Plant Biotechnology 35, 243–248 (2018)
DOI: 10.5511/plantbiotechnology.18.0529a

Original Paper

Dissecting promoter of *InMYB1* gene showing petal-specific expression

Mirai Azuma[1,2], Yoshimi Oshima[3], Shingo Sakamoto[3], Nobutaka Mitsuda[3], Masaru Ohme-Takagi[3,4], Shungo Otagaki[1], Shogo Matsumoto[1], Katsuhiro Shiratake[1,*]

[1] Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, Aichi 464-8601, Japan; [2] Department of Agricultural Bioscience, College of Bioresource Sciences, Nihon University, Kameino, Fujisawa, Kanagawa 252-0880, Japan; [3] Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology, Higashi, Tsukuba, Ibaraki 305-8566, Japan; [4] Institute for Environmental Science and Technology, Saitama University, Sakura, Saitama 338-8570, Japan
* E-mail: shira@agr.nagoya-u.ac.jp Tel & Fax: +81-52-789-4026

Received March 8, 2018; accepted May 29, 2018 (Edited by M. Otani)

Abstract We had previously reported that the *InMYB1* promoter, the 1023 bp upstream region of *InMYB1*, works petal-specifically in various dicot plants by recognizing petal identity at a cellular level. To determine the petal-specific region in the *InMYB1* promoter, Arabidopsis plants harboring *InMYB1*_1023b::*GUS* (*β-glucuronidase*), *InMYB1*_713b::*GUS*, *InMYB1*_506b::*GUS*, *InMYB1*_403b::*GUS*, *InMYB1*_332b::*GUS*, *InMYB1*_200b::*GUS* and *InMYB1*_140b::*GUS* were produced and confirmed a shortest region, which has the petal-specific promoter activity by using histochemical GUS assay. Petal-specific GUS staining was not observed in the Arabidopsis plants transformed with *InMYB1*_200b::*GUS* and *InMYB1*_140b::*GUS*, but observed in transgenic Arabidopsis plants harboring from *InMYB1*_1023b::*GUS* to *InMYB*1_332b::*GUS*. cDNA sequence of *InMYB1* shows that 120 bp upstream region of *InMYB1* is 5′ untranslated region, suggesting that the 332-121 bp upstream region of *InMYB1* contains an important element for petal-specific gene expression. In the Arabidopsis harboring the *InMYB1*_332-121b×3_TATA_Ω::*GUS*, petal-specific GUS staining was observed and the staining was stronger than in the Arabidopsis harboring *InMYB1*_1023b::*GUS*. This result shows that the 332-121 bp region is enough and essential for the petal specificity and the *InMYB1*_332-121b×3_TATA_Ω could be used for the molecular breeding of floricultural crops.

Key words: *cis*-element, *InMYB1* promoter, petal-specific gene expression.

Introduction

Genetic transformation has been used to produce novel crops with superior traits and to analyze gene function (Fukui et al. 2003; Katsumoto et al. 2007; Sasaki et al. 2014, 2016; Tanaka et al. 2013). For the genetic transformation, not only transgenes but also promoters, which control transgene expression in desired tissues and timings, are important (Oshima and Mitsuda 2016; Sasaki et al. 2016). Although cauliflower mosaic virus 35S (CaMV35S) promoter is used most frequently to drive constitutive transgene expression (Benfey and Chua 1990; Odell et al. 1985), tissue-specific promoters is also conventionally employed. For instance, *2A11* and *E8* promoters were used as fruit-specific promoters in tomato (Deikman and Fischer 1988; Pear et al. 1989). Seed-specific promoters for soybean, broad bean, and carrot and endosperm-specific promoters for rice and corn were also reported (Abbadi et al. 2004; Karunanandaa et al. 2005; Peremarti et al. 2010; Raclaru et al. 2006; Zhu et al. 2008). We had previously reported that the *InMYB1* promoter from Japanese morning glory (*Ipomoea nil*) functions petal-specifically in various dicot plants (Azuma et al. 2016a, b). The *InMYB1* promoter (*InMYB1*_1023b) is an ideal tool for molecular breeding of floricultural crops, because it can restrictively modify phenotype in petals without inducing undesirable changes in other organs or tissues (Azuma et al. 2016a, b).

