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Gene Note

Isolation of anthocyanin-related *MYB* gene, *GbMYB2*, from *Gynura bicolor* leaves

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Abstract The leaves of field-cultivated *Gynura bicolor* DC. are reddish purple on the abaxial side and green on the adaxial side. The leaves of cultured *G. bicolor* plantlets, however, appear almost completely green on both sides. Cultured plantlet leaves treated with sucrose accumulated anthocyanins on the abaxial side. In this study, to investigate the anthocyanin-accumulation mechanism in *G. bicolor* leaves, we isolated the *MYB* transcription regulatory gene from the leaves using a degenerate PCR method. The isolated gene, *GbMYB2*, was approximately 10-fold up-regulated in sucrose-treated leaves. In addition, co-expression of GbMYB2 and bHLH-type transcription factor, GbMYC1, activated *GbDFR* and *GbANS* promoters in tobacco leaf protoplasts. These results suggest that GbMYB2 might regulate anthocyanin biosynthesis genes in *G. bicolor* leaves.

Key words: Anthocyanin, bHLH, *Gynura bicolor*, R2R3 MYB, transcription factor.

Gynura bicolor DC. is a traditional vegetable cultivated commercially in the Ishikawa and Kumamoto prefectures in Japan. The leaves of *G. bicolor* cultivated in soil are reddish purple on the abaxial side, but are green on the adaxial side. In contrast, the leaves of plantlets cultured on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) are green on both sides. Cultured plantlets, however, began to again accumulate anthocyanin on the abaxial side of leaves after they were potted and grown under greenhouse conditions. We have demonstrated that anthocyanin accumulation in cultured plantlet leaves was induced by sucrose treatment. We cultured excised leaves on a high concentration of sucrose solution, and the treated green leaves turned reddish purple mainly on the abaxial side. In leaves treated with sucrose, the expression of anthocyanin biosynthesis genes was up-regulated (Shimizu et al. personal communication). Anthocyanins contained in *G. bicolor* leaves were highly acylated and stable (Shimizu et al. 2010; Yoshitama et al. 1994). In addition, these anthocyanins have high antioxidant activity and might be useful as a food colorant (Shimizu et al. 2010); therefore, it is important that the regulatory mechanisms of accumulation of stable anthocyanins in *G. bicolor* are understood in detail.

Anthocyanin is a plant pigment that contributes to the red, blue, and purple colors in the tissues, leaves, shoots, roots, flowers, and fruits of plants. Various factors, such as UV irradiation, sucrose, phytohormones, drought, and nutrient deficiency, are related to anthocyanin accumulation (Chalker-Scott 1999; Dixon and Paiva 1995; Harborne and Williams 2000). In various plants, anthocyanin pigmentation has been studied well, and it has been shown that the expression of anthocyanin structural genes is regulated by transcription factors such as a R2R3 MYB, a basic helix-loop-helix (bHLH) transcription factor, and a WD-40 protein (Broun 2005; Koes et al. 2005). In particular, in *Arabidopsis*, it has been revealed that the regulation of anthocyanin biosynthesis is carried out by Production of Anthocyanin Pigment 1 (PAP1), PAP2, MYB113, and MYB114 as MYB proteins; Transparent Testa8 (TT8), Glabra3, and Enhancer of Glabra3 (EGL3) as bHLHs; and TTG1 as a WD-40 protein (Borevitz et al. 2000; Gonzalez et al. 2008; Nesi et al. 2000; Payne et al. 2000; Tohge et al. 2005).

To study the genetic regulation of anthocyanin biosynthesis of *G. bicolor*, we isolated two anthocyanin-related *MYB* genes from *G. bicolor* using the degenerate PCR method. One of them, *GbMYB1*, was isolated from roots treated with methyl jasmonate (MJ); the other, *GbMYB2*, was isolated from leaves treated with sucrose. It was found that *GbMYB1* was related to MJ-inducible anthocyanin accumulation in *G. bicolor* roots (Shimizu et al. personal communication). In this paper, we analyze

Abbreviations: ANS, anthocyanidin synthase; bHLH, basic helix-loop-helix; 4CL, 4-coumarate:CoA ligase; C4H, cinnamate 4-hydroxylase; CHS, chalcone synthase; DFR, dihydroflavonol reductase; PAL, phenylalanine ammonia lyase

