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Original Paper

Utilization of a floral organ-expressing *AP1* promoter for generation of new floral traits in *Torenia fournieri* Lind

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Abstract To establish an efficient way to create novel floral traits in horticultural flowers, we have introduced many chimeric repressors of *Arabidopsis* transcription factors into torenia. Among them, we found a transgenic torenia exhibiting unopened flower buds and glossy dark green leaves with curled margins as a consequence of overexpression of *Arabidopsis MYB24* with a transcriptional repression domain (*MYB24-SRDX*). Petals inside the flower buds exhibited a distinct coloration pattern. To bring out this favorable petal trait without inducing the unfavorable phenotypes due to the constitutive expression of chimeric repressors by the cauliflower mosaic virus 35S (35S) promoter, we tested the ability of a floral organ-specific *Arabidopsis APETALA1* (*AP1*) promoter, which was found to be active in both petals and flower buds of torenia. As expected, *AP1* pro:*MYB24-SRDX* transgenic torenias resulted in the opening of flowers and a normal leaf phenotype. Furthermore, these *AP1* pro:*MYB24-SRDX* torenias exhibited wavy petals with a characteristic configuration. This is a good example of the utilization of a floral organ-specific promoter for creating distinct flower phenotypes without causing unfavorable morphological and physiological changes in other organs.

Key words: AP1, MYB24, promoter analysis, torenia, transcription factor.

Developments in genetic engineering have allowed the production of flowers with novel traits, such as blue carnations (Fukui et al. 2003) and blue roses (Katsumoto et al. 2007), which could not be generated by traditional breeding (Shibata 2008). Currently, these transgenic flowers are produced for commercial purposes in Japan. While the product life of horticultural flowers is generally short because of shifts in consumer preference (for review Ohtsubo 2011 in this issue), generation of novel floral traits using either genetic engineering or classical breeding takes a longer time and the production of desirable floral traits using either method remains difficult. Therefore, technological development is required to efficiently create numerous novel floral traits within a short time. As an approach to solve this problem, we screened transgenic torenias modified by chimeric repressors of *Arabidopsis* transcription factors to isolate novel floral traits and accumulate information on desirable floral traits (Mitsuda et al. 2008 and 2011 in this issue; Shikata et al. 2011 in this issue).

The chimeric repressors, in which transcription factors are fused to SRDX, dominantly interfere with the activity of target transcription factors and prevent the expression of their downstream genes, even if the transcription factors have functionally redundant endogenous counterparts (Hiratsu et al. 2003; for review Mitsuda and Ohme-takagi 2009). The strong gene-silencing system specific to transcription factors, designated chimeric repressor gene-silencing technology (CRES-T), has greatly contributed to the study of transcription factor functions not only in *Arabidopsis* (for review Mitsuda and Ohme-takagi 2009) but also in other plant species, such as rice (Mitsuda et al. 2006), tomato (Itkin et al. 2009), and torenia (Narumi et al. 2008; Sasaki et al. 2010). Furthermore, CRES-T has been utilized to create novel floral traits in various horticultural flower species including torenia (Mitsuda et al. 2008 and 2011 in this issue). To further efficiently create floral traits, a mixture of chimeric repressors of *Arabidopsis* transcription factors was collectively

Abbreviations: AP1, Arabidopsis APETALA 1; CRES-T, chimeric repressor gene-silencing technology; CT, collective transformation; GUS, β-glucuronidase.

This article can be found at http://www.jspcmb.jp/

introduced into *Agrobacterium* and used to co-transform torenia. This procedure is called the collective transformation (CT) system (Shikata et al. 2011 in this issue). This transformation resulted in the isolation of various types of flowers. However, utilization of the 35S promoter occasionally produced unfavorable phenotypes or growth inhibition in transgenic plants (Shikata et al. 2011 in this issue). For example, 35S:*MYB24-SRDX* transgenic torenia exhibited unfavorable phenotypes such as unopened flower buds and curled leaf margins (Shikata et al. 2011 in this issue). These problems are supposed to be caused by interference with paralogous transcription factors, which are expressed in developmental processes, such as the formation of leaves and calli (or adventitious shoot initiation). On the other hand, petal coloration changed in unopened flower buds of 35S:*MYB24-SRDX* torenia, suggesting that *MYB24-SRDX* was useful for changing petal traits. We therefore utilized an organ-specific promoter to express *MYB24-SRDX* instead of the 35S promoter to change floral traits without affecting leaf phenotypes.