Promoter consists of three parts: core promoter, proximal region and distal enhancer (Dey et al. 2015). Core promoter usually contains TATA-box element which is bound by TATA-box binding protein and the protein complex containing RNA polymerase. Promoter

Abbreviations: CaMV35S, cauliflower mosaic virus 35S; GUS, *β*-glucuronidase; PCR, polymerase chain reaction; UTR, untranslated region.
This article can be found at http://www.jspcmb.jp/
Published online July 11, 2018

cann't work by core promoter element alone, because binding of transcription factors (TFs) to *cis*-elements in proximal region is necessary to give spatio-temporal preference to the gene expression. Distal enhancer, which sometimes presents far upstream of proximal region, is also bound by TFs and modify the gene expression. Several databases, such as Plant CARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and PLACE (https://sogo.dna.affrc.go.jp/cgi-bin/sogo.cgi?lang=en&pj=640&action=page&page=newplace), provide information about *cis*-elements in plants (Higo et al. 1999; Lescot et al. 2002).

In this study, we attempted to identify the petal-specific region of *InMYB1* promoter by deleting the upstream region. Our results showed that the 332-121 bp upstream region of *InMYB1* is an essential element for petal-specific promoter function. Furthermore, we investigated the promoter activity of a combination of three-tandem repeat of the 332-121 bp upstream region of *InMYB1*, the TATA-box element of CaMV35S promoter, and translational enhancer Ω and showed that the modified system can promote higher gene expression than the original *InMYB1* promoter.

Materials and methods

Construction of vectors

To produce the vector harboring *InMYB1*_1023b:: *GUS* (*β-glucuronidase*), *InMYB1*_713b::*GUS*, *InMYB1*_506b::*GUS*, *InMYB1*_403b::*GUS*, *InMYB1*_332b::*GUS*, *InMYB1*_200b::*GUS* or *InMYB1*_140b::*GUS*, 1023 bp, 713 bp, 506 bp, 403 bp, 332 bp, 200 bp or 140 bp upstream region of *InMYB1* was amplified by PCR from a genomic clone (DDBJ Accession number: AB232773; Morita et al. 2006) using the primers described in Table 1 and then replaced the CaMV35S promoter in pBI121 (Figure 1). To produce the vector harboring *InMYB1*_332-121b×3_TATA_Ω::*GUS*, 332-121 bp upstream region of *InMYB1* was amplified by PCR using the primer described in Table 1 and then three these fragments were concatenated by In-fusion HF cloning Kit (TaKaRa, Shiga, Japan). The obtained three-tandem repeat fragment was fused to TATA element and Ω enhancer of pTATA-LUC-HSP vector (Yoshida et al. 2013). After that, the *InMYB1*_332-121b×3_TATA_Ω replaced the CaMV35S promoter in pBI121 (Figure 4).

Plant transformation

*InMYB1*_1023b::*GUS*, *InMYB1*_713b::*GUS*, *InMYB1*_

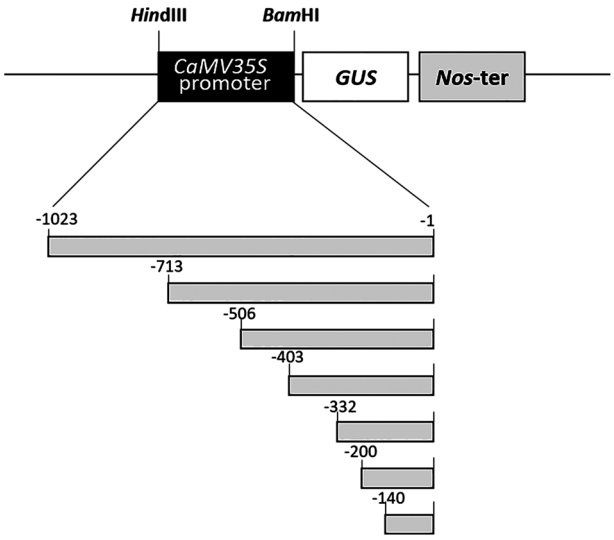

Figure 1. Schematic diagram of the *InMYB1* promoter::*GUS* vectors. The upstream regions of *InMYB1* were amplified and replaced the CaMV35S promoter in pBI121. The numbers of constructs indicate the distance in basepairs from the start codon (ATG). *Hin*dIII and *Bam*HI: restriction enzyme sites. *GUS*: *β*-glucuronidase gene. *Nos*-ter: nopaline synthase terminator.

Table 1. List of primers used in this study.

