3 Biotech (2023) 13:75
https://doi.org/10.1007/s13205-023-03490-6

ORIGINAL ARTICLE

PlgMYBR1, an R2R3-MYB transcription factor, plays as a negative regulator of anthocyanin biosynthesis in *Platycodon grandiflorus*

Eunhui Kim[1] · **Tae Kyung Hyun**[1]

Received: 21 October 2022 / Accepted: 19 January 2023 / Published online: 3 February 2023

Abstract

Floral color plays a major role in pollinator specificity, and changes in color may result in pollinator shifts and pollinator-mediated speciation. In the purple flowers of *Platycodon grandiflorus*, anthocyanins are the major pigment metabolites, whereas white flowers result due to the absence of anthocyanins. The lack of anthocyanins may be due to the inhibition of the anthocyanin biosynthesis pathway. However, the molecular mechanism of anthocyanin biosynthesis in *P. grandiflorus* is not fully understood. Hence, we identified R2R3-MYB transcription factor, PlgMYBR1, as a negative regulator for anthocyanin biosynthesis using sequence homology and tissue-specific expression pattern analyses. A heterologous co-expression assay suggested that PlgMYBR1 inhibited the function of AtPAP1 (*Arabidopsis thaliana* production of anthocyanin pigment 1), indicating that PlgMYBR1 plays as a repressor of anthocyanin biosynthesis in *P. grandiflorus*. Our results provide a foundation for future efforts to understand the anthocyanin biosynthesis in *P. grandiflorus* and, thereby, to improve flower color through genetic engineering.

Keywords Anthocyanin · Flower color · R2R3-MYB transcription factor · *Platycodon grandiflorus*

Introduction

Platycodon grandiflorus is a monotypic species belonging to the Campanulaceae family, which is a widely used ornamental, edible, and traditional Chinese medicinal herb in East Asia (Zhang et al. 2015). In traditional Chinese medicine, rhizomes of *P. grandiflorus* are administered to treat cough, phlegm, chest tightness, sore throats, and other disorders (Ji et al. 2020). Although 75 triterpenoid glycosides have been identified from *P. grandiflorus*, platycodin D is considered to be the main active compound, which possesses multiple biological effects, such as anticancer, anti-inflammatory, antiobesity, and antidiabetic properties (Jeon et al. 2019; Ji et al. 2020). In addition, flavonoids (e.g., luteolin-7-*O*-glucoside, flavoplatycoside, and apigenin-7-*O*-glucoside), phenolic acids (e.g., caffeic acid, ferulic acid, and iobetyolin), polyacetylene (e.g., lobetyolinin and lobetyolin), and sterols (e.g., β-sitosterol, spinasterol, and betulin) have been isolated from this plant (Ji et al. 2020). Although considerable attention has been focused on the pharmacologic properties of the roots and rhizomes of *P. grandiflorus*, EtOH extract of *P. grandiflorus* flowers have also been shown to exhibit antioxidant, cholesterol adsorption inhibitory, and α-glucosidase inhibitory activities (Kang et al. 2019), indicating the potential of flowers as a source of dietary health supplements.

In Korea, two varieties with purple flowers (PFs) or white flowers (WFs) are more common, and there is a difference in pharmaceutical properties between these varieties (Park et al. 2007; Han et al. 2014). Therefore, SSR (sequence-characterized amplified region) and SNP (single nucleotide polymorphism) markers were developed for predicting flower color in *P. grandiflorus* (Park et al. 2007; Yu et al. 2021). Although these markers facilitate the analysis of genetic diversity and marker-assisted selection breeding, the molecular mechanisms of flower color variation in *P. grandiflorus* are still not fully understood.

Flower color is mainly due to the accumulation of pigments such as flavonoids/anthocyanins, carotenoids, and chlorophylls (Grotewold et al. 2006). Among them, anthocyanins are major pigmentation compounds, which primarily

✉ Tae Kyung Hyun
taekyung7708@chungbuk.ac.kr

[1] Department of Industrial Plant Science and Technology, College of Agricultural, Life and Environmental Sciences, Chungbuk National University, Cheongju 28644, Republic of Korea

cause the formation of red, orange, blue, and purple flowers (Grotewold et al. 2006). In higher plants, anthocyanin accumulation is strongly correlated with the expression of anthocyanin structural genes controlled by MYB-bHLH-WD40 (MBW) activation complex, whereas R2R3-MYB with R2 and R3 repeats and R3-MYB with only R3 repeat are verified to be anthocyanin repressors via interfering with MBW complex formation (Mekapogu et al. 2020). This indicates that MYB transcription factors are the most specific and conspicuous regulators of anthocyanin biosynthesis among the MBW complex (Zimmermann et al. 2004; Mekapogu et al. 2020). Although the regulatory mechanisms by which MYBs affect plant color have been widely investigated in eudicot plants such as Petunia, Arabidopsis, Mimulus, grapevine, apple, and peach (Yuan et al. 2014; Liu et al. 2015; Hu et al. 2016; Tohge et al. 2017; Ma et al. 2018; Ding et al. 2020), the functional analysis of MYBs regulating anthocyanin biosynthesis are still relatively unknown in *P. grandiflorus*.

In this study, we aimed to investigate the molecular mechanism responsible for the different flower colors of *P. grandiflorus* and elucidate the role of MYB transcription factors by analyzing the expression pattern in different tissue and flower development stages. Using transient color assays, we performed functional characterization of *P. grandiflorus* MYB repressor 1 (PlgMYBR1). Taken together, these results suggest that the variation in *PlgMYBR1* expression level between two varieties affects the accumulation of anthocyanins and flower color.