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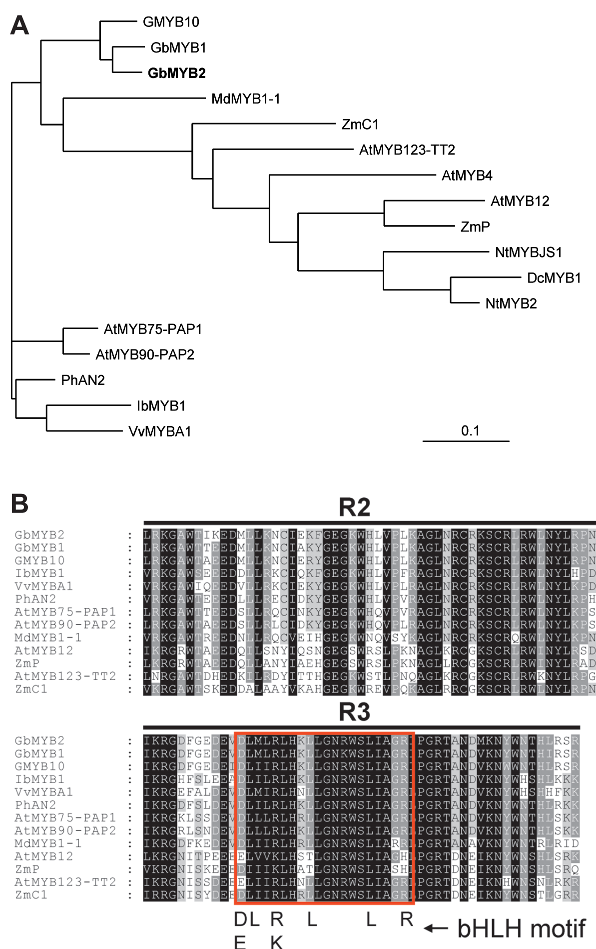

Figure 1. Protein sequence analysis of GbMYB2. (A) Phylogenetic analysis of the R2R3 MYB domain among plant MYBs. (B) Protein sequence alignment of the R2R3 MYB domains of GbMYB2 and other anthocyanin-related MYB proteins. The R2 and R3 domains are underlined in black. Motif for interaction with bHLH proteins (bHLH motif) is boxed in red in the R3 domain. Gene (accession numbers) and genus nomenclature are as follows: AtMYB75-PAP1 (NP176057), *Arabidopsis thaliana*; AtMYB90-PAP2 (NP176813), *A. thaliana*; AtMYB12 (NP182268), *A. thaliana*; AtMYB123-TT2 (NP18405), *A. thaliana*; AtMYB4 (BAA21619), *A. thaliana*; ZmC1 (AAA33482), *Zea mays*; ZmP (AAC49394), *Z. mays*; NtMYB2 (BAA88222), *Nicotiana tabacum*; NtMYBJS1 (BAE93149), *N. tabacum*; DcMYB1 (BAE54312), *Daucus carota*; PhAN2 (AAF66727), *Petunia hybrida*; MdMYB1-1 (ABK58136), *Malus domestica*; IbMYB1 (BAE94389), *Ipomoea batatas*; VvMYBA1 (BAD18977), *Vitis vinifera*; GMYB10 (CAD87010), *Gerbera hybrida*. Sequence alignment and preparation of a phylogenetic tree were performed with the neighbor-joining algorithm of CLUSTALW.

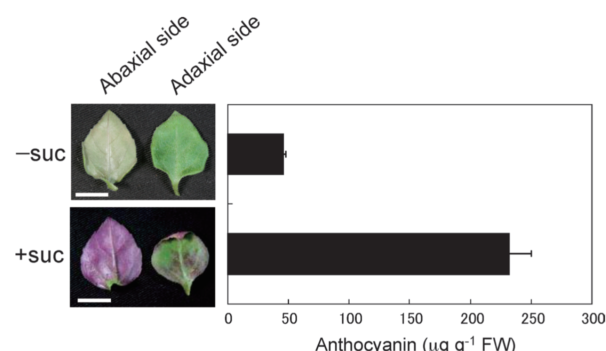

Figure 2. Anthocyanin accumulation induced in sucrose-treated leaves. Leaves cultured on water without sucrose (−suc) and 9% sucrose solution (+suc) for 5 days are shown at the left side of the figure (bar=1 cm). Leaves cultured on 9% sucrose solution for 5 days (+suc) mainly accumulated anthocyanin in the abaxial side of the leaf. Anthocyanin contents were shown at the right side of the figure. Bars indicate standard errors (n=3), FW=fresh weight.

the results of isolation of the *GbMYB2* gene.