MYB24 is a plant-specific R_2R_3-MYB transcription factor (Romero et al. 1998), which comprises a large transcription factor family (for review Mitsuda and Ohme-takagi 2009 and Dubos et al. 2010). MYB24 belongs to subgroup 19 of this family together with MYB21 (Stracke et al. 2001; Dubos et al. 2010). Both *MYB21* and *MYB24* are mainly expressed in flowers (Shin et al. 2002; Yang et al. 2007) and are induced by jasmonate in *Arabidopsis* (Mandaokar et al. 2006). The *myb24* mutant is phenotypically normal, while *myb24* mutation exacerbated *myb21* mutant phenotype that exhibited defects in jasmonate-mediated stamen development (Mandaokar et al. 2006). Furthermore, the *myb21 myb24* double mutant exhibits short petals and unopened flowers. A recent study has revealed that MYB24 acts downstream of MYB21 together with MYB108 in a transcriptional cascade that mediates stamen and pollen maturation in *Arabidopsis* (Mandaokar and Browse 2009).

In this study, we examined the ability of a combination of a floral organ-specific promoter and *MYB24-SRDX* to change floral traits without causing phenotypical changes in the other organs of torenia. Because the promoter of *Arabidopsis APETALA1* (*AP1*), which is expressed in the floral organs of *Arabidopsis* (Gustafson-Brown et al. 1994; Mandel et al. 1992; Urbanus et al. 2009), was also active in the floral organs of torenia, we used this promoter to express *MYB24-SRDX*. The resultant *AP1* pro:*MYB24-SRDX* transgenic torenias exhibited characteristic petal phenotypes without affecting the leaf phenotype. The possible use of floral organ-specific promoters in creating novel floral traits is further discussed on the basis of the results obtained using *AP1* pro:*MYB24-SRDX* torenias.

Materials and methods

Plant materials

Torenia (*Torenia fournieri* Lind. 'Crown Violet') and *Arabidopsis* (*Arabidopsis thaliana*, Col-0 accession) were used as plant materials. A 35S:*MYB24-SRDX* transgenic torenia was isolated in the study of Shikata et al. (2011 in this issue). This transgenic torenia contains a transgene encoding a chimeric repressor of *Arabidopsis MYB24* under the control of the 35S promoter. Plant materials were aseptically maintained in a plant box supplemented with 1/2 Murashige and Skoog medium containing 0.32% gellan gum. These torenias were vegetatively reproduced by herbaceous cutting and grown at 25°C under fluorescent light with long-day conditions (16L/8D, 85 μmol m^{-2} s^{-1}), according to Aida and Shibata (2001). *Arabidopsis* plants were grown at 22°C under long-day conditions (16L/8D, 70μmol m^{-2} s^{-1}).

Plasmid construction for transgenic torenias

A 1.7-kb promoter region of the *Arabidopsis AP1* gene (AT1G69120) was amplified using a set of forward (5′-AAAaagcttAAATTTGGTTATATAACCACGACC-3′; the underlined part indicates the *Hind*III site) and reverse (5′-AAAggatccCATTTTTGATCCTTTTTTAAGAAACTTG-3′; the underlined part indicates the *Bam*HI site) primers. The amplified fragment was digested with *Hin*dIII and *Bam*HI and cloned into the corresponding site of p35SSRDXG (Mitsuda et al. 2006) to produce p*AP1*proSRDXG. The coding region of the β-glucuronidase gene *GUS* (S69414) was digested with *Bam*HI and *Sac*I of the pBI121 binary vector and cloned into the corresponding site of p*AP1*proSRDXG to produce the p*AP1*pro:*GUS* vector. A coding region of *Arabidopsis MYB24* (AT5G40350) was amplified using a set of forward (5′-AAAggatccATGGAGAAAAGAGAAAGTAG-3′; the underlined part indicates the *Bam*HI site) and reverse (5′-ATTACCATTATATATATTCATG-3′) primers. The amplified fragment was digested with *Bam*HI and cloned into the *Bam*HI and *Sma*I sites of p*AP1*proSRDXG to produce the p*AP1*pro:*MYB24-SRDX* vector. After confirmation of the sequence, the region corresponding to each transgene was transferred into the pBCKK plant binary vector using the Gateway system (Invitrogen) to produce pBCKK-*AP1*pro:*GUS* and pBCKK-*AP1*pro:*MYB24-SRDX*.

Generation of transgenic torenia and Arabidopsis

In torenia, the binary vectors mentioned above and pBI121, which was used for 35S:*GUS* transgenic torenias, were introduced into the *Agrobacterium tumefaciens* strain EHA105 by electroporation. After infection of torenia leaf discs by *Agrobacterium*, transgenic torenias were screened and regenerated according to Aida and Shibata (1995).

