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Review

Torenia fournieri (torenia) as a model plant for transgenic studies

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Abstract Torenia is an annual plant of the family Scrophulariaceae that is used as an ornamental summer bedding plant. Torenia is also an experimental plant with several useful characteristics, *i.e.*, ease of genetic transformation, ability to differentiate adventitious structures, protruding embryo sac, and capacity for *in vitro* flowering. Genetic transformation of torenia was first reported in 1995, and it has been used in various transgenic studies. Torenia is a useful model plant for transgenic studies on ornamental plant characteristics such as the color, shape, and longevity of flowers. In this paper, the characteristics of torenia as an experimental plant and the transgenic studies performed with torenia are reviewed.

Key words: Flower color, ornamental plants, transformation, *Torenia fournieri.*

Recently, transgenic plants have been commercialized in various crops, especially *Glycine max* (soybean), *Zea mays* (corn), *Brassica napus* (oilseed rape), and *Gossypium hirsutum* (cotton). The major target characters have been resistances to herbicides and insects. Among ornamental plants, lines of transgenic *Dianthus caryophyllus* (carnation) with a bluish color have been commercialized, and bluish *Rosa hybrida* (rose) lines are to be released soon. In addition to these commercial uses, transgenic plants are commonly used in many kinds of fundamental studies, including plant physiology and genetics. For example, *Arabidopsis thaliana*, *Nicotiana tobacum* (tobacco), *Solanum lycopersicum* (tomato), *Oryza sativa* (rice), and *Lotus japonicus* are relatively popular model plants used in fundamental studies.

Genetic transformation of *Torenia fournieri*, an ornamental plant, was first reported by Aida and Shibata (1995), and since then, it has been used for various transgenic studies. Torenia is easy to transform and, as such, is useful as a model plant, especially for transgenic studies on ornamental characteristics such as flower color and shape. In this paper, the characteristics of torenia as an experimental plant and the transgenic studies performed with torenia are reviewed for promoting torenia as a model plant in a broad range of future studies.

Distribution and horticultural use of torenia

The genus *Torenia*, which belongs to the family Scrophulariaceae, contains about 40 species, and almost all of them occur in tropical and subtropical Asia and Africa (Huxley et al. 1997). Torenia is a common horticultural name for several species of the genus *Torenia* (for example, *T. fournieri*, *T. concolor*, *T. baillonii*, and their hybrids). In this article, "torenia" refers to *T. fournieri*, the most important ornamentally used species in the genus. Torenia, an annual from tropical Indochina, is used especially as a summer bedding plant (Figure 1). However, only erect type torenia with violet or white flower color had been available until the 'Crown' series, that contained pink, blue, and reddish-purple flower color lines, was released in 1988 from PanAmerican Seed (West Chicago, IL, USA). Subsequently, creeping type interspecific hybrids (*T. fournieri*×*T. concolor*), the 'Summerwave' series were released in 1995 from Suntory Ltd. (Osaka, Japan). Currently, *T. baillonii*, having a yellow corolla, is becoming popular. Based on the release of these cultivars, *Torenia* is becoming increasingly popular for horticultural use in Japan and around the world.

Torenia as an experimental plant

Torenia is known as an experimental plant in addition to its horticultural use. Based on reports indicating its notable ability to differentiate adventitious structures from tissues, torenia has been used for morphogenetic response studies (Tanimoto and Harada 1990). Also, its mechanism of anthocyanin synthesis and degradation of chlorophyll has been analyzed using regenerated shoots (Nagira et al. 2006). In addition, torenia has been used in the analysis of fertilization of flowering plants, because of its protruding embryo sac. Remarkable results have been obtained on plant fertilization with torenia

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(Higashiyama et al. 2001, 2006). Besides the aforementioned studies, torenia has been used for experimental genetic transformation purposes as described in the following sections.

Torenia plants flower and produce seeds under *in vitro* culture conditions if artificial pollination is performed (Aida and Shibata 2001), an important feature of experimental plants. Special equipment, such as an enclosed greenhouse, is not essential to observe flower characteristics and to obtain offspring. However, repeated self-pollination may lead to inbreeding depression because torenia has a nature of outcrossing. The 'Summerwave' interspecific hybrids have sterile male and female reproductive organs; however, plants in the genus *Torenia* are easily propagated by vegetative cutting, which is another useful feature in experimental studies of torenia.