To identify the regulatory mechanism of sucrose-induced anthocyanin accumulation in *G. bicolor* leaves, we isolated the *MYB* gene and then analyzed the expression in *G. bicolor* sucrose-treated leaves. The cDNA-encoding MYB protein was isolated from *G. bicolor* sucrose-treated leaves using a degenerate PCR method with primer pairs, 5′-TGYATHRAYAARTAY-GGIGARGGIAARTGG-3′ as the sense primer and 5′-GTRTTCCARTARTTYTTIACRTCRTTNGC-3′ as the antisense primer, designed in the R2R3 MYB domain. Total RNA was isolated with RNeasy Plant Mini kit (Qiagen, Hilden, Germany) and reverse transcripted into cDNA using TaKaRa RNA PCR kit (TaKaRa, Tokyo, Japan). Amplified cDNA fragments by PCR were cloned into the pCRII-TOPO vector (Invitrogen, Carlsbad, CA, USA). The full-length sequence of the cDNA was determined by 5′ and 3′ rapid amplification of the cDNA ends (5′-RACE and 3′-RACE) using a GeneRacer kit (Invitrogen). Isolated *MYB* gene was termed *GbMYB2* (accession number, AB550245). *GbMYB2* encoded a predicted protein of 276 amino acids. This protein shared high homology with MYBs, PAP1 in *Arabidopsis*, GMYB10 in *Gerbera*, and AN2 in *Petunia*, all of which regulate anthocyanin biosynthesis gene expression. Phylogenetic analysis showed that GbMYB2 is part of a group of MYB proteins that regulate anthocyanin biosynthesis (Figure 1A). We have found that GbMYB1, which has high homology with anthocyanin-related MYB proteins in the R2R3 domain, was up-regulated in MJ-treated roots and coordinate action of GbMYB1 with GbMYC1, bHLH transcription factor, activated *GbDFR* promoter, and *GbANS* promoter activity in tobacco protoplasts (Shimizu et al. personal communication). GbMYB2 shared 93.3% similarity with GbMYB1 in the R2R3 MYB domain, and it showed 60.8% similarity to GbMYB1 over the entire protein. In addition, GbMYB2 shared 91.3% similarity with GMYB10, 82.7% similarity with AN2, and 77.8% similarity with AtPAP1 in the R2R3 MYB domain, and has the amino acid residues $[DE]Lx_2[RK]x_3Lx_6Lx_3R$ that interact with bHLH (Grotewold et al. 2000; Zimmermann et al. 2004) (Figure 1B). This suggests that GbMYB2 might be related to the regulation of anthocyanin accumulation in *G. bicolor* leaves.

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Table 1. Name and accession numbers of genes and sequences of primers for standard RT-PCR analysis and quantitative real-time RT-PCR analysis

Gene (Accession No.)	Sense primer (5′ to 3′)	Antisense primer (5′ to 3′)
GbPAL (AB550238)	TCGCTTTCGAGAATGACACA	ATAGCCGTGAACACCCTGTC
GbCHS (AB550239)	TGTCAAGTGCGTGTGTTTTG	ATTGTGGTTGGGAGGCTATG
GbDFR (AB550240)	TTATGGAAGGCGGATTTGAC	CCCTTCGATTGTTGGCTTTA
GbANS (AB550241)	TCCACGAACCTTCCAACAAC	AATGACCCGAGCAACAACAT
GbMYB2 (AB550245)	GACGCCGTCAATGATACCTT	CAATTCCCTTCTCCTGGTCA
GbMYB1 (AB550244)	TTGATTGCCGGAAGAATACC	TCCGGGTGTTTAGGTTCTTG
GbMYC1 (AB550246)	ACGAGTGTTTGAGACAATGAGC	CGTTGACGCTGAACAACTCTTT
GbACTIN (AB550242)	TGAACTTCGTGTTGCTCCTG	CATAGCGGGAACATTGAAGG

For sucrose-treatment experiments, plantlets were cultured on MS solid medium (pH 5.7) containing 3% sucrose and 0.22 (w/v) Gelrite at 25°C under 14 h of light (70 μmol m^{-2} s^{-1})/day for 6 weeks, and leaves excised from the stem were incubated in a 9% sucrose solution for 5 days. Anthocyanins were extracted with 0.5% H_2SO_4 from treated leaves, and absorbance of the extract was measured. Anthocyanin content was calculated as cyanidin 3-*O*-glucoside equivalent from a standard curve using cyanidin 3-*O*-glucoside. Anthocyanins in leaves of cultured *G. bicolor* plantlets were barely accumulated; however, excised sucrose-induced leaves accumulated anthocyanins in the abaxial side (Figure 2).