In *Arabidopsis*, the binary vectors pBCKH-35S:*MYB21-SRDX* and pBCKH-35S:*MYB24-SRDX*, which were produced in Shikata et al. (2011 in this issue), were introduced into the *A. tumefaciens* strain GV3101 by electroporation. The method of transformation followed was according to a previous report (Clough and Bent 1998).

Expression analysis by RT-PCR

Total RNA was prepared using TRIzol (Invitrogen). cDNA was synthesized from total RNA using a cDNA synthesis kit (Toyobo) and was used to amplify the transcripts. RT-PCR was performed using KOD Plus 2 (Toyobo). The sequence of each specific primer is described in Supplementary Table S1. Quality and quantity of each cDNA sample were checked using the torenia *ACTIN3* gene (*TfACT3*; AB330989) as an internal control.

Histochemical and fluorometric GUS assays

GUS activity was histochemically and fluorometrically analyzed according to Kosugi et al. (1990), with some modification. For histochemical GUS staining, plant tissues were incubated in the GUS reaction mixture containing 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, 50 mM potassium phosphate buffer (pH 7.0), 10–20% (v/v) methanol, and 1 mM dithiothreitol at 37°C for approximately 16–20 h. After the reaction was stopped by replacing the GUS reaction buffer with 70% ethanol, pigments and chlorophylls were removed by repeated 70% ethanol treatments.

For the quantitative analyses, each plant organ was homogenized in the GUS assay buffer (50 mM potassium phosphate, 10 mM EDTA, 0.1% Triton X-100, 0.1% Sarkosyl, and 2 mM DTT), and an aliquot of the supernatant was incubated in the buffer with 4-methylumbelliferyl-β-D-glucronide as a substrate at 37°C for 30 min. The amount of 4-methylumbelliferone formed in each GUS reaction was determined using a fluorescence spectrophotometer (VersaFluor™ fluorometer; Bio-Rad). Protein concentration was determined using a Coomassie protein assay kit (Bio-Rad) and BSA as the standard. In this study, three independent transgenic torenias were used for quantitative analyses shown in Figures 3 and 6. The data are shown as average with standard deviations of four independent experiments using each transgenic torenia.

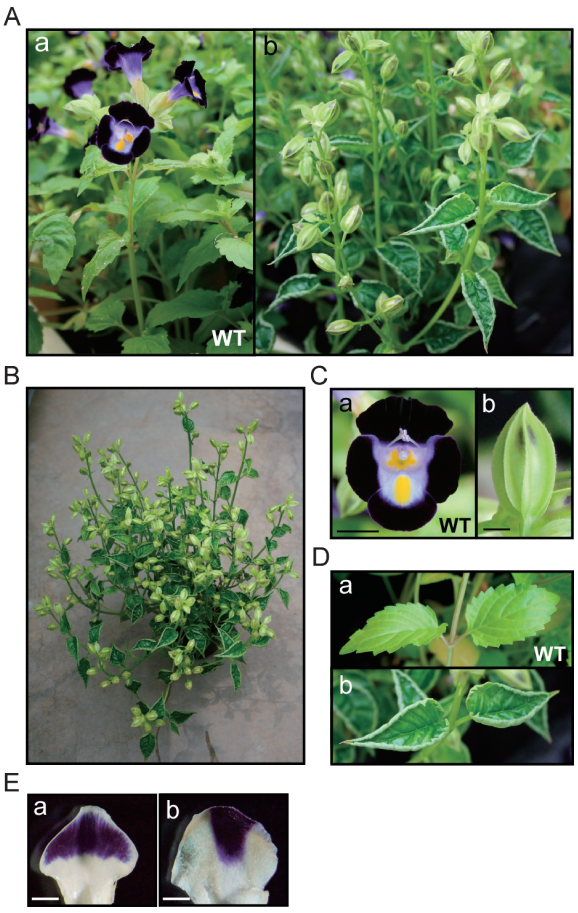

Figure 1. Photographs of a 35S:*MYB24-SRDX* transgenic torenia. (A) Photographs of a wild-type plant from the lateral side (a) and a 35S:*MYB24-SRDX* transgenic torenia (b). (B) Photograph of a whole 35S:*MYB24-SRDX* transgenic torenia from above. (C) Photographs of a flower of a wild-type plant (a) and a flower bud of a 35S:*MYB24-SRDX* torenia (b). (D) Photograph of leaves of a wild-type plant (a) and a 35S:*MYB24-SRDX* torenia (b). (E) Photographs of an immature petal of a wild-type plant (a) and a 35S:*MYB24-SRDX* torenia (b). Scale bar = 5 mm in C-a, 2.5 mm in C-b, and 1 mm in E.