Construction	Forward primer	Reverse Primer
InMYB1_1023B::GUS	5′-CGAAGCTTCTCAATCAATAATTCTGGGG-3′	5′-CAAGGATCCGGCAGGCTGACGTAAATT-3′
*InMYB1*_713b::GUS	5′-CGAAGCTTCCGACGTTGTATAGTTTTG-3′	5′-CAAGGATCCGGCAGGCTGACGTAAATT-3′
*InMYB1*_506b::GUS	5′-CGAAGCTTGCTTTAATTTAACTTTTTGTCC-3′	5′-CAAGGATCCGGCAGGCTGACGTAAATT-3′
*InMYB1*_403b::GUS	5′-CGAAGCTTCGGTATAGAGAATATTTTTGC-3′	5′-CAAGGATCCGGCAGGCTGACGTAAATT-3′
*InMYB1*_332b::GUS	5′-CGAAGCTTCTCAATCAATAATTCTGGGG-3′	5′-CAAGGATCCGGCAGGCTGACGTAAATT-3′
*InMYB1*_200b::GUS	5′-CGAAGCTTCTCAATCAATAATTCTGGGG-3′	5′-CAAGGATCCGGCAGGCTGACGTAAATT-3′
*InMYB1*_140b::GUS	5′-CGAAGCTTAACTAGGTCCTAGTGCTGTG-3′	5′-CAAGGATCCGGCAGGCTGACGTAAATT-3′
*InMYB1*_332-121 b (1)	5′-ACTGGCCGGTACCTGCACGACTTGGATGTT-AATG-3′	5′-AACATCCAAGTCGTGCACAGCACTAGGACC-TAGTT-3′
*InMYB1*_332-121 b (2)	5′-CACGACTTGGATGTTAATG-3′	5′-AACATCCAAGTCGTGCACAGCACTAGGACC-TAGTT-3′
*InMYB1*_332-121 b (3)	5′-CACGACTTGGATGTTAATG-3′	5′-ATCTGATATCCTCGACACAGCACTAGGACC-TAGTT-3′
Gene expression	Forward primer	Reverse Primer
AtEF1α	5′-CTGGAGGTTTTGAGGCTGGTAT-3′	5′-CCAAGGGTGGAAAGCAAGAAGA-3′
GUS	5′-CGCTCACACCGATACCATCA-3′	5′-CGGCTGATGCAGTTTCTCCT-3′

Upper: list of primers used to amplify upstream regions of *InMYB1* for constructing vectors. Lower: list of primers used for real-time PCR to analyze the *GUS* expression level.

506b::*GUS*, *InMYB1*_403b::*GUS*, *InMYB1*_332b::*GUS*, *InMYB1*_200b::*GUS*, *InMYB1*_140b::*GUS* or *InMYB1*_332-121b×3_TATA_Ω::*GUS* vectors were transformed into *Agrobacterium tumefaciens* GV3101. *Arabidopsis thaliana* (Col-0) was transformed using the Agrobacterium harboring each vector by floral dip method (Clough and Bent 1998). The T1 generation of 8–22 independent transgenic lines for each construct were analyzed by histochemical GUS assay.

Histochemical GUS assay

We analyzed the promoter activity of *InMYB1* upstream regions by histochemical GUS assay following the method of Jefferson (1987) with partial modification. Samples were soaked in X-Gluc buffer [12 mM potassium ferricyanide, 12 mM potassium ferrocyanide, 0.3% (v/v) Triton X- 100 and 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-glucuronide] and the buffer was infiltrated into the samples under vacuum. Following staining for 2 days at 37°C, the samples were washed in 70%