The possible change in the transcriptional levels of anthocyanin biosynthesis genes and transcription factor genes *GbMYB2*, *GbMYB1,* and *GbMYC1* in the sucrose-treated leaves of cultured plantlets was tested by standard RT-PCR analysis and quantitative real-time RT-PCR (qRT-PCR) analysis. Total RNA was isolated from sucrose-treated leaves using an RNeasy Plant Mini kit. cDNA was synthesized from the isolated total RNA sample (1 mg) with a ReverTra Ace qPCR kit (Toyobo, Osaka, Japan). The synthesized cDNA was diluted 10 times in H_2O, and the diluted cDNA and gene-specific primers listed in Table 1 were used for standard RT-PCR and qRT-PCR analyses. Standard RT-PCR and qRT-PCR were performed using a SYBR Green Real-time PCR Master Mix-Plus- (Toyobo). The *actin* gene was used as the control for RT-PCR and qRT-PCR analyses.

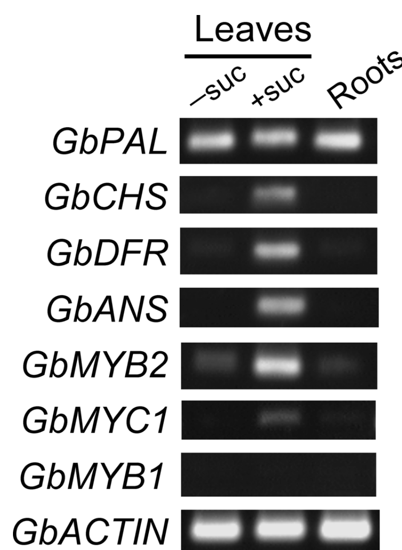

Figure 3. Expression of anthocyanin biosynthesis genes and *GbMYB2* in sucrose-treated leaves. Expression of anthocyanin biosynthesis genes and regulatory genes was detected by standard RT-PCR analysis. The *actin* gene of *G. bicolor* was also amplified as the control. RNA samples were prepared from leaves, which were cultured on water (−suc) or 9% sucrose solution (+suc) for 5 days, and roots of plantlets cultured on solid MS medium for 6 weeks. The thermal cycler condition for all reactions was 15 s at 95°C, 15 s at 58°C, 45 s at 72°C (for 28 cycles). Amplified products for *GbMYB1* were barely detected at 28 cycles.

Analysis of the expression of anthocyanin biosynthesis and regulatory genes revealed that the structural genes of the anthocyanin biosynthesis pathway were expressed more strongly in the sucrose-treated leaves (+suc) than in the untreated leaves (−suc) (Figures 3, 4). Expression of *GbCHS*, *GbDFR,* and *GbANS* genes was up-regulated strongly in sucrose-treated leaves. In particular, the *GbANS* expression level increased approximately 3600-fold with the sucrose treatment (Figure 4). In addition, expression of anthocyanin biosynthesis genes, *GbCHS* and *GbDFR,* was very low in roots (Figures 3, 4); however, the phenylpropanoid biosynthesis gene, *GbPAL,* was expressed at almost the same level in both leaves and roots (Figures 3, 4). These results indicate that anthocyanin accumulation in sucrose-treated leaves of cultured *G. bicolor* was influenced by transcriptional regulation of flavonoid biosynthesis genes. Anthocyanins of red *Perilla* (*P. frutescens*) and red cabbage (*Brassica oleracea* var. *captitata*) are used as food colorants. In *P. frutescens*, two formae, an anthocyanin-producing red forma and an anthocyanin nonproducing green forma, are known. Anthocyanin biosynthesis genes are activated in red forma, but not in green forma (Gong et al. 1997). In red forma, MYB-P1, MYB-RP, and PFWD participate in the regulation of the anthocyanin biosynthesis gene (Gong et al. 1999a; Gong et al. 1999b; Sompornpailin et al. 2002). In red cabbage, anthocyanin biosynthesis genes are activated during all stages of vegetative growth, and expression of regulatory genes, *bHLH* and *MYB*, are coordinately activated with structural genes (Yuan et al. 2009). In various other plants, it has been