Materials and methods

Plant materials

Seeds of *P. grandiflorus* with WFs and PFs, obtained from the Danong, Co., Ltd, in South Korea, and of tobacco (*Nicotiana tabacum* cv. Petit Havana) were germinated and grown in a growth chamber with a long photoperiod (16-h light/8-h dark). To analyze the anthocyanin content and gene expression patterns, buds and flowers were harvested by stages according to bud diameter and open flower and immediately frozen in liquid nitrogen.

Determination of anthocyanin and chlorophyll contents

Total anthocyanin content was quantified as described by Shin et al. (2007). The absorbance was determined at 530 nm (A530) and 657 nm (A657), respectively, and the content was calculated using the following formula: $(A530 - 0.33 \times A657)$/g of fresh weight.

Ten milligrams of samples were extracted in 95% ethanol at 80 °C for 30 min, and an aliquot of the ethanolic extracts was used for the determination of chlorophyll (Chl) contents. The contents of Chl a and b and total Chl (Chl a + b) were calculated according to Czyczyło-Mysza et al. (2013). Total anthocyanin and Chl contents in each sample were determined in triplicates.

Isolation and expression pattern of MYB repressors (MYBRs) in *P. grandiflorus*

To enlist the complete family of MYBR in *P. grandiflorus*, BLAST searches ($p < 0.01$) were performed on *P. grandiflorus* (variety with WFs) sequencing data (Kim et al. 2020). The amino acid sequences of candidate genes were analyzed to examine the presence of the characteristic conserved domains using Simple Modular Architecture Research Tool (http://smart.embl-heidelberg.de/).

To analyze the tissue-specific expression pattern of *P. grandiflorus,* MYB repressors (PlgMYBRs), RNA-Seq data derived from eight different tissues were downloaded from NCBI GenBank (variety with WFs, SRR8712510–SRR8712517). As described by Kim et al. (2020), transcription levels of each gene were estimated in fragments per kilobase of transcript per million mapped read values.

RNA isolation and quantitative real-time PCR

Total RNA from *P. grandiflorus* and tobacco samples was extracted using the FavorPrep Plant Total RNA Mini Kit, and reverse-transcribed into cDNA using the cDNA synthesis kit (Toyobo, Co., Ltd., Osaka, Japan). Quantitative real-time PCR (qRT-PCR) was performed using the Toyobo SYBR-Green Master Mix. Specific primer pairs for each gene (Table S1) were used, and the transcription levels of target genes were normalized to *PlgActin* or *NtGAPDH*. All analyses were performed in three biological replicates and two technical replicates.

Cloning and transient expression of *PlgMYBR1* in tobacco

The full-length *PlgMYBR1* gene amplified using gene-specific primers was cloned into the pENTR/D-TOPO vector and subcloned into a gateway binary vector pGWB505. For transient *PlgMYBR1* expression, *Agrobacterium tumefaciens* GV3101 containing 35S:*PlgMYBR1* or 35S:*Arabidopsis thaliana* production of anthocyanin pigment 1 (*AtPAP1*) construct was inoculated into 3 mL LB medium with antibiotics at 28 °C, and agro-infiltration was performed as described by Ji et al. (2021). Leaf color and anthocyanin

content were monitored at 7 days after infiltration. Transient expression was assessed in three biological replicates.

Results and discussion

The expression levels of anthocyanin biosynthetic genes in two flower color varieties of *P. grandiflorus*

As described earlier, the chemical compounds determining the flower colors mainly include flavonoids/anthocyanins, carotenoids, and chlorophylls. In the PF of *P. grandiflorus*, delphinidin and its polyacylated derivatives including platyconin (delphinidin 3-dicaffeylrutinoside-5-glucoside) were identified as a major anthocyanin (Kondo et al. 2021; Lv et al. 2021). This suggests that anthocyanins are major pigmentation compounds, although a comparative analysis between WF and PF varieties of *P. grandiflorus* is still needed. Hence, we initially determined the contents of total anthocyanin and total Chl from petals of four different flowering stages of WF and PF. As shown in Fig. 1, the total anthocyanin contents of PF and WF were similar in S1 and S2. However, total anthocyanin content in PF increased at S3 and reached the maximum level at S4, whereas total anthocyanin content in WF remained unchanged in all stages. Total Chl content in both PF and WF increased to the highest at S1 and decreased as the flowers bloomed (Fig. 1).

Differences in anthocyanin contents between two flower color varieties prompted us to investigate whether this phenomenon is mediated by the altering expression of genes involved in the anthocyanin biosynthetic pathway. As shown in Fig. 2, the expression levels of anthocyanin biosynthetic genes in PF were significantly higher than those of WF. In PF, the expression level of these genes increased initially, then decreased, and finally reached a peak at S3, whereas these genes were expressed at low levels and were almost unchanged or slightly decreased during the developmental process in WF (Fig. 2). Similarly, the differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation underlie color divergence of flowers, fruits, and leaves in various plants (Wei et al. 2011, 2015; Zhang et al. 2017; Naing et al. 2018; Le Maitre et al. 2019; Ye et al. 2021). Taken together, this indicates that anthocyanins are the main contributors to the coloration of *P. grandiflorus* petals. Thus,