Results and discussion

35S:MYB24-SRDX torenia showed unopened flower buds and phenotypically changed leaves

A main characteristic of the 35S:*MYB24-SRDX* torenia is that unopened flower buds are continuously formed (Figure 1A-b, B, C-b). In addition, the 35S:*MYB24-SRDX* torenia exhibits glossy dark green leaves with curled margins (Figure 1D-b), unlike the wild-type plants (Figure 1D-a). As in this case, utilization of the 35S promoter occasionally produced unfavorable phenotypes in transgenic plants, particularly when used for chimeric repressors (Shikata et al. 2011 in this issue). Because petals in the unopened flower buds (Figure 1C-b) lacked color at both sides of the petal (Figure 1E-b), this petal phenotype appeared to be useful for changing petal traits. We tested a combination of a floral organ-specific promoter and *MYB24-SRDX* in order to change floral traits without causing phenotypical changes in the leaves of torenia.

On the other hand, a chimeric repressor construct of *Arabidopsis MYB21* produced similar phenotypes to that in the 35S:*MYB24-SRDX* torenia. These torenias also showed glossy dark green leaves with curled margins (Shikata et al. 2011 in this issue; Supplementary Figure S1), and most of them blasted before the flowers opened (data not shown). The similarity in phenotypes of the 35S:*MYB21-SRDX* and 35S:*MYB24-SRDX* torenias suggested that these two MYBs functioned similarly in torenia. The unopened-flower phenotype has also been observed in the *myb21 myb24* double mutant of *Arabidopsis* (Mandaokar et al. 2006). These results suggested that torenia also has functionally orthologous MYB(s) similar to *Arabidopsis* MYB21 and/or MYB24.

35S:*MYB24-SRDX* and 35S:*MYB21-SRDX* transgenic plants of *Arabidopsis* exhibited epinastic and serrated rosette leaves and short petals (Supplementary Figure S2). The short-petal phenotype is also observed in the

myb21 myb24 double mutant (Mandaokar et al. 2006). However, *MYB24* and *MYB21* expression is restricted to the floral organs of *Arabidopsis* (Shin et al. 2002; Yang et al. 2007), and these changes in the leaves of 35S:*MYB24-SRDX* and 35S:*MYB21-SRDX* torenias and *Arabidopsis* were not observed in the *myb21 myb24* double mutation (Mandaokar et al. 2006). These results suggest that these leaf phenotypes of transgenic torenia and *Arabidopsis* may be caused by the functional interference with other endogenous paralogous MYBs through ectopic expression of these chimeric repressors.

AP1 promoter is active in the floral organs of torenia

With regard to a floral organ-specific promoter, the 1.7-kb promoter of *Arabidopsis AP1* expressed in the floral organs of *Arabidopsis* (Gustafson-Brown et al. 1994; Mandel et al. 1992; Urbanus et al. 2009) also effectively modifies petal phenotypes in torenia (T. Niki and T. Nishijima personal communication; National Institute of Floricultural Science). Therefore, we decided to use it to express *MYB24-SRDX* in torenia. To utilize the *AP1* promoter for *MYB24-SRDX* expression in the flowers of torenia, the promoter activity was examined in floral organs of torenia in detail. The *AP1* promoter was fused to *GUS*, which is used as a reporter gene, and *AP1* pro:*GUS* transgenic torenias were generated. Introduction of the transgene was confirmed by GUS staining of the flower buds and *GUS* expression in these buds was detected by RT-PCR analysis (Supplementary Figure S3). Detailed GUS staining was performed using various organs of isolated transgenic torenias exhibiting representative staining patterns. Among the organs examined, GUS staining was mainly detected in petals and flower buds (Figure 2A, B) but not in other organs, including the leaves (Figure 2C). In petals, strong and moderate GUS staining was observed in dorsal and lateral petals, respectively (Figure 2A, red arrowhead). In flower buds, GUS staining was mainly detected in the tip (Figure 2B, red arrowhead). To examine whether the *AP1* promoter was active in the early stage of petal formation, GUS staining was performed using petals in three developmental stages, after removing the sepals (Figure 2D). The results indicated that the *AP1* promoter is active in petals at all the three developmental stages examined (left in Figure 2E). In addition, GUS staining was observed in two different developmental stages in flower buds (Figure 2B, F). We next performed GUS staining after cutting the flower buds longitudinally. In both early (Figure 2G) and late stages (Figure 2H), GUS staining was observed in the tip and around the basal portion of the flower buds. When the basal portion was magnified, GUS staining was observed around the basal portion where the petals and sepals were fused (Figure 2I). Even after removing the petals and carpels of late-stage flower buds, the basal portion of both petals and sepals was stained (Figure 2E, J). In the floral organs of torenia, *AP1* promoter activity was mainly detected in petals and sepals. Because *AP1* is also expressed in the petals and sepals of *Arabidopsis* (Mandel et al. 1992), the *AP1* promoter was supposed to function in a similar manner in the floral organs of torenia.