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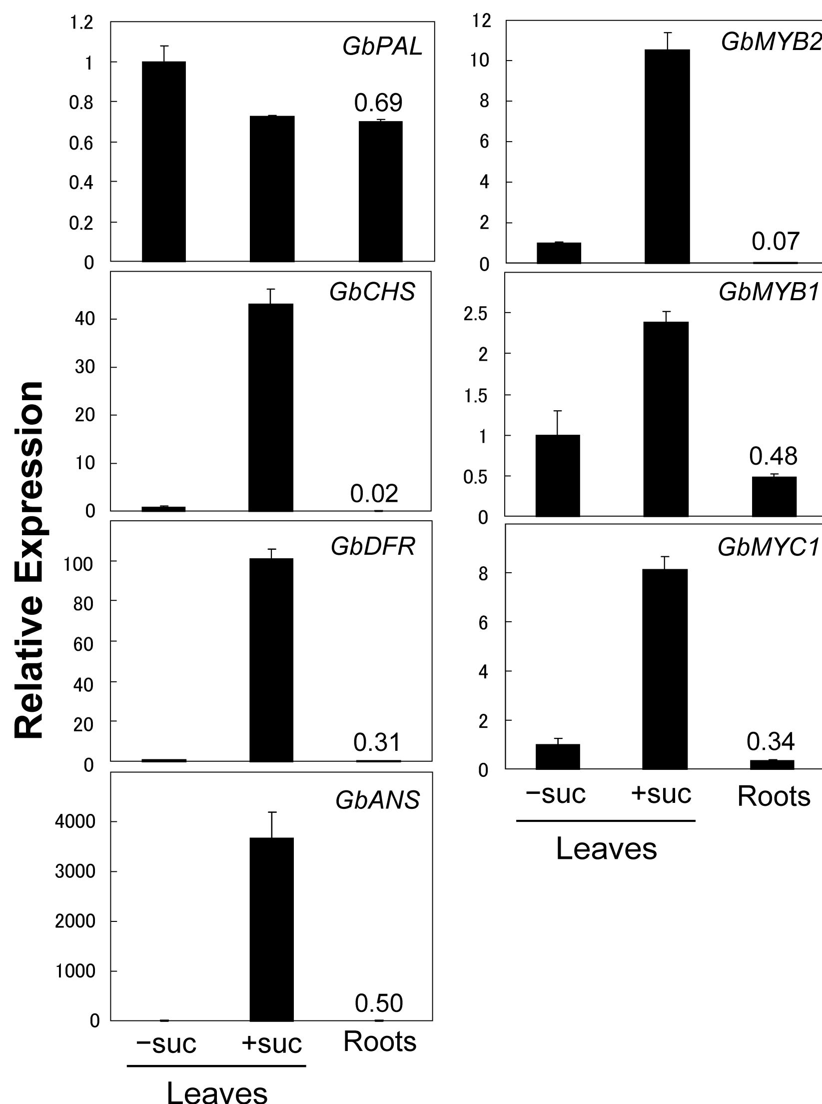

Figure 4. Expression levels of *GbMYB2* and anthocyanin biosynthesis genes in leaves treated with sucrose. Expression level of anthocyanin biosynthesis genes and regulatory genes was quantified using quantitative real-time RT-PCR. The *actin* gene of *G. bicolor* was used as an endogenous control for quantification. RNA samples were prepared as described in Figure 3. Expression levels of genes were shown as ratios relative to the sample of untreated leaves set as 1. The numbers on the columns indicate the ratio relative to expression of leaves untreated with sucrose (−suc). Error bars indicate standard error of three replicate reactions.

reported that the MYB transcription factors are related to the regulation of anthocyanin biosynthesis genes. In *Arabidopsis*, it was shown that AtPAP1 regulates anthocyanin accumulation in sucrose-treated leaves (Solfanelli et al. 2006). Expression analysis of regulatory genes revealed that *GbMYB2* was expressed strongly in sucrose-treated leaves and was approximately 10-fold up-regulated compared with that in untreated leaves (Figures 4). In roots, expression of *GbMYB2* was low, 0.07-fold, compared with that in untreated leaves (Figure 4). Both *GbMYB2* and *GbMYC1* were up-regulated in sucrose-treated leaves; latter up to 8.1-fold (Figure 4), suggesting that these regulatory factors might be related to anthocyanin regulation in *G. bicolor* sucrose-treated leaves. This also suggests that expression of these structural genes might affect the expression of GbMYB2 in *G. bicolor*. On the other hand, expression of *GbMYB1* was very low in both leaves and roots, and a drastic expression change of *GbMYB1* was not observed, suggesting GbMYB1 might not be involved in the regulation of anthocyanin biosynthesis genes expression in sucrose-treated leaves (Figures 3, 4).

