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Combinatorial expression of transcription factor genes *B-Peru* and *mPAP1* enhances anthocyanin accumulation in transgenic *Petunia hybrid*

Trinh Ngoc Ai[a,b], Muthukrishnan Arun[a], Aung Htay Naing[a], Sun-Hyung Lim[c], Chang Kil Kim[a,*]

[a] Department of Horticultural Science, Kyungpook National University, Daegu 702-701, South Korea
[b] School of Agriculture and Aquaculture, Tra Vinh University, Tra Vinh, Vietnam
[c] National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, South Korea

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ABSTRACT

The present study aimed to determine the role of transcription factors (bHLH and MYB) in enhancing anthocyanin production in *Petunia* 'Mirage rose'. Initially, we optimized an *Agrobacterium*-mediated transformation protocol to over-express transcription factors (*B-Peru*, *mPAP1*, and *B-Peru* + *mPAP1*) in *Petunia.* Phosphinothricin (PPT) concentrations of 0.5, 1.0, and 1.5 mg l^{-1} were found to be ideal for the selection and regeneration of transformed shoots at different developmental stages. Clavamox (250 mg l^{-1}) efficiently eliminated *Agrobacterium* after co-cultivation (2 days) and favored maximum shoot regeneration. In addition, incubation for 30 min in *Agrobacterium* suspension increased the number of transformed cells and resulted in improved regeneration in the selection medium. The transformed plants were successfully developed through a direct organogenesis system. The transformed plants were selected using BASTA® and the presence of transgenes was assessed using PCR. Visible anthocyanin accumulation was evident only in plantlets (shoots, stem, leaves, and roots) carrying the gene combination *B-Peru* + *mPAP1*. The expression of biosynthetic genes involved in the flavonoid pathway was analyzed using quantitative real-time PCR. Expression levels of *PAL*, *CHS*, *CHI*, *F3H*, *DFR*, and *ANS* were higher in the young and mature leaves of plants transformed with *B-Peru* + *mPAP1* compared to those transformed with *B-Peru*, *mPAP1*, and/or non-transformed plants. Furthermore, the highest level of anthocyanin was recorded in the leaves, stem, and roots of plants transformed with *B-Peru* + *mPAP1*. These results validate the combinatorial requirement of *B-Peru* and *mPAP1* to enhance anthocyanin content in *Petunia hybrida* 'Mirage rose'.

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1. Introduction

Ornamental plants with novel/improved flower color and fragrance are of great importance in the horticulture and industrial sectors. However, these characteristics are extensively influenced by environmental factors (Zhao and Tao, 2015). For instance, elevated temperature was found to significantly reduce floral pigmentation in *Petunia* (Shvarts et al., 1997), *Chrysanthemum* (Nozaki et al., 2006), rose (Dela et al., 2003), and oriental lily (Lai et al., 2011). This deleterious effect could considerably decrease the market value of flowering plants. Anthocyanins are the most diverse group of natural plant pigments that are responsible for the blue, purple, red, and orange colors in plants (He et al., 2010). Enriching plant anthocyanins through the use of metabolic engineering could reduce the adverse effects of various stresses on flower fading. In addition, improved anthocyanin accumulation in ornamental plants could result in the development of flowers with improved colors that are of commercial importance. *Petunia* (*Petunia* × *hybrida*) is a popular ornamental bedding plant of high commercial interest, which is widely cultivated throughout the world. Currently, numerous cultivars of petunia with diverse flower colors and growth habits are available in commercial floricultural industries. Hence, improving the anthocyanin content by manipulating flavonoid biosynthesis in plants such as *Petunia* is a promising research priority that will help to maintain its economic values.

* Corresponding author. Fax: +82 53 950 5722.
E-mail addresses: aunghtaynaing2005@gmail.com (A.H. Naing), ckkim@knu.ac.kr (C.K. Kim).

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Fig. 1. *Agrobacterium*-mediated genetic transformation and transfer of *B-Peru*, *mPAP1*, and/or *B-Peru + mPAP1* into *Petunia* 'Mirage Rose' to enhance anthocyanin content. (a) Multiple shoots induced from non-infected leaf explants after 30 days of culture on SIM. (b–d) Multiple shoots induced from infected leaf explants after 30 days of culture on SIM containing 250 mg^{-1} Clavamox and 0.5 mg^{-1} PPT. (e) Elongated shoots in SEM after 21 days of culture. (f–h) Elongated shoots after 21 days of culture in SEM supplemented with 250 mg^{-1} Clavamox and 1.0 mg^{-1} PPT. (i) Rooted shoot on RM after 21 days of culture. (j–l) Rooted shoots in RM amended with 1.5 mg^{-1} PPT after 21 days of culture. (m–p) Fertile *Petunia* plant grown in a greenhouse (m NT plant; **n–p** Putative transformants).

Transcription factors belonging to two classes, namely the MYB family and the basic helix–loop–helix (bHLH) family, along with WD40 domain-containing proteins, regulate the expression of biosynthetic genes (*PAL*, *CHS*, *CHI*, *F3H*, *DFR*, and *ANS*) in the anthocyanin pathway (Koes et al., 2005). Transcription factors required for the regulation of anthocyanin biosynthesis have been reported

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