The chromosome number of torenia is 2n=18 and genome size is estimated at 1.71×10^8 bp (Kikuchi et al. 2006). This relatively small genome size is comparable to that in *Arabidopsis thaliana* (1.57×10^8 bp), but much smaller than that of *Lotus japonicus* (4.69×10^8 bp) or *Oryza sativa* (4.89×10^8 bp), suggesting that torenia is a practical experimental plant for molecular studies.

Transformation system of torenia

Agrobacterium-mediated transformation of torenia has been reported (Aida and Shibata 1995, 2001), and a revised transformation system has been developed as follows. Leaf segments of *in vitro* plants were co-cultured with *A. tumefaciens* having a vector plasmid for 7 days at 22°C under dark condition on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) containing $1\,mg\,l^{-1}$ BA, $1\,mg\,l^{-1}$ IAA, and $100\,\mu M$ acetosyringon. Subsequent culture at 25°C under a 16-h photoperiod regime with fluorescent light on an MS medium containing $1\,mg\,l^{-1}$ BA, $300\,mg\,l^{-1}$ carbenicillin, and a selective agent ($300\,mg\,l^{-1}$ kanamycin or $20\,mg\,l^{-1}$ hygromycin), which allowed selection of transformants. Leaf segments of shoots that regenerated from green compact calli were cultured on the selection medium to observe resistance to the selective agent (leaf test). The segments from putative transformants regenerated shoots, while those from the wild-type control and from non-transformed escapees failed to survive on the medium. Using this method, transformants occur at approximately 5% frequency (transformants/explants). The cauliflower mosaic virus (CaMV) 35S promoter is the most popular one for transgene expression in plants and it works well in torenia. In addition, a translational enhancer derived from the 5′-untranslated region of the tobacco *alcohol dehydrogenase* gene has enhanced transgene expression by 50–100 times in torenia (Aida et al. 2008). Transgenic torenia plants were also obtained using the selectable marker gene, *phosphomannose isomerase*, which encodes the enzyme phosphomannose isomerase to enable selection of transformed cells on media supplemented with $20\,g\,l^{-1}$ mannose and $5\,g\,l^{-1}$ sucrose as carbon sources (Li et al. 2007). Their reported transformation efficiency ranged from 7% to 10%.

Mendelian inheritance of introduced transgenes from primary transformants to their progenies has been demonstrated (Aida and Shibata 1995). Although developmentally regulated transgene silencing was reported in one experiment (Aida and Shibata 1998), expression of transgenes seemed to be generally stable. The relatively easy transformation system of torenia makes the plant valuable as a tool for fundamental studies. In the following sections, results with transgenic torenia plants are reviewed, with emphasis on ornamental characteristics: flower color, flower shape, and flower longevity.

Modification of flower color

Flower color in torenia has been changed by transferring the *chalcone synthase* (*CHS*) or *dihydroflavonol 4-reductase* (*DFR*) genes in sense or antisense orientations (Aida et al. 2000a; Figure 2). Transformants incorporating antisense transgene(s) tended to change to a uniformly light color over the whole corolla, while transformants having sense transgene(s) tended to show a greater degree of color lightening in the tube than in the lip. When anthocyanins in plants create complexes with copigments such as flavones and flavonols (copigmentation), the visible absorption maximum of the flowers is shifted so that it becomes longer, *i.e.*, the flowers look bluer. Torenia transformants with the antisense *DFR* gene produced bluer flowers than plants with the antisense *CHS* gene, and inactivation of the *DFR* gene caused accumulation of flavones, which might have acted as copigments and resulted in bluer torenia flowers (Aida et al. 2000b). The typical modified phenotype among torenia with an introduced antisense gene is a uniformly lighter-colored corolla. Of the 67 plants in which an antisense *CHS* gene was introduced, only a single line showed a wavy pattern on the flower lip. In flowers of this plant, the inner part of the corolla lip was pigmented more deeply than the outer part and formed a wave-like pattern that does not exist in normal cultivars. The segregation ratio of the flower color patterns of the offspring and a Southern blot analysis demonstrated that one of the two detected transgene loci may cause the wavy phenotype; the other locus was never associated with the wavy phenotype, but alone it could produce a typical antisense type pattern (Aida et al. 2001).

