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THE MOLECULAR BREEDING OF ORNAMENTAL FLOWERING PLANTS; FLOWER COLOR MODIFICATION OF *Torenia hybrida*

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White and blue/white varieties of Torenia hybrida cv. Summerwave (SWB) were successfully obtained from the blue variety of by cosuppressing gene expression of two of the enzymes involved in anthocyanin biosynthesis; chalcone synthase (CHS) and dihydroflavonol 4-reductase (DFR). Such molecular breeding is the only precise and efficient way to widen the flower color variation of SWB due to its male and female sterility. Flower color and the degree of suppression varies depending on the transgenic lines. The dorsal and ventral petal lobes and corolla tube consistently lose anthocyanins prior to lateral petal lobes. A pink variety was also obtained by cosuppressing the flavonoid 3'5'-hydroxylase (F3'5'H) gene. Yellow torenia was obtained from T-33, an in-house cultivar that contained both carotenoids and anthocyanins, by blockage of anthocyanin biosynthesis with cosuppressing CHS or DFR genes.

INTRODUCTION

Molecular breeding is a powerful method of plant breeding because it can change a specific characteristic of a plant without changing other desirable characteristics. Flower color is predominantly influenced by two types of pigments; flavonoids and carotenoids. The anthocyanin biosynthetic pathways of many plants have been well established (1) and conserved (Fig. 1). Chalcone synthase (CHS) and dihydroflavonol 4-reductase (DFR) are the first specific enzymes in flavonoid and anthocyanin biosynthesis, respectively. The presence of flavonoid 3'5'-hydroxylase (F3'5'H), cytochrome P-450 (2), is almost critical to the production of blue to purple anthocyanins. The flower color is reddish in its absence.

Torenia (Torenia fournieri), belonging to Scrophulariaceae, is one of the most important bedding plants. Suntory Ltd. successfully developed a new type of torenia cultivar Summerwave (T. hybrida), which has many characteristics superior to common torenia cultivars. Summerwave originally had one flower color (Blue, SWB). Because its male and female sterility predicted the difficulty of conventional breeding, a molecular approach was applied to widen its flower color variation.

Gene silencing is a common phenomenon in transgenic plants and it affects both transgenes and endogenous genes (3). Both constitutive

expressions of a sense (4) and antisense (5) petunia CHS gene in transgenic petunia results in an altered flower pigmentation. The flower color patterns varied significantly among inter- and intra-transgenotes. The suppression pattern and stability were also varied. Although suppression of anthocyanin biosynthesis has been reported in some species (6), none of them were seemed to have commercial value. In this report, we made transgenic torenia plants harboring sense cDNAs encoded CHS, DFR and F3'5'H enzymes isolated from torenia SWB and observed flower color change with new and simple patterns.

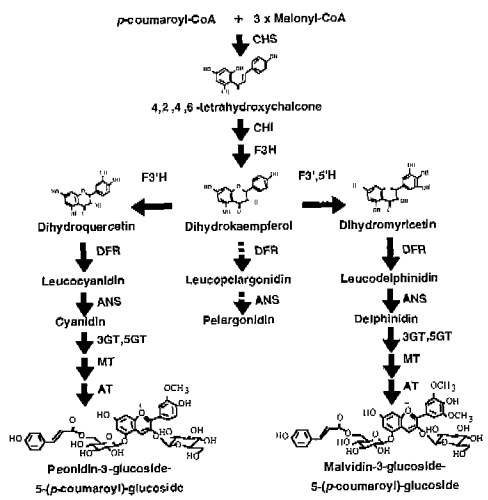

Fig. 1. Schematic representation of the biosynthesis pathway for anthocyanin pigment production in Torenia hybrida cv. Summerwave Blue. The reactions shown by dotted arrows do not naturally occur in SWB. CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavanone 3-hydroxylase; F3'H: flavonoid 3'-hydroxylase; F3'5'H: flavonoid 3'5'-hydroxylase; DFR: dihydroflavonol 4-reductase; ANS: anthocyanidin synthase; 3GT: UDP-glucose:flavonoid-3-O-glucosyltransferase; 5GT: UDP-glucose:flavonoid-5-O-glucosyltransferase; MT: anthocyanin O-methyltransferase; AT: anthocyanin acyltransferase.

MATERIALS AND METHODS

Plant material and genetic transformation. Torenia hybrida cv. Summerwave Blue, commercialized by Suntory Ltd., and T. hybrida inbred line T-33 were grown under standard greenhouse conditions. In vitro cultured shoots used for transformation were maintained on a MS agar medium. SWB and T-33 were transformed using the method of Agrobacterium tumefaciens mediated transformation of T. fournieri (7). Pigment analysis. Extraction and HPLC analysis of anthocyanin were used as described in Tanaka et al (8). Molecular analysis. A SWB petal cDNA library constructed in the UNI-ZAP (Stratagene) was screened with rose CHS (unpublished result), DFR (D85102) and gentian F3'5'H (D85184) and their counterpart cDNAs were obtained.