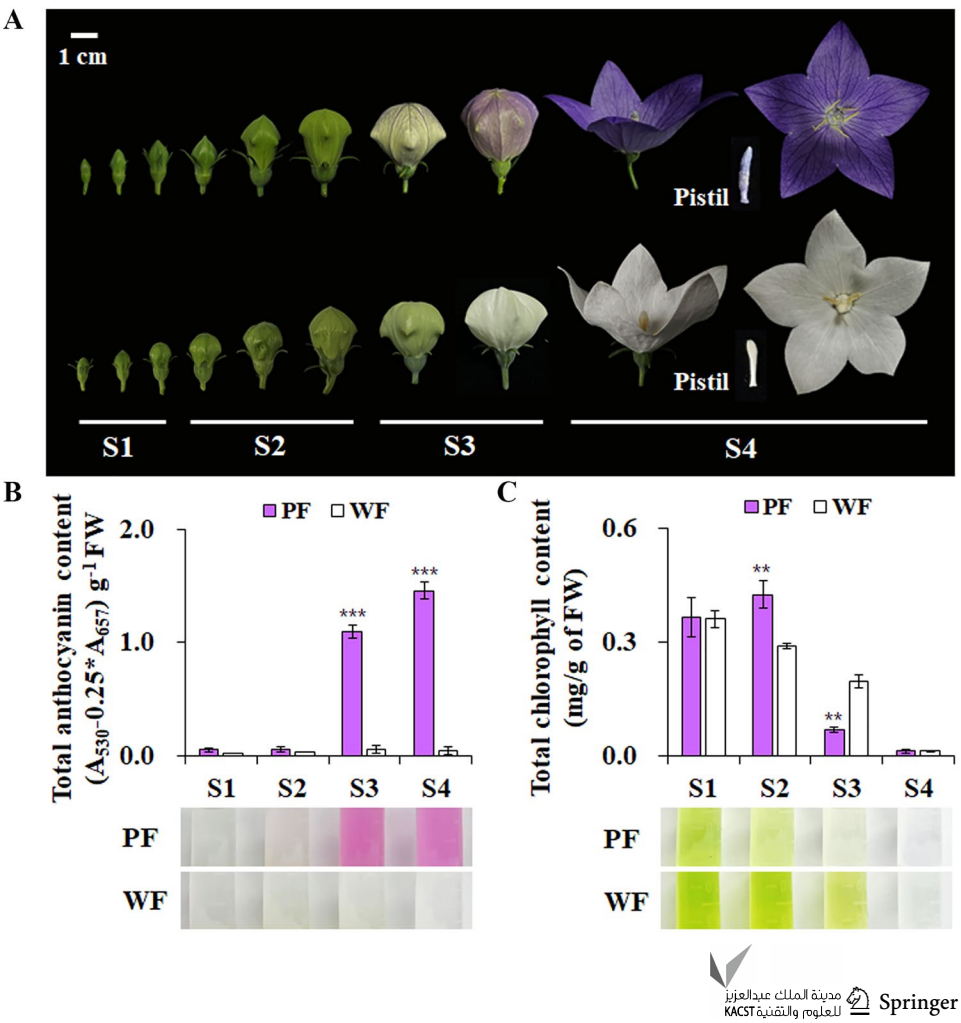

Fig. 1 Change in the color, anthocyanin content, and chlorophyll (Chl) content during flower development of *P. grandiflorus*. **A** The images of the flowers of *P. grandiflorus* varieties. S1: < 1 cm diameter, S2: 1–2 cm diameter, S3: 2–3 cm diameter, and S4: fully open. Analyses of anthocyanin accumulation (**B**) and total Chl contents (**C**) during the flower development in both varieties. The data are representative of three independent experiments (mean ± SE). $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, as compared with those of purple petals

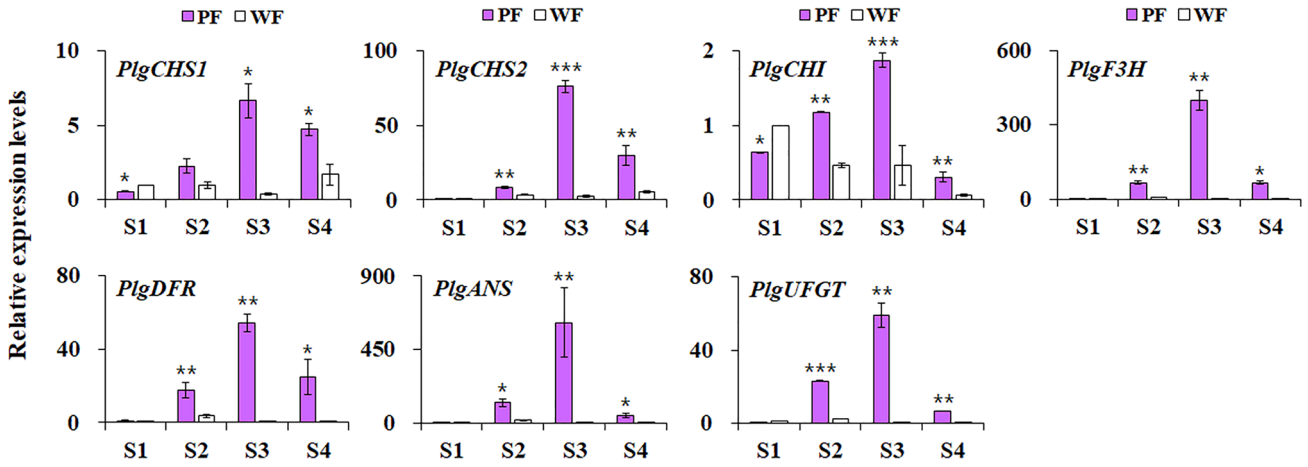

Fig. 2 The expression levels of genes involved in anthocyanin biosynthesis in the petal of two *P. grandiflorus* varieties. The expression levels of genes from the white petals were compared with those of the purple petals. The data are representative of three independent experiments (mean ± SE). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ as compared with those of the purple petals. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone hydroxylase; DFR, dihydroflavanol-4-reductase; ANS, anthocyanin synthase; UFGT, flavonoid-3-O-glucosyltransferase

we focused on the differential expression levels of anthocyanin biosynthetic genes in the two contrasting flower color varieties of *P. grandiflorus*.