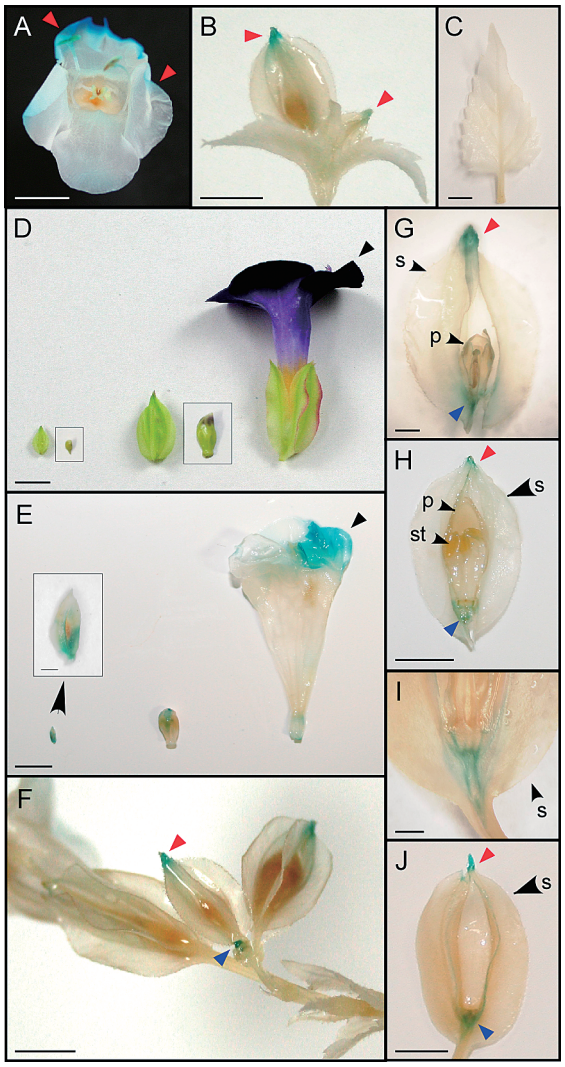

Figure 2. Histochemical GUS staining of various organs of *AP1* pro:*GUS* transgenic torenias. GUS staining of (A) a flower, (B) flower buds, and (C) a leaf of *AP1* pro:*GUS* torenias. (D) Photograph of the floral organs of transgenic torenias in three developmental stages. Photographs in the inset boxes on the right side show flower buds after removing sepals. (E) GUS staining was performed on these three floral organs, and the early-stage flower was magnified as shown in the inset box. Black arrowheads in D and E indicate dorsal petals. (F) GUS staining of flower buds in various stages. GUS staining of longitudinal sections of (G) early-stage and (H) late-stage flower buds. (I) Basal portion of late-stage petals was magnified. (J) Late-stage flower bud after removing petals including stamens. Scale bar=5 mm in A, B, C, D, E, F, H, and J; 0.5 mm in the inset box in E; and 1 mm in G and I. p; petal, s; sepal, st; stamen.

To further examine whether *AP1* promoter activity is specific to floral organs, the activity was determined

using the leaves, flower buds, and petals of transgenic torenias. In addition, to evaluate the levels of *AP1* promoter activity in torenia, this activity was compared with that of the 35S promoter in these organs. Introduction of the 35S:*GUS* transgene was confirmed by GUS staining of the leaves; strong blue staining was observed in all transgenic torenias (Supplementary Figure S4). In *AP1* pro:*GUS* transgenic torenias, GUS activity was high in petals whereas it was low in leaves and flower buds (Figure 3A). The results seem contradictory because the leaves were not stained while the flower buds were partially stained (Figure 2B, C). However, this may be because GUS activity in leaves was distributed throughout the entire leaf, while that in flower buds was localized. Thus, we observed localized GUS staining in flower buds but not in the leaves of *AP1* pro:*GUS* torenias. In 35S:*GUS* torenias, strong GUS activity was observed in leaves and petals (Figure 3B). 35S promoter activity observed in the leaves of torenia was consistent with that previously reported (Aida et al. 2008). However, GUS activity in flower buds was low and only approximately twice that of *AP1* promoter activity. In this study, the pBI121 plasmid was used for GUS analysis of the 35S promoter, while pBCKH, which was used in the transgenic torenia shown in Figure 1, additionally contained an omega element as a translational enhancer (Gallie 1993). Therefore, GUS activity resulting from the pBCKH construct containing the omega element may be approximately five to ten times higher than that resulting from the pBI121 construct (Holtorf et al. 1995). On the other hand, *AP1* promoter activity in petals was approximately half that of 35S promoter activity, and it was also detected in the early developmental stages. Furthermore, because *AP1* promoter activity in leaves was low, we expected that the *AP1* promoter could be used for *MYB24-SRDX* expression in torenia without changing the leaf phenotypes.