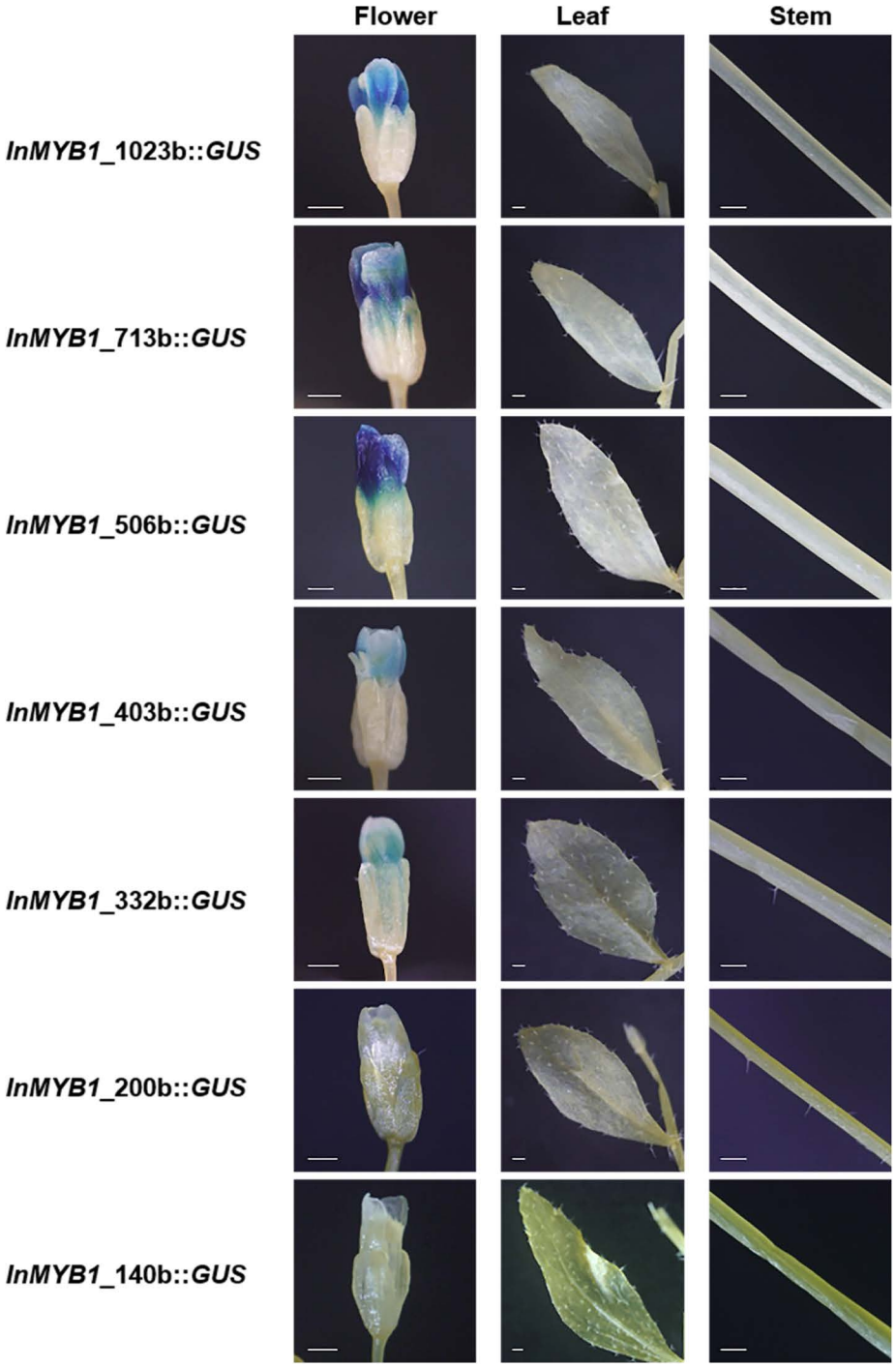

Figure 2. Histochemical assay for GUS activity in flowers, leaves, and stems of Arabidopsis harboring *InMYB1*_1023b::*GUS*, *InMYB1*_713b::*GUS*, *InMYB1*_506b::*GUS*, *InMYB1*_403b::*GUS*, *InMYB1*_332b::*GUS*, *InMYB1*_200b::*GUS* and *InMYB1*_140b::*GUS*. Scale bars=500 μm.

(v/v) ethanol and then photographed using a stereomicroscope (VB-G25; Keyence, Osaka, Japan) and a CCD camera (VB-7010; Keyence).

Measurement of promoter activity

Level of *GUS* mRNA was detected by real-time RT-PCR using the primers in Table 1. *Elongation factor-1α* (*AtEF1*) was used as an internal control. Total RNA was isolated from stage-3 flower buds (Azuma et al. 2016b) using Trizol (Thermo Fisher Scientific, MA, USA) and reverse-transcribed using PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, Shiga, Japan). Real-time PCR was carried out using obtained cDNA and SYBR Premix EX Taq (TaKaRa, Shiga, Japan) with StepOnePlus real-time PCR system (Thermo Fisher Scientific, MA, USA). The PCR cycle was 95°C for 5 s, 58°C for 20 s and 72°C for 20 s. This cycle was repeated 40 times.