Identification of *MYBR* genes in *P. grandiflorus*

In *Arabidopsis*, *R2R3-MYB* genes belonging to subgroup 6 (e.g., *Arabidopsis thaliana MYB75*, *90*, and *114*) are known as MYB activators that positively regulate the anthocyanin biosynthetic pathway, whereas the subgroup 4 *R2R3-MYB* genes (e.g., *Arabidopsis thaliana MYB3*, *4*, *7*, and *32*) play as MYBRs that inhibit the anthocyanin biosynthesis (Ma and Constabel 2019; Deng et al. 2020). Similarly, three apple MYBs (*Malus domestica* MYB1, 10, and MYBA) regulate anthocyanin accumulation by directly activating the expression of anthocyanin structural genes, whereas *Malus domestica* MYB16, 111, and 308 negatively regulate pigmentation in apple (Lin-Wang et al. 2010; Ma and Constabel 2019; Wang et al. 2022). This indicates that the biosynthesis of anthocyanin is coregulated by MYB activators and MYBRs, and the identification of both regulators is important to understand flower or fruit color variation. To identify MYB activators and MYBRs in *P. grandiflorus* genome, amino acid sequences of MYB activators and MYBRs from various plants were used as queries, and the redundant sequences were removed, resulting in a total of 10 candidate *R2R3-MYBR* genes. However, we could not find MYB activators in the draft genomic data of *P. grandiflorus*, probably due to sequencing errors, the missing sequence that included exons or parts of exons, and misassemblies. As shown in Fig. 3, the sequence alignment of these candidate PlgMYBR proteins indicated that all had the conserved

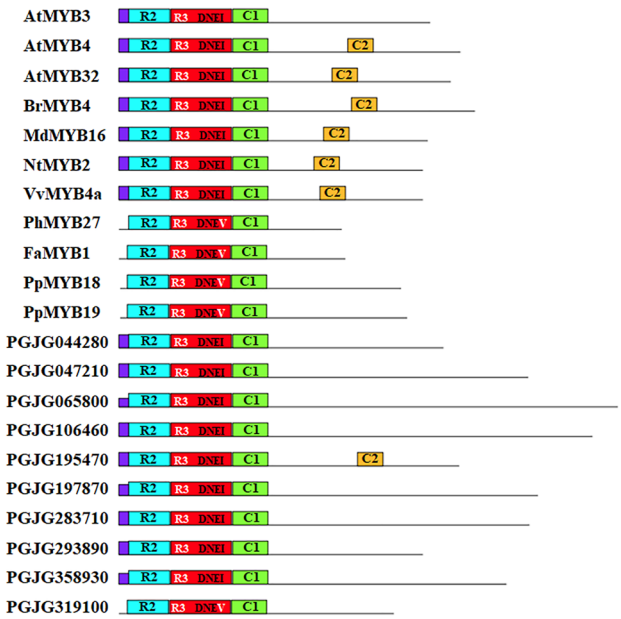

Fig. 3 Comparison of putative PlgMYB repressors with other plant R2R3 type MYB repressors. Motif distributions were investigated using the MEME web server

R2 and R3 domains in the N-terminal, together with C1/GIDP motif. The subgroup 4 R2R3 type MYBRs are characterized by two conserved motifs: C1/GIDP motif and C2/EAR (pdLNLD/EL), indicating that PGJG195470 belongs to the subgroup 4 R2R3 type MYB family. However, it is uncertain whether these C1/GIDP and C2/EAR motifs are the sole determinant of the repressive activity. In addition, *Arabidopsis thaliana* MYB3, *Petunia hybrid* MYB27, and

Fragaria×*ananasa* MYB1 do not contain C2/EAR motif, although they are well characterized as R2R3-type MYBRs (Yan et al. 2021). R2R3-type MYBRs are divided into two clades: *Arabidopsis thaliana* MYB4-like clade (inhibition by direct binding to the promoter of anthocyanin biosynthetic genes) and *Fragaria*×*ananasa* MYB1-like clade (acting on bHLH and interfering with the proper assembly of the MBW complex) (Yan et al. 2021). MYBRs belonging to *Fragaria*×*ananasa* MYB1-like clade usually possess TLLLFR motif, which seems to be their suppression role (Matsui et al. 2008; Yan et al. 2021). Another difference between *Arabidopsis thaliana* MYB4-like and *Fragaria*×*ananasa* MYB1-like clades is the DNEI and DNEV sequences in the R3 domain, respectively (Chen et al. 2019). As shown in Fig. 3 except PGJG319100, all putative PlgMYBR proteins have a DNEI sequence, indicating that they are more closely related to the *Arabidopsis thaliana* MYB4-like clade and may play as MYBR by directly binding to the promoters of the target genes.

Expression pattern of putative *PlgMYBRs* in various organs and flower developmental stages

The analysis of organ-specific expression patterns should be helpful to determine whether target genes play a role in defining the function of given organs. A PF has purple petals with a purple pistil, while a WF has white petals with a white pistil (Fig. 1). Thus, we hypothesized that the difference in the anthocyanin accumulation between PF and WF is due to differentially expressed *PlgMYBRs* in petals and pistil. To test this, we analyzed the expression patterns of *PlgMYBRs* in different organs using RNA-seq data generated from *P. grandiflorus* variety with WFs (Kim et al. 2020), and expression patterns were organized by hierarchical clustering into 5 clusters (Fig. 4a). Cluster 1 contained genes that were expressed in root and stem, and cluster 3 genes were highly expressed in most organs, except petal and pistil. Interestingly, only cluster 4 genes were highly expressed in petal and pistil. Similar to our findings, MYBRs such as chrysanthemum MYB7 (CmMYB#7) and *Freesia hybrida* MYBx were markedly differentially expressed between colored and noncolored petals or tissues (Xiang et al. 2019; Li et al. 2020). This indicates the role of the two MYBRs, PGJG106460 (PlgMYBR1) and PGJG195470, in the biosynthesis of anthocyanins, considering their high transcript levels in white petals and pistil. Further, we compared the expression levels of *PlgMYBR1* and *PGJG195470* in PFs and WFs. As shown in Fig. 4b, the expression of *PlgMYBR1* decreased during flower development, and *PlgMYBR1* was highly expressed in WFs compared to that in PFs. The expression pattern of *PGJG195470* was highly correlated with the accumulation pattern of anthocyanins in PFs. In chrysanthemum, the changes of *Chrysanthemum morifolium MYB#7* transcript levels induced alterations in anthocyanin content, resulting in the production of red or white petals (Xiang et al. 2019). This suggests that PlgMYBR1 is an important factor in the two contrasting flower color varieties of *P. grandiflorus* too.