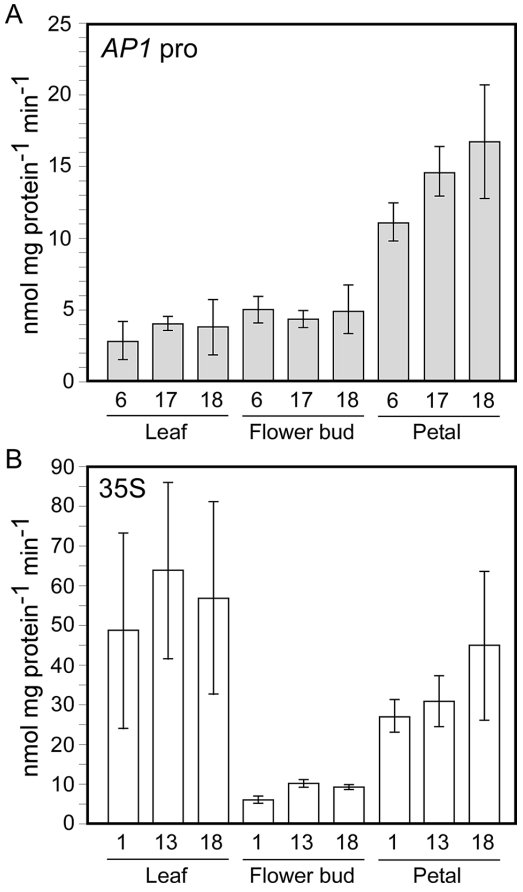

Figure 3. Quantitative GUS activity in various organs of *AP1* pro:*GUS* transgenic torenias. GUS activity in leaves, flower buds, and petals of three independent (A) *AP1* pro:*GUS* and (B) 35S:*GUS* transgenic torenias, respectively. The data are shown as average with standard deviations of four independent experiments using each transgenic torenia.

AP1 pro:MYB24-SRDX transgenic torenias bloomed without affecting the leaf phenotype

AP1 pro:*MYB24-SRDX* transgenic torenias were generated to examine whether the *AP1* promoter could produce characteristic floral traits without affecting the leaf phenotype. Introduction of the transgene was confirmed by *MYB24-SRDX* expression in flower buds with RT-PCR analysis (Supplementary Figure S5). The flower buds of *AP1* pro:*MYB24-SRDX* transgenic torenias opened and showed a characteristic petal phenotype (Figure 4A), and most of them had a normal leaf phenotype (Figure 4B). Although 18.8% (6 of 32 isolated transgenic plants) of *AP1* pro:*MYB24-SRDX* torenias sometimes showed curled leaf margins, the surface of the curled leaves seemed normal (Supplementary Figure S6). This weak leaf phenotype was observed only in young leaves due to the low *AP1* promoter activity in leaves (Figure 3A). Since even the low *AP1* promoter activity caused a weak phenotype in leaves, the 35S promoter, even without an omega element, will presumably cause a strong leaf phenotype. While *MYB24* is involved in stamen development in *Arabidopsis* (Mandaokar et al. 2006; Mandaokar and Browse 2009), no notable phenotypical change was found in the stamens of *AP1* pro:*MYB24-SRDX* torenias (data not shown). This corresponds to undetectable levels of *AP1* promoter activity in the stamens of torenia (Figure 2H). The result demonstrated that utilization of the *AP1* promoter for expression of *MYB24-SRDX* is useful for opening of flowers with characteristic petals without causing unfavorable phenotypes in leaves.