To investigate the transcriptional activation ability of GbMYB2 from *GbDFR* and *GbANS* promoters, PEG-mediated transient expression analyses were carried out with tobacco leaf protoplasts, as described by Takeuchi et al. (2000) in *Arabidopsis* cultured cells with some modification. *GbDFR* and *GbANS* promoters fused to a firefly luciferase (*Luc*) gene were prepared as reporter constructs (pGbDFRpro-Luc and pGbANSpro-Luc)

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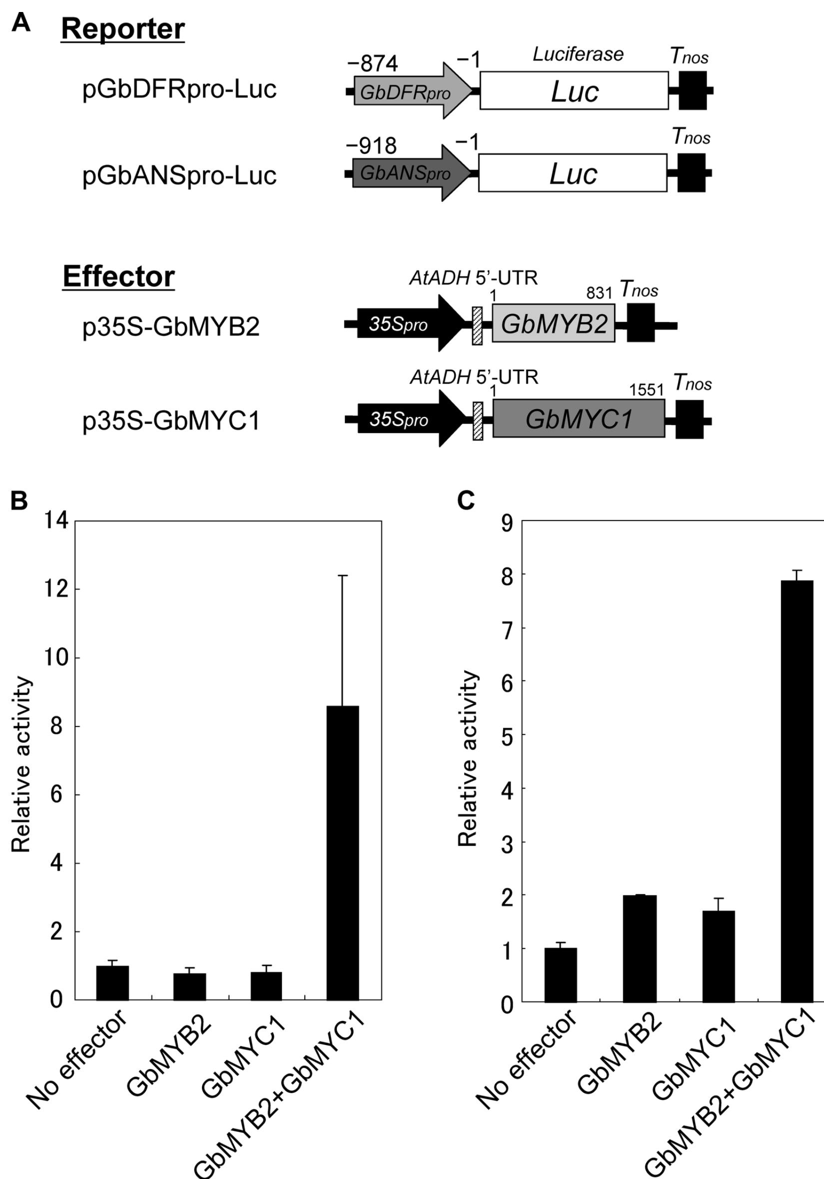

Figure 5. Transient expression analysis in tobacco leaf protoplasts using *GbDFR* and *GbANS* promoters and a combination of GbMYB2 and GbMYC1. (A) Schematic drawing of the reporter and effector constructs. In reporter constructs, the putative translation starting site is numbered +1. *GbDFRpro* and *GbANSpro* indicate *GbDFR* and *GbANS* promoter. *35Spro* and T_{nos} indicate cauliflower mosaic virus *35S* promoter and *Nos* terminater. (B) Activation of *GbDFR* promoter activity, and (C) *GbANS* promoter by co-expression of GbMYB2 and GbMYC1. The normalized promoter activity by Rluc was shown as relative activity set to 1 of the activity of the reporter construct only. Error bars indicate standard error of three independent measurements.