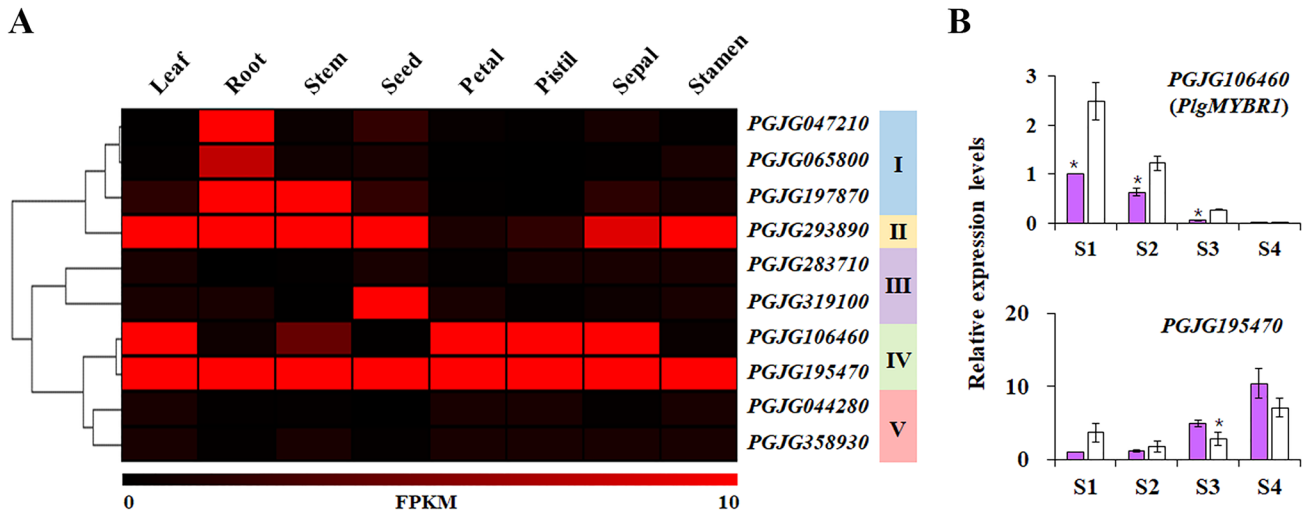

Fig. 4 Expression pattern of putative PlgMYB repressors (*PlgMYBRs*). **A** Tissue-specific expression pattern of *PlgMYBRs*. Data represent FPKM values of RNA-Seq data generated from eight different tissues of *P. grandiflorus* with white flower. **B** The expression patterns of the selected *PlgMYBRs* were analyzed using qRT-PCR. The expression levels of genes from the white petals were compared with those of the purple petals. *$p < 0.05$. FPKM, fragments per kilobase of transcript per million mapped reads

Functional characterization of putative PlgMYBR1 in tobacco

Transient color assays in tobacco leaves have been commonly used to characterize the function of MYB activators and MYBRs in various plants (Wang et al. 2017; Tirumalai et al. 2019; Xiang et al. 2019; Zhou et al. 2019). To characterize the function of PlgMYBR1, we co-infiltrated *PlgMYBR1* with a well-known MYB activator, *AtPAP1*. As shown in Fig. 5a, transient expression of *AtPAP1* resulted in the production of abundant anthocyanins after 7 days, whereas PlgMYBR1 significantly inhibited AtPAP1-induced anthocyanin accumulation. Interestingly, *PlgMYBR1* sequence obtained from PFs exhibited a difference in single nucleotide sequence that changed the amino acid sequence, W211C, compared with *P. grandiflorus* genomic data (Fig. S1). Thus, we hypothesized that this inconsistent protein sequence seemed important for catalytic activity. To test this, we compared the inhibitory effect of PlgMYBR1 with that of PlgMYBR1(W211C). As shown in Fig. 5a, there was no difference in inhibitory effects between PlgMYBR1 and PlgMYBR1 (W211C) variants. In addition, the expression levels of AtPAP1-induced anthocyanin biosynthetic genes were significantly reduced by PlgMYBR1 and PlgMYBR1 (W211C). These indicated that PlgMYBR1 is a negative regulator of anthocyanin biosynthesis and floral color variation in *P. grandiflorus* is associated with the transcript level of *PlgMYBR1* rather than the single amino acid difference between WFs and PFs.

Transposon insertions and deletions in promoter regions of MYBs lead to changes in the transcript levels of MYBs, resulting in flower or skin color variation between cultivars of apple, grape, and dahlia (Poudel et al. 2008; Espley et al. 2009; Ohno et al. 2011). Therefore, one possible explanation should be that transposable element influences on the expression of *PlgMYBR1*. In addition, the transcript levels of *Malus domestica MYB1* change by DNA methylation in its promoter region, resulting in determining anthocyanin content in the fruit peel of two apple cultivars (Ma et al. 2018). Similarly, histone H3K9 demethylase JMJ25 directly affects the expression of poplar *MYB182* (anthocyanin repressor) to modulate the biosynthesis of anthocyanins in poplar (Fan et al. 2018). Based on these findings, we hypothesized that altering the expression levels of *PlgMYBR1* by epigenetic modification modulate anthocyanin biosynthesis in flowers of two *P. grandiflorus* varieties, as other possible explanations.