AP1 pro:*MYB24-SRDX* torenias showed the characteristic floral phenotype with wavy and crispate dorsal and lateral petals (Figure 4D). On the other hand, at the beginning of the flowering period, ventral petals were lost or crispate in *AP1* pro:*MYB24-SRDX* torenias (Figure 4E, F). Although these phenotypes were not

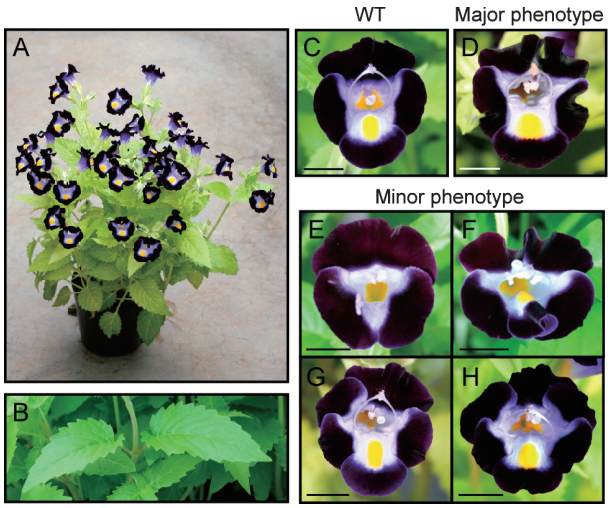

Figure 4. Phenotype of *AP1* pro:*MYB24-SRDX* transgenic torenias. (A) Photograph of a whole *AP1* pro:*MYB24-SRDX* transgenic torenia from above. (B) Photograph of the leaves of an *AP1* pro:*MYB24-SRDX* torenia. (C) A flower of wild-type torenias. (D) A major flower phenotype of *AP1* pro:*MYB24-SRDX* torenias. Minor flower phenotypes in *AP1* pro:*MYB24-SRDX* torenias, such as ventral petals were lost (E) or crispate (F) at the beginning of the flowering period. These phenotypes were not observed thereafter, and several flowers in every *AP1* pro:*MYB24-SRDX* transgenic line exhibited increased one (G) or two (H) petals as minor phenotypes. Scale bar=5 mm in C, D, E, F, G, and H.

observed thereafter, several flowers in every *AP1* pro:*MYB24-SRDX* transgenic line showed increased petal number as minor phenotypes (Figure 4G, H). A significant difference was observed in the petal number between *AP1* pro:*MYB24-SRDX* torenias and wild-type plants ($P<0.02$; data not shown). While these two irregular flower phenotypes, such as decreased or increased petal number, seem contradictory, we presumed that these phenotypes were attributed to one of the *MYB24-SRDX* functions in the development and/or differentiation of petals. Indeed, the *AP1* promoter was active in the petals of torenia (Figure 3A), and *AP1* pro:*MYB24-SRDX* torenias showed distinct petal configuration as a representative phenotype (Figure 4D).

Because the wavy petal phenotype in *AP1* pro:*MYB24-SRDX* torenias was difficult to recognize when observed from the front side (Figure 5A), flowers exhibiting this typical phenotype (Figure 4D) were observed from various angles. By observation from the top and lateral side, we could recognize that the dorsal and lateral petals were wavy and protruded forward (red arrowheads; Figure 5B, C, D), whereas the flowers of wild-type plants were flat (Figure 5F, G, H). Because the *AP1* promoter was preferentially active in dorsal and lateral petals (Figure 2A), the phenotypical changes in these organs were consistent with *AP1* promoter activity. On the other hand, scanning electron microscopy analysis indicated that the wavy petals of *AP1* pro:*MYB24-SRDX* torenias show no remarkable changes in cell size, shape, or

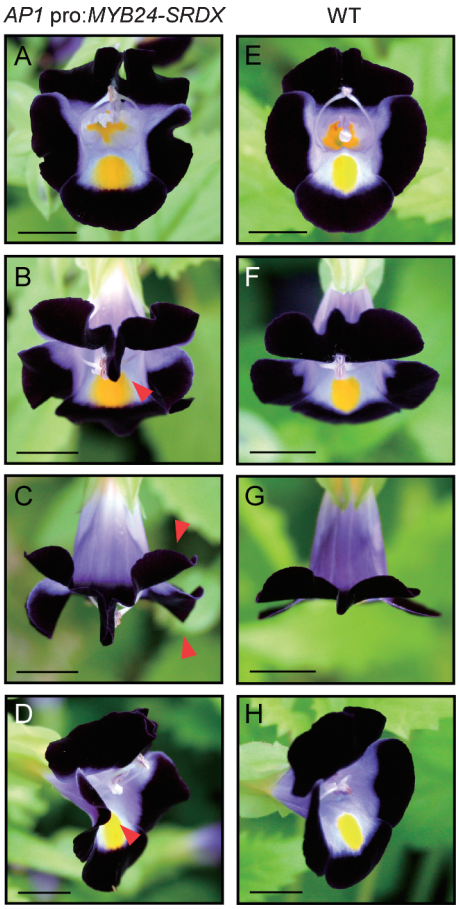

Figure 5. Flowers of an *AP1* pro:*MYB24-SRDX* transgenic torenia. Photographs of the flowers of an *AP1* pro:*MYB24-SRDX* torenia (A) from the front side, (B) and (C) oblique view from above along the dorsal–ventral axis, and (D) oblique view from the lateral side. Red arrowheads indicate points that were not observed in wild-type plants. Photographs of the flowers of wild-type plants (E) from the front side, (F) and (G) oblique view from above along the dorsal–ventral axis, and (H) oblique view from the lateral side. Scale bar=5 mm.