(Figure 5A). *GbMYB2* or *GbMYC1* cDNA driven by cauliflower mosaic virus 35S (CaMV35S) promoter were prepared as effector constructs (p35S-GbMYB2 or p35S-GbMYC1), which included *Arabidopsis ADH1* 5′-UTR as a translational enhancer sequence downstream of the CaMV35S promoter (Figure 5A). As a reference, a renilla luciferase (*Rluc*) gene, which was under the control of the same promoter, was cotransfected (p35S-Rluc). Protoplasts were cotransfected with p35S-GbMYB2, p35S-GbMYC1, pGbDFRpro-Luc or pGbANSpro-Luc, and p35S-Rluc. After culturing in the dark at 25°C for 22 h, Luc and Rluc activities were measured using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). The Luc activity relative to Rluc was calculated to show activation of the *GbDFR* or *GbANS* promoter by GbMYB2 and GbMYC1 (Figure 5B, C). Some anthocyanin-related MYB proteins require a partner protein, bHLH transcription factor, for activation of the target gene promoter (Grotewold et al. 2000; Spelt et al. 2000; Zimmermann et al. 2004). It was revealed in our previous study that co-expression of GbMYB1 and GbMYC1 activates *GbDFR* and *GbANS* promoters in tobacco leaf protoplasts (Shimizu et al. personal communication). Amino acid sequences

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between GbMYB2 and GbMYB1 were highly conserved in the R2R3 MYB domain, and in particular, they were conserved completely in the bHLH motif (Figure 1B), and both *GbMYB2* and *GbMYC1* expressed in cultured sucrose-treated plantlet leaves (Figures 3, 4). This suggests that GbMYC1 might act as a partner protein of GbMYB2 for activation of expression of anthocyanin biosynthesis genes, even in leaves; therefore, GbMYC1 was tested as a partner protein for transient expression assay. As a result, in transient expression analysis, it was shown that co-expression of GbMYB2 and GbMYC1 could activate *GbDFR* and *GbANS* promoters (Figure 5B, C). These results suggest that GbMYB2 acts as an anthocyanin regulator in *G. bicolor* leaves treated with sucrose. The sole expression of GbMYB2 could not completely activate *GbDFR* and *GbANS* promoters, *GbDFR* promoter was not activated by sole expression of GbMYB2 (Figure 5B), whereas *GbANS* promoter activity increased approximately 2-fold by sole expression of GbMYB2 (Figure 5C). Differences of *cis*-elements between *GbDFR* and *GbANS* promoters might give rise to the difference in these results. Further analyses about the relationship between *cis*-elements in the promoter regions of these genes and transcription regulatory factors will be required.

In this study, it was shown that GbMYB2 might be related to the regulation of anthocyanin biosynthesis in *G. bicolor* leaves. In *Arabidopsis*, it has been shown that PAP1 regulates both MJ-and sucrose-inducible anthocyanin accumulation (Loreti et al. 2008; Solfanelli et al. 2006). In *G. bicolor*, *GbMYB2* might be related to sucrose-inducible anthocyanin accumulation in leaves and *GbMYB1* might be related to MJ-inducible anthocyanin accumulation in roots (Shimizu et al. personal communication). The expression of *GbMYC1* was upregulated by MJ treatment (Shimizu et al. personal communication) and sucrose treatment (Figure 4), suggesting that *GbMYC1* might be related to both anthocyanin-accumulation phenomena. We are currently creating plants in our laboratory that overexpress these regulatory genes to analyze the regulatory mechanisms of anthocyanin biosynthesis in *G. bicolor*.