Results and discussion

Identification of the petal-specific region by deleting the upstream region of InMYB1

To identify the petal-specific region of *InMYB1* promoter, *InMYB1*_1023b::*GUS*, *InMYB1*_713b::*GUS*, *InMYB1*_506b::*GUS*, *InMYB1*_403b::*GUS*, *InMYB1*_332b::*GUS*, *InMYB1*_200b::*GUS* or *InMYB1*_140b::*GUS* transgenic Arabidopsis plants were produced and analyzed by histochemical GUS assay. In all organs of *InMYB1*_200b::*GUS* and *InMYB1*_140b::*GUS* transgenic plants, no GUS staining was observed (Figure 2). On the other hand, petal-specific GUS staining was observed in *InMYB1*_1023b::*GUS*, *InMYB1*_713b::*GUS*, *InMYB1*_506b::*GUS*, *InMYB1*_403b::*GUS* and *InMYB1*_332b::*GUS* transgenic plants (Figure 2). To know the extent of promoter activity, ratio of GUS-staining flowers was determined. In the *InMYB1*_1023b::*GUS* transgenic plants, 97% of the flowers were stained and more than 85% of the flowers were stained in the *InMYB1*_713b::*GUS* and *InMYB1*_506b::*GUS* transgenic plants (Figure 3). In the *InMYB1*_403b::*GUS* or *InMYB1*_332b::*GUS* transgenic plants, it was decreased to 50% or 30%, respectively, and no GUS staining was observed in the *InMYB1*_200b::*GUS* and *InMYB1*_140b::*GUS* transgenic plants (Figure 3). We previously reported that some kinds of *cis*-element such as CAAT-box, which increases promoter activity, exist in the 1023-332 bp upstream region of *InMYB1* (Azuma et al. 2016b). Although the petal-specific promoter activity became weaker and unstable by deletion of the several *cis*-elements, the 332 bp upstream region of *InMYB1* still functioned as a petal-specific promoter.

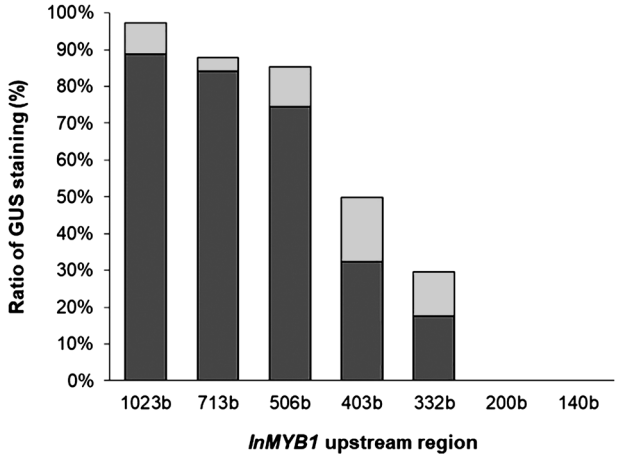

Figure 3. The ratio of GUS-staining flowers of Arabidopsis harboring *InMYB1*_1023b::*GUS*, *InMYB1*_713b::*GUS*, *InMYB1*_506b::*GUS*, *InMYB1*_403b::*GUS*, *InMYB1*_332b::*GUS*, *InMYB1*_200b::*GUS* and *InMYB1*_140b::*GUS*. Light gray bars show the proportion of weak GUS-staining flowers while dark gray bars show the proportion of strong GUS-staining flowers. Each value represents mean of 11–22 independent transgenic lines.

Determination of 5′UTR region of InMYB1 promoter

To determine 5′ untranslated region (UTR), which shouldn't contain *cis*-elements, cDNA sequence of *InMYB1* was searched in the Gene Index database of *Ipomoea nil* (http://compbio.dfci.harvard.edu/cgi-bin/tgi/Blast/index.cgi). The obtained cDNA sequence of *InMYB1* showed that the 120 bp upstream region of *InMYB1* is 5′UTR. Together with the data obtained by promoter-deletion analysis, we hypothesized that the 332-121 bp upstream region of *InMYB1* is essential for the petal-specificity.

Improvement of InMYB1 promoter for molecular breeding

It has been reported previously that the promoter activity including three-tandem repeat of *cis*-element was stronger than that of the native promoter (Espley et al. 2009). We thought that the three-tandem repeat of the 332-121 bp upstream region of *InMYB1* is possibly more stable and stronger than a single of 332-121 bp upstream region of *InMYB1*. Therefore, to demonstrate that the 332-121 bp upstream region is enough for the petal-specific expression, we investigated the promoter activity and petal-specificity of *InMYB1* 332-121b×3_TATA_Ω, a combination of three-tandem repeat of the 332-121 bp upstream region of the *InMYB1* gene, the TATA-box element of CaMV35S promoter, and translational enhancer Ω (Figure 4). We examined 8 independent lines of *InMYB1*_332-121b×3_TATA_Ω::*GUS* transgenic Arabidopsis plants and strong GUS staining in petals was observed in the all lines, indicating that the 332-121 bp upstream region is enough and essential for the petal-specific expression (Figure 4).