arrangement (data not shown), indicating that the petal phenotype was not derived from changes in cell traits. The wavy petal phenotype may represent a part of the intrinsic function of the torenia *MYB24* ortholog. Isolation of torenia *MYB24* orthologs and functional analysis using the native promoter would help us understand the mechanisms causing wavy petal phenotypes and the intrinsic functions of this transcription factor in torenia.

GUS activities of AP1 and 35S promoters in calli

In the case of the CT system, we failed to obtain transgenic plants carrying certain types of chimeric repressor constructs (Shikata et al. 2011 in this issue). This might be because the chimeric repressors interfere with the activity of target transcription factors necessary for differentiation and/or developmental processes, including callus formation and/or adventitious shoot initiation. In such cases, elucidation of their native

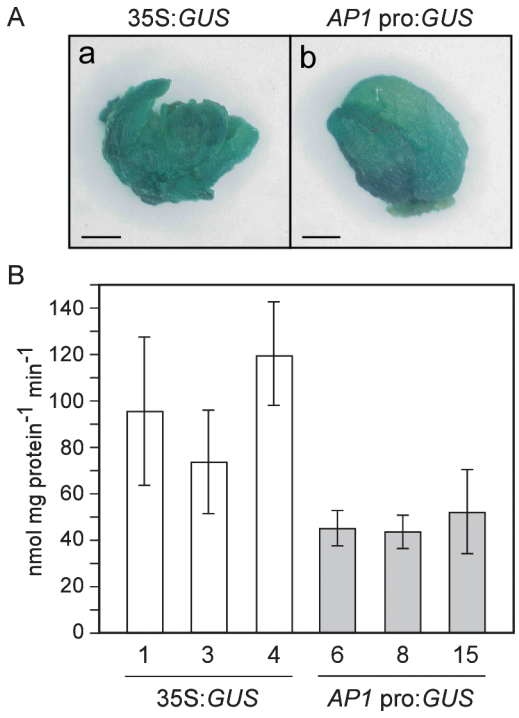

Figure 6. GUS analyses in calli of *AP1* pro:*GUS* transgenic torenias. (A) Histochemical GUS staining of calli of 35S:*GUS* (a) and *AP1* pro:*GUS* (b) transgenic torenias. (B) Quantitative GUS activity in calli of three independent 35S:*GUS* and *AP1* pro:*GUS* transgenic torenias, respectively. The data are shown as average with standard deviations of four independent experiments using each transgenic torenia. Scale bar = 0.5 mm.

functions, particularly in floral organs *in vivo*, is extremely difficult. We therefore examined *AP1* promoter activity in calli, in particular, to determine whether the promoter can be utilized to avoid such problems. Surprisingly, histochemical GUS analysis revealed that both *AP1* and 35S promoters are active in calli (Figure 6A). To understand the levels of promoter activity in calli, we next quantified *AP1* promoter activity. Among the organs examined (Figure 3), both *AP1* and 35S promoters showed the highest activity in calli (Figure 6B), although *AP1* promoter activity was approximately half that of the 35S promoter. While a floral organ-specific promoter would avoid defects in plant regeneration, the *AP1* promoter would not be utilized for this purpose because of the high promoter activity in calli (Figure 6B). Isolation of a floral organ-specific promoter, which is not active in calli and adventitious shoots, is a subject for future study.

In this study, we demonstrated that utilization of a floral organ-expressing promoter is effective in creating a distinct flower phenotype without causing unfavorable phenotypical changes in other organs. Various floral organ-specific promoters with diverse expression patterns could contribute to the creation of novel floral traits. In fact, we have observed that the chimeric repressor constructs of *Arabidopsis TCP3* fused to several floral organ-expressing promoters led to the creation of more varied floral traits than expected (in preparation). Floral organ-specific promoters that are not active in calli and adventitious shoots could also be used to express chimeric repressor constructs in order to suppress these unintended developmental processes. In addition, expression of chimeric repressors such as *MYB24-SRDX*, which affect the leaf phenotype, using a leaf-specific promoter would be also effective for changing the leaf phenotype without affecting the floral phenotype. The combination of various promoters and transcription factors, including chimeric repressors, would accelerate the development of biotechnology to efficiently produce numerous fascinating flowers, which have never been seen before.

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