RESULTS AND DISCUSSION

Figure 1 shows the anthocyanin biosynthetic pathway which was determined on the basis of structural analysis of anthocyanins in the petals and molecular cloning of many of the genes involved in the pathway. It is known that the pathways to anthocyanidin-3-glucosides are well conserved and that further modification of them is variable among plant species. Malvidin-3-glucoside-5-(p-coumaroyl)-glucoside, derived from delphinidin, was the main product and occupied over 80 % of the total anthocyanins in the petals of SWB. Torenia CHS (AB012923), DFR (AB012924) and F3'5'H (AB012925) cDNAs were isolated and sequenced. For the reduction of these enzyme activities we generated transgenic plants with cosuppression binary constructs.

Torenia SWB was transformed with the binary vector containing whole torenia CHS sense cDNA or partial torenia DFR cDNA lacking 300 bp in the 5' region under the control of the enhanced CaMV35S promoter (9). Many of the

transgenic plants had petals with reduced amounts of anthocyanins. Both transgenic plants of SWB CHS and SWB DFR showed the same phenotypic pattern. The degree of the reduction varied with each transgenic plant, and the petals had a range of colors from blue, to blue and white, and pure white. Interestingly, anthocyanin production was more consistently suppressed in the dorsal and ventral petal lobes, and the corolla tube, than in the lateral petal lobes. The degree of suppression of petal colors was classified into six groups, A to F described in Table 1. Jorgensen et al (10) reported that 63% and 11 % of the 185 sense CHS transgenic plants studied had corollas with altered colors and white flowers, respectively. SWB CHS transgenic plants showed phenotypic change with a frequency similar to petunia (Table 1). Interestingly, variegated pigmentation was not observed in SWB. The frequency of CHS suppression with sense CHS gene was very low and no variegated phenotypes were observed in chrysanthemum (11), rose and carnation (6). Sense suppression phenomena may be different among plant species.

Table 1. Frequency of flower color change of transgenic plants. a: pure white; b: pale yellow; c: pure pink. In the case of SWB CHS and DFR transformants, phenotype A: a small region of pale blue or white color specifically in the inside of the dorsal and ventral petal lobes, B: a white color region was larger in the dorsal and ventral petal lobes, and the corolla tube was colored pale blue, C: a blue color in the lateral petal lobes and was almost completely white in other parts, except for a little pale blue color still visible in the corolla tube, D: the only lateral petal lobes showing blue color, E: a white color in all regions, with the exception of the tip of the lateral petal lobes, F: a pure white color, UC: unchanged flower color, IC: irregularly changed flower color.

Host	Transformed	No. of Trnsgenic Plants								
Plant	Gene	Total	UC	IC	A	B	C	D	E	F
SWB	CHS	121	91	0	4	0	3	0	4	19[a]
SWB	DFR	115	63	1	9	4	8	12	10	8[a]
T33	CHS	96	64	0	10	9	10	2	0	1[b]
T33	DFR	80	46	0	12	4	9	3	4	2[b]
SWB	F3′5′H	105	88	1	0	3	0	9	0	4[c]

In order to study the correlation between gene suppression and anthocyanin accumulation, CHS and DFR transcripts in the petals were analyzed. Petals in phenotype A-B had more mRNA than the control line expressing GUS protein. As with phenotype A to F, messages were decreased and finally phenotype F rarely showed the signal. In the flower of SWB, anthocyanin pigments accumulated only in the epidermal cell layer of the petal. Many genes of the flavonoid biosynthesis enzyme were expressed specifically in this cell layer (12). The transgene driven by the enhanced CaMV35S promoter might be expressed in all cell layers of the petals. Most of the message detected in our results may have been derived from the transgene. In phenotype F, expression of the endogenous CHS gene in epidermal cells should be very low. We concluded that the high level of transcription of the transgene caused the suppression of endogenous mRNA, as the result of cosuppression.

The binary vector contained the partial torenia F3′5′H cDNA containing only about 600 bp in

the 3′ region with sense direction was used to transforme SWB. Some transgenic plants changed flower color from blue to pink. This change was consistently observed to be stronger in the dorsal and ventral petal lobes and the corolla tube compared to the lateral petal lobes seen in SWB CHS and SWB DFR plants. The plants showing intermediate phenotypes had pink colored flowers only in the corolla tube. No anthocyanins derived from delphinidin were produced in completely pink flowers. An increased amount of cyanidin and peonidin and a small amount of pelargonidin were observed. The suppression of the F3′5′H gene clearly caused a change from the delphinidin pathway to the cyanidin pathway in anthocyanin biosynthesis (Fig. 1).

Torenia T-33 petals, containing both anthocyanins and carotenoids, were dark brown and purple. To make yellow flower varieties from T-33, by specific suppression of biosynthesis of anthocyanins, T-33 was transformed with CHS or DFR cDNA with same methods used with SWB. About 40 % of the transgenic plants changed flower color (Table 1). The patterns of anthocyanin reduction in each construct were observed to be same as SWB/CHS and SWB/DFR, except for the pale yellow lines (phenotype F) which were isolated with a lower frequency than SWB (Table 1). Further molecular breeding to widen flower color of SWB is in progress.

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