chalcone 4'-O-glucosyltransferase* (*4'CGT*) and *aureusidin synthase* (*AS1*) were isolated from *A. majus* (yellow) using PCR, specific primers and cloned into pCAMBIA1304 and pBI121 expression vectors, respectively. The recombinant pCAMBIA1304+*AS1* and pBI121+*4'CGT* vectors were verified by Colony PCR, restriction enzyme cutting, sequencing, and submitting their alignment in the GeneBank database. Binary vectors were constructed and transformed together into *Agrobacterium tumefaciens* (strain *LBA4404*) by electroporation. They then were investigated using transient expression and stable transformation gene in the petals of *Saintpaulia*. In transient expression, *Agrobacterium*-mediated suspension carry gene construct was injected using a syringe at the base of the petals. Next, the expression of transferred genes (*4'CGT* and *AS1*) was studied by various analysis. Morphological results after 3 days clearly showed changing the color of white to yellow (pale yellow) petals. PCR analysis and observation with light microscopy confirmed gene expression and phenotypic changes, respectively. In stable transformation, inoculation of leaf explants was performed with *Agrobacterium* carry gene structure and transgenic plants were also regenerated from inoculation medium on selective culture media containing kanamycin and cefotaxime antibiotics. Finally, 15 positive transgenic plants were obtained from 60 leaf explants through stable transformation via 20 to 30% transformation efficiency with yellow flowers indicating the presence of both genes, and all transgenic plants were kept in the greenhouse. The results of morphological investigation of transgenic plants showed that most of the petals were completely yellow. Nevertheless, no differences in petal coloration were observed between not-transformed (NT) plants.Transition accuracy, expression and integration of genes were confirmed in T0 transgenic plants by PCR, Southern blot, RT-PCR and qRT-PCR. Transgenic plants were examined using a light microscope and HPLC-DAD-MSn to change the cross-sectional color of the petals and to identify the *Aureusidin 6-O-glucoside* composition, respectively. In general, in the first, color changing and expression of genes were confirmed by transient expression and through morphological, molecular and microscopic analyzes in transgenic plants; that was a simple method and low-cost for effective analysis of genes in limited-time. Consequently, the creation of the aurone biosynthesis pathway was achieved only by co-expression transfer of genes (*4'CGT+AS1*) in the conversion of *Saintpaulia* petals from white to yellow. These results can provide an effective and efficient strategy to create a new yellow color in ornamental plant species that lacks this type of color.

Key words: Anthocyanin, *Saintpaulia ionantha*, Flower color, Tissue culture, Genetic engineering.