Furthermore, to compare the promoter activity of *InMYB1*_332-121b×3_TATA_Ω with the original

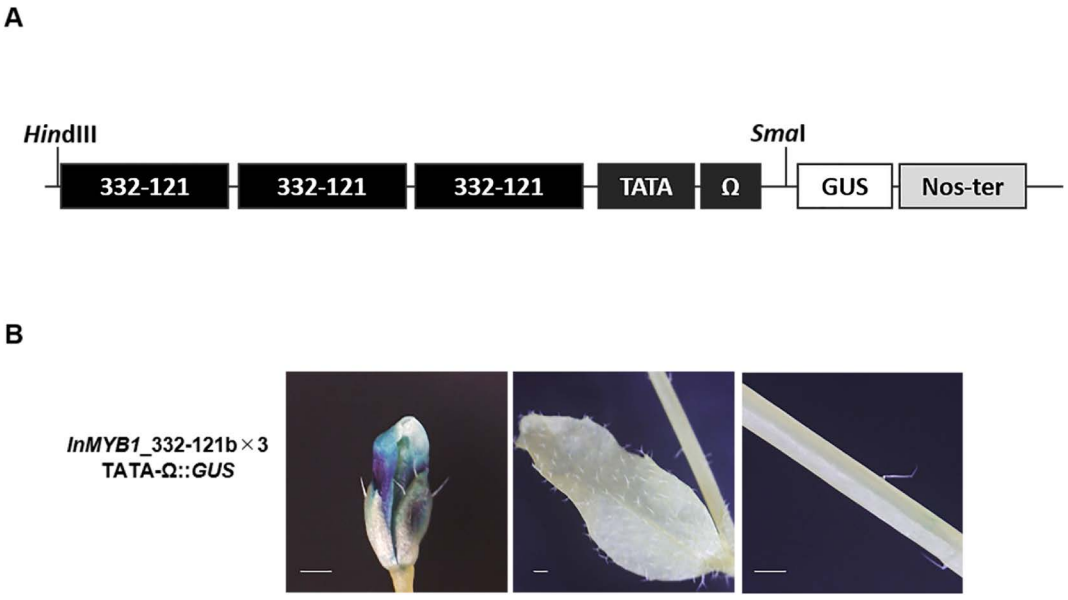

Figure 4. Histochemical assay for GUS activity in the *InMYB1*_332-121b×3_TATA_Ω::*GUS* transgenic Arabidopsis. A: Schematic diagram of the *InMYB1*_332-121b×3_TATA_Ω::*GUS* vector. The *InMYB1*_332-121b×3_TATA_Ω replaced the CaMV35S promoter in pBI121. 332-121: the 332-121b upstream region of *InMYB1*. TATA: TATA box. Ω: omega leader of the 5′UTR sequence of tobacco mosaic virus RNA, enhancing translation. *Hin*dIII and *Sma*I: restriction enzyme sites. *GUS*: *β*-glucuronidase gene. Nos-ter: nopaline synthase terminator. B: Histochemical assay for GUS activity in flower, leave, and stem of *InMYB1*_332-121b×3_TATA_Ω::*GUS* transgenic Arabidopsis. Scale bars=500 *μ*m.

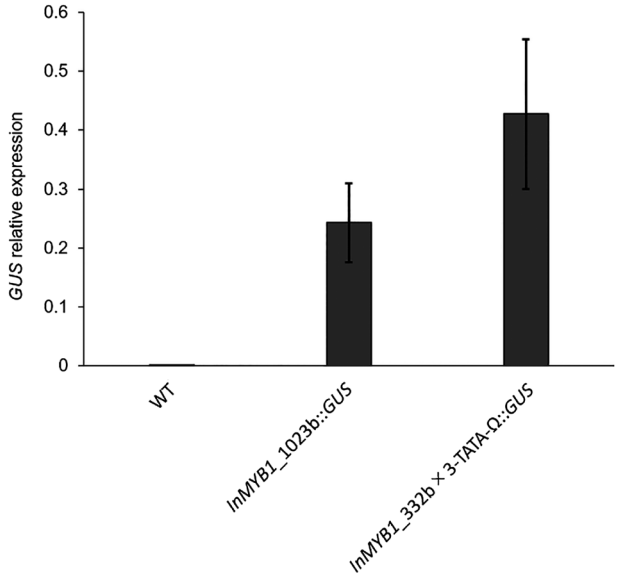

Figure 5. Expression of *GUS* in *InMYB1*_1023b::*GUS* and *InMYB1*_332-121b×3_TATA_Ω::*GUS* transgenic Arabidopsis. Gene expression was determined by real-time RT-PCR. *Elongation factor-1α* (*AtEF1*) was used as an internal control. Each value represents mean±standard error of three independent transgenic lines.