Conclusion

In this study, we identified that PlgMYBR1 as a negative regulator in anthocyanin biosynthesis by analyzing sequence homology and expression patterns. In addition, the heterologous co-expression assay suggested that PlgMYBR1 inhibited the function of AtPAP1, indicating that the transcript level of *PlgMYBR1* determines anthocyanin contents in the petal of two *P. grandiflorus* varieties. Although the function of PlgMYBR1 has been inferred in heterologous systems

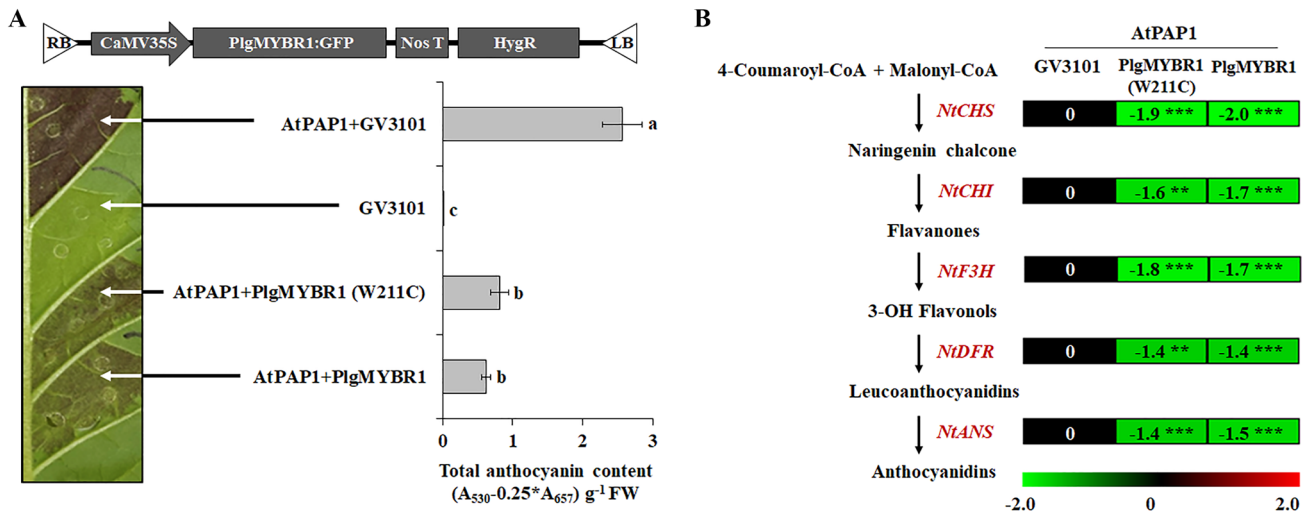

Fig. 5 Transient activation of PlgMYBR1 in tobacco leaves. **A** Transient expression of *PlgMYBR1*-expressing constructs in tobacco introduced through agro-infiltration. *AtPAP1* infiltration led to high accumulation of anthocyanin, whereas PlgMYBR1 inhibited the accumulation of AtPAP1-induced anthocyanin. **B** Effect of PlgMYBR1 on the expression of AtPAP1-induced genes involved in the anthocyanin biosynthetic pathway. The level of expression is represented as log2 ratio. Data are means (±SE) of three biological replicates per construct. Different letters indicate statistically significant differences between the samples by Duncan multiples: $^{**}p < 0.01$, $^{***}p < 0.001$

and further experiments are required to understand the variation of its expression levels, our results have provided insights into the function of PlgMYBR1, which is predicted to regulate flower color formation through the regulation of anthocyanin biosynthesis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13205-023-03490-6.

Author contributions EK and TKH conceived and designed the experiments. EK performed the experiments. EK and TKH wrote the manuscript.

Data availability The data presented in this study are available on request from the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Chen L, Hu B, Qin Y, Hu G, Zhao J (2019) Advance of the negative regulation of anthocyanin biosynthesis by MYB transcription factors. Plant Physiol Bioch 136:178–187. https://doi.org/10.1016/j.plaphy.2019.01.024

Czyczyło-Mysza I, Tyrka M, Marcińska I, Skrzypek E, Karbarz M, Dziurka M, Hura T, Dziurka K, Quarrie SA (2013) Quantitative trait loci for leaf chlorophyll fluorescence parameters, chlorophyll and carotenoid contents in relation to biomass and yield in bread wheat and their chromosome deletion bin assignments. Mol Breed 32:189–210. https://doi.org/10.1007/s11032-013-9862-8

Deng J, Wu D, Shi J, Balfour K, Wang H, Zhu G, Liu Y, Wang J, Zhu Z (2020) Multiple MYB activators and repressors collaboratively regulate the juvenile red fading in leaves of Sweet potato. Front Plant Sci 11:941. https://doi.org/10.3389/fpls.2020.00941

Ding B, Patterson EL, Holalu SV, Li J, Johnson GA, Stanley LE, Greenlee AB, Peng F, Bradshaw HD Jr, Blinov ML, Blackman BK, Yuan YW (2020) Two MYB proteins in a self-organizing activator-inhibitor system produce spotted pigmentation patterns. Curr Biol 30:802–814. https://doi.org/10.1016/j.cub.2019.12.067

Espley RV, Brendolise C, Chagné D, Kutty-Amma S, Green S, Volz R, Putterill J, Schouten HJ, Gardiner SE, Hellens RP, Allan AC (2009) Multiple repeats of a promoter segment causes transcription factor autoregulation in red apples. Plant Cell 21:168–183. https://doi.org/10.1105/tpc.108.059329

Fan D, Wang X, Tang X, Ye X, Ren S, Wang D, Luo K (2018) Histone H3K9 demethylase JMJ25 epigenetically modulates anthocyanin biosynthesis in poplar. Plant J 96:1121–1136. https://doi.org/10.1111/tpj.14092