References

Borevitz JO, Xia Y, Blount J, Dixon RA, Lamb C (2000) Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. *Plant Cell* 12: 2383–2394

Broun P (2005) Transcriptional control of flavonoid biosynthesis: a complex network of conserved regulators involved in multiple aspects of differentiation in *Arabidopsis*. *Curr Opin Plant Biol* 8: 272–279

Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. *Photochem Photobiol* 70: 1–9

Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. *Plant Cell* 7: 1085–1097

Gong Z, Yamazaki M, Sugiyama M, Tanaka Y, Saito K (1997) Cloning and molecular analysis of structural genes involved in anthocyanin biosynthesis and expressed in a forma-specific manner in *Perilla frutescens*. *Plant Mol Biol* 35: 915–927

Gong Z, Yamazaki M, Saito K (1999a) A light-inducible *Myb*-like gene that is specifically expressed in red *Perilla frutescens* and presumably acts as a determining factor of the anthocyanin forma. *Mol Gen Genet* 262: 65–76

Gong Z, Yamagishi E, Yamazaki M, Saito K (1999b) A constitutively expressed *Myc*-like gene involved in anthocyanin biosynthesis from *Perilla frutescens*: molecular characterization, heterologous expression in transgenic plants and transactivation in yeast cells. *Plant Mol Biol* 41: 33–44

Gonzalez A, Zhao M, Leavitt JM, Lloyd AM (2008) Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in *Arabidopsis* seedlings. *Plant J* 53: 814–827

Grotewold E, Sainz MB, Tagliani L, Hernandez JM, Bowen B, Chandler VL (2000) Identification of the residues in the Myb domain of maize C1 that specify the interaction with the bHLH cofactor R. *Proc Natl Acad Sci USA* 97: 13579–13584

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. *Phytochemistry* 55: 481–504

Koes R, Verweij W, Quattrocchio F (2005) Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. *Trends Plant Sci* 10: 236–242

Loreti E, Povero G, Novi G, Solfanelli C, Alpi A, Perata P (2008) Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in *Arabidopsis*. *New Phytol* 179: 1004–1016

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15: 473–497

Nesi N, Debeaujon I, Jond C, Pelletier G, Caboche M, Lepiniec L (2000) The *TT8* gene encodes a basic helix-loop-helix domain protein required for expression of *DFR* and *BAN* genes in *Arabidopsis* siliques. *Plant Cell* 12: 1863–1878

Payne CT, Zhang F, Lloyd AM (2000) GL3 encodes a bHLH protein that regulates trichome development in *Arabidopsis* through interaction with GL1 and TTG1. *Genetics* 156: 1349–1362

Shimizu Y, Imada T, Zhang H, Tanaka R, Ohno T, Shimomura K (2010) Identification of novel poly-acylated anthocyanins from *Gynura bicolor* leaves and their antioxidative activity. *Food Sci Technol Res* 16: 479–486

Solfanelli C, Poggi A, Loreti E, Alpi A, Perata P (2006) Sucrose-specific induction of the anthocyanin biosynthetic pathway in *Arabidopsis*. *Plant Physiol* 140: 637–646

Sompornpailin K, Makita Y, Yamazaki M, Saito K (2002) A WD-repeat-containing putative regulatory protein in anthocyanin biosynthesis in *Perilla frutescens*. *Plant Mol Biol* 50: 485–495

Spelt C, Quattrocchio F, Mol JN, Koes R (2000) Anthocyanin1 of petunia encodes a basic helix-loop-helix protein that directly activates transcription of structural anthocyanin genes. *Plant Cell* 12: 1619–1632

Takeuchi M, Ueda T, Sato K, Abe H, Nagata T, Nakano A (2000) A dominant negative mutant of sar1 GTPase inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus in tobacco and *Arabidopsis* cultured cells. *Plant J* 23: 517–525

Tohge T, Nishiyama Y, Hirai MY, Yano M, Nakajima J, Awazuhara M, Inoue E, Takahashi H, Goodenowe DB, Kitayama M et al. (2005) Functional genomics by integrated analysis of

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metabolome and transcriptome of *Arabidopsis* plants over-expressing an MYB transcription factor. *Plant J* 42: 218–235

Yoshitama K, Kaneshige M, Ishikura N, Araki F, Yahara S, Abe K (1994) A stable reddish purple anthocyanin in the leaf of *Gynura aurantiaca* cv. 'Purple Passion'. *J Plant Res* 107: 209–214

Yuan Y, Chiu LW, Li L (2009) Transcriptional regulation of anthocyanin biosynthesis in red cabbage. *Planta* 230: 1141–1153

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

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