InMYB1 promoter, we investigated *GUS* mRNA level of each transgenic Arabidopsis plant by real-time RT-PCR. *GUS* mRNA level in *InMYB1* 332-121b×3_TATA_Ω::*GUS* transgenic Arabidopsis was almost double of that in *InMYB1*_1023b::*GUS* transgenic Arabidopsis (Figure 5).

In previous studies, several flower-specific promoters had been reported (Gustafson-Brown et al. 1994; Imai et al. 2013; Liu et al. 2011; Mandel et al. 1992; van der Meer et al. 1990). However, these promoters suffer from some drawbacks such as low petal specificity or restricted activity during the flowering stage. In addition, petal-specific promoter activity of these promoters was not tested in a wide range of plant species. We previously reported that the *InMYB1* promoter functions petal-specifically in various dicots plants by recognizing petal identity at a cellular level (Azuma et al. 2016a, b). In this study, we showed the promoter activity of *InMYB1*_332-121b×3_TATA_Ω is higher than that of the original *InMYB1* promoter, suggesting that the *InMYB1*_332-121b×3_TATA_Ω is a useful tool for molecular breeding of floricultural crops.

Conclusion

In this study, we revealed that the 332-121 bp upstream region of *InMYB1* is enough and essential element for the petal-specific promoter function. Furthermore, we showed that the modified artificial promoter, i.e. *InMYB1*_332-121b×3_TATA_Ω, can promote higher gene expression than the original *InMYB1* promoter in petal and therefore the artificial promoter could be a useful tool for molecular breeding of floricultural crops.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI [grant nos. 21658009, 23658027,

and 15H04449] and the Japan Science and Technology Agency (JST). We thank Ms. Ayli Chong for the critical reading of our manuscript.

References

Abbadi A, Domergue F, Bauer J, Napier JA, Welti R, Zähringer U, Cirpus P, Heinz E (2004) Biosynthesis of very-long-chain polyunsaturated fatty acids in transgenic oilseeds: Constraints on their accumulation. *Plant Cell* 16: 2734–2748

Azuma M, Mitsuda N, Goto K, Oshima Y, Ohme-Takagi M, Otagaki S, Matsumoto S, Shiratake K (2016a) The petal-specific *InMYB1* promoter functions by recognizing petaloid cells. *Plant Cell Physiol* 57: 580–587

Azuma M, Morimoto R, Hirose M, Morita Y, Hoshino A, Iida S, Oshima Y, Mitsuda N, Ohme-Takagi M, Shiratake K (2016b) A petal-specific *InMYB1* promoter from Japanese morning glory: A useful tool for molecular breeding of floricultural crops. *Plant Biotechnol J* 14: 354–363

Benfey PN, Chua N-H (1990) The cauliflower mosaic virus 35S promoter: Combinatorial regulation of transcription in plants. *Science* 250: 959–966

Clough SJ, Bent AF (1998) Floral dip: A simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16: 735–743

Deikman J, Fischer RL (1988) Interaction of a DNA binding factor with the 5′-flanking region of an ethylene-responsive fruit ripening gene from tomato. *EMBO J* 7: 3315–3320

Dey N, Sarkar S, Acharya S, Maiti IB (2015) Synthetic promoters in planta. *Planta* 242: 1077–1094

Espley RV, Brendolise C, Chagné D, Kutty-Amma S, Green S, Volz R, Putterill J, Schouten HJ, Gardiner SE, Hellens RP, et al. (2009) Multiple repeats of a promoter segment causes transcription factor autoregulation in red apples. *Plant Cell* 21: 168–183

Fukui Y, Tanaka Y, Kusumi T, Iwashita T, Nomoto K (2003) A rationale for the shift in colour towards blue in transgenic carnation flowers expressing the flavonoid 3′,5′-hydroxylase gene. *Phytochemistry* 63: 15–23

Gustafson-Brown C, Savidge B, Yanofsky MF (1994) Regulation of the Arabidopsis floral homeotic gene *APETALA1*. *Cell* 76: 131–143

Higo K, Ugawa Y, Iwamoto M, Korenaga T (1999) Plant *cis*-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Res* 27: 297–300