Grotewold E (2006) The genetics and biochemistry of floral pigments. Annu Rev Plant Biol 57:761–780. https://doi.org/10.1146/annurev.arplant.57.032905.105248

Han E-H, Son Y-W, Kim M-B, Shin Y-W, Cho Y-S, Lee S-W (2014) Establishment of tissue culture and acclimation of white balloon flower (*Platycodon grandiflorum* DC. cv. Jangback) for the raising of in vitro propagated seedlings. J Plant Biotechnol 41:134–139. https://doi.org/10.5010/JPB.2014.41.3.134

Hu DG, Sun CH, Ma QJ, You CX, Cheng L, Hao YJ (2016) MdMYB1 Regulates anthocyanin and malate accumulation by directly facilitating their transport into vacuoles in apples. Plant Physiol 170:1315–1330. https://doi.org/10.1104/pp.15.01333

Jeon D, Kim SW, Kim HS (2019) Platycodin D, a bioactive component of *Platycodon grandiflorum*, induces cancer cell death associated with extreme vacuolation. Anim Cells Syst 23:118–127. https://doi.org/10.1080/19768354.2019

Ji MY, Bo A, Yang M, Xu JF, Jiang LL, Zhou BC, Li MH (2020) The Pharmacological effects and health benefits of *Platycodon grandiflorus*-a medicine food homology species. Foods 9:142. https://doi.org/10.3390/foods9020142

Ji HS, Bang SG, Ahn MA, Kim G, Kim E, Eom SH, Hyun TK (2021) Molecular cloning and functional characterization of heat stress-responsive superoxide dismutases in garlic (*Allium sativum* L.). Antioxidants 10:815. https://doi.org/10.3390/antiox10050815

Kang JR, Kang MJ, Cho AR, Shin JH (2019) Physiochemical characteristics of *Platycodon grandiflorum* flower ethanolic extracts. Korean J Food Preserv 26:785–795. https://doi.org/10.11002/kjfp.2019.26.7.785

Kim J, Kang SH, Park SG, Yang TJ, Lee Y, Kim OT, Chung O, Lee J, Choi JP, Kwon SJ, Lee K, Ahn BO, Lee DJ, Yoo SI, Shin IG, Um Y, Lee DY, Kim GS, Hong CP, Bhak J, Kim CK (2020) Whole-genome, transcriptome, and methylome analyses provide insights into the evolution of platycoside biosynthesis in *Platycodon grandiflorus*, a medicinal plant. Hortic Res 7:112. https://doi.org/10.1038/s41438-020-0329-x

Kondo T, Hagihara S, Takaya Y, Yoshida K (2021) Polyacylated anthocyanins in bluish-purple petals of Chinese bellflower *Platycodon grandiflorum*. Int J Mol Sci 22:4044. https://doi.org/10.3390/ijms22084044

Le Maitre NC, Pirie MD, Bellstedt DU (2019) Floral color, anthocyanin synthesis gene expression and control in *Cape erica* species. Front Plant Sci 10:1565. https://doi.org/10.3389/fpls.2019.01565

Li Y, Shan X, Gao R, Han T, Zhang J, Wang Y, Kimani S, Wang L, Gao X (2020) MYB repressors and MBW activation complex collaborate to fine-tune flower coloration in *Freesia hybrida*. Commun Biol 3:396. https://doi.org/10.1038/s42003-020-01134-6

Lin-Wang K, Bolitho K, Grafton K, Kortstee A, Karunairetnam S, McGhie TK, Espley RV, Hellens RP, Allan AC (2010) An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae. BMC Plant Biol 10:50. https://doi.org/10.1186/1471-2229-10-50

Liu J, Osbourn A, Ma P (2015) MYB Transcription factors as regulators of phenylpropanoid metabolism in plants. Mol Plant 8:689–708. https://doi.org/10.1016/j.molp.2015.03.012

Lv Y, Tong X, Zhang P, Yu N, Gui S, Han R, Ge D (2021) Comparative transcriptomic analysis on white and blue flowers of *Platycodon grandiflorus* to elucidate genes involved in the biosynthesis of anthocyanins. Iran J Biotechnol 19: e2811. https://doi.org/10.30498/ijb.2021.239899.2811

Ma D, Constabel CP (2019) MYB repressors as regulators of phenylpropanoid metabolism in plants. Trends Plant Sci 24:275–289. https://doi.org/10.1016/j.tplants

Ma C, Jing C, Chang B, Yan J, Liang B, Liu L, Yang Y, Zhao Z (2018) The effect of promoter methylation on MdMYB1 expression determines the level of anthocyanin accumulation in skins of two non-red apple cultivars. BMC Plant Biol 18:108. https://doi.org/10.1186/s12870-018-1320-7

Matsui K, Umemura Y, Ohme-Takagi M (2008) AtMYBL2, a protein with a single MYB domain, acts as a negative regulator of anthocyanin biosynthesis in Arabidopsis. Plant J 55:954–967. https://doi.org/10.1111/j.1365-313X.2008.03565.x