Suntory Ltd. developed and commercialized the 'Summerwave' series of interspecific hybrids. Numerous studies have been performed, mainly by researchers of

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Suntory Ltd, on modification of flower color by genetic transformation with 'Summerwave' as a model plant. Suzuki et al. (2000) produced white and blue/white varieties from the blue 'Summerwave' by suppressing *CHS* or *DFR* with its sense gene. A pink variety was obtained by suppressing the *flavonoid 3′,5′-hydroxylase* (*F3′,5′H*) gene, and a yellow variety was obtained from a cultivar that contained both carotenoids and anthocyanins by suppression of *CHS* or *DFR* genes. Ueyama et al. (2002) reported that a 'Summerwave' whose *F3′,5′H* expression had been suppressed was further transformed with the *flavonoid 3′-hydroxylase* (*F3′H*) sense gene. Some of the transgenic plants had an elevated amount of cyanidin-type anthocyanins and exhibited redder flower color. Suppression of the *flavone synthase II* (*FNSII*) gene successfully decreased the amount of flavones and increased the amount of flavanones, and yielded paler flower color. Fukusaki et al. (2004) demonstrated that the *CHS* gene was suppressed by RNA interference (RNAi) in 'Summerwave'. When the coding and the 3′-untranslated regions of the *CHS* gene were used as RNAi targets, the blue flower color changed to white and pale colors, respectively. They showed that gene-specific silencing was induced with a gene-specific sequence of the 3′-untranslated region among the *CHS* gene family. Nakamura et al. (2006) showed suppression of the *anthocyanidin synthase* gene using three methods: antisense suppression, sense suppression, and RNAi in transgenic 'Summerwave'. About half of the transgenic plants produced white flowers following RNAi suppression of the gene, while antisense and sense suppressions yielded few and no white flowers, respectively. The white flower color obtained by RNAi has been stable for three years in a greenhouse. Ono et al. (2006) demonstrated that the expression of *Antirrhinum majus chalcone 4′-O-glucosyltransferase* and *A. majus aureusidin synthase* genes resulted in the accumulation of aureusidin 6-*O*-glucoside in transgenic 'Summerwave'. Furthermore, their expression, combined with suppression of an anthocyanin biosynthesis gene (*DFR or flavanone 3-hydroxylase*) by RNAi, resulted in yellow flowers.

Figure 1. Flowering torenia. Torenia is popular, especially as a summer ornamental bedding plant.

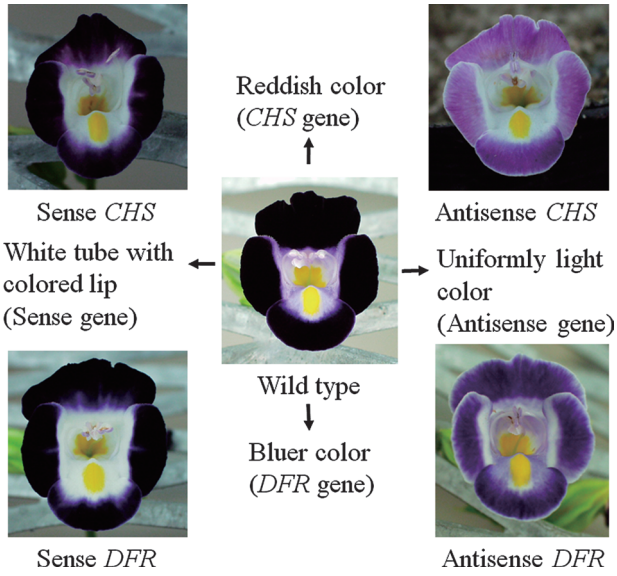

Figure 2. Flower color modification in transgenic torenia. Transformants incorporating antisense transgene(s) tended to change to a uniformly light color on the whole corolla (right-side images), while transformants having sense transgene(s) tended to show a greater degree of lightening in the tube than in the lip (left-side images). The torenia transformants with the antisense dihydroflavonol 4-reductase (*DFR*) gene produced bluer flowers (lower-right image) than plants with the antisense chalcone synthase (*CHS*) gene (upper-right image).

Torenia is an easy model plant to introduce transgene(s), and appears to easily change its flower color characteristics. Based on the results of the aforementioned studies, it is obvious that torenia is useful for examining flower color modification by genetic transformation.