Imai A, Takahashi S, Nakayama K, Satoh H (2013) The promoter of the carotenoid cleavage dioxygenase 4a-5 gene of *Chrysanthemum morifolium* (*CmCCD4a-5*) drives petal-specific transcription of a conjugated gene in the developing flower. *J Plant Physiol* 170: 1295–1299

Jefferson RA (1987) Assaying chimeric genes in plants: The GUS gene fusion system. *Plant Mol Biol Report* 5: 387–405

Karunanandaa B, Qi Q, Hao M, Baszis SR, Jensen PK, Wong YH, Jiang J, Venkatramesh M, Gruys KJ, Moshiri F, et al. (2005) Metabolically engineered oilseed crops with enhanced seed tocopherol. *Metab Eng* 7: 384–400

Katsumoto Y, Fukuchi-Mizutani M, Fukui Y, Brugliera F, Holton T, Karan M, Nakamura N, Yonekura-Sakakibara K, Togami J, Pigeaire A, et al. (2007) Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. *Plant Cell Physiol* 48: 1589–1600

Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P, Rombauts S (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res* 30: 325–327

Liu Y, Lou Q, Xu W, Xin Y, Bassett C, Wang Y (2011) Characterization of a chalcone synthase (*CHS*) flower-specific promoter from *Lilium orential* 'Sorbonne'. *Plant Cell Rep* 30: 2187–2194

Mandel MA, Gustafsonbrown C, Savidge B, Yanofsky MF (1992) Molecular characterization of the Arabidopsis floral homeotic gene *APETALA1*. *Nature* 360: 273–277

Morita Y, Saitoh M, Hoshino A, Nitasaka E, Iida S (2006) Isolation of cDNAs for R2R3-MYB, bHLH and WDR transcriptional regulators and identification of c and ca mutations conferring white flowers in the Japanese morning glory. *Plant Cell Physiol* 47: 457–470

Odell JT, Nagy F, Chua N-H (1985) Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature* 313: 810–812

Oshima Y, Mitsuda N (2016) Enhanced cuticle accumulation by employing MIXTA-like transcription factors. *Plant Biotechnol* 33: 161–168

Pear JR, Ridge N, Rasmusgen R, Rose RE, Houck CM (1989) Isolation and characterization of a fruit-specific cDNA and the corresponding genomic clone from tomato. *Plant Mol Biol* 13: 639–651

Peremarti A, Twyman RM, Gómez-Galera S, Naqvi S, Farré G, Sabalza M, Miralpeix B, Dashevskaya S, Yuan D, Ramessar K, et al. (2010) Promoter diversity in multigene transformation. *Plant Mol Biol* 73: 363–378

Raclaru M, Gruber J, Kumar R, Sadre R, Lühs W, Zarhloul MK, Friedt W, Frentzen M, Weier D (2006) Increase of the tocochromanol content in transgenic *Brassica napus* seeds by overexpression of key enzymes involved in prenylquinone biosynthesis. *Mol Breed* 18: 93–107

Sasaki K, Kato K, Mishima H, Furuichi M, Waga I, Takane K, Yamaguchi H, Ohtsubo N (2014) Generation of fluorescent flowers exhibiting strong fluorescence by combination of fluorescent protein from marine plankton and recent genetic tools in *Torenia fournieri* Lind. *Plant Biotechnol* 31: 309–318

Sasaki K, Yamaguchi H, Kasajima I, Narumi T, Ohtsubo N (2016) Generation of novel floral traits using a combination of floral organ-specific promoters and a chimeric repressor in *Torenia fournieri* Lind. *Plant Cell Physiol* 57: 1319–1331

Tanaka Y, Oshima Y, Yamamura T, Sugiyama M, Mitsuda N, Ohtsubo N, Ohme-Takagi M, Terakawa T (2013) Multi-petal cyclamen flowers produced by AGAMOUS chimeric repressor expression. *Sci Rep* 3: 2641–2646

van der Meer IM, Spelt CE, Mol JNM, Stuitje AR (1990) Promoter analysis of the chalcone synthase (*chsA*) gene of Petunia hybrida: A 67 bp promoter region directs flower-specific expression. *Plant Mol Biol* 15: 95–109

Yoshida K, Sakamoto S, Kawai T, Kobayashi Y, Sato K, Ichinose Y, Yaoi K, Akiyoshi-Endo M, Sato H, Takamizo T, et al. (2013) Engineering the *Oryza sativa* cell wall with rice NAC transcription factors regulating secondary wall formation. *Front Plant Sci* 4: 383–394

Zhu C, Naqvi S, Breitenbach J, Sandmann G, Christou P, Capell T (2008) Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. *Proc Natl Acad Sci USA* 105: 18232–18237