Mekapogu M, Vasamsetti BMK, Kwon OK, Ahn MS, Lim SH, Jung JA (2020) Anthocyanins in floral colors: biosynthesis and regulation

in chrysanthemum flowers. Int J Mol Sci 21:6537. https://doi.org/10.3390/ijms21186537
Naing AH, Park DY, Park KI, Kim CK (2018) Differential expression of anthocyanin structural genes and transcription factors determines coloration patterns in gerbera flowers. 3 Biotech 8:393. https://doi.org/10.1007/s13205-018-1408-7
Ohno S, Hosokawa M, Hoshino A, Kitamura Y, Morita Y, Park KI, Nakashima A, Deguchi A, Tatsuzawa F, Doi M, Iida S, Yazawa S (2011) A bHLH transcription factor, DvIVS, is involved in regulation of anthocyanin synthesis in dahlia (*Dahlia variabilis*). J Exp Bot 62:5105–5116. https://doi.org/10.1093/jxb/err216
Park CG, Bang KH, Kim OT, Jin DC, Kim DH, Sung JS, Seong NS, Park HW, Lee SC (2007) Development of SCAR marker for discriminating between violet flowered lines and white flowered lines in Chinese bellflower (*Platycodon grandiflorum* A.). Korean J Med Crop Sci 15:1–5
Poudel PR, Goto-Yamamoto N, Mochioka R, Kataoka I, Beppu K (2008) Expression analysis of UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT) gene in an interspecific hybrid grape between *Vitis ficifolia* var. ganebu and *Vitis vinifera* cv. Muscat of Alexandria. Plant Biotechnol Rep 2:233. https://doi.org/10.1007/s11816-008-0069-0
Shin J, Park E, Choi G (2007) PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in Arabidopsis. Plant J 49:981–994. https://doi.org/10.1111/j.1365-313X.2006.03021.x
Tirumalai V, Swetha C, Nair A, Pandit A, Shivaprasad PV (2019) miR828 and miR858 regulate VvMYB114 to promote anthocyanin and flavonol accumulation in grapes. J Exp Bot 70:4775–4792. https://doi.org/10.1093/jxb/erz264
Tohge T, de Souza LP, Fernie AR (2017) Current understanding of the pathways of flavonoid biosynthesis in model and crop plants. J Exp Bot 68:4013–4028. https://doi.org/10.1093/jxb/erx177
Wang S, Chu Z, Ren M, Jia R, Zhao C, Fei D, Su H, Fan X, Zhang X, Li Y, Wang Y, Ding X (2017) Identification of anthocyanin composition and functional analysis of an anthocyanin activator in *Solanum nigrum* fruits. Molecules 22:876. https://doi.org/10.3390/molecules22060876
Wang S, Zhang Z, Li LX, Wang HB, Zhou H, Chen XS, Feng SQ (2022) Apple MdMYB306-like inhibits anthocyanin synthesis by directly interacting with MdMYB17 and MdbHLH33. Plant J 110:1021–1034. https://doi.org/10.1111/tpj.15720
Wei YZ, Hu FC, Hu GB, Li XJ, Huang XM, Wang HC (2011) Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of *Litchi chinensis* Sonn. PLoS One 6:e19455. https://doi.org/10.1371/journal.pone.0019455
Wei H, Chen X, Zong X, Shu H, Gao D, Liu Q (2015) Comparative transcriptome analysis of genes involved in anthocyanin biosynthesis in the red and yellow fruits of sweet cherry (*Prunus avium* L.). PLoS One 10:e0121164. https://doi.org/10.1371/journal.pone.0121164
Xiang L, Liu X, Li H, Yin X, Grierson D, Li F, Chen K (2019) CmMYB#7, an R3 MYB transcription factor, acts as a negative regulator of anthocyanin biosynthesis in chrysanthemum. J Exp Bot 70:3111–3123. https://doi.org/10.1093/jxb/erz121
Yan H, Pei X, Zhang H, Li X, Zhang X, Zhao M, Chiang VL, Sederoff RR, Zhao X (2021) MYB-mediated regulation of anthocyanin biosynthesis. Int J Mol Sci 22:3103. https://doi.org/10.3390/ijms22063103
Ye LJ, Möller M, Luo YH, Zou JY, Zheng W, Wang YH, Liu J, Zhu AD, Hu JY, Li DZ, Gao LM (2021) Differential expressions of anthocyanin synthesis genes underlie flower color divergence in a sympatric *Rhododendron sanguineum* complex. BMC Plant Biol 21:204. https://doi.org/10.1186/s12870-021-02977-9
Yu GE, Shin Y, Subramaniyam S, Kang SH, Lee SM, Cho C, Lee SS, Kim CK (2021) Machine learning, transcriptome, and genotyping chip analyses provide insights into SNP markers identifying flower color in *Platycodon grandiflorus*. Sci Rep 11:8019. https://doi.org/10.1038/s41598-021-87281-0
Yuan YW, Sagawa JM, Frost L, Vela JP, Bradshaw HD Jr (2014) Transcriptional control of floral anthocyanin pigmentation in monkeyflowers (Mimulus). New Phytol 204:1013–1027. https://doi.org/10.1111/nph.12968
Zhang L, Wang Y, Yang D, Zhang C, Zhang N, Li M, Liu Y (2015) Platycodon grandiflorus—an ethnopharmacological, phytochemical and pharmacological review. J Ethnopharmacol 164:147–161. https://doi.org/10.1016/j.jep.2015.01.052
Zhang L, Xu B, Wu T, Yang Y, Fan L, Wen M, Sui J (2017) Transcriptomic profiling of two Pak Choi varieties with contrasting anthocyanin contents provides an insight into structural and regulatory genes in anthocyanin biosynthetic pathway. BMC Genom 18:288. https://doi.org/10.1186/s12864-017-3677-7
Zhou H, Lin-Wang K, Wang F, Espley RV, Ren F, Zhao J, Ogutu C, He H, Jiang Q, Allan AC, Han Y (2019) Activator-type R2R3-MYB genes induce a repressor-type R2R3-MYB gene to balance anthocyanin and proanthocyanidin accumulation. New Phytol 221:1919–1934. https://doi.org/10.1111/nph.15486
Zimmermann IM, Heim MA, Weisshaar B, Uhrig JF (2004) Comprehensive identification of Arabidopsis thaliana MYB transcription factors interacting with R/B-like BHLH proteins. Plant J 40:22–34. https://doi.org/10.1111/j.1365-313X.2004.02183.x