Modification of flower shape

Flower shape is an important character in ornamental

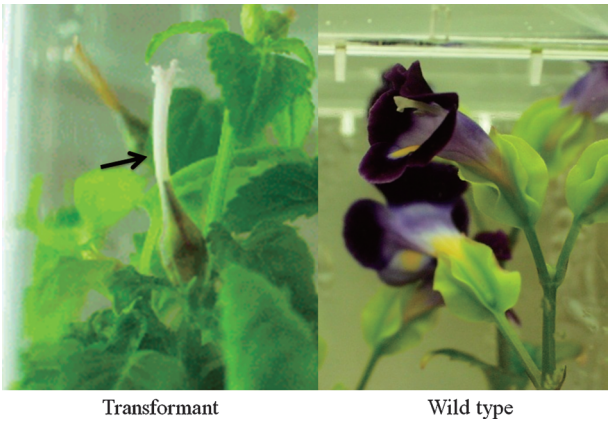

Figure 3. Flower shape modification in transgenic torenia. Constitutive expression of the C gene from *Rosa rugosa* changed torenia sepal (wild type) to carpeloid structure (transformant). Both plants flowered *in vitro*. Arrow indicates the carpeloid structure of a transformant.

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plants. The creation of new flower shapes has been a major breeding target. The ABC model (Coen and Meyerowitz 1991) and its modified version (Theißen 2001) are known to be applicable to a broad range of plants (Kim et al. 2005). The ABC model proposed that three functionally different genes, *i.e.*, A, B, and C, specified the four-whorl structure of flowers. Gene A is responsible for sepal development in the first (outermost) whorl. Genes A and B together specify the petals in the second whorl. Genes B and C determine the stamens in the third whorl, and gene C alone specifies the carpels in the fourth whorl (Coen and Meyerowitz 1991). Constitutive expression of the C gene from *Rosa rugosa* in torenia resulted in a carpeloid structure in place of sepals (Kitahara et al. 2004; Figure 3). Constitutive expression of *Antirrhinum majus* B genes *DEF* and *GLO* in transgenic torenia resulted in the conversion of sepals to petals (Dr. Takashi Handa, personal communication). These studies indicate that the functions of homeotic genes involved in flower organ development were maintained in torenia.

Application of a synthetic cytokinin, forchlorfenuron (CPPU), to inflorescences of torenia has induced flower shape modification, such as serrate petals, incised petals, a paracorolla, and an increased number of floral organs (Nishijima and Shima 2006). These morphological changes occurred systematically depending on the floral stage at the time of CPPU application. This indicates that torenia would be a useful plant for determining the relation between cytokinin and flower shapes.

Extension of flower longevity

In many plants, including torenia (Goto et al. 1999), flower senescence is triggered by a phytohormone ethylene. Ethylene biosynthesis starts with methionine, from which *S*-adenosylmethionine (SAM) is synthesized by the action of SAM synthase. SAM is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase, and ACC is converted to ethylene by the action of ACC oxidase (Bleecker and Kende 2000). The inhibition of ethylene biosynthesis is a way to delay flower senescence, and transgenic torenia plants with extended flower longevity were produced by introducing a fragment of the *ACC oxidase* gene in either sense or antisense orientations (Aida et al. 1998). Among the primary transformants, sense gene-introduced 8 plants and antisense gene-introduced 3 plants showed significantly greater longevity, *i.e.*, 2.7–7.1 days in the former and 2.5–2.7 days in the latter, respectively, than wild-type plants (2.0 days). Analysis of the offspring suggested that the introduced gene had been inherited, and that the extended flower longevity was linked to the existence of the gene.

Ethylene perception and signal transduction have been well established (reviewed by Chen et al. 2005), and suppression of ethylene perception or the signal transduction pathway has been reported for several ornamental plants. Addition of ethylene insensitivity by inhibition of ethylene perception and/or the signal transduction pathway is a practical method to delay senescence of flowers, and torenia would be useful for such flower longevity analysis.

Future prospects

As suggested in the above review, torenia is a useful model plant, especially for studies of ornamental characteristics (*i.e.*, flower color and flower shape) and for analysis of physiological mechanisms (*i.e.*, plant fertilization and morphogenesis). Recently, Narumi et al. (2008) showed that a gene silencing technology targeting transcription factor, Chimeric REpressor gene-Silencing Technology (CRES-T; Hiratsu et al. 2003), functioned in torenia. The CRES-T system could become a powerful tool for suppressing transcription factor genes in torenia. Sasaki et al. (2008) demonstrated that a combination of genetic transformation and heavy-ion beam irradiation was useful to shorten the required time for breeding in torenia, indicating that the risk–cost/benefit ratio of genetically modified ornamental plants could be reduced.

Based on the development of new technologies and the increasing accumulation of fundamental data, torenia is an attractive plant for fundamental studies. Thus, we anticipate that torenia will become more popular in both research fields and summer gardens.

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